Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.113

RESEARCH ARTICLE

Effects of Lemuru Fish Oil (Sardinella Sp.) on Estrous Response, Hormonal Profile and Conception Rates in Garut Ewes

Aeni Nurlatifah[1,3], Herdis Herdis[1*], Jean-Baptiste Menassol[2], Lilis Khotijah[3], Raden Iis Arifiantini[4], Mashitah ShikhMaidin[5], Dewi Apri Astuti[3] and Pradita Iustitia Sitaresmi[1]

[1]Research Center for Animal Husbandry, National Research and Innovation Agency, Jalan Raya Jakarta-Bogor, 16915, Indonesia; [2]SELMET, Institut Agro Montpellier, CIRAD, INRAE, Univ Montpellier, F-34000 Montpellier, France; [3]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia; [4]Department of Veterinary Clinics, Reproduction and Pathology, Faculty of Veterinary Medicine, IPB University, Bogor, 16680, Indonesia; [5]Institute of Tropical Agriculture and Food Security (ITAFos), Universiti Putra Malaysia, 43400, Malaysia
*Corresponding author: herd001@brin.go.id

ARTICLE HISTORY (24-327)

Received:	June 15, 2024
Revised:	July 11, 2024
Accepted:	July 14, 2024
Published online:	July 27, 2024

Key words:
Estrous expression
Fish oil
Flushing
Hormonal profile
Reproduction
Sheep

A B S T R A C T

The aim of this study was to determine the effects of short-term feed supplementation of Lemuru fish oil on the reproductive performance of 20 primiparous Garut ewes. For this purpose, the effects of four diets were compared: a control diet (C) and three diets with various oil supplements. These diets were: 6% palm oil (P1), 3% palm oil with 3% Lemuru fish oil (P2), and 6% Lemuru fish oil (P3). Experimental ewes were subjected to estrous synchronization using two injections of PGF2α 11 days apart. The hormonal profiles and blood metabolites were monitored for animals of each group. Estrous expression was observed at mating, and embryo counts were recorded individually using trans-rectal ultrasonography 20 days after mating. Results showed that plasma concentrations of cholesterol were significantly ($P<0.05$) higher for P2 and P3 (44.90±10.51 and 49.81±14.37 mg/dL, respectively) than control and P1 groups. Similarly, blood estradiol concentrations were significantly higher for P3 than P1 and P2 groups before estrus, while during estrus P3 had higher blood estradiol than control group ($P<0.05$). All treatment diets significantly enhanced the onset of estrus in comparison with control animals ($P<0.05$), while receptivity was higher for P3 (21.80±2.86 times) than for control group ($P<0.05$). The proportion of ewes pregnant after the first estrus was the highest for P3 (100%) compared to all other diets. However, there was no effect of diets on the number of embryos. In conclusion, feed supplementation of Lemuru fish oil improves reproductive performance of ewes following natural mating by stimulating onset of estrus and increasing pregnancy rates, making Lemuru oil an attractive feed supplement.

To Cite This Article: Nurlatifah A, Herdis H, Menassol JB, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA and Sitaresmi PI, 2024. Effects of Lemuru fish oil (Sardinella sp.) on estrous response, hormonal profile and conception rates in garut ewes. Pak Vet J, 44(3): 739-744. http://dx.doi.org/10.29261/pakvetj/2024.113

INTRODUCTION

The reproductive performance of our animals is still quite low in many developing countries with large human population such as Indonesia; which adversely affects the success of livestock farming. Meeting livestock nutritional requirements is important to improve their productive and reproductive performance, increase livestock population and improve food security (Makela *et al.*, 2022). Nutrition acts on the reproductive axis through two components, the static and dynamic effects of nutrition, which are mediated through different neuro-hormonal pathways (Abecia *et al.*, 2006). In sheep, short-term feed supplementation or flushing mobilizes the dynamic components of nutrition to act directly on the ovary and improve folliculogenesis (Scaramuzzi *et al.*, 2006). This effect is mediated through metabolic hormones and growth factors, including insulin, IGF-I, leptin and growth hormone (GH), acting within the follicular environment to modulate gonadotrophin-stimulated folliculogenesis (Scaramuzzi *et al.*, 2006; Alves *et al.*, 2019). Thus, flushing practices aim at boosting the nutritional sensing

pathways that act on the functioning of the ovaries. When performed at the timing of reproduction, this can be viewed as an effective on-farm technique to improve reproductive performances in small ruminants.

