Buletin Peternakan 48 (2): 166-175, August 2024

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v48i3.93637

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

Dilla Mareistia Fassah, Mila Taniasari, Fanny Rahmasari Daeli, Didid Diapari, Dewi Apri Astuti, and Lilis Khotijah*

Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia

Article history
Submitted: 29 January 2024
Accepted: 24 July 2024

* Corresponding author:
E-mail: liliskh@apps.ipb.ac.id

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, Fushing frequency, Growth, Performance, Pre-weaning

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, $CaCO_3$, 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.
[1]TDN (%): -37.3039 +(1.3048 x %CP) + (1.3630 x %NFE) + (2.1302 x %EE) – (0.1034 x %CF) (Wardeh, 1981).

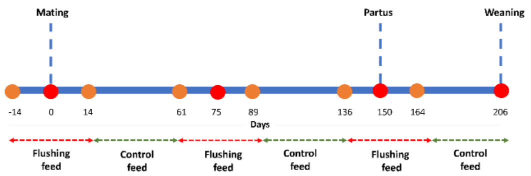

Figure 1. Flushing management program during experiment.

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 -20ºC 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 -20ºC 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 ± standard deviation. Results were considered statistically significant at $p<0.05$, while trends were identified for $0.05<p\leq0.10$.

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 ± 150.15	1100.81 ± 55.31	1078.60 ± 159.79	1049.80 ± 124.58	0.76
Crude protein	163.07 ± 26.25	182.55 ± 10.27	179.56 ± 25.54	173.23 ± 23.85	0.79
Crude fiber	141.74 ± 19.40	163.61 ± 6.26	158.76 ± 25.88	151.87 ± 20.54	0.66
Crude fat	10.40 ± 1.47[b]	11.94 ± 0.49[b]	11.61 ± 1.85[b]	24.44 ± 4.32[a]	0.003
NFE	581.51 ± 92.00	653.24 ± 35.16	641.56 ± 92.76	608.61 ± 82.91	0.75
TDN	690.14 ± 108.59	776.11 ± 41.18	761.87 ± 110.71	751.55 ± 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), [1]Kaneko *et al.* (2008), [2]Varanis *et al.* (2021a).

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), [1]Varanis *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.

Funding statement

This research was supported by the Indonesian Ministry of Technology and Higher Education through the International Collaboration Project and IPB University Research Institute.

Acknowledgement

Authors would like to acknowledge Indonesian Ministry of Technology and Higher Education for funding assistance of this study through the International Collaboration Project (Contract No. 2492/IT3.L1/PN/2020) in 2020 and IPB University Research Institute (Contract No. 264) in 2021.

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).

References

Abecia, J. A., S. Luis, and F. Canto. 2021. Implanting melatonin at lambing enhances lamb growth and maintains high fat content in milk. Veterinary Research Communications 45: 181–188. https://doi.org/10.1007/s11259-021-09799-y

Abuzahra, M., D. Wijayanti, M. H. Effendi, I. Mustofa, and L. A. Eid. 2024. Estimate the effect of non-genetic factors on the reproductive traits of Afec-Assaf strain in Bani Naim Farm, Palestine. Jurnal Ilmu Ternak dan Veteriner 29: 9–15. https://doi.org/10.14334/jitv.v29i1.3213

Ahmed, S. 2017. Pre and post-natal nutrition of ewes on the performances of native Bengal Ewes and their lambs. Animal and Veterinary Sciences 5: 33. https://doi.org/10.11648/j.avs.20170502.12

Antunović, Z., M. Šperanda, J. Novoselec, M., Didara, B. Mioč, Ž. Klir, and D. Samac. 2017. Blood metabolic profile and acid-base balance of dairy goats and their kids during lactation. Croatia Veterinarski Arhiv 87: 43–55.

AOAC. 2019. Official Methods of Analysis of AOAC International 21st edn. G. W. Latimer (ed). AOAC International, Washington DC.

