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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

Table 1
Criteria for selecting the experimental animals.

Diet/Group	Number of animals	Parameters (Mean ± SEM)		
		Age (months)	Parity	Body weight (kg)
n-3 PUFA-rich diet (TRT group)	n = 8	19.2 ± 0.52[a]	1.56 ± 0.18[a]	166.43 ± 15.80[a]
Control diet (CON group)	n = 8	20.4 ± 0.71[a]	1.50 ± 0.19[a]	163.45 ± 12.52[a]
Overall mean		19.8 ± 0.44[a]	1.53 ± 0.13[a]	164.94 ± 10.08[a]

[a, b]Values bearing common superscripts within a column did not differ significantly (P > 0.05).

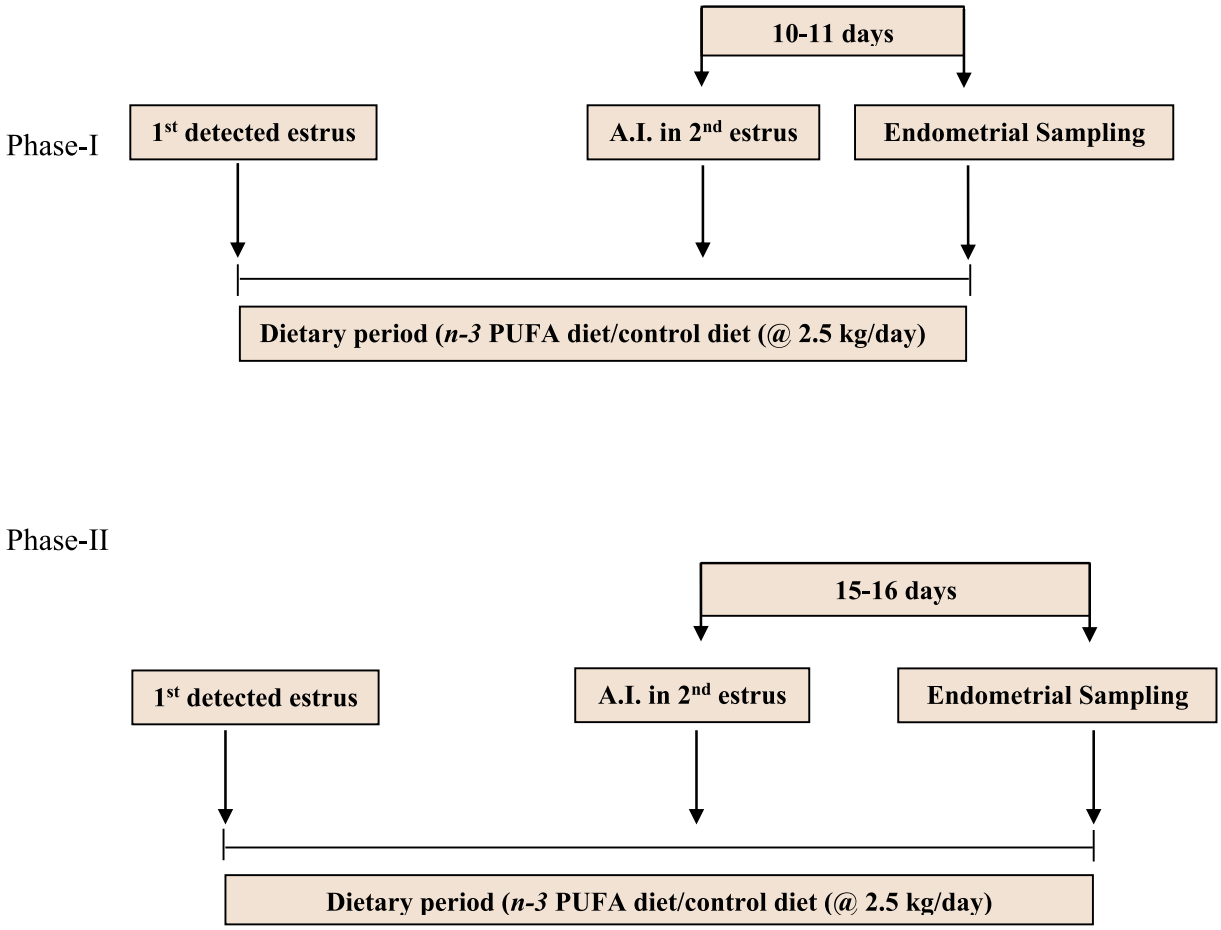

Fig. 1. Schematic representation of the experimental design. Selected sows were assigned randomly but equally to one of the diets containing *n-3* PUFA source (TRT group) or standard control diet (CON group). Throughout the experimental period, sows were monitored for estrous activity and artificially inseminated on second estrus (AI performed twice at 12 and 24 h after standing reflex detection using good quality liquid boar semen). Endometrial sampling was performed after slaughter on days 10–11 and 15–16 post-mating in Phase-I and Phase-II, respectively.