Among the various feed and nutritional supplements that can be used in a flushing program, Lemuru fish oil is of particular interest. According to Kosasih *et al.* (2021),.Lemuru fish oil contains 20 to 26% of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Like other oils, Lemuru fish oil is an energy-dense supplement and considered as an economically competitive alternative source of omega-3 fatty acids. Thus, in goats, a diet containing 1.75% fish oil increased the number of pre-ovulatory follicles, an effect mediated by Omega-3 fatty acids (Hammiche *et al.*, 2011). According to Kia and Safdar (2015), Omega-3 fatty acids rich diets inhibit cyclooxygenase activity and inhibit the synthesis of prostaglandins (PGF2α), thereby preventing early embryonic death. Accordingly, sheep diet supplemented with Omega-3 fatty acids showed higher pregnancy and twin birth rates than sheep diet supplemented with linoleate (Kia and Safdar, 2015). Moreover, feeding Lemuru fish oil as source of Omega-3 fatty acids in ewes can produce twin lambs with good growth performances (Nurlatifah *et al.*, 2022). However, the specific effects of nutritional flushing with Omega-3 fatty acids rich supplements containing EPA and DHA on estrous behaviour and pregnancy rates in ewes are not clearly known. Therefore, this study was conducted to evaluate the effect of Lemuru fish oil flushing at the timing of reproduction on estrous response, hormone profiles and pregnancy rates in Garut ewes.

MATERIALS AND METHODS

Experimental animals and diets: The current research protocol was approved by the Animal Care and Use Committee (ACUC) at IPB University No. 119-2018 IPB. This study was conducted during the period from October 2022 to March 2023. The animals included in the study were 20 primiparous Garut ewes, aged 12-14 months, with a body condition score (BCS) of 2.69±0.10. These ewes were divided into 4 treatment groups (n=5 per group) on the basis of their live weight (LW), as follows: 28.84±2.52kg (control group), 29.26±2.65kg (P1 group), 28.50±1.91kg (P2 group) and 28.88±2.61kg (P3 group). Ewes were kept in individual pens equipped with feeding and water troughs.

The base daily diet for the ewes consisted of Napier grass (*Cenchrus purpureus*) and concentrate. Daily feed amount was adjusted to 3.5% of individual LW with a 30:70 ratio of Napier grass to concentrate, based on DM, as recommended by the NRC (2007). This diet remained unchanged for the control group (Group C) with protein content of 14.79% and TDN of 71.34%. Flushed groups received additional concentrate feed designed to be isoenergetic and isoproteic with total protein content of 17% and TDN of 75%. The different experimental groups were then subjected to different dietary treatments as follows: P1=Flushing concentrate with 6% palm oil, P2=Flushing concentrate with 3% palm oil and 3% Lemuru fish oil, and P3=Flushing concentrate with 6% Lemuru fish oil. Flushing period began 14 days before mating and continued till 14 days after mating (28 days in total). Drinking water was provided *ad libitum*.

Estrous synchronization: Synchronization of estrus was performed by an intramuscular injection of prostaglandin-F2α (Lutalyse®, Zoetis, US) at a dose of 2.0 mg dinoprost per animal. It was administered using the double injection method, with an interval of 11 days between each injection (Sözbilir *et al.*, 2006).

Estrous observation, mating and pregnancy diagnosis: Experimental ewes were closely monitored daily for onset of estrus after the 2nd PGF2α injection. Estrous observations were conducted by placing ewes and ram in the same flock. The estrous response was calculated from the number of estrus ewes in one treatment group. The onset of estrus was taken as the time period (hours) when the ewe showed first estrus symptoms and accepted the male for mating after the 2nd PGF2α injection. The duration of estrus (hours) was calculated starting from the first expression of estrus symptoms until the ewe refused to accept the male for mating. Receptivity was determined as the number of mounts one ewe received from the start until the end of its estrous period.

Ewes in estrus were allowed to mate with fertile rams of good semen quality. At the timing of estrus, one fertile ram was presented with 4 ewes (one from each treatment group). Using trans-rectal ultrasonography on day 20 after mating, ewes that were diagnosed as non-pregnant were presented to the ram during their next estrus. In this way, mating of non-pregnant ewes was allowed for three successive estrous cycles.

