The effects of dietary omega fatty acids on pregnancy rate, plasma prostaglandin metabolite levels, serum progesterone levels, and milk fatty-acid profile in beef cows

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Abstract

The objectives were to determine the effects of feeding supplements rich in omega-6 or omega-3 fatty acids (FA) during the late gestation to the early postpartum and breeding periods on reproduction and milk FA profile in beef cows. For each of two years, at the beginning of period 1 (mid-December), 72 beef cows, calving in January or February, were assigned to diets supplemented with roasted flaxseed (Flax) or roasted soybean (Soybean). For each of two years, after 11 wk (end of period 1), 18 cows of 36 in the Flax group were switched to the soybean supplement and 18 cows of 36 in the Soybean group were switched to the flax supplement (start of Period 2). Cows were bred by timed artificial insemination (TAI) in week 5 of period 2. The FA composition of the milk reflected the FA profile of the oilseed supplements. There were no differences in pregnancy rates among the 4 groups. The treatments had no effect on plasma prostaglandin metabolite levels or ratios at 4 to 11 d postpartum. At 5 to 6 d post-TAI, pregnant cows fed Flax in period 1 had lower ($P < 0.05$) plasma prostaglandin F metabolite (PGFM) levels and PGFM to prostaglandin E metabolite (PGEM) ratio than cows fed Soybean, but there were no significant differences at 19 to 20 d post-TAI. Cows pregnant from TAI and fed Flax in period 2 had higher ($P < 0.05$) serum progesterone levels at 5 to 6 d post-TAI than cows fed Soybean, but there was no difference at 19 to 20 d post-TAI. The dietary treatments had no effect on pregnancy rates, but there were some effects on plasma PGFM levels, PGFM to PGEM ratios, and serum progesterone levels. The FA supplements influenced the FA composition of milk.

Résumé

Les objectifs de la présente étude étaient de déterminer les effets de suppléments alimentaires riches en acides gras (FA) omega-6 ou omega-3 lors de la période fin de gestation au début du postpartum et lors des périodes d'accouplement sur la reproduction et les profils de FA chez les vaches d'embouche. Pour chacune des deux années, au début de la période 1 (mi-décembre), 72 vaches d'embouche, devant vêler en janvier ou février, ont été assignées à des rations supplémentées avec de la graine de lin rôtie (Flax) ou des graines de soya rôties (Soya). Pour chacune des deux années, après 11 semaines (fin de la période 1), 18 des 36 vaches dans le groupe Flax ont été changées au supplément de soya et 18 des 36 vaches du groupe soya ont été changées pour le groupe Flax (Début de la période 2). Les vaches ont été saillies par insémination artificielle minutée (TAI) lors de la semaine 5 de la période 2. La composition en FA du lait représentait le profil de FA des suppléments alimentaires. Il n'y avait pas de différence dans les taux de gestation parmi les 4 groupes. Le traitement n'avait pas d'effet sur les niveaux ou ratios plasmatiques des métabolites des prostaglandines du jour 4 au jour 11 postpartum. Aux jours 5 à 6 post-TAI, les vaches gestantes nourries au Flax durant la période 1 avaient des niveaux significativement ($P < 0,05$) *plus bas de métabolite de la prostaglandine F (PGFM) et des ratios de PGFM au métabolite de la prostaglandine E (PGEM) que les vaches nourries avec le Soya, mais il n'y avait pas de différence significative aux jours 19 à 20 post-TAI. Les vaches gestantes suite à la TAI et nourries avec Flax durant la période 2 avaient des niveaux sériques de progestérone plus élevés* ($P < 0,05$) *aux jours 5 à 6 post-TAI que les vaches nourries au Soya, mais il n'y avait plus de différence aux jours 19–20 post TAI. Les traitements alimentaires n'avaient aucun effet sur les taux de gestation, mais il y avait des différences sur les niveaux plasmatiques de PGFM, les ratios PGFM/PGEM, et les niveaux sériques de progestérone. Les FA des suppléments ont influencé la composition en FA du lait.*

(Traduit par Docteur Serge Messier)

Feeding cows supplemental fats high in omega-6 (n-6) fatty acids (FA) (e.g., linoleic acid, C18:2n-6) can increase tissue arachidonic acid levels (1). High arachidonic acid levels can, in turn, increase uterine prostaglandin-F synthesis (1–3). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) plays an important role in enhancing uterine defence mechanisms in the early postpartum period (1–3). Feeding omega-6 FA to dairy cows in late pregnancy and early lactation has been reported to increase $PGF_{2\alpha}$ production, enhance immune competency, reduce uterine infections, and assist normal uterine involution in the postpartum period, with consequent beneficial effects on fertility. In

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addition, there is evidence of positive effects from feeding these FA on follicular growth, embryo quality, and pregnancy rates during this period (1–3).