were set at 250 °C and the split ratio was 25:1. Peaks were identified using purified standards and different fatty acids were identified by comparing their relative FAME peak retention times and peak areas with respect to the internal standard (C13:0). The ingredient composition, nutrient composition and fatty acid profiles of different diets are shown in Tables 2A and 2B

2.3. Sample collection, total RNA isolation and cDNA synthesis

Endometrial tissue samples were collected after slaughter from sows of both the groups during days 10–11 and 15–16 post-A.I. Pregnancy was confirmed by the presence of apparently normal tubular or filamentous conceptuses in uterine flushings along with the morphological examination of corpora lutea and endometrium. Uterine flushings were obtained by flushing with 60 ml of phosphate buffered saline (pH 7.4, 30 ml per uterine horn) and examined under stereomicroscope (Olympus SZX16). Tissue samples (about 4 cm^2, n = 8) from individual sows were collected from different parts of the middle portion of the uterine horn, dissected from the myometrium and other tissues at the anti-mesometrial side. Collected endometrial samples from each sow were pooled together and thoroughly mixed to reduce the heterogeneity of gene expression and processed as per the standard procedures. Total RNA was isolated from endometrial tissue using RNAzol®RT (Sigma-Aldrich® Chemical Co., USA) according to the manufacturer's protocol. Samples were treated with DNase I (Thermo Fisher Scientific Inc., USA) to eliminate genomic DNA. The mixture was incubated at 37 °C for 30 min and then at 65 °C for 10 min after addition of EDTA (according to manufacturer's instructions). Quantity and quality of total RNA sample was determined by $A_{260/}$

Table 2A
Ingredient and nutrient compositions of diets fed to experimental sows.

Parts (Control diet)		Parts (Treatment diet)
Ingredient composition		
Maize	56.0	40.0
Soyabean Meal with hulls	14.0	9.0
Wheat bran	14.5	36.5
Flax seed oil supplement	–	4.0
Ground nut, sol extracted	14.0	9.0
Mineral and Vitamin pre-mix	1.0	1.0
Salt	0.5	0.5
Total (g)	100	100
Nutrient composition		
DM (g/kg)	899[a]	898[a]
CP (%)	16.2[a]	16.3[a]
Ash (g/kg of DM)	45.60[a]	49.50[a]
ME (Kcal/kg)	3226.0[a]	3306.0[a]
Total oil content (%)	4.30[a]	7.35 [b]

*Abbreviations: DM = Dry matter; CP = Crude Protein; ME = Metabolizable Energy.[a, b]Values on the same row with different superscript are statistically significant at 5% level.

Table 2B
Fatty acid profiles of treatment and control diets fed to experimental sows.

Fatty acid profiles (% of total fatty acids)	Control diet	Treatment diet
Total saturated fatty acids (SFA)	19.91[a]	16.53[a]
Total monounsaturated FA (MUFA)	40.72[a]	24.66[a]
Total polyunsaturated FA (PUFA)	33.37[a]	58.80[b]
Total *n-3* polyunsaturated FA (*n-3* PUFA)	1.31[a]	33.84[b]
Palmitic acid (C16:0)	16.20[a]	11.86[a]
Stearic acid (C18:0)	3.71[a]	4.67[a]
Oleic acid (C18:1n9)	40.72[a]	24.66[a]
Linoleic acid (LA) (C18:2n6)	32.06[a]	24.96[a]
Alpha linolenic acid (ALA) (C18:3n3)	1.31[a]	33.84[b]

[a, b]Values on the same row with different superscript are statistically significant at 5% level.

$_{280}$ ratio using BioPhotometer® plus, (Eppendorf AG, Hamburg, Germany) and Hellma® TrayCell™ (Hellma GmbH & Co. KG, Mullheim, Germany). RNA quality was verified by Agarose Gel Electrophoresis (1%) and by ensuring that all RNA samples had an absorbance ($A_{260/280}$) of between 1.8 and 2.1 and 200 ng of RNA was used for cDNA synthesis.

