Lower Pregnancy Losses in Lactating Dairy Cows Fed a Diet Enriched in α -Linolenic Acid

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ABSTRACT

The objectives were to determine if a diet enriched in α -linolenic acid (ALA) would influence ovarian function, early embryo survival, conception rates, and pregnancy losses in lactating dairy cows. Beginning 28 d before breeding, Holstein cows (55 ± 22 d postpartum; mean $\pm$ SD) were assigned to diets supplemented with either rolled flaxseed (FLAX; 56.7% ALA, $n = 62$) or rolled sunflower seed (SUNF; 0.1% ALA, $n = 59$) to provide approximately 750 g of oil/d. Diets continued for 32 d after timed artificial insemination (TAI, d 0) following a Presynch/Ovsynch protocol. Barley silage- and barley grain-based TMR were formulated to meet or exceed National Research Council requirements. Metabolizable protein and net energy for lactation concentrations were similar in the 2 diets. Based upon a mean dry matter intake of 22 kg/d, cows fed FLAX or SUNF consumed >410 g or <1 g of ALA, respectively. Pregnancy was confirmed by ultrasound 32 d after TAI. Nonpregnant cows were placed on a second Ovsynch regimen and reinseminated 42 d after first TAI, and received oilseeds for 32 d after second TAI. Relative to prediet levels, FLAX increased the ALA content of milk by 187%. Ovarian ultrasonography was performed in 8 cows per diet; the mean diameter of ovulatory follicles was larger in cows fed FLAX compared with SUNF (16.9 ± 0.9 vs. 14.1 ± 0.9 mm), but follicle number, corpus luteum size, and plasma progesterone concentrations remained unaffected. Presumptive conception (progesterone < 1 ng/mL on d 0 and >1 ng/mL on d 21) rates to first TAI were greater in FLAX than in SUNF (72.6 vs. 47.5%). Pregnancy losses were lower in cows fed FLAX (9.8%) compared with those fed SUNF (27.3%). Including flaxseed in the ration of dairy cows increased the size of the ovulatory follicle and reduced pregnancy losses.

Key words: flaxseed, α -linolenic acid, conception rate, pregnancy loss

INTRODUCTION

Reproductive inefficiency is a major economic problem in dairy production, particularly during the phase of negative energy balance that occurs in early lactation. One way of improving energy status, and thereby reproductive performance, is to increase the energy density of the diet with fat supplementation. Reproductive performance is enhanced by dietary fat independent of energy status (Staples et al., 1998). In that regard, it has been hypothesized that dietary long-chain fatty acids (LCFA), particularly polyunsaturated fatty acids (PUFA), may improve reproductive performance of dairy cattle. Flaxseed is an excellent source of fat and PUFA, but it is not commonly fed to cattle. Previous studies have shown that 2 unique fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), present in substantial concentrations in fish oil, escape biohydrogenation in the rumen (Gulati et al., 1999), reduce PGF_{2 α} synthesis in the endometrium (Thatcher et al., 1997), delay luteolysis, and improve pregnancy rates (Burke et al., 1997) in lactating dairy cows. Alpha-linolenic acid (ALA, C18:3n-3), a precursor to the fatty acids EPA and DHA, is a major component of flaxseed oil. Feeding whole or processed flaxseed or infusing flaxseed oil into the abomasum of dairy cows increased LCFA concentration in milk (Kennelly and Khorasani, 1993), including that of EPA (Hagemeister et al., 1991). In one study, 30 dairy cows were inseminated after feeding a formaldehyde-treated, flaxseed-based diet or a control diet containing calcium salts of palm oil; conception rates were higher in cows given flaxseed compared with those given the control diet (88 vs. 50%; Petit et al., 2001). Studies with larger numbers of cows are needed to determine if these findings are repeatable.

We hypothesized that the high ALA content of flaxseed would improve reproduction by enhancing ovarian function, embryonic development, or both. The objec-

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tives were 1) to determine if a diet supplemented with flaxseed influenced ovarian function; and 2) to compare early embryo survival, conception rates, and pregnancy losses in dairy cows receiving a diet enriched in ALA vs. a control diet (very low in ALA). We also determined the effects of diets on milk yield, milk composition, milk fatty acid profiles, and on plasma concentrations of cholesterol, glucose, NEFA, and triglycerides.

