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Dietary n-3 Polyunsaturated Fatty Acid Affects the Onset of Prostaglandin $F_{2\alpha}$ Induced Oestrus in the Goat (*Capra hircus*)

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ABSTRACT

The objective of the present study was to examine the effect of dietary n-3 PUFA supplementation on oestrus response in the goat following synchronization with prostaglandin analogue. Parous cycling goats (n=17/group) were fed a concentrate diet supplemented with either fish oil (FO) or palm oil (PO). The FO provided n-3 EPA and DHA @ 156 mg kg^{-1} body weight while PO was given @ 0.6 ml kg^{-1} body weight to make the diet isocaloric. Oestrus was synchronized using two dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at 11 days apart, with first PG on day 25 of oil supplementation. Goats were observed for oestrus twice a day using a vasectomized teaser buck following second injection of $PGF_{2\alpha}$. The number and diameter of preovulatory follicle (POF) on the day of oestrus was studied using transrectal ultrasonography. The mean interval from $PGF_{2\alpha}$ administration to the onset of oestrus was significantly (χ^2_{df1}=7.003, *P*=0.008) longer in FO than PO supplemented goats (48.71±3.78 vs 37.41±1.75 h). The proportion of goats showed oestrus within 48 h was 94.11% in the PO group (16/17), while it was 58.82% in the FO group (10/17). However, the oestrus duration was not affected by the FO supplementation. The number of POF was higher in the FO group than the PO (2.23±0.14 vs. 1.82±0.15; *P*=0.054); however the diameter of POF did not differ among the group (6.90±0.10 vs. 6.77±0.14; *P*>0.05). In conclusion, the supplementation of goats with n-3 PUFA rich FO delayed the onset of $PGF_{2\alpha}$ induced oestrus and increased the POF number on the day of oestrus.

Keywords: Fish oil, Goat, n-3 PUFA, Oestrus, $PGF_{2\alpha}$

Dietary fat supplementation has been used to increase the energy density of feeds to alleviate postpartum negative energy balance, improve milk production and fertility in the dairy cattle (Staples *et al.*, 1998; Santos *et al.*, 2008). There is consensus that the effect of supplemented fat on reproduction is not only due to improvement in the energy status of animal but also the composition of fatty acid in the fat which has a crucial role in determining the effect on reproduction (Mattos *et al.*, 2000; Lucy, 2001). Therefore, attention has been now focused on identifying the potential role of various fatty acids on reproduction, particularly long-chain polyunsaturated fatty acids (PUFAs) like omega-3 (n-3) and omega-6 (n-6) PUFAs (Wathes *et al.*, 2007; Santos *et al.*, 2008). PUFAs play an important role in the female reproductive events like ovulation, fertilization, and parturition (Abayasekara and Wathes, 1999). Several reports in dairy cattle have indicated positive effects of n-3 PUFA supplementation on different reproductive processes like follicle turnover and growth, ovulation, corpus luteum (CL) size, steroidogenesis and conception rate (Thatcher and Staples, 2000; Petit *et al.*, 2002; Ambrose *et al.*, 2006). Animals cannot synthesize n-3 or n-6 PUFAs *de novo* due to lack of desaturase enzyme, therefore, need to be supplied in the diet (Wathes *et al.*, 2007). The proportion of different PUFA in the diet alters the cell membrane phospholipid composition which influences various cellular processes. The PUFAs act as the precursors for eicosanoids including prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX) and leukotrienes (LT) in the cell (Abayasekara and

Wathes, 1999). Eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), long chain n-3 PUFA are the precursor of 3-series eicosanoids which are anti-inflammatory. In contrast, arachidonic acid (AA, 20:4 n-6) gives rise to proinflammatory eicosanoids of 2-series. The possible effects of n-3 PUFAs EPA and DHA on reproduction is principally explained by the inhibition of 2- series PG and favouring the production of the less bioactive 3-series PG through competitive inhibition of AA synthesis, exclusion of AA in the phospholipid bilayer and competitive inhibition of cyclooxygenase-2 (COX-2) enzyme (Thatcher and Staples, 2000).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a derivative of n-6 AA, is central to the control of estrous cycle by virtue of its luteolytic effect. Uterine endometrium secretes $PGF_{2\alpha}$ which induces an irreversible degeneration of the CL, characterized by a dramatic drop in progesterone (P_4) concentrations in the blood. The dietary n-3 PUFA reduces uterine $PGF_{2\alpha}$ secretion during critical stage of embryonic development thus preventing the onset of luteolysis and facilitating the establishment of pregnancy in cattle (Gulliver *et al.*, 2012). Mattos *et al.* (2000) proposed that n-3 PUFAs may also reduce the sensitivity of the CL to $PGF_{2\alpha}$ and delay the luteolysis and onset of subsequent oestrus. To the best of our knowledge, no study could be traced on the effect of n-3 PUFA on goat reproduction. Therefore, the objective of the present study was to investigate the effect of dietary supplementation of n-3 PUFA rich fish oil on oestrus response following the administration of exogenous $PGF_{2\alpha}$ and follicular development *vis-à-vis* number and diameter of pre-ovulatory follicle (POF) in the goat.

