Tropical Animal Health and Production (2024) 56:190
https://doi.org/10.1007/s11250-024-04060-5

REGULAR ARTICLES

The benefits of flushing with Lemuru fish oil as a source of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on the performance of reproductive parameters in Garut ewes

Aeni Nurlatifah[1] · Herdis Herdis[1] · Lilis Khotijah[2] · Iis Arifiantini[3] · Mashitah ShikhMaidin[4,5] · Dewi Apri Astuti[2] · Pradita Iustitia Sitaresmi[1] · Tri Puji Priyatno[1] · Puji Lestari[1] · Santoso Santoso[1] · Fitra Aji Pamungkas[1] · Anita Hafid[1] · Umi Adiati[1]

Received: 3 July 2023 / Accepted: 20 June 2024 / Published online: 29 June 2024

Abstract
This study aimed to evaluate and analyze the effects of a flushing diet containing Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) from Lemuru (*Sardinella sp*) fish oil on the reproductive performance parameters of Garut ewes. Forty ($n = 40$) primiparous Garut ewes aged 12–14 months with an average body weight of 28.92 ± 4.94 kg were assigned into four experimental treatment groups. The experimental diets contained roughage: concentrate (30:70%) designated as control concentrate (CNT), flushing concentrate with 6% palm oil (PO), flushing concentrate with 3% palm oil mixed with 3% lemuru oil as DHA and EPA sources (PFO), and flushing concentrate with the addition of 6% lemuru oil (FO). Treatment animals were fed two weeks before and after conception and parturition (8 weeks of total flushing treatment). The addition of fish oil at either 3% (PFO) or 6% (FO) resulted in significantly higher reproductive performance of ewes by increasing the litter size, as reflected by the birth of multiple kids ($P < 0.05$) compared to CNT and PO. Adding fish oil (PFO and FO) also maintains gestation, resulting in increased lamb yield, especially in the FO treatment, which yields the highest lamb yield (0% single lamb birth). The lamb male ratio was also higher with fish oil supplementation (PFO and PO) ($P < 0.05$). This research revealed a positive effect of 6% Lemuru oil on decreasing embryo loss and increasing the proportion of twin births. These findings thus support the hypothesis that ration flushing with double the required DHA and EPA from 6% Lemuru fish oil (FO) resulted in significantly higher reproductive performance in Garut sheep.

Keywords Lemuru fish oil · Docosahexaenoic acid (DHA) · Eicosapentaenoic acid (EPA) · Flushing · Reproductive performance

Introduction

Garut sheep from West Java Province, Indonesia, represent an Indonesian sheep germplasm with excellent potential to meet the national animal protein demand in addition to lamb products, and this breed is relatively easy to maintain. These sheep have a higher economic value than other local sheep breeds in Indonesia because of their favorable traits such as their physique, excellent reproductive performance, and large round horns. The weight of an adult ram ranges from 60 to 100 kg. In addition, Garut sheep have cultural functions that can be leveraged to attract regional tourism; however, based on data from 2021, it has been estimated that the national sheep population will decrease by 10.77%, from 17.52 million in 2020 to 15.62 million. This was because the

✉ Aeni Nurlatifah
aeni001@brin.go.id

1 Research Center for Animal Husbandry, Cibinong Science Center, National Research and Innovation Agency, Jalan Raya Jakarta-Bogor, Bogor 16915, Indonesia

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

3 Department of Veterinary Medicine, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia

4 Department of Biology, Faculty of Science, Universiti Putra Malaysia, Selangor 43400, Malaysia

5 Institute of Tropical Agriculture and Food Security (ITAFos), Universiti Putra Malaysia, Serdang, Selangor, Malaysia

increase in lamb consumption was not in line with population growth. The traditional management of small ruminants and farmers' assumptions about sheep behavior contribute to this decrease. Sheep are fed by letting them graze, typically without providing sufficient concentrates. This has rendered conservation management efforts inadequate. As a result, the potential for energy and nutrients obtained from the sheep decreased. In addition, tropical climates tend to contribute to a high crude-fiber content with low protein and energy (Agus and Widi 2018). This imbalance in energy and nutrients has led to a decrease in the reproductive performance and has prevented adequate reproduction (Mekuriaw 2023).

Nutrient deficiency can lead to an increase in non-esterified fatty acids and blood urea nitrogen (Yıldırır et al. 2022), resulting in decreased secretion of reproductive hormones (gonadotropin-releasing hormone [GnRH], follicle-stimulating hormone, luteinizing hormone, estrogen, and testosterone (Sammad et al. 2022). This can also contribute to the disruption of folliculogenesis, decreased sperm quality, and, most importantly, decreased prophylactic ability, which may lead to a decline in livestock populations (Nechifor et al. 2022). One way to improve reproductive performance is to provide a flushing diet before mating. A feeding technique known as "flush feeding" has been used to increase the ovulation rate before mating by increasing feed intake or switching it to a different kind of feed energy. Short feeding bursts can influence reproductive performance, which can be attained by stimulating GnRH secretion or ovarian activities through enhanced food intake and absorption, thus affecting the ovaries, mitogenesis, and growth (Mallmann et al. 2020; Yıldırır et al. 2022).

Flushing can be conducted by administering oil as a supplementary energy source. Polyunsaturated fatty acids (PUFA), particularly long-chain fatty acids, improve reproductive performance. Linoleic acid (C18:2–6) and linolenic acid (C18:3–3) are the only two omega-6 fatty acids that are generally considered necessary (Ballard and Byrd 2018). Supplementing the energy source with linoleic acid up to threefold the basal requirement during pregnancy can improve the reproductive performance of prolific ewes. This can be achieved by improving steroid hormone synthesis, the number of ovulations, pregnancies, multiple births, and the male-to-offspring ratio (Astuti et al. 2020; Khotijah et al. 2015). In addition, a previous study found that supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oil (FO) can increase the size of the preovulatory follicle and the number of mRNA transcripts of interferon-stimulated genes, which can increase pregnancy rates (Telle-Hansen et al. 2019). The long-chain PUFAs EPA and arachidonic acid are precursors of eicosanoids, including prostaglandins (PG), thromboxanes, leukotrienes, and prostacyclins. Eicosanoids are signaling molecules associated with the production of the PG series-3. Series-1 and series-3 PGs are less inflammatory than series-2 (Gulliver et al. 2012). Increased plasma EPA concentrations are associated with increased levels of peroxisome proliferator-activated receptors PPARs (MacLaren et al. 2006). This may also be associated with reduced progesterone clearance (Galbreath et al. 2008).

