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Different Flushing Frequency on Blood Metabolites Profile of Ewes and Their Lambs at Pre-Weaning Period

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ABSTRACT

This study determined the effects of different flushing frequency on performance and blood metabolite profile of ewes and their suckling lambs at pre-weaning period. Twelve multiparous Garut ewes (2 years-old, BW 30.06 ± 6.20 kg) and 18 lambs born to experimental ewes (lambing weight 2.49 ± 0.56 kg) were used in this study. Ewes were randomly assigned into four treatment groups in a complete block design, namely: without flushing (T0: control), flushing at the beginning of mating (T1: 2 weeks before and after mating), two times flushing (T2: T1 + 4 weeks flushing at mid-gestation), and three times flushing (T3: T2 + 2 weeks flushing at before and after parturition). Three times flushing increased ($p < 0.05$) the crude fat intake, while different flushing frequencies did not affect ($p > 0.05$) dry matter intake and intakes of crude protein, crude fiber, nitrogen-free extract, and total digestible nutrients of ewes at the pre-weaning period. Different flushing frequencies did not change ($p > 0.05$) the productive performances of ewes during the pre-weaning period. Flushing application improved ($p < 0.05$) the average daily gain of pre-weaning lambs at 14 days, but it did not affect the weaning weight of lambs. Two times flushing showed no pre-weaning mortality rate ($p < 0.05$). Flushing application tended to decrease ($p = 0.08$) blood plasma triglyceride of ewes at 21- days, while two times flushing frequency tended to increase blood plasma cholesterol ($p = 0.05$) and triglyceride ($p = 0.08$) of lambs at 21 days. In conclusion, increased flushing frequency supports ewes and their twin lamb growth performance and blood metabolite profile at the pre-weaning period.

Keywords: Blood metabolites, Flushing frequency, Growth, Performance, Pre-weaning

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Introduction

Pregnancy and lactation are challenging states that affect maternal and lamb health. Energy demand increases at the end of pregnancy since more energy and protein are required to support rapid fetal development. Meanwhile, early lactation requires metabolic adjustment in mammary and non-mammary tissues to support milk production (Roche, 2018). To cope with those challenges, ewes enhance their feeding frequency, increase lipolysis and proteolysis, and reduce lipogenic activity (Opsomer *et al.*, 2017). However, ewe with multiple pregnancy which is not supported by adequate nutrition during pregnancy often associates with low birthweight and growth rate, as well as high pre-weaning mortality rate lambs (Ridler *et al.*, 2022; Sudarman, 2019). Therefore, providing a high energy diet for ewes during pregnancy and lactation is essential to support lamb growth (Behrendt *et al.*, 2019).

Flushing is a management program to improve the body condition so that the ewe is ready for reproduction. Flushing diet, a high-quality ration

can be fed to ewe for two to three weeks prior to and during the breeding season to fulfill ewe's energy requirement (Farrag, 2019; Mostafa and Farghal, 2022). Flushing rations may contain high amounts of carbohydrates or fat, increasing energy intake and improving body condition and fertility. Flushing can be given at certain periods, such as before mating, and at early, mid, and late gestation (Khotijah *et al.*, 2022). Flushing using a diet containing DHA and EPA before mating and during lactation increases milk yield and positively affects reproductive performance of sheep (Nguyen *et al.*, 2019). Flushing at 3 weeks before and 2 weeks after mating and late gestation increased the ewe's milk production and improved lamb weaning weight (Nugroho *et al.*, 2020).

Lemuru fish oil and coconut oil are two fat source ingredients used in this study. Lemuru fish oil contains 36.61% poly-unsaturated fatty acid (PUFA), 12.85% mono-unsaturated fatty acid (MUFA), and 28.57% saturated fatty acid (SFA) (Dari *et al.*, 2017), while coconut oil mostly contains of SFA, such as 32.73% lauric acid and 28.55% myristic acid (Novilla *et al.*, 2017). Lemuru fish oil

contains high omega-3 PUFA reached about 20-26% combination of EPA and DHA (Kosasih *et al.*, 2021). Aidismen *et al.* (2018) reported that omega-3 poly-unsaturated fatty acid on flushing diet extend estrous period and improve the prolific index. A combination of lemuru fish oil and coconut oil on flushing diet increases ewe's BCS, cholesterol, and glucose concentration at early pregnancy (Nurlatifah *et al.*, 2020). A two times provision of flushing diet containing lemuru fish oil (at 2 weeks before and after mating, and 2 weeks before and after parturition) improves the immune system of ewes (Nurlatifah *et al.*, 2022). Supplementation of 2% fish oil rich in EPA and DHA in late gestation enhances ewe's milk quality and lamb's suckling ability (Nickles *et al.*, 2019). Averós *et al.* (2022) reported that PUFA supplementation at final stage of gestation improves the immune response of lambs at early stages of life.