MATERIALS AND METHODS

Location and Animals

This study was conducted at the dairy research unit of the University of Alberta, Edmonton, Alberta, Canada (53°34'N, 113°31'W). Cows were cared for according to the Canadian Council of Animal Care Guidelines (Olfert et al., 1993); all experimental procedures were approved by the Faculty Animal Policy and Welfare Committee, University of Alberta. Cows were housed and fed individually in a tie-stall barn equipped with an in-stall pipeline milking system. Cows were milked twice daily at 0430 and 1600 h and let out for exercise in a dirt-based covered area twice daily, for approximately 1 h each time. Cows were weighed monthly and body condition was scored once every 3 wk using a 5-point scale (1 = emaciated, and 5 = obese; Wildman et al., 1982).

Experimental Diets

Lactating, nonpregnant dairy cows ($n = 121$) with no overt clinical illnesses were randomly assigned to 1 of 2 dietary treatments starting 55 ± 22 d (mean $\pm$ SD) after calving. Rations were formulated to meet or exceed the requirements of a 650-kg cow at 50 DIM, producing 45 kg/d of milk with 3.5% fat (NRC, 2001). Diet composition and chemical analysis of the TMR are presented in Table 1. Diets were isocaloric and provided equal amounts of MP. Dry matter, protein, fat, and fatty acid content of the oilseeds are presented in Table 2. The concentrate mixtures were formulated to provide approximately 750 g of oil/d per cow from either rolled flaxseed (**FLAX**) or rolled sunflower seed (**SUNF**). Flaxseed and sunflower seed were processed in a roller mill (Lethbridge Industries Ltd., Lethbridge, AB, Canada) equipped with rollers that were 22.9 cm in diameter and 76.2 cm long, with 12 grooves per 2.54 cm. The rollers were adjusted to provide the coarsest roll possible, while ensuring that the seed coat was broken. Cows received their respective diets for at least 8 wk. Diets were delivered individually, once daily at 1000 h, with a self-propelled machine that mixed, weighed and dispensed rations (Data Ranger; American Calan Inc.,

Table 1. Ingredients and chemical composition of the experimental diets fed to dairy cows

Item	Sunflower seed	Flaxseed
Ingredients, % of DM		
Alfalfa hay, chopped	8.0	8.1
Alfalfa silage	25.3	25.1
Barley silage	8.0	8.1
Barley grain, dry rolled	16.1	16.4
Corn grain, ground	6.4	6.5
Molasses, beet	0.6	0.6
Corn gluten meal, dried	4.6	4.7
Soybean meal, solvent 48% CP	1.1	0
Sunflower seed, rolled	8.7	0
Flaxseed, rolled	0	9.0
Dried corn distillers grains with solubles	19.3	19.6
Minerals/vitamin premix	2.0	2.0
Composition, chemical analysis ¹		
CP, % of DM	23.6	22.6
NE _L , ² Mcal/kg of DM	1.72	1.72
ADF, % of DM	21.7	20.6
NDF, % of DM	32.6	32.7
Crude fat, % of DM	7.15	7.23
Crude fat, added, % of DM	3.36	3.21

¹Nutrient content of the TMR was calculated from chemical analyses of the forages and the oilseed concentrate mixtures; ADF and NDF of the concentrate mixtures were calculated using the chemical analyses provided in NRC (2001).

²Calculated using published values of feed ingredients (NRC, 2001).

Northwood, NH). To determine daily intake, orts were weighed before commencement of each feeding.

Ovarian Ultrasonography

Transrectal ultrasonography (Aloka-500V scanner with 7.5-MHz linear transducer, Aloka Co., Tokyo, Japan) was performed in a subset of 16 cows ($n = 8$ per diet) on alternate days, from d 0 to 21 (d 0 = day of insemination), to monitor ovarian follicular dynamics

Table 2. Dry matter, protein, and fat content, and fatty acid methyl esters present in oilseed

	Flaxseed	Sunflower seed
DM, %	94.7	94.6
CP, %	23.4	15.8
Crude fat, % of DM	35.7	38.8
Fatty acid, % of total lipid		
C16:0	5.8 ^a	7.0 ^b
C18:0	3.7 ^a	5.0 ^b
C18:1	16.7 ^a	12.2 ^b
C18:2	16.5 ^a	74.1 ^b
C18:3	56.7 ^a	0.12 ^b
C20:0	0.18 ^a	0.57 ^b
C20:3	0.13 ^a	0.75 ^b
C20:1	0.22 ^a	0.12 ^b
C24:0	0.09 ^a	0.21 ^b

^{a,b}Means within the same row followed by different superscripts differ ($P < 0.002$).