The first strand cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) according to the manufacturer's instructions. Reverse transcription was performed in thermal cycler (Veriti™ Thermal Cycler, Applied Biosystems, CA, USA) and the thermal cycling conditions were as follows: 10 min at 25 °C, followed by 120 min at 37 °C, 5 s at 85 °C. Random primers were used for cDNA synthesis (10X RT Random Primers, 2.0 μL and 25X dNTP (100 mM) mix, 0.8 μL for a total reaction volume of 20.0 μL). Required quantity of the resultant cDNA was determined and standardized (200 ng) for a total reaction volume of 20 μl used in the qRT-PCR. The confirmation of amplification of specific PCR amplicons was performed by 8.0% (w/v) non-denatured polyacrylamide gel electrophoresis and the resultant bands were visualized under white light, size estimated and recorded on a gel documentation system (Gel Doc™ XR+, Biorad, CA, USA).

2.4. Quantitative Real-Time PCR

All candidate and reference gene-specific primer sequences used to detect endometrial gene expression (Table 3) in the present study were designed using the NCBI Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) based on available nucleotide sequences in the NCBI (http://www.ncbi.nlm.nih.gov/). The specificity, compatibility and potential dimers of the designed primers were also determined by NCBI Primer-BLAST (Basic Local Alignment Search Tool) before being synthesized. The designed primers were obtained from a commercial supplier (GCC Biotech Pvt. Ltd., Kolkata, India). Transcript abundance of β-actin (ACTB) was found to be stable between different experimental groups, and thus used as suitable stable reference gene for endometrial tissue to determine the relative gene expression levels and to normalize gene expression data. Data were normalized to the calibrator sample using the ΔΔCt method with correction for amplification efficiency [29]. The expression profiles of microsomal Prostaglandin E Synthase-1 (mPGES-1), Prostaglandin F Synthase (PGFS) and Carbonyl Reductase-1 (CBR-1) genes in the porcine endometrium were analyzed through quantitative Real-Time PCR technique in a StepOnePlus™ Real-Time PCR instrument (Applied Biosystems, CA, USA) using QuantiTect® SYBR® Green PCR master mix (Qiagen GmbH, Hilden, Germany) and designed primers according to the manufacturer's instructions. For each candidate gene, twenty-four cDNA samples per experimental group were analyzed. Each sample was run in triplicate, and each reaction contained 10 μl of 2x QuantiTect® SYBR® Green PCR Master Mix, 20 pmol of forward and reverse primers each and 100 ng of cDNA in a final volume of 20 μl. A negative control without cDNA template was run in each assay. Common thermal cycling conditions (15 min at 95 °C for initial denaturation, 35 cycles of 15 s at 94 °C for denaturation, 30 s at respective annealing temperatures viz. 54 °C for mPGES-1, 57 °C for PGFS and 55 °C for CBR1, and 30 s at 72 °C for elongation) were used to amplify each transcript. Product identities were verified by sequencing (EMBL-EBI Accession No's viz. LT594963, LT594964 and LT594965 for mPGES-1, PGFS and CBR-1 respectively). Melting curve analysis of the PCR product(s) was performed in all PCR runs using built-in StepOne™ RT-PCR Software v2.1 (Applied Biosystems, CA, USA) for verifying the specificity.

2.5. Statistical analysis

All statistical analyses were performed using the statistical software package SAS (SAS version 9.3 for Windows; SAS Institute, Cary, NC). Data were checked for adherence to a normal distribution using the UNIVARIATE procedure of SAS. The fatty acid profiles and diet compositions were analyzed using independent sample *t*-test (PROC TTEST). Endometrial mRNA expression data were analyzed using appropriate procedures of the SAS. Differences at the 95% confidence level (P value < 0.05) were considered statistically significant and $P \leq 0.10$ but >0.05 were considered trends approaching significance.

Table 3
Porcine primer accession numbers, sequences and amplicon sizes for targeted genes.

Gene	Accession Number	Forward primer (5′-3′ direction)	Reverse primer (5′-3′ direction)	Amplicon (bp)
mPGES-1	AY857634	TTCCTGGGCCTCGTCTACTC	TTCCCCAGGTAGGCTATGGT	110
PGFS	AY863054	TTGGACTTGGCACTCTCGTC	CCCACCTCCTCCTCGTTTTC	130
CBR1	M80709	CTGGTGGGGCTCATGAACAA	CTGGACAACACAGAGACCCC	116
ACTB	AF309819	GAGATCGTGCGGGACATCAA	TCGTTGCCGATGGTGATGA	140

Abbreviations: mPGES-1 (microsomal Prostaglandin E Synthase-1); PGFS (Prostaglandin F Synthase); CBR1 (Carbonyl Reductase-1); ACTB (β-actin); bp (base pairs).