MATERIALS AND METHODS

Experimental animals and design

The study was conducted on local goats of Rohilkhand region during the breeding season (July–November) at Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. The experiment was approved by the Institute Animal Ethics Committee (IAEC). All the experimental goats were maintained under intensive housing system. The goats were vaccinated against Foot and Mouth disease and Peste des petits ruminants as per the prophylactic calendar. The goats were also dewormed before the start of experiment.

Apparently normal, cycling goats (n=34) of 1.5-2.5 years age and 1-2 parity were used in the study. The mean±SEM weight was 18.62±0.62 kg. The goats were randomly allocated to a concentrate diet supplemented with either (i) fish oil (FO; n=17), rich in n-3 PUFA or (ii) palm oil (PO; n=17), as a control. The FO contained 16% EPA and 10% DHA (total 26% n-3 PUFA) and was supplemented to provide 156 mg kg^{-1} body weight EPA and DHA (0.6 ml kg^{-1} body weight FO). The PO, rich in saturated fatty acids (51%) and mono-unsaturated fatty acids (39%), with negligible amount of n-3 PUFA, was supplemented @ 0.6 ml kg^{-1} body weight to make the diets isocaloric in both the groups. The feeding of oil supplemented diets was initiated following an acclimatization period of 7 days. The acclimatization period was started on the day of oestrus and during this period increasing amount of n-3 rich FO was supplemented to make the animal adjusted to the new diet. The goats were offered oil supplementation individually after thorough mixing in the concentrate feed daily between 1100-1200 h. Each goat was offered 0.25 kg DM of concentrate daily to meet the maintenance requirement. To fulfill the nutritional requirement, maize green (*Zea mays*) was given @ 0.5 kg/goat/day, while wheat straw was offered *ad libitum*. The ingredient composition and chemical analysis of the concentrate as well as the chemical analysis of the maize green and wheat straw is presented in Table 1. Goats of either group continued to receive the respective diets for 40 days (Fig. 1).

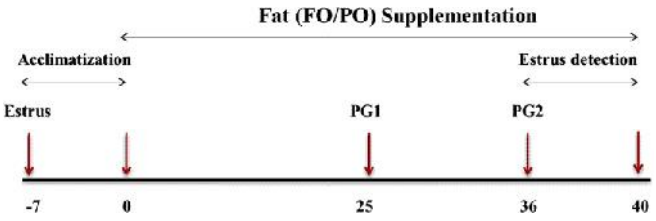

Fig. 1: Schematic representation of n-3 PUFA supplementation, oestrus synchronization and heat detection in the experimental goat

Oestrus synchronization and oestrus detection

Experimental goats were administered two intramuscular injection of Cloprestenol sodium @125µg/goat/dose; Vetmate® ($PGF_{2\alpha}$ analogue) at 11 days apart. First dose was given on day 25 of initiation of dietary

supplementation of FO or PO followed by second dose on day 36. The experimental flock was observed for oestrus twice a day (0600 and 1800 h) using a vasectomized teaser buck combined with inspection for 30 minutes following second injection of $PGF_{2\alpha}$ (Fig. 1). The oestrus was confirmed retrospectively by the estimation of P_4 concentration in serum (<1 ng ml^{-1}). The midpoint between the last unobserved and first observed oestrus signs was considered as the time of onset of oestrus, while the mid-point between the last observed and first unobserved oestrus signs was considered as the time of end of oestrus. The interval from second $PGF_{2\alpha}$ injection to onset of oestrus (oestrus response interval) and oestrus duration were calculated.

