

Effects of different polyunsaturated fatty acid supplementations during the postpartum periods of early lactating dairy cows on milk yield, metabolic responses, and reproductive performances¹

E. Dirandeh,^{*2} A. Towhidi,^{*} S. Zeinoaldini,^{*} M. Ganjkhanelou,^{*} Z. Ansari Pirsaraei,[†]
and A. Fouladi-Nashta[‡]

^{*}Department of Animal Science, University of Tehran, Karaj, Alborz, Iran; [†]Animal Science Department, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran; and [‡]Department of Veterinary Basic Sciences, The Royal Veterinary College, Hatfield, North Mymms, Hatfield, UK

ABSTRACT: In spite of the difficulties in delivering PUFA to ruminants, studies have generally indicated that the PUFA of the omega-6 (linoleic acid) and omega-3 [α -linolenic acid; eicosapentaenoic (EPA), C20:5 omega-3; docosahexaenoic (DHA), C22:6 omega-3] families are the most beneficial to improving reproduction in cows. The objectives were to determine if a diet enriched in α -linolenic acid (omega-3) or linoleic acid (omega-6) would influence milk production and composition, metabolic status, and reproductive performance in lactating dairy cows. High-yielding multiparous Holstein dairy cows ($n = 120$) with no overt clinical illnesses were blocked according to calving date and parity. Cows were assigned randomly to be fed 1) soybean whole roast (Soy, omega-6, $n = 40$) or 2) linseed (Lin, omega-3, $n = 40$) or 3) palm oil as a source of SFA (PO, $n = 40$) from calving until first heat after 40 d postpartum (dpp), and then half of the cows in each treatment group were switched to receive either Lin or SFA (PO) from first heat after d 40 to 120 dpp. Blood was collected from a subsample of cows.

Blood was collected at 14 d intervals for 12 wk, starting on the day of calving. Results showed milk yield and DMI were not affected. Milk compositions were similar ($P > 0.08$) among diets, except concentration and yield of milk fat percentage, which was less in cows fed Lin ($P < 0.05$). Uterine involution in cows fed Soy occurred earlier ($P < 0.05$). Diets affected day to first estrus and day to first insemination in cows ($P < 0.05$). There were no differences among treatments for percent heat detection, percent pregnancy per first insemination, and percent conception per AI at estrus. Also, there is a trend of pregnancy by 120 d, which is 66.7% for the Lin group vs. 50.91% for the PO group ($P < 0.08$). Of the 4 pregnancy losses, 2 occurred in PO-PO group and 2 occurred in Soy-PO group, and none occurred in the other 4 treatments. In conclusion, our study showed feeding omega-6 fatty acids during 40 dpp could be a good treatment for early postpartum periods, and a shift to omega-3 fatty acids until 40 d after AI can be considered as a strategy for improving fertility in lactating dairy cows

Key words: dairy cows, milk yield, omega-3 fatty acids, omega-6 fatty acids, reproductive performance

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INTRODUCTION

During negative energy balance (NEB), the blood concentrations of NEFA increase at the same time

that IGF-I, glucose, and insulin decrease (Santos, 2001). These shifts in blood metabolites and hormones might compromise ovarian function and fertility. It has also been reported that energy balance and DMI might affect plasma concentrations of progesterone (Vasconcelos et al., 2003), which may interfere with follicle development and maintenance of pregnancy. Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation (Santos et al., 2004). Mechanisms for the role of fats in the reproduction of

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²Corresponding author: dirandeh@gmail.com

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dairy cows may include improved dietary energy density (Ferguson et al., 1990), altered follicle development (Staples and Thatcher, 2005), increased concentrations of progesterone (Staples et al., 1998), prevention of luteolytic signals around maternal recognition of pregnancy (Mattos et al., 2000), and improved embryo quality (Cerri et al., 2004).

Uterine synthesis of $\text{PGF}_{2\alpha}$ is regulated in part by substrate availability, and arachidonic acid (C20:4 omega-6) is the precursor for $\text{PGF}_{2\alpha}$ synthesis, so it is plausible to suggest that increments of arachidonic acid content of endometrial tissue should enhance uterine $\text{PGF}_{2\alpha}$ secretion, which may affect uterine health (Cullens et al., 2004; Silvestre et al., 2011). Heravi Moussavi et al. (2007) demonstrate that dietary supplementation with fish meal or omega-3 fatty acids in early lactating dairy cows significantly increased uterine omega-3 fatty acid concentrations.