Astuti, A., R. Rochijan, B. P. Widyobroto, and C. T. Noviandi. 2022. Nutrient status, hematological and blood metabolite profile of mid-lactating dairy cows during wet and dry seasons raised under Indonesian tropical environmental conditions. Journal of Animal Behaviour and Biometeorology 10: 1–6. https://doi.org/10.31893/jabb.22007

Averós, X., I. Granado-Tajada, J. Arranz, I. Beltrán de Heredia, L. González, R. Ruiz, A. García-Rodríguez, and R. Atxaerandio. 2022. Pre-partum supplementation with polyunsaturated fatty acids on colostrum characteristics and lamb immunity and behavior after a mild post-weaning aversive handling period. Animals 12: https://doi.org/10.3390/ani12141780

Behrendt, R., J. E. Hocking Edwards, D. Gordon, M. Hyder, M. Kelly, F. Cameron, J. Byron, M. Raeside, G. Kearney, and A. N. Thompson. 2019. Offering maternal composite ewes higher levels of nutrition from mid-pregnancy to lambing results in predictable increases in birthweight, survival, and weaning weight of their lambs. Animal Production Science 59: 1906–1922. https://doi.org/10.1071/AN18505

Bianchi, A. E., T. Zortea, C. J. Cazzarotto, G. Machado, L. G. Pellegrini, N. S. P. Dos Santos Richards, M. D. Baldissera, A. S. Da Silva, A. C. Galvão, and V. D. P. Macedo. 2018. Addition of palm oil in diet of dairy ewes reduces saturated fatty acid and increases unsaturated fatty acids in milk. Acta Scientiae Veterinariae 46: 10. https://doi.org/10.22456/1679-9216.89180

Chay-Canul, A. J., E. Aguilar-Urquizo, G. M. Parra-Bracamonte, Á. T. Piñeiro-Vazquez, J. R. Sanginés-García, J. G. Magaña-Monforte, R. A. García-Herrera, and N. López-Villalobos. 2019. Ewe and lamb pre-weaning performance of Pelibuey and Katahdin hair sheep breeds under humid tropical conditions. Italian Journal of Animal Science 18: 850–857. https://doi.org/10.1080/1828051X.2019.1599305

Coleman, D. N., K. D. Murphy, and A. E. Relling. 2018. Prepartum fatty acid supplementation in sheep. II. Supplementation of eicosapentaenoic acid and docosahexaenoic acid during late gestation alters the fatty acid profile of plasma, colostrum, milk and adipose tissue, and increases lipogenic gene expression of adipose tissue1. Journal of Animal Science 96: 1181–1204. https://doi.org/10.1093/jas/skx013

Conte, G., T. Giordani, A. Vangelisti, A. Serra, M. Pauselli, A. Cavallini, and M. Mele. 2021. Transcriptome adaptation of the ovine mammary gland to dietary supplementation of extruded linseed. Animals 11: 2707. https://doi.org/10.3390/ani11092707

Cordero, M. O., C. A. M. Herrera, J. M. V. García, C. A. Stewart, C. A. R. Nieto, A. E. O. Alfaro, I. W. Purvis, V. C. Reyes, H. A. L. Rangel, and G. B. Martin. 2019. Pregnancy and litter size, but not lamb sex, affect feed intake and wool production by merino-type ewes. Animals 9: 214. https://doi.org/10.3390/ani9050214

Countouris, M. E., C. Holzman, A. D. Althouse, G. G. Snyder, E. Barinas-Mitchell, S. E. Reis, and J. M. Catov. 2020. Lactation and maternal subclinical atherosclerosis among women with and without a history of hypertensive disorders of pregnancy. Journal of Women's Health 29: 789–798. https://doi.org/10.1089/jwh.2019.7863

Dari D. W., M. Astawan, N. Wulandari, and H. Suseno. 2017. Karakteristik minyak ikan sardin (Sardinella sp.) Hasil pemurnian bertingkat. Jurnal Pengolahan Hasil Perikanan Indonesia 20: 455–467.

