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Dietary supplementation of *n-3* polyunsaturated fatty acid alters endometrial expression of genes involved in prostaglandin biosynthetic pathway in breeding sows (*Sus scrofa*)

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ABSTRACT

The present investigation was designed to study the effect of dietary supplementation of omega-3 (*n-3*) PUFA on endometrial expression of fertility-related genes in breeding sows. Sixteen crossbred sows were randomized to receive diets containing 4% (wt/wt) flaxseed oil as *n-3* PUFA source (TRT group) or iso-nitrogenous, iso-caloric standard control diet (CON group), starting from the first day of estrus up to 40 days and were artificially bred on the second estrus. Endometrial samples were collected during days 10–11 and 15–16 post-mating for studying relative expression profile of candidate genes viz. Prostaglandin F Synthase (PGFS), microsomal Prostaglandin E Synthase-1 (mPGES-1) and Carbonyl Reductase-1 (CBR-1) using quantitative Real-Time PCR. Expression level of mPGES-1 gene transcript was 2.1-fold higher ($P < 0.05$) during 10–11 days of pregnancy and 1.4-fold higher ($P > 0.05$) during 15–16 days of pregnancy in TRT group as compared to CON group. Relative expression of PGFS gene transcript was significantly lower ($P < 0.05$) during 10–11 days of pregnancy in TRT group while there was no significant effect ($P > 0.05$) of dietary supplementation during 15–16 days of pregnancy. Endometrial mRNA level of CBR1 was significantly lower ($P < 0.05$) with 3.93-fold decrease in TRT group during 10–11 days of pregnancy whereas 2.82-fold reduction in expression ($P > 0.05$) was observed subsequently during 15–16 days of pregnancy as compared to CON group. Collectively, these results indicate that dietary *n-3* PUFA supplementation can modulate gene expression of key enzymes in prostaglandin biosynthetic pathway during early gestation, which in turn might have beneficial impact on overall reproductive response in breeding sows. These findings partly support strategic dietary supplementation of plant-based source of *n-3* PUFA with an aim to improve overall reproductive performance in sows.

1. Introduction

Improving litter size has a substantial impact on the efficiency of swine production. A major physiological constraint for increasing litter size in pig is the early embryonic mortality that occurs during day 10–35 of gestation. Fertilization rate in pig is generally high and has been estimated to be 95% [1] suggesting that it is unlikely to be the limiting factor with respect to number of piglets born at term. However, embryonic and fetal losses are very high and more than 40% of the embryos are lost before farrowing. Thus, early embryonic mortality in sows significantly limits litter size and has a negative economic effect on the swine industry [2]. Further, the effects of the maternal diet in early gestation are particularly important as inadequate nutrition to the conceptus directly affects their survivability [3]. Diet composition may alter the maternal environment of reproductive system through metabolic as well as endocrine modifications favouring higher fertility [4].

In recent years, there has been an increasing interest on

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identifying potential benefits of omega-3 polyunsaturated fatty acid (*n-3* PUFA) supplementation in animals and there is emerging evidence that dietary *n-3* PUFA can improve reproductive performance independent of their role as energy substrates [5–7]. Animal tissue can synthesize oleic acid family of fatty acids; however, PUFAs like linoleic acid and α-linolenic acid (ALA) are nutritionally essential fatty acids and cannot be synthesized endogenously due to absence of desaturases [8]. Long chain *n-3* PUFAs viz. docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) are essential for many body functions and can be made available directly from the diet or produced within the body from the precursor ALA [9]. Dietary *n-3* fatty acid supplements may be of either plant or marine origin. Flaxseed or linseed (*Linum usitatissimum*) oil contains high levels of *n-3* PUFA constituting approximately 55% of oil's total fatty acids. Studies in dairy cattle have shown that dietary flaxseed supplementation had positive effects on milk production and conception [10,11].

It is well established that *n-3* PUFAs can support important cellular processes including membrane stability, gene transcription, and cell proliferation [12]. Few studies in cattle have also validated complex nature of alterations in gene transcriptional regulation process in the endometrium following *n-3* PUFA supplementation which may positively influence the uterine environment [13,14]. Furthermore, *in vitro* studies in cattle suggest that *n-3* PUFAs may play significant roles by modulating uterine prostaglandins which are majorly involved in the control of estrous cycle and in early embryonic survival [15].