Blood metabolites have been referred to as important indicators of livestock nutritional and metabolic status since they represent the final results of complex processes taking place within the cell (Astuti *et al.*, 2022; Ghaffari *et al.*, 2017; Goldansaz *et al.*, 2017). Blood biochemical composition accurately mirrors the livestock metabolic conditions providing insights of animal adaptation to nutritional or physiological challenges and specific health status on certain period (Hernández *et al.*, 2020). Pregnancy and lactation lead to significant changes in several metabolites associated with energy, mineral, and acid-base balances (Antunović *et al.*, 2017; Khayat *et al.*, 2017; Pesántez-Pacheco *et al.*, 2019a). The metabolic state of ewe plays a crucial role in determining birth weight of their offspring. Challenges in maternal metabolism may affect health and productivity of the lambs. When appropriately fed, prolific ewes can meet the metabolic requirements of both pregnancy and milk production, leading to improved pre-weaning performance in lambs (Pesántez-Pacheco *et al.*, 2019b).

Generally, flushing rations containing fat sources have only been provided before and after mating and late pregnancy. Limited information about the effect of flushing frequency on ewes and lambs' performance and blood metabolite profiles is available. Therefore, this study aims to evaluate the effect of different flushing frequencies, namely before and after mating, before and after mating continuing to the middle of pregnancy, and before and after mating, middle of pregnancy, and late pregnancy on ewes and lambs' performance and blood metabolites at pre-weaning period.

Materials and Methods

This study was performed at the Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Indonesia, from January until March 2021. This study was approved by The Animal Care and Use

Committee of IPB University (Ethical approval Number 120-2020/IPB).

Animals and diets

Twelve multiparous Garut ewes (2 years old, BW 30.06 ± 6.20 kg) and 18 lambs born to experimental ewes (lambing weight 2.49 ± 0.56 kg) were used in this study. Ewes were classified according to their body weight into light, medium, and heavy. Then they were randomly assigned into four treatment groups in a completely randomized block design consisting of four feeding treatments and 3 replications. The feeding treatments were namely: without flushing (T0: control), flushing at the beginning of mating (T1: 2 weeks pre-mating until 2 weeks just after-mating), two times flushing (T2: T1 + 4 weeks flushing at mid-gestation), and three times flushing (T3: T2 + 2 weeks flushing at the end of gestation and 2 weeks after parturition). Each treatment consisted of three ewes. Eighteen lambs were divided into four treatment groups following the group of maternal ewes. Ewes and lambs were kept in individual cages. Lambs were allowed to suckle from birth until weaning (56 days old).

During the study, ewes were fed a ration at 3.5% of BW (DM basis). Ration consisted of forage (*Pennisetum purpureum*) and concentrate with a ratio of 30:70 (DM basis). The concentrate diet of the control group was composed of soybean meal, pollard, dried cassava, molasses, CaCO₃, NaCl, and premix. About 2% of coconut oil and 5% of Lemuru fish oil were used in flushing concentrate to obtain twice the maintenance requirement of EPA and DHA (Nurlatifah *et al.*, 2022). The chemical composition of diets was analyzed according to AOAC (2019). The composition of experimental concentrates is shown in Table 1, and the chemical composition of the experimental diet is shown in Table 2. Feed was provided three times daily at 07.00 am, 11.00 am, and 03.00 pm, and water was served *ad libitum*. The flushing concentrate was provided in accordance with the flushing management program (Figure 1). Flushing at the beginning of mating was administered 2 weeks before and after mating. Flushing at mid-gestation was provided at 2 weeks before the 75th day of pregnancy and 2 weeks after the 75th day of pregnancy. Meanwhile, flushing at late pregnancy was given at 2 weeks before and after parturition. Subsequently, ewes were fed with a control ratio throughout pregnancy and lactation.