During most of the gestation period, pregnancy in cattle is maintained by progesterone, which is secreted by the corpus luteum (CL) on the ovary. Prostaglandin $F_{2\alpha}$ is the main hormone responsible for luteolysis (4). In contrast, prostaglandin E_2 (PGE_2) is luteotropic (5) and increased production by the uterus would support pregnancy. The relative production of $PGF_{2\alpha}$ and PGE_2 by the uterus may be more important in the maintenance of pregnancy than the absolute production of each prostaglandin (6). Feeding supplements high in omega-3 (n-3) FA (e.g., linolenic acid, C18:3n-3) during the breeding season and early gestation has been reported to reduce uterine $PGF_{2\alpha}$ production and improve embryo quality and pregnancy maintenance. Pregnancy rates may be increased by enhanced progesterone production and decreased embryo mortality (2,3,6).

Dairy cattle have been used in most studies on the dietary effects of FA on bovine reproduction. Studies on beef cattle have yielded variable and inconsistent results (7,8). In addition, to the authors' knowledge, there is no information on sequential feeding using different fats during the late gestation to the early postpartum and breeding periods in beef cows.

The effects of dietary supplementation with FA on milk composition has been studied extensively (9,10). The FA composition of the diet can affect milk FA profile, which in turn can influence calf plasma and adipose tissue profiles (11). Fatty acids are important in metabolic regulatory functions, vigor, and immune response in calves (11–15).

The objectives of this study were to determine the effects of feeding supplements high in omega-6 or omega-3 FA during the late gestation to the early postpartum and breeding periods on pregnancy rates, plasma prostaglandin metabolite levels and ratios, serum progesterone levels, and milk FA profile in beef cows. Our hypotheses were that increasing omega-6 FA in the diet would result in increased $PGF_{2\alpha}$ production by the uterus, and that increasing omega-3 FA in the diet would result in decreased $PGF_{2\alpha}$ production. These could affect reproductive efficiency and progesterone production, as described previously. We also hypothesized that dietary FA would be reflected in the milk FA profile.

The 144 cows used in this study were British-breed crossbred beef cows in good body condition housed at the Agriculture and Agri-Food Canada Research Farm, Nappan, Nova Scotia. The mean ± standard deviation (SD) body condition score of the cows was 5.7 ± 0.9 in year 1 and 5.6 ± 0.7 in year 2, on a 9-point scale, and the mean ± SD body weight was 708 ± 95 kg in year 1 and 704 ± 66 kg in year 2. Seventy-two cows (mean ± SD age of 5.0 ± 1.5 y in both years) were used for each of the 2 y and all cows calved in January or February. All animals in this study were cared for in accordance with the guidelines suggested by the Canadian Council on Animal Care (16).

In each year, on December 15 (beginning of period 1), 72 cows were randomly assigned to 1 of 2 dietary treatment groups: Flax or Soybean. The basal diet of first-cut timothy (70%, dry matter basis) and red clover (30%, dry matter basis) silage (dry matter 32%) was fed free choice using Calan gates (American Calan, Northwood, New Hampshire, USA) for individual feeding, with a weighback maintained of about 5% (as fed basis). Free-choice minerals and salt blocks were available in each pen. The soybean supplement consisted of 2 kg of whole roasted soybean (containing linoleic acid, C18:2n-6, 57.7%). The flax supplement consisted of 1 kg of roasted flaxseed (containing linolenic acid, C18:3n-3, 58.5%) and 1 kg of soybean meal to give similar lipid and protein contents. The oilseeds were roasted in a fluidized bed roaster (Sweet Manufacturing, Springfield, Ohio, USA). Supplements were top dressed for each cow daily. For each year, cows on each treatment were assigned randomly to 6 pens for a total of 6 cows per diet in each pen. One week after breeding, cows were group fed according to the dietary supplement in 2 larger pens to allow for breeding with bulls. The basal silage was mixed with the dietary supplement at a rate of 2 kg per head.

