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The effects of omega-3 α-linolenic acid from flaxseed oil supplemented to high-yielding dairy cows on production, health, and fertility

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ABSTRACT

Among the long-chain fatty acids (FA), the omega-3 (n-3) FA have the most potent immunomodulatory activities. In the present large-scale study, we tested the effects of supplementation of α-linolenic acid (ALA) from flaxseed commenced at the dry period on milk yield, health and fertility of dairy cows. Cows in a large commercial dairy farm were randomly divided into two groups 21 days before expected calving. During the dry period, cows in the treatment group were fed a diet that contained, on a DM basis, 40 g/kg of an extruded flaxseed supplement (EFLX; $n = 276$); their postpartum diet contained 50 g/kg of the same supplement. The cows were group-fed, and based on average cow's intake, the EFLX cows consumed 80 and 220 g ALA/day per cow prepartum and postpartum, respectively. The control cows received a diet with a different composition but a similar content of nutrients (CTL; $n = 240$). A veterinarian routinely examined the cows 7 to 10 days after calving, treated them according to the farm's routine practice, and determined their body condition score at that visit and at peak lactation. Milk yield was 4.5% greater (1.8 kg/day; $P < 0.0001$), and fat ($P < 0.0001$) and protein ($P = 0.002$) contents were lower in the EFLX vs. CTL group. The proportion of n-3 FA in milk was 3.9 times higher in EFLX than in CTL cows ($P < 0.0001$), and the omega-6 (n-6):n-3 ratio in the milk fat decreased from 13.0 in the CTL cows to 4.1 in the EFLX cows ($P < 0.0001$). The unsaturated FA content in milk fat was 20.1% greater in EFLX than in CTL cows ($P < 0.0001$). Ketosis incidence was lower in the EFLX vs. CTL group, 23.5 and 31.2%, respectively ($P = 0.05$), and ketosis was less severe in the former ($P = 0.03$). The mortality rates in EFLX and CTL cows were 0.7 and 4.6%, respectively ($P = 0.005$). No differences were observed in conception rates at first or second insemination, but days from first service to conception and calving to conception were 17 ($P = 0.07$) and 18 days ($P = 0.09$) less in the EFLX cows, respectively. In conclusion, supplementation of EFLX to dairy cows from the dry period increased milk yield, decreased incidence of ketosis and severe metritis, reduced mortality, and tended to enhance fertility performance. Overall, ALA supplementation improved milk quality and was beneficial to the cow's health and fertility.

1. Introduction

In recent years, fatty acids (**FA**) have been recognized as major regulators in biological tissues; they are the main components of cell membranes and their composition influences cell functioning. In westernized societies, consumption of omega-6 (**n-6**) polyunsaturated FA (**PUFA**) is on the rise; the n-6:omega-3 (**n-3**) FA ratio has increased from 2.4 in primitive human diets to 12.0 in the modern human diet (Simopoulos, 2002). Diets with lower n-6:n-3 ratios are considered healthier for humans. Traditionally, milk and milk products have been among the main contributors to human nutrition. Linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3; **ALA**) are the most prominent PUFA in milk fat; in the liver, these two essential FA may be converted to longer-chain FA by desaturation and elongation enzyme systems.

Fatty acids are involved in several biological systems and processes: the immune system (Prescott and Calder, 2004; Raphael and Sordillo, 2013; Sordillo, 2016); blood coagulation and vascular resistance; enzyme activities; cell proliferation and differentiation; and receptor expression (Clandinin et al., 1991). The types of FA within the membrane phospholipid can affect both membrane fluidity and function of immune cells (Raphael and Sordillo, 2013). The n-3 FA are the most potent participants in the above pathways (Deckelbaum et al., 2006); the long chain length and the presence of double bonds at carbon 3 endow these FA with distinct characteristics that confer unique biological capabilities. Sordillo (2016) thoroughly reviewed studies that found direct or indirect involvement of long-chain FA in many

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immune-system activities.

Our group has demonstrated beneficial effects of n-3 supplementation on the reproductive system in dairy cows (Zachut et al., 2010 and 2011; Moallem et al., 2013; Moallem, 2018) and in bulls (Moallem et al., 2015). Feeding n-3 PUFA attenuated endometrial prostaglandin $F_{2\alpha}$ (Mattos et al., 2002), and cows fed rolled flaxseed exhibited decreased pregnancy loss rates and larger ovulatory follicles (Ambrose et al., 2006).

In ruminants, biohydrogenation activity of the microflora largely converts unsaturated FA reaching the rumen to saturated FA (SFA), and Chouinard et al. (1997) suggested that heat treatment of grains, such as extrusion at high temperature, might protect PUFA from biohydrogenation activity in the rumen. Flaxseed and its oil form the most widely available botanical source of n-3 FA that contain >50% ALA (Moallem, 2018). Although many sophisticated studies have reported the beneficial effects of n-3 FA supplementation on health and fertility of dairy cows, no large-scale study, begun during the dry period and continuing through lactation, has ever been performed. We hypothesized that feeding dairy cows with n-3 FA from the dry period will help to prepare the immune system and consequently benefit postpartum health and fertility. Therefore, the objectives of the present study were to determine the outcomes of extruded flaxseed (EFLX) supplementation—which provided ALA—that was initiated 3 weeks before expected calving, on milk yield, milk composition, and health and fertility of dairy cows.

