The Effects of Feeding Fish Oil on Uterine Secretion of $\text{PGF}_{2\alpha}$, Milk Composition, and Metabolic Status of Periparturient Holstein Cows

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ABSTRACT

The objectives were to determine the effect of dietary fish oil (FO) on uterine secretion of $\text{PGF}_{2\alpha}$, milk production, milk composition, and metabolic status during the periparturient period. Holstein cows were assigned randomly to diets containing FO ($n = 13$) or olive oil (OO, $n = 13$). Cows were fed prepartum and postpartum diets that provided approximately 200 g/d from 21 d before the expected parturition until 21 d after parturition. The FO used contained 36% eicosapentaenoic acid (EPA, C20:5, n-3) and 28% docosahexaenoic acid (DHA, C22:6, n-3). Blood samples were obtained from 14 d before the due date until d 21 postpartum. A total of 6 FO and 8 OO cows without periparturient disorders were used in the statistical analyses of $\text{PGF}_{2\alpha}$ -metabolite (PGFM) and metabolite concentrations. Length of prepartum feeding with OO or FO did not differ. Proportions of individual and total n-3 fatty acids were increased in caruncular tissue and milk of cows fed FO. The combined concentrations of EPA and DHA in caruncular tissue were correlated positively with the number of days supplemented with FO. Cows fed FO had reduced concentrations of plasma PGFM during the 60 h immediately after parturition compared with cows fed OO. Concentrations of prostaglandin H synthase-2 mRNA and protein in caruncular tissue were unaffected by diet. Production of milk and FCM were similar between cows fed the two oil diets. However, cows fed FO produced less milk fat. Feeding FO reduced plasma concentrations of glucose. Dietary fatty acids given during the periparturient period can reduce the uterine secretion of $\text{PGF}_{2\alpha}$ in lactating dairy cows and alter the fatty acid profile of milk fat.

(**Key words:** fish oil, prostaglandin, uterus, n-3 fatty acid)

Abbreviation key: CLA = conjugated linoleic acid, DA = displaced abomasum, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, FO = fish oil, G3PDH = glyceraldehyde 3-phosphate dehydrogenase, OO = olive oil, PGHS-2 = prostaglandin H₂ synthase 2, PGFM = 13, 14 dihydro, 15-keto $\text{PGF}_{2\alpha}$, PUN = plasma urea nitrogen.

INTRODUCTION

Fish oil (FO) contains relatively high concentrations of two polyunsaturated fatty acids of the n-3 family: eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). These fatty acids can be supplied only by the diet because EPA and DHA cannot be synthesized de novo in mammalian systems. Eicosapentaenoic acid and DHA have inhibited secretion of $\text{PGF}_{2\alpha}$ in different animal cell culture systems (Achard et al., 1997), including bovine endometrial cells (Mattos et al., 2001). Inhibiting uterine secretion of $\text{PGF}_{2\alpha}$ in vivo by feeding EPA and DHA may reduce endometrial secretion of $\text{PGF}_{2\alpha}$ to possibly induce an antiluteolytic effect during early pregnancy and increase fertility rates (Staples et al., 1998; Mattos et al., 2000). Several studies documented the effects of dietary marine products on $\text{PGF}_{2\alpha}$ secretion. Feeding fish meal, which contained approximately 8% FO (DM basis), to lactating multiparous cows for 47 to 51 d reduced the secretion of $\text{PGF}_{2\alpha}$ induced by estradiol and oxytocin injected on d 15 of a synchronized estrous cycle (Mattos et al., 2002). In humans, consumption of large amounts of FO was reported to prolong gestation (Olsen et al., 1992), possibly due to suppressed secretion of $\text{PGF}_{2\alpha}$ delaying parturition. Also, infusing ewes with 3 mL/kg of BW per day (i.v.) of an emulsion of FO containing 30% EPA and 20% DHA blocked a betamethasone-induced increase in plasma concentrations of prostaglandin E_2 and delayed

Received December 19, 2002.

Accepted August 8, 2003.

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Table 1. Number of experimental cows and incidence of health disorders.

	Olive oil	Fish oil	Total
Animals starting the experiment			
Primiparous, n	5	4	9
Multiparous, n	8	9	17
Total animals, n	13	13	26
Animals used in statistical analyses			
Primiparous, n	1	2	3
Multiparous, n	7	4	11
Total animals, n	8	6	14
Health disorders			
Dystocia, %	46 (6/13)	38 (5/13)	42 (11/26)
Stillborn calf, %	8 (1/13)	15 (2/13)	12 (3/26)
Retained fetal membranes, %	15 (2/13)	15 (2/13)	15 (4/26)
Toxic metritis, %	15 (2/13)	8 (1/13)	12 (3/26)
Displaced abomasum, %	23 (3/13)	38 (5/13)	31 (8/26)

occurrence of parturition in sheep (Baguma-Nibasheka et al., 1999).

A very intense secretion of uterine $\text{PGF}_{2\alpha}$, as evidenced by dramatic increases in concentration of plasma $\text{PGF}_{2\alpha}$, is characteristic of the immediate postpartum period in the cow (Guilbault et al., 1984a). Plasma concentrations of 13, 14 dihydro, 15-keto $\text{PGF}_{2\alpha}$ (**PGFM**), the metabolite of $\text{PGF}_{2\alpha}$ used to estimate $\text{PGF}_{2\alpha}$ secretion, peaked at concentrations >1 ng/mL within 1 to 3 d after parturition and decreased progressively until d 15 postpartum (Guilbault et al., 1984a, 1985). This period of intense secretion of uterine $\text{PGF}_{2\alpha}$ was used in this experiment to test inhibitors of prostanoid synthesis.

