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Effects of polyunsaturated fatty acids from plant oils and algae on milk fat yield and composition are associated with mammary lipogenic and SREBF1 gene expression

J. Angulo[1,2], L. Mahecha[2], K. Nuernberg[3], G. Nuernberg[4], D. Dannenberger[3], M. Olivera[1], M. Boutinaud[5], C. Leroux[6], E. Albrecht[3] and L. Bernard[6†]

[1]*Group Biogenesis, Faculty of Agricultural Science, University of Antioquia, AA 1226 Medellin, Colombia;* [2]*Group GRICA, Faculty of Agricultural Science, University of Antioquia, AA 1226 Medellin, Colombia;* [3]*Research Unit of Muscle Biology and Growth, Leibniz Institute for Farm Animal Biology, 18196 Dummerstorf, Germany;* [4]*Research Unit of Genetics and Biometry, Leibniz Institute for Farm Animal Biology, 18196 Dummerstorf, Germany;* [5]*INRA, UMR1080 Dairy Production, F-35000 Rennes, France;* [6]*INRA, UR1213 Herbivores, Site de Theix, F-63122 Saint-Genès-Champanelle, France*

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The main aim of the present study was to examine the effects of long-term supplementing diets with saturated or unprotected polyunsaturated fatty acids from two different plant oils rich in either n-3 or n-6 fatty acids (FAs) plus docosahexaenoic acid (DHA)-rich algae on mammary gene expression and milk fat composition in lactating dairy cows. Gene expression was determined from mammary tissue and milk epithelial cells. Eighteen primiparous German Holstein dairy cows in mid-lactation were randomly assigned into three dietary treatments that consist of silage-based diets supplemented with rumen-stable fractionated palm fat (SAT; 3.1% of the basal diet dry matter, DM), or a mixture of linseed oil (2.7% of the basal diet DM) plus DHA-rich algae (LINA; 0.4% of the basal diet DM) or a mixture of sunflower oil (2.7% of the basal diet DM) plus DHA-rich algae (SUNA; 0.4% of the basal diet DM), for a period of 10 weeks. At the end of the experimental period, the cows were slaughtered and mammary tissues were collected to study the gene expression of lipogenic enzymes. During the last week, the milk yield and composition were determined, and milk was collected for FA measurements and the isolation of milk purified mammary epithelial cells (MECs). Supplementation with plant oils and DHA-rich algae resulted in milk fat depression (MFD; yield and percentage). The secretion of de novo *FAs in the milk was reduced, whereas the secretion of* trans*-10,*cis*-12-CLA and DHA were increased. These changes in FA secretions were associated in mammary tissue with a joint down-regulation of mammary lipogenic enzyme gene expression (stearoyl-CoA desaturase,* SCD1*; FA synthase,* FASN*) and expression of the regulatory element binding transcription factor (*SREBF1*), whereas no effect was observed on lipoprotein lipase (*LPL*) and glycerol-3-phosphate acyltransferase 1, mitochondrial (*GPAM*). A positive relationship between mammary* SCD1 *and* SREBF1 *mRNA abundances was observed, suggesting a similar regulation for these genes. Such data on mammary gene expression in lactating cows presenting MFD contribute to strengthen the molecular mechanisms that govern milk fat synthesis in the mammary glands. In purified MEC, the dietary treatments had no effect on gene expressions. Differences between mammary tissue and milk purified MEC gene expression were attributed to the effect of lipid supplements on the number of milk purified MEC and its RNA quality, which are determinant factors for the analysis of gene expression using milk cells.*

Keywords: lactating cow, plant oils, algae, milk fatty acid, lipogenic gene expression

Implications

Milk fatty acids (FAs) output (impacting milk quality for consumers) and lipogenic gene expression from mammary tissue and milk cells were evaluated in lactating dairy cows supplemented with rumen-stable fractionated palm fat or a combination of plant oils (linseed or sunflower oils) plus docosahexaenoic acid (DHA)-rich algae for 10 weeks. Plant oils plus DHA-algae in the diet decreased milk fat yield and *de novo* FA secretion, responses that occurred together with decreases in the mRNA levels of two lipogenic enzymes and a transcription factor in mammary tissue. These data contribute to decipher the molecular mechanisms underlying nutritional regulation of mammary lipogenesis in ruminants.