2. Material and methods

2.1. Cows and treatments

The experimental study protocol was approved by the Animal Care Committee of the Volcani Center, Rishon LeZion, Israel. The experiment was conducted on a large commercial dairy farm (Darom Farm, Kibbutz Gut, Israel). Multiparous Israeli-Holstein cows ($n = 516$) were used in this 18-month study; the cows were randomly divided into two groups—EFLX, and control (CTL)—3 weeks before the expected calving. The average parameters (mean ± SD) of the cows at the previous lactation of the control and EFLX cows, respectively, were: milk during 305 days of lactation – 12,574 ± 1643 and 12,459 ± 1717 kg; average milk fat percentage – 3.66 ± 0.28 and 3.64 ± 0.26; average milk protein percentage – 3.32 ± 0.15 and 3.33 ± 0.14, and parity number - 1.8 ± 1.3 and 1.9 ± 1.4. During the dry period, the cows received diets that were similar in nutrients content, but the diet of the EFLX group contained extruded flaxseed (Valomega 160; Valorex, Combourtille, France) at 40 g/kg of diet DM. Valomega 160 is a specific extruded supplement that comprises flaxseed at 700 g/kg and wheat bran at 300 g/kg. The supplement is produced by Tradi-Lin technology (patent no. EP 1,155,626 A1), which is characterized by a preliminary maturing step of specific duration—between 10 and 30 min—and temperature around 80 °C, and by the level of incorporation of steam and mounting of the extruders.

After calving, the cows were housed in two loose barns with adjacent outside yards that were oriented parallel to each other. The pens were identical in size (100 cows in each), design, bedding and ventilation. In addition, in Israel there are four seasons, and the study continued 18 mo, in which the cows in both groups experienced 6 seasons in these pens. Both groups were fed lactating cow rations (Table 1) with similar contents, but that of the EFLX group contained Valomega 160 at 50 g/kg of diet DM. The cows were moved after calving from the dry-cow's barn to the milking-cow's barns and remained in the EFLX barn until they were detected as pregnant by the veterinarian, and then they were moved to a different barn. The ingredients and chemical composition of representative diets are presented in Table 1; however, because the study lasted 18 months, the dietary composition was changed from time to time depends on the availability and relative cost of the ingredients. The FA profile of representative diets are presented in Table 2. The cows were fed a total mixed ration (**TMR**) twice daily at 0900 and 1300 h; it comprised 107% of the expected intake, adjusted according to the previous day's intake. The TMR was sampled every month, composited and analyzed for FA profile.

Table 1
Ingredients and chemical composition of lactating cow's diet.

Ingredients	g/kg of DM	
	CTL[1]	EFLX[1]
Corn grain, ground	122	127
Wheat grain, ground	44	44
Rapeseed meal	22	52
Wheat bran	11	0
Corn gluten feed	102	89
Whole cottonseed	25	0
Wheat silage	98	98
Rye + whole cottonseed	0	12
Sorghum silage	161	154
Dried distiller's grain, gold	45	45
Oat hay	22	30
Clover hay	35	35
Dough, bakery waste	56	38
Corncob peels	105	76
Whey	128	128
Valomega 160	0	50
Calcium salts of fatty acids[2]	8	7
Urea (NPN)	5	4
Calcium bicarbonate	5	5
Vitamins and minerals[3]	11	11
Limestone	8	8
Salt	0.3	5
Chemical composition		
NE_L, MJ/kg[4]	7.41	7.41
Crude protein, g/kg	163	163
NDF[5], g/kg	290	289
NDF – forage[6], g/kg	170	170
Ether extract, g/kg	62.5	65
Ca, g/kg	0.09	0.09
P, g/kg	0.04	0.04

[1] CTL, control; EFLX, extruded flaxseed.
[2] Calcium salts of palm oil distillate.
[3] Comprising (per kg) 16,000,000 IU vitamin A, 3200,000 IU vitamin D, 48,000 IU vitamin E, 24.0 g Mn, 24.0 g Zn, 24.0 g Fe, 12.8 g Cu, 1.44 g I, 0.32 g Se, 0.32 g Co.
[4] Calculated using NRC (1989) values.
[5] From forage plus other ingredients.
[6] From forage ingredients only.

Cows were milked thrice daily and milk production was recorded electronically. Milk samples from two consecutive milkings were collected monthly, pooled into one sample per cow and analyzed at the laboratories of the Israeli Cattle Breeders' Association (Caesarea, Israel) according to standard IDF 141C, 2000, for milk fat, protein, lactose and urea contents. Somatic cell counts (**SCC**) were also determined at the same laboratory.

During milk sampling, further milk samples were taken randomly to determine milk fat FA profile from 24 cows from each group that were at least 50 days in lactation. In addition, milk of both groups was collected into separate tankers, from which samples were collected weekly for FA composition analysis for 42 weeks—through completion of the study. Cows were weighed automatically after each milking with a walk-in electronic scale (S.A.E. Afikim, Kibbutz Afikim, Israel). Every 3 months (six times in total), blood samples were taken after the morning milking at 0800 h from six cows that were randomly selected for FA profile analysis. They were collected from the coccygeal tail vein into vacuum tubes containing lithium heparin (Becton Dickinson Systems, Cowley, UK) and were immediately put on ice. Plasma was then collected after centrifugation at 1500 × g for 20 min, and stored at −32ºC pending analysis.

Table 2
Fatty acid (FA) composition of postpartum diets (g per 100 g FA).

	Diets	
FA	CTL[1]	EFLX[1]
C16:0	32.88	24.65
C16:1	0.46	0.48
C18:0	4.42	4.21
C18:1n-9	33.26	30.19
C18:2n-6	24.75	24.17
C18:3n-3	1.89	14.45
C20:0	0.51	0.49
C20:1n-9	0.11	0
C20:2n-6	0.4	0.18
C20:4	0.41	0.28
C22:0	0.23	0.29
C22:2n-6	0.16	0.16
C22:5n-3	0.12	0.10
C24:0	0.28	0.22
C24:1	0.13	0.11
SFA[2]	38.32	29.86
MUFA[3]	33.96	30.78
PUFA[4]	27.73	39.34
n-3[5]	2.01	14.55
n-6[6]	25.31	24.51
n-6:n-3	12.59	1.68

[1] CTL, control; EFLX, extruded flaxseed.
[2] Sum of saturated FA.
[3] Sum of monounsaturated FA.
[4] Sum of polyunsaturated FA.
[5] Sum of n-3 FA.
[6] Sum of n-6 FA.