Feeding omega-3 fatty acids (linolenic acid, 18:3, omega-3, linseed) beginning at 40 d postpartum (**dpp**) could reduce $\text{PGF}_{2\alpha}$ secretion, which would increase fertility and reduce pregnancy losses. Therefore, our study aimed to determine the best fatty acid feeding strategy for postpartum periods associated with lactation and reproductive performances in dairy cows. A diet with whole roast soybean (**Soy**) was expected to induce greater plasma $\text{PGF}_{2\alpha}$ concentrations because of its greater omega-6; a diet of linseed oil (**Lin**) was expected to decrease $\text{PGF}_{2\alpha}$ concentrations because of its greater omega-3 content, and the palm oil diet (**PO**) with SFA was the control.

MATERIALS AND METHODS

This experiment was performed according to the procedures established by the Iranian Ministry of Agriculture (experimental permission 858).

Experimental Design

Three diets were formulated to have equal concentrations of DM, ME, and CP but to have different ratios of omega-3/omega-6 PUFA (Tables 1, 2). Fatty acid analyses were made for Soy, Lin, and PO before

the study began to predict dietary formulations, and we repeated the analysis again in the total mixed ration (**TMR**) diets every 2 wk to be sure of the quality of fat supplements and to check the level of dietary fatty acids.

High-yielding multiparous Holstein dairy cows ($n = 120$) with no overt clinical illnesses were blocked according to calving date and parity. There was no difference among groups (mean \pm SEM) in parity (3.2 ± 1.90) or BCS at calving (3.2 ± 0.07). The frequency distribution of cows among the BCS was the same for the subsample of cows used for metabolite measurements.

Cows were assigned randomly to be fed 1) SOY as a source of omega-6 ($n = 40$) or 2) Lin as a source of omega-3 ($n = 40$) or 3) PO as a source of SFA ($n = 40$) from calving until first estrus after 40 dpp, and then one-half of the cows in each treatment group were switched to receive either the diet containing Lin (omega-3) or PO (SFA) from first heat after 40 to 120 dpp (Table 1). Supplementation of fatty acids was at 1.5% of dietary DM. Blood samples were collected from 8 cows per treatment.

Cows in the Soy-Lin group were fed Soy (omega-6) from calving until the first estrus after d 40 and were then fed Lin (omega-3) until 120 dpp. Cows in the PO-Lin group were fed Soy and then Lin until 120 d postpartum. Cows in the Lin-Lin group were fed Lin until 120 dpp, and cows in the PO-PO group were fed PO until 120 dpp. Cows in the Soy-PO group were fed Soy from calving until the first estrus after d 40 and were then fed PO until 120 dpp.

Reproductive Management

The ovarian status of cows was synchronized for ovulation beginning on 30 dpp with 2 intramuscular injections of $\text{PGF}_{2\alpha}$ (Synchromate, 150 μg cloprostenol sodium, Aburaihan Company, Tehran, Iran) given 14 d apart. All cows showed estrus 1 to 3 d after the second $\text{PGF}_{2\alpha}$ injection except 10 cows [PO, $n = 6$; Lin (omega-3), $n = 62$; Soy (omega-6), $n = 62$], which were removed from the experiment. Cows were artificially inseminated at the second estrus postpartum that was at least 20 d after dietary change, provided they had been on their appropriate diet for at least 20 d. Insemination was repeated by 1 technician at any subsequent estrus until the end of the experiment at 120 dpp. Estrus was detected using a combination of behavioral observations, pedometer activity monitoring, and ultrasonography.

Nonpregnant cows were injected with 500 mg of $\text{PGF}_{2\alpha}$ (Synchromate, 150 μg cloprostenol sodium, Aburaihan Company) and then injected with 100 μg of GnRH 56 h later. A timed AI (**TAI**) was performed 16 h after the GnRH injection for the second insemination after diet change. Cows were examined by trans-rectal ultrasonography 32 d after the second TAI. Pregnancy

Table 1. Experimental design and treatment

Period 1: Calving to first heat after 40 d postpartum		Period 2: First heat to 120 d postpartum
Diet Soy (omega-6 fatty acid, $n = 40$)	20	Group 1: One-half of cows in each treatment fed diet Lin (omega-3 fatty acid, $n = 60$)
Diet Lin (omega-3 fatty acid, $n = 40$)	20	
Diet PO (SFA, $n = 40$)	20	Group 2: One-half of cows in each treatment fed diet C (SFA, $n = 60$)
	20	
	120	

was evaluated at 40 d after AI, and pregnant cows had their pregnancy reconfirmed at 60 d after AI and pregnancy losses determined.