Ultrasound examination

The transrectal ultrasonographic examination of the ovaries was carried out on the day of second $PGF_{2\alpha}$ injection to ascertain the presence of CL using a real time B-mode ultrasound scanner (Aloka SSD 500, Japan) provided with a linear array transducer of 7.5 MHz frequency. The maximum diameter of CL was also measured. On the day of oestrus, the number and diameter of the POF was examined.

Progesterone assay

Blood samples were collected by jugular venipuncture on the day of second dose $PGF_{2\alpha}$ injection and the day of oestrus onset for the estimation of serum P_4 concentration. The serum was harvested and stored at -20° C until assay. The serum P_4 concentration was estimated using RIA kit (Immunotech®, France).

Statistical analysis

Kaplan-Meier survival analysis was done to see the interval from second dose $PGF_{2\alpha}$ injection to the onset of oestrus. A log rank test was run to determine the statistical difference in the oestrus onset in FO and PO groups. Pearson chi-square test was applied to see the association between the treatment and oestrus response interval. Oestrus response interval of 48 h from second $PGF_{2\alpha}$ injection was kept as cut-off point for the Pearson chi-square test. Interval of ≤48 h was considered as early response while >48 h as a delayed response. Odds ratio was also computed for the association study.

Normality of dependent variables (oestrus duration, P_4 concentration, CL diameter, POF number and its diameter) was tested by Shapiro-Wilk test. Comparison between FO and PO group for oestrus duration, CL size, POF number and its diameter was made by Independent-sample *t* tests and data were presented as mean±SE. Serum P_4 concentrations were compared by means of Mann-Whitney U tests and data was presented as mean±SD. Data analysis was done with SPSS software (IBM® SPSS® statistics, version 20.0).

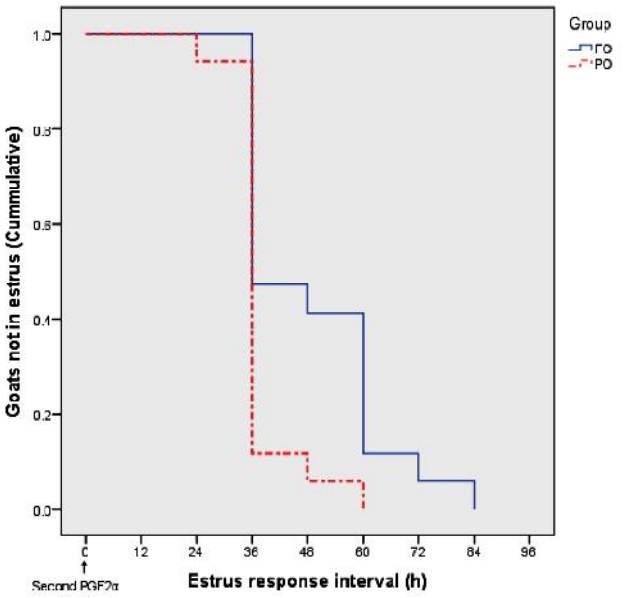

Fig. 2: Kaplan-Meier survival analysis showing the onset of oestrus following the second $PGF_{2\alpha}$ injection in the goats of FO and PO group. The proportion of goats that came into oestrus within 48 h was 58.82% and 94.11% in FO and PO groups, respectively. Log Rank test showed a statistically significant difference in the onset of oestrus (χ^2_{df1}=7.003, P=0.008).

RESULTS AND DISCUSSION

Onset of oestrus and oestrus duration

Goats of both FO and PO group showed behavioral oestrus within 84h following second $PGF_{2\alpha}$ injection. Mean interval from $PGF_{2\alpha}$ administration to the onset of oestrus was 48.71±3.78 h (range 36-84 h) and 37.41±1.75 h (range 24-60 h) in FO and PO groups, respectively. Higher proportion of goats was observed in oestrus (Fig. 2) within 48 h of second $PGF_{2\alpha}$ injection in PO group