2.2. Health and reproductive management

Cows were routinely examined by a veterinarian 7 to 10 days after calving, treated according to the farm management routine, and clinical events and treatments were recorded. For estrus quantification by locomotion assessment, the cows were fitted with a pedometer system (AfiFarm System; SAE AFikim, Israel). Pedometer readings were taken thrice daily at 8-h intervals in the milking parlor, and analyzed automatically by the herd management computer program. Estrus was detected solely by pedometers, unless cows were observed for estrus behavior signs. The cows were inseminated from 70 days in milk (**DIM**); cows that did not exhibit behavioral estrus by 70 DIM were examined and treated by the veterinarian according to routine protocols. All data on postpartum health disorders and reproductive performance (number of inseminations, date of conception, etc.) were obtained from the dairy farm database (NOA, Israeli Cattle Breeders Association). Cows that did not return in estrus 45 days after artificial insemination (AI) were checked by a veterinarian by rectal palpation for presence of pregnancy. A positive pregnancy diagnosis required the presence of an amniotic vesicle with viable embryo detectable heartbeat. Days from first service to conception and calving to conception (days open), number of AI per conception, conception rates at first and second AI, as well as health disorders (ketosis, retained placenta, metritis, milk fever, and mastitis) were analyzed according to treatment groups (CTL or EFLX). Metritis and ketosis events were categorized as mild, moderate, or severe, according to diagnosis and number of treatments until recovery: recovery after one treatment session was defined as mild, after two or three treatments as moderate, and after three and more treatments as severe. One veterinarian performed the diagnosis and treatment of cows throughout almost the entire study period. The body condition score (**BCS**) of cows (scale 1–5; Edmonson et al., 1989) was determined by the veterinarian 7 to 10 days postpartum and at peak lactation.

2.3. Chemical analysis

Diets were sampled each month and DM was determined. Feed samples were dried at 65ºC for 24 h and then ground to pass through a 1.0-mm Retsch (Haan, Germany) SM 100 screen. At the end of the study, the monthly diet samples from each group were combined for further analysis. The ground combined samples were dried at 100ºC for 24 h and analyzed for N by method 984.13 of the Association of Official Analytical Chemists (**AOAC**) (1990). Neutral detergent fiber (**aNDF**) and acid detergent fiber contents were determined with Ankom Technology equipment (Fairport, NY, USA). The aNDF content was determined using α-amylase and sodium sulfite, with ash included for calculations (Van Soest et al., 1991). The National Research Council (1989) (NRC) values, as accepted in Israel, were used for net energy for lactation (**NE$_L$**). AOAC (1990) methods 935.13 and 964.06 were used to determine Ca and P, respectively. Samples were dried at 550ºC for 3 h for ash determination.

2.4. Fatty acid analysis

Milk fat and plasma FA composition were determined by gas chromatography (GC) after extraction according to AOAC method 996.06 (AOAC, 1990). The analyses were performed at Milouda Laboratories (Haifa, Israel) with an Agilent Technologies 6890 N GC instrument (Santa Clara, CA, USA) equipped with a capillary column (60 *m* × 0.25 mm, 0.25 µm) (Quadrex, Woodbridge, CT, USA) and a flame ionization detector. The process temperature was increased from 60 °C by 5 °C/min to 225 °C isothermal, at which it was maintained. Hydrogen was used as the carrier gas, with a linear velocity of 1.6 m/min; injection volume was 1 µl, with a split ratio of 1:100.

2.5. Statistical analysis

Continuous variables (milk and milk solids) were treated as repeated measurements with the PROC MIXED procedure, version 9.2 of SAS (2002). The model used for yield (Y) was:

$$Y_{ijkl} = \mu + T_i + L_j + C(T{*}L)_{ijk} + DIM_{ijkl} + E_{ijklm}$$

Where μ = overall mean; T_i = treatment effect, $_i$ = 1 or 2; L_j = parity, $_j$ = 2 or >2; $C(T^*L)_{ijk}$ = cow$_k$ nested in treatment$_i$ and cow nested in parity$_j$; DIM$_{ijkl}$ = DIM as continuous variable; and E_{ijklm} = random residual.

The autoregressive order 1 (AR 1) was used as a covariance structure in the model, because it resulted in the lowest Bayesian information criterion (BIC) for most of the variables that were tested. Means were estimated using the LSMEANS procedure of SAS by using the Tukey-Kramer procedure.

The FA profiles in plasma and milk were analyzed with the general linear models (GLM) procedure, version 9.2 of SAS (2002). Distributions of health disorders and conception rates were analyzed with the PROC FREQ procedure, version 9.2 of SAS (2002). When relevant, least-squares means and adjusted standard error means (SEM) are presented in the tables. Significance was considered at $P \leq 0.05$ and tendencies were reported for $0.05 < P < 0.10$.

3. Results

3.1. Fatty acid profile of total mixed ration

Table 2 presents the FA profiles of the TMR. Proportions of palmitic acid (C16:0) and oleic acid (C18:1n-9), respectively, were 25% and 9% lower in the EFLX vs. CTL diet. The proportion of ALA was 7.6 times greater in the EFLX vs. CTL diet, and total PUFA was 41% greater in EFLX than in CTL. The n-6:n-3 ratio was 12.59 in CTL compared with 1.68 in the EFLX diet.

Table 3
Mean treatment effects on milk yield and composition.

	Treatment[1]			
	CTL	EFLX	SEM	*P*-value
Milk, kg/day	39.6	41.4	0.21	<0.0001
Fat, g/kg	36.9	32.0	0.04	<0.0001
Protein, g/kg	32.2	31.6	0.01	0.002
Lactose, g/kg	47.9	47.9	0.011	0.92
Fat, kg/day	1.47	1.33	0.02	<0.0001
Protein, kg/day	1.29	1.32	0.01	0.04
Lactose, kg/day	1.96	2.04	0.02	0.006
SCC (x 10^3)	498	398	40	0.08

[1] Cows were randomly divided into two groups 21 days before the expected calving. EFLX cows (n = 276) received a diet containing: precalving, 4% of an extruded flaxseed supplement providing α-linolenic acid (ALA) and postpartum, 5% of the same supplement. CTL cows (n = 240) received a diet without the flaxseed supplement but with similar nutrient content.

