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Effect of supplementing calcium salts of n-3 and n-6 fatty acid to pregnant nonlactating cows on colostrum composition, milk yield, and reproductive performance of dairy cows

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ABSTRACT

The objective of this study was to determine the effect of dietary supplementation with calcium salts of n-3 and n-6 fatty acids (FA) during the last 3 weeks of gestation on colostrum composition and productive and reproductive performance of nulliparous and parous Holstein cows during the entire subsequent lactation. During the last 3 weeks of pregnancy, cattle (n = 120), within parity, were randomly assigned to 1 of 3 treatments, no fat supplement (CON), a Ca-salt supplement enriched in C18:2n-6 (CSO), or a Ca-salt supplement enriched in eicosapentaenoic and docosahexaenoic acid FA (CFO). After calving, all cows received a common lactation diet. As expected, cows fed CSO or CFO produced colostrum with higher ($P < 0.01$) contents of n-6 or n-3 FA, respectively. Colostrum yield and composition were not affected by treatments, but cows fed fat produced colostrum with higher ($P < 0.01$) IgG concentration. The major impact of treatments on productive and reproductive performance was observed for the contrast comparison of supplemental fat (CSO and CFO) compared with CON. Body weight was not affected by treatments, but at 14 weeks after parturition, cows fed fat tended ($P = 0.07$) to have a greater body condition score compared with CON cows. Milk yield and 4% fat corrected milk were higher ($P < 0.001$) for cows fed fat compared with CON (37.8, 34.9 versus 35.2, 33.0 kg/d, respectively) during the experimental period. Milk fat content was higher ($P < 0.001$) in CON cows compared with cows fed fat (36.1 vs. 34.9 g/kg, $P < 0.001$). Plasma cholesterol concentrations were higher ($P \leq 0.02$) for both fat supplement groups during the pre- and postpartum period, but plasma concentration of non-esterified FA was lower ($P < 0.01$) for fat-supplemented compared with CON-supplemented cows during postpartum. Fat-supplemented cows had shorter days to first estrus and first insemination ($P < 0.04$). Total reproductive disorders tended ($P = 0.06$) to be lower in cows fed supplemental fat than in those fed CON. Cows fed supplemental fat had lower ($P = 0.01$) incidences of total health disorders compared with those fed CON diet. In summary, prepartum fat supplementation increased milk yield during the

Abbreviations: AI, artificial insemination; BCS, body condition score; BUN, blood urea nitrogen; BW, body weight; CFO, Ca-salts of fish oil; CSO, Ca-salts of soybean oil; DHA, docosahexaenoic acid; DIM, days in milk; DM, dry matter; DMI, dry matter intake; EPA, eicosapentaenoic acid; FAT, contrast of fat supplement versus no fat supplement; (CSO + CFO vs. CON)FCM, 4% fat corrected milk; NEFA, non-esterified fatty acid; PUFA, polyunsaturated fatty acids; SCC, somatic cell count; SFO, contrast of fatty acid source (CSO vs. CFO); TMR, total mixed ration

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entire lactation and improved reproductive performance. Our findings suggest that the benefits of supplemental polyunsaturated FA during the prepartum period only is independent of the n-type of FA.

1. Introduction

At the onset of lactation, high producing dairy cows drastically increase their demand for nutrients to cope with the demands of the mammary gland. Thus, a proper nutritional program during the dry period, and most critically during the last 3 weeks of gestation is crucial to attaining a successful lactation with high levels of milk production, lower incidence of disease, and good reproductive efficiency. Supplemental fats are included in lactating dairy cattle diets to increase energy density, which may improve the energy balance of early lactating dairy cows (Ballou et al., 2009). Although the benefits of supplemental fat have been most commonly evaluated in lactating diets (Rabiee et al., 2012), more recently some studies have investigated the effects of supplemental fats during the dry and lactation periods (Juchem et al., 2010; Caldari-Torres et al., 2011). However, the effect of supplementing long chain fatty acids (FA) during the close-up period only and its effect on the subsequent lactation is scarce.

The discovery of the essentiality of C18:2n-6 by Burr and Burr (1930) and later that of C18:3n-3, due to the major role of its derivative C22:6n-3 in brain development (Neuringer et al., 1988; Anderson and Connor, 1989), led the scientific community to investigate the potential productive and reproductive benefits of essential FA and/or their elongated derivatives on dairy cow performance. Fatty acids exert critical roles in different biological processes, one of the primary mechanisms is as a component of cell membranes providing stability and regulating signal transduction (Simons and Ikonen, 1997). Several studies in humans and rodents have reported that n-3 compared with n-6 FA are more potent activators of genes involved in lipid oxidation, more potent reducers of genes encoding enzymes for lipid synthesis, and important regulators of immune response (Clarke, 2000; Sampath and Ntambi, 2005). In dairy cattle, the supplementation of a specific type of FA has increased its concentrations and/or that of its derivatives in plasma and milk (Greco et al., 2015) and the endometrium (Bilby et al., 2006).

A recent meta-analysis, which included 38 studies where 36 of them fed supplemental fat during the postpartum only, concluded that cows fed supplemental fat produced more milk (+ 1 kg/d) compared with cows fed diets without supplemental fat (Rabiee et al., 2012). Duske et al., (2009) is the only study, referred by Rabiee et al. (2012), who supplemented fat during prepartum. Duske et al. (2009) supplemented a rumen-protected fat (2.8 and 4.7% of dietary DM during wk 8 to 4 and from wk 3 to parturition, respectively) enriched in C16:0 and C18:1 and reported unfavorable results due to fat supplementation, i.e., dry matter intake (DMI) prepartum was depressed, milk production during the first four weeks of lactation was reduced, and prepartum circulating nonesterified fatty acid (NEFA) was increased. Furthermore, supplementing diets rich in C18:3n-3 (flaxseed) compared with diets rich in C18:2n-6 (micronized soybean) did not impair DMI prepartum (Petit and Benchaar, 2007). Petit and Benchaar (2007) continued supplementing similar FA for ~ 120 d postpartum and reported that cows fed flaxseed ate 9% more dry matter (DM), and although milk production or milk components were not affected by the type of FA, cows fed flaxseed had higher conception rate. The findings in the aforementioned studies suggest that the impact of supplemental fat can differ depending on the type of FA fed and on the timing of supplementation.

The benefits of supplemental FA postpartum on reproductive efficiency of lactating dairy cows have been extensively investigated. Postpartum supplementation of diets enriched in C18:3n-3, or its elongated derivatives, reduced pregnancy loss (Ambrose et al., 2006), enhanced embryo development (Thangavelu et al., 2007), increase pre-ovulatory follicle size (Ambrose et al., 2006), and increased percentage of pregnancy (Sinedino et al., 2017). The mechanisms for these observed beneficial effects of n-3 FA on reproduction may include improved dietary energy density, altered follicle development, increased concentrations of progesterone, prevention of luteolytic signals around the time of maternal recognition of pregnancy, and improved embryo quality (Dirandeh et al., 2013).

Although extensive research has been conducted to evaluate the impact of n-3 and n-6 FA supplemented during the lactation period alone or the dry period and through early or mid-lactation (Rabiee et al., 2012; Santos et al., 2008), studies evaluating the productive and reproductive impact of feeding long chain FA during the transition period only are scarce. A recent study evaluated the supplementation of n-3 FA during the whole dry period only (8 wk before calving), during the far-off only (first 5 wk), or the close-up only (last 3 wk) and found no effects on milk yield and reproductive efficiency in the subsequent lactation (Badiei et al., 2014). Another study feeding seed oils rich in n-6 or n-3, during the last 35 d prepartum reported a higher incidence of diseases in cows fed n-6-rich diets but reported no dietary effect on reproductive efficiency (Salehi et al., 2016a). Therefore, we hypothesized that feeding rumen-protected long chain FA during the last 3 weeks of gestation would enhance reproductive traits and production response of lactating dairy cows in their subsequent lactation. The objective was to evaluate the effect of supplementing rumen-protected n-3 and n-6 FA to Holstein dairy cows during the last 3 wk of pregnancy on the FA profile of colostrum and productive and reproductive responses from calving throughout the entire lactation.