Artificial inseminations were conducted by 1 technician in all groups. Uterine involution (uterine horns and cervix <40 mm in diameter) was considered for every cow by a veterinarian and confirmed by a transrectal ultrasound scan at 10 d intervals starting at 20 dpp using an Aloka scanner equipped with a 5-MHz linear array transducer (Aloka Co., Ltd., Tokyo, Japan). Pregnancy was confirmed by a veterinary surgeon using rectal palpation on d 40 and 60 postinsemination. This analysis only includes cows diagnosed as pregnant at the first check that either sustained or lost a pregnancy at the second check.

Body Condition Score

All cows were evaluated for BCS at the day of parturition, at 40 dpp, and at the first TAI. Scores were given by 2 veterinarians on the basis of a 1 (thin) to 5 (obese) scale using a quarter-point system (Edmonson

et al., 1989). Changes in BCS were obtained by subtracting BCS at 40 dpp from BCS at parturition and BCS at TAI from BCS at 40 dpp. The BCS gain or loss was used as an indicator of energy status.

Milk Yield and Composition

Milk yield, feed intake, and pedometer activity were recorded daily throughout the experiment in 16 cows per treatment in period 1 and 24 cows per treatment in period 2. Milk samples were taken weekly (Monday and Thursday mornings) and analyzed for fat, protein, and lactose by infrared analysis at the National Milk Records Laboratory (Sari, Iran) using AOAC reference method 972.16 (AOAC, 1990).

Blood Sampling and Analysis

Blood samples were collected from a subsample of cows (8 cows per treatment, a total of 48 cows) at 14 d intervals for 12 wk starting on the day of calving to determine metabolites in plasma. During

Table 2. Ingredients and chemical composition of experimental diets

Item	Calving to first heat \geq 40 dpp			First heat to 120 dpp partum	
	PO ¹	Lin ²	Soy ³	PO	Lin
Ingredients, % DM					
Alfalfa hay mid	27.20	27.20	27.20	15.0	15.0
Corn silage	21.15	21.15	21.15	22.22	22.22
Beet pulp, dehydrated	3.02	3.02	3.02	13.35	13.35
Corn grain, ground	9.75	9.75	9.75	9.11	8.96
Barley grain, rolled	9.75	9.75	9.75	14.94	15.05
Molasses	0.78	0.78	0.78	—	—
Meat meal	—	—	—	0.90	0.90
Wheat bran	0.33	0.21	0.71	1.87	1.33
Soybean meal, 48%	8.81	8.07	3.03	6.84	5.44
Soybean whole, roast	0	0	7.31	—	—
Corn gluten meal, 60%	1.86	1.86	1.86	—	—
Cottonseed meal	3.39	3.14	3.14	3.33	3.37
Cottonseed whole	1.51	1.51	1.51	4.28	2.17
Linseed, extruded ⁴	0	4.03	0	—	3.04
Canola meal solvent	4.82	3.31	4.57	4.51	5.04
Fish meal	—	—	—	0.66	0.66
Wheat	2.69	2.69	2.69	—	—
Palm oil	1.41	0	0	—	0.53
Chemical composition, % DM					
NE _L , Mcal/kg of DM	1.58	1.58	1.58	96.87	98.51
Fat	4.00	4.00	4.00	—	—
CP	17.90	17.85	17.85	—	—
NDF	34.40	33.90	34.10	—	—
ADF	20.40	20.50	20.40	—	—
Omega-6/omega-3	3.2	1.2	4.2	—	—

¹PO = SFA.

²Lin = omega-3.

³Soy = omega-6.