(Kassa et al., 2002). The number of class 1 (<6 mm), class 2 (6 to 9 mm), and class 3 (>9 mm) follicles located in each ovary was recorded. The relative locations of class 2 and class 3 follicles, and the diameter of the dominant follicle(s), were also recorded at each examination. The size of the corpus luteum was determined using built-in calipers, and recorded.

Collection of Milk and Blood Samples

Milk samples (for fatty acid analysis) were obtained from 8 cows of each dietary group 1 wk before (wk 0) and 4 wk after the initiation of diets. Samples were collected at 2 consecutive milkings, mixed in proportion to yield to obtain a composite sample, and stored at -20°C until processed for fatty acid analysis.

Blood samples were collected from these 16 cows on d -10, -3, daily from d 0 to 7, and on d 14, 16, 18, 21, and 24 to determine progesterone concentrations in plasma. Additional blood samples were collected from cows at 14-d intervals for 6 wk, starting on the day of initiation of diets (4 samples per cow at 0, 2, 4, and 6 wk after start of diets) to determine plasma concentrations of cholesterol, glucose, NEFA, and triglycerides. Blood samples were collected from all remaining cows on d -10, -3, 0, 21, and 24 for determination of plasma progesterone concentrations. Blood samples were obtained on d 7 from 92 of the 121 cows. All blood samples (7 to 10 mL) were collected by coccygeal venipuncture into evacuated tubes (Vacutainer, Becton Dickinson and Co., Franklin Lakes, NJ) containing sodium heparin and were chilled immediately after collection, and centrifuged ($1,500 \times g$, 20 min) within 2 h; plasma was harvested, and stored at -20°C until further processing.

Reproductive Management

To eliminate the variations associated with estrus detection, and for managerial convenience, timed artificial insemination (TAI) was performed on all cows following a protocol for synchronization of ovulation (Ovsynch; Pursley et al., 1995). Ovarian status was presynchronized by 2 injections of $\text{PGF}_{2\alpha}$ (500 μg of cloprostenol; Estrumate, Schering Canada Inc., Pointe Claire, QC, Canada) given 14 d apart, and the Ovsynch protocol was initiated 12 d after the second of the 2 presynchronizing $\text{PGF}_{2\alpha}$ treatments. The Ovsynch protocol involved 2 GnRH treatments (gonadorelin acetate, 100 μg ; Fertiline, Vetoquinol NA Inc., Lavaltrie, QC, Canada) given 9 d apart with a $\text{PGF}_{2\alpha}$ treatment given 7 d after the first GnRH treatment. Cows were TAI approximately 16 h after the second GnRH treatment.

Presumptive conception rate at d 24 was assessed based on plasma progesterone concentrations at 0, 21,

and 24 d after TAI. Cows were presumed conceived on d 24 only if the progesterone concentration was <1 ng/mL on d 0 and ≥ 1 ng/mL on both d 21 and 24. Pregnancy diagnosis was performed by transrectal ultrasonography 32 d after TAI, and reconfirmed by transrectal palpation of uterine contents approximately 90 d after TAI. Cows were monitored for return to estrus and bred again if detected in estrus. Once pregnancy was confirmed at 32 d, pregnant cows were removed from the experimental diets. Nonpregnant cows were immediately placed on the Ovsynch protocol for a second time, and rebred by TAI 10 d later (42 d after the first TAI). In these cows, the experimental diets continued until pregnancy diagnosis, 32 d after second TAI.

Cows with progesterone concentrations <1 ng/mL on d -10, -3, and 0 were considered anestrous. Cows with progesterone concentrations ≥ 1 ng/mL on d -3 and <1 ng/mL on d 0 were considered responders to the synchronization treatment. When d 7 samples were available ($n = 92$), the progesterone concentrations of d 7 (≥ 1 ng/mL) were considered in addition to that of d -3 and 0 (as described above), for determining the response to synchronization treatment.