Lemuru (*Sardinella* sp.) fish oil is rich in omega-3 fatty acids and is produced locally from fish processing waste. Lemuru FO contains a combined total of 20–26% EPA and DHA (Kosasih et al. 2021). However, the amounts of EPA and DHA required to improve the reproductive performance and support the growth and health of ewes during pregnancy remain unknown. Further studies on the effects of EPA and DHA on the reproductive performance and productivity of ruminants, especially in the tropics, are required. Therefore, this study was designed to test the hypothesis that feeding Garut ewes with flushing rations containing DHA and EPA fatty acids from lemuru FO during the pre- and post-mating and pre- and post-partum periods would influence their reproductive performance. The objective of this study was to evaluate the effects of a flushing diet containing DHA and EPA from lemuru FO on nutrient utilization, metabolic effects, and reproductive performance in Garut ewes.

Materials and methods

Experimental site and animal

This research was conducted at the Meat and Draught Animal Laboratory, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University (IPB) (6°18'0"–6°47'10" S and 106°23'45"–107°13'30" E) at an altitude of 500–600 mASL. Forty primiparous Garut sheep (*Ovis aries*) aged 12–14 months with body weights of 28.92 ± 4.94 kg and BCS 2.69 ± 0.10 were divided into four treatments ($n = 10$ replication).

Experimental treatment

The ration for the ewes consisted of Napier grass, soybean meal, pollard, dried cassava, molasses, salt, and premix (Table 1). Ewes were fed a total dry matter ratio of 3.5% of body weight. The ratio of Napier grass (*Pennisetum purpureum*) to concentrate was 30:70 based on dry matter. Therefore, the ewe fed Napier grass 1,5 kg/h/d and concentrate 1 kg/h/d. The total concentrate and forage consumed is calculated every day. Ration designed to fulfil dry matter, crude protein, and TDN required by pregnant ewe according to the NRC (2007). EPA and DHA contents were adjusted to

Table 1 Composition of experimental feeds (%) by additional oil as a feed additive in DM basis

Ingredients	Treatment			
	CNT	PO	PFO	FO
	%			
Penisetum Purpureum	30	30	30	30
Basal Concentrate	70	64	64	64
Palm Oil	-	6	3	-
Lemuru oil	-	-	3	6

CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm and DHA EPA by addition fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by addition lemuru oils

the omega-3 fatty acid requirements necessary for optimal reproduction as determined by Pudelkewicz et al. (1968) during the flushing time. The EPA and DHA content in the PO ration was 0%, in the PFO ration 0.47%, and in the FO ration 0.94%, depending on the percentage of lemuru oil fed with an addition of 6% palm oil (PO), 3% each of palm and lemuru fish oil (PFO), and 6% of fish oil (FO) to meet these requirements. The composition ratio data are described in Table 1. The treatment of the flushing concentrate with the addition of oil is as follows: CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm mixture DHA EPA by addition of fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by adding lemuru oils (Table 1). Total flush concentrate during pregnancy was supplemented two weeks before mating, two weeks at the beginning of pregnancy (early gestation), two weeks before parturition (last gestation), and two weeks after lambing (Fig. 1). Excluding the flushing time, all ewes were given the same feed as the control. Table 2 shows the proximate analysis for each feed ingredient used in this study.

Estrus synchronization, mating procedures, and monitoring of pregnancy

At two weeks after flushing, prostaglandin PGF2α (Lutalyse®, Zoetis, US) was injected intramuscularly at 2 mg dinoprost per animal for synchronization (Fig. 1). To confirm each ewe's estrus, the injections were repeated 11

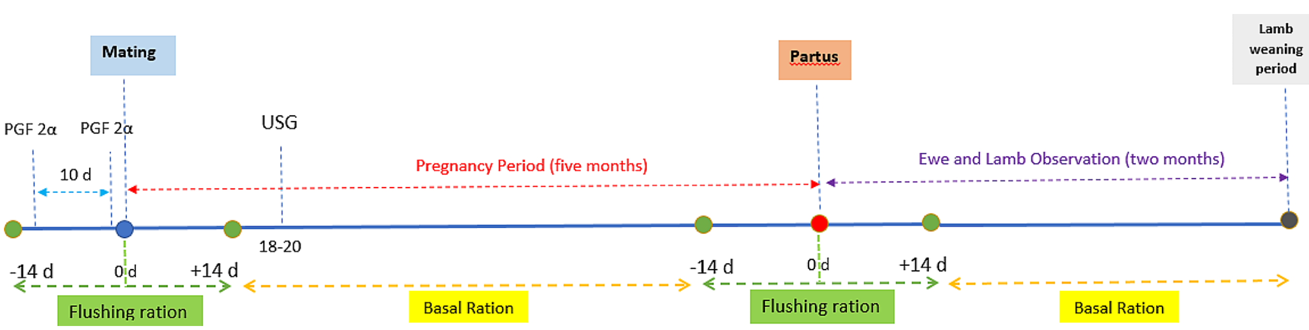

Fig. 1 Experimental procedures

Table 2 Nutrient composition in each treatment feed based on the dry weight

Nutrient Contents	CNT	PO	PFO	FO	Penisetum purpureum
	%				
DM Basis	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
EPA and DHA	**-**	**-**	**0.67**	**1.34**	-
Palmintat acid	-	2.61	1.30	-	-
Linoleat	-	0.69	0.73	0.08	-
Linolenat	-	0.01	0.02	0.01	-
Laurat	-	0.01	0.01	0.01	-
Crude fiber	9.92	9.47	9.12	9.25	32.57
NDF	63.33	54.15	54.22	54.44	45.03
TDN[b]	71.34	73.16	73.24	72.93	49.48
Ca	0.73	0.82	0.82	0.82	0.46
P	0.65	0.56	0.56	0.56	1.1

Test results in the laboratory of Feed Science and Technology, IPB University (2019) CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm and DHA EPA by addition fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by addition lemuru oils. [b]TDN = total digestible nutrient; calculated by Wardeh formula. (1981) TDN = 2,6407 + (0,6964 x %CP) + (1,2159 x %CF) – (0,1043 x %CF) + (0,9194 x %NFE) days after the first injection. The occurrence, duration, and response to estrus were observed in ewes' behavior for three days. In communal cages, rams mated ewes at a 5:1 ratio for four days. An ALOKA ultrasonic device model SSD-500 (ALOKA Co. Ltd., Japan) with a 7.5 GHz linear probe was used to monitor pregnancy on day 28.

