Postpartum responses of dairy cows supplemented with n-3 fatty acids for different durations during the peripartal period

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ABSTRACT

The objective of this study was to determine the effect of different durations of n-3 supplementation during the peripartal period on production and reproduction performance of Holstein dairy cows. Thirty-two Holstein dry cows (16 multiparous and 16 primiparous) were blocked within parity for similar expected calving dates 8 wk before calving. Cows within blocks were assigned randomly to 1 of 4 treatments: (1) control without n-3 fatty acid (FA) supplementation during the dry period; (2) n-3 FA supplementation during the whole dry period (8 wk); and (3) n-3 FA supplementation during the early dry period (first 5 wk; far-off), or (4) n-3 FA supplementation during the late dry period (last 3 wk; close-up). All cows received the same diet without n-3 FA after calving for the first 6 wk of lactation. Ovaries of each cow were examined 10, 17, 24, and 34 d from calving (calving = d 0) by transrectal ultrasonography to determine follicular development. Blood samples were collected at 14-d intervals starting on the first day of the dry period (8 wk before expected calving) to determine plasma concentrations of glucose, β -hydroxybutyrate, nonesterified fatty acids, urea N, aspartate aminotransferase, and insulin. Blood samples were also collected on d 1, 10, 17, 24, 31, and 38 postpartum for determination of progesterone concentration. Milk yield was recorded daily throughout the experiment and samples were taken twice weekly (Monday and Thursday mornings) for analysis of fat, protein, and lactose. Yields of milk and 4% fat-corrected milk and milk composition were similar among treatments except for fat proportion, which tended to be lower in cows that were fed n-3 FA throughout the dry period. We observed no differences among treat-

ments for plasma concentrations of metabolites and hormones. The cows that were fed in the 3 n-3 FA treatments had larger ovulatory follicles compared with those fed the controlled diet. Treatments did not differ significantly in terms of the number of days open, day to first service, or number of services per pregnancy. In conclusion, n-3 FA supplementation throughout the dry period or in the early or late prepartal period had no carryover reproductive postpartum benefits and no effect on the production of Holstein dairy cows.

Key words: dairy cow, fatty acid, ovarian function, progesterone, uterine response

INTRODUCTION

Postpartum supplementation of the dairy cow diet with n-3 PUFA helps to improve fertility by influencing follicular growth and ovulation. Indeed, feeding fat supplements such as calcium salts of PUFA increases the number of large follicles (>10 mm) after d 25 postpartum in a synchronized estrous cycle (Lucy et al., 1993). Moreover, Dirandeh et al. (2013a) reported increased diameter and number of follicles in cows fed n-3 PUFA. Cows fed rolled flaxseeds, which are rich in the n-3 α -linolenic acid (56.7% of total FA), had larger mean diameter of the ovulatory follicle than those fed rolled sunflower seeds, which are rich in n-6 PUFA (16.9 vs. 14.1 mm; Ambrose et al., 2006). Conversely, cows fed flaxseed had fewer small follicles than those fed soybeans, which are rich in n-6 PUFA (Ponter et al., 2006). Recent studies have shown a 23% increase in the diameter of large follicles (>10 mm) in cows fed diets rich in n-3 PUFA compared with those fed MUFA (Ambrose et al., 2006; Bilby et al., 2006b; Santos et al., 2008).

Although these studies indicate that the effect of fat sources on follicular growth depends on FA profile, very few studies have been conducted on the effects of prepartal FA supplementation on follicular development,

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resumption of cyclicity, and reproductive performance of dairy cows. One study has shown that cows fed diets enriched in linoleic or linolenic acid during the prepartal period had a reduced interval from calving to first ovulation compared with those fed a diet enriched in oleic acid, with no beneficial effect on fertility (Colazo et al., 2009). Conversely, continuous supplementation with a source of n-3 FA, such as flaxseed, from the prepartum period until d 50 of pregnancy improves fertility of dairy cows, as shown by lower embryo mortality and increased conception rate (Petit and Benchaar, 2007). Moreover, feeding flaxseed compared with no lipids or a source of SFA from 6 wk before calving may be a useful strategy to prevent the development of fatty liver in the transition dairy cow, as indicated by increased hepatic concentrations of glycogen and decreased liver concentrations of triglycerides after calving (Petit et al., 2007). Higher DMI for cows fed flaxseed than for those fed soybeans (as a rich source of n-6 FA) or calcium salts of palm oil (Petit and Benchaar, 2007) may contribute to improving energy balance; this explains, in part, the enhanced fertility of cows supplemented with n-3 FA.