Analyses of Feed, Milk, and Plasma

Feed samples were dried at 60°C for 72 h, and ground through a 1-mm screen (Thomas-Wiley mill, model 4; Thomas Scientific, Swedesboro, NJ). Samples were analyzed for CP ($6.25 \times \text{N}$; Leco FP-428 nitrogen determinator, Leco Corp., St. Joseph, MI), NDF, and ADF (Ankom filter bag technique, Ankom Company, Macedon, NY). Dry matter was determined by drying samples overnight at 135°C .

Concentrations of fat and protein in milk were determined using a midinfrared analyzer (Milko Scan 605, A/S N Foss Electric, Hillerød, Denmark) at the Central Milk Testing Laboratory in Edmonton (AB, Canada).

Milk fat extraction and transmethylation were performed according to the procedure of Chouinard et al. (1999) and fatty acid methyl esters were analyzed on a Varian 3600 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a septum programmable injector and flame-ionization detector. Procedures for fatty acid analysis of oilseeds were as described by Sukhija and Palmquist (1988).

Plasma progesterone concentrations were determined using a solid-phase radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA). The intra- and interassay coefficients of variation were 5.3 and 8.0%, respectively. Plasma concentrations of glucose, NEFA, triglycerides, and cholesterol were analyzed by photometric methods using a clinical chemistry system (Dade Dimension XL, Dade

Behring, Inc., Mississauga, ON, Canada) in a commercial laboratory (Central Veterinary Pathology Laboratory Ltd., Edmonton, AB, Canada).

Statistical Analyses

Data were analyzed with ANOVA for a completely randomized design. Repeated measures on DMI, BCS, BW, ovarian follicles, concentrations of hormones and metabolites in plasma, and that of milk fatty acids were analyzed using the MIXED procedure of SAS (SAS Institute, 2000), 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 time j , $(\alpha\beta)_{ij}$ is the effect of treatment by time interaction, and e_{ijk} is the residual error. The Kenward-Roger procedure was used for approximating the degrees of freedom (Kenward and Roger, 1997). Animal nested within treatment was considered as the subject on which repeated measures were taken and covariance structures modeled. Based on the smallest values of fit statistics for Akaike's information criterion, Akaike's information criterion corrected, and Bayesian information criterion, the covariance structure of the repeated measurements for each variable was modeled separately and an appropriate structure fitted (Littell et al., 2000). Variables with only 2 time-point comparisons (wk 0 and 4), such as yields of milk, fat, and protein, and concentrations of fat and protein, were analyzed using a model similar to the one described above, but time and interaction with time were not considered. Preplanned treatment comparisons were made with the PDIFF option and declared significant at $P < 0.05$. Categorical data (presumptive conception rate at 24 d post-TAI, actual conception rate at 32 d, estimated embryo survival, proportion of cows calving, and calf sex) were analyzed using a χ^2 test. The influence of cow, parity, DIM, BCS, BW, and month of TAI on pregnancy and embryo survival was tested using a linear model procedure. The influence of the above variables on pregnancy was tested using a stepwise regression (forward selection) procedure. Odds ratio estimates for pregnancy risk were computed using the LOGISTIC procedure of SAS (SAS Institute, 2000). To determine if cows were distributed evenly between the 2 dietary groups by parity and DIM, frequency tables were generated, and results analyzed by a t -test. For all data, differences were considered significant if a probability value of ≤ 0.05 was obtained. Probability values of 0.05 to 0.10 were considered as trends.

Table 3. Least squares means of DMI, BW, BCS, milk yield, and milk components in dairy cows fed a TMR that included either rolled flaxseed (FLAX) or rolled sunflower seed (SUNF)

Variable	FLAX	SUNF	SEM
DMI, kg/d	22.5 ^a	21.3 ^b	0.50
BW, kg	612.4	609.9	5.60
BCS	2.81	2.82	0.03
Milk yield, kg/d	36.7	36.0	0.94
Milk fat, %	3.69	3.37	0.07
Milk fat yield, kg/d	1.33	1.20	0.04
Milk protein, %	3.01	2.97	0.08
Milk protein yield, kg/d	1.09	1.06	0.04

^{a,b}Means with different superscripts within the same row tended to differ ($P = 0.08$).

RESULTS

Intake, BW, and BCS

Dry matter intake tended ($P = 0.08$) to be lower for cows on SUNF compared with those on FLAX diet. Body weight increased with time ($P < 0.01$), but was similar between diets (Table 3). There were no differences in BCS between diets.