3.2. Yields of milk and milk solids

The average daily milk production was 4.5% (1.8 kg/day) higher in the EFLX vs. CTL cows (Table 3). Fat and protein contents were lower in EFLX vs. CTL cows. The fat yields (kg/day) were lower and the protein yields higher in the EFLX vs. CTL group. The SCC tended to be lower in the EFLX vs. CTL group (Table 3).

3.3. Plasma and milk FA profile

Table 4 presents plasma FA composition of all blood samples taken throughout the study period. Palmitic acid content was lower and that of stearic acid (C18:0) tended to be lower in blood of the EFLX vs. CTL cows. The content of ALA was 3.4 times higher in the EFLX vs. CTL group. Total SFA was lower and PUFA tended to be higher in the EFLX vs. CTL cows. Total n-3 FA were 1.7 times higher in EFLX than in CTL, and EFLX supplementation decreased the plasma n-6:n-3 ratio (Table 4).

Fifty FA and their isomers were detected in the milk fat; in Table 5, we present the main ones. The proportions of palmitic acid and total oleic acid were, respectively, 21% lower and 20% greater in the EFLX vs. CTL cows. The proportion of stearic acid was 13% higher in the EFLX vs. CTL cows. Total linoleic acid (C18:2) and conjugated linoleic acid (CLA) contents were higher in EFLX than in CTL cows (Table 5). The proportion of ALA was 3.5 times higher and those of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) also were higher in the EFLX vs. CTL cows. The proportion of total SFA was 7.3 percentage points lower and those of monounsaturated FA (**MUFA**) and PUFA were, respectively, 5.7 and 1.68 percentage points higher in the EFLX than in the CTL cows. The proportion of total n-3 fatty acids was 3.6 times higher and the n-6:n-3 ratio was lower in the EFLX vs. CTL cows (Table 5).

In addition to the individual cows' milk analyses, we obtained the results from 42 pooled milk samples from each group—samples that we collected weekly from the milk tankers. We identified 50 FA and their isomers, and the same trend that appears in Table 5 for individual cows also appeared in the tanker results: lower palmitic acid, higher ALA, lower SFA, higher MUFA and PUFA contents, and lower n-6:n-3 ratio in the EFLX vs. CTL milk fat (data not presented).

3.4. Health event incidence and body condition

Table 6 presents incidences of health events. Incidence of cows without metritis, or with mild or moderate metritis, did not differ between groups, but occurrence of severe metritis was numerically higher in the CTL vs. EFLX cows. Incidence of cows without ketosis was 7.6 percentage points (11%) higher in the EFLX vs. CTL cows, and that of

Table 4
Mean treatment effects on plasma fatty acid (FA) composition (g per 100 g FA) in cows supplemented with extruded flaxseed.

	Treatment[1]			
Fatty acid	CTL	EFLX	SEM	*P*-value
C14:0	0.90	0.78	0.05	<0.12
C16:0	17.10	15.8	0.4	<0.04
C16:1	1.38	1.20	0.14	0.38
C16:2	0.57	0.62	0.05	0.54
C16:3	0.36	0.36	0.05	0.98
C18:0	13.12	12.30	0.36	0.12
C18:1n-9	8.95	8.41	0.38	0.32
C18:1n-7	0.87	1.05	0.24	0.59
C18:2n-6	45.9	46.8	0.93	0.51
C18:3n-6	0.97	0.48	0.10	0.002
C18:3n-3	1.44	4.88	0.12	0.0001
C20:1n-9	0.41	0.32	0.04	0.12
C20:3	1.80	1.17	0.09	<0.0001
C204n-6	2.82	2.33	0.13	<0.02
C20:4n-3	0.35	0.44	0.06	0.26
C20:5n-3	0.42	0.43	0.08	0.92
C22:1n-9	0.18	0.15	0.04	0.65
C22:5n-3	0.28	0.28	0.07	0.97
C22:6n-3	2.14	2.12	0.25	0.94
SFA[2]	31.1	28.9	0.71	0.03
MUFA[3]	11.8	11.1	0.48	0.34
PUFA[4]	57.1	59.9	1.1	0.07
n-3[5]	5.05	8.48	0.34	<0.0001
n-6[6]	49.7	49.6	0.90	0.93
n-6:n-3	11.1	6.07	0.51	<0.0001

[1] Cows were randomly divided into two groups 21 days before expected calving. EFLX cows (n = 276) received a diet containing: precalving, 4% of an extruded flaxseed supplement providing α-linolenic acid (ALA) and postpartum, 5% of the same supplement. CTL cows (n = 240) received a diet without the flaxseed supplement but with similar nutrient content. In total 33 blood samples from the CTL cows and 35 from the EFLX were analyzed.
[2] Sum of saturated FA.
[3] Sum of monounsaturated FA.
[4] Sum of polyunsaturated FA.
[5] Sum of n-3 FA.
[6] Sum of n-6 FA.

severe ketosis was higher in the CTL vs. EFLX animals. Milk fever incidence did not differ between groups, and incidence of mastitis events that were treated by the veterinarian was also similar between groups. Cow mortality during the study period was 4.6 and 0.7% in the CTL vs. EFLX groups (Table 6). Measured BCS in the first week and at peak lactation were higher in the EFLX than in CTL cows; however, no differences were observed between groups in the BCS changes from the first week to peak lactation (~0.62 BCS unit for both groups). In addition, the average BW during the study period was 12.1 kg higher in the EFLX vs. CTL animals (Table 6).

3.4. Fertility performance

Table 7 presents fertility performance. Groups did not differ in days to first service or in conception rates from first and second AI. The interval from first service to conception was 17 days shorter and the number of days open were 18 days less in EFLX than in CTL cows. In addition, the number of AI per conception tended to be lower in the EFLX cows. Pregnancy rates at 120 and 150 DIM were not significantly different between groups (Table 7).

