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Effect of Feeding Whole, Unprocessed Sunflower Seeds and Flaxseed on Milk Production, Milk Composition, and Prostaglandin Secretion in Dairy Cows[1]

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ABSTRACT

Four multiparous Holstein cows were used in a 4 × 4 Latin-square design experiment to study the effects of different fat sources on milk production and composition, N utilization, follicular development, and prostaglandin secretion. Cows were fed 4 total mixed rations (TMR) based either on calcium salts of palm oil (Megalac), whole flaxseed, whole sunflower seed, or no supplementary fat (control). Feed intake and digestibilities were generally similar among treatments, except that ether extract digestibility was the lowest for cows fed the control diet. Milk yields were greater for cows fed whole flaxseed and Megalac (32.1 and 31.5 kg/d, respectively) than for those fed sunflower seed and control (25.9 and 24.8 kg/d, respectively). Milk protein concentration was significantly lower for cows fed Megalac (3.68%) compared with those fed flaxseed (3.87%) or control (3.92%). Concentrations of n-3 fatty acids and the n-6 to n-3 fatty acids ratio in milk were the highest and lowest, respectively, for cows fed whole flaxseed. There was an interaction between treatment and time for levels of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in plasma; they were greater 30 and 45 min after the oxytocin challenge for cows that were fed sunflower seed compared with those fed either Megalac, flaxseed, or control. Moreover, when concentrations of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in plasma were expressed as the area under the overall response curve from 0 to 120 min after the oxytocin injection, it tended to be greater for cows that were fed the sunflower diet compared with those fed either Megalac or flaxseed. In general, follicle dynamics were similar among treatments. These results suggest that feeding diets with high proportions of n-6 fatty acids (61% of total fatty acids for the sunflower seed diet) tended to increase the secretion of series 2 prostaglandins in blood.
(**Key words:** flaxseed, fatty acids, ovarian function, prostaglandins)

Abbreviation key: CL = corpus luteum, **CON** = concentrate without fat, **FA** = fatty acids, **FLA** = concentrate based on whole flaxseed, **MEG** = concentrate based on calcium salts of palm oil (Megalac), **PG** = prostaglandins, **PGFM** = 13,14-dihydro-15-keto-$PGF_{2\alpha}$, **SUN** = concentrate based on whole sunflower seed.

INTRODUCTION

Dietary polyunsaturated fatty acids (**FA**) are perceived to be healthier than saturated FA. As a result, there has been a great deal of interest in manipulating the FA profile of milk fat to respond to consumers' demands. Feeding oilseeds to lactating dairy cows is one method to change the proportion of unsaturated FA in milk fat, with increases as high as 40% (Casper et al., 1990; Stegeman et al., 1992; Kim et al., 1993), although extensive biohydrogenation occurs normally in the rumen (Palmquist and Jenkins, 1980). Sunflower seed and flaxseed would be a good choice from a consumer point of view, as both are rich in polyunsaturated FA, with sunflowers being a source of linoleic acid (66% of total fatty acids), which is an n-6 FA, whereas flaxseed is rich in linolenic acid (56% of the total fatty acids), which is an n-3 FA. In fact, both whole flaxseed and whole sunflower seed are acceptable fat sources for mid-lactating cows, as they result in similar milk yields when fed at a rate of 13 to 15% of the DM (Petit, 2003), but there is no information on the effects of feeding whole sunflower seed on milk yield of early lactating cows. Moreover, sunflower seed oil supplementation (6% of DM) dramatically reduces protozoa numbers in rumen fluid within 5 d (Ivan et al., 2001), suggesting that oilseeds are potential feed ingredients to control protozoa populations in ruminants and to increase the efficiency of dietary protein utilization.

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Linoleic acid (C18:2n-6) is converted to arachidonic acid (C20:4n-6), which is the precursor of the dienoic (2-series) prostaglandins (**PG**), such as $PGF_{2\alpha}$. The same elongase and desaturase enzymes also convert the main dietary n-3 FA (α-linolenic acid; C18:3n-3) to eicosapentaenoic acid (C20:5n-3), the precursor of the trienoic (3-series) PG, such as $PGF_{3\alpha}$ (Abayasekara and Wathes, 1999). Competition between n-3 and n-6 precursors for desaturation and elongation, as well as at the site of PG synthetase, means that increasing the supply of n-3 FA will decrease production of dienoic PG (Barnouin and Chassagne, 1991). In many cases, the trienoic PG have lower biological activity than the corresponding dienoic PG (Fly and Johnston, 1990), and this may directly affect aspects of fertility. For example, treatments that reduce ovarian and endometrial synthesis of $PGF_{2\alpha}$, at the expense of $PGF_{3\alpha}$, may contribute to a reduction in embryonic mortality (Mattos et al., 2000). Feeding a diet rich in linoleic acid could then contribute in increasing secretion of $PGF_{2\alpha}$, compared with feeding a diet rich in linolenic acid, which could have negative effects on reproduction of cattle. There is, however, no information on the effects of feeding whole sunflower seed, which are rich in n-6 FA, on prostaglandin secretion. Therefore, the objectives of the present experiment were to study the effects of feeding different fat sources on prostaglandin secretion and dietary N utilization.