⁴Omegalin, Nutri advance, Ploufragan, France.

the synchronized estrous cycle blood samples were collected at d 6, 8, 10, 12, 14, 16, 18, and 20 for progesterone assay. Also, we performed ultrasonography to make sure all cows were in similar stages of the cycles for progesterone samples. All blood samples were collected by coccygeal venipuncture. Samples were collected in evacuated glass tubes containing EDTA and were centrifuged ($1500 \times g$, 20 min at 4°C) within 2 h. Plasma was harvested and stored at -20°C until further analysis. The plasma glucose, cholesterol, triglyceride, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were measured using a spectrophotometer and commercial kits (Pars Azmoon, Tehran, Iran). Intra- and interassay CV were $<5\%$. Blood progesterone concentrations were analyzed by ELISA kits following the manufacturer's instructions (Diaplus, North York, ON, Canada).

Statistical Analysis

Repeated measures on milk yield data, DMI, BCS, and concentrations of progesterone and metabolites (glucose, cholesterol, triglyceride, HDL, and LDL) in plasma were analyzed in periods 1 and 2 separately using the repeated measures responses of the mixed model procedure (SAS Inst. Inc., Cary, NC) with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk},$$

where μ is the population mean, α_i is a population parameter corresponding to treatment (diet) i , β_j is the fixed effect of sampling day or time j , $(\alpha\beta)_{ij}$ is the interaction effects of treatment and sampling d or time, and e_{ijk} is the residual error. Differences between means were tested using Duncan's test. Differences were considered significant at $P < 0.05$. Data are presented as least squares means \pm SE.

Data were tested for normal distribution of the residuals by the PROC UNIVARIATE procedure of SAS. Residuals were considered to be normally distributed when the Shapiro-Wilk statistic was equal to or greater than 0.90 and were log transformed if required. For each dependent variable, the autoregressive 1 covariance structure was selected because it had the best relative goodness of fit based on penalty criteria (Bayesian criterion).

All of the reproductive responses (binary responses), such as heat detection (0, 1), conception to detected heats (0, 1), pregnancy to first insemination (0, 1), pregnancy to all inseminations (0, 1), and pregnancy losses (0, 1), were analyzed by Glimmix using a binary distribution and a logit odds ratio link to examine period 1 (early postpartum diets, PO, Soy, and Lin), period 2 (diet effects after 40 d, PO and Lin), and period 1 \times

period 2 interactions with the specific contrasts. To examine effects of treatments in period 1 on length of time, such as day from calving to uterine involution, day from calving to first estrus, and day from calving to first insemination, survival analysis was performed.

RESULTS

Dry Matter Intake, BCS, Milk Production, and Composition

Chemical compositions and ingredients of diets are presented in Table 1. The omega-6/omega-3 FA ratio was greatest in the Soy group (omega-6, 4.20), intermediate for the PO group (SFA, 3.2), and least for the Lin group (omega-3, 1.0) supplements (Table 2). The greater omega-6/omega-3 ratio of the Soy (omega-6) supplement was due to the greater quantities of linoleic acid in this supplement (58.7%). The low omega-6/omega-3 ratio in the Lin (omega-3) supplement was due to the greater concentrations of linolenic acid (29.70%) compared with those of the other FA supplements. Fatty acid compositions of the TMR were characterized by greater proportions of linoleic acid and α -linolenic acid in diets supplemented with Soy and Lin, respectively.

There was no treatment effect on intakes of DM. The frequency distribution of cows among different BCS quartiles did not differ at the time of initiation of diets postpartum (median BCS of 3.2) and was not affected by diets from 0 to 8 wk postpartum.

Milk yield was not affected by diet in periods 1 and 2 ($P > 0.05$, Table 3). Also, interactions between periods 1 and 2 were not significant ($P > 0.08$). Milk yield increased over time ($P < 0.001$), whereas no treatment \times d interactions was detected in periods 1 and 2 ($P = 0.35$). Milk composition was similar among diets in both periods (Table 3), except milk fat percentage and yield of milk fat, which were less in the Lin (omega-3) group than in the other groups in periods 1 ($P = 0.002$) and 2 ($P = 0.01$, Table 3). Interactions between periods 1 and 2 were not significant ($P > 0.08$). Milk fat concentration and yield of milk fat did not change during period 1 in cows fed PO and Soy, whereas milk fat concentration declined from 3.84% to 3.50% ($P < 0.05$) from wk 0 to 8 in cows fed Lin.