2. Materials and methods

2.1. Cows and treatments

This study was carried out on a commercial dairy farm (Zarin Khoshe Dairy Farm, Arak district, Iran) from July 2016 to October

2017. All experimental procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. The Animal Experiment Committee approved all procedures and guidelines involving animals at Tehran University, Karaj, Iran. One hundred twenty nonlactating pregnant Holstein cattle were enrolled in the study and were randomly assigned, within parity, to 1 of 3 treatments at 3-weeks before expected calving date; enrollment was completed in 4 consecutive days. Forty cattle (14 nulliparous, 10 primiparous, and 16 s or more lactation) were assigned to each treatment group (1 pen per treatment). The experimental period started from 3 weeks (21 ± 4 d) before the expected calving date and ended with drying off, and the treatments were fed only during the prepartum period. All cattle were housed in free stall barns with access to exercise lots. Prepartum pens were of the same size with similar bunk space, stall design, flooring, water accessibility, and animal density. After calving all cows were moved to a fresh pen and then moved to the early, mid, and late-lactation pens respectively, until the end of the experiment. The basal diets for the prepartum period (Table 1) were formulated to meet nutrient demands recommended by the National Research Council (2001). The basal prepartum diet was formulated to have low concentrations of total FA, linoleic acid, and α-linolenic acid; thus fat supplements replaced corn ground in the control treatment (Tables 2, 3). Prepartum cattle were fed 1 of the following 3 diets: (1) no fat supplement (CON), (2) 1.15% of dietary DM as Ca-salts of soybean oil (CSO, 140 g/cow/daily) supplement (Persiafat, Kimiya Danesh Alvand Co. Tehran, Iran), or (3) 1.15% of dietary DM as Ca salts of fish oil (CFO, 140 g/cow/daily) supplement (Persiafat, Kimiya Danesh Alvand Co. Tehran, Iran). In the subsequent lactation, all cows received the same lactation diet formulated to meet their nutritional requirements (NRC, 2001). Cattle were fed once daily (1100 h) during the prepartum period and three times daily (0600, 1400 and 2200 h) during the postpartum period. The total mixed ration (TMR) was offered to allow 10% refusals on an as-fed basis, and water was always available.

2.2. Feed, colostrum, milk, body weight, and body condition score

Samples of offered TMR were collected weekly, dried at 55 °C and moisture loss recorded and used to determine DM concentration. Weekly dried TMR were ground in a hammer mill with a 1 mm screen (Arthur Hill Thomas Co., Philadelphia, PA) and analyzed in triplicate for DM, ash, crude protein, and ether extract according to AOAC (1990) procedures 930.15, 924.05, 984.13 and 954.02, respectively. Neutral detergent fiber was analyzed according to Van Soest et al. (1991), assayed with sodium sulphite, without alpha-amylase, and expressed with residual ash. Average individual daily feed intake was estimated by subtracting orts from offered TMR, per pen, and dividing by the number of cow's present in a given treatment pen, and corrected by DM content, as reported by others (Mandebvu et al., 2003; Roodbari et al., 2016). Cows were milked with a cow-side vacuum pump within 1 h after calving and colostrum weight was recorded using a commercial scale (model 4010, Pelouze Scale Co, Bridgeview, IL). Colostrum samples, first mammary gland secretion obtained within 1 h after calving, were collected and analyzed for concentrations of fat, CP, and lactose by infrared spectroscopy (Foss Electric, Hillerod, Denmark). Colostrum samples were also measured for bovine total IgG concentration using radioimmunodiffusion (Garcia et al., 2014). Colostrum FA were extracted as described by Folch et al. (1957). The

Table 1
Ingredient of experimental diets fed to pregnant Holstein animals starting 3 wk before calculated parturition date.

Item	Prepartum diet[a]		
	CON	CSO	CFO
Ingredient (g/kg DM)			
Alfalfa hay	312.5	312.5	312.5
Corn silage	271.0	271.0	271.0
Wheat straw	37.5	37.5	37.5
Corn, ground	86.2	74.7	74.7
Barley, ground	115.2	115.2	115.2
Extruded soybean seed	11.4	11.4	11.4
Soybean meal	63.5	63.5	63.5
Wheat Bran	51.5	51.5	51.5
Calcium carbonate	4.0	4.0	4.0
Vitamin–mineral premix[b]	26.8	26.8	26.8
White salt	0.4	0.4	0.4
Monensin sodium 10%	0.2	0.2	0.2
Glucose precursors	14.4	14.4	14.4
Toxin binder	1.2	1.2	1.2
Dicalcium phosphate	2.1	2.1	2.1
Rumen protected choline[c]	2.1	2.1	2.1
Ca-salts of fish oil[d]	0	0	11.5
Ca-salts of soybean oil[d]	0	11.5	0

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk).
[b] Vitamin–mineral premix contained per kg: Ca 160 g; P 40 g; Mg 50 g; Fe 5 g; Cu 6 g; Zn 14 g; Mn 10 g; I 0.2 g; Co 0.08 g; Se 0.02 g; vitamin A 1600,000 IU; vitamin D 250,000 IU; vitamin E 7000 IU.
[c] Balchem Inc., Slate Hill, NY, USA.
[d] all produced by Persiafat, Kimiya Danesh Alvand Co. (Tehran, Iran).

Table 2
Chemical composition (g/kg DM unless otherwise noted) of experimental diets fed to pregnant Holstein animals starting 3 wk before calculated parturition date.

Item (g/kg DM)	Prepartum diet[a]		
	CON	CSO	CFO
Nutrient composition (g/kg DM)			
NE_L[b] (Mj/kg DM)	5.94	6.15	6.15
DM (g/kg diet)	530.0	531.0	531.0
CP	141.2	140.2	140.2
OM	896.0	896.0	896.0
NDF	403.0	403.0	403.0
ADF	246.0	246.0	246.0
NFC	349.0	340.5	340.5
Ether extract	28.7	38.0	38.0
Ca	13.2	13.5	13.5
P	4.0	4.0	4.0
FA	18.7	28.0	28.0
C16:0	3.5	4.9	5.4
C16:1	0.10	0.10	0.10
C18:0	0.50	0.80	1.9
C18:1	2.0	4.6	4.6
C18:2n-6	8.4	13.0	8.7
C18:3n-3	3.6	3.8	4.1
EPA[c] & DHA[d]	0	0	1.5
Omega-6/omega-3	2.33	3.42	1.55

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk).
[b] Net energy of diets calculated using 12 kg/d of DMI (CPM-Dairy software; University of Pennsylvania, Kennett Square, PA).
[c] Eicosapentaenoic acid (C20:5).
[d] Docosahexaenoic acid (C22:6).

Table 3
Fatty acid profile (g/100 g of FA) of prepartum fat supplements and diet.