3. Results and discussion

The confirmation of amplification of specific PCR amplicons was done by polyacrylamide gel electrophoresis and representative samples are shown in Fig. 2. Electrophoresis of samples revealed specific amplicons of 110, 130 and 116 bp for microsomal Prostaglandin E Synthase-1 (mPGES-1), Prostaglandin F Synthase (PGFS) and Carbonyl Reductase-1 (CBR-1) genes, respectively.

In the present study, quantitative RT-PCR demonstrated significantly higher ($P < 0.05$) endometrial expression of mPGES-1 transcripts during early pregnancy in TRT group sows, and expression level was 2.1-fold greater during 10–11 days of pregnancy in sows receiving *n-3* PUFA supplemented diet compared with those receiving control diet (Fig. 3). However, 1.4-fold increase in mPGES-1 expression, although statistically non-significant ($P > 0.05$), was observed during 15–16 days of pregnancy in TRT group sows. Uterine synthesis of prostaglandins plays a significant role in regulation of estrous cycle and establishment of pregnancy in many domestic species. PGES enzyme catalyzes synthesis of prostaglandin E_2 (PGE_2) which plays important roles as luteoprotective factor, local mediator of embryo signal on uterus and is also critically required in early gestation especially for vascular permeability and placental development in pigs [30,31]. It has also been reported that, besides estrogens, PGE_2 as an anti-luteolytic factor could be involved in maternal recognition of porcine pregnancy [32,33]. Recent studies have demonstrated complex nature of alterations in transcriptional regulation process in bovine endometrium following dietary *n-3* PUFA supplementation which could positively influence the uterine environment [13,14]. The present finding of higher endometrial mPGES-1 expression in TRT group suggests that *n-3* PUFA supplementation stimulates synthesis of Prostaglandin E Synthase (PGES) enzyme during early porcine pregnancy.

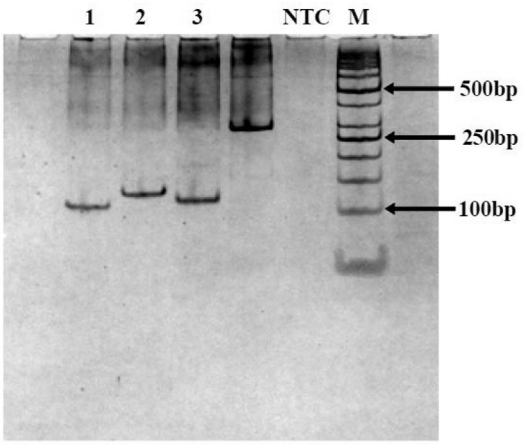

Fig. 2. Polyacrylamide gel (8.0%) electrophoresis showing specific PCR products of candidate genes amplified using designed primers. Electrophoresis of representative samples revealed specific amplicons of 110, 130 and 116 bp for mPGES-1, PGFS and CBR1 genes, respectively. Lane 1 : mPGES-1 (110 bp) Lane 2 : PGFS (130 bp) Lane 3 : CBR1 (116 bp) NTC : No Template Control Lane M: 50 bp DNA ladder

Interestingly, relative expression levels of PGFS transcripts in the present study were significantly lower (12.5 fold, $P < 0.05$) during 10–11 days of pregnancy in TRT group as compared to the CON group (Fig. 3). However, there was no significant effect ($P > 0.05$) of dietary treatment on PGFS expression during 15–16 days of pregnancy as relative expression levels of 0.364 ± 0.04 and 0.288 ± 0.02 fold changes were observed in control and treatment group, respectively. PGFS enzyme converts prostaglandin H_2 (an unstable intermediate in the prostaglandin biosynthetic pathway) into $PGF_{2\alpha}$. It is established that uterine $PGE_2/PGF_{2\alpha}$ ratio plays an important role especially during 11–15 days after mating-the critical period for establishment of pregnancy in pigs [17,18]. The apparent changes observed in mPGES-1 and PGFS expression in the endometrium during this period might provide a sufficient stimulus to modulate $PGE_2/PGF_{2\alpha}$ ratio in the uterus as well as in systemic circulation [19]. In agreement with the present findings, it is therefore plausible to infer that dietary *n-3* PUFA supplementation may alter endometrial expression of genes regulating $PGF_{2\alpha}$ synthesis, possibly leading to a reduction in uterine $PGF_{2\alpha}$ production. Reduction in uterine $PGF_{2\alpha}$ secretion can in turn inhibit or delay regression of corpus luteum thereby possibly improves preimplantation embryonic survival.