Pregnancy diagnosis and the number of embryos per ewe were determined by trans-rectal ultrasonography 20 days after mating, using the ultrasound scanner ALOKA model SSD-500 (ALOKA Co. LTD, Japan) equipped with a 7.5 MHz linear-array probe.

Blood collection and hormone assays: Blood samples were collected at 3 days before estrus (assuming that estrus would occur 48 hours after the 2nd PGF2α injection), estrous day, and then 3, 6, 9, 12 and 15 days after estrus. Blood was collected from the jugular vein in the morning before feeding using sterile tubes that contained EDTA as an anticoagulant. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes (4°C) and plasma was stored at 4°C until used for hormones assay. The estradiol and progesterone assays were carried out following the procedure provided by the company providing the commercial ELISA kits (DRG International Inc., Germany). Plasma estradiol concentrations were determined using the estradiol ELISA Kit No. 1907 with recovery standard of 99.84%. Plasma progesterone concentrations were determined using the progesterone ELISA Kit No. 60126 with recovery standard of 97.97% and R^2 of 0.99%. Minimum detection limit (sensitivity) for progesterone was 0.045 ng/ml and for estradiol it was 9.714 pg/ml. Inter-assay and intra-assay CV for estradiol was 2.14 to 6.02% and for progesterone it was 6.98 to 8.35%.

Blood metabolites: For blood metabolites (glucose and cholesterol) determination, samples were collected from

the jugular vein of each ewe using EDTA tubes one day before mating in the morning 2 hours after morning feeding. Blood samples were immediately centrifuged at 3000 rpm for 10 minutes to collect plasma. Analysis of total blood glucose was conducted using glucose Kit (Cat. No. 112191, Greiner, AU), while total cholesterol Kit (Cat. No. 101592, Greiner, AU) was used for blood cholesterol analysis. Enzymatic Kit techniques and a spectrophotometer at 546 nm (Genesys 10S UV-Vis, USA) were used to measure blood metabolites concentrations.

Experiment design and data analysis: The experiment followed a randomized complete block design (RCBD), taking body weight as block due to its CV of >10%, which might affect nutrition consumption and influence reproductive performance. The percentages of pregnant ewes per group were analyzed using quantitative descriptive analysis. All other data were statistically analyzed using ANOVA and the differences between treatments were tested using the Duncan's multiple range test. All analyses were performed using the SAS software and differences were considered as significant whenever $P<0.05$. Data are presented as mean±SEM.

RESULTS

Feed intake: There was non-significant differences between experimental groups in terms of total dry matter intake, crude fiber, nitrogen free extract and total digestible nutrients (Table 1). However, ewes of groups P1 and P2 showed significantly higher ($P<0.05$) crude protein intake compared to control and P3 groups, the difference between the latter two groups was non-significant. Crude fat intake was significantly lower in control group than the other treatments, while it was the highest in ewes of groups P1 and P2 ($P<0.05$).

Body condition score: Compared to control, all three short term nutritional supplementations using palm oil or Lemuru fish oil for four weeks did not affect BCS (Table 2). However, BCS after supplementation increased by 7.5-13.5% compared to BCS before supplementation.

Blood metabolites: While there was non-significant difference between groups in terms of plasma glucose concentrations, plasma cholesterol concentrations were higher ($P<0.05$) in P2 and P3 groups compared to control and P1 groups (Table 3). However, differences in plasma cholesterol levels between P2 & P3, as well as control & P1 groups, were non-significant.

Steroid hormones profiles: Short-term nutritional supplementation significantly affected ($P<0.05$) plasma estradiol levels (Fig. 1). The highest ($P<0.05$) plasma estradiol levels were found for the animals in the P3 group. However, 3 days before estrus, this difference was only significant when compared with the animals of the P1 and P2 groups and, at the timing of estrus, it was only significantly different when compared with the animals from the control group. When plasma progesterone was considered, there was non-significant difference among all four groups on different days before and after estrus (Fig. 2).