Although the postpartal effects of n-3 FA supplementation on fertility have been well documented, no information has been found on the effect of the duration of dry-period supplementation with n-3 FA on postpartal reproductive parameters and energy balance of dairy cows. It is also unknown whether the length of the supplementation period results in any carryover reproductive postpartum benefits. Therefore, the objectives of the present experiment were to determine the effects of different periods (early, late, or whole dry) of prepartal supplementation with n-3 FA on follicular development, number of days open, and resumption of cyclicity in dairy cows. The hypotheses were that different durations and periods of supplementation would have a differential influence on follicular development and carryover effects on fertility.

MATERIALS AND METHODS

Cows and Treatments

Thirty-two Holstein dry cows (multiparous $n = 16$, 714 ± 51 kg of BW; primiparous $n = 16$, 568 ± 34 kg of BW) were blocked within parity for similar expected calving dates, for 9 blocks of multiparous cows and 7 blocks of primiparous cows. The experiment was conducted from 8 wk before calving until wk 6 of lactation and the treatments were fed only during the peripartal period. The cows were dried off 8 wk before expected calving and the cows within blocks were assigned randomly to 1 of 4 treatments: (1) control without n-3 FA

supplementation during the dry period; (2) supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Worksop, UK) during the whole dry period (8 wk); (3) supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk; far-off period); or (4) supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk; close-up period). The FA profile of Optomega, expressed as a percentage of total FA, included 22% SFA, 45% MUFA, and 33% PUFA (4% *cis*-6 18:2, 2% *cis*-3 18:3, 2% 18:4, 2% 20:4, 8% 20:5, 3% 22:5, and 12% 22:6). All cows received the same diet without n-3 FA after calving for the first 6 wk of lactation (Table 1 and Table 2). Body weight and BCS [based on 1 (thin) to 5 (obese) scale; Edmonson et al., 1989] were determined on the first day of the experiment and on the day of calving.

Diets were fed once daily (1500 h) for ad libitum intake (10% refusals on as-fed basis) during the whole experiment. Cows were fed individually and milked 3 times daily at 0615, 1430, and 2300 h. Milk production was recorded at every milking. Milk samples were taken weekly (Monday and Thursday, twice a day, milk samples were pooled on a yield basis to give one sample per week) and analyzed for fat, protein, and lactose by infrared spectroscopy (AOAC International, 2000; method 972.16). Total mixed rations were sampled every 2 wk and pooled on a 4-wk basis. Feed samples were dried at 65°C for 24 h and then ground to pass through a 1-mm screen (Retsch SM 100, Retsch GmbH, Haan, Germany). Samples were analyzed for DM, CP, NDF, and ADF according to the methods of the AOAC International (2000). This experiment was carried out according to the procedures laid out by the Iranian Ministry of Agriculture (experimental permission No. 923).

Fatty Acid Analysis

Fatty acids in diets were extracted by using the method of Folch et al. (1957) with some modifications. Chloroform:methanol (2:1, vol/vol) containing 0.005% butylated hydroxytoluene (as antioxidant) was added (usually 5 mL of solvent added to 50–100 μ L of sample), mixed vigorously for 1 min, and then left at 4°C overnight. One milliliter of 0.9% NaCl was added and the solution was mixed again. The lower chloroform phase was transferred to a clean tube and care was taken not to transfer any remaining aqueous phase along with the chloroform. Residue in original tubes was extracted twice with 2.0 mL of chloroform and left to separate; then, the chloroform phase was combined after each extraction. Fatty acid methyl esters were prepared by methods similar to those described previously (Morrison and Smith, 1964), using boron