FA, g/100 g of total FA	Fat supplement[a]		Prepartum diet[b]		
	CSO	CFO	CON	CSO	CFO
C16:0	15.0	20.0	18.7	17.5	19.3
C16:1	–	–	0.53	0.35	0.35
C18:0	5.0	15.0	2.70	2.85	6.80
C18:1	25.0	25.0	10.7	16.4	16.4
C18:2n-6	50.0	5.0	44.9	46.4	31.1
C18:3n-3	2	5.0	19.2	13.6	14.7
Σ EPA[c] & DHA[d]	–	14.0	0	0	5.40
Other	3.0	16.0	3.3	2.9	5.95
Σ SFA	25.0	40.0	21.5	20.4	26.1
Σ UFA	75.0	60.0	75.0	76.8	68.2
Σ PUFA	52.0	30.0	64.0	60.0	51.2

[a] Fat supplements for prepartum diets: Ca-salts of soybean oil supplement (CSO) and Ca-salts of fish oil supplement (CFO), both produced by Persiafat, Kimiya Danesh Alvand Co. (Tehran, Iran).
[b] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk).
[c] Eicosapentaenoic acid (C20:5).
[d] Docosahexaenoic acid (C22:6).

fatty acid profile of extracted lipids was assessed by gas chromatography as previously described by Cruz-Hernandez et al. (2007). Milk production was recorded at every milking. Cows were milked 3 times daily at 0500, 1300, and 2100 h. Milk was sampled twice per week at each milking time. Weekly milk samples were pooled on a yield basis and analyzed for fat, protein, and lactose by infrared spectrophotometry (Foss Electric, Hillerod, Denmark). Somatic cell count (SCC) were analyzed with an automatic counter (Fossmatic 5000 Automatic Counter, Foss Electric), at the same sampling times as milk composition but only during the first 3 mo of lactation. Cows were weighed and scored for their body condition after the morning milking and before the morning meal on -21, 0, +21, +63 and 98 days in milk (DIM). Body condition scores (BCS) were attained by two trained individuals using a 5-point scale (1 = thin to 5 = obese; Wildman et al., 1982) throughout the study.

2.3. Blood sampling and analysis

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) on d 21, 14 and 7 prior to the expected calving date, immediately after parturition, and on weeks 3, 9 and 14 postpartum to determine plasma concentrations of glucose, cholesterol, non-esterified FA (NEFA), triglycerides, blood urea nitrogen (BUN), total protein, albumin and insulin. Samples were immediately chilled, and then plasma was obtained by centrifugation at 3000 × *g* for 15 min and stored at −20 °C. Plasma concentrations of glucose, total protein, albumin, cholesterol, and triglycerides were determined using an automatic microplate Reader (EON-BIOTEK, America) and commercial kits (Pars Azmoon, Tehran, Iran). Enzymatic assays were employed for analyses of plasma insulin (DRG, Marburg, Germany), NEFA (Randox Laboratories Ltd., UK, Cat # FA 1 are15 and 30 t) and BUN (Pars Azmoon, Tehran, Iran) in an automatic spectrophotometer (Clima Plus, RAL, Madrid, Spain) following the manufacturer's instructions.

2.4. Reproductive management

Cows were enrolled in a PreSynch/Ovsynch protocol starting at 30 DIM (Moore and Thatcher, 2006). Briefly, cows were given 2 injections of PGF2α (500 μg, Cloprostenol Sodium, i.m.; Estroplan, Parnell technologies PTY. LTD., Alexandria, Australia), 14 d apart. Fourteen days after the second PGF2α injection cows were intramuscularly-injected with GnRH (100 μg gonadorelin acetate, Gonabreed, Parnell Technologies PTY. LTD., Alexandria, Australia), followed by another PGF2α injection 7 d later. An additional GnRH was injected 48 h after PGF2α injection. Artificial insemination (AI) occurred 16 h after GnRH injection. Cows showing signs of estrus following the second injection of PGF2α were inseminated and excluded from the protocol. Estrus was detected using a combination of behavioral observations (two times daily) and pedometer activity. Cows were inseminated by one technician and pregnancy diagnosis performed by ultrasonography (ECM, IMAGO.S; France) 30 d after timed AI.

2.5. Evaluation of uterine diseases

Twenty-four hours after parturition, an examination was carried out to check for the expulsion of the fetal membranes. If the fetal membranes were not expelled by 24 h after calving, they were considered retained (Palomares et al., 2010). All cows with retained placenta received an intrauterine infusion of 40 ml of oxytetracycline (5% solution). Cows were scored for dystocia according to the degree of assistance provided. Recognized dystocia scores were 1 = no problem, 2 = slight problem, 3 = needed assistance, 4 = considerable force, and 5 = extreme difficulty (Atashi et al., 2012). For the current study, dystocia scores of 1 or 2 were defined as easy calving, and scores ≥3 were defined as difficult calving. During the first 14 d of lactation, the herd veterinarians examined all cows for signs of metritis. Metritis was characterized by the presence of watery, fetid vaginal discharge and rectal temperature > 39.5 °C. Cows with metritis received ceftiofur hydrochloride for 5 consecutive days at a dose of 2.2 mg/kg body weight (BW) (ceftiofur hydrochloride sterile suspension; Excenel RTU EZ; Pfizer Animal Health, New York, NY, USA). Clinical endometritis was evaluated at 20 ± 0.5 and 40 ± 2 DIM (mean ± SD) by assessing vaginal mucus, as previously described (Sheldon et al., 2006). Cows having mucopurulent or purulent discharge were classified as having clinical endometritis (Sheldon et al., 2006). Inactive ovary was considered as a condition in which the ovaries were quiescent without signs of cyclicity or cycle-related ovarian structures. Ovarian cysts in dairy cattle are generally defined as follicular structures of at least 1.7 cm in diameter that persist for at least 10 d in the absence of a corpus luteum (Dirandeh et al., 2009). Total reproductive disorders were defined as the sum of retained fetal membranes, metritis, inactive ovaries, and ovarian cyst incidence. Total health disorders were defined as the sum of retained fetal membranes, metritis, inactive ovaries, ovarian cyst, mastitis, and dystocia.

2.6. Statistical analysis

Continuous data were analyzed using the MIXED procedure of SAS (SAS, 2002), in a completely randomized block design with time as repeated measures. Daily milk yields were collapsed into monthly averages. Preplanned contrast analysis was used to compare means for fat supplement versus no fat supplement (FAT) and CSO versus CFO (SFO). Single degree of freedom contrasts of the 2 treatment contrasts (FAT and SFO) with parity were performed. The mixed model equation for repeated measures was defined as:

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

where Y_{ijk} is the dependent variable, μ is the population mean, α_i is the treatment effect, βj 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. Cow within treatments was the random error term for all data analyses. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P < 0.10$. Tukey's adjustment was used for multiple comparison tests.

Before analysis of the reproductive data, 5 cows were removed from the data set (2, 0 and 3 cows from CON, CSO, and CFO, respectively). These cows were culled after d 50 postpartum because of physical injury. The interval between calving and first AI and the number of days open and to the first estrus were analyzed using survival analysis and the product limit method of the