(94.11%; 16/17) than that of FO group (58.82%; 10/17). A log rank test on the Kaplan-Meier survival curve showed a statistically significant difference in the onset of oestrus (χ^2_{df1}=7.003, *P*=0.008). Similarly, Pearson's chi-square test revealed that there was a significant association between n-3 PUFA supplementation and oestrus onset interval (χ^2_{df1}=5.88, *P*=0.015). The odds ratio of delayed oestrus (>48 h) in FO supplemented goat was 11.20 times higher (95% confidence interval, 1.19-105.132) than that of PO supplementation. The delayed oestrus response following n-3 PUFA supplementation in the dairy cow supports the results of present study. Burke *et al.* (1997) found that the proportion of cows with plasma P_4 concentration of >1 ng ml^{-1} 48 h post- $PGF_{2\alpha}$ injection was greater by 25% when n-3 PUFA rich fish meal was fed than the control diet. Similarly, Zachut *et al.* (2010) reported that the interval from $PGF_{2\alpha}$ injection to behavioral oestrus was longer in the cows supplemented n-3 PUFA rich flaxseed oil than with n-6 PUFA rich sunflower oil. Peripartum supplementation of n-3 rich linseed diet numerically increased the onset of $PGF_{2\alpha}$ induced oestrus by 3.6 h (*P*=0.1) postpartum and the plasma LH and E_2 peak were longer (6.5 h and 5.3 h, respectively) in other study by the same group (Zachut *et al.*, 2011).

Several reports indicated that feeding n-3 PUFA such as EPA and DHA induce an antiluteolytic effect by reducing the endometrial secretion of $PGF_{2\alpha}$ (Staples *et al.*, 1998; Mattos *et al.*, 2000); thus, delay the luteal regression and onset of the oestrus. The mechanism by which EPA and DHA inhibit secretion of uterine prostanoids is not fully understood. When n-3 PUFA rich diet is supplemented in the feed, EPA and DHA are incorporated into cellular lipid pools and may compete with AA for processing by the COX-1 and COX-2 and reduce the synthesis and activity of cyclooxygenases (Caldari-Torres *et al.*, 2006). This inhibits the synthesis of $PGF_{2\alpha}$ and leads to increased synthesis of prostanoids of 3- series at the expense of 2-series (Mattos *et al.*, 2004). The mechanism by which dietary n-3 PUFA delay the luteal regression and onset of oestrus following luteolytic doses of exogenous $PGF_{2\alpha}$ is not known. It is possible that EPA and DHA might reduce the sensitivity of the CL to $PGF_{2\alpha}$ as hypothesized by Mattos *et al.* (2000). It is reported that n-3 PUFAs such as EPA and DHA serve as precursor for the formation of the vasodilatory PGI_3 (Needleman *et al.*, 1979), which might be accumulated in the luteal cells following dietary supplementation of fish oil, and delay the CL regression mediated by a partial neutralization of the vasoconstrictive action of $PGF_{2\alpha}$. In addition, it has been demonstrated that $PGF_{2\alpha}$ increases the mRNA expression of the pro-inflammatory cytokines TNFα, IL-1β, and IFNγ that has the potential role in the mechanism of luteolysis (Neuvians *et al.*, 2004). Therefore, it is also possible that EPA and DHA may alter the process of luteolysis, as these fatty acids are the precursors of anti-inflammatory prostanoids.

Table 1: Ingredient compositions and chemical analysis of the concentrate and forage

	Concentrate	Green	Straw
(A) Ingredients (g kg^{-1})			
Wheat bran	520	—	—
Maize	250	—	—
Deoiled soya bean meal	200	—	—
Common salt	10	—	—
*Mineral mixture	20	—	—
(B) Chemical composition (g kg^{-1})			
DM	958	202	901.3
CP	197.2	69.1	33.9
ADF	128.1	430.1	509.9
NDF	459.6	735.4	762.2
EE	31.2	40.4	10.4
Ash	73.4	84.9	97.9
GE (Mcal kg^{-1})	3.82	3.47	3.9

Abbreviations DM: dry matter, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre, EE: ether extract, GE: gross energy

*Mineral mixture contained per kg of supplement: 220 g Ca, 120g P, 60 g Mg, 1000 mg Cu, 1200 mg Mn, 8000 mg Zn, 4000 mg Fe, 120 mg Co, 260 mg I and 700 mg F

The mean oestrus duration did not differ significantly (*P*>0.05) in both the groups (Table 2). Contrary to our finding, Zachut *et al.* (2011) observed that the duration of behavioral oestrus was longer in the flaxseed supplemented cows than the control (18.6±0.8 vs 15.8±0.9 h; *P*<0.04). The increased oestrus duration was due to longer interval from the onset of behavioral oestrus to LH peak (7.7±0.5 vs 6.4±0.6 h, *P*<0.1). It is reported that PGE_2 enhances GnRH release from the hypothalamus (Ojeda *et al.*, 1979) and the delay in LH surge may be due to alterations in n-3 PUFA mediated PGE_2. Supplementation of flaxseed

oil for 40 days to cycling sows significantly increased the duration of oestrus by 17 h as compared to control (Gokuldas, 2015). A lack of significance (*P*=1.00) on the duration of oestrus in FO supplemented goat might be due to twice-a-day oestrus detection schedule.