Our hypothesis was that feeding EPA and DHA using dietary FO would increase the proportion of these fatty acids in the uterine tissue of dairy cows and reduce the spontaneous secretion of uterine $\text{PGF}_{2\alpha}$ during the periparturient period. Our objectives were to determine the effect of dietary FO on spontaneous secretion of $\text{PGF}_{2\alpha}$ by the uterus during the periparturient period and to evaluate the effect of diet on milk production, milk composition, and indicators of metabolic status such as plasma concentrations of glucose, NEFA, BHBA, and plasma urea nitrogen (**PUN**). In addition, the effect of diet on fatty acid composition and concentrations of prostaglandin H_2 synthase-2 (**PGHS-2**) protein and mRNA in caruncular tissue were determined.

MATERIALS AND METHODS

Cows and Diets

The study was conducted from January 31 to April 7, 2001. Pregnant Holstein cows ($n = 17$) and heifers ($n = 9$) were assigned randomly to diets containing FO ($n = 13$) (Arista Industries, Wilton, CT) or olive oil (**OO**, $n = 13$) (Classico, Bertolli, Italy). A ration containing the oils was supplied from 21 d before the expected due

date until parturition. It was then replaced by rations containing either FO or OO, which were fed until cows reached 21 d postpartum. During the prepartum period, cows were housed in sod-based pens and were moved to a free-stall barn at calving. Cows ($n = 6$) and heifers ($n = 6$) that had moderate to severe dystocia, that were diagnosed with displaced abomasum (**DA**), that retained fetal membranes, or that had toxic metritis within 10 d after parturition were removed from the analyses of the effects of diet on plasma concentrations of PGFM, PUN, glucose, BHBA, and NEFA. Therefore, a total of three heifers (1 OO, 2 FO) and 11 cows (7 OO, 4 FO) were used in the statistical analyses because their health status likely did not interfere with the normal secretion of uterine $\text{PGF}_{2\alpha}$ (Table 1).

Rations were formulated to provide approximately 200 g/d of oil. Assumed average DMI were 10.2 and 11.6 kg/d for the close-up and early postpartum periods, respectively. Because of anticipated lower DMI in the close-up period, nonlactating cow rations contained 2% oil (DM basis), whereas lactating cow rations were formulated to contain 1.8% oil. The FO selected contained 36% EPA and 28% DHA (Table 2). Therefore, the combined intake of EPA (72 g) and DHA (56 g) was expected to be 128 g/d. Fish oil was fed at 1.8% of dietary DM postpartum in this study in order to deliver approximately 50 to 60 g/d of EPA plus DHA. This was assuming a ruminal escape of biohydrogenation of 65% and an intestinal absorption of 70%.

Ingredient and chemical composition of the diets are described in Tables 3 and 4. At parturition, rations were adjusted to supply nutrients required during early lactation.

The concentrate portions of the diets were mixed and stored in metal bins of 1.8-tonne capacity. Oils were premixed using ground corn as a carrier. Concentrate mixtures and forage sources were mixed in a weighing and mixing unit (American Calan, Inc.) and offered

Table 2. Fatty acid profile of fish oil and olive oil.

Fatty acid ¹	Diet	
	Olive oil	Fish oil
	(g/100 g of fatty acids)	
C14	0.00	0.41
C14:1	0.00	0.09
C15:0	0.00	0.26
C16:0	16.62	7.03
C16:1	1.86	6.33
C18:0	2.70	1.00
tC18:1	0.00	1.44
C18:1, n9	61.28	4.46
C18:2, n6	16.47	2.48
CLA t9t11	0.00	0.71
CLA c9t11	0.00	0.00
C18:3, n3	0.61	1.95
C20:0	0.46	0.56
C21:0	0.00	6.36
C20:5, n3	0.00	35.81
C22:0	0.00	0.24
C22:1	0.00	2.36
C22:6, n3	0.00	28.42
C24:0	0.00	0.09

¹Expressed as number of carbons:double bonds.

Table 3. Ingredient composition of experimental diets containing olive oil or fish oil fed to Holstein cows during late pregnancy and early lactation.

Feedstuff	Nonlactating		Lactating	
	% of dietary DM			
Corn silage	37.6		28.0	
Alfalfa hay	6.3		11.9	
Bermuda hay	6.4		—	
Cottonseed hulls	—		4.8	
Ground corn	23.4		22.4	
Soybean meal	9.2		14.1	
Citrus pulp	6.9		7.8	
Oil	2.0		1.8	
Whole cottonseed	1.0		1.9	
Pro-Lak ¹	1.2		2.2	
Mineral mix ²	5.5		—	
Mineral mix ³	—		4.7	
Trace mineralized salt ⁴	0.5		—	
Feedphos ⁵	—		0.3	

¹A marine and animal protein supplement containing 68% RUP (H. J. Baker & Bro., Inc., Stamford, CT).

²Mineral and vitamin mix containing 22.8% CP, 2.1% fat, 22.89% Ca, 0.16% P, 2.77% Mg, 0.75% Na, 0.20% K, 2.42% S, 8.03% Cl, 146.7 mg/kg of Mn, 95.0 mg/kg of Zn, 26.6 mg/kg of Fe, 112.5 mg/kg of Cu, 10.7 mg/kg of Co, 7.9 mg/kg of I, 6.9 mg/kg of Se, 268,130 IU/kg of vitamin A, 40,000 IU/kg of vitamin D, and 1129 IU/kg of vitamin E (DM basis).

³Mineral and vitamin mix containing 26.4% CP, 1.74% fat, 10.15% Ca, 0.90% P, 3.1% Mg, 8.6% Na, 5.1% K, 1.5% S, 4.1% Cl, 2231 mg/kg of Mn, 1698 mg/kg of Zn, 339 mg/kg of Fe, 512 mg/kg of Cu, 31 mg/kg of Co, 26 mg/kg of I, 7.9 mg/kg of Se, 147,756 IU/kg of vitamin A, 43,750 IU/kg of vitamin D, and 787 IU/kg of vitamin E (DM basis).

⁴Minimum concentrations of 40% Na, 55% Cl, 0.25% Mn, 0.2% Fe, 0.033% Cu, 0.007% I, 0.005% Zn, and 0.0025% Co (DM basis) (Flint River Mills, Inc., Bainbridge, GA).