Table 1. Ingredient and chemical composition of diets

Item	Treatment ¹			
	Control	Whole DP	Far-off	Close-up
Ingredient (% of DM)				
Alfalfa hay (mid bloom)	30.00	30.00	22.67	22.67
Corn silage	23.35	23.35	26.02	26.02
Barley meal	3.15	3.15	0	0
Rolled barley grain	2.10	2.10	22.01	22.01
Wheat straw	26.17	26.17	3.30	3.30
Wheat bran	6.36	6.36	1.68	1.68
Soybean meal (48% CP)	0	0	2.44	2.44
Pea	0	0	1.70	1.70
Corn gluten meal (60% CP)	0	0	3.43	4.33
Cottonseed meal	0	0	2.44	2.44
n-3 FA supplement	0	1.53	0	1.53
Canola meal (solvent)	3.15	3.15	0	0
Sunflower meal	2.20	2.20	0	0
Palm oil ²	0.63	0	0.51	0
Monensin	0	0	0.15	0.15
Vitamin and mineral premix ³	0.054	0.054	3.95	3.95
Glycolaine ⁴	0	0	1.52	1.52
Toxin binder	0	0	0.08	0.08
Salt	0.28	0.28	0	0
Sodium bicarbonate	0	0	1.14	1.14
Chemical composition				
NE _L (Mcal/kg of DM)	1.41	1.42	1.56	1.57
Fat (% of DM)	3.20	3.20	3.61	3.70
CP (% of DM)	14.3	14.3	16.11	16.20

¹Control = no supplementation with n-3 FA during the dry period; whole DP = supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Worksop, UK) during the whole dry period (8 wk); far-off = supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk); and close-up = supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk).

²Energizer-RP10 (IFFCO, Johor BahruJohor, Malaysia).

³Contained (per kilogram): 16,000,000 IU of vitamin A; 3,200,000 IU of vitamin D; 48,000 IU of vitamin E; 24.0 g of Mn; 24.0 g of Zn; 24.0 g of Fe; 12.8 g of Cu; 1.44 g of I; 0.32 g of Se; and 0.32 g of Co.

⁴DSL Chemical (Shanghai) Co. Ltd., Shanghai, China.

trifluoride (BF₃):methanol reagent (1 mL of 14% BF₃/methanol reagent). Lipid samples were mixed with 1 mL of hexane in 16-mL glass tubes with Teflon-lined caps. The BF₃/methanol reagent (1 mL) was added and the mixture was heated at 90 to 110°C in a metal block for 1 h and cooled to the room temperature;

methyl esters were extracted in the hexane phase after 1 mL of H₂O was added. The samples were allowed to stand for 20 to 30 min, and then the upper hexane layer was removed and concentrated under nitrogen. Tubes were mixed by vortex and the hexane phase was transferred to GLC vials and dried under a stream of

Table 2. Fatty acid profile (g/100 g of FA) of diets

FA	Treatment ¹			
	Control	Whole DP	Far-off	Close-up
<C16:0	14	2.14	3.73	1.12
C16:0	24.12	23.67	24.01	24.99
C16:1	0	1.02	1.5	1.30
C18:0	3.35	3.37	3.48	3.72
C18:1	21.0	24.71	23.90	24.25
C18:2	32.95	36.86	35.49	35.72
C18:3	4.58	5.57	5.93	6.49
Eicosapentaenoic acid (C20:5)	0	1.17	1.01	1.28
Docosahexaenoic acid (C22:6)	0	1.49	0.95	1.13

¹Control = no supplementation with n-3 FA during the dry period; whole DP = supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Worksop, UK) during the whole dry period (8 wk); far-off = supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk); and close-up = supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk).

nitrogen. Fatty acid methyl esters were analyzed by gas chromatography using a fully automated HP5890 system equipped with a flame-ionization detector (Agilent Technologies, Wilmington, DE), as described previously (Kang et al., 1992). The column parameters were as follows: an initial column temperature of 195°C was maintained for 2 min, the temperature was then programmed at 2°C/min to 205°C, 1°C/min to 214°C, 15°C/min to 240°C, and held at this temperature for 10 min. Injector and detector temperatures were 250°C and 300°C, respectively. Helium was used as the carrier gas at an initial flow rate of 1 mL/min for 8 min, which increased to 1.3 mL/min for 4.2 min and then to 1.9 mL/min. Peaks were identified by comparison with FA standards (Nu-Chek Prep, Elysian, MN), and area and percentage of each resolved peak were analyzed using a Perkin-Elmer M1 integrator (Perkin-Elmer, Waltham, MA).