Milk Yield, Composition, Fatty Acid Profile, and Plasma Metabolites

Milk yield and milk protein concentration and yield were not significantly affected by either diet (Table 3) or time. Concentration and yield of milk fat did not change from wk 0 to 4 in cows fed FLAX, whereas milk fat concentration and yield declined from 3.54 to 3.20% ($P < 0.05$) and from 1.25 to 1.14 kg/d ($P < 0.05$), respectively, from wk 0 to 4 in cows fed SUNF.

Dietary treatments had a significant impact on milk fatty acid composition (Table 4). The treatments had a differential effect on short-chain fatty acids in milk; they were reduced ($P < 0.05$) by SUNF, but not by FLAX feeding. In general, both treatments depressed ($P < 0.05$) medium-chain fatty acids and increased LCFA in milk. Of the 18-carbon fatty acids, ALA (18:3) content nearly tripled (187% increase) with FLAX ($P < 0.05$), and increased by 22% with SUNF ($P < 0.05$). Linoleic acid (18:2) content was increased by 122% with SUNF ($P < 0.05$) and by 74% with FLAX ($P < 0.05$). The proportion of milk 18:2 *cis*-9, *trans*-11 (conjugated linoleic acid) doubled with FLAX and tripled with SUNF ($P < 0.01$).

Plasma concentrations of glucose, triglycerides, cholesterol, and NEFA did not differ ($P > 0.10$) between diets (Table 5), but glucose concentrations were higher at wk 6 than in earlier weeks in both dietary groups.

Table 4. Effect of feeding flaxseed (FLAX) or sunflower seed (SUNF) on milk fatty acid (FA) composition in dairy cows¹

Fatty acids, % of total	FLAX		SUNF		SEM
	Before	After	Before	After	
Unsaturated:saturated	0.53 ^a	0.70 ^b	0.58 ^c	0.88 ^d	0.03
SCFA ²	7.46	6.99	6.80 ^c	5.69 ^d	0.27
MCFA ³	46.45 ^a	37.08 ^b	44.68 ^c	34.62 ^d	0.68
LCFA ⁴	43.37 ^a	51.15 ^b	45.74 ^c	56.28 ^d	0.82
Monounsaturated FA ⁵	30.98 ^a	33.89 ^b	32.84 ^c	38.77 ^d	0.94
Polyunsaturated FA ⁵	2.74 ^a	5.27 ^b	2.77 ^c	6.25 ^d	0.15
C18:2	1.89 ^a	3.29 ^b	1.94 ^c	4.31 ^d	0.09
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.47 ^a	0.98 ^b	0.43 ^c	1.43 ^d	0.06
C18:3n-3	0.31 ^a	0.89 ^b	0.32 ^c	0.39 ^d	0.01

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

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

¹Before = milk samples were taken 1 wk before cows were placed on experimental diets; After = milk samples were taken 3 wk after cows were placed on experimental diets.

²Short-chain FA: C_{4:0} to C_{10:0}.

³Medium-chain FA: C_{11:0} to C_{17:0}.

⁴Long-chain FA: >C_{18:0}.

⁵C12:1, C14:1, C16:1, C17:1, C18:1 *trans*-11, C18:1n-9, C18:1n-7, C18:1n-6, C20:1n-12, C20:1n-9, C22:1n-9.

⁶C18:2, C18:2 *cis*-9, *trans*-11, C18:3n-3, C20:4n-6.

Ovarian Characteristics, and Plasma Progesterone Concentrations

The ovulatory follicle (before the first AI, d 0) was larger in cows fed FLAX compared with those fed SUNF (16.9 ± 0.91 vs. 14.1 ± 0.86 mm; $P < 0.05$). Mean number of class 1, 2, or 3 follicles, growth rate of nonovulatory dominant follicle, or size of corpus luteum was not affected ($P > 0.10$) by diet (data not shown). There was no effect ($P > 0.10$) of diet, or diet $\times$ day interaction for plasma progesterone concentrations (FLAX 4.52 ± 0.45 ; SUNF 4.13 ± 0.44 ng/mL). Although mean serum progesterone concentrations were not different between diets, mean progesterone concentration was higher at the time of insemination (d 0) in cows fed FLAX compared with those fed SUNF (0.41 ± 0.06 vs. 0.15 ± 0.06 ng/mL; $P < 0.05$) in the subset of 16 cows.

