



Effect of n-3 PUFA rich fish oil supplementation on the reproductive performance of seasonally acyclic goats

DUSHYANT YADAV¹, AMIT KUMAR SINGH², AAMIR SALAM TEELI³, PUNEETH KUMAR⁴, BRIJESH KUMAR⁵, SANJAY KUMAR SINGH⁶, HARENDRA KUMAR⁷, GYANENDRA SINGH⁸, SACHIN KUMAR⁹, BHAWANA TYAGI¹⁰, MED RAM VERMA¹¹ and NARAYANAN KRISHNASWAMY¹²

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

In the present study, effect of dietary supplementation of fish oil during the non-breeding season on the reproductive performance of the goats was investigated. Experimental does were fed an isocaloric diet of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) rich fish (FO; n=12) or palm oil (PO; n=12) for 71 days. Periodical ovarian scanning for 21 days from the day of supplementation (day 0) confirmed acyclicity. Estrus induction was done by intra-vaginal progesterone (P₄) sponge for 14 days from day 22 to 35 of supplementation. Dietary FO did not affect serum P₄ throughout the period of supplementation. Ovarian scanning studies revealed that neither the number of surface follicles nor the diameter of largest surface follicle was significantly different between the groups at any point of supplementation. Similarly, the concentration of serum estradiol (E₂) and P₄ on the day of induced estrus was comparable between the groups. However, supplementation of FO decreased the PGFM significantly on day 16–18 post-estrus. In conclusion, supplementation of EPA and DHA rich FO for about 10 weeks inhibited the endometrial PGF_{2α} production during the luteolytic window at induced estrus; however, it did not improve the ovarian function and fertility in the seasonally acyclic goat.

Key words: Fertility, Fish oil, Goat, Non-breeding season, Palm oil, PGFM, PUFA

Dietary supplementation of fat in general and polyunsaturated fatty acid (PUFA) in specific is known to improve the reproduction in dairy cows. Besides improvement in the conception rate (CR), a positive effect of n-3 PUFA supplementation on different reproductive processes like size of preovulatory follicle (Mendoza *et al.* 2011), quality of oocyte (Zeron *et al.* 2002), corpus luteum (Petit *et al.* 2002), and embryo (Childs *et al.* 2008) has been reported. Diets rich in n-3 PUFAs like α -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) favour the production of series-3 PG at the expense of series-2 PG through competitive inhibition of

$\Delta 6$ desaturase, exclusion of arachidonic acid (AA) from the phospholipid bilayer and competitive inhibition of cyclooxygenase-2 (COX-2) enzyme (Calder 2013). Though the significance of n-6 AA acid on the ovulation, implantation, luteolysis and parturition of the farm animals is known (Weems *et al.* 2006), the role of n-3 PUFA in reproductive processes is emerging it is generally accepted that the inhibitory effect of dietary n-3 PUFA on endometrial PGF during the late luteal phase of the fertile estrous cycle (Gulliver *et al.* 2012) thus resulting antiluteolytic effect favours the conceptus survival (Mattos *et al.* 2000).

In goats, dietary EPA and DHA rich FO @ 72 mg/kg body weight for 8 weeks inhibited oxytocin induced PGF_{2α} pulses and estradiol (E₂) during the luteolytic window of estrous cycle (Verma *et al.* 2018). Further, supplementation of FO @ 156 mg/kg body weight for 10 weeks during the breeding season increased twinning by 25% and improved embryo survival by decreasing the PGFM and E₂ concentration during the window of maternal recognition of pregnancy (Mahla *et al.* 2016, 2017) through the down regulation of COX-2, cPLA2 and PGFS transcripts (Chaudhari *et al.* 2018). Therefore, present study was undertaken to explore n-3 PUFA rich FO supplementation in acyclic goats in an attempt to increase ovulation and fertility by improving the utero-ovarian functions.

Present address: ^{1,4}PhD Scholar (drdushyantyadav52@gmail.com, puneethargo@gmail.com), ²Research Associate (shrinet.amit@gmail.com), ³MVSc Scholar (teeliaamir7@gmail.com), ⁵Scientist (drbrijeshvet02@gmail.com), ^{6,7}Principal Scientist (hkumar1960@gmail.com), Division of Animal Reproduction; ⁸Principal Scientist (gyanendra@ivri.res.in), Division of Veterinary Physiology and Climatology; ¹¹Principal Scientist (medramverma@rediffmail.com, vetnanny@gmail.com), Division of Livestock Economics, Statistics and Information Technology. ¹⁰Scientist (bhawana_biochem@rediffmail.com), ⁶Research Associate (arensachin@gmail.com), Division of Dairy Cattle Nutrition.