Ultrasonography Examination and Estrus Detection

Ovaries of each cow were examined on d 10, 17, 24, and 34 after calving (calving = d 0) by transrectal ultrasonography using a real-time linear scanning ultrasound diagnostic system (ECM AL, Agrosca, Angoulême, France) previously described by Dirandeh et al. (2009). All follicles ≥ 3 mm were counted. Follicles were grouped into 3 diameter classes for analyses: small (3.0 to 4.9 mm), medium (5.0 to 9.9 mm), and large (≥ 10 mm). Diameter of each follicle was calculated as the average length and width of the antrum. Size of the corpus luteum and locations and diameters of the largest and second-largest follicles on each ovary were recorded. The dominant follicle of each follicular wave was defined as the largest newly emerged ovarian follicle that was 2 mm greater than the second-largest follicle that grew linearly (Dirandeh et al., 2009). The cows were observed visually around the estrus period for estrus detection twice daily at approximately 12-h intervals for a minimum of 30 min per period. Detection of estrus was confirmed by the disappearance of the dominant follicle and the subsequent appearance of a corpus luteum as determined by ultrasonography examination (Dirandeh et al., 2009).

Blood Sampling and Analysis

Starting on the first day of the experiment (d 1 = 6 wk before calving), blood samples were collected at 14-d intervals for 12 wk to determine plasma concentrations of glucose, cholesterol, NEFA, BHBA, triglycerides, BUN, aspartate aminotransferase (AST), and insulin. Blood samples were collected on d 1, 10, 17, 24, 31, and

38 postcalving for determination of progesterone. All blood samples were withdrawn 2 h after the morning meal from the caudal vein into EDTA-evacuated tubes (10.5 mg, Monoject, Sherwood Medical, St. Louis, MO). The samples were maintained on ice until plasma was separated by centrifugation ($1,500 \times g$ for 20 min at room temperature) within 1 h of collection. Plasma was harvested and stored at -20°C for further analysis. Plasma concentrations of glucose (kit no. 2135, Pars Azmoon Co., Tehran, Iran), NEFA (kit no. 3749, Pars Azmoon Co.), BHBA (kit no. 7637, Pars Azmoon Co.), cholesterol (kit no. 5412, Pars Azmoon Co.), and triglycerides (kit no. 6752, Pars Azmoon Co.) were determined enzymatically, using a spectrophotometer (Shimadzu 2100, Kyoto, Japan). Intra- and interassay coefficients of variation were $<5\%$. Plasma insulin and progesterone concentrations were analyzed by ELISA, following the manufacturer's instructions (DRG, Marburg, Germany).

Statistical Analysis

Data were tested for normal distribution of the residuals by the PROC UNIVARIATE procedure of SAS (version 8.0, 2000; SAS Institute, Cary, NC). Data on milk yield, intake, BCS, and plasma concentrations of progesterone and metabolites were analyzed as repeated measurements, using PROC MIXED of SAS (SAS Institute Inc.) with the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + (\alpha\beta)_{ij} + (\alpha\tau)_{ik} + (\beta\tau)_{jk} + (\alpha\beta\tau)_{ijk} + e_{ijk},$$

where y_{ijk} is the dependent variable, μ is the population mean, α_i is the treatment effect, β_j is the fixed effect of parity, τ_k is the effect of sampling day or time, $(\alpha\beta)_{ij}$ is the interaction effect of treatment and parity, $(\alpha\tau)_{ik}$ is the interaction effects of treatment and sampling day or time, $(\beta\tau)_{jk}$ is the interaction effect of parity and sampling day or time, $(\alpha\beta\tau)_{ijk}$ is the interaction effect of treatment, parity, and sampling day or time, and e_{ijk} is the residual error. Significance was declared at $P < 0.05$. When a significant F-test was detected, treatment means were separated using a Tukey new multiple range test.