Blood metabolites

Blood samples were collected in day 25, 75, and 135 after mating. Blood taken from the jugular vein in the morning before feeding time using a 3-ml syringe with 21-G Venoject needle (BD Vacuntainer, USA) and collected in sterile tubes containing EDTA anticoagulant. Blood samples were collected to measure blood metabolites and hematology using a hematology and UV Vis KIT assay.

Progesterone assay

Blood samples for progesterone measurement were collected every three days in two weeks before, during, and two weeks after lambing. Hormone analysis was performed to determine the concentration of the hormone progesterone in blood plasma using the ELISA Kit Progesterone (DRG, Germany) (Sitaresmi et al. 2017). The absorbance was measured using an ELISA microplate reader (Thermo Fisher Scientific, USA).

Reproductive performance

The number of embryos was determined using an ultrasound machine (USG; ALOKA model SSD-500; ALOKACo. Ltd., Japan; Fig. 1). Embryo loss was determined based on the difference between the number of embryos detected using the USG and the number of lambs born. The litter size was calculated as the number of lambs born to one ewe. The ratio of twins to single-type lambs and the ratio of males to females were observed at birth.

Statistical analysis

The animals were distributed in a completely randomized design with four treatment groups and ten individual replicates. Statistical analyses were performed using SPSS software (SPSS ver. 25). Differences among treatments were tested using one-way ANOVA. Duncan's post-hoc test was performed to compare treatment means. Statistical significance is reported at $P < 0.05$. Lamb sex ratio (male: female) and birth type ratio (twins: singletons) were tested using a chi-square ($\chi 2$) test.

Results

Nutrient utilization and metabolic effects

Nutrient intake of ewes during pregnancy is presented in Table 3. The experimental treatments had no significant effect on the total dry matter, crude protein, crude fiber, neutral detergent fiber, or total digestible nutrients. The treatment influenced crude fat consumption only in the last stages of pregnancy ($P < 0.05$), which was related to the fact that the flushing concentrate was fed at the beginning and end of the gestation period. Crude fat intake was greater ($P < 0.05$) in the palm oil (PO) treatment compared to that of PFO and FO (77,46 ± 10,01 g/h/d vs. 60,22 ± 11,01 g/h/d vs. 59,50 ± 9,98 g/h/d, respectively). Fat intake was lowest ($P < 0.05$) in the control (17,33 ± 5,50 g/h/d).

The results of blood metabolite tests in ewes during gestation are shown in Table 4. In general, flushing and supplementation with 3–6% lemuru FO had no significant effect on the blood profile of ewes ($P > 0.05$). Only the triglyceride concentration was significantly different at mid-gestation ($P < 0.05$). Triglyceride levels were higher ($P < 0.05$) in the control (CNT) compared to those in FO (50.13 ± 6.71 mg/dL vs. 37.59 ± 5.70 mg/dL, respectively). Triglycerides levels in the PO and PFO treatments did not differ from those in the CNT and FO treatments. The triglyceride levels in ewes flushed with lemuru FO were much lower than those in the other treatments. In addition, flushing with PO or lemuru FO resulted in significantly lower triglyceride levels than those in the control. Flushing with PO resulted in relatively higher levels of all blood biochemical parameters, although these were not significantly higher than those of the CNT and lemuru FO (PFO and FO; $P > 0.05$).

Reproductive performance outcomes

The progesterone hormone levels in the flush-treated ewes did not differ from those in the control from 15 days before lambing to 6 days after lambing (Table 5).

The effects of flushing ewes with fatty acid supplements (DHA and EPA) on reproductive performance are shown in Table 6. Lemuru FO supplementation resulted in significantly lower embryo loss than in the CNT ($P < 0.05$). Embryo loss was highest in the PO treatment (40.00%, followed by C (14.29%), PFO (8.32%), and FO (0.00%). No lambs in the PFO and FO groups were born as singletons. Moreover, FO group ewes produced quadruplets. The PFO and FO treatments resulted in 100% of the births being twins. Interestingly, the PO, PFO, and FO treatments significantly increased the number of male lambs born compared to that of female lambs. The treatments had no significant effect on lamb birth weight or gestation length.

Table 3 Effects of flushing with fatty acids (DHA and EPA) of lemuru oil origin on nutrient consumption of ewes

Nutrients (g/head/day)	Pregnancy stages	Treatment			
		CNT	PO	PFO	FO
Dry matter	Early	984.76 ± 187.91	1023.35 ± 129.72	916.76 ± 98.18	934.88 ± 129.83
	Middle	928.25 ± 191.40	979.99 ± 115.85	879.57 ± 99.17	911.48 ± 175.00
	Last	1225.3 ± 352.87	1177.5 ± 159.96	931.24 ± 164.25	1008.9 ± 170.99
Crude protein	Early	126.73 ± 23.57	131.89 ± 17.21	118.36 ± 12.53	120.25 ± 17.34
	Middle	120.29 ± 24.17	127.33 ± 14.92	114.15 ± 13.21	117.62 ± 23.71
	Last	161.91 ± 43.59	184.05 ± 24.17	149.27 ± 26.88	151.92 ± 25.47
Crude Fat	Early	14.49 ± 2.86	15.03 ± 1.87	13.43 ± 1.47	13.76 ± 1.81
	Middle	13.52 ± 2.90	14.21 ± 1.70	12.78 ± 1.45	13.36 ± 2.38
	Last	17.33 ± 5.50 [c]	77.46 ± 10.01 [a]	60.22 ± 11.01[b]	59.50 ± 9.98 [b]
Crude Fiber	Early	160.75 ± 33.27	166.35 ± 19.25	148.06 ± 17.18	152.84 ± 18.83
	Middle	148.01 ± 33.58	154.82 ± 18.96	139.52 ± 16.99	147.43 ± 23.85
	Last	182.21 ± 65.83	171.40 ± 26.64	130.30 ± 23.17	160.78 ± 29.82
NFE	Early	272.70 ± 107.22	595.82 ± 77.20	534.44 ± 56.73	543.51 ± 77.66
	Middle	542.67 ± 109.72	574.05 ± 67.42	514.80 ± 59.20	531.18 ± 105.81
	Last	726.95 ± 199.01	613.97 ± 82.42	487.14 ± 86.39	522.01 ± 88.02
Total digestable nutrient	Early	641.67 ± 119.98	6676.62 ± 86.63	598.90 ± 63.54	608.95 ± 87.18
	Middle	608.24 ± 122.83	643.49 ± 75.54	577.04 ± 66.43	592.22 ± 118.86
	Last	815.56 ± 222.52	800.06 ± 106.25	636.07 ± 113.53	667.94 ± 112.24

CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm and DHA EPA by addition of fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by addition lemuru oils [abc] significant different in each column or the treatment ($P < 0.05$). Early : 0–2 weeks in the beginning of pregnancy (flushing period); Middle : 4–16 weeks in the middle of pregnancy (nonflushing period); Early : 19th week until the end of pregnancy (flushing period);

Table 4 Effect flushing using supplementation fatty acids (DHA Dan EPA) from Lemuru oil during pregnancy in ewes

Nutrients	Pregnancy stages	Treatment			
		CNT	PO	PFO	FO
Glucose (mg/dL)	Early	60.70 ± 8.67	60.41 ± 5.47	57.26 ± 7.64	55.15 ± 4.01
	Middle	58.17 ± 1.73	64.57 ± 10.19	50.77 ± 5.23	54.56 ± 2.32
	Last	61.30 ± 3.08	62.94 ± 5.76	50.95 ± 17.92	60.55 ± 6.70
Triglycerides (mg/dL)	Early	56.67 ± 2.09	63.97 ± 16.80	51.03 ± 2.71	51.92 ± 6.54
	Middle	50.13 ± 6.71[a]	42.32 ± 4.64[ab]	42.04 ± 7.58[ab]	37.59 ± 5.70[b]
	Last	64.90 ± 5.70	82.73 ± 14.65	71.22 ± 6.69	75.31 ± 7.81
Cholesterol (mg/dL)	Early	29.60 ± 7.14	30.61 ± 13.26	28.88 ± 2.50	31.04 ± 3.77
	Middle	19.00 ± 4.67	29.09 ± 6.57	21.76 ± 5.05	21.07 ± 12.75
	Last	33.65 ± 12.23	31.92 ± 20.07	36.85 ± 15.80	37.50 ± 10.92

CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm and DHA EPA by addition of fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by addition lemuru oils [abc] significant different in each column or the treatment ($P < 0.05$) Early : 0–2 weeks in the beginning of pregnancy (flushing period); Middle : 4–16 weeks in the middle of pregnancy (nonflushing period); Early : 19th week until the end of pregnancy (flushing period);

Discussion

The sheep were fed fat sources to enhance the energy density of their diets. Many aspects of sheep production, particularly reproductive performance, are affected by dietary fat supplementation (Hervás et al. 2021; Mirzaei-Alamouti et al. 2018). The total dry matter intake in this study met the recommendations of the NRC (NRC 2007), which is 970 g/head/day for pregnant ewes weighing 20 kg. The fact that the rate of dry matter consumption did not differ from that of the control group suggests that the addition of PO and lemuru FO as sources of EPA and DHA to the concentrate did not affect palatability, even though the PFO and FO of lemuru FO are waste products of marine biomass and tend to have a fishy odor. Interestingly, these results also suggest that increasing energy density using oil supplementation through concentrate flushing does not reduce the rate of ration consumption. The addition of oil only affected crude

Table 5 Effects of flushing on maternal progesterone during pre- and post-lambing days (lambing day = day 0)

Progesterone (ng/ml)	Treatment			
	CNT	PO	PFO	FO
−15 d	49.9 ± 16.24	48.01 ± 2.84	44.89 ± 41.97	40.54 ± 31.57
−12 d	31.61 ± 15.45	27.07 ± 16.49	69.30 ± 15.20	35.66 ± 21.69
−9 d	39.12 ± 7.55	27.07 ± 16.49	11.50 ± 4.35	35.66 ± 21.69
−6 d	25.09 ± 8.63	29.16 ± 17.24	20.02 ± 9.88	67.85 ± 21.77
−3 d	35.06 ± 5.22	4.70 ± 6.62	41.51 ± 35.01	39.44 ± 14.45
0	1.66 ± 1.86	0.64 ± 0.32	1.10 ± 0.39	2.20 ± 1.83
3 d	0.12 ± 0.14	0.88 ± 1.41	0.70 ± 1.01	0.34 ± 0.26
6 d	0.89 ± 0.52	0.22 ± 0.05	0.20 ± 0.06	0.42 ± 0.31

CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm and DHA EPA by addition of fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by the addition of lemuru oils

Table 6 Effects of flushing with fatty acid supplementation (DHA and EPA) of lemuru oil on reproductive performance of Garut ewes

Parameters	Treatment			
	C	PO	PFO	FO
Gestations days	147.5 ± 2.12	145.25 ± 4.99	144.5 ± 4.20	145.00 ± 10.23
Litter size	1.6 ± 1.34	2.00 ± 1.00	2.50 ± 0.58	2.80 ± 0.83
Embryo loss (%)	14.29[b]	40.00[a]	8.32[c]	0.00[d]
Birth type (%)				
Single	40	30	0	0
Twin	40	60	50	40
Triplet	20	30	50	40
Quadruplet	0	0	0	20
Birth Ratio * (twins: singletons)	60:40	80:20	100:0	100:0
Lambs sex ratio (male: female) *	50:50	83:17	83:17	68:32
Birth weight (kg)	2.70 ± 0.75	2.29 ± 0.71	2.16 ± 0.50	2.41 ± 0.41

CNT = control concentrate; PO = flushing concentrate with palm oil; PFO = flushing concentrate with palm and DHA EPA by addition of fish lemuru oil as needed; FO = flushing concentrate with DHA EPA twice as required by addition lemuru oils

[abc] significant different in each treatment $P < 0.05$. The data showed significant differences in reproductive performance with PFO and FO (containing supplementation fish oils) elevated almost all parameters, increasing litter size and maintaining the pregnancy, which showed in highest twin and triplet kid yield means had the highest kid yields, especially in FO treatment

*significant differences in each column using chi-square analysis

fat consumption. These data are similar to those of previous studies on sheep, in which an oil-fat treatment was also used (Behan et al. 2019; Candyrine et al. 2016; Núñez De González et al., 2020); however, these findings differ from those of Dauber et al. (2022) who found that the addition of oils such as rice, canola, linseed, and safflower oil caused significant differences in dry matter intake. The reason for this discrepancy remains unclear. Although previous studies noted that adding oil in the range of 3–3.5% does not affect palatability, the results of this study showed that a higher rate of fat consumption in late pregnancy can be attributed to the crude fat concentration during this period. Flushed concentrate had a crude fat content of 7%, whereas the control concentrate was 1.22%. Crude fat consumption in early to mid-pregnancy was not affected by concentrate flushing because the animals received the same initial crude fat concentration. Protein consumption by ewes is considered sufficient to meet their nutritional needs during pregnancy if it meets the NRC (2007) recommendation of 103 g/day. Crude protein is essential for supporting the amino acid requirements for organogenesis and fetal muscle growth. High crude protein consumption before parturition is also required to support milk production.