Estrous expression and mating behaviour: The characteristics of estrous expression are presented in Table 4. There were non-significant differences between groups in the proportion of estrus response and duration of estrus. However, the onset of estrus was significantly earlier for the animals in P1, P2 and P3 groups when compared with the animals in the control group ($P<0.05$). The flushing treatments increased the receptivity to the males in comparison with animals in the control group, but this difference was only significant ($P<0.05$) for the animals in the P3 group.

Pregnancy rate and number of embryos: The pregnancy rate cumulated over three consecutive estrus cycles (Fig. 3) was high in this study, with variable results between the experimental groups. While the animals in the P1 and P2 groups had a pregnancy rate of 80% (at the second and first estrus cycles, respectively), the animals in the control and P3 groups showed pregnancy rate of 100%. This pregnancy rate was reached at the first estrus cycle for the animals in the P3 group and at the third estrus cycle for the animales in the control group. The effects of our experimental treatments on the number of embryos observed by ultrasonography at 20 days after mating were non-significant (Fig. 4). However, number of embryos tended to be slightly higher for the animals in the groups supplemented with fish oil (P2 and P3) compared to animals of control and P1 groups.

Table 1: Nutrient intake in ewes of each experimental treatment

Consumption (g/head/d)	Treatments			
	Control	P1	P2	P3
Dry matter	857.71±6.25	922.66±75.38	886.82±44.32	804.92±39.87
Crude protein	110.00±8.00^{b}	139.76±11.56^{a}	136.15±6.49^{a}	119.29±5.40^{b}
Crude fat	12.68±0.93^{c}	57.69±4.81^{a}	53.62±2.49^{a}	46.26±1.98^{b}
Crude fiber	141.59±10.65	146.42±11.68	139.96±7.88	133.57±8.25
Nitrogen free extract	557.35±40.57	614.48±5.30	589.64±28.63	527.58±24.71
Total digestible nutrients	497.54±3.22	476.31±39.06	457.68±22.55	414.35±19.94

C=Control; P1=supplementation concentrate with 6% palm oil; P2=supplementation concentrate with 3% palm oil and 3% Lemuru oil, P3=supplementation concentrate with 6% Lemuru oil. Values with different superscripts in the same row indicate significant difference due to different flushing treatments ($P<0.05$).

Table 2: Effects of the experimental treatments on body condition score before and after supplementation in ewes

Body condition score	Treatments			
	Control	P1	P2	P3
Before supplementation	2.59±0.18	2.80±0.20	2.80±0.12	2.60±0.10
After supplementation	2.94±0.18	3.09±0.17	3.05±0.12	2.84±0.10

C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3=Supplementation concentrate with 6% Lemuru oil.

Table 3: Mean plasma concentrations of glucose and cholesterol for ewes of each experimental group

Blood metabolites (mg/dL)	Treatments			
	Control	P1	P2	P3
Glucose	61.04±1.66	61.88±1.95	56.50±2.42	60.88±3.28
Cholesterol	25.70±3.15[b]	34.80±3.59[b]	44.90±4.71[a]	49.81±6.44[a]

C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3 = Supplementation concentrate with 6% Lemuru oil. Values with different superscripts in the same row indicate significant difference due to different flushing treatments (P<0.05).

Table 4: Effect of nutritional supplementation on estrus expression and mating behaviour in ewes

Parameters	Treatments			
	Control	P1	P2	P3
Estrous response (%)	100±0.00	100±0.00	100±0.00	100±0.00
Onset of estrus (hours)	68.11±1.67 [b]	48.04±2.03 [a]	48.09±1.99 [a]	47.88±2.14 [a]
Length of estrus (hours)	31.75±4.44	36.60±3.02	34.01±3.71	40.94±4.53
Receptivity (times)	12.80±1.53 [b]	17.60±1.87 [ab]	18.00±2.05 [ab]	21.80±1.28 [a]

C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3=Supplementation concentrate with 6% Lemuru oil. Values with different superscript letters within a row differ significantly (p<0.05).