Douh, M., A. Hicher, A. Saddek, and C. Aissaoui. 2019. Lambing and mortality rate in Ouled-Djellal sheeps in Tebessa region-Algeria. Iraqi Journal of Veterinary Sciences 32: 267–273. https://doi.org/10.33899/ijvs.2019.153863

Dove, H., M. Freer, and J. Z. Foot. 2018. The nutrition of grazing ewes during pregnancy and lactation: Relationships between herbage, supplement and milk intakes, and ewe and lamb liveweight and body composition. Animal Production Science 58: 1253–1270. https://doi.org/10.1071/AN16541

Farrag, B. 2019. Productive characteristics and reproductive responses to estrus synchronization and flushing in abou-delik ewes grazing in arid rangelands in halaieb - shalateen - abouramad triangle of Egypt. Journal of World's Poultry Research 9: 201–210. https://doi.org/10.36380/scil.2019.wvj26

Farrag, B., K. A. El-Bahrawy, H. A. Shedeed, and M. A.-H. El-Rayes. 2024. Effect of protected fatty acid supplementation on ovarian activity, reproductive hormone profiles and reproduction of Barki ewes under semiarid conditions. Archives Animal Breeding 67: 111–122. https://doi.org/10.5194/aab-67-111-2024

Flinn, T., D. O. Kleemann, A. M. Swinbourne, J. M. Kelly, A. C. Weaver, S. K. Walker, K. L. Gatford, K. L. Kind, and W. H. E. J. van Wettere. 2020. Neonatal lamb mortality: major risk factors and the potential ameliorative role of melatonin. Journal of Animal Science and Biotechnology 11: 107. https://doi.org/10.1186/s40104-020-00510-w

Getahun, D., T. Alemneh, D. Akeberegn, M. Getabalew, and D. Zewdie. 2019. Urea metabolism and recycling in ruminants. Biomedical Journal of Scientific & Technical Research 20: 14790-14796. https://doi.org/10.26717/BJSTR.2019.20.003401

Ghaffari, M. H., J. A. R. MacPherson, H. Berends, and M. A. Steele. 2017. Diurnal variation of NMR based blood metabolites in calves fed a high plane of milk replacer: a pilot study. BMC Veterinary Research 13: 271. https://doi.org/10.1186/s12917-017-1185-2

Goldansaz, S. A., A. C. Guo, T. Sajed, M. A. Steele, G. S. Plastow, and D. S. Wishart. 2017. Livestock metabolomics and the livestock metabolome: A systematic review. PLoS ONE 12: e0177675. https://doi.org/10.1371/journal.pone.0177675

Harjanti, W. A., D. W. Harjanti, P. Sambodho, and S. A. B. Santoso. 2017. Pengaruh suplementasi baking soda dalam pakan terhadap urea darah dan urea susu sapi perah laktasi. Jurnal Peternakan Indonesia 19: 65. https://doi.org/10.25077/jpi.19.2.65-71.2017

Hernández, J., J. L. Benedito, and C. Castillo. 2020. Relevance of the study of metabolic profiles in sheep and goat flock. Present and future: A review. Spanish Journal of Agricultural Research 18: 1–14. https://doi.org/10.5424/sjar/2020183-14627

Huang, G., J. Wang, K. Liu, F. Wang, N. Zheng, S. Zhao, X. Qu, J. Yu, Y. Zhang, and J. Wang. 2022. Effect of flaxseed supplementation on milk and plasma fatty acid composition and plasma parameters of Holstein Dairy cows. Animals 12: 1898. https://doi.org/10.3390/ani12151898

Kaneko, J., J. Harvey, and M. L. Bruss. 2008. Clinical Biochemistry of Domestic Animals 6th edn. Academic Press, Burlington.