Table 4
Yield, composition, and fatty acids (FA) content in colostrum from Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item	Dietary treatment[a] and parity (P)						SEM	*P*-value[b]				
	CON		CSO		CFO							
	Null	Parous	Null	Parous	Null	Parous		FAT	SFO	P	P × FAT	P × SFO
Colostrum yield and composition												
Yield (kg)	5.53	6.33	6.68	7.03	6.23	7.20	0.73	0.20	0.85	0.26	0.91	0.68
IgG, (g/L)	95	101	122	137	115	133	7.8	< 0.01	0.51	0.05	0.41	0.84
Fat (g/kg)	70.2	67.5	69.5	61.0	64.6	60.5	1.01	0.57	0.78	0.53	0.84	0.81
Protein (g/kg)	131.3	135.5	129.7	133.3	131.5	134.8	0.48	0.80	0.74	0.37	0.92	0.97
Lactose (g/kg)	30.3	32.5	31.3	32.3	30.8	32.8	0.20	0.82	0.98	0.31	0.86	0.78
Fatty acid profile, g/100 g of total fatty acids												
C4:0	1.43	1.17	1.54	1.17	1.32	1.30	0.16	0.91	0.20	0.05	0.90	0.94
C8:0	1.31	1.01	1.04	0.80	1.17	0.93	0.14	0.20	0.39	0.04	0.81	0.98
C9:0	0.14	0.10	0.18	0.10	0.22	0.12	0.05	0.52	0.64	0.16	0.65	0.88
C10:0	0.55	0.25	0.60	0.32	0.55	0.28	0.16	0.80	0.81	0.04	0.95	0.99
C12:0	1.48	1.04	1.24	0.88	1.34	0.97	0.23	0.48	0.71	0.05	0.86	0.97
C13:0	3.08	2.40	2.30	2.06	2.49	2.34	0.36	0.21	0.54	0.23	0.47	0.90
C14:0	13.0	11.2	12.5	11.4	11.7	10.4	0.67	0.27	0.19	0.01	0.63	0.83
C14:1 *cis*-9	1.34	1.79	0.86	1.02	1.22	1.55	0.31	0.18	0.19	0.25	0.72	0.78
C15:0	0.91	0.93	0.82	0.90	0.85	0.95	0.092	0.63	0.66	0.38	0.66	0.88
C15:1 *trans*	0.12	0.16	0.20	0.30	0.14	0.21	0.12	0.27	0.13	0.44	0.81	0.83
C16:0	33.9	37.5	34.5	37.1	34.1	36.5	1.29	0.91	0.68	0.01	0.62	0.91
C16:1 *cis*-9	1.90	2.30	2.02	2.46	2.30	2.70	0.21	0.18	0.25	0.03	0.99	0.90
C17:0	0.76	1.00	0.56	0.76	0.63	0.91	0.156	0.26	0.50	0.07	0.98	0.78
C17:1	0.60	0.76	0.56	0.76	0.49	0.55	0.13	0.48	0.33	0.25	0.88	0.62
C17:2	0.28	0.48	0.30	0.50	0.17	0.34	0.128	0.64	0.27	0.08	0.95	0.91
C18:0	6.40	5.28	7.27	5.85	8.10	7.26	0.69	0.04	0.13	0.05	0.99	0.68
C18:1 *trans*-4	0.015	0.009	0.024	0.158	0.022	0.015	0.003	0.04	0.79	0.03	0.72	0.88
C18:1 *trans*-5	0.005	0.013	0.020	0.014	0.020	0.013	0.003	0.03	0.86	0.03	0.78	0.81
C18:1 *trans*-9	0.14	0.11	0.24	0.18	0.23	0.19	0.023	< 0.01	0.94	0.03	0.60	0.74
C18:1 *trans*-10	0.19	0.15	0.32	0.26	0.28	0.23	0.033	< 0.01	0.30	0.06	0.88	0.84
C18:1 *trans*-12	0.22	0.18	0.35	0.31	0.31	0.27	0.033	< 0.01	0.30	0.17	0.86	0.89
C18:1 *cis*-9	16.9	17.8	17.2	18.0	16.0	16.7	1.27	0.73	0.35	0.47	0.96	0.95
C18:1 *cis*-11	0.22	0.13	0.18	0.14	0.21	0.12	0.072	0.78	0.95	0.23	0.83	0.74
C18:2n-6 *cis*	2.00	2.36	4.56	5.32	2.70	2.97	0.27	< 0.01	< 0.01	0.05	0.74	0.38
C20:0	0.41	0.23	0.44	0.32	0.28	0.22	0.082	0.97	0.16	0.08	0.55	0.74
C20:1	0.17	0.11	0.13	0.07	0.21	0.13	0.048	0.89	0.19	0.11	0.92	0.86
C18:3n-3 *cis*	0.77	0.58	0.60	0.96	0.57	0.66	0.11	0.86	0.12	0.40	0.08	0.29
C22:0	0.20	0.16	0.13	0.11	0.18	0.16	0.056	0.47	0.40	0.58	0.91	0.97
C22:1	0.05	0.05	0.07	0.08	0.06	0.07	0.029	0.44	0.74	0.71	0.84	0.98
C18:2 *cis*-9 *trans*-11	0.20	0.24	0.14	0.22	0.18	0.16	0.052	0.36	0.82	0.38	0.97	0.41
C18:2 *cis*-9 *cis*-11	0.18	0.13	0.18	0.08	0.17	0.07	0.053	0.56	0.90	0.08	0.55	0.97
C18:2 *cis*-12 *trans*-10	0.16	0.08	0.15	0.07	0.14	0.07	0.055	0.83	0.97	0.08	0.97	0.94
C18:2 *cis*-10 *trans*-12	0.05	0.10	0.11	0.19	0.08	0.13	0.054	0.31	0.43	0.17	0.87	0.79
C18:2 *cis*-8 *cis*-10	0.49	0.15	0.18	0.06	0.16	0.11	0.17	0.21	0.96	0.27	0.44	0.84
C18:2 *trans*-10 *cis*-12	0.12	0.09	0.26	0.18	0.21	0.13	0.076	0.21	0.52	0.31	0.74	0.99
C18:2 *cis*-10 *cis*-12	0.16	0.14	0.12	0.20	0.15	0.18	0.047	0.77	0.88	0.28	0.67	0.61
C18:2 *cis*-11 *cis*-13	0.04	0.06	0.03	0.07	0.03	0.05	0.018	0.82	0.56	0.06	0.82	0.60
C18:2 *cis*-11 *trans*-13	0.08	0.14	0.09	0.13	0.10	0.16	0.035	0.75	0.57	0.07	0.75	0.82
C18:2 *trans*-8 *cis*-10	0.06	0.09	0.06	0.12	0.06	0.08	0.048	0.92	0.78	0.32	0.93	0.74
C20:2n-6	0.00	0.00	0.36	0.21	0.00	0.00	0.036	< 0.01	< 0.01	0.09	0.24	0.04
C20:5n-3	0.00	0.00	0.00	0.00	1.23	0.92	0.055	< 0.01	< 0.01	0.03	0.14	< 0.01
C22:6n-3	0.00	0.00	0.00	0.00	1.60	1.20	0.082	< 0.01	< 0.01	0.05	0.19	0.02
C24:0	0.00	0.00	0.00	0.00	0.48	0.21	0.054	< 0.01	< 0.01	0.06	0.20	0.03
C24:1	0.00	0.00	0.00	0.00	0.34	0.16	0.030	< 0.01	< 0.01	0.03	0.14	0.01
Other[c]	8.91	7.46	8.51	6.86	8.28	6.70	0.55	0.27	0.77	< 0.01	0.87	0.94
Σ SFA	62.7	63.3	62.3	62.8	62.2	62.8	1.28	0.67	0.97	0.61	0.99	0.99
Total CLA	1.22	1.06	1.29	1.21	1.26	1.13	0.17	0.58	0.71	0.38	0.81	0.88
Σ MUFA	21.9	23.5	22.2	23.7	21.8	22.9	1.46	0.96	0.66	0.26	0.89	0.87
Σ PUFA	4.61	4.20	6.94	7.86	7.88	7.42	0.36	< 0.01	0.50	0.97	0.33	0.07
Σ n-6 PUFA	2.00	2.36	4.92	5.52	2.70	2.97	0.27	< 0.01	< 0.01	0.06	0.86	0.53
Σ n-3 PUFA	0.77	0.57	0.60	0.96	3.40	2.78	0.20	< 0.01	< 0.01	0.38	0.85	0.03

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk).
[b] FAT = Con vs. (CSO + CFO), SFO = CSO vs. CFO.
[c] Includes small proportions of identified and unidentified FA.

Kaplan–Meier model using the LIFETEST procedure of the SAS. Number of AI per pregnancy, pregnancy at the first AI and by 150 and 250 DIM, and incidence of productive and reproductive disorders were analyzed by the GENMOD procedure using a Poisson distribution. Binomially distributed data such as conception and ovulation rate were analyzed by the GENMOD procedure using a binary distribution and a logit odds ratio link.

