Effects of dietary polyunsaturated fatty acids on ovarian function and prostaglandin secretion in lactating dairy cows

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Abstract

As lactating cows in severe negative energy balance have poor reproductive performance, the effect of dietary fat supplementation (fish oil, soybean oil) on PGFM secretion, ovarian function and blood metabolites is investigated. In this experiment, the effects of dietary polyunsaturated fatty acids on plasma metabolites, ovarian function and prostaglandin secretion of 20 primiparous Holstein cows was studied. The cows were randomly allocated to one of four groups that were fed either: 1) a control diet; 2) a diet with 3% (Feed dry matter basis) fish oil; 3) a diet with 3% soybean oil; or 4) a diet with 1.5% fish oil and 1.5% soybean oil. Groups were synchronized using the heat-synch method and were fed their respective diets for 35 days, allowing 14 days for dietary adaptation and 21 days for data collection. Concentration of plasma glucose, triglycerides and low density lipoprotein (LDL) cholesterol were not affected by the treatments, but plasma total cholesterol and high density lipoprotein (HDL) cholesterol concentrations were significantly higher in cows that consumed the oil-containing diets ($p < 0.05$).The number of follicles, corpus luteum size and plasma estradiol, progesterone and prostaglandin $F_{2\alpha}$ metabolite (PGFM) concentrations were similar across all treatments. However, the size of the largest follicle was significantly greater in cows that consumed a diet containing fish oil or soybean oil ($p < 0.05$). These results suggest that polyunsaturated fatty acids can influence both ovarian and uterine function in cows, but further studies are required to test their effects on dairy cow reproduction.

Introduction

Since lactating cows in severe negative energy balance have poor reproductive performance (Butler and Smith, 1989), dietary fat supplementation could increase net energy intake and thus decrease the duration and magnitude of negative energy balance (Oldick *et al.,* 1997). Additional dietary fat has been found to increase levels of cholesterol (Staples *et al.,* 1998) and progesterone and the lifespan of induced corpora lutea (CLs) in cattle (Williams and Stanko, 1999). Cholesterol serves as a precursor for the synthesis of progesterone, which in turn prepares the uterus for implantation of the embryo and also helps to maintain pregnancy. Increased concentration of plasma progesterone has been associated with improved conception rates in lactating ruminants (Staples *et al.,* 1998). Increased concentrations of cholesterol from fat supplementation may lead to an increase in progesterone synthesis (Staples *et al.,* 1998) or reduced rate of its clearance from the blood (Hawkins *et al.,* 1995). It has been suggested that the fatty acids themselves, not the additional energy provided, stimulated ovarian function (Lucy, 2001).

There are two main families of essential fatty acid, omega-3 and omega-6, which have been linked to fertility. The main source of omega-6 fatty acid is dietary linoleic acid (C18:2n-6), which *inter alia* is the precursor of the dienoic (2-series) prostaglandins, such as $PGF_{2\alpha}$ (Abayasekara and Wathes, 1999). In addition, excess linoleic acid can be converted to a shunt metabolite, eicosadienoic acid (C20:2), rather than to arachidonic acid (Kaduce *et al.,* 1982), thereby reducing synthesis of series 1 and 2 prostaglandins. The inhibition mechanism is thought to involve competition between linoleic acid and arachidonic acid for binding with the key enzyme, cyclooxygenase. Eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) also have been shown to inhibit cyclooxygenase activity (Smith and Marnett, 1991). Competition between omega-3 and omega-6 for the binding site on prostaglandin

synthetase means that increasing the concentration of omega-3 fatty acids will decrease production of dienoic prostaglandins (Barnouin and Chassagne, 1991). Both EPA and DHA have been found to inhibit secretion of $PGF_{2\alpha}$ in different animal cell culture systems (Achard *et al*., 1997), including bovine endometrial cells (Mattos *et al*., 2004). Treatments that reduce endometrial synthesis of $PGF_{2\alpha}$ may reduce embryonic mortality (Mattos *et al*., 2000). Soybean oil, which is rich in linoleic acid, has been used as a source of omega-6 fatty acid; and fish oil, which is rich in omega-3 fatty acids, is an excellent source of EPA and DHA. $PGF_{2\alpha}$ secretion can be measured from plasma concentrations of 13, 14 dihydro, 15-keto $PGF_{2\alpha}$ (PGFM), the metabolite of $PGF_{2\alpha}$. Changes in the amounts or ratios of dietary polyunsaturated fatty acids may affect production of prostaglandins in the bovine reproductive system (Thatcher *et al*., 1994).