⁵Contains 21% P and 15 to 18% Ca (Southeastern Minerals, Inc., Bainbridge, GA).

twice daily at 0700 and 1230 h to allow 5 to 10%orts (as-fed basis). Orts from each diet were collected once daily and weighed. Daily ort weights for each diet were subtracted from the ration offered and divided by the number of cows fed to determine daily average individual cow intakes. The DM concentration of silage was monitored once weekly (55°C for 48 h) to maintain the proper forage-to-concentrate ratio of diets. Samples of forages and concentrate mixes were collected weekly, composited monthly, and analyzed for CP, NDF, ADF, ether extract, and minerals (Dairy One, DHIA Forage Testing Laboratory, Ithaca, NY) using wet chemistry.

Cows were milked 3× d at 0500, 1200, and 1900 h. Calibrated electronic milk meters were used at each milking to record milk weights. Body condition scores and BW were determined at the introduction of diets, calving, and at d 21 postpartum.

Collection of Blood Samples

Blood (10 mL) was obtained once daily at 1730 h from 14 d before due date until parturition and from d 15 until d 21 postpartum. Between the day of parturition (d 0) and d 14 postpartum, blood samples were collected 2× per day at 0800 and 1730 h. Blood was collected from the coccygeal vessels into evacuated blood tubes containing EDTA as an anticoagulant (10.5 mg, Monoject, Sherwood Medical, St. Louis, MO). Samples were maintained in ice until plasma was separated by centrifugation (2600 × g, 30 min) at 4°C within 1 h of collection and stored at −20°C until analyzed.

PGFM and Metabolite Assays

Samples collected between d 5 prepartum and d 21 postpartum were analyzed for concentrations of PGFM using a modification of the radioimmunoassay procedure described by Mitchell et al. (1976). The PGFM standard solutions were made by serial dilutions in a buffer of a stock solution (1 µg/mL in 10% ethanol and 90% PBS buffer) of authentic PGFM (Sigma, St. Louis, MO). Standards (100 µL) were run in duplicates at the following concentrations: 15.6, 31.2, 62.5, 125, 250, 500, 1000, 2000, 4000, and 8000 pg/mL. The standard curve included 100 µL of prostaglandin-free plasma, which was obtained from a cycling nonlactating beef cow pretreated twice with flunixin meglumine (Banamine, Schering-Plough, 1 g/i.m. injection), a PGHS inhibitor. Injections were given 16 h apart. Blood was collected 4 h after the second injection, and the plasma was separated by centrifugation. Samples of prostaglandin-free plasma had undetectable concentrations (<10 pg/mL) of PGFM when assayed utilizing standard curves in 0.05 M PBS buffer. The PBS buffer contained 2.3 g/L

Table 4. Chemical composition of experimental diets containing either olive or fish oil fed to Holstein cows during late pregnancy and early lactation.

Chemical	Olive oil nonlactating	Fish oil nonlactating	Olive oil lactating	Fish oil lactating
CP, % of DM	14.03	13.96	16.7	17.20
NE _L , Mcal/kg of DM ¹	1.68	1.68	1.73	1.74
NDF, % of DM	31.17	30.85	30.49	28.40
ADF, % of DM	19.39	19.11	19.43	18.75
Ether extract, % of DM	5.32	4.77	5.28	5.36
NFC ²	41.4	42.4	39.2	40.7
Ca, % of DM	2.20	2.06	1.48	1.44
P, % of DM	0.39	0.37	0.48	0.53
Mg, % of DM	0.44	0.42	0.40	0.43
K, % of DM	1.38	1.37	1.57	1.58
Na, % of DM	0.26	0.27	0.41	0.48
S, % of DM	0.27	0.28	0.22	0.24
Cl, % of DM	1.22	1.18	0.42	0.42
Fe, ppm of DM	206	210	250	242
Zn, ppm of DM	65	64	112	114
Mn, ppm of DM	51	49	88	114
Cu, ppm of DM	16	12	25	38

¹Calculated by DHI Forage Testing Laboratory, Ithaca, NY.²Calculated as (% NFC = 100% - % CP - % NDF - % fat - % ash).

NaH₂PO₄ H₂O, 4.76 g/L Na₂HPO₄ 7H₂O, 1 g/L sodium azide, and 8.41 g/L NaCl. The buffer pH was adjusted to 7.5 with NaOH.

Activity and volume of radioactively labeled PGFM (Amersham Pharmacia Biotech, Piscataway, NJ) used were 18,000 dpm and 100 μ L, respectively. For unknown samples, final assay volume was 400 μ L; it comprised 100 μ L of sample, 100 μ L of rabbit antiserum to PGFM (1:10,000; Meyer et al., 1995), 100 μ L of buffer, and 100 μ L of labeled PGFM. After a 12-h incubation at 4°C, free PGFM was separated using 750 μ L of a solution of charcoal-coated dextran (1.25% dextran [Sigma, St. Louis, MO], 12.5% activated charcoal [Sigma, St. Louis, MO] in a PBS buffer). After centrifugation for 20 min at 3565 $\times$ g, the supernatant was transferred to scintillation vials and mixed with 4.5 mL of scintillation fluid (Scintiverse II, Fisher Scientific, Pittsburgh, PA). Activity was measured using a liquid scintillation counter (model LKB 1219, Wallac Inc., Gaithersburg, MD). Accuracy of the assay was determined by determining known quantities of exogenous PGFM previously added to prostaglandin-free cow plasma. Added masses achieved final concentrations of 125, 250, 500, and 1000 pg/mL. Recovery of added (X) versus measured (Y) PGFM concentrations was described by linear regression ($Y = 1.1957X - 56.72$, $R^2 = 0.98$). Sensitivity of the assay was established at 15.6 pg/mL, because an antibody dilution of 1:10000 and an assay volume of 100 μ L of plasma permitted detection of a minimum mass of 1.56 pg. Dilution in prostaglandin-free plasma of a high (826.6 pg/mL) PGFM plasma sample to 100, 50, and 25% of original volume resulted in an inhibition curve that was parallel to the standard

inhibition curve (heterogeneity of regression, $P > 0.1$). Inter- and intraassay coefficients of variation were 9.8 and 11.9%, respectively.