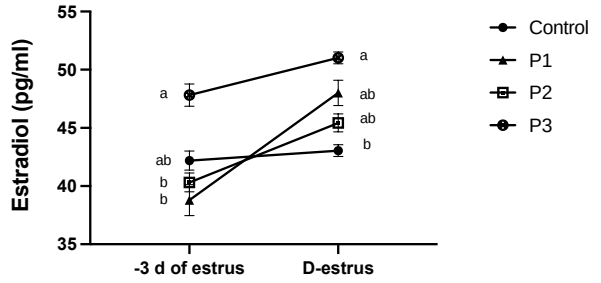

Fig. 1: Effect of nutritional supplementation on plasma estradiol profiles on 3 days before estrus and day of estrus. C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3=Supplementation concentrate with 6% Lemuru oil. Different superscripts in the figure indicate significant difference due to different flushing treatment on 3 days before estrus and day of estrus (P<0.05).

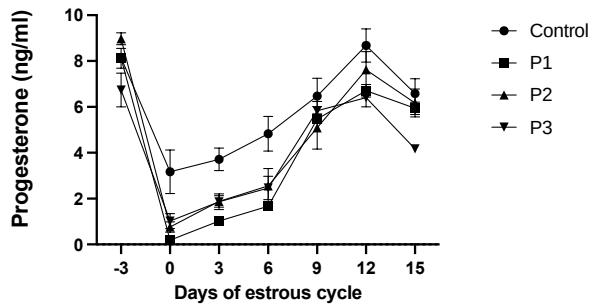

Fig. 2: Effect of nutritional supplementation on plasma progesterone profiles on 3 days before estrus and different days after estrous expression (mating day=day 0). C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3=Supplementation concentrate with 6% Lemuru oil.

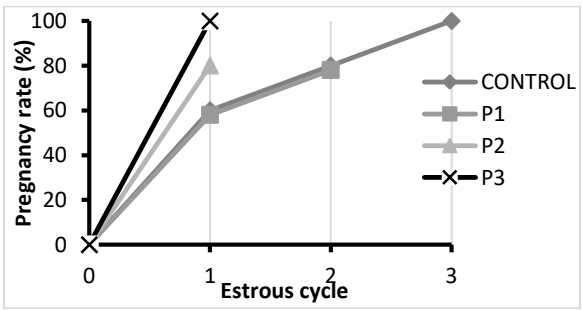

Fig. 3: The dynamics of pregnancy rate following three consecutive estrous cycles in the control and flushed groups. C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3=Supplementation concentrate with 6% Lemuru oil.

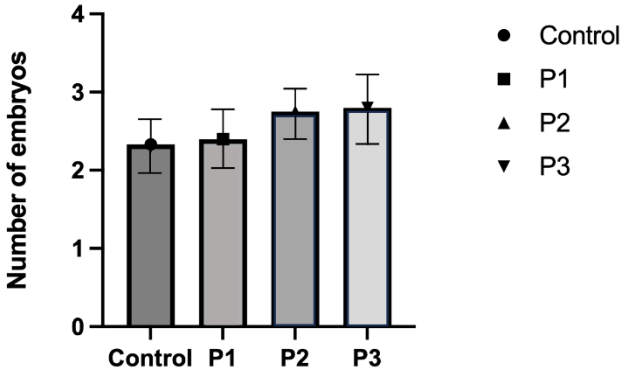

Fig. 4: Number of embryos in each experimental group. C=Control; P1=Supplementation concentrate with 6% palm oil; P2=Supplementation concentrate with 3% palm oil and 3% Lemuru oil; P3=Supplementation concentrate with 6% Lemuru oil.

DISCUSSION

Total DMI did not differ in ewes of different group in this experiment, which indicates that oil supplementation of diet did not affect its palatability. The total DMI in this study met the recommendations of NRC (2007). Since the protein content of the flushing diets was higher when compared with the control diet (17% vs 14%), the higher protein intake in these groups was expected. However, this effect was only evident for the P1 and P2 groups, probably because the animals in the P3 group tended to have relatively lower dry matter intake. The total protein intake in this study also met the recommendations of NRC (2007). In terms of crude fat intake, animals in the control group showed significantly lower crude fat intake than animals in the flushed groups. Since the flushing treatment was a mix of concentrate feed and oil supplement, this result was expected. As previously stated, significantly higher (P<0.05) levels of crude fat intake for animals in P1 and P2 groups compared with the animals in the P3 group could be explained by the tendency of the animals in this latter group to have lower dry matter intake.