As there is minimal data on the effect of dietary omega-3 and omega-6 fatty acids on bovine reproductive function, the aim of our investigation was to study the influence of dietary fish oil, soybean oil or their blend on PGFM secretion, ovarian function and blood metabolites.

Materials and Methods

Animals and diets

A population of 20 Holstein primiparous cycling cows (47 ± 11 days in milk or DIM) were selected and divided into four groups. Each group was fed one of the diets in a randomized design over a total period of 35 days. The first two weeks was used to allow cows to adjust to the experimental diets and data was collected during weeks three, four, and five. Dietary treatments consisted of either no additional fat (control diet, C), 3% (feed dry matter basis) fish oil (FO), 3% soybean oil (SO) or a blend of 1.5% fish oil and 1.5% soybean oil (FO+SO). Cows were housed in tie-stall barn and fed a total mixed ration (TMR) four times daily at approximately 0800, 1000, 1400, and 1600 h in amounts that resulted in 5% orts. The TMR (Table 1) was formulated to contain more than adequate amounts of major nutrients (NRC, 2001). Cows were milked three times daily at 0830, 1600, and 2400 h.

Reproductive management

At the beginning of experiment, cows' estrous cycles were synchronized with two injections of $PGF_{2\alpha}$ (2 ml D. Cloprostenol, Abureihan Co., Iran) 14 days apart. This was followed by administration of 8 μg of a GnRH agonist (Gonadrolin; Aborayhan Co., Iran) on day 12 of the next cycle (day -10 in heatsynch), followed by an injection of $PGF_{2\alpha}$ and an injection of estradiol benzoate (1 mg; Aboreihan Co., Iran) on day 19 and 20 respectively (day -3 & -2) (Stevenson *et al.,* 2004). Cows that did not respond to the first dose of $PGF_{2\alpha}$ were given a second injection, and regression of the CL was confirmed by ultrasonography. Ultrasonography was carried out transrectally using a Concept/MCV ultrasound scanner equipped with a linear-array 7.5 MH_z probe (Tokyo Keiki Co. Ltd., Tokyo, Japan) on day 6, 8, 10, 12, 14, 16, 18 and 20 of the estrous cycle. The size of the largest ovarian follicle was measured 24 and 72 h after PGF2α administration as the average vertical and horizontal diameter, using electronic calipers. Follicle size was then monitored at 6-h intervals until it disappeared (ovulation) or until 102 h after PGF2α injection (Stevenson, *et al.,* 2004). The size and number of all follicles > 3 mm were recorded on detailed follicular maps. Follicles were grouped into three classes for analysis: class 1 (3.0 to 4.9 mm), class 2 (5.0 to 9.9 mm) and class 3 ($\geqslant$ 10 mm). The size of CL was also recorded. On day 15 of the following estrus cycle (at the end of the luteal phase and start of the follicular phase), cows were injected intravenously with oxytocin (100 IU) to stimulate uterine secretion of $PGF_{2\alpha}$ (Oldick *et al.,* 1997).