4. Discussion

Previous reports by our group and others, of both sophisticated *in-vitro* and small-scale *in-vivo* studies, have demonstrated beneficial effects of n-3 FA supplementation on cow fertility and health. Here, we demonstrate several positive effects of n-3 FA supplementation from

Table 5
Mean treatment effects on milk fatty acid (FA) components (g per 100 g FA) in cows supplemented with extruded flaxseed.

	Treatment[1]			
Fatty acid	CTL	EFLX	SEM	*P*-value
C4:0	2.96	2.89	0.1	0.64
C6:0	1.94	1.78	0.08	0.17
C8:0	1.17	1.01	0.06	0.06
C10:0	2.93	2.45	0.14	0.02
C12:0	3.20	2.74	0.13	0.02
C14:0	10.36	8.96	0.23	< 0.0001
C15:0	1.36	1.09	0.04	< 0.0001
C16:0	30.98	25.65	0.38	< 0.0001
C16:1	1.75	1.86	0.10	0.45
C18:0	8.47	9.57	0.36	0.04
C18:1t10	3.49	4.17	0.48	0.32
C18:1t11	0.99	1.25	0.06	< 0.007
Total C18:1t	5.69	6.78	0.55	0.17
C18:1c9	19.10	22.39	0.4	< 0.0001
C18:1c11	1.41	1.89	0.05	< 0.0001
Total C18:1c	21.7	26.1	0.43	< 0.0001
Total C18:1	27.44	32.90	0.74	< 0.0001
C18:2n6t	0.10	0.20	0.01	< 0.0001
C18:2ct	0.11	0.13	0.00	0.10
C18:2tc	0.05	0.06	0.00	0.20
C18:2n-6	3.19	3.40	0.10	0.15
Total C18:2c	3.43	4.20	0.12	< 0.0001
CLA c9t11	0.40	0.53	0.02	< 0.0001
Total CLA	0.42	0.55	0.02	< 0.0001
Total C18:2	4.06	5.13	0.13	< 0.0001
C18:3n-3	0.24	0.84	0.03	< 0.0001
C20:4n6	0.18	0.13	0.01	0.007
C20:5n-3	0.0	0.05	0.00	< 0.0001
C22:5n-3	0.06	0.10	0.00	< 0.0001
C20:1n-9	0.41	0.32	0.04	0.12
Total C:4 to C:15	25.06	21.96	0.65	0.002
Total C:4 to C:16	57.79	49.48	0.83	< 0.0001
SFA[2]	64.78	57.5	0.90	< 0.0001
MUFA[3]	30.63	36.3	0.80	< 0.0001
PUFA[4]	4.59	6.27	0.16	< 0.0001
n-3[5]	0.26	0.94	0.03	< 0.0001
n-6[6]	3.47	3.59	0.11	0.43
n-6:n-3	13.8	4.06	0.38	< 0.0001

[1] Cows were randomly divided into two groups 21 days before expected calving. EFLX cows (n = 276) received a diet containing: precalving, 4% of an extruded flaxseed supplement providing α-linolenic acid (ALA) and post-partum, 5% of the same supplement. CTL cows (n = 240) received a diet without the flaxseed supplement but with similar nutrient content.
[2] Sum of saturated FA.
[3] Sum of mono-unsaturated FA.
[4] Sum of poly-unsaturated FA.
[5] Sum of n-3 FA.
[6] Sum of n-6 FA.

Table 6
Incidence of health events by treatment groups in cows supplemented with extruded flaxseed.

	Treatment[1]			
Event	Control	EFLX	SEM	*P*-value
Without metritis,%	64.6 (155/240)	67.8 (187/276)		NS
Mild metritis,%	10.4 (25/240)	8.3 (23/276)		NS
Moderate metritis,%	12.1 (29/240)	14.9 (41/276)		NS
Severe metritis,%	12.9 (31/240)	9.1 (25/276)		0.15
Without ketosis,%	68.9 (166/240)	76.5 (211/276)		0.05
Mild ketosis,%	11.7 (28/240)	8.7 (24/276)		NS
Moderate ketosis,%	8.3 (20/240)	7.6 (21/276)		NS
Severe ketosis,%	10.8 (26/240)	7.2 (20/276)		0.03
Udder edema,%	5.8 (14/240)	2.2 (6/276)		0.03
Mastitis[2],%	5.0 (11/240)	5.1 (14/276)		NS
Milk fever,%	0.8 (2/240)	1.1 (3/276)		NS
Mortality,%	4.6 (11/240)	0.7 (2/276)		0.005
BCS post-calving	3.45	3.65	0.03	< 0.0006
BCS peak lactation	2.84	3.03	0.03	< 0.0003
BW (kg)	648.7	660.8	0.5	< 0.0001

[1] Cows were randomly divided into two groups 21 days before expected calving. EFLX cows (n = 276) received a diet containing: precalving, 4% of an extruded flaxseed supplement providing α-linolenic acid (ALA) and post-partum, 5% of the same supplement. CTL cows (n = 240) received a diet without the flaxseed supplement but with similar nutrient content.
[2] Mastitis events that were treated by the veterinarian.

Table 7
Fertility performance by treatment groups in cows supplemented with extruded flaxseed.

	Treatment[1]			
Item	CTL	EFLX	SEM	*P*-value
Days from calving to 1st AI[2]	101.4	102.7	2.8	0.75
Conception rate 1st AI (%)	26.6 (38/143)	27.2 (46/169)		NS
Conception rate 2nd AI (%)	23.7 (23/97)	22.9 (27/118)		NS
Conception rate 1st + 2nd AI (%)	25.4 (61/240)	25.4 (73/287)		NS
Days from 1st AI to conception	66.6	49.7	6.6	0.07
Days open	166.1	148.5	7.3	0.09
AI per conception	2.99	2.51	0.2	0.10
Pregnancy rate, 120 DIM[2] (%)	25.3	29.0		0.46
Pregnancy rate, 150 DIM (%)	26.7	42.4		0.29

[1] Cows were randomly divided into two groups 21 days before expected calving. EFLX cows (n = 276) received a diet containing: precalving, 4% of an extruded flaxseed supplement providing α-linolenic acid (ALA) and post-partum, 5% of the same supplement. CTL cows (n = 240) received a diet without the flaxseed supplement but with similar nutrient content.
[2] AI, artificial insemination; DIM, days in milk.