For each of two years, after 11 weeks on the experimental diets (end of Period 1), 18 of the 36 cows in the Flax group, and 18 of the 36 cows in the Soybean group were switched to the Soybean and Flax diets, respectively (start of Period 2). This resulted in four treatment groups: Flax-Flax, Flax-Soybean, Soybean-Flax, and Soybean-Soybean. On the 11th wk of periods 1 and 2, calves were separated from cows for 12 h, returned to nurse to remove all available milk, and then removed until after milk samples were collected for FA analysis 24 h later. For the period 2 milking, milk samples were also collected from all cows for milk fat and protein analysis. Milk yield was also recorded at this time.

The percentage of milk fat and protein was determined (FOSS Milko Scan FT 6000; FOSS, Eden Prairie, Minnesota, USA). For FA analysis, milk samples were centrifuged at 4°C, 15300 × *g* for 30 min (Eppendorf centrifuge model 5804R, Eppendorf rotor model F45-30-11; Eppendorf Canada, Mississauga, Ontario) and a weighed aliquot of the milk fat layer was removed for methylation and gas chromatography analysis (Agilent 5890, Agilent Technologies Canada, Mississauga, Ontario) (17).

In weeks 4 and 5 of period 2, estrus was synchronized by treating each cow with gonadotropin-releasing hormone (GnRH, 100 μg Cystorelin IM; Mérial Canada, Baie d'Urfé, Québec) and a progesterone releasing intravaginal insert (CIDR 1380, 1.38 g progesterone USP; Pfizer Canada, Kirkland, Quebec) on day 0, prostaglandin (Estrumate, 500 μg cloprostenol, IM; Schering Plough Canada, Pointe-Claire, Quebec) on day 7, and a second GnRH injection at the time of insemination. All cows were bred by timed artificial insemination (TAI) 53 to 56 h after CIDR removal. Inseminations were done by 5 and 4 experienced technicians in year 1 and year 2, respectively, using frozen-thawed semen from sires with proven fertility. Technicians and semen were balanced as much as possible across treatments. Bulls were put in with the cows 2 d after TAI.

The cows were pregnancy tested by transrectal palpation or ultrasonography (Aloka SSD-500V with a 5.0 MHz linear array transducer; IMAGO, Vaudreuil, Dorion, Quebec) 68 to 69 d after TAI. Cows were categorized into those bred by TAI (68 to 69 d of gestation), those bred by the bull (50 d of gestation or less), and those with no detectable pregnancy. The occasional cow may have been bred by a bull shortly after TAI, but this applied equally to all groups so the results would not be affected.

Blood was collected for plasma prostaglandin metabolites [13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) and 13,14-dihydro-15-keto prostaglandin E_2 (PGEM)] levels 4 to 11 d after calving, and 5 to 6 d and 19 to 20 d post-TAI. Blood was drawn from the jugular vein

Table I. Percentage of selected fatty acids (FA) (g/100 g of total FA) in milk fat of beef cows at the end of periods 1 and 2. Minor FA have been excluded from table, but are included in totals

