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Effect of dietary supplementation of omega-3 polyunsaturated fatty acid (PUFA) rich fish oil on reproductive performance of the goat (*Capra hircus*)

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ABSTRACT

Dietary supplementation of n-3 PUFA decreases the luteolytic $PGF_{2\alpha}$ and improves the pregnancy rate in the dairy cow. However, its effect in the goat is not known. Accordingly, we studied the effect of supplementation of n-3 PUFA rich Fish oil (FO) on different reproductive events in the goat. Cycling goats (n = 30) were divided into two equal groups and fed an isocaloric and isonitrogenous diet supplemented with either FO (TRT; n = 15) or palm oil (PO) (CON; n = 15) @ 0.6 mL/kg body weight for 72 days during the breeding season. Estrus synchronization was done on day 25 and 36 of supplementation using two PG regimen and the goats in estrus were bred. Mean interval from $PGF_{2\alpha}$ administration to the onset of estrus was 12 h longer ($P < 0.05$) in the TRT group than that of CON. The number of preovulatory follicles (POF) and ovulation rate was significantly higher in FO supplemented goats ($P < 0.05$) by 39.64 and 41.35%, respectively. Though the corpus luteum diameter was significantly higher ($P < 0.05$) on day 5, 8 and 11 post-breeding in the TRT group, mean serum progesterone (P_4) did not differ significantly ($P > 0.05$). Mean concentration of serum estradiol (E_2) was significantly ($P < 0.01$) lower in the FO supplemented group during day 0–60 post-breeding which could be due to significantly low serum cholesterol ($P < 0.01$). Though the serum concentration of $PGF_{2\alpha}$ metabolite (PGFM) and PGE_2 metabolite (PGEM) in the pregnant goats was significantly ($P < 0.05$) lower in the TRT group on day 16 and 17 post-breeding, the ratio of PGEM to PGFM remained unaffected suggesting a favourable effect of FO supplementation on the early pregnancy. The number of embryos, twinning rate and kidding rate were high in FO supplemented group though it was non-significant. However, gestation length, birth weight of kids and neonatal behaviour were comparable between the groups ($P > 0.05$). In conclusion, supplementation of n-3 PUFA rich FO significantly increased the number of POF and ovulation rate with numerical increase in the kidding rate. Further, it decreased the serum E_2 and PGFM during the critical window of pregnancy recognition in the doe.

1. Introduction

Increasing the litter size by maximizing the ovulation rate and minimizing post-fertilization wastage is one of the approaches to augment reproductive rate of goat flock [1]. Various genetic selection and hormonal strategies have been attempted to increase the prolificacy of goat. Improvement of reproductive efficiency in the goat by genetic selection is slow and sometimes difficult as reproductive traits have low heritability [2]. Hormones have the limitations of high cost and require sound technical knowledge and physical intervention to the animal. Further, the animal industry is moving into practices that minimize or completely avoid chemical and hormonal treatments, and practices that do not compromise the welfare of the animals. Therefore, natural, hormone-free

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methods like nutritional strategies gain importance that offer innovative ways of augmenting fertility in the food animals. The use of such "clean, green and ethical" tools can be cost-effective and enhance productivity and improve the image of animal industry [1].

One such nutraceutical approach that has attracted the attention of researchers for improving the reproductive function in domestic animals is dietary supplementation of fatty acids particularly long-chain polyunsaturated fatty acids (PUFAs) like omega-3 (n-3) and omega-6 (n-6) fatty acids [3,4] which is well studied in the dairy cow. PUFAs are integral component of the membrane lipid bilayer and the dietary consumption is reflected by the proportion of different PUFAs in the different tissues including that of reproductive tract [3]. PUFAs can influence the reproductive processes through a variety of mechanisms; they act as the precursors for prostaglandins (PG) synthesis and can modulate the expression patterns of many key enzymes involved in both PG and steroid metabolism [3]. The possible effects of n-3 PUFA like eicosapentaenoic acid (EPA) and docosohexaenoic acid (DHA) on the reproduction is principally explained by the inhibition of $PGF_{2\alpha}$ and favouring the production of 3-series PG through competitive inhibition of Δ6 desaturase to prevent arachidonic acid (AA) synthesis, exclusion of AA in the phospholipid bilayer and competitive inhibition of cyclo-oxygenase-2 (COX-2) enzyme [5]. Dietary supplementation of n-3 PUFA is shown to improve different reproductive outcomes in the human as well animals [3,6,7]. Supplementation of n-3 PUFA enriched diet improves the pregnancy rate in the cow and buffalo [8] which is explained by a reduction in the uterine $PGF_{2\alpha}$ secretion and/or decrease in the sensitivity of the CL to $PGF_{2\alpha}$ during critical stage of embryonic development, preventing the onset of luteolysis and facilitating the establishment of pregnancy [9]. Besides improvement in the conception rate, several reports in the cow indicate a positive effect of n-3 PUFA supplementation on different reproductive processes like follicle turnover and growth, ovulation, CL size and steroidogenesis [5,10–12]. Increasing the maternal supply of long-chain n-3 PUFA during the late pregnancy marginally increases the gestation length and improves the neonatal behaviour as evidenced by a reduction in latency to stand and suckling in the lamb [13] and piglet [14].

