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The endometrial expression of prostaglandin cascade components in lactating dairy cows fed different polyunsaturated fatty acids

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ABSTRACT

Feeding n-6 polyunsaturated fatty acids (PUFA) increases the endometrial percentages of linoleic and arachidonic acids (AA), enhances the synthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and improves uterine health. In contrary, the n-3 PUFA, eicosapentaenoic acid, and docosahexaenoic acid may play pivotal roles by suppressing the synthesis of uterine $PGF_{2\alpha}$, a component being centrally involved in the control of the bovine estrous cycle and in early embryo survival. The objectives of the present study were to determine the effect of feeding a diet enriched in either α-linolenic acid (n-3) or linolenic acid (n-6) on the uterine expression of genes related to prostaglandin cascade and uterine release of $PGF_{2\alpha}$ (measured as 13, 14-dihydro-15-keto $PGF_{2\alpha}$ [PGFM]). From calving to 60 days in milk, cows ($n = 24$) were fed isonitrogenous, isocaloric, and isolipidic diets that differed in the ratio of n-3/n-6 PUFA. Treatments including palm oil ([PLM]; saturated FA, $n = 8$), soybean whole roast ([SOY]; n-6, $n = 8$), and linseed extruded ([LIN]; n-3, $n = 8$). At 30 days in milk, the ovulatory cycles of cows were synchronized using 2 injections of $PGF_{2\alpha}$ with a 14-day interval. On day 15 postovulation, cows were injected with oxytocin and blood samples were collected to monitor the uterine release of $PGF_{2\alpha}$ (measured as PGFM) and uterine endometrial biopsies were prepared to evaluate the expression of genes related to prostaglandin cascade (prostaglandin F synthase [PGFS], prostaglandin E synthase [PGES], prostaglandin endoperoxide synthase-2 [PGHS-2]), phospholipase A2 (PLA2), peroxisome proliferator-activated receptors [PPAR]). Results showed that uterine endometrial PPAR-δ genes were higher in cows fed LIN (3.17-fold) compared with cows fed PLM or SOY ($P < 0.05$). The messenger RNA (mRNA) level of PGES in the LIN group was threefold as high as those found in SOY and PLM diets ($P < 0.05$). The mean relative gene expression of PLA2 and PGFS was increased in animals fed the SOY diet (2.4- and 1.7-fold, respectively) compared with LIN and PLM diets ($P < 0.05$). The expression of mRNA for the PGHS-2, PPAR-α, and PPAR-γ was not influenced by the diet effect. Dietary inclusion of soy FAs was associated with an increase in the PGFM concentration, possibly through an increase in the expression of genes involved in prostaglandin cascade. The uterine concentration of PGFM, however, was decreased in cows fed diets containing n-3 FAs.

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1. Introduction

The endometrium plays a critical role in regulating the estrous cycle and establishment of pregnancy, primarily through the processing of arachidonic acid (AA) and synthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [1]. Studies on a variety of species have shown that dietary polyunsaturated fatty acids (PUFA) may modulate prostaglandin synthesis and metabolism [1,2]. The most biologically active two series prostaglandins are derived from AA, but the less active three series prostaglandins are enzymatically produced from eicosapentaenoic acid by the same enzymes [2]. The AA released by phospholipid hydrolysis is converted to prostaglandin endoperoxide synthase (PGHS)-2 prostaglandin H2 by PGHS, which is then converted to PGF by a reductase. Two forms of PGHS have been characterized, a constitutively expressed PGHS-1, and an induced PGHS-2 [3]. In cattle, the synthesis and activity of PGHS-2 must be attenuated to maintain pregnancy [4].

Peroxisome proliferator-activated receptors (PPAR) are a group of ligand-activated transcription factors regulating multiple physiologic processes [5]. MacLaren et al. [6] reported a comparable endometrial messenger RNA (mRNA) expression of PPAR-α and PPAR-δ in cyclic and pregnant Holstein cows. Agonists of PPAR-δ and PPAR-α had a profound stimulatory effect on PGH synthase (PGHS-2) mRNA levels and the synthesis of $PGF_{2\alpha}$ and PGE2, which seems to be mediated, at least in part, through PPAR-δ [5,6].