Data on the number and diameter of follicles were analyzed as repeated measurements using the mixed procedure of SAS (SAS Institute Inc.). The interval between calving and first AI and the number of days open were analyzed by survival analysis, using the product limit method of the Kaplan-Meier model by the LIFETEST procedure of SAS (Dirandeh et al.,

Table 3. Least squares means (and SEM) for milk production and composition, feed intake, and BCS during the experiment

Item	Treatment ¹				SEM	P-value
	Control	Whole DP	Far-off	Close-up		
DMI (kg/d)	14.27 ^a	12.40 ^b	13.52 ^{ab}	13.45 ^{bc}	0.3	0.04
Milk yield (kg/d)	29.0	32.9	31.3	31.0	1.4	0.34
4% FCM ² (kg/d)	27.4	30.6	29.3	28.7	1.4	0.57
Milk composition (%)						
Fat	3.95	3.60	3.75	3.71	0.18	0.07
Protein	2.88	2.80	2.83	2.81	0.07	0.87
Lactose	4.47	4.33	4.48	4.51	0.09	0.54
Milk yield (kg/d)						
Fat	1.14	1.18	1.17	1.15	0.12	0.69
Protein	0.83	0.92	0.88	0.87	0.28	0.75
Lactose	1.29	1.42	1.40	1.39	0.31	0.80
SCC ($\times 1,000$ cells/mL)	172.06 ^a	63.39 ^b	108.21 ^b	82.29 ^b	20.36	0.05
BW difference ³ (kg)	21.25	29.68	28.75	18.75	5.53	0.15
BCS	0.13	0.16	0.15	0.12	0.02	0.41

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Control = no supplementation with n-3 FA during the dry period; whole DP = supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Worksop, UK) during the whole dry period (8 wk); far-off = supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk); and close-up = supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk).

²Fat-corrected milk = $[0.4 \times \text{milk production (kg/d)}] + [15 \times \text{fat yield (kg/d)}]$.

³Body weight loss at end of experiment compared with starting BW.

2013b). Significance and tendencies were declared at $P < 0.05$ and $P < 0.15$, respectively, unless otherwise indicated.

RESULTS AND DISCUSSION

DMI and BCS

Body weight and BCS were similar among diets (Table 3). We detected an interaction ($P = 0.08$) between treatment and week for prepartal DMI and DMI increasing over time until calving. Prepartum DMI differed ($P = 0.04$) among diets, and the highest DMI was obtained for cows fed the control diet (Table 3) during the whole experiment. Higher amounts of unsaturated FA reaching the small intestine were likely responsible for the decrease in DMI of cows supplemented with n-3 FA. Indeed, abomasal infusion of unsaturated FA has been shown to decrease DMI of dairy cows (Drackley et al., 1992). Hayirli et al. (2011) reported that prepartal dietary FA source (canola seed, linola seed, or flaxseed at 8% of dietary DM) had no effect on mean DMI both prepartum and in early lactation. Similarly, Chilliard et al. (2009) reported that supplementation of the diet with 14.8% extruded flaxseed decreased DMI of late lactating dairy cows, although feeding 12.4% untreated whole flaxseed in the diet had no effect. Conversely, for the first 100 d of lactation, Zachut et al. (2010) reported higher postpartum intakes of DM and energy in dairy cows fed 7.9% of the diet as extruded flaxseed compared with those fed calcium salts of palm oil. However,

in a Latin square design with 28-d periods, similar DMI was obtained for cows fed no flaxseed and those fed 12.7% raw, extruded, or micronized flaxseed in the diet (Gonthier et al., 2005). Discrepancies between these studies could be due in part to the differences in the amounts, forms, and palatability of lipid supplements and the length of the experiments (short vs. long term).

Milk Production and Composition

Except for DMI, prepartal n-3 FA had negligible effects on performance and lactation variables. This may be explained by the provision of isocaloric and isonitrogenous diets prepartum, discontinuation of the oilseed supplementation after parturition, and the ruminal biohydrogenation process. Moreover, oilseeds are rich in amino acids, which could compensate for suppressed microbial protein synthesis due to the adverse effect of high fat on fiber degradation and contribute to milk protein concentration (Cant et al., 1993).