Plasma concentrations of NEFA and BHBA were measured in samples collected on d -5, -3, -1, 1, 4, 7, 10, 13, 16, and 19, relative to the day of parturition. A kit assay was used to measure plasma concentrations of BHBA (BHBA, kit 310-A, Sigma Diagnostics, St. Louis, MO). Inter- and intraassay coefficients of variation were 7.3 and 17.6%, respectively. Plasma concentrations of NEFA were measured with the Wako NEFA C test kit (Wako Chemicals USA, Richmond, VA). Inter- and intraassay coefficients of variation were 2.9 and 5.4%, respectively.

Concentrations of plasma glucose and urea nitrogen were determined daily from d -5 to d 21 with an automated colorimetric procedure, which utilized an autoanalyzer (AutoAnalyzer II, Bran+Luebbe, Buffalo Grove, IL). The glucose procedure is a modification (Technicon Industrial Method #339-19, Bran+Luebbe, Buffalo Grove, IL) of that described by Gochman and Schmitz (1972). The procedure for determination of urea nitrogen (Industrial Method US-339-01, Bran+Luebbe, Buffalo Grove, IL) is a modification of the carbamido-diacetyl reaction procedure (Coulombe and Favreau, 1963).

Milk Fat Isolation and Analysis

Milk samples for the determination of fat, protein, and somatic cells (Southeast Milk Inc., Belleview, FL) were collected weekly from two consecutive milkings. Milk fat and protein concentrations were analyzed by the mid-infrared spectroscopic method. Milk samples

also were collected during 2 consecutive milkings on the last experimental day (d 21) for analysis of fatty acids using GLC. Samples for fatty acid analysis were refrigerated at 4°C and subsequently composited according to the milk yield recorded for each of the milkings. Milk fat was extracted using a detergent solution containing 3% Triton X-100 (wt/vol) and 7% sodium hexametaphosphate in distilled water. A sample of milk fat was methylated in 0.5 M sodium methoxide in methanol followed by a second methylation in acetyl chloride:methanol (1:10, vol/vol) as described by Kramer et al. (1997). Fatty acid methyl esters in milk were separated by GLC (HP 5890, Agilent Technologies, Palo Alto, CA) on a 30 m × 0.25 mm × 0.2 μm-film thickness SP2380 capillary column (Supleco, Inc., Bellefonte, PA). The column oven was programmed to rise 4°C/min from an initial temperature of 50°C (held for 2 min) to a final temperature of 250°C (held for 15 min). Temperatures of the injector (100:1 split) and detector (flame ionization) ovens were 250° and 260°C, respectively. Helium was the carrier gas (20 cm/s).

Efficiency of transfer of EPA and DHA from diet to milk was calculated by dividing the mean mass of fatty acid secreted in milk daily (average daily milk production × average milk fat percentage × average proportion of EPA or DHA in milk fat) by the mean mass of that fatty acid consumed daily with the diet (average daily DMI × estimated concentration of EPA or DHA in the diet). Milk fat and fish oil were corrected for glycerol, assuming 10% by weight.

Collection of Caruncles

Caruncles were collected from 23 cows by manual extraction through the vagina within 12 h of parturition. To minimize bacterial contamination of the reproductive tract, the perineal area was washed with a disinfectant solution and dried with a paper towel. The technician used a shoulder-length sleeve and sterile lubrication. After collection, caruncles were washed with water, plunged in liquid nitrogen, and stored at -80°C. Subsamples of frozen caruncles were subsequently used for total RNA extraction, preparation of tissue lysates for protein analyses, and determination of fatty acid composition. Approximately 7 g of caruncular tissue were freeze-dried for 24 h using a lyophilizer (Labconco, Kansas City, MO) to obtain a final mass of approximately 1.5 g of DM. Fatty acids in dry caruncular tissue were methylated and separated by gas chromatograph using the same techniques described for milk fatty acid composition. The only change was the temperature program of the gas chromatograph, which increased from 140°C (3 min) to 220°C (20 min) at 3.7°C/min.

Isolation of mRNA from Placentomes

Total RNA was isolated using the RNeasy total RNA isolation system (Promega, Madison, WI). Placentomes were ground briefly in a mortar and pestle cooled with liquid nitrogen. Approximately 30 mg of tissue was transferred to a fresh tube containing 900 μL of denaturing solution and homogenized for 20 s using a polytron tissue grinder. Next 90 μL of 2 M sodium acetate and 900 μL of a solution containing phenol, chloroform, and indole-3-acetic acid was added to the lysate and incubated on ice for 15 min. Samples were centrifuged for 20 min at 14,000 × *g* in a refrigerated microcentrifuge. The supernatant was transferred to a fresh tube and RNA was precipitated with an equal volume of isopropanol and incubated at -20°C for 1 h. Samples were then centrifuged at 14,000 × *g* for 10 min to pellet RNA and washed with 1 ml of 70% ethanol. The RNA pellet was dried and resuspended in 30 μL of diethylene pyrocarbonate-treated water. The RNA purity and quantity was measured by absorbance at 260/280 nm in a spectrophotometer.