The mean BCS before supplementation was lower than the recommended target BCS of 3.00 in all groups at the timing of reproduction in sheep (Maurya *et al.*, 2009). Other studies in sheep suggest that animals with BCS ranging from 3.00 to 3.50 show high reproductive performance (Sejian *et al.*, 2010). However, higher BCS may show adverse effects on ovulation, embryo loss and

conception rates, which in turn affect pregnancy success (Kenyon *et al.*, 2014). According to Scaramuzzi *et al.* (2006), a low BCS at the timing of nutritional supplementation is expected to increase the positive effects of flushing on ovulation rate. There was a trend for the animals within each experimental group to gain BCS during the study that could be explained by our experimental conditions despite the fact that flushing protocols were not designed to increase the BCS or body weight of the animals.

The higher plasma cholesterol concentrations in ewes of P2 and P3 groups than those of control and P1 groups is likely due to the nature of the oil used for supplementation. Indeed, contrary to vegetable-based oils, animal-based oils like Lemuru fish oil, contain cholesterol-type sterols (Clifton, 2002). High levels of plasma cholesterol are important to support the biosynthesis of steroid hormones involved in the growth of follicles, estrous behaviour and ovulation (Schade *et al.*, 2020). Cholesterol is known to be a precursor to the biosynthesis of steroid hormones such as progesterone, cortisol, corticosterone, and estradiol (Strauss and FitzGerald, 2019).

The high plasma concentrations of estradiol for the animals in the P3 group is in line with the high plasma cholesterol levels recorded for the animals in this same group, and could be due to the high-fat content of EPA and DHA in the feed (Mirzaei-Alamouti *et al.*, 2018). Similarly, higher plasma concentrations of estradiol were reported when a diet supplemented with 3% fish oil was compared with a diet supplemented with sunflower seed oil and calcium soap from palm oil in Afshari sheep (Mirzaei-Alamouti *et al.*, 2018). However, a decrease in plasma estradiol levels following the administration of 0.6 mL/kg BW of fish oil was found in goats (Mahla *et al.*, 2017). According to Verma *et al.* (2018), administration of fish oil in low (<0.3 mL/kg feed) doses increases the blood estrogen response but medium and high (0.6-1.2 mL/kg feed) doses induce a decrease in blood estrogen levels in goats. In our study, the doses of fish oil could be considered as high, since these reached 2.1 and 4.2 mL/kg feed in the diets of P2 and P3 groups, respectively. Altogether, these differences might be attributable to differences in oil concentrations and species-specific responses. Our data emphasizes that the addition of fish oil at the appropriate dose in the diet promotes a favorable estrogen response compared to vegetable oil and a control situation.

Plasma estradiol concentrations exhibited a drastic increase between the two blood sample collections in animals of flushed groups. A similar increase in plasma estradiol concentrations in ewes supplemented with fish oil has been previously reported (Mirzaei-Alamouti *et al.*, 2018). The fact that this increase was observed in all flushed groups, including the animals in the P1 group that had a vegetal-based oil supplementation, would argue in favor of an interaction between oil supplementation and the lipid metabolism, thereby boosting the secretion of ovarian steroids (Meza-Villalvazo *et al.*, 2018). This argument is favored by the higher levels of crude fat intake by animals in these groups compared to animals of control group, as fatty acids are crucial in steroidogenesis (Khajeh *et al.*, 2017). Indeed, fatty acids are involved in the control of estrogen levels in livestock (Zeng *et al.*, 2023).

Non-significant difference in plasma progesterone concentration was reported in sheep given 3% fish oil (Mirzaei-Alamouti *et al.*, 2018) or supplemented with flaxseed oil four days before mating (Soydan *et al.*, 2020). Altogether, this suggests that the nutrient-sensing pathways involved in mediating the effects of flushing on the ovarian functions, in this case concentrate as well as vegetal and animal-based oils, did not interfere with the pathways controlling progesterone secretion.

In this experiment, onset of estrus was earlier for the animals in the supplemented groups when compared with the animals in the control group. This highlights the fact that nutritional supplementation can influence the dynamics of follicular development. Habibizad *et al.* (2015) found that nutritionally supplemented sheep showed earlier symptoms of estrus in comparison with the animals given the control diet. Furthermore, providing high energy diets for a short period of time can stimulate follicular growth and ovulation (Zabuli *et al.*, 2010; Alves *et al.*, 2019; Domingues *et al.*, 2020).