Sampling and analysis

Daily dry matter intake (feed offered minus orts) and milk production were recorded for each cow from day 0 to 18 of the estrous cycle (approximately day 15 to 33 of the experimental period). Samples were stored at -20° C until analyzed. Feed and ort samples were dried in a forced-air oven at 60° C for 48 h, and dry weights were used to determine feed intake. Subsamples of feed and ort were dried at 105° C for 24 h to correct to 100% dry matter (DM). After being ground through a 1 mm screen, feed samples were composited by week and analyzed for DM, crude protein (CP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), Ca, P, and Mg (AOAC, 2000). Fatty acid analysis of TMR, fish oil, soybean oil and milk fat samples was carried out using the method described by Sukhija and Palmquist (1998). Blood samples were taken via the coccygeal vein on day 6, 10, 12, and 14 of the estrous cycle. All blood samples were collected in sodium heparinized tubes, immediately placed on ice, and centrifuged at 3000g at 4°C for 15 min. Plasma samples were analyzed by colorimetric methods for triglycerides (kit no. 10-525; ZiestChem Diagnostics Co., Tehran, Iran), total cholesterol (kit no. 10-508), HDL cholesterol (kit no. 10-507), and glucose (kit no. 10-505). All plasma samples were analyzed in duplicate for progesterone (kit no. EIA-1561; DRG Instruments GmbH, Germany) and estradiol (kit no. EIA-2693) by competitive ELISA assay (Rosenfeld *et al.,* 2001). Oxytocin was injected on day 15 of the estrous cycle, as previously described, and blood samples were taken every 15 min from 60 min pre-injection to 240 min post-injection (Oldick *et al.,* 1997). Plasma was harvested, stored at -20ºC and later assayed in duplicate for PGFM by radioimmunoassay (Guilbault *et al.,* 1984).

Statistical analysis

The means of intake and production variables were obtained for each treatment for each cow for statistical analysis. Data were analyzed by a least squares ANOVA assay using the general linear model procedure of the SAS (SAS Institute, 1999). A Duncan's multiple range test was used for comparing means, and significance was declared at $p < 0.05$. Data on follicular development, hormones, blood metabolites and size of the CL were analyzed as repeated measurements with time using PROC MIXED of SAS. Scheffe's test was used to determine the effect of treatments on the size of corpus luteum, the difference in size between the largest and second largest follicle and the class and number of follicles. PGFM levels were analyzed as repeated measurements with time and also as the mean concentration over the 5-h sampling period.

Results

The chemical composition of the four diets is shown in Table 1. The control diet contained 2.5% EE (DM basis), and the EE content of FO, SO and FO+SO was 5.4, 5.1, and 5.2%, respectively. Consequently, the diets containing supplemental fat had a higher net energy for lactation (NE_L) concentration (1.62 to 1.66 compared with 1.57 Mcal/kg for the control diet).

The fatty acid compositions of TMR, fish oil and soybean oil are presented in Table 2. Soybean oil contained the highest concentration of linoleic acid (53.2 g/100 g fatty acid). Fish oil was characterized by an abundance of long chain (>C20) fatty acids and was particularly rich in EPA and DHA, whose concentrations were found to be 11.5 and 10.3 g/100 g of fatty acid, respectively. Results of milk yield, milk composition, milk fatty acid profiles and feed intake have been published in a parallel experiment (Fatahnia *et al.,* 2008).

The effect of experimental diets on plasma metabolites concentrations are shown in Table 3. Plasma glucose, LDL cholesterol and triglycerides concentrations were not significantly affected by supplemental fat. While, plasma concentrations of total cholesterol and HDL cholesterol were significantly higher for the cows that fed fat containing diets ($P < 0.05$). The effect of dietary

Table 1: Ingredient and nutrient content of experimental diets.

Item	Experimental diet[1]			
	C	FO	SO	FO + SO
Ingredient	% of DM			
Alfalfa hay	20.76	20.79	20.79	20.79
Corn silage	20.76	20.79	20.79	20.79
Ground barley	19.03	9.33	9.33	9.33
Ground corn	9.31	9.33	9.33	9.33
Soybean meal	8.38	9.33	9.33	9.33
Canola meal	5.59	5.60	5.60	5.60
Cottonseed meal	6.99	9.33	9.33	9.33
Beet pulp	6.99	10.36	10.36	10.36
Fish oil	0.00	3.00	0.00	1.50
Soybean oil	0.00	0.00	3.00	1.50
Sodium bicarbonate	0.40	0.40	0.40	0.40
Mineral and vitamin[2]	0.58	0.58	0.58	0.58
Calcium carbonate	0.93	0.93	0.93	0.93
Salt	0.23	0.23	0.23	0.23
Chemical composition				
CP	17.0	17.1	17.3	17.2
Ether extract	2.5	5.4	5.1	5.2
NDF	33.2	33.5	33.9	33.7
NFC [3]	40.7	37.0	37.3	37.1
Ash	6.6	7.0	6.4	6.8
Ca	0.81	0.85	0.85	0.85
P	0.38	0.37	0.37	0.37
Mg	0.22	0.23	0.23	0.23
NE_L,[4] Mcal/kg	1.57	1.66	1.62	1.64