Table 1. Ingredients composition of concentrates

Feed ingredients	Concentrate	
	Control	Flushing
Soybean meal (%)	25.00	28.00
Wheat pollard (%)	28.29	24.00
Cassava meal (%)	34.00	28.30
Coconut oil (%)	-	2.00
Lemuru fish oil (%)	-	5.00
Molasses (%)	10.00	10.00
CaCO ₃ (%)	1.00	1.00
Premix (%)	1.00	1.00

Table 2. Chemical composition of experimental diet

Nutrients	Control	Flushing
DM (%)	73.30	72.35
CP (%)	15.86	15.95
EE (%)	1.17	6.17
CF (%)	16.47	14.92
NFE (%)	54.79	50.62
TDN ¹ (%)	66.52	71.03
Ca (ppm)	11.05	2.57
P (ppm)	5.80	6.35

DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, TDN: total digestible nutrient.
¹TDN (%) = $-37.3039 + (1.3048 \times \%CP) + (1.3630 \times \%NFE) + (2.1302 \times \%EE) - (0.1034 \times \%CF)$ (Wardeh, 1981).

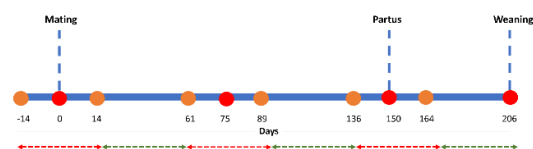


Figure 1. Flushing management program during experiment.

Nutrient intake and growth performance

The feed consumption and nutrient intake were measured just after parturition until 56 days. Samples of forage, concentrate, and their residues were collected daily throughout the experimental period and stored at -20°C until analysis. Daily intakes of concentrate and forage were calculated by removing the residual feed from the offered feed. The nutrient intake was calculated by multiplying the feed intake by each feed's nutrient content. The body weights of ewes and lambs were measured within 24 hours after lambing. After that, the body weight was measured every 2 weeks before morning feeding to determine the average daily gain (ADG) until the end of the experiment.

Blood metabolites

Blood samples of ewes were taken at parturition, 21 days, and 56 days after lambing, while the blood samples of lambs were taken at 21 days and 56 days after lambing. Blood was collected from the jugular vein of ewes and lamb using a 1-mL syringe into a 10 mL EDTA tube for measuring blood metabolites. Subsequently, blood samples were centrifuged at 3000 g for 15 minutes to separate plasma and supernatants. The collected plasma samples were stored at -20°C until analysis. The blood glucose, triglyceride, and cholesterol were analyzed using commercial kit number 112191, 16392, and 101592 from Greiner GMBH (Bahlingen, Germany), whereas the blood

urea nitrogen concentration was analyzed using commercial kit number 10505 from Human (Wiesbaden, Germany) followed the company instructions.

Statistical analysis

All data were analyzed as a completely randomized block design using the analysis of variance performed in SPSS 26.0 (SPSS, Chicago, IL). Ewes or lambs were used as the experimental units. Any statistical difference was analyzed using Duncan's multiple-range test. All data are presented as the mean \pm standard deviation. Results were considered statistically significant at $p < 0.05$, while trends were identified for $0.05 < p \leq 0.10$.

Results and Discussion

Performance of Ewes and Lambs after Lambing

Three times flushing significantly increased ($p < 0.05$) the crude fat intake of ewes after lambing, whereas the different flushing frequency did not affect ($p > 0.05$) intake of other nutrients (Table 3). Different flushing frequencies did not affect ($p > 0.05$) ewes' performance after lambing, including weight at weaning, percentage of weight changes, and average daily gain (Table 4). The different flushing frequencies did not affect ($p > 0.05$) the weaning weight of lambs. Though, all flushing treatments significantly affected ($p < 0.05$) to the average daily gain of lambs at day 14 compared to the control (without flushing) (Table 4). Two times flushing (T2) showed no pre-weaning mortality rate ($p < 0.05$).