The present study showed that flushing treatments did not affect the duration of estrus, which is in alignment with previous reports in sheep fed moderate and high nutrition (Farrag, 2019). The flushing treatments with 6% Lemuru oil (P3 group) increased the receptivity to the males compared to control group. This supports the fact that animals in this group had higher levels of plasma estrogen concentrations during estrus when compared with the animal in the control group. Indeed, estrogen contributes to the establishment of mating behavior (Juengel *et al.*, 2020). It has been established that this effect is mediated through central pathways, where estrogen stimulates catecholaminergic neurons involved in the central control of the mating behavior (Wersinger and Rissman, 2000). Another plausible pathway involves the influence of estrogen on the production of pheromones (Bakker *et al.*, 2010). Therefore, the increased plasma estradiol concentrations for the ewes in the Lemuru oil flushed group could have increased their attractivity (Rekwot *et al.*, 2001).

The pregnancy rate at first estrus was highest in P3 group, which coincides with our results for estradiol concentrations at estrus and receptivity to males. Similar effect was previously observed in fat-tailed Iranian Afshari ewes supplemented with fish oil (Mirzaei-Alamouti *et al.*, 2018). The number of embryos did not differ among flushed and non-flushed groups in this experiment, although it tended to be higher for the animals in the groups supplemented with fish oil (P2 and P3 groups). Similarly, Mahla *et al.* (2017) reported that goats given fish oil tended to have a higher number of embryos than those given palm oil but this difference was not statistically significant.

Conclusions: A short-term nutritional flushing including supplementation with vegetal (palm oil) or animal-based (Lemuru fish) oils improves reproductive performance of ewes following natural mating after estrus synchronization. However, this response was further improved when using animal-based oil supplementation, especially the Lemuru fish oil. These results highlight the

positive effect of specific components of animal-based oils, like DHA and EPA, and suggest that Lemuru fish oil constitutes an interesting feed supplement for ewes. It could be an interesting suggestion to increase the reproductive performance of sheep, specifically in Indonesia where this supplement is readily available and can be locally-produced.

Conflict of interests: The authors declare that they have no conflicts of interest.

Acknowledgments: We would like to extend our thanks to talent management BRIN and the Indonesian Ministry of Technology and Higher Education for funding our study through research grant No. 1/AMD/E1.KP.PTNBH/2020.

Authors contributions: AN, LK, RIA, MS and DAA conceived the idea and designed the study. AN, RIA, HH and DAA executed the experiment and analyzed samples. HH, AN, PIS and JBM analyzed the data. HH, AN, JBM and PIS prepared the manuscript. JBM, HH, RIA, AN, PIS, LK, MS and DAA reviewed the manuscript.

REFERENCES

Abecia JA, Sosa C, Forcada F, *et al*., 2006. The effect of undernutrition on the establishment of pregnancy in the ewe. Reprod Nutr Dev 46(4): 367–78.

Alves JPM, Fernandes CCL, Rossetto R, *et al*., 2019. Impact of short nutrient stimuli with different energy source on follicle dynamics and quality of oocyte from hormonally stimulated goats. Reprod Domest Anim 54(9): 1206–16.

Bakker J, Pierman S and González-Martínez D, 2010. Effects of aromatase mutation (ArKO) on the sexual differentiation of kisspeptin neuronal numbers and their activation by same versus opposite sex urinary pheromones. Horm Behav 57(4–5): 390–95.

Clifton P, 2002. Plant sterol and stanols—comparison and contrasts. Sterols versus stanols in cholesterol-lowering: Is there a difference? Atheroscler Suppl 3(3): 5–9.

Domingues RR, Ginther OJ, Toledo MZ, *et al*., 2020. Increased dietary energy alters follicle dynamics and wave patterns in heifers. Reproduction 160(6): 943–53.

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. World's Vet J 9(3): 201–10.

Habibizad J, Riasi A, Kohram H, *et al*., 2015. Effect of feeding greater amounts of dietary energy for a short-term with or without eCG injection on reproductive performance, serum metabolites and hormones in ewes. Anim Reprod Sci 160: 82–89.

Hammiche F, Vujkovic M, Wijburg W, *et al*., 2011. Increased preconception omega-3 polyunsaturated fatty acid intake improves embryo morphology. Fertil Steril 95(5):1820–23.