EFLX initiated in the dry period and administered through lactation in a large-scale study conducted on a commercial farm.

4.1. Milk yield and composition

Milk yield of the EFLX cows was higher than that of the CTL animals, similar to findings in other studies that used the same supplement (Moallem, 2009; Zachut et al., 2010), whole flaxseed as compared to sunflower seeds (Petit et al., 2004) or prilled fat (Petit et al., 2007). However, other studies found no effect on milk yield from feeding whole (Kennelly, 1996; Mustafa et al., 2003) or extruded (Gonthier et al., 2005) flaxseed. The inconsistency among the effects of the various flaxseed products on milk yield might be attributed to differences among the various studies' diets in terms of type of delivery, amount, or composition.

The extruded flaxseed supplementation used in the present study decreased the milk fat content by 4.9 g/kg. Decreases in fat percentage that resulted from feeding the same supplement at a rate of 40 g/kg of diet DM (Moallem, 2009) or 92 g/kg (Zachut et al., 2010) of DM were 2.2 or 4.0 g/kg, respectively. Mustafa et al. (2003) also observed lower milk fat content in cows fed unheated flaxseed. However, other studies that involved feeding whole (Petit et al., 2005) or extruded (Gonthier et al., 2005) flaxseed did not find a lower fat percentage. Conjugated linoleic acid (CLA; Baumgard et al., 2001) and C18:1trans (Piperova et al., 2004) were associated with low fat contents in milk; these specific isomers depress FA synthesis in the mammary gland, mainly affecting short-chain FA, up to C:16. Indeed, we found that the proportions of a few C18:1trans and CLA isomers were higher in EFLX vs. CTL cows (Table 5). Moreover, in an in-vitro study, Lee and Jenkins (2011) demonstrated the formation of several CLA isomers from linolenic acid in a continuous culture fermenter with mixed rumen microbes, which is consistent with our present findings. Furthermore, in our study, the proportion of total short-chain FA in milk—C:4 to

C:15—was 3.1 g per 100 g FA points (14%) lower in the EFLX vs. CTL cows, which indicates less de-novo synthesis of FA in the mammary gland of cows fed EFLX. The relatively large depression in milk fat in the present study may be attributed to the diet composition; this included high proportions of byproducts and ether extracts and low forage content, which could interact with the provided supplement to generate intermediate isomers and suppress milk fat production. The protein percentage in the milk was lower in the present study than those found in other studies that used the same supplement (Moallem, 2009; Zachut et al., 2010), whole flaxseed (Petit et al., 2004, 2007), or extruded flaxseed (Gonthier et al., 2005). We do not have an explanation for the present reduction in milk protein content. Part of the decrease in protein percentage can be explained by the increase in milk yields.

4.2. Plasma FA profile

In the present study, the proportion of ALA in the plasma was 3.4 times greater in EFLX vs. CTL cows, similar to the proportions found in milk fat, which was 3.5 times as great in the EFLX cows. Gonthier et al. (2005) obtained similar results, with the increase in blood ALA being 2 to 3 times higher in flaxseed treatments than in controls, which was correlated with the increase of ALA in the milk fat. Blood lipoproteins are the main source of the long-chain FA that are incorporated into milk fat, which could explain the close association between ALA proportions in plasma and milk. However, further research is required to elucidate the association between FA profiles in plasma and that in milk fat in dairy cows.

4.3. Milk fat FA composition

As expected, the FA profile in milk fat changed in response to extruded flaxseed supplementation. In general, other studies have found trends similar to those in the present study: higher proportions of oleic acid, linoleic acid and ALA, and lower contents of myristic and palmitic acids in cows supplemented with flaxseed (Mustafa et al., 2003; Gonthier et al., 2005; Petit et al., 2007; Moallem, 2009; Zachut et al., 2010). Moreover, other studies (Gonthier et al., 2005; Moallem, 2009; Zachut et al., 2010) found lower SFA and higher unsaturated FA contents in cows given diets supplemented with flaxseed products. In the present and other studies (Moallem, 2009; Zachut et al., 2010), n-3 FA in milk increased and the n-6:n-3 ratio decreased as a result of feeding extruded flaxseed. Milk and milk products are among the most important and widely used components of the modern human diet. Simopoulos (2002) suggested that a lower n-6:n-3 FA ratio is desirable for reducing the risk of many of the chronic diseases that are highly prevalent in western societies. The reduction in the n-6:n-3 ratio in milk fat observed here was accompanied by a reduction in SFA content, and enhanced proportions of MUFA and PUFA, all of which are desirable in human nutrition.

4.4. Effects on incidence of disease events

In the present study, we observed several beneficial effects on cow health, with a considerable impact on cow mortality rate. In another, small-scale ($n = 35$) study, Petit et al. (2007) found less sick animals among cows fed whole flaxseed than among those fed a control diet or supplemented with energy booster. Several studies have reported beneficial effects of n-3 FA on many health aspects of both animals and humans: metabolic syndrome, brain metabolic abnormalities (Simopoulos, 2013) inflammatory diseases (Calder, 2006) and immune system (Miles and Calder, 1998). Abnormalities in the FA composition of membranes, especially a deficiency in n-3 FA, may be involved in the pathogenesis of disorders linked to insulin resistance (Storlien et al., 1991; Borkman et al., 1993), which may be associated with the periparturient metabolism of high-yielding dairy cows. Because insulin is an anabolic hormone, disruption of its intercellular signaling in insulin resistance adipose tissue will encourage increased lipolysis (Murri et al., 2014) and triggers lipids breakdown and mobilization of adipose tissue in peripartum dairy cows. This disorder could be prevented by inclusion of n-3 FA in the diet (Storlien et al., 1991), which might be the case in our study. However, further mechanistic research should be conducted to establish this theory.