The relative changes in endometrial expression of CBR1 gene transcripts were also evaluated in the present study. Relative mRNA level of CBR1 gene was significantly decreased ($P < 0.05$) by more than 3.93-fold during 10–11 days of pregnancy following dietary *n-3* PUFA supplementation (Fig. 3). However, the degree of down-regulation was not statistically significant ($P > 0.05$) during 15–16 days of pregnancy with 2.82-fold decrease in expression observed in *n-3* PUFA supplemented group as compared to control group.

Carbonyl Reductase-1 (CBR1), also known as Prostaglandin E_2 9-Ketoreductase, can modulate both prostaglandin concentrations as it reversibly converts PGE_2 into $PGF_{2\alpha}$ [34–36]. Interestingly, a potential mechanism by which the conceptus can contribute to prevention of luteolysis during maternal recognition of porcine pregnancy is by changing prostaglandin synthesis in favor of PGE_2 [19]. Further, it has also been proposed that early pregnancy signalling events are linked to lipid signalling system consisting of lysophosphatic acid (LPA), $PGF_{2\alpha}$ and PGE_2 [37]. Higher expression of PGES in endometrium is responsible for down-regulation of PGFS and CBR1 in favor of PGE_2 which in turn support uterine and luteal functions for establishment and maintenance of porcine pregnancy [37]. These findings concur with the results of the present study and suggest that an apparent decrease in endometrial CBR1 expression might provide sufficient stimulus to modulate $PGE_2/PGF_{2\alpha}$ ratio thereby favouring more optimal conditions for successful pregnancy in *n-3* PUFA supplemented sows.

Recent evidence in animal models clearly indicates that *n-3* fatty acids have distinct and important bioactive properties as compared to other groups of fatty acids [38]. Long-chain *n-3* PUFAs are known to mediate its effects on numerous molecular and cellular processes including reproductive processes through modulation of gene expression and regulation of transcription factor activity [39–41]. The mechanisms through which dietary *n-3* PUFA affect gene