Early gestation and peri-implantation period, especially during 10–16 days of pregnancy is one of the most critical period in pregnant sows, as this period covers major events like maternal recognition of pregnancy, initiation of conceptus attachment and period of luteal maintenance. Periodic expression of important genes involved in prostaglandin synthesis, embryo survival and development occurs during this period. It is established that the regulation of prostaglandin signaling and metabolism are important for pregnancy recognition and early embryonic survival in the pig [16]. Moreover, endogenous prostaglandins like $PGF_{2\alpha}$ and PGE_2 play important role especially during the early period of pregnancy establishment in pigs [17,18]. PGFS, mPGES-1 and CBR1 are the key genes related to the terminal enzymes in the critical prostaglandin biosynthetic pathway. The apparent changes observed in the endometrial expression of these key genes involved in prostaglandin synthesis during early porcine pregnancy period might provide a sufficient stimulus to modulate $PGE_2/PGF_{2\alpha}$ ratio in the uterus and systemic circulation [19].

There have been earlier studies on gene expression changes in endometria during early pregnancy and implantation period in pigs [20–23] and major genes differently expressed at one stage of pregnancy have been identified. Lin et al. [24] identified comprehensive transcriptomic profile in the endometrium, which could be useful for targeted studies of genes and pathways potentially involved in abnormal endometrial receptivity and embryonic mortality during early pregnancy in pigs. Correspondingly, earlier studies in sows indicate that *n-3* PUFA rich diets can delay the onset of farrowing, reduce pre-weaning mortality of piglets [25] and enhance production performance [26]. Nevertheless, the use of flaxseed oil as a dietary fat supplementation and its subsequent effect on specific reproductive responses has been poorly studied in breeding sows. Focus on possible interaction of exogenous fatty acid components on prostaglandin synthesis and other endogenous regulatory factors during early pregnancy in sows and their role in embryonic development warrants detailed investigation. The main objective of this study was thus to investigate the effect of supplementing sow diet with flaxseed oil, a plant-based source of *n-3* PUFA, on endometrial expression of certain key genes related to prostaglandin bio-synthesis pathway during early pregnancy in breeding sows.

2. Materials and methods

The present study was conducted at the Institute Farm Complex, ICAR-National Research Centre on Pig, Guwahati, Assam, India. Agro-ecologically, experimental site is located in the central Brahmaputra valley (26.01° Lat. N., 91.34° Long. E, 56 m above MSL) having humid sub-tropical climate where the ambient temperature varied from 13.1 to 27.3 °C (with relative humidity ranging from 42 to 77% and an average rainfall of 107.84 mm) during the experimentation period.

2.1. Experimental animals

Sixteen healthy crossbred sows (cross between Hampshire and Ghungroo-a registered indigenous pig breed of India) with eight sows in each experimental group were selected for the present study. Sows randomly assigned to receive treatment and control diets were similar in age, parity and body weight (Table 1). Selected animals were maintained under similar managemental conditions during the experimental period. Estrus detection in experimental sows was by twice-daily checks and primarily performed through Back Pressure test (standing reflex) and also by observing important signs of estrus viz. red and swollen vulva, discharge from vulva, restlessness, mounting other females, frequent urination, ear cocking etc. Sows in estrus were artificially inseminated using good quality liquid boar semen. All procedures involving the use of animals were conducted in accordance with the national guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India and were duly approved by the Institute Animal Ethics Committee (IAEC).

2.2. Experimental diets and feeding schedule

Schematic representation of the experimental design is shown in Fig. 1. Selected sows were randomized to receive diets containing 4% (wt/wt) cold-pressed flaxseed oil as *n-3* PUFA source [Treatment (TRT) group, n = 8] or standard control diet, iso-nitrogenous and iso-caloric to treatment diet with 16% CP content, Maize-Wheat bran-Soybean meal diet containing 3.22 Mcal ME/kg [Control (CON) group, n = 8]. Sows in both the groups were fed with 2.5 kg/day of respective diets and given *ad-libitum* access to water. Dietary period started from the first day of detected estrus up to 40 days and sows were artificially inseminated on the second estrus using good quality liquid boar semen.

Representative samples of the experimental diets were also collected for compositional analyses using Kjeldahl method [27] and fatty acid analysis using Gas Liquid Chromatography [28]. For fatty acid analysis, samples were subjected to lipid extraction in Hexane and extracted lipid samples were further processed for *trans*-esterification. Methylated fatty acids were extracted with Hexane and the retention time was confirmed by injecting Supelco® 37-Component FAME Mix (Sigma Aldrich® Chemical Co., USA). Fatty acid composition of samples were measured by capillary Gas Chromatography on a SP™-2330 (30 m × 0.32 mm x 0.2 μm film thickness) fused silica capillary column installed on gas chromatograph (Agilent 7820 A, Agilent Technologies, Inc., California, USA) with a flame ionization detector (GC-FID) as described by Burns et al. [28]. The initial oven temperature was set at 140 °C and held for 5 min, later increased to 230 °C @ 4 °C/min, and finally held for 12 min to facilitate optimal separation. Hydrogen was used as carrier gas with a flow rate of 1.5 ml/min and the column head pressure was maintained at 280 kPa. Both the injector and detector