Determination of PGHS-2 mRNA Concentrations in Placentomes

Evaluation of PGHS-2 mRNA was accomplished using glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA as an internal control. Primers for G3PDH were designed from the published sequences and yielded the expected 106-base pair product. The PGHS-2 primers yielded the expected 484-base pair fragment and were designed as described previously (Tsai et al., 1996). Reverse transcription was carried out with 19 μL of 1× master mix (1× reverse transcription buffer, 0.2 mM deoxynucleotides, 100 pmol random primer, and 40 U of reverse transcriptase) and 1 μL of sample for 1.5 h at 37°C. For PCR, 4 μL of reverse transcription reaction was added to 1× PCR master mix (1× thermophilic buffer supplied with enzyme, 1.5 mM MgCl₂, 0.2 mM deoxynucleotides, 0.4 μM each of forward and reverse primer pairs, and 1 U of Taq DNA polymerase) for 28 cycles (95°C for 30 s, 57°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 5 min). Reaction products were separated on a 5% PAGE gel and stained with ethidium bromide. For each sample, PGHS-2 and G3PDH products were quantified using the Collage imaging system (Fotodyne Hartland, WI; Tsai et al., 1996).

Determination of PGHS-2 Protein Concentrations in Placentomes

The PGHS-2 protein was analyzed by immunoblot. Frozen uterine tissue (~60 mg) was homogenized in 400

μL of cold homogenization buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mg/mL leupeptin, 1 mg/mL aprotinin, 1% Triton X-100, and 0.25% deoxycholate) using a polytron tissue grinder. Lysate was centrifuged twice at $16,000 \times g$ for 10 min to obtain a clear lysate. An equal volume of $2\times$ loading buffer was added, and samples were steamed for 5 min. Samples (20 to 30 μL) and 6.25 to 100 ng of PGHS-2 standards (Cayman Chemical, Ann Arbor, MI) were loaded on a 10% SDS-PAGE gel and proteins were separated at 120 mA for 1.5 h. Proteins were transferred to polyvinylidene fluoride membrane using the mini-protean II gel transfer system (Bio-Rad, Hercules, CA). Following transfer, blots were incubated in blocking buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween 20, and 5% NDM) overnight at 4°C . Immunoblotting proceeded by incubating blots with antiPGHS-2 antibody (Cayman Chemical, Ann Arbor, MI) at 1:2000 dilution for 2 h at 25°C , followed by $3\times$ washing (10 mM Tris, pH 7.4, 150 mM NaCl, and 0.1% Tween 20). The antiPGHS-2 antibody does not cross-react with PGHS-1. Antirabbit horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) was added at 1:5000 dilution for 1 h at 25°C , followed by $3\times$ washing. Specific proteins were detected with enhanced chemiluminescent reagent (NEN Life Science Products, Boston, MA). Blots were exposed to X-ray film for 1 min and quantified using the Collage imaging system. The PGHS-2 standards were used to generate a standard curve ($Y = 2859X + 24969$, $R^2 = 0.99$) to determine the amount of PGHS-2 present in the uterine samples. To verify equal protein loading, membranes were stripped and reprobed with anti-actin primary antibody (Sigma, St. Louis, MO) at 1:20,000 dilution and antimouse-Horseradish Peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:5000 following the same procedure as for PGHS-2.

Statistical Analyses

Plasma concentrations of PGFM, glucose, NEFA, BHBA, and PUN were analyzed using the repeated measures analysis of the mixed procedure of SAS (Littell et al., 1996). Covariance structures tested included compound symmetry, autoregressive 1, and unstructured. The model included effects of treatment, cow within treatment, time, and treatment $\times$ time interaction. Differences among treatment means of the treatment $\times$ day interaction were tested using the slice option of the mixed procedure.

Milk and 4% FCM production, percentages of fat and protein in milk, and yields of protein and fat were analyzed using the mixed procedure of SAS. The model included the effects of treatment, parity, time, cow

within treatment $\times$ parity, and second-order interactions between treatment, parity, and time.

Data on milk fatty acid profiles and steady-state levels of PGHS-2 mRNA and protein in caruncular tissue were analyzed using the GLM procedure. The model for analysis of milk fatty acid profiles, PGHS-2 mRNA, and PGHS-2 protein included the effects of treatment. The steady-state levels of G3PDH mRNA and β -actin protein were included as a covariate in the model for analyses of PGHS-2 mRNA and PGHS-2 protein, respectively, to adjust for loading. Effects of treatment on the number of days in feed prepartum and the difference in days between the expected due date and actual calving date also were tested using the GLM procedure. The model included the effect of treatment only. Data on differences were considered significant at the 5% level.

RESULTS AND DISCUSSION

The length of prepartum feeding with OO (22.5 ± 2.8) or FO (21.8 ± 3.3) did not differ ($P > 0.05$). The range of days fed in the prepartum period was 10 to 39. Length of feeding did not affect the PGFM response of experimental cows ($P > 0.05$). Concentrations of EPA and DHA in caruncular tissue were increased in cows fed FO ($P < 0.01$), indicating that the dietary fatty acids were incorporated into uterine tissue (Table 5). The combined concentrations of caruncular EPA and DHA were correlated positively with the number of days supplemented with FO ($r^2 = 0.64$, $P < 0.01$), suggesting that introducing FO before d 21 before the expected due date could have further increased the concentrations of EPA and DHA in the uterus. Results from other studies support the concept that this period of feeding was sufficient to allow incorporation of dietary EPA and DHA into membrane phospholipids of the uterine caruncles. Intravenous infusion with n-3 fatty acids reduced plasma concentrations of prostaglandin E_2 after 4 d in sheep (Baguma-Nibasheka et al., 1999). In another study, feeding rats with a diet rich in n-3 fatty acids for 3 wk resulted in a 50% replacement of the uterine phospholipid pool of n-6 fatty acids with n-3 fatty acids. The reverse was true when the supply of n-3 fatty acids was limited. Changes in uterine fatty acid composition were detected 3 wk after the introduction of treatment diets (Howie et al., 1992).

Cows fed FO had reduced concentrations of plasma PGFM during the period of maximum secretion in the early postpartum period compared with cows fed OO. Differences were significant ($P < 0.05$) at 0, 0.5, 2, and 2.5 d postpartum (Figure 1). The pattern of postpartum concentrations of plasma PGFM was similar to what was previously reported. The inhibitory effects of FO on concentrations of plasma PGFM are in agreement

Table 5. Fatty acid profile of caruncles of Holstein cows fed diets containing olive or fish oil (% of total fatty acid in tissue).