The higher crude fat intake in T3 was due to an increase in the frequency of flushing. This result is in accordance with Bianchi *et al.* (2018) and Rizzo *et al.* (2024), who reported that the inclusion of feed sources rich in specific fatty acids, such as sunflower oil and palm oil could lead to an increase in ether extract intake. High feed fat content increases feed energy density which is useful for increasing steroid hormone (Farrag *et al.*, 2024). It also may influence the gene regulation leading to mammary gland development (Conte *et al.*, 2021). In this study, lemuru fish oil used as a fat source contains n-3 n-polyunsaturated fatty acid, i.e. EPA 3.70% and DHA 22.47%. Studies have shown the crucial role of EPA and DHA in modulating gene expression, lipid metabolism, and inflammatory

Table 3. Nutrient intake of ewes fed with different flushing frequency at pre-weaning period

Parameters	Treatment				P-value
	T0	T1	T2	T3	
	g/head/day				
Dry matter	974.81 \pm 150.15	1100.81 \pm 55.31	1078.60 \pm 159.79	1049.80 \pm 124.58	0.76
Crude protein	163.07 \pm 26.25	182.55 \pm 10.27	179.56 \pm 25.54	173.23 \pm 23.85	0.79
Crude fiber	141.74 \pm 19.40	163.61 \pm 6.26	158.76 \pm 25.88	151.87 \pm 20.54	0.66
Crude fat	10.40 \pm 1.47 ^b	11.94 \pm 0.49 ^b	11.61 \pm 1.85 ^b	24.44 \pm 4.32 ^a	0.003
NFE	581.51 \pm 92.00	653.24 \pm 35.16	641.56 \pm 92.76	608.61 \pm 82.91	0.75
TDN	690.14 \pm 108.59	776.11 \pm 41.18	761.87 \pm 110.71	751.55 \pm 104.40	0.77

NFE: nitrogen free extract, TDN: total digestible nutrient, T0: without flushing, T1: flushing at the beginning of mating, T2: two times flushing (T1 + 4 weeks flushing at mid gestation), T3: three times flushing (T2 + 2 weeks flushing at the end of gestation and 2 weeks after parturition).

Table 4. Different flushing frequency effect on productive performance of ewes and lambs' at pre-weaning period

Parameters	Treatment				P-value
	T0	T1	T2	T3	
Ewes					
Weight at weaning	36.17 ± 10.30	41.33 ± 4.93	41.67 ± 5.86	38.88 ± 6.91	0.21
Weight changes (%)	5.64	8.50	12.27	10.54	-
Average daily gain (g/day)	-36.11 ± 17.35	-63.89 ± 29.27	-97.22 ± 33.68	-108.34 ± 52.04	0.37
Lambs					
Weaning weight (kg)	10.82 ± 1.86	11.16 ± 1.46	11.25 ± 1.38	11.34 ± 0.74	0.97
ADG at 14 days (g/day)	66.57 ± 62.92 ^a	181.00 ± 45.93 ^b	163.42 ± 43.38 ^b	197.71 ± 41.20 ^b	0.02
ADG at 28 days (g/day)	156.50 ± 68.83	157.47 ± 7.31	144.21 ± 28.94	167.79 ± 17.96	0.78
ADG at 42 days (g/day)	149.17 ± 39.41	151.41 ± 8.36	141.58 ± 20.06	155.91 ± 17.78	0.78
ADG at 60 days (g/day)	139.42 ± 27.66	147.02 ± 15.10	145.49 ± 20.49	145.63 ± 6.35	0.96
Mortality (%)	25	33.33	0	25	-

T0: without flushing, T1: flushing at the beginning of mating, T2: two times flushing (T1 + 4 weeks flushing at mid gestation), T3: three times flushing (T2 + 2 weeks flushing at the end of gestation and 2 weeks after parturition).

pathways, which ultimately affect the health and productivity of ewes (Oscarsson and Hurt-Camejo, 2017; Peng *et al.*, 2020). Supplementing pregnant ewes with EPA and DHA modifies the fatty acid profile of plasma, colostrum, and milk which may enhance lipogenesis (Coleman *et al.*, 2018; Nguyen *et al.*, 2018; Nurlatifah *et al.*, 2022). Thus, colostrum's fatty acid composition changes may improve lambs' health and growth (Nurlatifah *et al.*, 2023).