MATERIALS AND METHODS

Animals and Diets

Four multiparous lactating Holstein cows, averaging 38 DIM (SEM = 7) and 654 kg of BW (SEM = 55 kg), were used in a 4 × 4 Latin-square design over four 35-d periods. The cows were kept in individual stalls and had free access to water. Cows were milked twice a day at 0730 and 1600 h. All cows were fed ad libitum (10% refusals) twice a day (0800 and 1600 h) 4 TMR based on a mixture of grass and corn silages (Table 1) and containing either 1) calcium salts of palm oil, Megalac (**MEG**), 2) whole unprocessed flaxseed (**FLA**), 3) whole, unprocessed sunflower seed (**SUN**), or 4) no fat in the concentrate (**CON**, Table 2). All fat-supplemented TMR (Table 3) were designed to yield similar fat intakes as a percentage of total DMI, and the 4 diets were calculated to be isonitrogenous and isoenergetic. The 4 diets were formulated to meet requirements for cows that were 640 kg of BW, producing 35 kg/d of milk with 3.8% fat (NRC, 1989). Animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Table 1. Chemical composition of grass and corn silages.[1]

Item	Grass silage	Corn silage
DM, %	33.4	41.4
ADF, % of DM	36.4	19.7
NDF, % of DM	43.8	36.1
CP, % of DM	17.3	8.0
Ether extract, % of DM	3.8	3.8
Fatty acids, % of total FA[2]		
C12:0	0	0.5
C14:0	1.5	0.6
C16:0	25.2	15.5
C16:1	0	0.5
C18:0	3.6	2.6
C18:1n9*cis*	3.0	26.1
C18:1*cis*11	0.5	0.8
C18:2n6*cis*	22.5	49.3
C18:3n6	0.4	0
C18:3n3	43.3	4.1

[1]Mean of 5-wk composite samples that were prepared from 3 weekly samples collected during each of 4 experimental periods.

[2]Mean of weekly composite samples that were prepared from daily samples collected on the digestibility week each of 4 experimental periods.

Experimental Procedures

Adaptation to diets was from d 1 to 14, milk sampling and total fecal and urine collection from d 14 to 20, and transrectal ultrasonography from d 20 to 35. Procedures for estrus synchronization, ultrasound scanning, and oxytocin challenge were the same as those previously described by Oldick et al. (1997) using a 4 × 4 Latin-square design with four 35-d periods. Feed intake and milk yield were measured daily. Yield of 4% FCM was calculated according to the equation of Tyrrell and Reid (1965). Feed ingredients and TMR were sampled 3 times each week (daily during the measurement of

Table 2. Composition of the TMR based either on Megalac (MEG), whole flaxseed (FLA), whole sunflower seed (SUN), or no fat supplement (CON).

Ingredient, % of DM	MEG	FLA	SUN	CON
Grass silage	33.2	33.2	33.5	31.5
Corn silage	23.2	20.1	21.1	20.2
Protein supplement[1]	10.7	4.4	6.3	7.8
Ground barley	26.1	30.8	27.7	38.7
Whole flaxseed	0	9.7	0	0
Whole sunflower seed	0	0	9.6	0
Megalac[2]	4.6	0	0	0
Premix[3]	1.5	1.5	1.5	1.5
Calcium carbonate	0	0.3	0.3	0.3
Ammonium phosphate	0.7	0	0	0

[1]Contained (as fed basis): 10% soybean meal, 25.3% corn gluten meal, 20.5% distillers' wheat, and 44.2% Soyplus.

[2]Church and Dwight, Princeton, NJ.

[3]Premix contained (DM basis): 400,000 IU of vitamin A/kg, 70,671 IU of vitamin D_3/kg, 245 IU of vitamin E/kg, 6.8% Ca, 6.7% P, 2000 mg/kg of Mn, 1865 mg/kg of Zn, 463 mg/kg of Cu, 2900 mg/kg of Fe, 58 mg/kg of I, 24 mg/kg of Co, and 18 mg/kg of Se.

Table 3. Chemical composition of the 4 total mixed diets based either on Megalac (MEG), untreated whole flaxseed (FLA), untreated whole sunflower seed (SUN), or no fat supplement (CON).[1]

Item	MEG	FLA	SUN	CON	SE
DM, %	45	45.6	46.7	47.4	0.5
OM, % of DM	92.4	92.7	92.7	93.1	0.1
ADF, % of DM	22.3	22.7	22.4	22.1	0.1
NDF, % of DM	33.7	35.2	34.7	34.8	0.3
CP, % of DM	16.7[a]	15.9[b]	16.1[b]	15.9[b]	0.1
Ether extract, % of DM	6.2[b]	6.6[b]	6.7[b]	3.6[a]	0.7
Fatty acids, % of total FA[2]					
C14:0	1.5	ND	ND	0.9	0.3
C14:1	ND	ND	ND	ND	ND
C16:0	34.7	11.7	11	21.4	5.5
C16:1	0.2	ND	ND	0.6	0.2
C18:0	3.8	3.3	3.3	2.7	0.2
C18:1n9*cis*	25.9	18.3	18.5	15.3	2.2
C18:1*cis*11	0.7	ND	ND	0.7	0.02
C18:2n6*cis*	25.2	28.3	60.6	43.6	8.1
C18:3n3	7.2	38.4	6.6	14.8	7.8

[a,b]Means within a row with no common superscript differ ($P < 0.05$).

[1]Mean of 5-wk composite samples that were prepared from one weekly sample collected during each of 4 experimental periods.

[2]Mean of weekly composite samples that was prepared from 7 daily samples collected on the digestibility week during each of 4 experimental periods.