Fatty acid ¹	Diet		SE	Treatment
	Olive oil	Fish oil		
	(g/100 g of fatty acids)			
C14	1.30	1.24	0.10	0.69
C14:1	0.27	0.18	0.05	0.25
C15:0	0.41	0.47	0.06	0.50
C16:0	19.84	21.79	0.74	0.09
C16:1	1.11	1.10	0.11	0.91
C18:0	24.97	25.53	0.47	0.04
tC18:1	1.09	2.44	0.21	<0.01
C18:1, n9	19.92	20.18	0.77	0.81
C18:2, n6	12.22	9.30	0.46	<0.01
CLA t9t11	0.35	0.27	0.07	0.40
CLA c9t11	0.36	0.35	0.05	0.88
C18:3, n3	0.51	0.44	0.08	0.52
C20:0	1.10	0.89	0.05	<0.01
C20:5, n3	0.23	1.66	0.02	<0.01
C22:0	5.09	4.09	0.20	<0.01
C22:6, n3	0.59	3.11	0.37	<0.01
C24:0	0.70	0.63	0.08	0.53

¹Expressed as number of carbons:double bonds.

with the study of Thatcher et al. (1997), which demonstrated that feeding diets containing 5.4% fish meal (DM basis) attenuated the plasma PGFM response to injections of estradiol-17 β and oxytocin given on d 15 of the estrous cycle of dairy cows. Mattos et al. (2002) described similar effects of increasing concentrations of dietary fish meal (0, 2.6, 5.2, and 7.8% of dietary DM) on secretion of uterine PGF_{2 α} induced by estradiol and oxytocin on d 15 of the estrous cycle. In the present study, the increased concentrations of inhibitory EPA and DHA in caruncular tissue of cows fed FO suggest that these fatty acids may be the active components reducing secretion of PGF_{2 α} . The correlation between caruncular concentrations of EPA and DHA and mean

concentration of plasma PGFM between parturition and d 5 tended to be significant ($r^2 = -0.14$, $P < 0.09$).

A sustained difference in plasma PGFM concentrations between cows fed OO and FO was not observed throughout the experimental period. Plasma PGFM concentrations of cows fed OO and FO converged at approximately d 5 and remained similar until the end of the experiment. The early reduction in plasma PGFM concentrations could be explained by an FO-induced reduction in PGF_{2 α} synthetic activity in caruncular tissue, which is a major source of PGF_{2 α} (Guilbault et al., 1984a, 1984b). With the normal early shedding of caruncular tissue (Gier and Marion, 1968) in both groups, the subsequent residual uterine production of PGF_{2 α} may not have been sufficient to detect possible differences attributed to the diets.

Diet did not affect the number of days between the expected due date and actual calving date. It was anticipated that reduced uterine PGF_{2 α} secretion could result in delayed parturition. Cows fed OO and FO calved 3.4 ± 1.8 and 3.3 ± 2.1 d before the due date, respectively ($P > 0.05$). However, correlation analysis indicated that late calving was associated with increasing concentrations of EPA and DHA in caruncular tissue ($r^2 = 0.52$, $P < 0.01$). Although it is tempting to speculate that increased EPA and DHA concentration in caruncular tissue cause delayed parturition, there is not a clear cause-effect relationship between the two variables. Late parturition due to causes unrelated to feeding could result in increased concentrations of EPA and DHA due to a longer feeding period and yield a significant correlation.

Figure 1. Pre- and postpartum plasma concentrations of prostaglandin F_{2 α} metabolite (PGFM) of cows fed fish oil ($\blacktriangle$, $n = 6$) or olive oil ($\square$, $n = 8$)(LSM + SE). The PGFM concentrations were lower in cows fed fish oil at 0, 0.5, 2, and 2.5 d after parturition (*, $P < 0.05$).

Table 6. Dry matter intake, milk production and milk composition of Holstein cows fed diets containing olive oil or fish oil.

Measurement	Olive oil	Fish oil	SE	<i>P</i>
Primiparous, n	1	2		
Multiparous, n	7	4		
DMI, prepartum kg/d	13.5	9.4	3.2 ¹	
DMI, postpartum kg/d	12.7	10.4	4.0 ¹	
Milk, kg/d	25.0	20.4	3.4	0.37
Milk fat, %	5.20	4.35	0.5	0.22
Milk protein, %	3.51	3.27	0.2	0.49
4% FCM, kg/d	26.1	20.1	3.6	0.27
Milk fat, kg/d	1.27	0.81	0.13	0.03
Milk protein, kg/d	0.86	0.63	0.11	0.18

¹Standard Deviation.

Feeding FO attenuated the rise in plasma PGFM concentrations that occurs in the postpartum period. The mechanism by which dietary EPA and DHA inhibit secretion of uterine prostanoids likely requires their absorption from the intestinal lumen and subsequent incorporation into cellular lipid pools of the endometrium. In this experiment, absorption of EPA and DHA from the diet was demonstrated by the increased concentrations of these fatty acids in milk fat. In addition, supplemental FO increased the concentrations of EPA and DHA in caruncular tissue. Similarly, feeding fish meal increased the proportion of EPA and DHA in endometrial lipids of beef cows (Burns et al., 2000), indicating that dietary changes can alter the fatty acid composition of the uterus.

Synthesis of PGF_{2α} involves cleavage of fatty acids from plasma membrane phospholipids. These cleaved fatty acids generally are unsaturated and include arachidonic acid and EPA, which can be converted to prostanoids of the 2 and 3 series, respectively. Increased availability of EPA in membrane phospholipids as a result of feeding FO could displace arachidonic acid, leading to increased synthesis of prostanoids of the 3 series at the expense of prostanoids of the 2 series, such as PGF_{2α}. Prostanoids of the 3 series are less bioactive (Needleman et al., 1979), and there appears to be no evidence for their role in ruminant luteolysis. Prostaglandin F_{3α} has only 25% affinity for the ovine luteal FP receptor compared with PGF_{2α} (Balapure et al., 1989).