Blood Metabolite Responses and Progesterone Concentrations

Plasma glucose concentrations in period 1 were affected ($P = 0.005$) by the diets (Table 4) and were greatest in the PO treatment (49.37 ± 0.62). Plasma glucose increased with days in milk (DIM; $P < 0.001$), but treatment \times time in period 1 was not significant. In

period 2, treatments did not affect glucose concentration ($P > 0.05$). Also, interactions between period 1 and 2 were not significant ($P > 0.08$).

Mean plasma LDL cholesterol in period 1 was greater ($P = 0.02$) in PO and Soy treatments than in the Lin (omega-3) treatment (Table 4), but in period 2 there were no significant differences between treatments ($P = 0.45$). There was no treatment ($P = 0.62$) effect on plasma cholesterol, triglycerides, and HDL cholesterol in both periods. The interaction between periods 1 and 2 was not significant ($P > 0.08$).

Mean serum progesterone concentrations were greater during the luteal phase (d 6 to 16 of cycle, $P < 0.03$) and at the time of insemination in cows fed Soy (omega-6, 0.65 ± 0.05 ng/mL) or Lin (omega-3, 0.80 ± 0.05 ng/mL) than in cows fed PO (0.15 ± 0.05 ng/mL, $P < 0.01$).

Reproductive Performance

Uterine involution in cows fed Soy (omega-6) occurred earlier ($P < 0.05$, Table 5). Diets affected day to first estrus and day to first insemination (Table 5) and were earlier in the Lin (omega-3) treatment compared with the PO treatment ($P < 0.05$), but there were no differences between Lin (omega-3) and Soy (omega-6) treatments in period 1.

There were no differences ($P > 0.08$) between treatments for percent heat detection, percent pregnancy per first insemination and percent conception per AI at

estrus. Also, There is a progressive trend of pregnancy by 120 d being 66.7% for the L (omega-3) group versus 50.91% for the C group ($P < 0.08$). Of the 4 pregnancy losses, 2 occurred in PO-PO, 2 occurred in Soy-PO and none in the other 4 groups ($P < 0.03$).

DISCUSSION

Average postpartum DMI was not affected by treatments in periods 1 and 2, whereas Zachut et al. (2010) showed the postpartum DMI and energy intake were greater in extruded flaxseed vs. control cows (3.8% and 5%, respectively). The increased intake of the extruded flaxseed cows is consistent with the results of Petit et al. (2007), who found that a diet containing a high proportion of SFA caused reduced feed intake than one rich in unsaturated fatty acids. However, several other studies have found that decreased intake resulted from abomasal infusion of unsaturated fatty acids (Bremmer et al., 1998) or from feeding cows increasing amounts of unsaturated fatty acids at the expense of SFA (Harvatine and Allen, 2006). However, Gonthier et al. (2005) did not observe an effect on DMI from feeding extruded flaxseed (12.7% of DM), whereas Chilliard et al. (2009) found that cows fed a supplement with 1 kg of extruded flaxseed (7.9% DM) per cow per day decreased DMI.

In this experiment, BCS did not differ between dietary treatments until 40 dpp. A greater proportion of cows gained body condition from 40 dpp to first

Table 3. Means \pm SE for milk production and composition, feed intake, and BCS during period 1 [calving to first estrus \geq 40 d postpartum (dpp)] and period 2 (first estrus to 120 dpp) and period 1 \times period 2 interactions

