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Effects of feeding a source of omega-3 fatty acid during the early postpartum period on the endocannabinoid system in the bovine endometrium

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ABSTRACT

A total of sixteen Holstein dairy cows (35 ± 1.1 kg/d of milk yield) were randomly assigned to consider the effects of feeding omega-3 sources on endometrial endocannabinoids system (ECS) genes expression to understand the effect mechanism of omega-3 on reproductive performances during the early postpartum period to evaluate to what extent can be intervened in reproduction, e.g. via nutrition to improve fertility. Experimental diets were 1) either protected palm oil (control) or 2) extruded linseed (linseed). Cows (n = 16) were fed from calving date to 70 days in milk (DIM). There was no difference between groups (mean ± S.E.M.) in parity (3.0 ± 1.00) or body condition score (BCS) at calving day (3.1 ± 0.25). At 30 DIM, the ovulatory cycles of cows were synchronized using two injections of prostaglandin F2α (PGF2α) with a 14-day interval. On day 15 of synchronized estrous cycle (d0 = ovulation) uterine endometrial biopsies were collected to evaluate the expression of genes related to ECS (endocannabinoid receptor (CNR2), N-acyl phosphatidylethanolamine phospholipase D (NAPEPLD), fatty acid amide hydrolase (FAAH), N-acylethanolamine acid amidase (NAAA), monoglyceride lipase (MGLL)) and PGF2α. Results showed that dry matter intake and milk yield were not affected by diets. Uterine endometrial NAAA (7.69 fold), and MGLL (1.96 fold) genes expression were greater (P < 0.05) in cows fed linseed compared with control ones. The messenger RNA (mRNA) levels of CNR-2 (4.26 fold), and NAPEPLD (20.0 fold) were decreased (P < 0.05) in animals fed linseed compared to control cows. The expression of mRNA for the FAAH was not influenced by the diets. First service conception rate was greater in cows fed linseed compared to control cows (75 vs. 25%). Pregnancy loss within 32–60 day after artificial insemination (AI) was lower in cows fed linseed compared to control cows (0 vs. 100%). In conclusion these data demonstrated that positive effect of omega-3 on reproduction may act through a mechanism involving the ECS. However, more studies to be undertaken to confirm these results.

1. Introduction

The endocannabinoid system (ECS) compromises a relatively small group of fatty acid-derived endogenous ligands of the cannabinoid receptors CB1 and CB2 and bind to G protein-coupled receptors [1]. The main endocannabinoids are arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG). Endocannabinoids are synthesized by the enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPEPLD) and released upon demand from lipid precursors in a receptor-dependent manner. They are transported into cells by an apparently specific uptake system and degraded primarily by three enzymes, fatty acid amide hydrolase (FAAH), N-acylethanolamine acid amidase (NAAA) and monoglyceride lipase (MGLL) resulting in the termination of their biological actions. The ECS is well-studied in non-ruminants (human and mouse) and reported to control feed intake and energy balance [2]. However, there is a paucity of literature about the role of the ECS on ruminant's reproduction. Weems et al. [3] reported endocannabinoid 1 and 2 (CB1; CB2) receptor agonists affect negatively cow luteal function in vitro. Indeed Tsutahara et al. [4] reported stimulation of either CB1 or CB2 receptors in vivo affected negatively luteal progesterone secretion by decreasing luteal mRNA for LH receptors. In addition, endocannabinoids decreased fertility in mice, which was reported to be

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via interference with implantation [5,6].

Numerous studies demonstrating the influence of dietary lipids on the ECS, because arachidonic acid (AA) and its biosynthetic precursor, linoleic acid (LA), are found in tissues, and diet to a great extent is a determinate, it is not surprising that intakes of polyunsaturated fatty acids (PUFA) from the omega-3 and omega-6 family can influence tissue levels of endocannabinoids [7].

It was reported that n-3 PUFA (a-linolenic, eicosapentaenoic acid, and docosahexaenoic acid) declined 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 [8,9]. Abolghasemi et al. [1] reported that feeding a diet enriched in conjugated linoleic acid (CLA) resulted in 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 prostaglandin-endoperoxide synthase 2 (PTGS2) that in turn can oxidate endocannabinoids and consequently resulted in increased plasma progesterone concentrations during early lactation.

Although the positive effects of diets supplemented with omega-3 fatty acid on reproductive performance have been reported extensively [10–13], it is unknown whether the effect of omega-3 fatty acid dietary supplementation on reproduction are mediated by the bovine endometrial ECS. Therefore, the objectives of this study was to evaluate the endometrial endocannabinoid system at gene level and its function in response to feeding a source of omega-3 fatty acids) during the early postpartum period. To our knowledge, this is the first study to determine the effect of dietary omega-3 fatty acids on endometrial ECS in dairy cows.