MATERIALS AND METHODS

Experimental animals and supplementation schedule: Normal, healthy and non-pregnant Rohilkhandi does (24) in 1–2 parity, were equally divided into treatment and control groups (n=12/group). Goats in the treatment group were fed concentrate feed supplemented with n-3 PUFA rich refined FO (Avestia Pharma, Mumbai). A dose of 156 mg/kg was chosen (Mahla *et al.* 2017, Verma *et al.* 2018). Palm oil (PO) was supplemented to the goats of control group according to the body weight to make the diet isocaloric. Feeding trial was done between December and February, which is the typical non-breeding season for the goat breeds in India (Agarwal *et al.* 1986). Experimental goats received the respective diets for 71 days that comprised of observation for acyclicity (21 days), progesterone (P_4) sponging (14 days) and post-breeding period (35 days).

Follicular development, estrus induction and breeding: To ensure acyclicity, the ovarian activity was examined sonographically (Aloka SSD 500, Japan) at 4-day interval till 21 days from the commencement of FO supplementation (day 0). Induction of estrus was done with intra-vaginal P_4 sponge, kept *in-situ* for 14 days in the vagina. Goats in estrus were bred twice with a fertile buck. To monitor the ovarian activity during the P_4 sponging, USG was done on the day of insertion (day 22 of feeding trial), day 7 of treatment (day 29 of feeding trial) and on the day of sponge removal (day 35 of feeding trial). At induced estrus, the diameter of largest follicle was recorded. Pregnancy diagnosis was done on day 35 by trans-rectal ultrasonography.

Endocrine changes and serum fatty acid: The serum concentration of EPA and DHA before and after the feeding trial was done by gas chromatography in a subset of experimental animals (n=4/group). Serum E_2 and P_4 were assayed by radioimmunoassay. To study the effect of FO supplementation on the modulation of endometrial $PGF_{2\alpha}$ Production, 13,14-dihydro-15 keto $PGF_{2\alpha}$ (PGFM) was assayed on day 16, 17 and 18 post-breeding using ELISA kit (Blue Gene Biotech, China).

Statistical analysis: The number of surface follicles, diameter of the largest follicle and number of CL and concentration of serum E_2 and P_4 at different points of time were analyzed by Proc Mixed procedure of SAS 9.3. Time interval from sponge withdrawal to the onset of estrus was tested by Kaplan-Meier survival curve analysis.

RESULTS AND DISCUSSION

Concentration of serum EPA and DHA at pre-and post-supplementation of FO: The concentration of serum EPA and DHA was significantly high ($P<0.05$) in the FO supplemented group as compared to PO at the end of the feeding trial (Fig. 1). The concentration of EPA and DHA increased 5.7 and 7.7 times, respectively in the FO group on day 71 of experiment with reference to pre-feeding and control group values; indicating the absorption from the gastrointestinal tract and bioavailability. Short chain (C18)

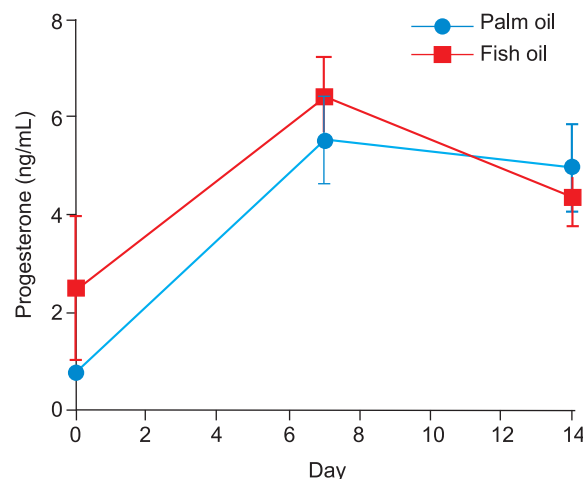


Fig. 1. Effect of dietary FO or PO on serum P_4 during the sponging period in seasonally anestrous Rohilkhandi doe ($P<0.0001$).

n-3 PUFA like α -linolenic acid or n-6 linoleic acid are biohydrogenated in the rumen to the extent of 70–85% (Juchem *et al.* 2007). In contrast, long chain n-3 PUFA such as EPA and DHA undergoes minimum biohydrogenation (Ashes *et al.* 1992). Collectively, the existing literature supports the notion that feeding protected or unprotected long chain n-3 PUFA can increase the serum EPA and DHA; alter the fatty acid profile in different tissues and improve fetal uptake.