Item	Period 1				Period 2			Interactions						
	PO ¹	Lin ²	Soy ³	SE	PO	Lin	SE	PO-PO	PO-Lin	Lin-PO	Lin-Lin	Soy-PO	Soy-Lin	SE
Milk yield, kg d ⁻¹	45.57	46.05	45.50	1.11	53.8	54.5	1.41	46.8	47.02	47.1	47.5	47.3	47.7	1.86
Milk fat, %	4.0 ^a	3.63 ^b	3.85 ^a	0.10	3.8 ^c	3.4 ^d	0.14	4.01 ^e	3.81 ^e	3.8 ^e	3.3 ^b	3.94 ^e	3.84 ^e	0.22
4% FCM, ⁴ kg d ⁻¹	45.52 ^a	43.47 ^b	44.45 ^a	0.73	56.32 ^c	52.85 ^d	0.99	46.7 ^e	45.65 ^e	45.54 ^e	42.4 ^h	46.8 ^e	46.53 ^e	1.2
Milk fat, kg d ⁻¹	1.82 ^a	1.67 ^b	1.75 ^a	0.06	2.32 ^c	2.07 ^d	0.08	1.87 ^e	1.79 ^e	1.78 ^e	1.56 ^h	1.86 ^e	1.83 ^e	0.08
Milk protein, %	2.78	2.8	2.81	0.22	3.21	2.99	0.09	3.01	2.91	3.27	2.95	3.34	2.99	0.18
Milk protein, kg d ⁻¹	1.26	1.28	1.27	0.07	1.72	1.62	0.07	1.4	1.36	1.54	1.4	1.57	1.42	0.08
Milk lactose, %	4.89	4.85	4.94	0.31	5.38	5.2	0.11	5.23	5.09	5.61	5.15	5.29	5.11	0.20
Milk lactose, kg d ⁻¹	2.22	2.23	2.24	0.07	2.89	2.83	0.92	2.44	2.39	2.64	2.44	2.5	2.43	0.9
SCC ⁵	520	470	450	60	470	490	55	600	560	500	400	530	510	70
DMI, kg d ⁻¹	23.3	24.1	23.6	0.5	27.5	28.1	0.62	24.3	24.45	24.9	25.2	24.6	24.8	0.67
BCS ⁶	2.86	2.93	2.75	0.1	2.53	2.41	0.19	2.42	2.45	2.51	2.54	2.48	2.5	0.18

^{a,b}Means within period 1 with different superscripts differ ($P < 0.05$).

^{c,d}Means within period 2 with different superscripts differ ($P < 0.05$).

^{e-h}Means within period 1 \times period 2 interactions with different superscripts differ ($P < 0.05$).

¹PO = SFA.

²Lin = omega-3.

³Soy = omega-6.

⁴FCM = fat-corrected milk, calculated as $[0.4 \times \text{milk production (kg d}^{-1})] + [15 \times \text{fat yield (kg d}^{-1})]$.

⁵SCC = somatic cell count ($\times 1,000$ cells/mL).

⁶Based on a 5-point scale.

Table 4. Means \pm SE plasma metabolites during period 1 [calving to first estrus \geq 40 d postpartum (dpp)] and period 2 (first estrus to 120 dpp) and period 1 \times period 2 interactions

Item	Period 1				Period 2			Interactions						
	PO ¹	Lin ²	Soy ³	SE ⁴	PO	Lin	SE	PO-PO	PO-Lin	Lin-PO	Lin-Lin	Soy-PO	Soy-Lin	SE
Glucose, mg/dL	49.37 ^a	47.41 ^a	42.19 ^b	1.2	43.54	46.15	2.1	43.12	49.78	41.36	45.66	46.15	43.02	3.1
Cholesterol, mmol/L	189.6	152.9	189.2	5.1	163.9	190.5	11.5	186.7	192.6	155.5	150.2	149.6	228.8	30.5
Triglyceride, mmol/L	6.9	8.1	6.9	0.6	8.05	6.6	0.7	6.6	7.3	11.1	5.1	6.3	7.4	1.2
HDL, ⁵ mmol/L	102.9	117.9	100.7	6.5	107.3	107.1	7.5	101.7	104.1	114.7	121.1	105.5	95.81	24.1
LDL, ⁶ mmol/L	100.6 ^a	49.79 ^b	91.79 ^a	3.5	71.64	89.85	5.2	111.3	89.9	47.7	51.8	55.8	127.7	12.5

^{a,b}Means within period 1 with different superscripts differ ($P < 0.05$).

¹PO = SFA.

²Lin = omega-3.

³Soy = omega-6.

⁴SE of the difference.

⁵HDL = high-density lipoproteins.

⁶LDL = low-density lipoproteins.

insemination day (i.e., approximately 80 dpp) when fed the Lin-Lin diet than when fed other diets.

