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Dietary conjugated linoleic acid supplementation alters the expression of genes involved in the endocannabinoid system in the bovine endometrium and increases plasma progesterone concentrations

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ABSTRACT

Endocannabinoids are derived from phospholipids and reduce fertility by interfering with implantation. Identification of changes in the expression of genes of the endocannabinoid system as a result of dietary inclusion of conjugated linoleic acid (CLA) is critical to the advancement of our understanding of the nutritional regulation of uterine function. An experiment was conducted on transition cows to evaluate the expression of key endocannabinoid genes in bovine endometrium in response to dietary supplementation with CLA. A total of 16 cows were randomly assigned to two treatments: (1) control (75 g/day palm oil) and (2) CLA (75 g/day CLA) from 21 days prepartum to Day 42 postpartum. Cows underwent uterine biopsy on days 21 and 42 postpartum. The abundance of mRNA encoding endocannabinoid receptor (*CNR2*), N-acyl phosphatidylethanolamine phospholipase D (*NAPEPLD*), fatty acid amide hydrolase (*FAAH*), N-acylethanolamine acid amidase (*NAAA*), and monoglyceride lipase (*MGLL*) was measured by real-time PCR. Results reported that relative levels of mRNA encoding *CNR2* and *NAPEPLD* were decreased ($P < 0.05$) compared with control cows between Days 21 and 42 postpartum. Relative levels of mRNA coding for *NAAA* and *MGLL* were not different ($P > 0.05$) in the same situation. Mean plasma progesterone concentrations were higher in CLA-fed cows compared with control cows at Day 42 postpartum (3.51 and 1.42 ng/mL, respectively, $P < 0.05$). In conclusion, we suggest that the beneficial effects of a diet enriched with CLA are the result of a decrease in relative gene expression of the endocannabinoid receptor (*CNR2*) and enzymes that synthesize fatty acid amides (*NAPEPLD*) and of an increase in the expression of *PTGS2* that in turn can oxidate endocannabinoids and consequently resulted in increased plasma progesterone concentrations during early lactation.

1. Introduction

The endocannabinoid system (ECS) and their G protein–coupled receptors are well studied in nonruminants. The endocannabinoids are a group of natural, endogenous lipid modulators that activate receptors such as the Δ9-tetrahydrocannabinol receptor [1,2]. They are amides and esters of long-chain polyunsaturated fatty acids (PUFAs) [3], and the most important endocannabinoids are N-arachidonoylethanolamine (AEA or anandamide) and 2-arachidonoyl glycerol (2-AG), both derived from arachidonic acid (AA) [4]. The ECS is reported to control feed intake, energy balance, metabolic processes, and reproduction [5–8]. High levels of endocannabinoids can restrain implantation by activating cannabinoid receptors and

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subsequently inhibition of Ca channels. On the other hand, low concentration of anandamide may activate the CNR1 receptors on the surface of the trophoblast and can have positive effects on smooth implantation by activating the extracellular signal-regulated kinase pathway [6]. Endocannabinoids have been reported to increase secretion of prostaglandin F2α (PGF2α) and inhibit corpus luteum (CL) function, leading to lost pregnancy [7].

Dietary fats are the only source of fatty acids required for the synthesis of endocannabinoids; therefore, the composition of the diet likely affects endocannabinoid levels, thus affecting appetite, energy intake, and body metabolism [8–10]. Endocannabinoid concentrations are regulated in part by the activity/expression of enzymes regulating endocannabinoid biosynthesis and degradation [11]. Dietary PUFAs can increase or decrease AA levels [12] and that a higher or lower abundance of (n-3) and (n-6) PUFA precursors in high-fat diets affects AEA and 2-AG levels [11]. The resulting endocannabinoid signaling from a diet having high n-6 PUFA content is hypothesized to be different from that for diets containing long-chain n-3 PUFA [12]. It was reported that n-3 PUFA (α-linolenic, eicosapentaenoic acid, and docosahexaenoic acid) reduces the amount of AA available in membrane phospholipids (PL) for the synthesis of AEA and 2-AG and, therefore, can exert anorexigenic effects on the peripheral ECS by acting as antagonists to n-6 PUFA [13]. It has been shown that feeding mice with a diet higher in n-3 PUFA content reduced AEA and 2-AG levels [14]. After 4 weeks of treatment, n-3 PUFA supplemented reduced levels of AEA and 2-AG in the visceral adipose tissue [11]. PUFA competes with the n-6 PUFA derivatives, anandamide and 2-arachidonoyl glycerol, which are major agonists for the ECS (CB1) [13]. Experiments *in vitro* definitely confirmed as dietary fatty acids influence endocannabinoid biosynthesis and then might modulate the ECS [11]. Matias et al. [11] reported that AA strongly increased 2-AG levels, docosahexaenoic acid decreased 2-AG, and no effect on 2-AG levels was observed after eicosapentaenoic acid treatment. When 3-week-old mice were fed diets containing 3% of either linoleic acid (LA) or CLA for 4 weeks, the amounts of 2-AG in the cerebral cortex were significantly decreased by CLA treatment compared with LA [15]. Pintus et al. [16] compared the effects of 90 g/day consumption of a naturally enriched sheep cheese in some fatty acids and a control cheese on plasma lipid and endocannabinoid profiles. In this 3-week crossover study, the enriched cheese in CLA decreased AEA levels. All these findings strongly support the significant role of dietary fatty acids in the physiological control of the ECS and their beneficial effects by reducing biosynthesis of endocannabinoids.