3. Results

3.1. Diets, nutrient composition, and FA profile

The estimation of individual intake based on group feeding, for which no statistical analysis was performed, was 12.7, 12.4, 12.2 kg/d for CON, CSO, and CFO, respectively. As per the design of the study, all diets were isonitrogenous (141, 140, and 140 g/kg CP for CON, CSO, and CFO, respectively), but CSO and CFO diets were 3.5% more energy-dense than the CON diet (6.15 vs. 5.94 Mj/kg DM, Table 2). The estimated prepartum energy intake was (75.4, 76.2, and 75.0 Mj/d for CON, CSO, and CFO, respectively). As expected, because the inclusion of fat supplements replaced corn, ether extract was higher in CSO and CFO compared with CON (38.0 vs. 29.0 g/kg DM). One evidence that the experimental cows were receiving the target treatments enriched with the target FA, measured in non-composited weekly samples of offered TMR, is the FA profile of the TMR, where CFO diets were rich in EPA and DHA, whereas these FA were undetectable in CON and CSO diets.

3.2. Fatty acid profile, yield, and composition of colostrum

Colostrum yield and chemical composition did not differ among treatments, but the concentration of IgG in colostrum was higher ($P < 0.01$, Table 4) in fat supplemented cows compared with control cows (126 vs. 98 g/l). Parous cows produced colostrum with higher IgG concentrations compared with nulliparous cows (123 vs. 110 g/l). Cattle fed fat before calving produced colostrum with higher C18:0 content compared with those fed CON (7.12 vs. 5.84% of FA; $P = 0.04$; Table 4). All 5 identified C18:1 *trans* isomers were higher ($P \leq 0.04$) in colostrum from cows fed fat compared with those fed CON. Similarly, C18:2n-6 and C20:2n-6 were higher ($P < 0.01$) in CSO-compared with CFO-fed cows. The concentrations of C20:5n-3, C22:6n-3, C24:0 and C24:1 were only detected in colostrum from cows fed CFO. Total n-6 FA was $\sim 1.8\times$ higher in colostrum of cows fed CSO instead of CFO. Contrary, total n-3 FA was $\sim 3.9\times$ higher in colostrum of cows fed CFO instead of CSO.

Parity had a great impact in colostrum FA profile, but it had minimal impact on the effect of supplemental fats (few interactions, Table 4). The most notorious interaction was observed in n-3 FA, and for cows fed CFO only, nulliparous cows had higher concentrations of C20:5n-3, C22:6n-3, C24:0 and C24:1. Thus, total n-3 FA was also higher in nulliparous cows fed CFO compared with parous cows fed CFO.

3.3. BCS, BW, and feed intake

Prepartum dietary supplementation of fat or a specific type of FA did not affect BW or BCS at -3 and +9 wk relative to calving (Table 5). Cows fed either CSO or CFO tended to have greater BCS ($P = 0.07$) at wk 14 postpartum, but BW was not different at any postpartum measurements.

Table 5
Body weight (BW) and Body condition score (BCS) from Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item	Dietary treatment[a] and parity (P)						SEM	*P*-value[b]		
	CON		CSO		CFO					
	Null	Parous	Null	Parous	Null	Parous		FAT	SFO	P
BW (kg)										
Prepartum[c], wk -3	605.3	799.2	599.3	788.0	591.6	783.3	9.5	0.19	0.55	< 0.01
Postpartum, wk 9	516.4	701.3	526.2	702.0	520.5	705.3	11.5	0.64	0.92	< 0.01
Postpartum, wk 14	489.1	667.9	511.4	684.2	510.9	678.0	13.0	0.13	0.79	< 0.01
BCS										
Prepartum[c], wk -3	3.60	3.65	3.66	3.73	3.68	3.74	0.10	0.39	0.87	0.47
Postpartum, wk 9	2.91	2.98	3.06	3.11	3.07	3.12	0.11	0.12	0.90	0.52
Postpartum, wk 14	2.74	2.79	2.90	3.02	2.95	3.04	0.13	0.07	0.77	0.41

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk), After calving all cows received a similar lactation diet.
[b] FAT = Con vs. (CSO + CFO), SFO = CSO vs. CFO. Interaction of treatment with parity were not significant ($P > 0.50$).
[c] LSM of wk −3 to 0.

3.4. Milk production and composition

Feeding different types of FA did not impact milk production or its components. Regardless of the type of FA, supplementing fat prepartum increased milk yield during the entire subsequent lactation (37.9 vs. 35.2 kg/d, $P < 0.01$, Table 6). Milk protein and lactose concentration did not differ among treatment groups ($P > 0.10$), but CON cows produced milk with higher fat content during the first 3 mo of the experiment ($P < 0.001$), and that effect was carried over the entire lactation. Fat yield was higher in cows fed any source of FA compared with CON cows, during mo 4–10 of lactation ($P < 0.001$). Protein and lactose yield were both higher in cows fed supplemental fat compared with CON cows, during the entire lactation. Milk SCC was ~100% and ~70% lower for CFO-cows compared with CSO-cows in the first month of the experiment and throughout the first 3 mo, respectively ($P < 0.001$).

3.5. Plasma metabolites

Prepartum supplementation of fat or specific FA had minimal effects on plasma concentrations of the analyzed metabolites and hormones, both prepartum and postpartum. Plasma cholesterol was higher ($P = 0.01$) in prepartum cows fed fat compared with CON (130.6 vs. 122 mg/dL, Table 7), a similar effect was observed during the postpartum period (159.2 vs. 153.5 mg/dl for fat and CON, respectively). Prepartum plasma NEFA concentrations did not differ with treatments, but during postpartum, it was lower ($P < 0.01$) for cows fed fat compared with CON (0.46 vs. 0.57 mmol/L).

3.6. Reproductive performance

Supplemental fat (CSO or CFO) shortened the days at first estrus (40.2 vs. 50.5 d, $P = 0.04$, Table 8) and first service (68.5 vs. 78.9 d, $P = 0.04$), thus tended to reduce ($P = 0.07$) the numbers of days open (96.1 vs. 110.8 d, P = 0.07) compared with cows fed CON. The percentage of pregnant cows tended to be higher ($P = 0.07$) for cows fed fat compared with CON (75.1 vs. 55.7%). No differences were detected among dietary treatments in the number of AI per pregnancy. Although prepartum fat supplementation or type of supplemental FA did not influence pregnancy at first AI or 150 DIM, the proportion of pregnant cows by 250 DIM tended to be higher in both fat-supplemented groups compared with CON ($P = 0.07$; Fig. 1).

Table 6
Yield, composition, and somatic cell count of milk from Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item	Month postpartum	Dietary treatment[a] and parity (P)						*P*-value[b]			
		CON Null	Parous	CSO Null	Parous	CFO Null	Parous	SEM	FAT	SFO	P
Milk yield (kg/d)	0-3	34.1	44.0	36.2	46.3	37.1	47.1	0.56	< 0.001	0.14	< 0.001
	4-10	28.1	34.3	30.8	36.5	31.9	37.6	0.89	< 0.001	0.26	< 0.001
	0-10	31.5	38.8	33.9	41.0	34.9	41.9	0.79	< 0.001	0.21	< 0.001
4% FCM[c] (kg/d)	0-3	31.6	40.6	32.8	41.4	33.1	41. 8	0.88	0.13	0.69	< 0.001
	4-10	27.1	32.7	29.4	34.5	30.5	35.2	0.71	< 0.001	0.23	< 0.001
	0-10	29.7	36.3	31.5	37.6	32.2	38.1	0.62	< 0.001	0.33	< 0.001
Fat (g/kg)	0-3	35.1	34.8	33.4	32.8	34.0	33.4	0.041	< 0.001	0.19	0.14
	4-10	37.3	37.5	37.2	36.9	37.2	36.2	0.053	0.24	0.58	0.26
	0-10	36.3	36.0	35.0	35.3	35.1	34.3	0.046	< 0.001	0.35	0.21
Fat yield (kg/d)	0-3	1.20	1.54	1.22	1.53	1.23	1.54	0.023	0.65	0.85	< 0.001
	4-10	1.05	1.26	1.13	1.32	1.18	1.35	0.027	< 0.001	0.21	< 0.001
	0-10	1.14	1.38	1.20	1.41	1.22	1.42	0.023	0.01	0.39	< 0.001
Protein (g/kg)	0-3	28.7	29.1	28.2	28.3	28.4	28.5	0.046	0.20	0.70	0.72
	4-10	30.6	30.9	30.4	30.7	30.2	30.4	0.031	0.31	0.39	0.35
	0-10	29.7	30.0	29.4	29.6	29.4	29.5	0.029	0.13	0.86	0.37
Protein yield (kg/d)	0-3	1.02	1.27	1.04	1.29	1.06	1.34	0.020	0.04	0.15	< 0.001
	4-10	0.86	1.05	0.93	1.11	0.95	1.13	0.023	< 0.001	0.32	< 0.001
	0-10	0.94	1.16	1.00	1.20	1.02	1.23	0.019	< 0.001	0.17	< 0.001
Lactose (g/kg)	0-3	45.4	45.5	45.1	45.5	45.1	45.3	0.036	0.51	0.84	0.53
	4-10	45.8	46.2	45.6	45.8	45.5	45.8	0.023	0.16	0.81	0.22
	0-10	45.8	45.5	45.4	45.6	45.3	45.6	0.019	0.18	0.76	0.16
Lactose yield (kg/d)	0-3	1.52	1.98	1.63	2.10	1.67	2.13	0.05	< 0.001	0.54	< 0.001
	4-10	1.29	1.58	1.41	1.67	1.45	1.72	0.036	< 0.001	0.29	< 0.001
	0-10	1.43	1.77	1.54	1.86	1.58	1.90	0.035	< 0.001	0.28	< 0.001
SCC (1000 cells/mL)	0-1	279	320	275	309	128	178	38	0.03	< 0.001	0.21
	0-3	290	320	281	306	190	228	24	0.01	< 0.001	0.13