Changes in ewe's live weight result from differences between energy intake and energy requirements. Among treatments, T3 showed a higher ewe's body weight loss percentage than control during lactation (Table 4). This result may be due to higher litter size. In line with Cordero *et al.* (2019), ewes carrying more than two lambs gain less weight during late pregnancy and lose more weight during the lactation/pre-weaning period. Moreover, the ewe's body weight loss may be influenced by higher lamb's total body weight/ewe (Chay-Canul *et al.*, 2019). Sarvinda *et al.* (2022) reported that lactating ewes may decrease body weight due to insufficient nutrient intake to support milk production. Thus, body fat mobilization resulted in weight loss in ewe during early lactation (Nugroho *et al.*, 2020). We conclude that three times flushing successfully increased the litter size; however, it still failed to maintain the ewe's body weight during the lactation/pre-weaning period. Further study is needed to determine the optimal coconut and Lemuru fish oil levels to enhance energy content and the EPA and DHA levels in the flushing ration on the lactation/pre-weaning period.

Different flushing frequencies did not influence the weaning weight of lambs. Meanwhile, all flushing treatments improved the lamb's daily gain at 14 days compared to the control. This result showed that flushing treatment may improve milk quality without changing ewes' milk yield, which is reflected in higher lamb daily gain. Milk yields of ewes increased gradually from week 1 to week 3 of lactation and declined gradually. The first few weeks of lambs' growth are crucially reliant on their mother's milk, although most of them have started to consume grass and concentrate (Ahmed *et al.*, 2017; Wang *et al.*, 2023). Thus, higher milk production resulted in a greater average daily gain of lambs (Chay-Canul *et al.*, 2019; Martins *et al.*, 2022). In addition, changes in milk quality, including fat and energy concentration, contribute to faster

lamb growth (Abecia *et al.*, 2021; Zdorovieva *et al.*, 2019). Our study confirms that flushing treatment may provide sufficient energy for ewes to produce milk reflected on the improvement of lamb body weight at 14 days (early lactation) compared to the control. However, the difference in flushing frequencies may not affect milk production quantity and subsequently the lamb body weights.

The mortality rate in this study was higher than Flinn *et al.* (2020), who reported that the pre-weaning mortality rate of lamb remained stable at 15-20% over the past 4 decades. However, there was no pre-weaning mortality rate on T2. A higher mortality rate may be caused by higher litter size, which may cause the nutrient competition of twin lambs. In this study, flushing frequency increases the litter size more than the duplet twin. The duplet-born lambs occurred in T1, T2, and T3 for about 50%, 100%, and 75%, respectively, whereas triplet-born lambs occurred in T1 at about 25%, and quartet-born lambs occurred in T3 at about 25%. Meanwhile, the mortality mostly occurred in quartet-born lambs. It has been highlighted that larger litter-sized lambs generally exhibit high mortality rates (Kenyon *et al.*, 2019; Whatford *et al.*, 2023). Factors such as the sex of the lamb, maternal age, and birth weight can impact the survival rates (Douh *et al.*, 2019). Additionally, infectious disease and failure of passive immunity could contribute to higher mortality rates in larger litter-size lambs (Abuzahra *et al.*, 2024). In this study, the nutrient on two times flushing management may be adequate to support ewe's milk production for twin-born lambs, which is reflected in the fact that there is no pre-weaning mortality rate. Further studies are needed to determine the optimum EPA and DHA content or in combination with other nutrients on a flushing diet to support ewes with triplet/quartet-born lambs. Our study concluded that different flushing frequencies did not affect ewe's performance during the lactation period, but it may increase the lamb's average daily gain at 14 days.

Blood plasma metabolite profiles of Ewes and Lambs at pre-weaning period

Glucose, cholesterol, triglyceride, and blood urea nitrogen may reflect animal nutritional status. Different flushing frequencies tended to decrease ($p=0.08$) the blood plasma triglyceride of ewes 21 days after lambing (Table 5). Different flushing