Conception Rates and Pregnancy Losses

Presumptive conception rate (d 24) to the first TAI was higher ($P < 0.01$) in cows fed FLAX (72.6%) than

in those fed SUNF (47.5%). Actual conception rate (d 32) to first TAI tended to be higher ($P < 0.07$) in cows fed FLAX (48.4%) compared with those fed SUNF (32.2%). In the stepwise regression analysis, the overall model accounted for 12.6% of the variation in pregnancy ($P = 0.02$), with cow, DIM, and parity contributing 9.5, 1.6, and 1.5%, respectively. Based on the odds ratio of 0.50, the pregnancy risk associated with FLAX diet was 2-fold (1/0.5) higher.

About 12% (14 of 121) of the cows were anestrus (based on plasma progesterone concentrations); they were equally distributed between the dietary groups. When only the cyclic cows ($n = 107$) were considered, d 32 conception rates to first TAI tended to increase slightly for FLAX vs. SUNF (53.6 and 37.3%, respectively; $P < 0.09$). The synchronization response to the Ovsynch treatment preceding first TAI, as determined by progesterone concentrations, was 83%.

There was, apparently, a higher rate of early (d 0 to 24) embryo survival in cows fed FLAX compared with those fed SUNF. However, the difference between pre-

Table 5. Effect of feeding flaxseed (FLAX) or sunflower seed (SUNF) on plasma metabolites in dairy cows

Plasma variable	FLAX	SUNF	SE	P-value		
				Diet	Week	Diet $\times$ Week
Cholesterol, mmol/L	6.29	5.39	0.47	0.18	0.52	0.81
Glucose, mmol/L	2.47	2.31	0.09	0.21	<0.01	0.60
NEFA, mEq/L	0.30	0.41	0.10	0.33	0.25	0.47
Triglycerides, mmol/L	0.15	0.16	0.01	0.80	0.31	0.55

Table 6. Pregnancy losses, and calving details, in dairy cows fed flaxseed (FLAX) or sunflower seed (SUNF) confirmed pregnant by ultrasonography 32 d after timed AI

Observation	Dietary treatment	
	FLAX, n (%)	SUNF, n (%)
Cows confirmed pregnant (32 d)	42	35
Early pregnancy loss (32 to 90 d)	2/42 (4.8)	4/35 (11.4)
Cows sold (after 90 d)	1/40 (2.5)	2/31 (6.5)
Late pregnancy loss (90 d to term)	2/39 (5.1)	5/29 (17.2)
Overall pregnancy loss (early and late)	4/41 (9.8) ^a	9/33 (27.3) ^b
Proportion of cows calving	37/41 (90.2) ^a	24/33 (72.7) ^b
Twin calving	2	2
Assisted calving	11	9
Calves born ¹	39	26
Male/female ratio	15/24	13/13
Stillbirths	1	1

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

¹Includes twins and stillborn calves.

sumptive (d 24) and actual (d 32) conception rates indicated that embryo survival rate from d 24 to 32 did not differ between SUNF (67.9%) and FLAX (66.7%). Cows were evenly distributed by parity and DIM between dietary groups. Neither parity nor DIM influenced conception rates; however, parity affected embryo survival, with older cows (>3 lactation) having a lower embryo survival rate than younger (≤ 3 lactation) cows (38.6 vs. 83.8%, $P < 0.02$). Conception rates to the second TAI (37.5% for FLAX and 41.0% for SUNF) and overall pregnancy rates (combined for both TAI; 67.7% for FLAX and 59.3% for SUNF) were not different between diets ($P > 0.10$).

Three pregnant cows (1 FLAX, 2 SUNF) left the herd before calving; therefore, calving data were unavailable from these animals. Pregnancy losses were lower ($P < 0.05$) in cows fed FLAX compared with those fed SUNF (Table 6).

DISCUSSION

Dietary PUFA and their influence on reproductive processes in cattle have been discussed by several authors in recent years (Abayasekara and Wathes, 1999; Thatcher et al., 1997, 2004). In a previous small-scale study (Petit et al., 2001), a flaxseed-based diet increased conception rate in dairy cows compared with control cows fed a diet containing Megalac, a calcium soap of palm oil. Although the diets used in this study and in that of Petit et al. (2001) were not identical, both studies had a similar hypothesis—that the high ALA content of flaxseed would have a positive influence on reproductive function. Sunflower seed was chosen for the control ration in the present study because of its very low ALA content.