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk), After calving all cows received a similar lactation diet.
[b] FAT = Con vs. (CSO + CFO), SFO = CSO vs. CFO. Interaction of treatment with parity were not significant ($P > 0.50$).
[c] FCM = fat-corrected milk, calculated as [0.4 × milk production (kg d^{-1})] + [15 × fat yield (kg d^{-1})].

Table 7
Concentration of plasma metabolites from Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item[a]	Dietary treatment[b] and parity (P)						SEM	*P*-value[c]				
	CON		CSO		CFO							
	Null	Parous	Null	Parous	Null	Parous		FAT	SFO	P	Week	Time × treatment
Prepartum[d]												
Glucose, mg/dL	62.1	63.0	65.6	63.4	65.7	64.1	2.45	0.32	0.86	0.62	< 0.001	0.98
Insulin, μIU/mL	12.2	12.7	14.0	13.0	13.8	12.5	1.17	0.40	0.77	0.53	< 0.001	0.82
NEFA, mmol/L	0.20	0.19	0.17	0.15	0.18	0.17	0.030	0.38	0.56	0.58	0.63	< 0.001
Cholesterol, mg/dL	120	124	129	130	129	133	3.84	0.01	0.75	0.43	< 0.001	< 0.001
Triglycerides, mg/dL	7.20	8.22	8.40	9.55	8.04	9.02	0.98	0.23	0.65	0.21	< 0.001	0.86
Total protein, g/dL	7.24	7.48	8.10	7.20	7.92	7.83	0.39	0.25	0.58	0.45	0.55	0.78
Albumin, g/dL	3.75	3.80	4.05	4.20	4.02	4.15	0.36	0.30	0.92	0.80	0.30	0.65
BUN, mg/dL	12.9	14.5	15.3	14.9	15.3	14.7	1.17	0.20	0.88	0.81	< 0.001	0.13
Postpartum[e]												
Glucose, mg/dL	53.4	53.0	55.8	55.0	56.5	53.5	2.26	0.32	0.86	0.46	0.84	0.98
Insulin, μIU/Ml	8.85	9.15	9.85	10.0	11.0	10.1	1.00	0.15	0.51	0.87	< 0.01	0.99
NEFA, mmol/L	0.57	0.56	0.51	0.44	0.49	0.42	0.025	< 0.01	0.58	0.01	< 0.001	0.79
Cholesterol, mg/dL	153	155	158	160	159	160	2.65	0.02	0.85	0.43	< 0.001	0.91
Triglycerides, mg/dL	12.4	12.9	13.5	14.1	14.1	14.7	1.06	0.14	0.56	0.51	0.37	0.96
Total protein, g/dL	6.58	6.75	7.00	7.14	7.12	7.30	0.38	0.17	0.71	0.62	< 0.001	0.98
Albumin, g/dL	3.58	3.62	3.75	3.92	3.98	3.84	0.24	0.23	0.77	0.92	0.26	0.96
BUN, mg/dL	19.2	18.5	19.7	19.3	19.2	19.5	0.73	0.39	0.90	0.44	< 0.001	0.55

[a] For all variables, no interaction between parity and treatment effect were detected ($P > 0.20$).
[b] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk), After calving all cows received a similar lactation diet.
[c] FAT = Con vs. (CSO + CFO), SFO = CSO vs. CFO.
[d] LSM of wk −3 to 0.
[e] LSM of wk +3, +9 and +14.

Table 8
Reproductive performance of Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item	Dietary treatment[a] and parity (P)						SEM	*P*-value[b]		
	CON		CSO		CFO					
	Null	Parous	Null	Parous	Null	Parous		FAT	SFO	P
Days to 1st estrus	45.6	55.4	43.2	46.6	41.3	45.9	3.70	0.04	0.72	0.05
Day to 1st service	73.8	84.0	65.2	73.2	62.2	73.35	5.87	0.04	0.80	0.05
Open days	108	113	91.0	103	81.4	108	8.98	0.07	0.83	0.04
AI per pregnancy	2.21	2.43	2.00	2.20	1.88	2.13	0.25	0.24	0.68	0.29
Preg. at 1st AI,[c] % (n/group)	35.7 (5/14)	25.0 (6/24)	50.0 (7/14)	38.5 (10/26)	42.8 (6/14)	34.8 (8/23)	0.48	0.24	0.50	0.20
Pregnant by 150 DIM, % (n/group)	50.0 (7/14)	41.7 (10/24)	64.3 (9/14)	57.7 (15/26)	57.1 (8/14)	56.5 (13/23)	0.46	0.32	0.43	0.37
Pregnant by 250 DIM, % (n/group)	57.1 (8/14)	54.2 (13/24)	85.7 (12/14)	69.2 (18/26)	71.4 (10/14)	73.9 (17/23)	0.51	0.07	0.21	0.25

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk), After calving all cows received a similar lactation diet.
[b] FAT = Con vs. (CSO + CFO), SFO = CSO vs. CFO. Interaction of treatment with parity were not significant ($P > 0.30$).
[c] Preg. per first AI = pregnancy per first insemination.

3.7. Postpartum health and reproductive disorders

The overall incidence of retained fetal membranes, metritis, inactive ovaries, and ovarian cysts, collectively referred as total reproductive disorders, tended to be higher ($P = 0.06$, Table 8) in cows fed CON diet during the prepartum period but did not differ between CSO and CFO treatments. Cows fed CON diet had higher ($P = 0.01$) incidence of total health disorders than fat-supplemented cows (Table 9).

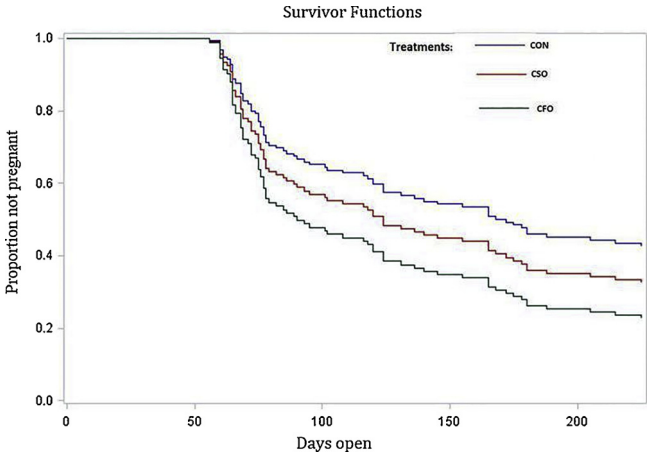

Fig. 1. Survival curves for interval from calving to pregnancy in cows at 250 DIM, (FAT, $P = 0.07$). Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition. After calving all cows received a similar lactation diet. Interactions of dietary treatments with parity were not significant, $P = 0.25$.