Kenyon, P. R., F. J. Roca Fraga, S. Blumer, and A. N. Thompson. 2019. Triplet lambs and their dams – a review of current knowledge and management systems. New Zealand Journal of Agricultural Research 62: 399–437. https://doi.org/10.1080/00288233.2019.1616568

Khayat, S., H. Fanaei, and A. Ghanbarzehi. 2017. Minerals in pregnancy and lactation: A review article. Journal of Clinical and Diagnostic Research 11: QE01–QE05. https://doi.org/10.7860/JCDR/2017/28485.10626

Khotijah, L., N. Arofah, K. Erlangga, S. H. Wijaya, D. Diapari, K. Komalasari, and D. A. Astuti. 2022. Reproductive performance of ewes, fed flushing diet at different management feeding program. Pakistan Journal of Biological Science 25: 827-834. https://doi.org/10.3923/pjbs.2022.827.834.

Kosasih, W., R. T. Rosmalina, C. Risdian, S. Priatni, W. Kosasih, and E. Saepudin. 2021. Production of omega-3 fatty acids by enzymatic hydrolysis from lemuru fish by-products. Sains Malaysiana 50: 2271–2282. https://doi.org/10.17576/jsm-2021-5008-11

Martins, A. S., D. M. Polizel, G. B. Oliveira, J. P. R. Barroso, M. V. de C. Ferraz Jr., A. A. Miszura, M. Baggio, E. M. Ferreira, and A. V. Pires. 2022. Narasin improves ewe milk yield efficiency and may affect performance of lambs. Scientia Agricola 79: 1-8. https://doi.org/10.1590/1678-992x-2020-0334

Mostafa, A. and M. Farghal. 2022. Effect of flushing with energy or protein sources on the reproductive performance in Ossimi ewes. Journal of Veterinary Medical Research 29: 38-42. https://doi.org/10.21608/jvmr.2022.133773.1053

Nguyen, Q., H. Le, D. Nguyen, P. Nish, J. Otto, B. Malau-Aduli, P. Nichols, and A. E. O. Malau-Aduli. 2018. Supplementing dairy ewes grazing low quality pastures with plant-derived and rumen-protected oils containing eicosapentaenoic acid and docosahexaenoic acid pellets increases body condition score and milk, fat, and protein yields. Animals 8: 241. https://doi.org/10.3390/ani8120241

Nguyen, Q. V., H. Van Le, D. V. Nguyen, B. S. Malau-Aduli, P. D. Nichols, and A. E. O. Malau-Aduli. 2019. Enhancement of dairy sheep cheese eating quality with increased n-3 long-chain polyunsaturated fatty acids. Journal of Dairy Science 102: 211–222. https://doi.org/10.3168/jds.2018-15215

Nickles, K. R., L. Hamer, D. N. Coleman, and A. E. Relling. 2019. Supplementation with eicosapentaenoic and docosahexaenoic acids in late gestation in ewes changes adipose tissue gene expression in the ewe and growth and plasma concentration of ghrelin in the offspring. J. Anim. Sci. 97: 2631–2643. https://doi.org/10.1093/jas/skz141

Novilla, A., P. Nursidika, and W. Mahargyani. 2017. Komposisi asam lemak minyak kelapa murni (virgin coconut oil) yang berpotensi sebagai anti kandidiasis. Educhemia 2: 161-173. https://doi.org/10.30870/educhemia.v2i2.1447

Nugroho, P., K. G. Wiryawan, W. Manalu, and D. A. Astuti. 2020. Blood hematology profile at postpartum in Ettawa grade does fed with different fatty acid flushing diets during the late gestation period and different litter sizes. E3S Web of Conferences 151: 01002. https://doi.org/10.1051/e3sconf/202015101002

Nurlatifah, A., L. Khotijah, R. I. Arifiantini, M. S. Maidin, and D. A. Astuti. 2022. Colostrum quality of ewe fed flushing diet containing epa and dha associated with lamb performance. Tropical Animal Science Journal 45: 348–355. https://doi.org/10.5398/tasj.2022.45.3.348

Nurlatifah, A., L. Khotijah, R. I. Arifiantini, M. S. Maidin, D. A. Astuti, and H. Herdis. 2023. Immunity and behaviour of lambs born from ewes fed a flushing diet containing EPA and DHA. Jurnal Ilmu Ternak dan Veteriner 28: 159–168. https://doi.org/10.14334/jitv.v28i3.3110