Glucose, cholesterol, and triglyceride levels are important for reproductive health. Glucose concentration in the blood reflects the nutritional status, which must meet the energy requirements for the maintenance of ewes and fetal development (Meikle et al. 2018; Soares et al. 2023). Cholesterol is a precursor of steroid hormones, which play an important role in livestock reproduction (Ying et al. 2013). Flushing rations with PO or lemuru FO had significantly lower triglyceride levels than those in the control group. This may be because the addition of PUFAs to PO and lemuru FO directly inhibits the development of blood triglycerides in general (Moallem 2018). The lower levels of triglycerides found in the FO group may be because EPA and DHA are poor substrates for the liver enzymes involved in triglyceride synthesis, which prevents the production of very low-density lipoprotein and triglycerides. Furthermore, they may prevent the esterification of other fatty acids in the body, which would otherwise be used to produce triglycerides (Teama and El-Tarabany 2016). Another explanation for the significantly higher triglyceride levels found in PO than in the other treatments is that PO used 10% higher BETN content in the diet (Table 2). Triglycerides are formed from carbohydrates and fats (Sitaresmi et al. 2020). Lower plasma triglyceride levels are associated with improved insulin sensitivity (Smith et al. 2020; Toro-Huamanchumo et al. 2019). EPA promotes PPARs (MacLaren et al. 2006), leading to improved insulin signaling and reduced inflammation (Bäck and Hansson 2019).

Progesterone is a steroid hormone secreted by the corpus luteum (CL) that is responsible for establishing and maintaining gestation (Widayati and Suranindyah 2019). The data of this study are comparable to those of Garut ewes supplemented with sunflower seed oil (Khotijah et al. 2015) or goats supplemented with PO (Tudisco et al. 2019). Contrary results were reported in Etawah goats supplemented with flaxseed and lemuru FO, in which the progesterone hormone level was significantly higher than that in the control group (Astuti et al. 2020). This can be interpreted as the addition of 3–6% lemuru FO to the concentrate not being in line with the hypothesis that the addition of EPA and DHA can increase progesterone hormone levels during pregnancy (Astuti et al. 2020; Moallem 2018) or reduce progesterone levels (Hess et al. 2008). These different results can be attributed to several factors, including the animals, experimental design, fat source, type of fatty acids used, and their concentrations (Moallem 2018). These results indicate that lemuru FO as a source of EPA and DHA (3–6%) and PO as a PUFA source in ewe rations did not interfere with the plasma progesterone profile during gestation. Another interesting finding of this study was that the PFO and FO treatments could maintain progesterone levels in the last period of gestation, which should drop sharply to zero immediately before delivery because of increasing luteolysis. Conversely, up to 6 days before delivery, progesterone concentrations in the PFO and FO groups remained high. This may be because lemuru FO, which is rich in DHA and EPA, can reduce luteal responsiveness through PGF2α activity and save 54% of CL from functional regression, thus maintaining progesterone concentration from 0 to 48 h before partus or act as an anti-luteolytic agent (Plewes et al. 2018).

Furthermore, data from this study showed that flushing with PO resulted in the highest embryo loss rate. Oil flushing increases ovulation and has the potential for multiple births (Greco et al. 2018); however, PO flushing, in contrast to lemuru FO supplementation, did not reduce the potential activity of prostaglandins (plant oils did not significantly inhibit PG in the endometrium; (Khotijah et al. 2022; Verma et al. 2018). The formed CL was possibly smaller than those in the FO treatment (Mahla et al. 2017), resulting in an inability to maintain pregnancy in the PO treatment; however, in the control group, pregnancy mostly resulted in a single embryo. Therefore, it is better to maintain pregnancy and lower the rate of embryo loss compared with PO. FO has a larger CL than PO and CNT, and for FO progesterone levels tended to be higher (Verma et al. 2018). This could have lowered embryo loss during the study. Lower embryo loss may also be correlated with the type of prostaglandins produced by the different fatty acids. EPA and DHA are eicosanoid precursors. Eicosanoids are signaling molecules associated with the production of PG series-3, which are less inflammatory than series-2 (Gulliver et al. 2012). This helps maintain gestation, as higher inflammatory conditions could potentially interfere with the normal progress of gestation. Both DHA and EPA promote normal blood flow and support placental development. A well-functioning placenta ensures embryonic survival. Increased plasma EPA concentrations are associated with increased levels of PPARs (MacLaren et al. 2006). This may also be associated with reduced progesterone clearance and improved maintenance of pregnancy (Galbreath et al. 2008).

The resulting sex ratio of males to females was 50:50 in the control group. Interestingly, flushing treatments (PO, PFO, and FO) changed the male-to-female ratio with a larger number of male lambs. This may be related to the amount of energy in the feed. Similar results have been reported in livestock fed high PUFA diets (Conway et al. 2018; Khotijah et al. 2015). The mechanism by which fatty acids affect offspring sex remains poorly understood. Studies on deer with oil supplementation showed a male birth ratio of 75% compared to 46% in control groups (Wauters and Lens 1995). Similar results have been reported in mice (Dama et al. 2011; Rosenfeld et al. 2003). High-energy feeding such as flushing results in a higher number of births among male lambs (Gharagozlou et al. 2016). In addition, the high number of male offspring can be attributed to an increase in the estrogen concentration in the plasma of ewes, which in turn can modulate the ewes' immune system and signaling in the oviduct (Gharagozlou et al. 2016). This high-fat diet is correlated with high estrogen levels (Mirzaei-Alamouti et al. 2018). High concentrations of estradiol in the culture medium increase the number of male embryos (Chang et al. 2022; Zhang et al. 2008). Cows with high estradiol concentrations have also been found to have a higher proportion of male calves (Emadi et al. 2014). In addition, the consumption of EPA and DHA has been associated with the inhibition of PGF2α synthesis (Kraisoon et al. 2018). Changes in the contraction rate of the fallopian tubes due to inhibition of PGF2 production can lead to changes in the sex ratio of the offspring (Emadi et al. 2014). Thus, a high proportion of male lambs is economically beneficial. In Indonesia, rams, especially Garut rams of the same body weight, are much more expensive than Garut ewes for religious and cultural reasons. Garut sheep are a meat-type sheep. This differs from dairy sheep, which are expected to have more female lambs.