Table 2: Effect of dietary fish oil (FO) and palm oil (PO) supplementation on oestrus response, corpus luteum diameter, progesterone concentration and preovulatory follicles in the goat

Dependent Variable	FO	PO	P-value
Oestrus response interval (h; Mean±SD)	48.71±3.78	37.41±1.75	0.008
Oestrus duration (h; Mean±SE)	31.06±2.01	31.06±2.26	1.00
CL diameter (mm; Mean±SE)	11.82±0.41	10.19±0.36	0.006
Serum progesterone concentration (ng ml^{-1}; Median)†			
On day of $PGF_{2\alpha}$ administration	7.41 (1.41-13.64)	7.89 (2.16-20.67)	0.973
On day of oestrus	0.47 (0.29-0.96)	0.41 (0.17-0.98)	0.660
POF diameter‡ (mm; Mean±SE)	6.90±0.10	6.77±0.14	0.460
Number of POF‡ (Mean±SE)	2.23±0.14	1.82±0.15	0.054

Abbreviations: CL= corpus luteum; POF= preovulatory follicle
*on day of $PGF_{2\alpha}$ administration
†Figures in the parenthesis indicate range value
‡on day of oestrus

Corpus luteum diameter and progesterone concentration

The mean difference CL diameter on the day of second $PGF_{2\alpha}$ was significantly high by 1.63 mm in the FO group (*P*=0.006) as compared to PO supplemented goats (Table 2). A significant increase in the CL diameter in FO supplemented goats is supported by the results in the cow (Petit *et al.*, 2002; Bilby *et al.*, 2006) and mare (Ravi, 2014). Proliferation of granulosa cells induced by n-3 PUFA might be the reason behind large diameter of CL (Burke *et al.*, 1997; Lucy, 2001).

The serum P_4 concentration on the day of second $PGF_{2\alpha}$ injection was comparable in both the groups which declined to <1 ng ml^{-1} on the day of oestrus (Table 2). Ambrose *et al.* (2006) and Ponter *et al.* (2006) also reported non-significant effect of n-3 PUFA on plasma P4 concentrations in cow. Diet high in n-3 PUFA is shown to the lower plasma cholesterol concentration, the precursor for P4 synthesis (Robinson *et al.*, 2002). In contrast, EPA supplementation is associated with an increase in the peroxisome proliferator activated receptors (PPARs) in the cow resulting in reduced clearance of P_4 (Galbreath *et al.*, 2008).

Number and diameter of preovulatory follicle

The mean diameter of largest POF did not differ significantly (*P*=0.46) in both the groups (Table 2). The finding is in concurrence with the earlier reports in dairy cattle (Petit *et al.*, 2002; Robinson *et al.*, 2002; Ponter *et al.*, 2006; Zachut *et al.*, 2011). In contrast, the greater diameter of the ovulatory follicle was observed in dairy cows fed diets rich in n-3 PUFA (Ambrose *et al.*, 2006; Bilby *et al.*, 2006). The number of POF in FO supplemented goats was higher (*P*=0.054) on the day of induced estrus than that of PO (Table 2). Earlier studies have also reported that cows fed with long chain fatty acids had an increased number of follicles (Lucy *et al.*, 1991; Ryan *et al.*, 1992) and enhanced development of smaller into larger follicles (Hightshoe *et al.*, 1991). It is also plausible that an increase in the time to onset of oestrus in goats would allow the growth of more number of small or medium sized follicles to reach preovulatory size.

CONCLUSION

From the present study, it may be concluded that EPA and DHA rich FO supplementation delayed the oestrus onset following exogenous $PGF_{2\alpha}$ administration and increased the POF number in the goat. The result supports the hypothesis that n-3 PUFA supplementation reduce the sensitivity of CL to exogenous $PGF_{2\alpha}$ through the accumulation of EPA and DHA in the luteal cells.

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