[1]C = No supplemental fat; FO = 3% fish oil; SO = 3% soybean oil; FO+SO = 1.5% fish oil + 1.5% soybean oil. [2]Mineral and vitamin mix contained 0.8% Ca, 0.7% P, 0.8% K, 0.4% Mg, 0.3% S, 1.4 mg/kg I, 100 mg/kg Mn, 100 mg/kg Zn, 0.3 mg/kg Co, 0.5 mg/kg Se, 99,450 IU/kg of vitamin A, 13,260 IU/kg of vitamin D, and 497 IU/kg of vitamin E. [3]NFC = Nonfiber carbohydrates, NFC = 100 – (CP + NDF + EE + ash). [4]Calculated according to NRC (1989) values.

Table 2: Fatty acid composition (g/100 g of fatty acids) of experimental diets, fish oil, and soybean oil.

Fatty acids[2]	Experimental diet[1]				Oil supplement	
	C	FO	SO	FO + SO	Fish	Soybean
C14:0	0.62	2.76	0.92	1.44	8.23	0.11
C16:0	27.48	30.36	25.63	29.04	16.65	11.83
C16:1	1.13	2.68	1.40	1.89	9.56	0.18
C18:0	3.62	4.06	4.64	4.38	3.68	3.75
C18:1c9	20.72	15.66	21.23	18.35	12.96	22.10
C18:2c9c12	34.44	25.56	35.97	30.50	1.38	53.20
C18:3 (n-3)	5.82	6.41	8.03	7.50	2.93	6.30
C20:0	0.68	1.02	0.72	0.82	0.46	0.32
C20:5 (n-3)	0.00	2.20	0.00	1.63	11.50	0.00
C22:6 (n-3)	0.00	1.76	0.00	0.91	10.30	0.00
Others	5.49	7.53	1.46	3.54	22.35	2.21
Unsaturated	62.26	54.27	66.88	60.78	48.63	81.78
Saturated	32.40	38.20	39.91	35.68	29.02	16.01
n-6/n-3 ratio	5.769	2.382	4.344	3.038	0.056	8.44

[1]Control = No supplemental fat; FO = 3% fish oil; SO = Soybean oil; FO + SO = 1.5% fish oil + 1.5% soybean oil. [2]Expressed as number of carbon atoms: number of double bonds.

Table 3: Plasma composition of Holstein cows fed experimental diets.

Item	Experimental diet[1]				SE
	C	FO	SO	FO + SO	
Glucose , mg/100 ml	65.17	62.10	65.69	66.83	4.62
Total cholesterol, mg/100 ml	214.60[b]	256.70[a]	292.00[a]	288.30[a]	16.32
HDL cholesterol, mg/100 ml	138.80[b]	167.20[a]	184.00[a]	176.50[a]	13.17
LDL cholesterol, mg/100 ml	76.8	89.53	108.02	111.74	16.55
Triglycerides, mg/100 ml	19.94	21.48	21.57	24.54	1.37

[ab]Means in the same row with different letters differ ($p < 0.05$). [1]Control = no supplemental fat; FO = 3% fish oil; SO = 3% soybean oil; FO+SO = 1.5% fish oil + 1.5% soybean oil.

Table 4: Reproductive data of Holstein cows fed experimental diets.

Item	Experimental diet[1]				SE
	C	FO	SO	FO + SO	
Class of follicles, no.					
Small, 3.0 to 4.9 mm	1.80	3.27	3.42	2.47	0.51
Medium, 5.0 to 9.9 mm	1.55	2.33	2.50	2.53	0.63
Large, = 10.0 mm	0.89	0.87	0.92	1.10	0.22
Total	4.24	6.47	6.84	6.10	1.00
Follicle diameter, mm					
Largest, F_1	10.51[b]	12.65[b]	11.66[b]	15.53[a]	0.92
Second largest, F^2	6.84	7.70	7.33	6.52	0.56
CL diameter, mm	18.90	20.66	19.85	21.68	0.96
Progesterone, ng/mL	6.96	6.47	8.30	9.62	1.94
Estradiol- 17β, pg/mL	22.20	21.86	23.83	26.50	2.90
PGFM, pg/mL	60.13	54.04	66.63	50.40	7.4