The number of twin or triplet births was relatively high when treated with both lemuru and PO. This is because the follicle diameter and quantity increased (Nugroho et al. 2021). The addition of lemuru FO resulted in the highest number of triplet and quadruplet births ($P<0.05$). This result is similar to that of FO supplementation in goats reported in previous studies (Mahla et al. 2017; Verma et al. 2018). This study showed that FO supplementation tended to produce more large follicles (de Graaf) and a larger number of total ovulations than PO supplementation. This study also supports data derived from cows (Moussavi et al. 2007); however, a detailed explanation of this phenomenon has yet to be provided. It is possible that the addition of EPA and DHA increases insulin sensitivity (Chanda et al. 2018), which, in turn, increases IGF-1 levels and follicular activity. The flushing ratio of the ewes had no significant effect on lamb birth weight. The birth weights of the lambs in this study ranged from 2.16 to 2.70 kg. These weights were almost identical to those of lambs administered a flushing ratio of sunflower oil (Khotijah et al. 2022).

In conclusion, this study revealed the positive effects of 6% lemuru FO on decreasing embryo loss and boosting the proportion of twin births. These findings support the hypothesis that flushing rations with double the required DHA and EPA of 6% lemuru FO significantly contribute to the increase in the reproductive performance of Garut sheep. However, this study had some limitations. First, it included a relatively small number of animals. Second, DHA and EPA levels in the blood and tissues of ewes have not yet been measured. Therefore, future studies should be conducted to obtain more comprehensive results.

Author contributions AN, H, PIS, LK and DAA mainly contributed to the study conception and design. AN, LK, IA, MSM, DAA performed material preparation and data collection. AN, H, PIS, peformed data analysis and wrote the initially manuscript. H, SS, PIS, TPP, PL supervised the drafted of manuscript. FAP, AH, UA helped the formatting manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding We would like to thank the National Research and Innovation Agency (BRIN) and the Indonesian Ministry of Technology and Higher Education for partially funding our study no. 1/AMD/E1.KP.PTNBH/2020.

Data availability The dataset that is generated or used to perform statistical analyzes in this study are available upon request to corresponding author.

Declarations

Ethical approval Current research approval was granted by the Animal Care and Use Committee (ACUC) at IPB University No. 119–2018 IPB.

Consent to participate Informed consent was obtained from the farm owners in this study.

Consent for publication We certify that all authors agree to submit the manuscript.

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

References

Agus A, Widi TSM (2018) Current situation and future prospects for beef cattle production in Indonesia—A review. Asian-Australasian J Anim Sci 31(7):976. https://doi.org/10.5713/ajas.18.0233

Astuti DA, Khotijah L, Maidin MS, Nugroho P (2020) Reproductive Profile of Etawah Crossbred Does Fed Flushing Diet containing different kinds of Plant Oil and Animal Fat. Pakistan J Biol Sciences: PJBS 23(5):650–657. https://doi.org/10.3923/pjbs.2020.650.657

Bäck M, Hansson GK (2019) Omega-3 fatty acids, cardiovascular risk, and the resolution of inflammation. FASEB J 33(2):1536–1539. https://doi.org/10.1096/fj.201802445R

Ballard M, Byrd AT (2018) Evaluation of a Rumen Protected Omega 3 supplement for Reproduction in dairy cows as determined in three large herd field trials. Open J Anim Sci 8(03):346. https://doi.org/10.4236/ojas.2018.83026

Behan AA, Loh TC, Fakurazi S, Kaka U, Kaka A, Samsudin AA (2019) Effects of supplementation of rumen protected fats on rumen ecology and digestibility of nutrients in sheep. Animals 9(7):400. https://doi.org/10.3390/ani9070400

Candyrine SCL, Jahromi MF, Ebrahimi M, Liang JB, Goh YM, Abdullah N (2016) In vitro rumen fermentation characteristics of goat and sheep supplemented with polyunsaturated fatty acids. Anim Prod Sci 57(8):1607–1612. https://doi.org/10.1071/AN15684

Chanda W, Joseph TP, Guo X, Wang W, Liu M, Vuai MS, Padhiar AA, Zhong M (2018) Effectiveness of omega-3 polyunsaturated fatty acids against microbial pathogens. J Zhejiang Univ Sci B 19(4):253. https://doi.org/10.1631/jzus.B1700063

Chang K-T, Su Y-T, Tsai Y-R, Lan K-C, Hsuuw Y-D, Kang H-Y, Chan W-H, Huang F-J (2022) High levels estradiol affect blastocyst implantation and post-implantation development directly in mice. Biomedical J 45(1):179–189. https://doi.org/10.1016/j.bj.2021.01.004

Conway MC, Mulhern MS, McSorley EM, Van Wijngaarden E, Strain JJ, Myers GJ, Davidson PW, Shamlaye CF, Yeates AJ (2018) Dietary determinants of polyunsaturated fatty acid (PUFA) status in a high fish-eating cohort during pregnancy. Nutrients 10(7):927. https://doi.org/10.3390/nu10070927

Dama MS, Singh NMP, Rajender S (2011) High fat diet prevents over-crowding induced decrease of sex ratio in mice. PLoS ONE 6(1):e16296. https://doi.org/10.1371/journal.pone.0016296

Dauber C, Carreras T, Casarotto Daniel G, Cabrera F, Liscano A, Vicente G, Britos A, Carro S, Cajarville C, Gámbaro A, Vieitez I (2022) Adding sunflower or soybean oil to goat's pasture-based diet improves the lipid profile without changing the sensory characteristics of milk. J Appl Anim Res 50(1):204–212. https://doi.org/10.1080/09712119.2022.2056464