Table 9
Postpartum reproductive and health disorders of Holstein cows (n = 120) fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item	Dietary treatment[a] and parity (P)						SEM	*P*-value[b]		
	CON		CSO		CFO					
	Null	Parous	Null	Parous	Null	Parous		FAT	SFO	P
No. of cows	14	24	14	26	14	23	–	–	–	–
Dystocia, % (n/group)	5/14 (35.7)	5/24 (20.8)	2/14 (14.3)	4/26 (15.4)	2/14 (14.3)	5/23 (21.7)	0.42	0.36	0.32	0.92
Retained fetal membranes, % (n/group)	1/14 (7.14)	3/24 (12.5)	0/14 (0.00)	2/26 (7.70)	1/14 (7.14)	2/23 (8.70)	0.72	0.18	0.82	0.18
Metritis, % (n/group),	1/14 (7.14)	4/24 (16.7)	2/14 (14.3)	5/26 (19.2)	2/14 (14.3)	6/23 (26.1)	0.49	0.81	0.35	0.22
Clinical endometritis, % (n/group)	3/14 (21.4)	8/24 (33.3)	2/14 (14.3)	5/26 (19.2)	2/14 (14.3)	7/23 (30.4)	0.42	0.38	0.60	0.19
Ovarian cyst, % (n/group)	2/14 (14.3)	7/24 (29.2)	2/14 (14.3)	2/26 (7.70)	2/14 (14.3)	5/23 (21.7)	0.47	0.24	0.75	0.66
Inactive ovaries, % (n/group)	1/14 (7.14)	2/24 (8.33)	0/14 (0.00)	1/26 (3.84)	0/14 (0.00)	2/23 (8.70)	1.18	0.99	0.28	0.13
Mastitis, % (n/group)	1/14 (7.14)	3/24 (12.5)	0/14 (0.00)	1/26 (3.84)	0/14 (0.00)	2/23 (8.70)	0.91	0.99	0.21	0.08
Total reproductive disorders,[c] % (n/group)	6/14 (42.8)	13/24 (54.2)	4/14 (28.6)	6/26 (23.1)	5/14 (35.7)	10/23 (43.5)	0.35	0.06	0.45	0.69
Total health disorders, % (n/group),	7/14 (50.0)	14/24 (58.3)	3/14 (21.4)	8/26 (30.8)	5/14 (35.7)	11/23 (47.8)	0.34	0.01	0.62	0.54

[a] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil (last 3 wk), After calving all cows received a similar lactation diet.
[b] FAT = Con vs. (CSO + CFO), SFO = CSO vs. CFO. Interaction of treatment with parity were not significant ($P > 0.20$).
[c] Total reproductive disorders = sum of retained fetal membranes, metritis, inactive ovaries and ovarian cyst incidence; total health disorders = sum of retained fetal membranes, metritis, inactive ovaries, ovarian cyst, clinical ketosis, mastitis, and dystocia.

4. Discussion

The authors acknowledge the limitation of the current study, with regards to the lack of individual DMI, particularly, during the prepartum period, when the dietary treatments were imposed to cows allocated in similar pens, but only having 1 pen per treatment. Nevertheless, as detailed before, all efforts were done to ensure experimental cows were allocated in pens with the same characteristics and that cows received their target diets. Indeed, the FA profile and ether extract of the experimental diets support the latter. Furthermore, strong evidence of cattle consuming their target dietary treatments is the finding that the FA profile of their colostrum resembles that of their diets.

Although the effect of parity was significant for most of the measured variables, it did not impact the effect of dietary treatments,

except for some FA in colostrum. Thus, the effect of parity will only be discussed for its importance on regulating colostrum FA, whereas for other variables only the main effect of dietary treatments will be discussed. Furthermore, direct comparison of findings in the current study with others will be limited to very few studies reporting supplementation of polyunsaturated fatty acids (PUFA) sources during the prepartum period only (Hayirli et al., 2011; Salehi et al., 2016a, b). Thus, we will also compare our findings with others feeding supplemental fat during prepartum and postpartum or postpartum only.

In the current study, cows fed any source of PUFA compared with non-fat supplemented cows produced colostrum with higher IgG content, without affecting yield and chemical composition. Garcia et al. (2014) reported that parous cows fed fat (saturated or rich in n-6 FA) produced colostrum with higher IgG concentration compared with parous cows non-fat supplemented. On the other hand, others have reported that cows fed n-6 but not n-3 produced colostrum with higher IgG content compared with non-supplemental cows (Salehi et al., 2016b). Our findings and those of others, are not in complete agreement on which type of FA promote higher contents of IgG in colostrum, but all agree on concluding that supplementing FA instead of non-FA-supplemented diets benefits colostrum quality.

Concentrations of short- to medium-chain FA in colostrum was not affected by treatments, which partly agrees with the lack of effect of linseed fed to prepartum cows on short-chain FA (10 carbon atoms and less) in colostrum (Santschi et al., 2009). Prepartum fat supplementation increased the proportion of C18:0 in colostrum compared with control. Similarly, prepartum feeding of seeds rich in n-6 or n-3 FA (Santschi et al., 2009; Salehi et al.,2016b) increased the proportion of C18:0 in colostrum compared with non-PUFA supplemented diets. The higher content of C18:1-all *trans* FA in colostrum of fat-supplemented cattle indicates that the Ca salt products fed were not completely ruminally inert, allowing some of the C18:2n-6 and C18:3n-6 to be metabolized by ruminal microorganisms (Lundy et al., 2004). Similarly, dairy cows supplemented with extruded linseed compared with a palm oil-based product produced colostrum with higher content of C18:1 *trans* isomers (Santschi et al., 2009). Furthermore, Garcia et al. (2014) reported an increase in concentrations of trans isomers of unsaturated monoene and diene FA detected in colostrum of cows fed a rumen bypass fat rich in C18:2n-6, during the transition period. In spite of a higher biohydrogenation of C18 FA, fat supplemented groups produced colostrum with more PUFA compared with CON cows, because they were offered higher proportions of dietary PUFA in the form of Ca salts which partly prevented its hydrogenation in the rumen (Klusmeyer and Clark, 1991). Hence, a higher amount of PUFA should have reached the intestine for absorption and potential transfer to the colostrum compared with CON. Indeed, in the current study FA from the n-6 family (C18:2n-6 and C20:2n-6) and the n-3 family (C20:5n-3, C22:6n-3) were higher in cows fed CSO and CFO, respectively. Our findings support that of others who also concluded that cows fed a particular profile of dietary FA produced colostrum with a FA profile that resembles that of the diet (Moallem and Zachut, 2012; Garcia et al., 2014; Salehi et al., 2016b).

Parity had a noticeable effect on the FA profile of colostrum. Nulliparous cows produced colostrum containing less C16:0 but more C18:0 compared with parous cows, and this response was not affected by dietary fat supplements. Garcia et al. (2014) reported similar effects of parity for C16:0 and C18:0, with lack of interaction with diets (fat supplements enriched in C18:0 or C18:2n-6). Total n-6 PUFA tended to increase in colostrum of parous cows fed supplemental fat, contrary total n-3 PUFA was higher for nulliparous compared with parous cows, in the CFO group. These findings are in partial agreement with those of Garcia et al. (2014), who reported that nulliparous cows produced colostrum with lower n-6 but with higher n-3 PUFA and concluded that the higher desaturase indices for C20:5n-3, and C22:6n-3 observed for nulliparous compared with parous cows, demonstrates less ability of older cows to synthesize essential FA derivatives. Furthermore, the fewer transfer of n-3 FA into colostrum of multiparous cows may suggest a potential greater requirement of these essential FA in older cows, but this hypothesis requires further investigation.