ND = Not detected.

digestibility) and pooled weekly. All samples were frozen at –20°C for subsequent chemical analyses. Milk samples were obtained from each cow for 14 consecutive milkings, pooled on a yield basis, and analyzed for N, lactose, fat, and fatty acids. Milk was collected 3 times a week, and samples were kept frozen to determine later progesterone concentration and confirm the beginning of a new estrus cycle. Feces were collected from a rubber mat placed behind the animals and stored in plastic containers. Daily feces were weighed and mixed thoroughly. A 10% subsample was taken and stored at –15°C for subsequent drying at 55°C. Total daily urine was collected in stainless steel containers via Gooch tube (BF Goodrich Co., Kitchener, ON, Canada) attached to the cow with a nylon netting covered with neoprene (Spall Bowan Ltd., Guelph, ON, Canada) affixed to the vulva. A 1% subsample was taken daily and kept frozen until analysis. Urine was acidified daily with 100 mL of 10 *N* H_2SO4.

Estrus cycles were synchronized in each period. On d 14, a GnRH agonist (Cystorelin; Rhône Mérieux, Victoriaville, QC, Canada) was administered (8 μg) i.m. to each cow, followed 7 d later by an i.m. injection of $PGF_{2\alpha}$ (25 mg; Lutalyse; Pharmacia and Upjohn Ltd., Orangeville, ON, Canada). This program synchronizes both follicular development and regression of the corpus luteum (**CL**) (Thatcher et al., 1989; Macmilland and Thatcher, 1991). Regression of the CL was confirmed using ultrasonography. Ovaries of each cow were examined by ultrasonography using an LS-300A ultrasound scanner equipped with a linear array 5 MHz probe (Tokyo Keiki Co. Ltd., Tokyo, Japan) in midmorning on each day from d 20 to 35 of each experimental period. Size and number of ovarian follicles >3 mm were recorded. Follicles were grouped into 3 diameter classes for analyses: class 1 (3.0 to 4.9 mm), class 2 (5.0 to 9.9 mm), and class 3 (≥10 mm). Size of CL also was recorded. On d 34, a catheter was inserted into the jugular vein. On the following day, cows were administered an oxytocin challenge to stimulate uterine production of $PGF_{2\alpha}$ as previously described by Oldick et al. (1997). At 0600 and 1000 h, respectively, estradiol (3 mg) and oxytocin (20 IU; Pfizer Canada, Inc.) were administered intravenously via the jugular catheter. Blood was collected in tubes containing lithium heparin (Becton Dickinson and Cie, Rutherford, NJ) at 15-min intervals for 1 h prior to the oxytocin injection and at 15-min intervals for 5 h after the oxytocin injection. The plasma was separated and frozen at –20°C for subsequent analysis of PGFM, the principal metabolite of $PGF_{2\alpha}$, and progesterone. Blood was also withdrawn at 0900 h into Vacutainer tubes (Becton Dickinson and Cie) containing either lithium heparin for determination of glucose, EDTA for fatty acids and NEFA analysis, or no preservative for cholesterol analysis.

Chemical Analysis

Dry matter of feed ingredients and TMR were determined by drying at 100°C for 48 h. Feed and fecal samples were ground through a 1-mm screen in a Wiley mill (model 3; Arthur M. Thomas, Philadelphia, PA). Total N determination was done by the Kjeldahl method (AOAC, 1990). Both ADF and NDF were measured according to the nonsequential procedures of Van Soest et al. (1991) without the use of amylase and sodium sulphate. Gross energy was measured by combustion in a Parr adiabatic bomb calorimeter (model 1241, Parr Instrument Co., Moline, IL). Ether extraction in feed ingredients, diets, and feces was conducted with a Soxlec system HT6 apparatus (Tecator, Fisher Scientific, Montreal, QC, Canada) according to the method No. 7.060 (AOAC, 1990). Concentration of lipid in Megalac was determined by combustion of organic matter at 550°C overnight in a muffle furnace (AOAC, 1990). Plasma NEFA was determined using a colorimetric kit (kit no. 9075401; Wako Pure Chemical Industries, Osaka, Japan), following modifications by Johnson and Peters (1993). Total and HDL cholesterol in plasma (kit no. 401 and 352, respectively; Sigma Chemical Co., St. Louis, MO) were determined as described by Seidel et al. (1983). Glucose in plasma was determined using a colorimetric kit (kit no. 14448668; Roche/Hitachi, Indianapolis, IN) according to the method of Trinder (1969).

Concentration of LDL cholesterol was calculated as the difference between total and HDL cholesterol concentrations. Plasma samples were assayed in duplicate for PGFM by radioimmunoassay according to Guilbault et al. (1984). The PGFM antibody was a gift from William Thatcher (University of Florida) and PGFM standards and competitor ([5,6,8,9,11,12,14(n)-^{3}H]13,14-dihydro-15-keto-PGF2α; 186 Ci/mmol) were purchased from Amersham (Piscataway, NJ). Plasma without detectable amounts of PGFM for use in standards was harvested from a cow treated twice (12 h apart) with an intravenous injection of a prostaglandin synthesis inhibitor: 10 mL of 50 mg/mL (500 mg) flunixin meglumine (Banamine; Schering-Plough Animal Health Corp., Kenilworth, NJ). Blood was collected into evacuated heparinized flasks 4 h after the second injection, placed on ice, and centrifuged at 5000 × *g* for 20 min at 4°C. Plasma was removed and stored at –20°C. Parallelism of a pool from cows was demonstrated for all assays and average recovery, which was calculated by the addition of different doses of unlabeled hormones to a pooled sample, varied between 99 and 113% for all assays. The sensitivity of the assay was 31.25 pg/mL for 200 uL of plasma, and the intra- and interassay coefficients of variation were 7.5 and 12.1%, respectively.