Conjugated linoleic acid (CLA) is an intermediate product of biohydrogenation of LA to stearic acid [17]. It is a component of an unsaturated fatty acids group that exists as positional and stereoisomers of octadecadienoate (18:2). CLA is available in foods derived from ruminants, such as beef or dairy products [18]. The CLA in milk and beef originates from two sources: one is from the biohydrogenation of LA in the rumen and the other is from biohydrogenation of unsaturated fatty acids from *trans*-11 C18:1 by mammalian tissues [19]. CLA supplementation has been studied as a tool to manage negative energy balance of transition cows and improve lactation and reproductive performance [20,21]. De Veth et al. [21] reported that dietary CLA supplementation during early lactation improved conception rates. This is consistent with the study of Castaneda-Gutierrez et al. [20] who found increased pregnancy rates in cows fed CLA before 140 days in milk.

Although the effects of supplementing the diet with CLA on reproductive performance have been reported, it is not known if fatty acid in the diet alters the expression of the ECS in bovine endometrium. Therefore, in the present study, our aim was to determine the effect of dietary CLA supplementation on the expression of key genes (*CNR2*, N-acyl phosphatidylethanolamine phospholipase D [*NAPEPLD*], *PTGS2*, monoglyceride lipase [*MGLL*], fatty acid amide hydrolase [*FAAH*], prostaglandin F2α synthase (*PGFS*), N-acylethanolamine acid amidase [*NAAA*], and *PTGS2*) involved endocannabinoid biosynthesis in dairy cows.

2. Materials and methods

2.1. Cows and treatments

Sixteen multiparous cows were randomly were assigned to two experimental diets, fed during a period between −21 and +42 days around calving (calving = Day 0). There were no differences between two groups in parity (3.1 ± 0.4) or body condition score at calving (3.1 ± 0.14). The cows received diets that included rumen-protected supplements of palm oil plus basal diet (saturated FA; n = 8), and CLA plus basal diet (Lutrell pure; BASF SE, Ludwigshafen, Germany), and the quantity of each fat in each diet was 75 g/day. Rumen-protected CLA provided 10 g/day each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 isomers. Cows were fed twice a day prepartum (8 AM and 4 PM) and four times a day postpartum (7 AM, 11 AM, 3 PM, and 7 PM) with an isonitrogenous and isoenergetic total mixed ration formulated on the basis of the NRC guidelines (2001) to meet all animal requirements. Fat supplements were manually mixed with 425 g specially formulated concentrate to ensure palatability and were top-dressed once daily on the morning total mixed ration feeding. The prepartum and postpartum diets were adjusted based on NRC guidelines (2001). Both diets were equal in concentration of dry matter, crude fat, and crude protein (Table 1).

Total mixed rations were sampled every 2 weeks and pooled each month. Feed samples were dried at 65 °C for 24 hours, ground, and then passed through a 1-mm screen (Retsch SM 100; Retsch GmbH, Haan, Germany) according to Dirandeh et al. [22]. Samples were analyzed for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber according to methods of the official methods of analysis of AOAC (2000). Values of net energy for lactation were calculated according to nutrient requirements of dairy cattle values (NRC, 2001). This experiment was carried out according to the procedures laid down by the Iranian Ministry of Agriculture (experimental permission No. 1036).

Ovaries were examined by ultrasonography (BCF Technology equipped with a 6- to 8-MHz linear transducer;