We observed no difference in milk yield among treatments ($P = 0.34$). Similar results have been reported for the cows supplemented with whole flaxseed, micronized soybeans, or calcium salts of palm oil in the last 6 wk of gestation (Petit and Benchaar, 2007). However, Hayirli et al. (2011) reported that lactation responses to prepartal FA supplementation were variable, with the highest milk yield for cows previously fed linola, a source of n-6 FA, compared with those previously fed canola seed or flaxseed at 8% of the diet. Fat proportion tended ($P = 0.07$) to be 11% lower for the cows supple-

mented with n-3 FA throughout the prepartal period compared with those fed no n-3 FA or supplemented with n-3 FA for a 3-wk period (Table 3). This agrees with the results of Petit et al. (2007), who reported lower milk fat proportion for cows fed whole flaxseed at 3.3% of the diet DM during the prepartum period, although milk production was higher for cows fed n-3 FA than for those fed SFA. Similar decreases in milk fat percentage have been observed for the cows fed flaxseed (Dirandeh et al., 2013b), 7% raw flaxseed (Mustafa and Seguin, 2003), and 14.8% extruded flaxseed (Chilliard et al., 2009) in the postpartum diet. On the contrary, Hayirli et al. (2011) reported that cows fed restricted prepartum had lower milk fat percentage ($P < 0.05$) in the subsequent lactation compared with those fed for ad libitum intake. The discrepancies between studies may be partly explained by the differences in the amounts and forms of n-3 FA supplements (e.g., treated vs. untreated flaxseed) and interactions with other diet ingredients, as suggested by Chilliard et al. (2009). Milk fat synthesis is depressed by FA isomers generated in the rumen during biohydrogenation of dietary unsaturated FA (Bauman and Griinari, 2003). Indeed, several studies reported higher concentrations of CLA and C18:1 *trans* isomers in milk fat when cows were supplemented with extruded flaxseed (Gonthier et al., 2005; Chilliard et al., 2009). Yields of fat and FCM were not affected by treatment.

We detected no difference in protein concentrations among treatments, which agrees with the results of Petit and Benchaar (2007) for cows fed calcium salts of palm oil, soybeans, or flaxseed during the prepartal period. Conversely, Chilliard (1993) reviewed the effects of fat (1 to 2% fish oil and soybean) on production and observed that milk protein proportion decreased in response to fat supplementation to a greater extent in early than in peak lactation (0.8 vs. 0.5 g/kg) and in short- compared with long-term experiments (1.0 vs. 0.5 g/kg), which may partly explain the discrepancies between the experiments. Proportion of milk lactose was similar among treatments.

The SCC was lower in cows fed the n-3 treatments compared with those fed the control group (Table 3), which could be due to a stronger immune system. This agrees with the findings of Staples et al. (2008), who reported that feeding n-3 FA before parturition resulted in antiinflammatory effects and lowered the number of neutrophils and white blood cells.

Blood Metabolites and Insulin and Progesterone Concentrations

Cow health status was not affected by treatment and parturition was normal for all cows except for one

that required minor assistance and antiinflammatory drugs. Treatments did not differ in plasma concentrations of metabolites, progesterone, or insulin. Dietary treatments had no effect on plasma concentrations of NEFA and BHBA (Table 4). Blood concentration of NEFA is an index of body fat mobilization (Roberts et al., 1981) and is related to the energy balance of dairy cows. Bertics et al. (1992) reported that DMI was inversely related to concentrations of NEFA and BHBA in plasma and liver. Thus, the lack of a significant difference in NEFA and BHBA concentrations from the current study may suggest that BW loss of cows was similar in all treatments. Serum total cholesterol (Table 4), low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol concentrations were similar among treatments. Increased plasma cholesterol concentrations were previously reported for the cows that were infused postruminally with free long-chain fatty acids (Drackley et al., 1992).