† E-mail: Laurence.Bernard@clermont.inra.fr

Introduction

Ruminant-derived foods (meat and milk) are the main source of conjugated linoleic acid (CLA) in the human diet (Lawson *et al*., 2001), with evidence from animal models that the predominant isomer, *cis*-9,*trans*-11, exhibits anticarcinogenic and antiatherogenic properties (Wahle *et al*., 2004). In milk, *cis*-9,*trans*-11-CLA is derived from the ruminal metabolism of 18:2n-6 and from endogenous synthesis via the action of Δ-9 desaturase on *trans*-11-18:1 (vaccenic acid, VA), an intermediate of linoleic (18:2n-6) and linolenic (18:3n-3) acid metabolism in the rumen (Bauman and Griinari, 2001). In addition, saturated fatty acids (SFA; mainly 14:0, 16:0), when consumed in excess, exert negative effects on human health (Parodi, 2005). Thus, there is considerable interest in developing nutritional strategies to increase the *cis*-9,*trans*-11-CLA content and decrease SFA in milk fat.

In bovines, a lot of studies have examined the effects of different nutritional strategies on the milk concentrations of *cis*-9,*trans*-11 and *trans*-11-18:1, mainly by supplementing the diet with plant or fish oil (FO; Bauman and Griinari, 2001). FO is more effective than plant oils in elevating milk fat *cis*-9,*trans*-11-CLA content (Offer *et al*., 1999) because of the inhibitory effect of docosahexaenoic acid (DHA) in FO on VA reduction to stearic acid in the rumen (Boeckaert *et al*., 2008). These increases in milk *cis*-9,*trans*-11-CLA and *trans*-11-18:1 can be further enhanced when FO is provided in combination with plant oils or oilseeds rich in 18:2n-6 (Whitlock *et al*., 2002; AbuGhazaleh *et al*., 2004). The combined use of FO to modify ruminal biohydrogenation and plant oils as a substrate for ruminal *trans*-11-18:1 formation is an established strategy for increasing milk fat *cis*-9,*trans*-11-CLA content. However, most of the studies reporting increased concentrations of *cis*-9,*trans*-11-CLA have been carried out with n-6-rich lipids and FO and during short-term periods (14 to 28 days; Whitlock *et al*., 2002; AbuGhazaleh *et al*., 2004; Shingfield *et al*., 2006). A considerable variation in the response of *cis*-9,*trans*-11-CLA was observed and was probably due to differences in the nature and composition of the plant oils as well as the duration of the treatment. Additionally, in bovines, the molecular mechanisms underlying the nutritional regulation of mammary lipogenesis are still poorly understood. To address these issues, the first aim of the present study was to evaluate the effects of sunflower oil (rich in n-6) and linseed oil (rich in n-3) combined with DHA-rich algae compared to rumen-stable fractionated palm fat (rich in C16:0) over a 70-day period in lactating Holstein cows on milk fat and fatty acid (FA) outputs and on the mammary lipogenic gene expression.

Most of the studies on the effect of nutrition on mammary lipid metabolism have been carried out using mammary tissue collected at the time of slaughtering or by biopsy, while only a few studies have been reported using milk cells from ruminants as a non-invasive method (Boutinaud *et al*., 2002 and 2008; Murrieta *et al*., 2006, Feng *et al*., 2007). The second aim of the present study was to investigate the effect of lipid-supplemented diets on the amount of milk purified mammary epithelial cells (MEC) and on the gene expression of lipogenic enzymes from MEC.

A reduction in milk fat yield due to sunflower oil (rich in n-6) and linseed oil (rich in n-3) combined with DHA-rich algae diets compared to rumen-stable fractionated palm fat (rich in C16:0) was expected. We hypothesised that reduction in milk fat yield would be due to an increase in rumen biohydrogenation intermediates and/or long-chain polyunsaturated fatty acids (PUFA) of lipid supplements that would down-regulate lipogenic gene expression, and would be associated with the transcription factor SREBF-1.

Materials and methods

Animals and diets

All experimental procedures were approved by the Animal Care Committee of the Leibniz Institute for Farm Animal Biology FBN in accordance with the Use of Vertebrates for Scientific Purposes Act of 1985. Eighteen primiparous German Holstein dairy cows in mid-lactation (days in milk, DIM = 91.6; standard error of the mean, s.e. = 7.8) were offered one of three experimental diets (SAT, LINA and SUNA) for 10 weeks, in three groups of six cows. The SAT diet included grass silage, maize silage, hay, a mineral feed mixture (Salvana 9522, Salvana, Sparrieshoop, Germany) and a concentrate mixture (MF 2000, Vollkraft, Güstrow, Germany) supplemented with rumen-stable fractionated palm fat, which is mostly saturated fat (SAT; Bergafat T-300, Dr. Pieper Technologie, Wuthenow, Germany) (3.1% of the basal diet dry matter, DM). The LINA diet included a combination of linseed oil (Dörnthaler Öhlmühle, Dörnthal, Germany) (2.7% of the basal diet DM) and DHA-rich marine algae (DHA Gold, Novus Europe, Brussels) (0.4% of the basal diet DM), whereas the SUNA diet included a combination of sunflower oil (Teutoburger Ölmühle, Ibbenbüren, Germany) (2.7% of the basal diet DM) and DHA-rich marine algae (DHA Gold, Novus Europe, Brussels) (0.4% of the basal diet DM). The diets were distributed as total mixed ration (TMR). The inclusion of dietary oils as 3.1% of DM was determined according to Cruz-Hernandez *et al.* (2007), who demonstrated that the level of rumenic acid could be retained when the level of oil in the diet is moderate (about 3% of the DM content). The ingredients and composition of the experimental treatments are shown in Table 1.