Ovarian functions during the first 21 days of treatment: Ovarian scanning at periodic intervals for first 21 days of the feeding revealed acyclicity; based on the presence of CL which was 83 (1/12) and 75% (3/12) in PO and FO group, respectively ($P=0.5091$). Neither period nor treatment effect was significant on the diameter of largest follicle, number of surface follicles and concentration of serum P_4 and E_2 during the pre-sponging period. However, there was a numerical decrease in the E_2 in FO group on day 20 of the feeding trial. Feeding trial (71 day) was grouped into pre- P_4 sponging (first 21 days), P_4 treatment (day 22 to 35 of feeding), sponge removal till the onset of estrus (day 36 to 38 of feeding) and at induced estrous cycle (day 36–38 to 71 of feeding). As estrus was spread over a period 24–72 h post-sponge removal, onset of estrus had a range from day 36–38 of feeding trial.

Ovarian functions during the period of P_4 sponging (Day 22 to 35 of feeding trial): No significant effect of FO feeding was observed either on the diameter of the largest follicle or mean number of surface follicles. A significant increase in the serum P_4 on day 7 and 14 post-insertion of the sponge ($P<0.05$) supports the efficacy of the sponge (Fig. 1). After the removal of P_4 sponge, ovarian scanning was done in the experimental goats at 24 h interval till the onset of estrus or up to six consecutive days, whichever was earlier. No effect of treatment or day was observed on the diameter of the largest follicle. During winter, 83.3% goats in the PO group and 75% in FO group were acyclic. Seasonal breeding is common in most breeds of goat in India; high frequency

of estrus occurs in October followed by June and lowest in March (Mishra and Biswas 1966). Anestrus in Jamunapari and Barbari breeds, and Marwari and Kutchi breeds of goat was observed from December to April (Agarwal *et al.* 1986) and May to October (Nandy *et al.* 2001), respectively. Seasonal variation in the breeding is observed in most breed of goats originating from hilly areas and in some local breeds of subtropical regions (Bodin *et al.* 2007). Presence of 4–6 mm diameter of follicles at seasonal anestrus is in accordance with the notion that follicular waves occur during acyclicity (Sarath *et al.* 2008). A decreasing trend in the serum E_2 in FO group does is consistent with previous reports (Mahla *et al.* 2017, Verma *et al.* 2018).

Effect of FO on reproductive variables at induced estrous cycle: No significant difference was found in the diameter of the largest follicle on the day of estrus in either group. Neither the concentration of serum E_2 nor P_4 on the day of induced estrus was significantly different between the groups ($P>0.05$). No significant difference in the diameter of the largest follicle on the day of estrus is supported by the finding that supplementation of FO did not increase the diameter of preovulatory follicle in the goat during breeding season (Mahla *et al.* 2017). Removal of P_4 sponge to estrus onset was not significantly different between the FO and PO fed goats, which could be due to the inimical effect of season and low sample size (8 goats in FO and 5 in PO). Further, no difference in the duration of estrus onset between FO and PO groups could be due to the lack of functional CL in the seasonally acyclic goats. Alpha-linolenic rich linseed diet increased the $PGF_{2\alpha}$ induced estrus by 3.6 h with longer plasma LH and E_2 peak in the cow (Zachut *et al.* 2011). Overall, the results indicate that the estrus onset and its duration were within the physiological limits in most studies. Supplementation of FO did not affect serum P_4 at estrus that could be due to decreased serum cholesterol level following feeding n-3 PUFA rich diet (Verma *et al.* 2017).

Kaplan-Meier survival curve analysis revealed that 63.6% doe in the FO group showed estrus within 72 h of sponge removal compared to 54.5% in the PO group (Fig. 2). The mean duration of induced estrus (h) was 38.4 ± 9 in PO, while it was 61.5 ± 12.3 in FO group ($P=0.2067$). Similarly, FO supplementation did not influence serum P_4 throughout the synchronized estrous cycle in the cyclic goat

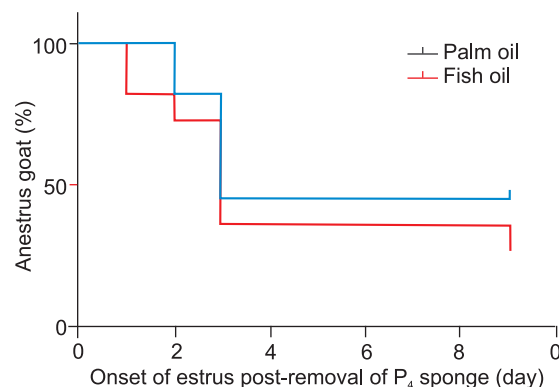


Fig. 2. Effect of dietary FO or PO on estrus onset from the time of sponge removal in seasonally anestrus Rohilkhandi doe ($P=0.4077$). Day 0 represents time of sponge removal. About 63.6% doe in the FO group showed estrus within 72 h while it was 54.50% in the control group ($P>0.05$).