Ovarian Function, Conception Rates, and Pregnancy Losses

Ovulatory follicles were significantly larger in cows of the FLAX group compared with those in the SUNF group, but there were no differences between diets in the number of class 1, 2, or 3 follicles, or in the total number of follicles. Higher fertility was reported in cows ovulating larger follicles even without an increase in progesterone concentrations in the subsequent luteal phase (Peters and Pursley, 2003). Therefore, the ovulation of larger follicles in FLAX cows may have contributed to the improvement in conception rate, perhaps due to a more viable oocyte. As in the present study, Petit et al. (2001) found no dietary effects on the number of class 1, class 2, or class 3 follicles. In another study (Robinson et al., 2002), the number of medium-sized (class 2 equivalent) follicles increased in cows fed diets enriched in protected ALA and linoleic acid, and the diameter of the first dominant follicle was larger in cows fed linoleic acid, but not in those fed ALA.

The overall plasma progesterone concentrations did not differ between dietary groups in the present study. In contrast, Robinson et al. (2002) reported lower progesterone concentrations in cows fed diets enriched in PUFA (either ALA or linoleic acid). The mean progesterone concentration at TAI (d 0, 64 to 68 h after PGF_{2 α} treatment) was higher in FLAX (0.41 ng/mL) than in the SUNF (0.15 ng/mL) in the subset of 16 cows subjected to intensive ovarian ultrasonography and frequent blood sampling. However, when all cows in the study were considered, there were no differences in plasma progesterone concentrations at d 0. Burke et al. (1997) reported significantly higher concentrations of progesterone 2 d after PGF_{2 α} injection in cows fed menhaden fish meal, suggesting delayed luteal regres-

sion in cows consuming the n-3 (omega-3) fatty acids, EPA and DHA. Similarly, supplementation with menhaden fish meal reduced $\text{PGF}_{2\alpha}$ secretion in dairy cows (Thatcher et al., 1997). Therefore, a diet enriched in ALA (due to the inclusion of flaxseed) can potentially suppress $\text{PGF}_{2\alpha}$ production, because ALA can be synthesized into EPA and DHA through desaturation and elongation (Abayasekara and Wathes, 1999).

Presumptive conception rate at 24 d post-TAI and the proportion of pregnancies carried to term were higher in FLAX than in SUNF cows. We recognize that early pregnancy determination (presumptive conception) based on plasma progesterone concentrations has some limitations. However, when progesterone concentrations in 3 (as in the present study) or more samples obtained between d 0 and 24 were considered, the pregnancy prediction was about 90% accurate (Kaul and Prakash, 1994). Although there was no difference in the embryo survival rate between d 24 and 32 post-TAI, the numerically increased conception rates in FLAX cows occurred early, apparently through a higher rate of embryonic survival before d 24 (increased presumptive conception rate). These results are consistent with those of Petit et al. (2001), who reported increased conception rates in cows given a ration enriched in ALA by supplementation with formaldehyde-treated flaxseed, compared with cows given a control ration (low in ALA) supplemented with Megalac. In their study, they excluded anestrous or cystic cows from insemination, and cows were inseminated only after detection of standing estrus. In contrast, no cow was excluded, and all cows received TAI without estrous detection in the present study. Furthermore, whereas the first insemination occurred at 83 d postpartum in the present study, inseminations began much later (at 105 d) in the other study (Petit et al., 2001), and continued until all cows were detected in estrus. Lower milk production levels (19 kg/d) in cows used by Petit et al. (2001) compared with that (36 kg/d) of cows in the present study could have also contributed to the higher fertility. Conception rates in the present study were within the current range reported for lactating Holstein cows managed by TAI in Canada and the United States. In a recent study using 983 beef heifers, Colazo et al. (2004) compared the effects of dietary inclusion of whole flaxseed and whole sunflower seed in a TAI program and found no effect of diets on conception rates. However, the quantity of oilseeds included was considerably lower (1 kg/d) than that used in the present study, suggesting that higher levels of supplementation, processing, or both, are necessary to enhance conception rates.