Milk yield did not differ between dietary groups in the present study. This is in disagreement with the results of Kennelly and Khorasani (1993), who fed whole flaxseed at 0%, 5%, 10%, or 15% of DMI without affecting milk yield. Petit (2003), who fed either whole untreated or whole formaldehyde-treated flaxseed or sunflower seed, also found no difference in milk yield among the diets. However, in a later study, milk yield was greater in cows fed flaxseed compared with those fed sunflower seed (Petit et al., 2004). However, feeding a CS of PO at 2.2% of dietary DM postpartum also improved milk yield compared with that from no fat supplementation (Garcia-Bojalil et al., 1998). No effects on milk yield by adding fish oil to diets have been reported (Abughazaleh et al., 2002; Mattos et al., 2002), although some studies have reported increases in milk yield when fish oil is fed at earlier stages of lactation (Bilby et al., 2006; Heravi Moussavi et al., 2007). These results disagree with findings from Petit et al. (2007), in which cows fed whole flaxseed at 3.3% and 11% of DM prepartum and postpartum, respectively, produced more milk than those fed a supplement rich in SFA. Zachut et al. (2010) reported milk yield until 100 DIM was 6.4% greater ($P < 0.004$) and fat content was 11% less in the extruded flaxseed fed cows than in the control cows ($P < 0.001$), whereas fat yield, fat-corrected milk, and milk energy output were not affected by treatment. Decreased milk fat percentage was also observed by Mustafa et al. (2003) in cows fed 7% raw flaxseed and by Chilliard et al. (2009) in cows fed a supplement containing 70% extruded flaxseed at 21.2% of the diet. Discrepancies between studies might be related to differences among the amounts and forms of supplemental flaxseed or to interactions with other diet components, as suggested by Chilliard et al. (2009).

Milk fat yield was less in cows fed Lin (omega-3) than in other cows, which is consistent with previous reports from Ramaswamy et al. (2001) and Whitlock et al. (2002) but inconsistent with studies on feeding fish meal (Polan et al., 1997; Mattos et al., 2002) or extruded flaxseed (Zachut et al., 2010). Abughazaleh et al. (2002) showed that milk fat percentages and yields were decreased only when 100% of soybean meal was replaced with fish meal in the diet. Milk fat concentration and yield were not affected by feeding flaxseed at 0%, 5%, 10%, or 15% of the DMI (Kennelly and Khorasani, 1993) or flaxseed and sunflower seed with or without formaldehyde treatment (Petit, 2003). Even though there was no difference in milk fat concentration and yield between cows fed flaxseed or sunflower seed in the work of Petit et al. (2004), cows fed flaxseed yielded more milk fat (1.14 kg/d) compared with those fed a no-fat control diet (0.85 kg/d). Milk fat synthesis might be depressed by unique FA isomers generated in the rumen during biohydrogenation of unsaturated fatty acids (Bauman and Griinari, 2003). Indeed, several studies reported greater concentrations of CLA and C18:1 *trans* isomer in milk fat from cows supplemented with extruded flaxseed (Mustafa et al., 2003; Gonthier et al., 2005; Chilliard et al., 2009).

Milk protein percentage and yield did not differ between the dietary groups in our study in either period. Petit (2003) reported a greater concentration of milk protein in cows fed flaxseed (3.38%) compared with those fed sunflower seed (3.21%), although inclusion of flaxseed in the diet did not change concentration or yield of milk protein in other studies (Kennelly and Khorasani, 1993; Petit et al., 2004). Ramaswamy et al. (2001) and Whitlock et al. (2002) did not observe a decrease in protein concentrations with fish oil or soybean oil supplementations. There was no

Table 5. Reproductive performance during period 1 [calving to first estrus ≥ 40 d postpartum (dpp)] and period 2 (first estrus to 120 dpp) and period 1 \times period 2 interactions