[ab]Means in the same row with different letters differ ($p < 0.05$). [1]Control = No supplemental fat; FO = 3% fish oil; SO = 3% soybean oil; FO+SO = 1.5% fish oil + 1.5% soybean oil.

treatments on follicles number, follicles and CL size and plasma concentrations of progesterone, estradiol-17β and PGFM are shown in Table 4. There were no significant effects of treatments on the mean numbers of follicles, mean size of the second largest follicle, mean CL size or plasma concentrations of progesterone, estradiol-17β and PGFM the size of the largest follicle was greater for cows on the 1.5% fish oil plus 1.5% soybean oil diet ($P < 0.05$).

Discussion

As there was no data on supplementary effect of omega-3 and omega-6 fatty acids on plasma metabolites, ovarian function and prostaglandin secretion, we aimed at understanding the effect of dietary polyunsaturated fatty acids on reproduction function in lactating dairy cows. Our results suggest that plasma glucose concentration was not affected by supplementary fat. Although this is consistent with some previous studies (Oldick *et al.,* 1997; Petit and Twagiramungu, 2006), other work suggested that fish oil decreases plasma glucose concentrations (Mattos *et al.,* 2004). The authors of that study concluded that this could be due to the reduced dry matter intake associated with that diet. Another possible explanation is that gluconeogenic enzymes are inhibited by components of fish oil, for example eicosapentaenoic acid was found to inhibit the expression of phosphoenolpyruvate carboxykinase (Murata *et al.,* 2001), an essential enzyme in glucose production. In the present study, it is possible that the eicosapentaenoic acid may did not have same inhibitory effect, therefore plasma glucose concentrations were similar among treatments. Plasma concentrations of total cholesterol and HDL-cholesterol were significantly higher in cows fed a fat containing diet, consistent with previous results (Oldick *et al.,* 1997; Thomas *et al.,* 1997; Petit and Twagiramungu, 2006). There was no effect on plasma LDL-cholesterol or triglyceride concentrations, in agreement with previous work (Petit and Twagiramungu, 2006; lammoglia *et al.,* 1996; Thomas *et al.,* 1997). In contrast, oldick *et al.* (1997) have been reported increased of plasma triacylglycerol concentration of cows that fed supplemental fat. Differences in plasma cholesterol values between studies could have been affected by the source of dietary fats, breed, reproductive status of the animal and differences in fat concentrations in the diets (Lammoglia *et al.,* 1997). In the current study, this increased cholesterol likely is due to need for increased absorption of fatty acids packaged in chylomicrons and very low density lipoproteins from the small intestine (Staples *et al.,* 1998). Although fish oil has been found to less cholesterologenic than soybean oil (Byers and Schelling, 1998), the present experiment showed no major differences in cholesterol and HDL-cholesterol concentrations between the diets. There were no significant effects of treatments on the mean numbers of follicles or the mean size of the second largest follicle, consistent with Petit *et al.* (2002). However, some previous studies reported an increase in the number of medium-sized follicles after administration of polyunsaturated fatty acid in the form of soybean oil or rice bran (Ryan *et al.,* 1992; Lammoglia *et al.,* 1996; Thomas *et al.,* 1997). In the current study, the size of the largest follicle was greater in cows fed the blend of fish oil and soybean oil (FO + SO diet). Dietary fat may enhance follicular development via metabolic hormones that act on the central nervous system to stimulate GnRH secretion; lipid-supplemented cows have been found to have increased basal LH concentrations (Hightshoe *et al.,* 1991). Another way in which dietary fat may affect follicular development is through metabolic hormones acting at the ovarian level. Plant oils such as soybean oil are rich in linoleic acid, which has been shown to increase ruminal propionate production, increasing gluconeogenesis and therefore insulin concentration (Chalupa *et al.,* 1986). Thomas and Williams (1996) found that follicular development, along with plasma insulin and follicular IGF-I concentrations, was enhanced by soybean oil. Another influential factor on follicular development is cholesterol. Fat supplements have been found to increase total and HDL-cholesterol concentrations (Thomas and Williams, 1996), which Bao *et al.* (1995) demonstrated stimulate cell division and IGF-I production in cultured granulosa cells. As cholesterol is the precursor of all steroids, increased substrate availability may increase follicular steroid synthesis (Carrol *et al.,* 1990). During ovarian follicle growth and differentiation, increasing amounts of estrogen are produced. This in turn upregulates the synthesis and release of LH, which promotes the final stage of follicle growth (Rosenfeld *et al.,* 2001). Therefore, the increase in plasma glucose and estradiol-17β concentrations in the current study probably increased the concentrations of insulin and LH, respectively. Although this may explain the large size of follicles in the diet containing fish oil and soybean oil, more research is required to better understand the mechanisms by which dietary fats stimulate follicular development. The mean size of the CL was not significantly affected by supplemental fats. Although this is in line with results from a previous study (Petit *et al.,* 2001), other studies reported an increase in CL size (Petit *et al.,* 2002; Petit and