Docosahexaenoic acid may act in a different manner and reduce expression of the PGHS enzymes (Achard et al., 1997), which could make these enzymes less available and further reduce prostanoid synthesis. Docosahexaenoic acid is not a substrate for the PGHS enzymes, but it is a strong competitive inhibitor of their activity (Corey et al., 1983).

However, concentrations of PGHS-2 mRNA in caruncles collected within 12 h of parturition were unaffected by diet. The steady-state levels of PGHS-2 were 37080 ± 6569 and 46597 ± 6094 densitometric units for OO

and FO cows, respectively (*P* = 0.19). This is in agreement with the findings described by Mattos et al. (2001), which indicated that polyunsaturated fatty acids such as EPA and DHA did not affect steady-state levels of PGHS-2 mRNA in bovine endometrial cells. In addition, concentrations of PGHS-2 protein in caruncular tissue were unaffected by diet (16.8 ± 5.3 vs. 16.4 ± 5.5 ng/mg tissue for cows fed OO or FO, respectively, *P* = 0.93).

In addition to competitive and inhibitory mechanisms, a lower efficiency of conversion of EPA to prostanoids of the 3 series may result in reduced total prostanoid synthesis. The PGHS-2 converts EPA into prostanoids of the 3 series in a less time-efficient manner than it converts arachidonic acid into prostanoids of the 2 series (Kulmacz et al., 1994).

Daily DMI was reduced by 30.3 and 18.1% in the prepartum and postpartum periods, respectively, when FO replaced OO in the diet (Table 6). In previous studies (AbuGhazaleh et al., 2002; Whitlock et al., 2002), FO fed at 2% of dietary DM was detrimental to DMI, whereas a similar intake of oil from extruded soybeans was not detrimental.

Average milk production between 0 and 21 d postpartum was unaffected (*P* > 0.05) by diet although least square means were different by 4.6 kg/d (Table 6). These results agree with those of AbuGhazaleh et al. (2002), Donovan et al. (2000), and Whitlock et al. (2002), who also reported no effect of feeding FO at 2% of dietary DM. Milk protein and fat concentrations also were unaffected (*P* > 0.05) by diet (Table 6). Others have reported no effect of feeding FO at 2% of dietary DM on milk protein concentration, but milk fat concentration has been reduced (Donovan et al., 2000; AbuGhazaleh et al., 2002; Whitlock et al., 2002). Production of 4% FCM and milk protein was not affected, whereas milk fat production was less by cows fed FO than by cows fed OO (*P* < 0.05). Parity × treatment interactions were not significant for each of these measurements. Changes in BW in the prepartum and postpartum periods were

Table 7. Milk fatty acid profile of Holstein cows fed diets containing olive oil or fish oil (% of total fatty acid in milk).

Fatty acid ¹	Diet		Normal Milk ²	SE	Treatment <i>P</i>
	Olive oil	Fish oil			
	— (g/100 g of fatty acids) —				
C4:0	2.01	1.85	3.29	0.13	0.40
C6:0	1.45	1.25	1.80	0.21	0.52
C8:0	0.69	0.61	1.20	0.13	0.66
C10:0	1.37	1.22	2.40	0.26	0.69
C12:0	1.45	1.30	2.69	0.25	0.67
C14:0	6.25	5.51	10.18	0.72	0.48
C14:1	0.59	0.53	—	0.04	0.31
C16:0	25.95	25.07	26.35	0.70	0.40
C16:1	1.61	1.79	2.40	0.12	0.30
C18:0	14.88	11.38	11.98	0.95	0.03
C18:1, n9	27.41	28.99	25.15	4.05	0.79
C18:2, n6	1.42	0.16	2.40	0.57	0.15
CLA t9t11	0.011	0.100	—	0.01	<0.01
CLA c9t11	0.46	0.68	—	0.09	0.12
CLA t10c12	0.00	0.021	—	0.009	0.12
Total CLA ³	0.469	0.799	—	0.10	0.04
C18:3, n3	0.084	0.162	1.50	0.005	<0.01
C20:1, n9	0.343	0.346	—	0.012	0.87
C20:5, n3	0.003	0.422	—	0.06	<0.01
C22:6, n3	0.013	0.467	—	0.08	<0.01
Other	6.68	10.75	—	0.75	<0.01
Total n-3 ⁴	0.100	1.050	—	0.15	0.01
Total n-6 ⁵	1.422	0.163	—	0.57	0.15
Total n-9 ⁶	33.50	34.84	—	1.66	0.59
Saturated	55.45	49.92	—	1.57	0.03
Unsaturated	37.69	39.18	—	1.84	0.59

¹Expressed as number of carbons:double bonds.²National Dairy Council, 1993.³Total CLA: cis-9, trans-11 C18:2 + trans-9, trans-11 C18:2 + cis-10, trans-12 C18:2.⁴n-3 fatty acids: C18:3 + C20:5 + C22:6.⁵n-6 fatty acids: C18:2.⁶n-9 fatty acids: trans-C18:1 + cis-C18:1 + C20:1 + C22:1.

not affected by diet ($P > 0.05$), suggesting that energy status was similar between the two groups.

Feeding FO increased the proportion of individual and total n-3 fatty acids (linolenic, EPA, and DHA) in milk when compared to cows fed OO ($P < 0.01$, Table 7). These results agree with previous reports (Cant et al., 1997; Donovan et al., 2000). The rates of transfer from diet to milk for EPA and DHA in this study were 5.4 and 7.6%, respectively. Transfer efficiency has been reported to be greater for DHA (16.2%) than for EPA (9.3%) (Cant et al., 1997). The increase in concentrations of n-3 fatty acids in milk from cows fed FO occurred in concert with decreased concentrations of saturated fatty acids ($P = 0.03$). This reduction is largely due to a reduction in concentrations of C18:0 in milk fat from cows fed FO. Similar effects of FO on total saturated fatty acids and on C18:0 have been reported (Franklin et al., 1999; Donovan et al., 2000). The proportion of total long-chain fatty acids (> 18 carbons) was not affected by diet ($P > 0.05$).