We detected no difference among treatments in plasma progesterone (P₄) concentration (Table 4). Similar P₄ concentrations have been reported for dairy cows fed a control diet and those fed a diet containing menhaden fish meal or calcium salts of fish oil during the luteal phase (Heravi Moussavi et al., 2007). In contrast, Hightshoe et al. (1991) reported higher blood cholesterol and plasma progesterone concentrations during the luteal phase when cows were fed calcium salts of palm oil compared with those fed a control diet with no fat supplement. Moreover, compared with cows fed no fat supplement, dairy cows fed calcium salts of palm oil or beef tallow had higher blood cholesterol concentrations (Marín-Aguilar et al., 2007) and cows fed diets enriched in n-3 or n-6 FA had higher plasma progesterone concentrations (Dirandeh et al., 2013a) have been reported. Burke et al. (1997) reported higher concentrations of progesterone 2 d after a PGF_{2α} injection in cows fed menhaden fish meal, suggesting delayed luteal regression. A previous study reported that cows on pasture had a significantly greater peak of plasma P₄ concentration than those managed in a freestall barn (7.0 vs. 5.0 ng/mL) during the first estrous cycle after calving, although this was not observed in the subsequent estrous cycles (Boken et al., 2005). Similar plasma insulin concentrations of cows fed the different diets may be due to the choice of FA fed in the present experiment. Indeed, dietary FA profile is important to modulate the whole-body response to insulin. Altogether, the lack of prepartal n-3 FA supplementation on metabolic profile may be attributed to the effect of FA biohydrogenation in the rumen and to discontinuation of FA supplementation after parturition (Petit et al., 2007).

Table 4. Least squares means (and SEM) for plasma metabolites and hormones during the experiment

Item	Treatment ¹				SEM	P-value
	Control	Whole DP	Far-off	Close-up		
Glucose (mg/dL)	62.65	60.53	58.24	57.71	1.8	0.22
NEFA (mmol/dL)	0.494	0.419	0.416	0.497	0.03	0.19
BHBA (mmol/dL)	0.508	0.517	0.466	0.458	0.07	0.90
Cholesterol (mg/dL)	135.15	141.75	143.09	138.52	11.2	0.96
Triglycerides (mg/dL)	16.53	17.46	17.0	16.93	1.17	0.88
MUN (mg/dL)	14.55	13.05	13.24	14.02	0.73	0.16
Aspartate aminotransferase (U/L)	72.40	63.67	67.63	70.42	4.29	0.56
Insulin (pmol/L)	13.07	13.02	12.42	12.28	1.71	0.92
Progesterone (ng/mL)	2.46	2.13	2.03	2.33	0.48	0.91

¹Control = no supplementation with n-3 FA during the dry period; whole DP = supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Workop, UK) during the whole dry period (8 wk); far-off = supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk); and close-up = supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk).

Follicle Number and Size

We found no significant effect of n-3 FA supplementation on the mean number and size of follicles (Table 5). Similarly, Petit (2002) and Petit et al. (2002) found little or no effect of diets supplemented with linseed, fish oil, flax, or sunflower seed on follicular dynamics. However, Bilby et al. (2006a) found that the number and size of developing follicles increased in cows fed calcium salts of *trans*-octadecenoic acids and linseed oil enriched in n-3 PUFA. Many studies have reported that dietary supplementation with n-3 FA improves fertility by influencing follicular growth and ovulation in dairy cows. For example, diameter and number of follicles (Robinson et al., 2002) and mean diameter of the ovulatory follicle increased (Ambrose et al. 2006) in cows fed rolled flaxseed. Bilby et al. (2006a) showed that size of the dominant follicle increased in cows fed diets rich in linoleic acid and linolenic acid compared with saturated FA.

Recent studies indicate an increase in the diameter of large follicles (>10 mm) when cows are fed diets rich in n-3 PUFA such as flax seed and oil, calcium salts of long-chain FA, and fish oil compared with cows fed MUFA (Ambrose et al., 2006; Bilby et al., 2006a; Santos et al., 2008; Dirandeh et al., 2013a). These studies clearly indicate the differential effects of FA profile on follicular growth. Cholesterol is the precursor of all steroids and increased substrate availability may increase follicular steroid synthesis (Carroll et al., 1990). However, we found no significant difference among treatments for plasma cholesterol concentration, which may explain the lack of effect of n-3 FA supplementation on follicular growth.