Emadi SR, Rezaei A, Bolourchi M, Hovareshti P, Akbarinejad V (2014) Administration of estradiol benzoate before insemination could skew secondary sex ratio toward males in Holstein dairy cows. Domest Anim Endocrinol 48:110–118. https://doi.org/10.1016/j.domaniend.2014.03.001

Galbreath CW, Scholljegerdes EJ, Lardy GP, Odde KG, Wilson ME, Schroeder JW, Vonnahme KA (2008) Effect of feeding flax or linseed meal on progesterone clearance rate in ovariectomized ewes. Domest Anim Endocrinol 35(2):164–169. https://doi.org/10.1016/j.domaniend.2008.05.002

Gharagozlou F, Youssefi R, Akbarinejad V (2016) Effects of diets supplemented by fish oil on sex ratio of pups in bitch. Veterinary Res Forum 7(2):105. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4959337/

Greco LF, Neto JN, Pedrico A, Lima FS, Bisinotto RS, Martinez N, Ribeiro ES, Thatcher WW, Staples CR, Santos JEP (2018) Effects of altering the ratio of dietary n-6 to n-3 fatty acids on spontaneous luteolysis in lactating dairy cows. J Dairy Sci 101(11):10536–10556. https://doi.org/10.3168/jds.2018-15065

Gulliver CE, Friend MA, King BJ, Clayton EH (2012) The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim Reprod Sci 131(1–2):9–22. https://doi.org/10.1016/j.anireprosci.2012.02.002

Hervás G, Toral PG, Fernández-Díez C, Badia AD, Frutos P (2021) Effect of dietary supplementation with lipids of different unsaturation degree on feed efficiency and milk fatty acid profile in dairy sheep. Animals 11(8):2476. https://doi.org/10.3390/ani11082476

Hess BW, Moss GE, Rule DC (2008) A decade of developments in the area of fat supplementation research with beef cattle and sheep. J Anim Sci 86(suppl14):E188–E204. https://doi.org/10.2527/jas.2007-0546

Khotijah L, Wiryawan KG, Setiadi MA, Astuti DA (2015) Reproductive performance, cholesterol and progesterone status of Garut ewes fed ration containing different levels of sun flower oil. Pak J Nutr 14(7):388–391. https://doi.org/10.3923/pjn.2015.388.391

Khotijah L, Arofah N, Erlangga K, Wijaya SH, Diapari D, Komalasari K, Astuti DA (2022) Reproductive Performance of Ewes, Fed Flushing Diet at different management feeding program. Pakistan J Biol Sciences: PJBS 25(9):827–834. https://doi.org/10.3923/pjbs.2022.827.834

Kosasih W, Rosmalina R, Risdian C, Priatni S, Saepudin E (2021) Production of omega-3 fatty acids by enzymatic hydrolysis from lemuru fish by-products. Sains Malaysiana 50(8):2271–2282. https://doi.org/10.1088/1755-1315/789/1/012019

Kraisoon A, Navanukraw C, Inthamonee W, Bunma T (2018) Embryonic development, luteal size and blood flow area, and concentrations of PGF2α metabolite in dairy cows fed a diet enriched in polysaturated or polyunsaturated fatty acid. Anim Reprod Sci 195:291–301. https://doi.org/10.1016/j.anireprosci.2018.06.007

MacLaren LA, Guzeloglu A, Michel F, Thatcher WW (2006) Peroxisome proliferator-activated receptor (PPAR) expression in cultured bovine endometrial cells and response to omega-3 fatty acid, growth hormone and agonist stimulation in relation to series 2 prostaglandin production. Domest Anim Endocrinol 30(3):155–169. https://doi.org/10.1016/j.domaniend.2005.07.003

Mahla AS, Chaudhari RK, Verma AK, Singh AK, Singh SK, Singh G, Sarkar M, Dutta N, Kumar H, Krishnaswamy N (2017) Effect of dietary supplementation of omega-3 polyunsaturated fatty acid (PUFA) rich fish oil on reproductive performance of the goat (Capra hircus). Theriogenology 99:79–89. https://doi.org/10.1016/j.theriogenology.2017.05.023

Mallmann AL, Arend LS, Oliveira GS, Mellagi AP, Ulguim RR, Bernardi ML, Bortolozzo FP, Knox RV (2020) Effects of flush feeding strategy before breeding on reproductive performance of modern replacement gilts: impacts on ovulation rate and litter traits. J Anim Sci 98(6):skaa186. https://doi.org/10.1093/JAS/SKAA186

Meikle A, de Brun V, Carriquiry M, Soca P, Sosa C, de Lourdes Adrien M, Chilibroste P, Abecia JA (2018) Influences of nutrition and metabolism on reproduction of the female ruminant. Anim Reprod 15(Suppl 1):899. https://doi.org/10.21451/1984-3143-AR2018-0017

Mekuriaw Y (2023) Negative energy balance and its implication on productive and reproductive performance of early lactating dairy cows: review paper. J Appl Anim Res 51(1):220–229. https://doi.org/10.1080/09712119.2023.2176859

Mirzaei-Alamouti H, Mohammadi Z, Shahir MH, Vazirigohar M, Mansouryar M (2018) Effects of short-term feeding of different sources of fatty acids in pre-mating diets on reproductive performance and blood metabolites of fat-tailed Iranian Afshari ewes. Theriogenology 113:85–91. https://doi.org/10.1016/j.theriogenology.2018.02.007

Moallem U (2018) Invited review: roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. J Dairy Sci 101(10):8641–8661. https://doi.org/10.3168/jds.2018-14772

Moussavi AH, Gilbert RO, Overton TR, Bauman DE, Butler WR (2007) Effects of feeding fish meal and n-3 fatty acids on ovarian and uterine responses in early lactating dairy cows. J Dairy Sci 90(1):145–154. https://doi.org/10.3168/jds.s0022-0302(07)72616-4

Nechifor I, Florea MA, Radu-Rusu R-M, Pascal C (2022) Influence of Supplemental Feeding on Body Condition score and Reproductive Performance dynamics in Botosani Karakul Sheep. Agriculture 12(12):2006. https://doi.org/10.3390/agriculture12122006

NRC (2007) Nutrient requirements of small ruminants: Sheep, goats, cervids, and new world camelids. National Academies, Washington DC