	Period 1				Period 2			
Fatty acids	Flax	Soybean	$S_{\bar{x}}$	*P*	Flax	Soybean	$S_{\bar{x}}$	*P*
C 10:0	2.36	2.28	0.051	0.306	2.36	2.21	0.057	0.054
C 12:0	2.76	2.62	0.064	0.118	2.71	2.54	0.067	0.089
C 14:0	9.06	8.31	0.143	0.0003	9.42	8.61	0.157	0.0004
C15:0	1.58	1.40	0.019	0.0001	1.37	1.25	0.015	0.0001
C16:0	22.6	22.3	0.260	0.461	22.4	22.1	0.328	0.426
C18:0	16.4	16.6	0.300	0.693	14.8	14.0	0.275	0.051
C18:1n-9	23.5	22.9	0.280	0.167	23.8	24.2	0.288	0.400
C18:2n-6	1.06	2.36	0.051	0.0001	1.12	2.30	0.057	0.0001
C18:1n-7t	2.43	3.62	0.079	0.0001	3.21	4.34	0.100	0.0001
c9, t11-CLA	0.79	1.12	0.022	0.0001	1.12	1.64	0.033	0.0001
C20:4n-6	0.09	0.11	0.003	0.0001	0.09	0.11	0.002	0.0001
C18:3n-3	0.96	0.97	0.012	0.628	1.04	1.02	0.016	0.245
C20:5n-3	0.12	0.09	0.002	0.0001	0.11	0.09	0.002	0.0001
C22:5n-3	0.17	0.13	0.004	0.0001	0.14	0.13	0.003	0.0001
C22:6n-3	0.03	0.02	0.002	0.009	0.03	0.02	0.001	0.288
SFA	60.3	58.9	0.34	0.007	58.3	55.8	0.40	0.0001
MFA	29.1	29.8	0.31	0.149	30.5	32.0	0.32	0.001
PUFA	3.30	4.95	0.077	0.0001	3.75	5.46	0.092	0.0001
n-3 FA	1.28	1.22	0.015	0.007	1.32	1.27	0.017	0.024
n-6 FA	1.23	2.60	0.052	0.0001	1.31	2.55	0.060	0.0001
n-3/n-6	1.09	0.49	0.019	0.0001	1.06	0.52	0.021	0.0001

SFA — saturated FA; MUFA — monounsaturated FA; PUFA — polyunsaturated FA; n-6 — omega-6 FA; n-3 — omega-3 FA; c9, t11-CLA — conjugated linoleic acid. $S_{\bar{x}}$ — standard error of the mean.

into 10 mL K2 EDTA tubes (Vacutainer tubes; Becton Dickinson, Mississauga, Ontario). The samples were cooled on ice and then centrifuged at 1000 × *g* for 25 min. The plasma was transferred into 2.0 mL cryovials (Fisher Scientific, Whitby, Ontario) and frozen on dry ice for transport to the laboratory where they were stored at −80°C until analyzed.

Plasma samples were assayed in triplicate for PGEM and PGFM by enzyme immunoassay using commercial kits (No. 514531 and 516671, respectively; Cayman Chemical Company, Ann Arbor, Michigan, USA). Inter-assay coefficient of variation for PGFM was 17.2%. Intra and inter-assay coefficients of variation for PGEM were 9.1% and 15.3%, respectively. The sensitivities of the PGEM and PGFM assays were 2 and 8.2 pg/mL of plasma, respectively.

Blood was collected for serum progesterone levels 5 to 6 d and 19 to 20 d after TAI. Blood was drawn from the jugular vein into 10 mL tubes without anticoagulant (Vacutainer tubes; Becton Dickinson). After clotting, samples were centrifuged and the serum was kept on ice for transport to the laboratory. Serum was stored at −20°C until analysis for progesterone concentration, which was done using a sequential competitive chemiluminescent enzyme immunoassay (Immulite Progesterone; Diagnostic Products Corporation, Los Angeles, California, USA).

Results (PGFM, PGEM, PGFM to PGEM ratios, FA composition) from samples collected during period 1 were analyzed using a 2-way analysis of variance (ANOVA) with dietary treatment and pen as the main effects. For the results (PGFM, PGEM, PGFM to PGEM ratios, FA composition, progesterone, milk yield, and percentages of milk fat and protein) from samples collected during period 2, ANOVA with period 1 treatment and period 2 treatment as main effects was done, as well as the interaction between the 2 treatment periods. In addition, pregnancy rates were analyzed using Fisher's exact test.

There were no significant differences among treatments in milk yield, and percentages of milk fat and milk protein at the end of period 2 ($P > 0.05$; data not shown). This was expected because all diets contained similar levels of oil.

The results of the milk FA analysis are presented in Table I. Because there was no significant interaction between dietary treatments and periods 1 and 2, the results were pooled for each period. In general, the FA composition of the milk reflected the major FA in the oilseed supplements with the milk from Flax-fed cows containing more omega-3 FA, and the milk of the Soybean-fed cows containing more omega-6 FA.

With respect to the omega-6 series of FA, there were significant differences in the levels of linoleic acid (C18:2n-6, the major FA in soybean), conjugated linoleic acid (CLA), and vaccenic acid, the precursor of CLA. This resulted in a significantly higher level of total omega-6 FA in the milk for cows fed the Soybean supplement.