Body weight was similar among treatments, and all cows had almost equal BW at the end of the study. These results are in accordance with Grummer, (1995), who reported no effect of prepartum tallow supplementation (2.8% of DM) on BW of dairy cows during the first 150 d of lactation. Furthermore, Garcia et al. (2014), found that prepartum dietary FA supplementation did not affect 4% fat corrected milk (FCM), BW gain, change in BCS, or gestation length in Holstein dairy cows. Regardless of parity, cows fed CSO and CFO tended to have greater BCS compared to CON cows at weeks 14 postpartum, this coupled with the higher milk production for cows fed fat, may indicate that cows fed fat had a higher DMI postpartum and were more efficient converting feed in milk, however, as we did not measure DMI or digestibility our suggestion cannot be confirmed.

Increasing the intake of PUFA, regardless of the omega family, improved milk and FCM during the entire experimental period (300 DIM). Although non-significant, cows fed CFO produced more milk and FCM than cows fed CSO (+ 981 and + 645 kg in 300 d) and both together produced + 838 and + 552 kg of milk and FCM, respectively, compared with cows fed CON prepartum. Contrary to our findings, Salehi et al. (2016a) reported that multiparous cows fed seed oil, regardless of the type of FA produced less milk during +1, +2, and +4 weeks postpartum, whereas Hayirli et al., (2011) found no effect of prepartum PUFA on milk yield in the subsequent lactation. Nevertheless, Greco et al. (2015) found that increasing the amounts of n-3 PUFA in replacement of n-6 PUFA during lactation, improved milk, and FCM yields. Greco et al., (2015) attributed a portion of the higher milk and FCM yields to higher caloric intake and the other portion, not accounted for the increased energy intake, to changes in tissue FA composition which may have influenced nutrient partitioning, thus favoring lactation. Muscle and sub-cutaneous fat of lambs suckling dams supplemented with Ca salts of palm oil, olive oil, or fish oil, resemble the composition of their dams' milk, which in turn resembled that of their diets (Gallardo et al., 2014).

Although cows fed CON produced milk with higher fat content, cows fed fat produced more milk and thus had a higher fat yield, along with higher protein and lactose yields. Duske et al. (2009) reported that cows fed diets supplemented with fat prepartum (rich in C18:0 and C18:1) produce milk with a lower content of lactose but higher fat content, and milk yield did not differ, during the first 4 wk postpartum. The findings of Duske et al. (2009), who fed a common lactation diet after calving, are in agreement with our findings that strategic supplementation of FA during prepartum only have a carryover effect on subsequent lactation, and also support

the finding that the dietary fat supplements should contain PUFA rather than saturated or monounsaturated FA.

The impact of prepartum diets rich in PUFA on lowering milk SCC was driven by the positive impact of n-3 PUFA and not by n-6 PUFA (305, 294, and 209 × 10^3 cells/mL, CON, CSO, and CFO, respectively). Others also reported that supplemental fish oil-FA reduced milk SCC (Badiei et al., 2014; Sinedino et al., 2017). Those authors concluded that the benefit of n-3 PUFA on reducing SCC might be related to changes in immune and inflammatory response, providing the n-3-supplemented cows with a more robust immune system.

The observed minimal effects of prepartum diets on prepartum metabolites is not unexpected, as the estimated peripartum DM and caloric intake suggest similar intakes among treatments and cows were not producing milk that could increase their nutrient demand. The only effect of fat supplements was towards increasing plasma cholesterol during the pre- and postpartum periods. Cows infused postruminally with free long-chain FA had higher plasma cholesterol concentrations (Drackley et al., 1992). The effect of fat on lowering cholesterol prepartum was maintained during the lactation period. An opposed effect of diets was observed for NEFA, where cows fed CON had higher postpartum NEFA concentrations. Studies supplementing fat during both the prepartum and postpartum or postpartum have found inconsistent results on plasma concentrations of NEFA, with some reporting fat supplements did not alter (Douglas et al., 2004, 2006) or increased (Grummer and Carroll, 1991; Ballou et al., 2009) NEFA concentrations. Reflecting the greater BCS by the end of the experimental period, cows fed fat compared with CON had lower concentrations of NEFA postpartum, which may be related to a less negative energy balance postpartum, as others have associated higher levels of NEFA with increased body fat mobilization and insufficient FA oxidation (Roberts et al., 1981; Vazquez-Anon et al., 1994).

Regardless of the type of FA, fat supplementation improved several reproductive parameters. Cows fed fat manifested heat 6 d earlier, were inseminated 10 d earlier, and were open for 14 d less compared with CON cows. Salehi et al. (2016a) reported that oilseed supplementation and type of oilseeds, supplemented prepartum only, had no effect on pregnancy at first AI or the proportion of pregnant cows by 150 DIM, but oilseed supplementation, regardless of the type of FA, tended to improve the proportion of pregnant cows by 250 DIM. In contrast to our findings and those of Salehi et al. (2016a); Badiei et al. (2014) reported no reproductive benefit of feeding n-3 FA during the prepartum period only, noteworthy is the small number of cows in Badiei et al., (2014), 8 per treatment, compared with our current study where 40 cows where used per treatment. The observed higher plasma cholesterol in cows fed fat might have benefited reproductive efficiency. Others have also found that fat supplementation increased plasma cholesterol and cholesterol content in follicular fluid and corpus luteum (Staples et al., 1998). Another potential mechanism for improved reproductive efficiency with fat supplementation may be related to the strategic deposition of FA in reproductive organs such as the endometrium (Mattos et al., 2003, 2004), which may have played a critical role on early resumption of heat and lower embryo death rate. Furthermore, Burke et al. (1997) reported higher concentrations of progesterone 2 d after PGF2α injection in cows fed menhaden fish meal, suggesting a delayed luteal regression in cows consuming n-3 FA (eicosapentaenoic and docosahexaenoic acid). A higher incidence of postpartum disorders or diseases has been highly associated with impaired reproductive efficiency (Fourichon et al., 2000; Ribeiro et al., 2013; Bollwein et al., 2017). In the current study, cows fed CON almost doubled the incidence of health and reproductive problems compared with cows fed fat, and it was coupled with a lower reproductive efficiency for in CON cows. Furthermore, Juchem et al. (2010) reported that calcium salts of linoleic and *trans*-octadecenoic acids supplemented from 25 d prepartum to 80 d of lactation reduced the incidence of puerperal metritis, which might contribute to their reported enhanced fertility. Contrary to our findings, Salehi et al. (2016a) reported no effect of prepartum feeding of oilseeds (n-6 or n-3) or specific FA on pregnancy rate but reported that cows fed oil seeds tended to have a higher incidence of total reproductive disorders and cows fed n-3 instead of n-6 FA had a higher incidence of total health disorders.

5. Conclusions

Strategic supplementation of PUFA during the close-up period only, improved productive and reproductive performance in the subsequent lactation evaluated up to 300 d. Fatty acid composition of colostrum resembled that of the dietary supplements. Contrary to our hypothesis, the type of PUFA (n-6 vs. n-3) supplemented prepartum did not impact performance. Despite the lower fat content in cows fed either calcium salts rich in n-6 or n-3, they had higher milk and FCM yields. Dietary treatments have a minor impact on plasma metabolites. Total health and reproductive disorders were decreased in cows fed supplemental fat prepartum, and it was coupled with earlier estrus and days to first service, hence lower days open and higher pregnancy rate by 250 DIM. Our findings suggest that prepartum supplementation of PUFA, during last 3 weeks of gestation, may be an effective strategy to increase the profitability of the dairy industry through improving productive and reproductive performance. Future studies should consider examining the effects of prepartum fat supplementation on the performance and health of the preweaned and postweaned calf.

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