Prolonged previous lactation, a long dry period (Vanholder et al., 2015), and precalving over-conditioning (Fronk et al., 1980) are among the management factors related to postpartum ketosis. Nevertheless, ketosis is closely associated with nutritional management; among recognized risk factors for ketosis in dairy cows are prepartum feed intake (Douglas, 2002), and postpartum inappetence and restricted diets (Dann et al., 2005). In the present study, we found lower prevalence of ketosis in the EFLX vs. CTL cows, It should be emphasized that we started the n-3 FA supplementation 3 weeks before expected calving, which may elicit positive effects on the metabolic status of the cows before calving, shown to be important by Vanholder et al. (2015). However, the present study was conducted on a commercial dairy farm, and therefore we had no records of feed intake to determine energy balance.

In the present study, there were no substantial differences between groups in metritis occurrence. The most frequent postpartum risk factors for metritis in cows include abortion, dystocia, stillbirth, twins, retained placenta, calf size, age, and calving season (Kaneene and Miller, 1995; Bruun et al., 2002; LeBlanc, 2008; Kumari et al., 2016). None of these factors are directly related to nutritional management, although LeBlanc (2008) mentioned the impact of low feed intake as a risk factor for metritis, which could explain the absence of inter-group differences in metritis incidence in the present study. However, the occurrence of severe metritis was 30% less in the EFLX vs. CTL group ($P < 0.15$), which may be attributed to improved immune system activity and faster healing in the former.

Only mastitis events that were treated by the veterinarian were recorded, and their occurrence was similar between groups. However, the groups differed markedly in mortality: cows in the present study in both groups died due to complications of milk fever, mastitis, and lameness and these findings indicate higher resilience of the EFLX cows. Involvement of FA, and especially PUFA, in the immune system has been well demonstrated by many in-vivo and in-vitro studies, as summarized by Sordillo (2016). Although we did not measure the immune function in this study, we can assume that the higher resilience of the EFLX cows may be associated with the PUFA supplementation. However, further research is required to establish the association between PUFA supplementation, the immune system, and the resilience of dairy cows to common diseases.

4.5. Fertility performance

In the present study, we did not observe any inter-group differences in conception rates, but we did observe a tendency toward fewer days from first AI to conception, open days, and number of AI per conception in the EFLX cows. Beneficial effects of feeding n-3 FA on the reproductive system can be found in other reports: attenuated endometrial prostaglandin $F_{2\alpha}$ production was found in dairy cows fed n-3 PUFA (Mattos et al., 2002). Ambrose et al. (2006) found 27.3% lower pregnancy losses in cows fed rolled flaxseed than in those fed rolled sunflower seed; they also found larger ovulatory follicles and lower pregnancy losses in cows fed rolled flaxseed. Previous studies in our laboratory have demonstrated beneficial effects of n-3 FA supplementation on cows' reproductive system (Zachut et al., 2010, 2011; Moallem et al., 2013). Zachut et al. (2011) reported greater length and intensity of behavioral estrus in cows supplemented with n-3 FA than in controls. Estrus detection rate is recognized as an important factor affecting reproductive efficiency, and Lopez et al. (2004) found considerably lower duration and intensity of estrous behavior in modern high-yielding dairy cows compared to cows of a few decades ago. Despite the lack of effect on conception rate in this study, the shorter intervals between first AI and conception and less open days in the EFLX vs. CTL cows suggest that higher detection of behavioral estrus in those cows was due to its greater length and intensity (Zachut et al., 2011); this promotes re-insemination of repeating cows and reduces the rate of missed estrus.

5. Conclusions

Supplementation of extruded flaxseed to dairy cows providing ALA from 3 weeks prepartum increased milk yield, reduced milk fat and protein percentages, and improved cows' body condition and BW. Extruded flaxseed supplementation decreased the incidence of ketosis and severe metritis, remarkably reduced mortality, and tended to decrease the interval between first AI and conception and number of open days, without affecting conception rates. Furthermore, enrichment of milk with n-3 FA and reduction in the n-6:n-3 ratio in milk fat in the EFLX group was accompanied by a reduction in SFA content, and enhancement of MUFA and PUFA contents, which are all desirable for human nutrition. These beneficial effects of n-3 FA on the animal per se came together with alterations in milk fat composition that are all desirable for human nutrition.

Declaration of Competing Interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Supplementary materials

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References

Ambrose, D.J., Kastelic, J.P., Corbett, R., Pitney, P.A., Petit, H.V., Small, J.A., Zalkovic, P., 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in α-linolenic acid. J. Dairy Sci. 89, 3066–3074.

Association of Official Analytical Chemists (AOAC) 1990. Official methods of analysis. pp. 80–88. AOAC, Arlington, VA, USA.

Baumgard, L.H., Sangster, J.K., Bauman, D.E., 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J. Nutr. 131, 1764–1769.

Borkman, M., Storlien, L.H., Pan, D.A., Jenkins, A.B., Chisholm, D.J., Campbell, L.V., 1993. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N. Engl. J. Med. 328, 238–244.

Bruun, J., Ersboll, A.K., Alban, L., 2002. Risk factors for metritis in Danish dairy cows. Prev. Vet. Med. 54, 179–190.

Calder, P.C., 2006. Polyunsaturated fatty acids and inflammation. PLEFA 75, 197–202.

Chouinard, P.Y., Lévesque, J., Girard, V., Brisson, G.J., 1997. Dietary soybeans extruded at different temperatures: milk composition and in situ fatty acid reactions. J. Dairy Sci. 80, 2913–2924.

Clandinin, M.T., Cheema, S., Field, C.J., Garg, M.L., Venkatraman, J., Clandinin, T.R., 1991. Dietary fat: exogenous determination of membrane structure and cell function. FASEB J 13, 2761–2769.

Dann, H.M., Morin, D.E., Murphy, M.R., Bollero, G.A., Drackley, J.K., 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J. Dairy Sci. 88, 3249–3264.

Deckelbaum, R.J., Worgall, T.S., Seo, T., 2006. N-3 fatty acids and gene expression. Am. J. Clin. Nutr. 83, 1520–1525 (suppl).