Animals of same parities, similar milk yields and similar DIM were selected for this study and the treatments were randomly assigned to these pre-selected animals.

The diets were isoenergetically and isonitrogenously calculated and were offered *ad libitum* as two equal meals at 0700 and 1700 h. The cows had free access to water throughout the experiment and were milked twice a day. The animals were slaughtered at the end of the experimental period.

Measurements and sampling

Samples of milk were collected from the morning milking on the last day of the experimental period for analysis of FA composition, according to the method described by

Table 1 *Ingredients and composition of experimental treatments offered in the mixed ration*

	Treatment[1]		
	SAT	LINA	SUNA
Ingredients (% of DM)			
Grass silage[2]	17.5	17.3	17.5
Maize silage[3]	35.3	35.4	35.3
Hay[4]	2.0	2.0	2.0
Concentrate[5]	41.5	41.5	41.5
Bergafat T-300[6]	3.1	–	–
Linseed oil[7] + algae DHA gold[8]	–	3.1	–
Sunflower oil[9] + algae DHA gold	–	–	3.1
Minerals[10]	0.7	0.7	0.7
Nutrient composition (% of DM)			
CP	16.6	16.6	16.6
Crude fibre	13.5	13.5	13.5
Crude fat	6.24	6.24	6.26
Net energy (MJ/kg DM)	7.38	7.37	7.37
FA intake (g/day)[11]			
C14:0	8.32[a]	5.46[b]	5.92[b]
C16:0	396.7[a]	102.6[b]	115.6[b]
C18:0	64.5[a]	23.6[b]	27.3[b]
cis-9-C18:1	230[a]	171.21[b]	168.41[b]
C18:2n-6	236.2[b]	261.5[b]	490.6[a]
C18:3n-3	85.96[b]	306.79[a]	94.68[b]
C22:6n-3	0.0[b]	5.6[a]	5.88[a]

DM = dry matter; DHA = docosahexaenoic acid; FAs = fatty acids.
Different letters indicate significant differences between treatments at $P \leqslant 0.05$.
[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower and algae (SUNA).
[2]Maize silage composition (g/kg DM unless otherwise stated) was DM (g/kg fresh weight), 352; CP, 87; net energy of lactation (MJ/kg DM), 7; fibre, 169; crude fat, 33; starch, 346; simple carbohydrates (sugars), 5; ashes, 34.
[3]Grass silage composition (g/kg DM, unless otherwise stated) was DM, 424 (g/kg fresh weight); CP, 191.3; net energy of lactation, 6; fibre, 242; crude fat, 35; sugars, 67; ashes, 106.
[4]Hay composition (g/kg DM, unless otherwise stated) was DM, 832 (g/kg of fresh weight); CP, 125; net energy of lactation, 5; fibre, 337; crude fat, 15; sugar, 60; ashes, 61.
[5]Concentrate: 33% soybean, 20% corn, 17% wheat gluten feed, 13% wheat, 8% rapeseed meal, 5% molasses, 2% $NaHCO_3$, 1.3% $CaCO_3$, 0.2% NaCl, 0.5% vitamins and minerals.
[6]Bergafat T-300 – rumen-stable fractionated palm fat (TG) contained (g/100 g of FAs) C14:0 (1.4), C16:0 (71.2), C18:0 (11.0), *cis*-9-C18:1 (12.8) and C18:2n-6 (3.2).
[7]Linseed oil contained (g/100 g of FAs) C14:0 (0.05), C16:0 (5.3), C18:0 (3.0), *cis*-9-C18:1(18.0), C18:2n-6 (16.0) and C18:3n-3 (55.6).
[8]DHA-rich algae contained (g/100 g of FAs) C14:0 (12.0), C16:0 (27.7), C18:0 (0.6), C18:1*cis*-9 (0.1), C18:2n-6 (0.04), C20:5n-3 (1.4), C22:5n-3 (0.3) and C22:6n-3 (53.1).
[9]Sunflower-seed oil contained (g/100 g of FAs) C14:0 (0.07), C16:0 (6.0), C18:0 (1.8), C18:1*cis*-9 (25.2), C18:2n-6 (64.1) and C18:3n-3 (0.09).
[10]Mineral feed mixture (Salvana 9522 declared as containing 92% ashes, 20% calcium, 5% phosphorous, 8% sodium, 6% magnesium).
[11]FA intake was estimated based upon TMR FA composition and dry matter intake.