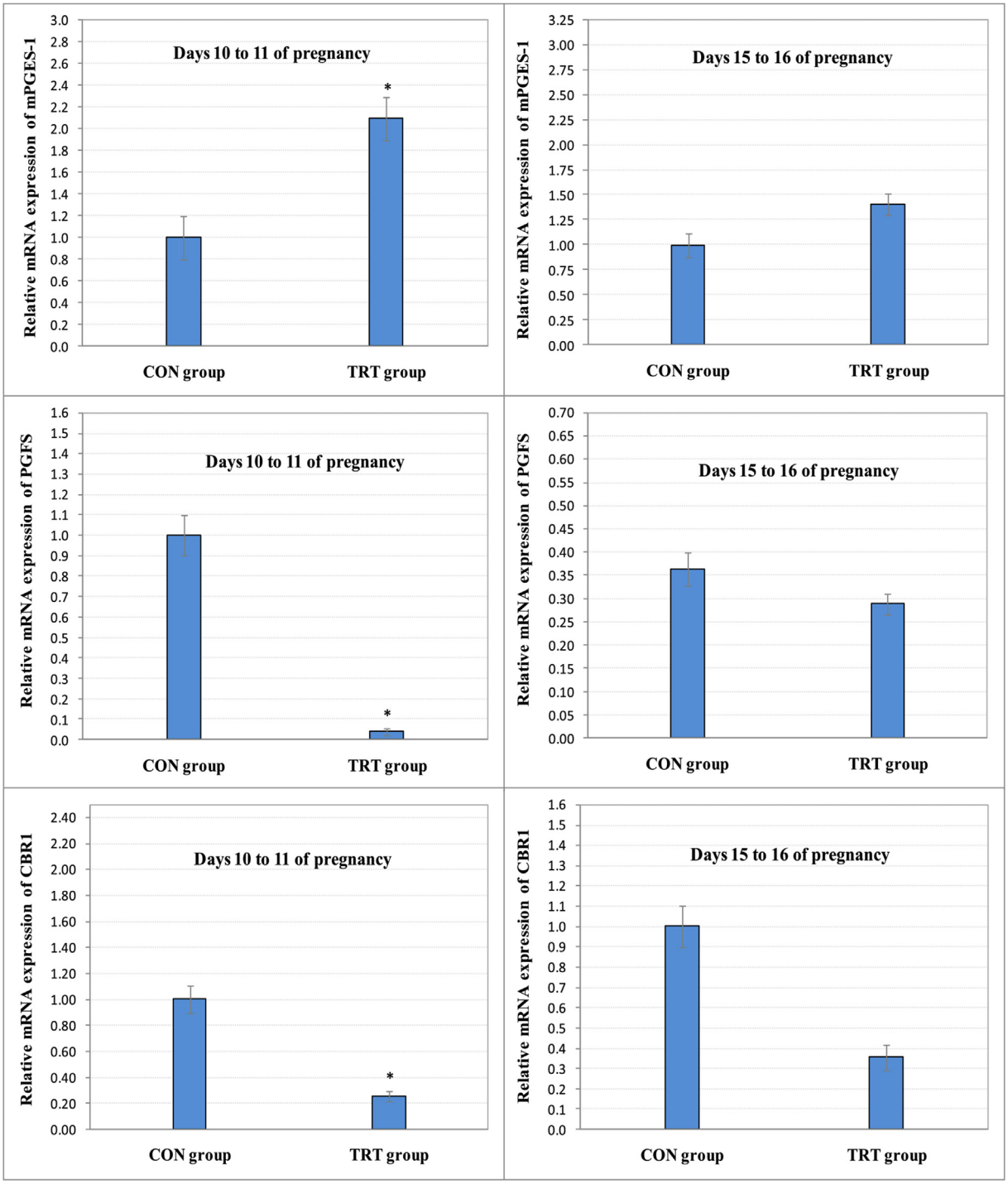

Fig. 3. Relative expression levels of mPGES-1, PGFS and CBR1 genes during 10–11 and 15–16 days of pregnancy in the endometrium of sows fed *n-3* PUFA supplemented diet (TRT group) and control diet (CON group). Data are presented as mean ± SEM relative to ACTB gene expression (*P < 0.05).

expression are complex and involve multiple processes [38]. Most of the effects are due to modulation of specific genes by *n-3* PUFA and cross-talk between these genes [42,43]. Interestingly, recent studies indicate that dietary *n-3* PUFAs can interact with transcription factors, like peroxisome proliferator-activated receptors (PPAR) that directly modulate the expression of target genes [44,45]. PPARs can be activated by natural ligands such as PUFAs or prostaglandin metabolites and are the key messengers responsible for translation of nutritional stimuli into changes for the expression of genes, particularly those involved in lipid metabolism [46].

As in any mammalian species, successful establishment of porcine pregnancy and embryonic development follows a specific pattern of endocrine changes and gene expression [47]. During early pregnancy in the litter-bearing pig, peri-implantation period is one of the most critical stages of conceptus development. Nutrition before and during this period has profound effects on embryonic survival and growth and therefore supplementing *n-3* PUFA during early pregnancy could improve embryonic survival,

consequently conception rate and litter size. Furthermore, supplementation of diets with *n-3* PUFA has previously been shown to improve reproductive performance in cattle and sheep. In the present study, it was hypothesized that dietary *n-3* PUFA would alter endometrial expression of certain genes involved in prostaglandin synthesis pathway during early pregnancy in sows. The study has not fully revealed the mechanisms of how dietary *n-3* fatty acids influence specific reproductive processes in sows, nevertheless the findings are closer to identifying significant dietary effects on expression of certain fertility-related genes during early gestation which might positively affect reproductive performance in sows. The apparent changes in endometrial PGFS and CBR1 mRNA expression observed in sows fed with *n-3* PUFA diet might have an influence on uterine prostaglandin synthesis, possibly leading to a reduction in $PGF_{2\alpha}$ production which in turn can significantly affect reproductive outcomes like estrus onset, embryonic survival and farrowing in sows. Collectively, these findings in the present study concur with the notion that maternal dietary *n-3* PUFA may have beneficial impact on improving reproductive response in sows.

In conclusion, the present results demonstrate that dietary *n-3* PUFA supplementation in breeding sow diets can have major influence on uterine endometrial expression of genes involved in prostaglandin bio-synthesis pathway and thus might have the potential to influence a wide array of reproductive events, including implantation and embryonic survival. Fortifying sow diets with *n-3* PUFA could provide new nutraceutical alternatives for development of nutritionally focussed management strategies to improve reproductive efficiency and further detailed studies are warranted to investigate the effects of dietary *n-3* PUFA on transcriptional regulation of important biological processes related to uterine and ovarian environment.

Conflicts of interest

The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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