Pregnancy losses were lower in the FLAX group, resulting in a higher proportion of pregnancies carried to

term. Even though cows received the experimental diets for only 60 d (i.e., from 28 d before TAI to 32 d after TAI), the ALA-enriched diet during early gestation improved maintenance of pregnancy. These findings could have major implications for nutritional management of reproductive function and warrant further investigation. Because ALA can be transformed to DHA through desaturation and elongation (Abayasekara and Wathes, 1999), it is plausible that there was an increased availability of DHA for transport to the embryo in FLAX-fed cows, thus enhancing embryonic development and reducing pregnancy losses. Although this remains a possibility, most of the benefit (in terms of embryo survival) in FLAX cows occurred earlier than 24 d, a period when placental development and attachment have barely begun. Alternatively, enhancement of early embryonic development by the FLAX diet may have occurred through an attenuation of $\text{PGF}_{2\alpha}$ secretion around the time of maternal recognition of pregnancy, or through other embryotrophic mechanisms. However, these remain speculations at this time.

High conception rates (Tenhagen et al., 2004) and improved pregnancy retention (Starbuck et al., 2004) have been reported in primiparous dairy cows. Parity did not influence conception rate in the present study; however, pregnancy losses were higher in older cows (exceeding 3 lactations) than in younger animals.

Intake, BW, and BCS

Inclusion of rolled flaxseed at approximately 9% on a DM basis had no negative effect on intake in the present study. Similarly, inclusion of whole flaxseed at levels of 5 to 15% (Kennelly and Khorasani, 1993) or feeding formaldehyde-treated flaxseed at up to 17% of DM (Petit et al., 2001) did not reduce DMI in dairy cows. In the present study, DMI was slightly reduced in SUNF cows, which is in agreement with the observation of Petit (2003), who found lower DMI in cows fed sunflower seed compared with those fed either whole untreated or whole formaldehyde-treated flaxseed.

Milk Yield and Milk Composition

Milk yield did not differ between dietary groups in the present study. This is in agreement with 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 higher in cows fed flaxseed compared with those fed sunflower seed (Petit et al., 2004). Concentration and yield of milk fat de-

clined over time in SUNF cows in the present study, whereas they remained unaffected in FLAX cows. 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 protein percentage and yield did not differ between the dietary groups in our study. Petit (2003) reported a higher 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).

Milk Fatty Acid Composition

The decrease in medium-chain fatty acids and increase in LCFA concentrations of milk observed for cows fed FLAX and SUNF is in general agreement with the results reported in several previous studies (Goodridge et al., 2001; Petit, 2003; Petit et al., 2004) of cows fed diets enriched in PUFA. Significant increases occurred in the proportions of both ALA and linoleic acid with both dietary treatments, the most notable being ALA, which nearly tripled on the FLAX diet. The highest concentration of ALA in milk after feeding flaxseed, in most previous studies, was similar to what was seen in the present study. Mean concentrations of ALA usually range from 0.9 to 1.1% of total fatty acids, although ALA concentrations as high as 3.7 to 6.4% of total fatty acids have been reported after feeding protected (formaldehyde-treated) flaxseed (Goodridge et al., 2001). Cows fed either FLAX or SUNF in the present study had substantial increases in milk PUFA, implying that sufficient quantities of PUFA present in the oilseeds escaped biohydrogenation in the rumen and ultimately were transferred into milk fat.

Plasma Metabolites

Dietary treatments did not significantly affect cholesterol, glucose, NEFA, or triglycerides in the present study. However, glucose concentrations were higher in wk 6 than in other weeks, in both FLAX and SUNF groups. Because of the inclusion of oilseeds in the diets, we expected a time-dependent increase in cholesterol as the duration of diets increased, but this did not occur. In a previous study, cows fed Megalac had a greater

increase in blood cholesterol than those fed flaxseed (Petit et al., 2001). The authors suggested that the cholesterol-depressing effect of ALA in flaxseed, as reported by Cunnane et al. (1993) in humans, could have contributed to the difference in the rate of increase in blood cholesterol between control (Megalac) and flaxseed-fed cows. Whether ALA depresses cholesterol in cattle remains to be determined.

CONCLUSIONS

Including flaxseed in the ration of dairy cows increased the diameter of the ovulatory follicle and reduced pregnancy losses. Although these findings add further strength to the concept that dietary fatty acids enhance reproductive processes in cattle, the underlying mechanisms through which an ALA-enriched diet may improve reproductive function remain unclear. Despite the promising nature of the present findings, larger field trials must be undertaken before advocating producers to include flaxseed in dairy rations as a nutritional strategy for improving reproductive performance.

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