Item	Period 1				Period 2			Interactions						
	PO ¹	Lin ²	Soy ³	SE ⁴	PO	Lin	SE	PO-PO	PO-Lin	Lin-PO	Lin-Lin	Soy-PO	Soy-Lin	SE
Day postpartum														
First estrus	46.16 ^a	43.75 ^{ab}	42.48 ^b	3.7	—	—	—	—	—	—	—	—	—	—
First insemination	73.13 ^a	70.43 ^{ab}	67.66 ^b	7.2	—	—	—	—	—	—	—	—	—	—
Uterine involution	42.35 ^a	40.36 ^b	38.58 ^c	1.9	—	—	—	—	—	—	—	—	—	—
HD, ⁵ %	83.3 (30/36)	86.1 (31/36)	87.5 (35/40)	7.3	81.8 (45/55)	89.5 (51/57)	8.6	82	84	83	88	80	95	4.3
CR, ⁶ %	63.3 (19/30)	64.5 (20/31)	77.1 (27/35)	4.5	62.2 (28/45)	74.5 (38/51)	5.5	57	68	60	68	68	84	3.7
Preg. per first AI, ⁷ %	30.5 (11/36)	36.1 (13/36)	37.5 (15/40)	3.0	28.6 (16/56)	40.3 (23/57)	2.9	29	31	27	44	30	45	2.5
Preg. per all AI, %	52.7 (19/36)	55.5 (20/36)	67.5 (27/40)	3.6	67 (38/57) ^c	82 (45/55) ^d	5.2	48 ^e	57 ^e	50 ^e	60 ^e	55 ^e	80 ^h	3.8
Pregnancy loss (d 40 to 60 after AI), %	5.5 (2/36)	0 (0/36)	5.1 (2/39)	0.8	7.1 (4/56) ^e	0 (0/57) ^d	1.2	11 ^e	0 ^h	0 ^h	0 ^h	10 ^e	0 ^h	0.6

^{a,b}Means within period 1 with different superscripts differ ($P < 0.05$).

^{c,d}Means within period 2 with different superscripts differ ($P < 0.05$).

^{e-h}Means within period 1 \times period 2 interactions with different superscripts differ ($P < 0.05$).

¹PO = SFA.

²Lin = omega-3.

³Soy = omega-6.

⁴SE of the difference for comparing treatment group means.

⁵HD = heat detection.

⁶CR = conception per AI at estrus.

⁷Preg. per first AI = pregnancy per first insemination.

difference in lactose, total solid, or solid non fat in the milk.

The overall plasma progesterone concentrations did not differ between Soy and Lin but were greater than control treatments. Addition of fat to cattle diets has consistently been shown to increase plasma cholesterol and cholesterol content in follicular fluid and in the corpus luteum (Staples et al., 1998). Burke et al. (1997) reported significantly greater concentrations of progesterone 2 d after PGF_{2 α} injection in cows fed menhaden fish meal, suggesting delayed luteal regression in cows consuming the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. In contrast to our results, Robinson et al. (2002) reported decreased progesterone concentrations in cows fed diets enriched in PUFA (either α -linolenic acid or linoleic acid).

There were no differences among treatments for percent heat detection, percent pregnancy per first insemination, or percent conception per AI at estrus. However, there is a trend of pregnancy by 120 d that is 66.7% for the Lin group vs. 50.91% for the PO group ($P < 0.08$). In agreement with this result, Silvestre et al. (2011) reported that the overall pregnancy per AI was greater in cows fed omega-6 followed by omega-3 at 60 d of pregnancy and that pregnancy loss was reduced in omega-3-fed cows. Dietary PUFA and their influence on reproductive processes in cattle have been discussed by several authors in recent years (Thatcher et al., 1997, 2004; Abayasekara and Wathes, 1999; Silvestre et al., 2011). In a previous small-scale study

(Petit et al., 2001), a flaxseed-based diet increased the conception rate in dairy cows compared with control cows fed a diet containing Megalac, a calcium soap of palm oil. Juchem et al. (2010) reported that feeding a calcium salt of linoleic and *trans*-octadecenoic acids during the transition period reduced the incidence of puerperal metritis. Juchem (2007) evaluated the effect of feeding pre- and postpartum cows Ca long-chain fatty acids of palm oil or a blend of C18:2 omega-6 and *trans*-octadecenoic fatty acid. Cows fed unsaturated fatty acids were 1.5 times more likely to be pregnant at 27 or 41 d after AI compared with cows fed palm oil. Improvements in pregnancy when cows were fed Ca salts of a mix of C18:2 omega-6 and *trans*-octadecenoic fatty acids were supported by increased fertilization and embryo quality in non-superovulated lactating dairy cows (Cerri et al., 2004).

In conclusion, feeding omega-6 PUFA after calving to the first estrous cycle and shifting to omega-3 PUFA after the first estrous cycle might be a nutritional strategy to improve reproductive performance and increase the percentage of pregnancies per all inseminations in lactating dairy cows. However, because of the small number of observations in this study, we suggest repeating this experiment on a large scale to reevaluate the findings.

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