Twagiramungu, 2006), possibly due to greater size of the largest follicle (Lucy, 2001; Vasconcelos *et al.,* 2001). Another possibility for the numerical larger CL in the oil containing diets may be the lower plasma concentrations of PGFM, a $PGF_{2\alpha}$ metabolite (Table 4). Plasma concentrations of progesterone and estradiol-17β were not affected by diet. The greater size of the CL and largest follicle may explain the numerical increases in plasma estradiol-17β and progesterone concentrations, respectively. Previous work suggested that elevated concentrations of these hormones resulted from a decrease in clearance rate from the circulatory system rather than an increase in their secretion (Hawkins *et al.,* 1995; Sangritavong *et al.,* 2002). Another factor that may explain the numerical increase in these hormones is the higher concentration of plasma total cholesterol and HDL-cholesterol because cholesterol is the precursor for all steroids (Carrol *et al.,* 1990). A positive correlation has been found between plasma progesterone concentration and pregnancy rate (Petit and Twagiramungu, 2006). Plasma PGFM concentrations during the oxytocin challenge were similar among treatments, in agreement with Petit and Twagiramungu (2006). There is conflicting evidence as to the effect of dietary fat on plasma PGFM concentration; although one study reported an increase (Petit *et al.,* 2002), two other studies reported a decrease (Thatcher *et al.,* 1997; Mattos *et al.,* 2000). The mechanism by which EPA and DHA inhibit secretion of uterine prostanoids is not fully understood. It is thought to require incorporation of EPA and DHA into cellular lipid pools and may involve competition with arachidonic acid for processing by the cyclooxygenase -1 and -2 enzymes. Feeding fish meal has been found to increase the proportion of EPA and DHA in endometrial lipids in beef cows (Burns *et al.,* 2000), indicating that dietary changes can alter the fatty acid composition of the uterus. EPA and arachidonic acid are broken down by cyclooxygenase to series 2- and 3 prostanoids, respectively. Because both EPA and arachidonic acid are substrates for cyclooxygenase, increased availability of dietary EPA could displace arachidonic acid, leading to greater synthesis of 3-series than 2-series prostanoids (Mattos *et al.,* 2004). Series 3 prostanoids are less bioactive (Needleman *et al.,* 1979), and there appears to be no evidence for their role in ruminant luteolysis. Besides of this competitive mechanism, EPA and DHA may reduce the expression of cyclooxygenase genes (Achard *et al.,* 1997), making it less available and reducing prostanoid synthesis. Additionally, cyclooxygenase convert EPA into 3-series prostanoids less efficiently manner than it converts arachidonic acid into 2-series prostanoids, possibly resulting in reduced total prostanoid synthesis. According to the results of current experiment, the four diets differed primarily in terms of their n-3 and n-6 polyunsaturated fatty acid content and ratio of n-3: n-6. Total plasma cholesterol and HDL-cholesterol concentrations and size of largest follicle increased in all fat supplemented groups but other parameters were not affected significantly. The non-significant decrease in plasma PGFM concentration in cows fed fish oil and soybean oil may support our hypothesis that the ratio of omega-3 to omega-6 fatty acids will affect synthesis of the dienoic PG ($PGF_{2\alpha}$), but requires further study.

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