Reproductive Performance

Despite the fact that our study did not have sufficient animals to make statistical interferences about days open and service rate, number of days open, ser-

Table 5. Least squares means (and SEM) for follicle number and size during the experiment

Item	Treatment ¹				SEM	P-value
	Control	Whole DP	Far-off	Close-up		
Left ovary						
Small follicles (no.)	0.28	0.44	0.34	0.15	0.14	0.55
Large follicles (no.)	0.37	0.55	0.50	0.57	0.16	0.81
All follicles (no.)	0.65	1.00	0.84	0.72	0.17	0.55
Diameter of dominant follicle (mm)	9.8	12.86	13.3	8.0	2.91	0.66
Right ovary						
Small follicles (no.)	0.20	0.26	0.46	0.11	0.12	0.30
Large follicles (no.)	0.45	0.28	0.33	0.71	0.10	0.06
All follicles (no.)	0.66	0.54	0.79	0.83	0.16	0.63
Diameter of dominant follicle (mm)	15.6	13.89	13.1	14.5	1.52	0.70
Both ovaries						
All follicles (no.)	1.32	1.54	1.64	1.55	0.22	0.76
Diameter of dominant follicle (mm)	16.68	16.63	17.18	17.37	1.56	0.90

¹Control = no supplementation with n-3 FA during the dry period; whole DP = supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Workop, UK) during the whole dry period (8 wk); far-off = supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk); and close-up = supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk).

Table 6. Reproductive performance during the experiment

Item	Treatment ¹				SEM	P-value
	Control	Whole DP	Far-off	Close-up		
AI per pregnancy	1.58	1.51	1.38	1.50	0.18	0.91
Days to first service	79.3	72.21	64.0	63.6	5.27	0.27
Open days	94.0	87.30	73.0	90.8	9.76	0.48

¹Control = no supplementation with n-3 FA during the dry period; whole DP = supplementation with 160 g/d of n-3 FA (Optomega, Optivite Co., Worksop, UK) during the whole dry period (8 wk); far-off = supplementation with 160 g/d of n-3 FA during the early dry period (first 5 wk); and close-up = supplementation with 160 g/d of n-3 FA during the late dry period (last 3 wk).

vices per pregnancy, and day to first service did not differ among treatments (Table 6). This agrees with the results of Dirandeh et al. (2013b), who reported no difference in heat detection percentage, pregnancy rate at first insemination, and conception rate per AI for cows fed soybeans, flaxseed, or palm oil. However, percentage of pregnancy at d 120 tended ($P = 0.08$) to be higher for cows fed n-3 FA than for those fed palm oil (66.7% vs. 50.91%; Dirandeh et al., 2013b). Juchem et al. (2010) reported that feeding calcium salts of linoleic and *trans*-octadecenoic acids during the transition period reduced the incidence of puerperal metritis, which might contribute to enhanced fertility. Juchem (2007) evaluated the effect of calcium salts of palm oil or a blend of C18:2 and *trans*-octadecenoic FA during the prepartum and postpartum periods and found that cows fed the unsaturated FA were 1.5 times more likely to become pregnant 27 or 41 d after AI compared with those fed the calcium salts of palm oil. In conclusion, feeding n-3 FA throughout the dry period or in the early or late prepartal period had no carryover reproductive benefits postpartum and no effects on milk production of Holstein dairy cows.

CONCLUSIONS

The objectives of the present experiment were to determine the effects of prepartal supplementation with n-3 FA in different periods (early, late, or whole dry) on milk production and composition, ovarian function, reproduction parameters, and blood parameters in the subsequent lactation. We showed that adding n-3 FA had a negative effect on DMI during dry period. Milk yield and milk protein and lactose concentrations did not differ among treatments. Fat proportion tended to be lower for cows supplemented with n-3 FA throughout the prepartal period compared with control group. Somatic cell count was significantly lower in cows fed the n-3 treatments compared with those fed the control diet, which could be of practical importance on farms with high SCC. Treatments did not differ in plasma concentrations of metabolites (NEFA, BHBA, serum

total cholesterol, low-density and high-density lipoprotein cholesterol), progesterone, and insulin, and n-3 FA supplementation had no effect on the mean number and size of follicles. The number of days open, services per pregnancy, and days to first service were not different among treatments, as these indices were lower in treatment groups. We did not have sufficient animals to make statistical interference about days open and service rate; therefore, these indices need future investigation with an adequate number of animals.

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