Juengel JL, Quirke LD, Hyslop K, *et al*., 2020. Association of fertility with group mating behavior in ewes. Anim Reprod Sci 216: 106359.

Kenyon P, Maloney S and Blache D, 2014. Review of sheep body condition score in relation to production characteristics. New Zealand J Agric Res 57(1): 38–64.

Khajeh M, Rahbarghazi R, Nouri M, *et al*., 2017. Potential role of polyunsaturated fatty acids, with particular regard to the signaling pathways of arachidonic acid and its derivatives in the process of maturation of the oocytes: Contemporary review. Biomed Pharmacother 94: 458–67.

Kia HD and Safdar AHA, 2015. Effects of calcium salts of fatty acids (CSFA) with different profiles (ømega-3 and ømega-6) during the flushing period on reproductive performance of 'Afshari'ewes. Small Rumin Res 126(8): 1–8.

Kosasih W, Rosmalina RT, Risdian C, *et al*., 2021. Production of omega-3 fatty acids by enzymatic hydrolysis from lemuru fish by-products. Sains Malaysiana 50(8): 2271–82.

Mahla AS, Chaudhari RK, Verma AK, *et al*., 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.

Makela B, Recktenwald E, Alves FC, *et al*., 2022. Effect of pre-conceptional nutrition and season on fetal growth during early pregnancy in sheep. Theriogenology 190: 22–31.

Maurya VP, Kumar S, Kumar D, *et al*., 2009. Effect of body condition score on reproductive performance of Chokla ewes. Indian J Anim Sci 79(11): 1136–38.

Meza-Villalvazo VM, Magaña-Sevilla H, Rojas-Marquez CA, *et al*., 2018. Corn oil enhances progesterone and estradiol plasma levels in tropical hair sheep. Ecosistemas y Recursos Agropecuarios 5(15): 583–89.

Mirzaei-Alamouti H, Mohammadi Z, Shahir MH, *et al*., 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.

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

Nurlatifah A, Khotijah L, Arifiantini RI, *et al*., 2022. Colostrum quality of ewe fed flushing diet containing EPA and DHA associated with lamb performance. Trop Anim Sci J 45(3): 348–55.

Rekwot PI, Ogwu D, Oyedipe EO, *et al*., 2001. The role of pheromones and biostimulation in animal reproduction. Anim Reprod Sci 65(3–4): 157–70.

Scaramuzzi RJ, Campbell BK, Downing JA, *et al*., 2006. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reprod Nutr Dev 46(4): 339–54.

Schade DS, Shey L and Eaton RP, 2020. Cholesterol review: A metabolically important molecule. Endocr Pract 26(12):1514–23.

Sejian V, Maurya VP, Naqvi SMK, *et al*., 2010. Effect of induced body condition score differences on physiological response, productive and reproductive performance of Malpura ewes kept in a hot, semi-arid environment. J Anim Physiol Anim Nutr (Berl) 94(2): 154–61.

Soydan E, Kuran M, Ocak N, *et al*., 2020. Effects of omega-3 and omega-6 fatty acids on some reproductive parameters in ewes. Large Anim Rev 26(6): 329–36.

Sözbilir NB, Maraşli Ş, Öztürkler Y, *et al*., 2006. Effects of double injections of PGF_ {2}\backslashalpha at different intervals on some reproductive traits in Tuj ewes. Turkish J Vet Anim Sci 30(2): 207–11.

Strauss JF and FitzGerald GA, 2019. Steroid hormones and other lipid molecules involved in human reproduction. In: Yen and Jaffe's Reproductive Endocrinology, pp:75–114. Elsevier.

Verma AK, Mahla AS, Chaudhari RK, *et al*., 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: 153–61.

Wersinger SR and Rissman EF, 2000. Dopamine activates masculine sexual behavior independent of the estrogen receptor α. J Neurosci 20(11): 4248–54.

Zabuli J, Tanaka T, Lu W, *et al*., 2010. Intermittent nutritional stimulus by short-term treatment of high-energy diet promotes ovarian performance together with increases in blood levels of glucose and insulin in cycling goats. Anim Reprod Sci 122(3–4): 288–93.

Zeng X, Li S, Liu L, *et al*., 2013. Role of functional fatty acids in modulation of reproductive potential in livestock. J Anim Sci Biotechnol 14(1): 24.