(Mahla *et al.* 2017). The EIR (%) of 66.7 and 41.7 in the FO and PO groups, respectively ($P>0.05$) was not statistically significant.

As compared to EIR, the CR (%) was low (33.3 and 16.7 in FO and PO groups, respectively) in the present study ($P>0.05$) (Table 1). Decreased CR in the FO is partly due to decreased tupping per cent, which is due to absence of standing estrus in 50% goat following induction of heat. It is reported that the CR did not differ in the estrus synchronized goat during breeding season (Mahla *et al.* 2017). An absence of treatment effect on CR is in agreement with the previous reports in the cow supplemented with fish meal (Burke *et al.* 1997) and flaxseed (Petit and Twagiramungu 2006, Fuentes *et al.* 2008). Similarly, flaxseed oil supplementation prior to mating did not affect the reproductive performance including pregnancy rate in the ewe (Akbarinejad *et al.* 2012).

An EIR of 42–67% during the non-breeding season in the experimental does indicates that inducing preovulatory LH surge through P_4 block is not as effective as estrus synchronization during the breeding season. A decrease in the LH pulse frequency by 54% during the non-breeding season (Chemineau *et al.* 1986) is likely to be the major reason behind low EIR and CR. Further, the P_4 treatment for 14 days (long-term treatment) might have adversely affected the folliculogenesis and oocyte competence; and for this reason, short term P_4 protocol is advocated (Rubaines and Menchaca 2003).

The concentration of serum P_4 (ng/mL) on day 8 post-breeding was 13.7 ± 0.90 and 14.2 ± 3.15 , in the pregnant goats of FO and PO group, respectively. With respect to endometrial function, a significant decrease in the serum PGFM was seen in the goats of FO group on day 16–18 post-estrus (Fig. 3) indicating that dietary n-3 PUFA inhibits endometrial $PGF_{2\alpha}$ production. The concentration of serum E_2 on the day of induced estrus was not significantly different between the groups. In contrast, supplementation of FO for 35–58 day significantly decreased serum E_2 on the day of estrus as well during diestrus in the cyclic goat

Table 1. Effect of dietary FO or PO supplementation on the reproductive performance at induced estrus in the seasonally anestrus Rohilkhandi goat

Reproductive variable	Treatment (n=12/group)		Statistical value	
	Palm oil	Fish oil	χ^2 (Yate's corrected)	P
Estrus induction rate (EIR, %)	41.66 (5/12)	66.66 (8/12)	0.6713	0.4126
Tupping %	80 (4/5)	50 (4/8)	0.1875	0.6650
Conception rate (CR, %)	33.33 (4/12)	16.66 (2/12)	0.2222	0.4714

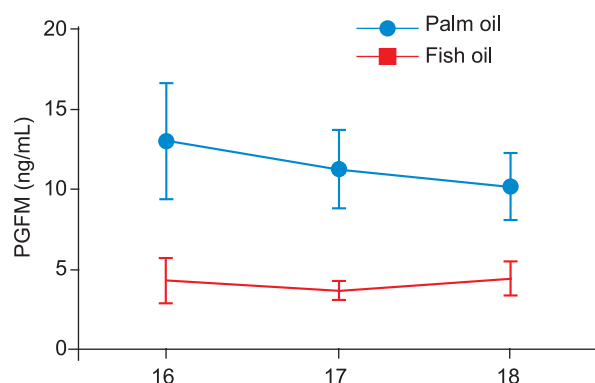


Fig. 3. Effect of dietary FO or PO on serum PGFM (ng/mL) during the maternal recognition period after induced estrus in the seasonally anestrous Rohilkhandi doe (Mean±SEM; n=4/group) (P=0.0371).

(Mahla *et al.* 2017, Verma *et al.* 2018).

With respect to endometrial function, a significant decrease in the serum PGFM was seen in the goats of FO group on day 16–18 post-estrus (Fig. 3), which is in accordance with our earlier report that dietary FO decreased PGFM as well as PGEM during the window of MRP in the cyclic goat (Mahla *et al.* 2017). Similarly, FO supplementation decreased the basal as well as OXT induced $\text{PGF}_{2\alpha}$ production during the late luteal phase in the goat (Verma *et al.* 2018).

The results suggest that the supplementation of n-3 PUFA rich FO for 10 weeks could inhibit endometrial $\text{PGF}_{2\alpha}$ production during the maternal pregnancy recognition window following estrus induction; however, it did not improve the ovarian function as well as fertility during the non-breeding season in the Rohilkhandi goat.

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