Most studies related to the effect of n-3 PUFA on reproduction in the ruminants focused either on biomarkers of reproductive success such as effects on circulating endometrial concentrations of PG [15–19], and expression pattern of different genes related to PG and steroid synthesis [20,21], or maturation, quality and chilling resistance of oocytes and quality of embryo *in vitro* [22–25]. The results from these studies provided important evidence of mechanisms linking the effect of n-3 PUFA on reproduction outcomes; however, experiments determining the effects of n-3 PUFA on reproduction success *in vivo* are required to confirm the mechanisms of action and its utility under production setting [7]. The effects of dietary supplementation of n-3 PUFA on the reproductive functions have not been investigated in the doe so far. Hence, the objective of the present study was to know whether supplementation of EPA and DHA rich fish oil (FO) could modulate uterine and ovarian functions as well as measurable reproductive performance.

2. Materials and methods

2.1. Experimental animals

The study was conducted on apparently normal, thirty cycling goats of Rohilkhand region (Uttar Pradesh, India). Reproductive ultrasound was done to ensure the presence of apparently normal ovaries and uterus. The experimental does were 1.5–2.5 years age, 1–2 parity with a mean body weight (kg) of 18.78 ± 0.68 and body condition score 3.0 to 3.5 on a scale of 5 and maintained under intensive system of management at ICAR-Indian Veterinary Research Institute, Izatnagar. The institute is situated at an altitude of 250 m above the mean sea level at 29.42°N latitude and 79.54°E longitude and agroclimatically, it is a hot sub-humid sub-tropical climate. The experiment commenced during the typical breeding season (October–November) of the Rohilkhand goats. The experiment was approved by the Institute Animal Ethics Committee (IAEC).

2.2. Experimental diet and feeding regime

The goats were randomly divided into two groups: (i) long chain n-3 PUFAs rich fish oil (FO; Avestia Pharma, Mumbai, India) supplemented treatment (TRT; n = 15) and (ii) palm oil (PO; Raag®, Adani Wilmar Ltd., Ahmedabad, India) supplemented control (CON; n = 15). Does of either group were offered their respective oil supplements at 0.6 mL/kg body weight in the concentrate feed daily at 1100 h. The oil constituted approximately 1.75% of the total DM offered. Each goat was offered concentrate 0.25 kg DM/day to meet the maintenance requirement. To meet the gut fulfillment, maize green (*Zea mays*) was given @ 0.3 kg/goat/day, while wheat straw was offered *ad libitum*. The feeds in both the groups were maintained isonitrogenous and isocaloric. All animals had free access to clean drinking water throughout the experimental period. The ingredient composition and chemical analysis of the feed is presented in Table 1. The fatty acid composition of the FO and PO is shown in Table 2.

The feeding was commenced after synchronization of estrus using cloprostenol sodium (Vetmate®, Vetcare, Provimi Animal Nurition India Ltd., Bangalore, @125μg/goat). Feeding trial commenced from the day of estrus for a period of 72 days excluding a week of acclimatization. During acclimatization period, increasing amount of respective oil supplements were offered to the goats of both the groups to make them adjusted to the new diet. The day of initiation of acclimatization period (day of estrus) and day of commencement of actual feeding are denoted as day −7 and day 0, respectively, until mentioned otherwise.

2.3. Estrus synchronization and breeding

Experimental goats were administered two intramuscular injection of $PGF_{2\alpha}$ analogue (Cloprestenol sodium @125μg/goat/dose) at 11 days apart, first on day 25 of supplementation followed by second on day 36 (Fig. 1). The experimental goats were subjected to estrus detection twice a day (0600 and 1800 h) following second injection of $PGF_{2\alpha}$ using a vasectomized teaser buck combined with visual observations for 30 min. The midpoint between the last unobserved and first observed estrus signs was considered as the time of onset of estrus, while the mid-point between the last observed and first unobserved estrus signs was considered as the time of end of estrus. Goats in estrus were mated twice at 12 h interval with two proven bucks. Out of 30 goats, 14 in TRT and 13 in CON responded to the two PG regimen of estrus synchronization. Thus, the data presented indicates the observation on 27 goats.

2.4. Ultrasonographic examination

A real time B-mode ultrasound scanner provided with a linear array transducer of 7.5 MHz frequency connected to B-Mode high resolution real-time echo camera (Aloka SSD 500, Japan) was used for the trans-rectal ultrasonographic examination of ovaries and uterus. Ultrasonographic scanning of the ovaries was done on the day of second $PGF_{2\alpha}$ injection (day 36) to ascertain the population of different classes of follicles. The follicles were counted, measured