Milk samples collected during the digestion trial were analyzed for crude protein (N × 6.38) and fat using the Kjeldahl and Roese-Gottlieb methods (AOAC, 1990), respectively. Concentration of lactose in milk was determined by a colorimetric method (kit 176 303; Boehringer Mannheim, Ville St. Laurent, QC, Canada), according to AOAC (1984, 1990). Milk concentrations of progesterone were measured by radioimmunoassay (Bulman and Lamming, 1978) in duplicate, and intra- and interassay coefficients of variation were 13 and 13%, respectively. Fatty acids in plasma and milk were extracted, methylated, and prepared according to the methods previously used by Petit (2002). Fatty acid methyl ester profiles were measured by GLC on a Hewlett-Packard 6890 chromatograph (Hewlett-Packard Ltd., Montreal, QC, Canada) with a G1315A autosampler equipped with a flame-ionization detector and a split-splitless injector, as described by Delbecchi et al. (2001).

Statistical Analysis

All results were subjected to ANOVA for a 4 × 4 complete Latin square allowing measurement of residual effects (Cochran and Cox, 1957) using the general linear model procedures of SAS (1999). Main sources of variation in the model were cow, period, and treatment. Probability values greater than 0.05 were considered nonsignificant. A tendency toward significance was declared at $0.05 < P < 0.15$ as previously done by Oldick et al. (1997) for a similar Latin square design. Data on follicular development and size of CL and data on PGFM were analyzed as repeated measurements across time using PROC MIXED of SAS (1999). Data on PGFM were also analyzed as peak value and area under the curve mean and as concentrations over the 4-h sampling period. Data on progesterone concentrations were analyzed from d 21 to 35 as repeated measurements across time using PROC MIXED of SAS (1999) and as peak value, area under the curve, and mean concentrations from d 21 to 35 when there was no interaction between sampling hour and treatment ($P > 0.10$). Multiple comparisons were done using Tukey's test after a significant *F*-test.

RESULTS AND DISCUSSION

Feed Composition

The chemical composition of the TMR (Table 3) was generally similar among diets, with the exception of CP, which was greater for MEG than for FLA, SUN, and CON. As expected, ether extract concentration was the lowest for the control diet. The FA composition of concentrates differed among TMR (Table 3), reflecting our formulation objectives. Concentrations of C16:0 and C18:1 were the highest for MEG, and C18:3 concentrations were the highest for FLA. Concentration of C18:2 was high for SUN, as sunflower seed is rich in linoleic acid. Similarly, CON contained a high percentage of C18:2, which would reflect the main fatty acid in corn silage.

Feed Intake

Total DMI was similar among treatments (Table 4). This would agree with the results of Allen (2002), who calculated that the addition of FA from oilseeds results in a quadratic effect on DMI with a minimum effect at approximately 3.0% added FA, which is the difference between ether extract concentration of the control diet (3.6%) and those containing oilseeds (6.6 and 6.7% for FLA and SUN, respectively). Similar DMI has been reported for early-lactating cows fed calcium salts of palm oil and for those fed diets of 10% whole flaxseed (Petit, 2002). Moreover, feeding about 10% of sunflower seed in the diet has no effect on DMI of cows (Rafalowski and Park, 1982), although greater DMI for midlactating cows fed whole flaxseed compared with those fed whole sunflower seeds have been reported previously (Petit, 2003).

Table 4. Feed intake, digestibility, milk production, and milk composition of Holstein cows fed a total mixed diet based either on Megalac (MEG), untreated whole flaxseed (FLA), untreated whole sunflower seed (SUN), or no fat supplement (CON).[1]

Item	MEG	FLA	SUN	CON	SE
Total DMI, kg/d	21.2	21.1	20.7	23.8	1.2
Digestibility, %					
DM	65.6	64.7	63.5	64.8	1.7
CP	70	65.6	68.4	65.7	1.8
ADF	48.9	50.5	46.2	47.7	2.9
NDF	49.4	50.9	47.4	47.9	2.6
Energy	64.7	62	61.6	62.8	1.9
Ether extract	79.2[a]	74.0[ab]	80.7[a]	67.9[b]	2.4
N intake, g/d	583	575	538	609	29
Output, g/d					
N in feces	176	198	170	210	15
N in urine	251[a]	213[ab]	196[b]	226[ab]	11
N in milk	181[a]	194[a]	150[b]	151[b]	10
Total	608[a]	605[a]	516[b]	587[ab]	26
N retention					
% of intake	–4.2	–6	3.6	3.9	4.1
g/d	–25	–30	22	22	27
Milk yield, kg/d	31.5[a]	32.1[a]	25.9[b]	24.8[b]	1
4% FCM, kg/d	28.4[a]	30.0[a]	23.5[b]	22.7[b]	1.3
Milk composition, %					
Fat	3.35	3.63	3.3	3.49	0.23
Protein	3.68[c]	3.87[ab]	3.74[bc]	3.92[a]	0.06
Milk components, kg/d					
Fat	1.05[ab]	1.14[a]	0.88[ab]	0.85[b]	0.07
Protein	1.21[a]	1.22[a]	0.97[b]	0.97[b]	0.05

[a,b,c]Means within a row with no common superscript differ ($P < 0.05$).

[1]Least square means with pooled SE.