frequencies did not affect ($p>0.05$) plasma glucose, cholesterol, and urea nitrogen of ewes until 60 days after lambing. In this study, a more frequent flushing diet increased the crude fat intake since the flushing diet contained high Lemuru fish oil. Still, a tendency of low blood triglyceride has occurred to ewes at 21 days of lactation. In accordance with Tharwat *et al.* (2023), blood triglyceride decreases during lactation due to it being a crucial source of long-chain fatty acids for milk production. Lactation may be associated with lower lipid levels and decreased insulin resistance post-partum (Countouris *et al.*, 2020). Over time, lactation makes peripheral cells more sensitive and responsive to insulin, reducing the use of lipid metabolites for gluconeogenesis and raising their levels during the mid and late stages of lactation (Rathod *et al.*, 2024). In this study, flushing application resulted in more litter size (twin and quartet-born lamb) than control which causes higher milk production in ewes suckling twins at early lactation (Dove *et al.*, 2018; Rosales Nieto *et al.*, 2018). Our results confirm that more frequent provision of flushing contributes to higher crude fat intake, subsequently decreasing blood triglyceride used to support adequate milk production during peak lactation.

In this study, the blood glucose of ewes was relatively stable during lactation. This result indicates gluconeogenesis continues to function efficiently to ensure a stable glucose supply during lactation. Net hepatic gluconeogenesis is a complex interplay of various pathways. The liver adapts to maintain glucose homeostasis even in the low blood triglyceride levels. Wang *et al.* (2023) reported that dietary protein levels during late gestation have a significant effect on blood triglyceride in lactation ewes. This suggests that dietary factors can impact metabolic parameters, but the body's inherent regulatory mechanisms maintain blood glucose levels through gluconeogenesis.

Different flushing frequency tended to increase blood plasma cholesterol ($p=0.05$) and triglyceride ($p=0.08$) of lambs at 21 days (Table 6). Different flushing frequencies did not affect ($p>0.05$) plasma glucose and urea nitrogen of lamb at 21 and 60 days. These results were in accordance with the results for blood metabolites of ewes. Silva *et al.* (2022) reported that up to 85% of blood glucose is used for milk lactose synthesis in the mammary gland. Therefore, the lamb will consume the ewe's milk lactose, influencing the lamb's blood glucose concentration. In line with our

Table 5. Different flushing frequency effect on blood plasma metabolite profiles of ewes at pre-weaning period

Parameters	Treatment				p-value	Reference
	T0	T1	T2	T3		
Glucose (mg/dL)						
Day-1 (lambing/early lactation)	65.31 ± 8.50	47.95 ± 6.76	50.53 ± 15.33	50.87 ± 9.56	0.25	50-80 ¹
Day-21 (peak lactation)	53.53 ± 1.44	54.32 ± 0.66	51.92 ± 3.53	54.36 ± 1.68	0.49	
Day-60 (late lactation)	49.17 ± 4.28	50.69 ± 4.26	56.73 ± 0.14	52.38 ± 2.61	0.10	
Cholesterol (mg/dL)						
Day-1 (lambing/early lactation)	31.35 ± 4.59	28.88 ± 3.19	29.17 ± 3.34	30.84 ± 4.76	0.84	13-117 ²
Day-21 (peak lactation)	31.32 ± 4.68	27.91 ± 5.28	24.86 ± 1.09	26.87 ± 2.74	0.29	
Day-60 (late lactation)	33.18 ± 6.40	32.42 ± 4.36	30.14 ± 1.37	34.70 ± 2.41	0.61	
Triglyceride (mg/dL)						
Day-1 (lambing/early lactation)	15.04 ± 1.77	15.93 ± 3.54	15.34 ± 1.02	18.29 ± 5.62	0.67	9-30 ¹
Day-21 (peak lactation)	21.62 ± 1.80	17.12 ± 1.56	20.72 ± 1.80	20.72 ± 2.38	0.08	
Day-60 (late lactation)	11.76 ± 0.84	12.52 ± 3.88	10.26 ± 2.54	9.80 ± 2.43	0.59	
Urea nitrogen (mg/dL)						
Day-1 (lambing/early lactation)	31.50 ± 1.87	29.47 ± 9.27	26.79 ± 4.07	26.75 ± 4.56	0.78	7-55.8 ²
Day-21 (peak lactation)	36.25 ± 0.95	39.77 ± 4.13	41.35 ± 2.00	39.02 ± 1.08	0.15	
Day-60 (late lactation)	33.40 ± 5.65	35.00 ± 5.31	29.08 ± 2.03	28.60 ± 4.77	0.26	

T0: without flushing, T1: flushing at the beginning of mating, T2: two times flushing (T1 + 4 weeks flushing at mid gestation), T3: three times flushing (T2 + 2 weeks flushing at the end of gestation and 2 weeks after parturition), ¹Kaneko *et al.* (2008), ²Varanis *et al.* (2021b).