2. Material and method

This study was carried out in a large commercial dairy herd (Mahdasht, Sari, Iran) with 2750 lactating cows. All experimental animal procedures were approved by the Iranian Ministry of Agriculture (experimental permission No. 2232). During the study period the mean ambient temperature and the relative humidity were 20–24 °C and 48–65%, respectively.

2.1. Animals, treatments

Sixteen multiparous Holstein cows producing 35 ± 1.1 kg of milk per day with 710 ± 43 Kg body weight, mean ± SD were randomly allocated to two experimental diet groups started from calving date. There was no difference between groups (mean ± S.E.M.) in parity (3.0 ± 1.00) or body condition score (BCS) at calving day (3.1 ± 0.25). Diets containing palm oil (Control; n = 8) or extruded linseed (omega-3; n = 8) were formulated to meet NRC (2001) nutrient requirements for a lactating dairy cow of 700 kg body weight (BW), producing 40 kg milk per day, according to NRC [14] guidelines. Diets were formulated to be isonitrogenous and isoenergetic (Table 1). Fatty acid analyses were done for palm oil and linseed before starting the study to assess dietary formulations (Table 2).

2.2. Dry matter intake and milk production

Diets were fed twice daily (7:00 a.m. and 4:00 p.m.) for ad libitum intake (10% of refusals on as fed basis) from calving date until 70 DIM. Cows were milked three times daily 7:00 a.m., 2:00 p.m., and 11:30 p.m. and yields were recorded automatically using electronic milk meters (BouMatic, Madison-Wisconsin, USA).

Table 1
Ingredient and chemical composition of experimental diets.

	Treatments	
	Control	Linseed
Ingredient (% of dry matter)		
Alfalfa hay (mid bloom)	26.72	26.72
Corn silage	22.1	22.1
Dehydrated beet pulp	2.97	2.97
Ground corn grain	9.74	9.74
Rolled barley grain	9.65	9.65
Molasses	0.78	0.76
Wheat bran	0.32	0.20
Soybean meal (48% crude protein)	8.81	7.93
Corn gluten meal (60% crude protein)	1.83	1.83
Cottonseed meal	3.39	3.08
Whole cottonseed	1.51	1.48
Extruded linseed	0	4.06
Canola meal (solvent)	4.6	3.26
Wheat rolled	2.64	2.69
Palm oil	1.41	0
Limestone	0.45	0.45
Vitamin and mineral premix[a]	0.89	0.89
Dicalcium phosphate	0.54	0.54
Magnesium oxide	0.20	0.20
Salt	0.45	0.45
Sodium bicarbonate	1.00	1.00
Chemical composition		
Net energy for lactation (Mcal/kg of dry matter)	1.58	1.58
Fat (% of dry matter)	4.00	4.00
Crude protein (% of dry matter)	17.90	17.85
Neutral detergent fiber (% of dry matter)	34.40	33.90
Acid detergent fiber (% of dry matter)	20.40	20.50

[ab] Means within a row with different superscripts differ ($P < 0.05$).
[a] Contained (per kilogram): 16,000,000 IU of vitamin A; 3,200,000 IU of vitamin D; 48,000 IU of vitamin E; 24.0 g of Mn; 24.0 g of Zn; 24.0 g of Fe; 12.8 g of Cu; 1.44 g of I; 0.32 g of Se; and 0.32 g of Co.

2.3. Body condition score

All cows were evaluated for BCS on the day of parturition, on the day 30 post-partum (dpp) and 70 dpp. Scores were given by one experienced veterinarian based on a 1 (thin) to 5 (obese) predetermined scales using a quarter point system [15]. Changes in BCS were obtained by subtracting BCS at 30 dpp from BCS at parturition and BCS at 70 dpp from BCS at 30 dpp.

2.4. Estrus synchronization and reproductive management

The ovulatory cycles of cows were synchronized using two injections of PGF2α with a 14-day interval starting at d 30 postpartum [16]. All cows ovulated at 46 ± 2 DIM. Ovarian follicular development was monitored daily from the day of the second PGF2α

Table 2
Fatty acid (FA) profile (g/100 g of FA) of milk samples in the Holstein dairy cows fed diets supplemented with palm oil, or linseed as a source of omega-3.

	Treatments	
Fatty acid	Control	Linseed
C < 12	0.41	0.30
C14:0	0.47	3.03
C16:0	33.01^{a}	12.41^{b}
C16:1	1.01	0.93
C18:0	16.28^{a}	2.80^{b}
C18:1	12.20	18.45
C18:2	26.78^{a}	30.93^{a}
C18:3	8.31^{a}	29.75^{b}
C20:0/1	1.49	1.51

[ab] Means within a row with different superscripts differ ($P < 0.05$).