Diet Digestibility and N Balance

The apparent digestibilities of DM, CP, ADF, NDF, and energy were similar among treatments (Table 4). Similar DM and CP digestibilities have been reported for early-lactating cows fed calcium salts of palm oil and whole flaxseed, although ADF and NDF digestibilities were lower for cows fed flaxseed (Petit, 2002). Moreover, Petit (2003) reported similar DM, ADF, and NDF digestibilities for midlactating cows fed whole sunflower seed and for those fed whole flaxseed, although CP digestibility was greater for cows fed the former diet. The lack of significant effects on digestibilities of DM, CP, ADF, and NDF agree with results from studies in which ruminally inert fat supplements were fed to cows (Grummer, 1988; Schauff and Clark, 1989) and indicates that fat supplements based on calcium salts of palm oil, whole flaxseed, and whole sunflower seed did not disrupt normal digestive processes. Significant lower digestibility of ether extract was observed with the CON diet compared with the MEG and SUN diets. The fat in the CON diet contained more nonnutritive lipid, such as waxes, compared with that in the MEG and SUN diets, which would have contributed to lower digestibility of ether extract. Supplementation with calcium salts of long-chain FA has been reported to augment fat digestibility in several experiments (Grummer, 1988; Schneider et al., 1988) due to dilution of highly digestible fats in the diets by the contributions of poorly digested lipids other than FA (Garcia-Bojalil et al., 1998). It seems unlikely that the lowest ether extract digestibility on the CON diet relates to impaired rumen function, since there were no effects on DMI. Petit (2002) reported that cows fed whole flaxseed had lower ether extract digestibility, compared with those fed calcium salts of palm oil. The same direction was observed in the current study, although the difference was not significant.

Intake and output of N in feces were similar among treatments (Table 4). Output of N in urine was significantly greater for cows fed MEG (251 g/d), compared with those fed SUN (196 g/d). Dietary supplementation of sunflower seed oil (6% of DM) reduced protozoa numbers in rumen fluid of sheep (Ivan et al., 2001), suggesting that sunflower oil is a potential feed ingredient to control protozoa populations in ruminants and to increase the efficiency of dietary protein utilization. Oil released from sunflower seed during digestion may have decreased dietary protein degradation by rumen microbes and caused the reduction in urinary N loss, as observed in the present experiment for cows fed SUN as compared with those fed rumen inert calcium salts of palm oil. Similar output of N in urine for cows fed

whole flaxseed and whole sunflower seed have previously been reported by Petit (2003).

Output of N in milk was greater on the FLA (194 g/d) diet than on the SUN (150 g/d) and CON (151 g/d) diets, partly as a result of greater milk yield for cows fed FLA. Total output of N was significantly lower for cows fed SUN compared with those fed the other diets, probably due to a combination of both a decrease in dietary N degradation by rumen microbes and in N output in milk following lower milk yield. Retention of N, expressed as a percentage of N intake or in grams per day, was similar among treatments.

Milk Production and Composition

Milk yield and 4% FCM yield (Table 4) were higher for cows fed the MEG and FLA diets, compared with those fed the SUN and CON diets. Intake of digestible energy (data not shown), calculated from digestibility data, averaged 59.4, 56.4, 55.0, and 61.9 Mcal/d, respectively, for cows fed MEG, FLA, SUN, and CON. Considering that cows fed MEG and FLA produced about 7 kg/d more milk than cows fed SUN and CON, they would require an extra 9 Mcal of DE per day to meet these requirements (NRC, 1989). Cows fed MEG and FLA lost, respectively, 25 and 30 g of N per day, which would represent slightly more than 1 kg of loss in BW when considering that 145 g of CP equals 1 kg of BW loss (NRC, 1989). Therefore, cows fed MEG and FLA would use their body reserve of protein to increase milk yield. This would agree with the results of Petit (2002), who suggested that greater fat mobilization could have contributed to increased milk yield of cows fed calcium salts of palm oil. It has been reported previously that midlactation cows with lower energy requirements and fed either whole flaxseed or sunflower seed produced similar milk yields (Petit, 2003), although early-lactating cows fed flaxseed produced 2.2 kg more milk per day than those fed calcium salts of palm oil (Petit, 2002).

Concentrations of fat and lactose in milk were similar among treatments, as previously reported for midlactating cows fed whole flaxseed and whole sunflower seed (Petit, 2003) and for midlactating cows fed Ca salts of palm oil and formaldehyde-treated flaxseed (Petit et al., 2002). Milk protein concentration was significantly reduced by feeding calcium salts of palm oil (treatment MEG) compared with when no fat was fed (3.68 and 3.92%, respectively) as previously reported by Garcia-Bojalil et al. (1998). Protein concentration in milk was similar for cows fed the FLA and SUN diets, and it was similar for those fed FLA and CON. Feeding whole flaxseed, as compared with calcium salts of palm oil, has previously been reported to increase milk protein percentage (Petit, 2002). On average, milk protein percentages were high for all treatments, perhaps because cows were carrying the B genetic variant for κ-casein. In general, yields of fat, protein, and lactose were significantly higher for cows fed the MEG and FLA diets, compared with those fed the SUN and CON diets, which would result in the greater milk yields observed using those treatments.