Feeding n-3 fatty acids (FA) may attenuate the endometrial $PGF_{2\alpha}$ production [7,8]. Dirandeh et al. [9] demonstrated that FA from flaxseed reduced plasma 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) concentrations compared with feeding soybean whole roast and palm oil (PLM) after an oxytocin challenge in dairy cows. Dairy cows fed fish oil during the transition period had greater eicosapentaenoic acid and docosahexaenoic acid concentrations in caruncular tissues and reduced postpartum concentrations of PGFM compared with cows fed olive oil [10]. Conversely, feeding fat sources rich in n-6 FA increased plasma concentration of PGFM after an oxytocin challenge [2]. Thus, supplemental lipids can either inhibit or stimulate prostaglandin secretion depending on their specific FA profile. The aim of the present study was to determine the effect of dietary supplementation with n-3 and n-6 PUFA on the mRNA expression of key genes (PGFS, PGES, PGHS-2, PLA2, PPARs) involved in prostaglandin biosynthesis and circulatory PGFM concentration in dairy cows to improve fertility in dairy cows.

2. Material and methods

2.1. Cows and treatments

Twenty-four multiparous cows were blocked according to calving date and parity, and were allocated randomly to three experimental diets fed during a period between calving through 60 days in milk. There was no difference among groups (mean ± standard error of the mean) in parity (3.1 ± 0.9) or body condition score at calving (3.2 ± 0.07).

The cows were fed on diets containing PLM (saturated FA; n = 8), soybean whole roast (n-6; n = 8; SOY), or linseed extruded (n-3; n = 8; LIN). The diets were isonitrogenous, isoenergetic, and isolipidic, and were formulated to meet or exceed NRC [11] nutrient requirements (Table 1). The omega-6 to omega-3 FA ratios were 4.2, 3.2, and 1.0 fortreatments SOY, PLM, and LIN, respectively (Table 2). Fatty acid profile (g/100 g of FA) of fat supplements presented in Table 3.

The total mixed ration was sampled weekly throughout the trial and their DM content was determined by drying at 110 °C for 18 hours. Diets were provided twice per day (0800 and 1600 hours) for *ad libitum* intake (10% of refusals on as-fed basis) from calving through day 70 postpartum. Total mixed ration were sampled each week and pooled each month. Compositional and ingredient analyzed for total mixed ration. Briefly, the dry matter of feed sample was determined by placing it in a drying oven at 100 °C for 48 hours (AOAC, 1990, Method 930.15). Crude protein was determined using the Dumas Method and a Leco FP-528 (LECO Corporation, St. Joseph's, MI, USA). Acid detergent fiber and neutral detergent fiber concentrations were determined [12]. The cows were milked 3 times per day at 0700, 1400, and 2300 hours, and the milk yields were recorded automatically.

2.2. Synchronization of the estrous cycle

The cows were synchronized for ovulation beginning on day 30 via two intramuscular injections of $PGF_{2\alpha}$ (Synchromate, 150 μg cloprostenol sodium, Aburaihan Company, Tehran, Iran) given with a 14-day interval [13].

2.3. Monitoring uterine conditions

On days 5, 10, 15, 20, and 30 postpartum, blood samples were collected from eight cows per treatment to determine PGFM concentrations, also on day 14 of the synchronized estrous cycle, a catheter was inserted in the jugular vein of cows that calved which showed no difference in blood PGFM concentrations among the experimental diets on days 5 and 10 postpartum along with no uterine problems (n = 8 per treatment). On day 15, the cows were injected intravenously at 1300 hours with 100 IU of oxytocin as described by Dirandeh et al. [9] to monitor the uterine secretion of PGFM. Blood samples were collected into vacutainer tubes containing EDTA (10.5 mg, Monoject; Sherwood Medical, St. Louis, MO, USA) at 15-minute intervals for 1 hour before oxytocin injection and at 15-minute intervals for 4 hours after the oxytocin injection. The blood sample was centrifuged at 2600× *g* for 30 minutes and the plasma was frozen at −20 °C for subsequent assay of PGFM using an ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) with an ELISA reader (Stat fax 2100, Awareness Technologies, UK). Inter- and intra-assay coefficients of variation for one reference sample (1.85 ng/mL; [9]) were 6.34% and 7.10%, respectively. The sensitivity of the assay was 0.02 ng/mL.

2.4. Uterine biopsy

2.4.1. Tissue sampling

On day 15 of the synchronized estrous cycle, uterine endometrial biopsies were collected immediately after the fulfillment of the oxytocin challenge and blood sampling