Douglas, G.N., 2002. Peripartal lipid metabolism and tissue fatty acid composition in Holstein cows fed supplemental fat during the dry period. PhD Thesis. University of Illinois at Urbana-Champaign, Champaign, IL, USA.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72, 68–78.

Fronk, T.J., Schultz, L.H., Hardie, A.R., 1980. Effect of dry period overconditioning on subsequent metabolic disorders and performance of dairy cows. J. Dairy Sci. 63, 1080–1090.

Gonthier, C., Mustafa, A.F., Ouellet, D.R., Chouinard, P.Y., Berthiaume, R., Petit, H.V., 2005. Feeding micronized and extruded flaxseed to dairy cows: effects on blood parameters and milk fatty acid composition. J. Dairy Sci. 88, 748–756.

Kaneene, J.B., Miller, R., 1995. Risk factors for metritis in Michigan dairy cattle using herd- and cow-based modelling approaches. Prev. Vet. Med. 23, 183–200.

Kennelly, J.J., 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60, 137–152.

Kumari, S., Kumaresan, A., Patbandha, T.K., Ravi, S.K., 2016. Risk factors for metritis and its effect on productive and reproductive performance in dairy cattle and buffaloes. Agric. Res. 5, 72–80.

LeBlanc, S.J., 2008. Postpartum uterine disease and dairy herd reproductive performance: a review. Vet. J. 176, 102–114.

Lee, Y.J., Jenkins, T.C., 2011. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. The J. Nut. 141, 1445–1450.

Lopez, H., Satter, L.D., Wiltbank, M.C., 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81, 209–223.

Mattos, R., Staples, C.R., Williams, J., Amorocho, A., McGuire, M.A., Thatcher, W.W., 2002. Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. J. Dairy Sci. 85, 755–764.

Miles, E.A., Calder, P.C., 1998. Modulation of immune function by dietary fatty acids. The Proc. Nutr. Soc. 57, 277–292.

Moallem, U., 2009. The effects of extruded flaxseed supplementation to high-yielding dairy cows on milk production and milk fatty acid composition. Anim. Feed Sci. Technol. 152, 232–242.

Moallem, U., 2018. Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. J. Dairy Sci. 101, 8641–8661.

Moallem, U., Neta, N., Zeron, Y., Zachut, M., Roth, Z., 2015. Dietary α-linolenic acid from flaxseed oil or eicosapentaenoic and docosahexaenoic acids from fish oil differentially alter fatty acid composition and characteristics of fresh and frozen-thawed bull semen. Theriogenology 83, 1110–1120.

Moallem, U., Shafran, A., Zachut, M., Dekel, I., Arieli, A., 2013. Dietary alpha-linolenic acid (ALA) from flaxseed oil improved folliculogenesis and IVF performance in dairy cows, similarly to eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids from fish oil. Reproduction 146, 603–614.

Murri, M., Insenser, M., Luque, M., Tinahones, F.J., Escobar-Morreale, H.F., 2014. Proteomic analysis of adipose tissue: informing diabetes research. Expert Rev. Proteomics 11, 491–502.

Mustafa, A.F., Chouinard, P.Y., Christensen, D.A., 2003. Effects of feeding micronized flaxseed on yield and composition of milk from Holstein cows. J. Sci. Food Agric. 83, 920–926.

National Research Council (NRC), 1989. Nutrient Requirements of Dairy Cattle, 6th revised edition. National Academy Press, Washington, DC, USA.

Petit, H.V., Germiquet, C., Lebel, D., 2004. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. J. Dairy Sci. 87, 3889–3898.

Petit, H.V., Ivan, M., Mir, P.S., 2005. Effects of flaxseed on protein requirements and N excretion of dairy cows fed diets with two protein concentrations. J. Dairy Sci. 88, 1755–1764.

Petit, H.V., Palin, M.F., Doepel, L., 2007. Hepatic lipid metabolism in transition dairy cows fed flaxseed. J. Dairy Sci. 90, 4780–4792.

Piperova, L.S., Moallem, U., Teter, B.B., Sampugna, J., Yurawecz, M.P., Morehouse, K.H., Luchini, D., Erdman, R.A., 2004. Changes in milk fat in response to dietary supplementation with calcium salts of trans-18:1 or conjugated linoleic fatty acids in lactating dairy cows. J. Dairy Sci. 87, 3836–3844.

Prescott, S., Calder, P., 2004. N-3 polyunsaturated fatty acids and allergic disease. Curr. Opin. Clin. Nutr. Metab. 7, 123–129.

Raphael, W., Sordillo, L.M., 2013. Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid biosynthesis. Int. J. Mol. Sci. 14, 21167–21188.

SAS, 2002. SAS User's guide: statistics, Version 9.2. Statistical Analysis Systems Institute Inc., Cary, NC, USA.

Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56, 365–379.

Simopoulos, A.P., 2013. Dietary omega-3 fatty acid deficiency and high fructose intake in the development of metabolic syndrome, brain metabolic abnormalities, and non-alcoholic fatty liver disease. Nutrients 5, 2901–2923.

Sordillo, L.M., 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci. 99, 4967–4982.

Storlien, L.H., Jenkins, A.B., Chisholm, D.J., Pascoe, W.S., Khouri, S., Kraegen, E.W., 1991. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle

phospholipid. Diabetes 40, 280–289.
Vanholder, T., Papen, J., Bemers, R., Vertenten, G., Berge, A.C.B., 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J. Dairy Sci. 98, 880–888.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Zachut, M., Arieli, A., Moallem, U., 2011. Incorporation of dietary n-3 fatty acids into ovarian compartments in dairy cows, and the effects on hormonal and behavioral patterns around estrus. Reproduction 141, 833–840.
Zachut, M., Dekel, I., Lehrer, H., Arieli, A., Arav, A., Livshitz, L., Yakoby, S., Moallem, U., 2010. Effects of dietary fats differing in n-6:n-3 ratio fed to high-yielding dairy cows on fatty acid composition of ovarian compartments, follicular status, and oocyte quality. J. Dairy Sci. 93, 529–545.