Nugroho P, Wiryawan KG, Astuti DA, Manalu W (2021) Stimulation of follicle growth and development during estrus in Ettawa Grade does fed a flushing supplement of different polyunsaturated fatty acids. Veterinary World 14(1):11. https://doi.org/10.14202/VETWORLD.2021.11-22

Núñez De González M, Attaie R, Woldesenbet S, Mora-Gutierrez A, Kirven J, Jung Y, Myers D (2020) Effect of feeding a Low Level of Encapsulated Fish Oil to dairy goats on milk yield, composition, and fatty acid Profile. J Am Oil Chemists' Soc 97(3):281–288. https://doi.org/10.1002/aocs.12318

Plewes MR, Burns PD, Graham PE, Bruemmer JE, Engle TE (2018) Influence of omega-3 polyunsaturated fatty acids from fish oil or meal on the structure of lipid microdomains in bovine luteal cells. Anim Reprod Sci 193:40–57. https://doi.org/10.1016/j.anireprosci.2018.03.036

Pudelkewicz C, Seufert J, Holman RT (1968) Requirements of the female rat for linoleic and linolenic acids. J Nutr 94(2):138–146. https://doi.org/10.1093/jn/94.2.138

Rosenfeld CS, Grimm KM, Livingston KA, Brokman AM, Lamberson WE, Roberts RM (2003) Striking variation in the sex ratio of pups born to mice according to whether maternal diet is high in fat or carbohydrate. Proceedings of the National Academy of Sciences, 100(8), 4628–4632. https://doi.org/10.1073/pnas.0330808100

Sammad A, Khan MZ, Abbas Z, Hu L, Ullah Q, Wang Y, Zhu H, Wang Y (2022) Major nutritional metabolic alterations influencing the reproductive system of postpartum dairy cows. Metabolites 12(1):60. https://doi.org/10.3390/metabo12010060

Sitaresmi PI, Widyobroto BP, Bintara S, Widayati DT (2017) Progesterone and biochemical profile of Ettawa-Saanen crossbreed goats in turi area, Yogyakarta-Indonesia. Int J Dairy Sci 12(4):289–294. https://doi.org/10.3923/ijds.2017.289.294

Sitaresmi PI, Widyobroto BP, Bintara S, Widayati DT (2020) Effects of body condition score and estrus phase on blood metabolites and steroid hormones in Saanen goats in the tropics. Veterinary World 13(5):833. https://doi.org/10.14202/vetworld.2020.833-839

Smith GI, Polidori DC, Yoshino M, Kearney ML, Patterson BW, Mittendorfer B, Klein S (2020) Influence of adiposity, insulin resistance, and intrahepatic triglyceride content on insulin kinetics. J Clin Investig 130(6):3305–3314. https://doi.org/10.1172/JCI136756

Soares PC, Carvalho CCD, da Cunha Mergulhão FC, da Silva TGP, de Araújo Gonçalves DN, de Oliveira Filho EF, de Mendonça CL, Afonso JAB (2023) Serum concentrations of folic acid and cobalamin and energy metabolism of ewes as a function of the energy density of the diet, peripartum period, and pregnancy toxemia. Trop Anim Health Prod 55(1):10. https://doi.org/10.1007/s11250-022-03423-0

Teama FEI, El-Tarabany AA (2016) Physiological and biochemical response to Omega-3 plus as a dietary supplement to growing goats under hot summer conditions. Revista Brasileira de Zootecnia 45:174–180.

Telle-Hansen VH, Gaundal L, Myhrstad MC (2019) Polyunsaturated fatty acids and glycemic control in type 2 diabetes. Nutrients 11(5):1067. https://doi.org/10.3390/nu11051067

Toro-Huamanchumo CJ, Urrunaga-Pastor D, Guarnizo-Poma M, Lazaro-Alcantara H, Paico-Palacios S, Pantoja-Torres B, Ranilla-Seguin delC, Benites-Zapata V, V. A., and, Group MSR (2019) Triglycerides and glucose index as an insulin resistance marker in a sample of healthy adults. Diabetes Metabolic Syndrome: Clin Res Reviews 13(1):272–277. https://doi.org/10.1016/j.dsx.2018.09.010

Tudisco R, Musco N, Pero ME, Morittu VM, Grossi M, Mastellone V, Cavaliere G, Wanapat M, Infascelli F, Lombardi P (2019) Influence of dietary hydrogenated palm oil supplementation on serum biochemistry and progesterone levels in dairy goats. Anim Nutr 5(3):286–289. https://doi.org/10.1016/j.aninu.2019.03.005

Verma AK, Mahla AS, Chaudhari RK, Singh AK, Khatti A, Singh SK, Dutta N, Singh G, Sarkar M, Kumar H (2018) Effect of different levels of n-3 polyunsaturated fatty acids rich fish oil supplementation on the ovarian and endometrial functions in the goat (Capra hircus). Anim Reprod Sci 195:153–161. https://doi.org/10.1016/j.anireprosci.2018.05.019

Wauters LA, Lens L (1995) Effects of Food Availability and Density on Red Squirrel (Sciurus Vulgaris) Reproduction. Ecology 76(8):2460–2469. https://doi.org/10.2307/2265820

Widayati T, Suranindyah Y (2019) Cortisol and Blood Urea Nitrogen profiles in fertile and repeat-breeder holstein-friesian crossbred cows. Pakistan J Biol Sciences: PJBS 22(7):356–360. https://doi.org/10.3923/pjbs.2019.356.360

Ying SJ, Xiao SH, Wang CL, Zhong BS, Zhang GM, Wang ZY, He DY, Ding XL, Xing HJ, Wang F (2013) Effect of nutrition on plasma lipid profile and mRNA levels of ovarian genes involved in steroid hormone synthesis in Hu sheep during luteal phase. J Anim Sci 91(11):5229–5239. https://doi.org/10.2527/jas.2013-6450

Yıldırır M, Çakır DÜ, Yurtman İY (2022) Effects of restricted nutrition and flushing on reproductive performance and metabolic profiles in sheep. Livest Sci 258:104870. https://doi.org/10.1016/j.livsci.2022.104870

Zhang L, Du W, Lin X, Zhang A, Chen H (2008) Progesterone and 17β-estradiol, but not follicle stimulating hormone, alter the sex ratio of murine embryos fertilized in vitro. Theriogenology 69(8):961–966. https://doi.org/10.1016/j.theriogenology.2008.01.009

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.