For the omega-3 series of FA, there were no significant differences in linolenic acid (C18:3n-3) level between the flax and the soybean supplemented cows. However, there were marked differences in the longer chain omega-3 FA, i.e., C20:5n-3 (EPA), C22:5n-3 (DPA), C22:6n-3 (DHA). Since none of these FA are found in plant material, it is obvious that the metabolism of dietary linolenic acid (C18:3n-3) was efficient, and the production of these longer omega-3 FA was,

Table II. Pregnancy rates of cows fed 1 of 4 sequential dietary treatments as expressed as absolute numbers and percentages

Supplement	Pregnant by TAI *n* (%)	Pregnant by bull *n* (%)	Not pregnant at 68 to 69 d post-TAI (%)
Flax-Flax (*n* = 32)	13 (40.6)	14 (43.8)	5 (15.6)
Flax-Soybean (*n* = 34)	17 (50.0)	12 (35.3)	5 (14.7)
Soybean-Flax (*n* = 32)	16 (50.0)	15 (46.9)	1 (3.1)
Soybean-Soybean (*n* = 35)	20 (57.1)	10 (28.6)	5 (14.3)

TAI — Timed artificial insemination.

Table III. Mean ± $S_{\bar{x}}$ plasma PGFM and PGEM levels (pg/mL) and PGFM to PGEM ratios of cows fed 1 of 4 sequential dietary treatments and pregnant at 5 to 6 and 19 to 20 d post-TAI

	5 to 6 d post-TAI			19 to 20 d post-TAI		
Supplement	PGFM	PGEM	PGFM/PGEM	PGFM	PGEM	PGFM/PGEM
Flax-Flax	156.0 ± 39.8	36.2 ± 3.6	4.8 ± 1.2	212.5 ± 49.8	36.2 ± 3.6	6.2 ± 1.4
Flax-Soybean	157.6 ± 35.2	38.1 ± 3.2	4.6 ± 1.0	218.4 ± 44.0	41.1 ± 3.1	5.7 ± 1.3
Soybean-Flax	233.8 ± 37.2	34.0 ± 3.4	9.0 ± 1.1	287.2 ± 46.6	34.7 ± 3.3	9.3 ± 1.3
Soybean-Soybean	227.4 ± 32.5	42.7 ± 3.0	5.9 ± 1.0	270.9 ± 40.7	36.8 ± 2.9	7.5 ± 1.2

PGFM — Prostaglandin F metabolites; PGEM — Prostaglandin E metabolites; TAI — Timed artificial insemination. $S_{\bar{x}}$ — standard error of the mean.

perhaps, limited by the amount of linolenic acid supplied in the supplement.

Unlike other studies (9,10), we did not have enough animals to have a control treatment with no fat supplement. However, it is obvious from the milk FA profile that much of the dietary FA were able to bypass the rumen and escape biohydrogenation due to the heat treatment of the oilseed supplements. This could positively affect calf health and vigor (11–15).

The results of pregnancy diagnosis are presented in Table II. No significant differences in pregnancy rates were found among the 4 treatment groups (Fisher's exact test, $P = 0.43$). This may be the result of an insufficient number of cows to demonstrate differences or the type of dietary fat may not influence pregnancy rates in a well-managed herd. Recent studies involving this herd with cows on 1 of 3 levels of postpartum metabolizable energy intake and using 2 methods of estrus synchronization have demonstrated good pregnancy rates (53% to 57%) from TAI (18) regardless of which diet was fed.

Mean ± $S_{\bar{x}}$ plasma levels of PGFM taken 4 to 11 d after calving were 1787.1 ± 164.2 pg/mL and 1546.3 ± 169.8 pg/mL for cows on the Flax and Soybean diets, respectively ($P > 0.05$). Mean ± $S_{\bar{x}}$ levels of PGEM taken at the same time were 148.4 ± 18.0 and 131.7 ± 18.6 pg/mL for cows on the Flax and Soybean diets, respectively ($P > 0.05$). In addition, there was no significant difference in the plasma PGFM to PGEM ratios between the 2 treatments (data not shown).