Table 5. Milk fatty acid composition of Holstein cows fed a total mixed diet based either on Megalac (MEG), untreated whole flaxseed (FLA), untreated whole sunflower seed (SUN) or no fat supplement (CON).[1]

Item	MEG	FLA	SUN	CON	SE
	% of Total fatty acids				
C10:0	3.4[c]	4.6[b]	3.3[c]	5.0[a]	0.2
C12:0	3.5[c]	4.7[b]	3.4[c]	5.8[a]	0.3
C14:0	11.2[c]	14.0[b]	11.1[c]	15.4[a]	0.5
C14:1	0.9[c]	1.1[b]	1.0[b]	1.3[a]	0.1
C16:0	38.0[a]	31.2[b]	24.3[c]	38.1[a]	1.6
C16:1	1.6	1.5	1.6	1.9	0.4
C18:0	10.2[c]	13.7[b]	15.7[a]	8.4[d]	0.8
C18:1n9*trans*	1.5[b]	1.4[b]	3.9[a]	1.0[b]	0.3
C18:1n9*cis*	24.8[b]	23.1[b]	29.8[a]	17.9[c]	1.2
C18:1*cis*11	0.58[b]	0.55[b]	0.70[a]	0.69[a]	0.04
C18:2n6*cis*	3.1[ab]	2.4[b]	3.8[a]	3.2[a]	0.2
C18:2n6*trans*	0.27[b]	0.33[b]	0.58[a]	0.30[b]	0.04
C18:3n3	0.6[b]	1.1[a]	0.5[b]	0.6[b]	0.1
C20:3n6	0.17	0.13	0.13	0.18	0.01
C20:4n6	0.18	0.19	0.19	0.23	0.01
Saturated[2]	66.3[c]	68.1[b]	57.8[d]	71.3[a]	1.4
Unsaturated	33.7[b]	31.9[b]	42.1[a]	28.7[c]	1.4
PUFA	4.3[b]	4.2[b]	5.2[a]	4.5[b]	0.2
n-6/n-3	6.8[b]	2.8[c]	9.9[a]	6.5[b]	0.8
n-3	0.55[b]	1.12[a]	0.50[c]	0.59[b]	0.06
n-6	3.75[b]	3.10[b]	4.74[a]	3.87[b]	0.22

[a,b,c,d]Means within a row with no common superscript differ ($P < 0.05$).

[1]Least square means with pooled SE.

[2]Saturated = 10:0 + 12:0 + 14:0 + 16:0 + 18:0, unsaturated = 14:1 + 16:1 + 18:1n9*trans* + 18:1n9*cis* + 18:1*cis*11 + 18:2n6*cis* + 18:2n6*trans* + 18:3n3 + 20:3n6 +20:4n6, and PUFA (polyunsaturated) = 18:2n6*cis* + 18:2n6*trans* + 18:3n3 + 20:3n6 +20:4n6.

Milk Fatty Acids

Milk fatty acid concentrations (Table 5) of C10:0, C12:0, and C18:0 were higher for cows fed FLA than for those fed MEG, whereas the inverse was observed for concentrations of C16:0. This is in general agreement with the results of Petit (2002), who compared whole flaxseed and Megalac. The proportion of C18:3 in milk was relatively high even for the MEG diet in which the concentrates supplied only low levels of C18:3, which would likely reflect the higher proportion of forage of these diets and higher levels of C18:3 in immature grass silage (Dewhurst and King, 1998). Changes in milk fat composition were typical of those induced by feeding or infusing fats (Cant et al., 1991; Drackley et al., 1992). Cows fed SUN had the highest

Table 6. Blood composition of Holstein cows fed a TMR containing a concentrate based either on Megalac (MEG), untreated whole flaxseed (FLA), untreated whole sunflower seed (SUN), or no fat supplement (CON).[1]

Item	MEG	FLA	SUN	CON	SE
NEFA, μeq/L	167.3	131.5	182	139.9	9.9
Total cholesterol, mg/100 mL	257[a]	270[a]	212[ab]	168[b]	16.1
HDL cholesterol, mg/100 mL	132.7	131	129.3	96.7	8.1
LDL cholesterol, mg/100 mL	124.8	139	82.9	71.3	13.4
Glucose, m*M*	3.72	3.47	3.62	3.54	0.04

[a,b]Means within a row with no common superscript differ ($P < 0.05$).

[1]Least square means with pooled standard error (SE).

Table 7. Plasma fatty acids composition of Holstein cows fed a total mixed diet based either on Megalac (MEG), untreated whole flaxseed (FLA), untreated whole sunflower seed (SUN), or no fat supplement (CON).[1]

Item	MEG	FLA	SUN	CON	SE
	% of Total fatty acids				
C14:0	0.9	1.4	1.2	1.5	0.1
C16:0	10.8	9.7	9.2	10.4	0.4
C16:1	0.9[b]	1.2[ab]	1.0[b]	1.3[a]	0.1
C18:0	10.7[c]	14.9[a]	14.4[a]	13.8[b]	0.6
C18:1n9*trans*	0.5[b]	0.7[b]	1.3[a]	0.6[a]	0.1
C18:1n9*cis*	5.9	5.5	5.2	5	0.3
C18:1*cis*11	0.4	0.7	0.6	0.8	0.1
C18:2n6*cis*	62.5[a]	50.1[b]	59.5[a]	57.2[a]	2
C18:2n6*trans*	0	0.04	0.17	0	0.03
C18:3n6	0.7[b]	0.9[b]	0.9[b]	1.3[a]	0.1
C18:3n3	3.0[b]	11.1[a]	3.0[b]	4.0[b]	0.9
C20:3n6	2.21[b]	2.13[b]	2.29[b]	2.95[a]	0.13
C20:4n6	1.2	1.1	1.0	1.1	0.1
C20:5n3	0.30[b]	0.56[a]	0.23[b]	0.10[b]	0.06
Saturated[2]	22.4	26.1	24.7	25.6	1.0
Unsaturated	77.6	73.9	75.3	74.4	1.0
n-6/n-3	20.5[a]	4.8[b]	23.9[a]	16.1[a]	2.5
PUFA	69.9	66.0	67.3	66.6	1.4
n-3	6.2[b]	22.9[a]	6.2[b]	8.1[b]	1.9
n-6	65.9[a]	53.3[b]	63.1[a]	61.2[a]	2.1

[a,b,c]Means within a row with no common superscript differ ($P < 0.05$).