Feeding FO increased the concentrations of total conjugated linoleic acids (**CLA**) ($P < 0.01$). Increased total CLA concentrations as a result of feeding n-3 fatty acids also have been reported (Cant et al., 1997; Franklin et al., 1999; Donovan et al., 2000). An increased conversion of dietary linoleic and linolenic acids to CLA caused by FO may explain the higher concentrations of CLA observed (Whitlock et al., 2002).

Plasma concentrations of NEFA were not affected by diet ($P > 0.05$, Figure 2). Concentrations of NEFA increased during the week before parturition, suggesting increased fat mobilization and a decreasing DMI. After parturition, NEFA concentrations decreased slowly from 900 to 600 $\mu\text{Eq/L}$ by d 21 postpartum. This profile of periparturient NEFA concentrations is similar to that reported by Garcia-Bojalil et al. (1998). Plasma concentrations of BHBA increased with increasing days postpartum ($P < 0.02$, Figure 3). Concentrations of BHBA tended to be greater in cows fed FO ($P = 0.08$, diet $\times$ day interaction). Pairwise compari-

Figure 2. Pre- and postpartum plasma concentrations of NEFA of cows fed fish oil ($\blacktriangle$, $n = 6$) or olive oil ($\square$, $n = 8$) (LSM $\pm$ SE). Concentrations were not affected by diet ($P > 0.05$).

sons of treatment means indicated differences on d 10 ($P = 0.05$) and 13 ($P = 0.10$). The timing of peak and range of BHBA concentrations of this study were similar to those reported by Vazquez-Añón et al. (1997).

Feeding FO reduced plasma concentrations of glucose ($P = 0.03$, Figure 4). Concentrations peaked at 1 d before parturition and declined progressively until d 13 to 15 postpartum. Duffield et al. (1998) reported similar profiles of postpartum glucose concentrations. The effect of FO on glucose concentrations could be due to the reduced DMI associated with that diet. Another possible explanation involves the inhibition of gluconeogenic enzymes by components of FO. Eicosapentaenoic acid inhibited the expression of phosphoenolpyruvate carboxykinase in rat hepatoma cells (Murata et al., 2001). This enzyme catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, which is a required step in the conversion of propionic acid to glucose. It is possible that EPA supplied in the FO diet may have inhibited

Figure 3. Pre- and postpartum plasma concentrations of BHBA of cows fed fish oil ($\blacktriangle$, $n = 6$) or olive oil ($\square$, $n = 8$) (LSM $\pm$ SE). Concentrations of BHBA were greater in cows fed fish oil at 10 and 13 d after parturition (*, $P < 0.10$; **, $P = 0.05$).

Figure 4. Profiles of plasma glucose concentrations of periparturient cows fed diets containing fish oil ($\blacktriangle$, $n = 6$) or olive oil ($\square$, $n = 8$) (LSM $\pm$ SE). (**, $P \leq 0.05$; *, $P \leq 0.1$). The main effect of the diet is significant ($P = 0.03$).

gluconeogenesis and therefore reduced plasma glucose concentrations.

Concentrations of PUN were greater in the prepartum period in cows fed FO, as determined by differences detected on d -5 and d -3 ($P < 0.1$, Figure 5). Postpartum PUN concentrations were similar to those reported by Roseler et al. (1993) for rations supplying 100% of the requirements of degradable intake protein and undegraded intake protein for lactating dairy cows (National Research Council, 2001).

The incidence of postpartum health disorders was unaffected by diet (Table 1). The incidence of DA (31%, 8/26) was considerably greater than what is considered normal for high producing herds. A survey of 61 high-

Figure 5. Profiles of plasma urea nitrogen of periparturient cows fed diets containing fish oil ($\blacktriangle$, $n = 6$) or olive oil ($\square$, $n = 8$) (LSM $\pm$ SE). Significant differences were detected at Day -5 and Day -3. (**, $P \leq 0.05$; *, $P \leq 0.1$).

producing dairy herds in the United States indicated a mean incidence of DA of 3.3%, with a range of incidence from 0 to 14% (Jordan and Fourdraine, 1993). The high incidence of DA likely was associated with feeding supplemental oils (OO and FO), which may have impaired ruminal fermentation and reduced DMI. Occurrence of DA was associated with increased concentrations of plasma PGFM in the postpartum period (1023 vs. 582 pg/mL, SE = 150, $P = 0.05$ for main effect of DA). Twenty-three cows were included in this analysis. Concentrations of plasma PGFM were increased before cows showed any signs of DA. Prostanoids have been shown to regulate gastrointestinal motility and secretion in other species (Eberhart and Dubois, 1995). Thus, increased PGFM could have had a direct effect on the incidence of DA, or it could be an indicator of an abnormal postpartum period that is a risk factor for the occurrence of DA. Change in BW did not differ between normal cows and cows that had DA ($P > 0.05$). High BCS has been associated with increased incidence of left DA. Incidence rates of DA increased with increasing BCS at parturition (Shaver, 1997). However, in the current study, BCS at calving was not different between cows that had DA (3.3) and cows that did not have DA (3.38; $P > 0.05$).

IMPLICATIONS

Uterine synthesis of $\text{PGF}_{2\alpha}$ may be reduced in dairy cows consuming elevated amounts of the n-3 fatty acids, EPA and DHA. If this same response initiated by select dietary fatty acids can be achieved around the time of pregnancy recognition, regression of the corpus luteum might be prevented and embryo survival enhanced in lactating dairy cows.

ACKNOWLEDGMENTS

This research was supported in part by the NRI Competitive Grant Program/USDA, grant 98-35203-6367. This is Florida Agriculture Experimental Station Journal Series No. R-09849.

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