Nurlatifah, A., L. Khotijah, K. Komalasari, and D. A. Astuti. 2020. The effect of flushing with fatty acid supplementation in ewes ration on folliculogenesis. IOP Conference Series: Earth and Environmental Science 411: 012036. https://doi.org/10.1088/1755-1315/411/1/012036

Opsomer, G., M. Van Eetvelde, M. Kamal, and A. Van Soom. 2017. Epidemiological evidence for metabolic programming in dairy cattle. Reproduction, Fertility and Development, 29: 52–57. https://doi.org/10.1071/RD16410

Oscarsson, J. and E. Hurt-Camejo. 2017. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. Lipids in Health and Disease 16: 149. https://doi.org/10.1186/s12944-017-0541-3

Parente, M. O. M., I. Susin, C. P. Nolli, E. M. Ferreira, R. S. Gentil, D. M. Polizel, A. V. Pires, S. P. Alves, and R. J. B. Bessa. 2018. Effects of supplementation with vegetable oils, including castor oil, on milk production of ewes and on growth of their lambs. J. Anim. Sci. 96: 354-363. https://doi.org/10.1093/jas/skx015

Peng, Z., C. Zhang, L. Yan, Y. Zhang, Z. Yang, J. Wang, and C. Song. 2020. EPA is more effective than DHA to improve depression-like behavior, glia cell dysfunction and hippcampal apoptosis signaling in a chronic stress-induced rat model of depression. Int. J. Mol. Sci. 21: 1769. https://doi.org/10.3390/ijms21051769

Pesántez-Pacheco, J. L., A. Heras-Molina, L. Torres-Rovira, M. V. Sanz-Fernández, C. García-Contreras, M. Vázquez-Gómez, P. Feyjoo, E. Cáceres, M. Frías-Mateo, F. Hernández, P. Martínez-Ros, J. V. González-Martin, A. González-Bulnes, and S. Astiz. 2019a. Influence of maternal factors (Weight, body condition, parity, and pregnancy rank) on plasma metabolites of dairy ewes and their lambs. Animals 9: 1–19. https://doi.org/10.3390/ani9040122

Pesántez-Pacheco, J. L., A. Heras-Molina, L. Torres-Rovira, M. V. Sanz-Fernández, C. García-Contreras, M. Vázquez-Gómez, P. Feyjoo, E. Cáceres, M. Frías-Mateo, F. Hernández, P. Martínez-Ros, J. V. González-Martin, A. González-Bulnes, and S. Astiz. 2019b. Maternal metabolic demands caused by pregnancy and lactation: Association with productivity and offspring phenotype in high-yielding dairy ewes. Animals 9: https://doi.org/10.3390/ani9060295

Rathod, P., S. S. Chaudhary, V. K. Singh, T. D. Manat, A. B. Joshi, and B. S. Siddhapara. 2024. Biochemical analytes during different stages of lactation in Surti buffaloes. Int. J. Adv. Biochem. Res. 8: 330–333. https://doi.org/10.33545/26174693.2024.v8.i5Se.1183

Ridler, A. L., K. J. Flay, P. R. Kenyon, H. T. Blair, R. A. Corner-Thomas, and E. J. Pettigrew. 2022. Factors associated with mortality of lambs born to ewe hoggets. Animals 12: https://doi.org/10.3390/ani12030319

Rizzo, F. A., A. C. Fluck, J. Schafhäuser Junior, R. B. Scheibler, J. L. Nörnberg, D. P. deVargas, L. A. Lourenço, A. P. B. de Souza, and J. L. S. da Silva. 2024. Sunflower oil supplementation in the diets of lactating cows: productive and nutritional performance. Semina: Ciências Agrárias 44: 329–344. https://doi.org/10.5433/1679-0359.2024v44n2p329