[1]Least square means with pooled SE.

[2]Saturated = 10:0 + 12:0 + 14:0 + 16:0 + 18:0; unsaturated = 14:1 + 16:1 + 18:1n9*trans* + 18:1n9*cis* + 18:1*cis*11 + 18:2n6*cis* + 18:2n6*trans* + 18:3n3 + 20:3n6 +20:4n6; and PUFA (polyunsaturated) = 18:2n6*cis* + 18:2n6*trans* + 18:3n3 + 20:3n6 +20:4n6.

C18:2 concentration in milk, which may result from the highest C18:2 concentration in the diet (Table 3). Concentrations of n-3 FA in milk were the highest for cows fed the FLA diet, as previously reported by Petit (2002) for cows fed whole flaxseed, and they were the lowest for those fed the SUN diet. Concentrations of n-6 FA in milk were greater for cows fed the SUN diet as compared with cows fed the other diets. The n-6 to n-3 FA ratio in milk was significantly affected by the diet, with treatments ranking from the highest to the lowest ratio as follows: SUN > CON = MEG > FLA. A decrease in the n-6 to n-3 FA ratio in milk has been reported by Petit (2002), when cows were fed whole flaxseed compared with calcium salts of palm oil, and whole flaxseed decreased the n-6 to n-3 FA ratio in milk compared with sunflower seed (Petit, 2003).

Plasma Analysis

Plasma concentrations of NEFA averaged 155 μeq/L and they were similar among treatments (Table 6), although NEFA concentrations in plasma usually increase when fat is supplemented (Grummer and Carroll, 1991). However, Johnson et al. (2002) found no significant difference in serum NEFA concentrations between cows fed a control diet and those fed a diet supplemented with either 4.0 or 5.6% of fat in the form of oilseeds. Blood concentrations of NEFA are an index of body fat mobilization (Roberts et al., 1981), and they are related to the energy balance of cows. Intake of DM was similar among treatments, although milk yield was greater for cows fed MEG and FLA than for those fed SUN and CON, suggesting a greater negative energy balance and better energy efficiency for cows fed FLA and MEG than for those fed SUN and CON. Activation of a group of nuclear hormone receptors, termed peroxisome proliferator-activated receptors, could have an important role in FA metabolism (Schoonjans et al., 1996; Desvergne et al., 1998). Their concentrations could be increased by feeding particular FA (Kersten et al., 1999), affecting hepatic FA oxidation and NEFA concentrations in blood. This would be corroborated with the fact that cows in a theoretical, more positive energy balance and fed calcium salts of palm oil had higher NEFA plasma concentration than those fed whole flaxseed (Petit, 2002). Serum total cholesterol concentration was significantly lower for cows fed the CON diet (168 mg/100 mL) compared with those fed the MEG (257 mg/100 mL) and FLA (270 mg/100 mL) diets, and those fed SUN (212 mg/100 mL) had values similar to cows fed the other 3 diets. Fat supplementation usually increases blood cholesterol (Garcia-Bojalil et al., 1998), but the type of fatty acid alters the effect. There were no effects among treatments in serum HDL and LDL cholesterol concentrations or plasma glucose concentrations.

Plasma concentrations of C14:0, C16:0, C18:1*cis*11, C18:2n6*trans*, C20:4n6, saturated FA, unsaturated FA, and polyunsaturated FA were similar among diets (Table 7). Cows fed FLA had the lowest concentration of C18:2n6*cis* and the highest concentrations of C18:3n3 and C20:5n3, which resulted in greater concentrations of n-3 and less n-6 FA in the plasma of cows fed FLA. As a result, cows fed FLA had the lowest n-6 to n-3 FA

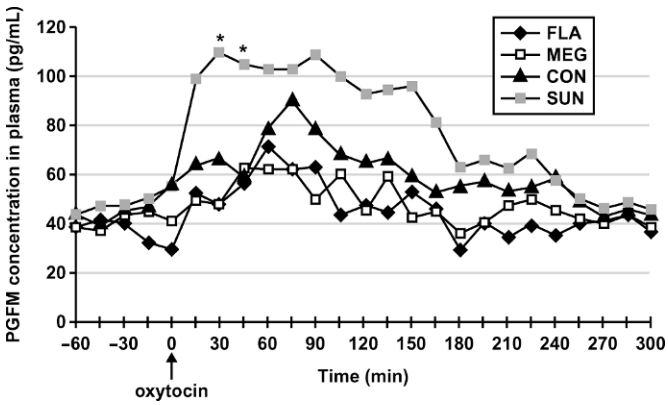

Figure 1. Mean plasma 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) concentrations following an oxytocin challenge on d 15 of a synchronized estrus cycle for lactating Holstein cows fed a total mixed diet based on Megalac (MEG), whole flaxseed (FLA), whole sunflower seed (SUN), or no fat supplement (CON). The PGFM response was higher in cows fed whole sunflower seed 30 and 45 min after injection of oxytocin (*, $P < 0.05$). SEM = 8.1 and n = 4.

ratio, as previously reported by Petit (2002). Concentrations of C18:2n6*cis*, C18:3n3, C20:5n3, and ratio of n-6 to n-3 FA in plasma were similar for cows fed the MEG, SUN, and CON diets.