Plasma levels of PGFM and PGEM, and PGFM to PGEM ratios of cows that were pregnant at 5 to 6 and 19 to 20 d post-TAI are presented in Table III. There were no significant differences ($P > 0.05$) in these levels in the nonpregnant cows (data not shown). In addition, the stage of the estrous cycle would be uncertain for nonpregnant cows 19 to 20 d post-TAI.

The combined effect of supplements fed in periods 1 and 2 had no significant effect ($P > 0.05$) on plasma PGFM or PGEM levels, or PGFM to PGEM ratios. However, at 5 to 6 d post-TAI, pregnant cows fed Flax in period 1 compared with those fed Soybean, had significantly lower plasma PGFM levels (157.5 ± 24.2 pg/mL and 230.9 ± 22.5 pg/mL, respectively; $P < 0.05$) and PGFM to PGEM ratio (4.7 ± 0.7 and 7.4 ± 0.8, respectively; $P = 0.02$), but there were no significant differences in PGFM levels at 19 to 20 d post-TAI. There was a tendency ($P = 0.08$) for cows pregnant 19 to 20 d post-TAI that were fed Flax in period 1 to have a lower plasma PGFM to PGEM ratio than cows fed Soybean during this period. Thus, Flax appeared to have a long-lasting effect on prostaglandin metabolite levels, which was not affected by the FA supplement fed in period 2.

The serum progesterone levels at 5 to 6 d and 19 to 20 d post-TAI are presented in Table IV. Values at 19 to 20 d for nonpregnant cows are not presented as their stage of the estrous cycle would be uncertain. Cows pregnant from TAI and fed Flax in period 2 had higher ($P < 0.05$) serum progesterone levels at 5 to 6 d post-TAI than cows fed Soybean in period 2 (2.95 ± 0.15 ng/mL and 2.52 ± 0.14 ng/mL, respectively). This effect was not seen in the nonpregnant cows at 5 to 6 d post-TAI or in pregnant cows at 19 to 20 d post-TAI.

Cows that were 19 to 20 d pregnant would be well past maternal recognition of pregnancy, which occurs at 15 to 16 d of gestation (4), and the corpora lutea would be well established by this time. However, the differences in progesterone results between pregnant and nonpregnant cows at 5 to 6 d post-TAI are difficult to explain, as the embryos of the pregnant cows would not be hatched from the zona pellucida and would be in the oviduct or tip of the uterine horn (4). The relatively low plasma PGFM and PGFM to PGEM ratio in cows pregnant 5 to 6 d post-TAI and fed Flax in period 1 may partially explain these progesterone results, but this does not account for the relatively high serum progesterone levels in the Soybean-Flax group.

Our hypotheses were partially supported by the results. The dietary treatments used in this trial had little effect on factors affecting pregnancy rates in this well-managed beef herd. However, feeding flax, as opposed to soybean, up to a month before breeding decreased plasma PGFM levels and the PGFM to PGEM ratio in pregnant cows at 5 to 6 d

Table IV. Mean ± $S_{\bar{x}}$ serum progesterone levels (ng/mL) at 5 to 6 and 19 to 20 d post-TAI of cows fed 1 of 4 sequential dietary treatments

	Progesterone levels at 5 to 6 d		Progesterone levels at 19 to 20 d
Supplement	Cows not pregnant from TAI	Cows pregnant from TAI	Cows pregnant from TAI
Flax-Flax	2.24 ± 0.24	2.99 ± 0.21[a]	7.94 ± 0.53
Flax-Soybean	2.04 ± 0.25	2.69 ± 0.19[a,b]	8.03 ± 0.48
Soybean-Flax	2.87 ± 0.25	2.92 ± 0.20[a]	8.66 ± 0.50
Soybean-Soybean	2.38 ± 0.25	2.35 ± 0.19[b]	8.08 ± 0.47

TAI — Timed artificial insemination. $S_{\bar{x}}$ — standard error of the mean.
[a,b] Means with different superscripts within the same column are significantly different ($P < 0.05$).

post-TAI. Feeding flax a month before TAI resulted in higher levels of serum progesterone in cows 5 to 6 d pregnant compared with cows fed soybean for the same period. In addition, the FA supplements in this study influenced the FA composition of milk, which could positively affect calf health and vigor. The effect of changes in FA composition of milk on calves requires further research.

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