Roche, J. R., C. R. Burke, M. A. Crookenden, A. Heiser, J. L. Loor, S. Meier, M. D. Mitchell, C. V. C. Phyn, and S. A. Turner. 2018. Fertility and the transition dairy cow. Reproduction, Fertility and Development 30: 85–100. https://doi.org/10.1071/RD17412

Rosales Nieto, C. A., M. B. Ferguson, C. A. Macleay, J. R. Briegel, D. A. Wood, G. B. Martin, R. Bencini, and A. N. Thompson. 2018. Milk production and composition, and progeny performance in young ewes with high merit for rapid growth and muscle and fat accumulation. Animal 12: 2292–2299. https://doi.org/10.1017/S1751731118000307

Sarvinda, T. D., S. Bintara, I. G. P. Budisatria, Kustantinah, and E. Baliarti. 2022. The effect of flushing premating with spirulina platensis supplementation on ewes postpartum estrus. Buletin Peternakan 46: 31-35. https://doi.org/10.21059/buletinpeternak.v46i1.67523

Silva, J. V. V., S. Ganesan, H. K. J. P. Wickramasinghe, N. Stepanchenko, C. A. Kaya, D. C. Beitz, and J. A. D. R. N. Appuhamy. 2022. Effects of branched-chain amino acids on glucose uptake and lactose synthesis rates in bovine mammary epithelial cells and lactating mammary tissue slices. Journal of Dairy Science 105: 1717–1730. https://doi.org/10.3168/jds.2021-20950

Sudarman, A., H. Fatmiati, and L. Khotijah. 2019. Formulasi susu pengganti dan evaluasi pengaruhnya terhadap performa anak domba kembar. Jurnal Sain Peternakan Indonesia 14: 228–236. https://doi.org/10.31186/jspi.id.14.3.228-236

Tharwat, L., A.-E. R. A.-E. M. Abd-El Raheem, and A. E.-S. A. Mohamed. 2023. Effect of transition period in buffalo cows on some biochemical parameters. SVU-International Journal of Veterinary Sciences 6: 46–62. https://doi.org/10.21608/svu.2023.170430.1234

Varanis, L. F. M., E. B. Schultz, K. A. Oliveira, L. F. Sousa, W. F. G. da Cruz, and G. de-L. M. Júnior. 2021a. Serum biochemical reference ranges for lambs from birth to 1 year of age in the tropics. Semina: Ciências Agrárias 42: 1725-1740. https://doi.org/10.14393/BJ-v37n0a2021-47695

Varanis, L. F. M., K. A, Oliveira, C. M. Araújo, W. F. G. da Cruz, and G. de-L. M. Júnior. 2021b. Serum biochemical reference ranges for pregnant sheep. Bioscience Journal 37: e37036. https://doi.org/10.14393/BJ-v37n0a2021-47695

Wang, X., Y. Wang, Q. Wang, C. Dai, J. Li, P. Huang, Y. Li, X. Ding, J. Huang, T. Hussain, and H. Yang. 2023. Effect of dietary protein on growth performance, and serum biochemical index in late pregnant Hu ewes and their offspring. Animal Biotechnology 34: 97–105. https://doi.org/10.1080/10495398.2021.1939042

Wardeh, M. F. 1981. Models for estimating energy and protein utilization for feeds [Dissertation, Utah State University]. https://doi.org/https://doi.org/10.26076/9026-5aad. Accessed 20 September 2020.

Whatford, L., B. D. Chivers, M. Rowe, and N. Blackie. 2023. A survey of the current farming practices and perceptions on adopting orphan lambs in the United Kingdom: How do “ewe” do it?. Ruminants 3: 468–482. https://doi.org/10.3390/ruminants3040038

Zdorovieva, E., G. Boryaev, E. Kistanova, A. Nosov, Y. Fedorov, and N. Semigodov. 2019. Protected fat in the diet of lactating ewes affects milk composition, lamb body weight and their biochemical parameters. Bulgarian Journal of Agricultural Science 25: 1277-1280.