There was a significant interaction ($P < 0.01$) between treatment and time; concentration of PGFM was significantly greater 30 and 45 min after the oxytocin injection for cows fed SUN than for those fed MEG < FLA = CON, and there was no difference among treatments for the other sampling times (Figure 1). Calculation of area under the overall response curve from 0 to 300 min was similar among treatments. However, Mattos et al. (2002) showed that the response of PGFM of cows fed fishmeal, which is a source of n-3 FA, was significantly affected early after the oxytocin injection. Therefore, the area under the overall response curve was reanalyzed from 0 to 120 min after the oxytocin challenge, and it tended ($P = 0.11$) to be greater for cows that were fed the SUN diet compared with those fed either the MEG or the FLA diet (Table 8). Filley et al. (2000) previously observed greater plasma PGFM concentrations for beef heifers supplemented with calcium salts of FA, which they explained by the greater concentration of linoleic acid in plasma. The relationship between plasma concentration of C18:2 and that of PGFM was not observed in the present experiment, as cows fed MEG, SUN, and CON had similar C18:2 concentrations but different PGFM concentrations. Peak PGFM concentration in response to the oxytocin challenge was similar among treatments. Results of the present experiment would partly support our hypothesis that increasing the ratio of n-6 to n-3 FA increases the synthesis of the dienoic PG ($PGF_{2\alpha}$), as feeding SUN effectively increased the n-6 to n-3 FA ratio in both milk and plasma relative to MEG, FLA, and CON, and tended to result in the highest PGFM response following an oxytocin challenge. However, although feeding FLA significantly lowered the n-6 to n-3 FA ratio in milk and plasma, secretion of PGFM was similar for cows fed FLA, MEG, and CON. This may suggest that

Table 8. Reproduction data of Holstein cows fed a total mixed diet based either on Megalac (MEG), untreated whole flaxseed (FLA), untreated whole sunflower seed (SUN), or no fat supplement (CON).[1]

Item	MEG	FLA	SUN	CON	SE
PGFM in plasma, ng/mL[2]					
Mean[3]	55.1[b]	55.5[b]	102.7[a]	71.4[ab]	10.7
Area under the curve[4]	1744[b]	2260[b]	5734[a]	2753[ab]	788
Peak	73	83	128	92	16
Progesterone in milk, ng/mL					
Mean, d 21 to 35	5.2	6	6.7	5.4	0.7
Area under the curve, d 21 to 35	61.1	61.2	80.4	66.5	9.7
Peak between d 21 to 35[5]	12.2[b]	18.9[ab]	20.8[a]	14.3[ab]	2.3
Follicles diameter, mm					
Largest (F1)	11.3	11.8	13.3	12.4	0.6
Second largest (F2)	7.1	7.5	8.3	6.9	0.8
F1-F2	4.2	4.3	5	5.5	0.8
Class of follicles, no					
small, 3.0 to 4.9 mm	2.5	1.8	2	2.3	0.3
medium, 5.0 to 9.9 mm	1.5[ab]	1.0[b]	1.1[b]	1.6[a]	0.2
large, ≥10.0 mm	1	1.2	1.1	1	0.1
Total	5.0[a]	4.0[b]	4.2[b]	4.9[ab]	0.3
CL diameter, mm	17.1	17.4	18.1	5.3	1

[1]Least square means with pooled standard error (SE).

[2]Measured between 0 and 120 min after the injection of oxytocin.

[3a,b,c]Means within a row with no common superscript differ, $P = 0.07$.

[4a,b,c]Means within a row with no common superscript differ, $P = 0.11$.

[5a,b]Means within a row with no common superscript differ, $P = 0.09$.

availability of n-3 FA in the form of linolenic acid has little or no effect on uterine prostaglandins production, although Petit et al. (2002) previously reported that a lower n-6 to n-3 FA ratio decreased the secretion of PGFM in cows fed flaxseed. Similar findings were reported by Mattos et al. (2002), who fed fishmeal to lactating dairy cows. Reduced levels of $PGF_{2\alpha}$ could contribute to improved fertility of cows fed α-linolenic acid (Petit et al., 2001) through reduced luteolysis (Thatcher et al., 1995) and vice versa.

Milk progesterone concentrations, expressed as mean values or as the area under the curve from d 21 to 35, were not significantly different among treatments (Table 8), and there was no interaction between day and treatment. Cows fed the MEG diet had values similar to those of cows fed FLA, as previously reported by Petit et al. (2002) for cows fed formaldehyde-treated flaxseed compared with those fed calcium salts of palm oil. Similarly, Mattos et al. (2002) found no difference in plasma progesterone concentration between cows fed a diet containing no fat supplement and those fed a source of n-3 FA, such as fish meal. Peak concentration of progesterone from d 21 to 35 tended ($P = 0.09$) to be greater for cows fed SUN than for those fed MEG (Table 8).

Ovarian Function

There was no significant treatment affect on the mean size of follicles (Table 8). Although the number of class 1 follicles over time was similar among diets, cows on the CON treatment had a greater number of class 2 follicles than those fed the FLA and SUN diets; the number of class 3 follicles was similar among treatments. The mean size of the CL did not differ among treatments, which agrees with findings of Petit et al. (2001), who compared diets based either on formaldehyde-treated flaxseed or calcium salts of palm oil.

CONCLUSIONS

The release of PGFM in response to a standard oxytocin challenge was greater for cows fed whole sunflower seed compared with those fed whole flaxseed. Whole flaxseed contains a high proportion of n-3 fatty acids, whereas sunflower seed has a greater percentage of n-6 fatty acids. Therefore, feeding diets with high proportions of n-6 fatty acids (61% of total fatty acids for the sunflower seed diet) could increase the secretion of series 2 prostaglandins in blood. Feeding polyunsaturated fatty acids had no effect on N retention, although cows fed whole sunflower seed had lower excretion of N in urine than those fed calcium salts of palm oil.

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