Table 6. Different flushing frequency affect on blood plasma metabolite profiles of lamb at 21 and 60 days

Parameters	Treatment				p-value	References
	T0	T1	T2	T3		
Glucose (mg/dL)						
Day-21 (peak lactation)	82.07 ± 12.17	98.36 ± 19.37	88.30 ± 10.32	86.49 ± 8.19	0.38	33-98.1 ¹
Day-60 (late lactation)	78.94 ± 9.68	79.60 ± 9.54	88.78 ± 5.86	76.43 ± 9.15	0.12	
Cholesterol (mg/dL)						
Day-21 (peak lactation)	84.71 ± 8.61	83.00 ± 13.69	96.61 ± 18.98	69.03 ± 9.86	0.05	15-139.3 ¹
Day-60 (late lactation)	91.93 ± 10.75	71.69 ± 19.80	74.81 ± 15.52	72.24 ± 15.41	0.35	
Triglyceride (mg/dL)						
Day-21 (peak lactation)	49.59 ± 6.15	61.68 ± 7.06	92.56 ± 31.41	78.86 ± 24.54	0.08	5-78 ¹
Day-60 (late lactation)	55.04 ± 10.50	45.38 ± 2.06	47.34 ± 10.69	57.48 ± 19.28	0.44	
Urea nitrogen (mg/dL)						
Day-21 (peak lactation)	19.75 ± 1.55	19.74 ± 4.75	21.92 ± 5.76	21.46 ± 2.79	0.83	12.8-100 ¹
Day-60 (late lactation)	21.92 ± 2.60	23.93 ± 4.14	23.71 ± 4.31	23.64 ± 2.80	0.88	

T0: without flushing, T1: flushing at the beginning of mating, T2: two times flushing (T1 + 4 weeks flushing at mid gestation), T3: three times flushing (T2 + 2 weeks flushing at the end of gestation and 2 weeks after parturition), ¹Varanis *et al.* (2021b).

study, the flushing diet containing specific fatty acids (EPA and DHA) may increase milk fat content and alter fatty acid composition (Coleman *et al.*, 2018; Parente *et al.*, 2018). Fatty acid supplementation contained in a flushing diet may raise blood triglyceride levels in lambs by transferring fatty acids from the maternal diet to the milk. Thus, higher milk fat may elevate the plasma triglyceride and cholesterol concentration of lamb consuming this milk (Huang *et al.*, 2022).

In this study, lambs' high blood cholesterol and triglyceride in flushing treatments may be associated with higher lamb's ADG than control. Elevated blood triglyceride and cholesterol levels are essential in lambs since these molecules store energy reserves (Nurlatifah *et al.*, 2022). The blood urea nitrogen in lamb is affected by milk protein (urea) consumed. Urea in milk originates from body protein degradation and feed protein degradation via the urea cycle, which is transported by the blood (Getahun *et al.*, 2019). In addition, blood urea nitrogen concentration is related to milk urea nitrogen (Harjanti *et al.*, 2017). From this study, it can be concluded that flushing application may in part improve the nutritional status of twin-bearing ewes and their lambs during the pre-weaning period. The different flushing frequencies may contribute in the blood metabolite profile of lactation ewes and lambs during the pre-weaning period.

Conclusion

Increasing flushing frequency up to three times (before and after mating, middle of pregnancy, and late pregnancy) may improve the nutritional status of twin-bearing ewes and their lambs during pre-weaning. Three times flushing increases the ewe's crude fat intake, subsequently enhancing their blood metabolite profile and potentially leading to better health outcomes for both the ewes and their lambs.

Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

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Author's contribution

The authors confirm their contribution to the paper as follows: Study conception and design: L. K., D. A. A., D. D., D. M. F.; Data collection: M. T., F. R. D., L. K., D. M. F.; Analysis and interpretation of results: M. T., F. R. D., L. K., D. A. A., D. D., D. M. F.; Draft manuscript preparation: D. M. F., L. K.;

All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

There are no human subjects in this article and informed consent is not applicable. This study was approved by The Animal Care and Use Committee of IPB University (Ethical approval Number 120-2020/IPB).

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