Duske *et al.* (2009). Briefly, the lipid fraction of milk was extracted with n-hexane/isopropanol (v/v = 3/2). FA methyl esters were prepared and subjected to GC-FID analysis. The temperature programme started with an initial oven temperature of 45°C held for 4 min; this temperature was increased to 150°C at a rate of 13°C/min, was held for 47 min, and then was increased to 215°C at 4°C/min and held for 35 min to identify the *trans*-C18:1 isomers. The individual isomers of CLA methyl esters were prepared from *cis*-9,*trans*-11 CLA, *trans*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, *cis*-9,*cis*-11 CLA and *cis*-11,*trans*-13 CLA purchased as free FA from Matreya (Pleasnet Gap, PA, USA). Identification and quantification analysis of the CLA isomers was performed by Ag+-HPLC using an HPLC system (LC 10A, Shimadzu, Japan) equipped with a pump (LC-10AD VP), an autosampler (SIL-10AF), a 50 μl injection loop, a photodiode array detector (SPD-M 10Avp, Shimadzu, Japan), and the Shimadzu CLASS-VP software system (Version 6.12 SP4). Four ChromSpher 5 Lipids analytical silver ion-impregnated columns (4.6 mm i.d. × 250 mm stainless steel; 5 μm particle size; Chrompack-Varian, Walnut Creek, CA, USA) were used in series. The mobile phase (0.1% acetonitrile in n-hexane) was prepared fresh daily and pumped at a flow rate of 1.0 ml/min. The detector was operated at 233 nm to identify CLA isomers based on the measurement of integrated area under the 233 nm peaks attributed to conjugated dienes. The identification of CLA isomers was made by the retention time of individual CLA methyl esters (*cis*-9,*trans*-11 CLA, *trans*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, *cis*-9,*trans*-11 CLA, *cis*-9,*cis*-11 CLA and *cis*-11,*trans*-13 CLA). The external calibration plots of the standard solutions were adapted to different concentration levels of individual CLA isomers in the lipid extracts, as described by Shen *et al.* (2011).

Milk yield was recorded daily. Milk aliquots were collected once weekly during morning milking for the analysis of fat, protein, lactose contents (Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg-Vorpommern e.V., Güstrow, Germany). Milk fat yield and milk FA yield (Glasser *et al.*, 2007) were calculated from milk data of the last experimental week. A representative sample of hay, maize silage, and concentrate was taken and stored at −20°C. The chemical composition of the feed ingredients was determined using standard procedures (Association of Official Analytical Chemists (AOAC), 1997). FA intake was calculated as dry matter intake (DMI) during the last experimental week multiplied by the estimated FA composition of the diet (TMR).

Mammary tissue

At the end of the experimental period (day 70), the cows were slaughtered by captive bolt stunning followed by exsanguinations in the abattoir of the Leibniz Institute for Farm Animal Biology in Dummerstorf (Germany). Immediately before slaughter, the animals were milked to remove most of the milk from the mammary glands. Immediately after slaughter, the skin of the udder was removed, and a slice of mammary tissue from the upper middle of the right rear quarter was collected. Approximately 100 g of mammary tissue was collected under sterile conditions in the secretory area. The samples were immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction.

Milk cells

Milk sampling was carried out at day 70 of the experimental period. The methodology described by Boutinaud *et al.* (2008)

was used to extract the milk cells. Two litres of fresh milk per cow ($n = 18$) from the morning milking were collected and skimmed by 15 min of centrifugation at 1500 × **g** at 4°C in 175 ml tubes (Capitol Scientific Inc., Austin, TX, Germany). The skim milk was removed, and the remaining total cell pellet was re-suspended in 225 ml of phosphate buffered saline (PBS; Gibco, Invitrogen, Karlsruhe, Germany). The cell suspension was washed twice in PBS and filtered through a 200-μm nylon membrane. After a final centrifugation (1000 × **g**, 10 min at 4°C), the cell pellet was re-suspended in 2 ml of PBS containing 1% bovine serum albumin (BSA; Sigma-Aldrich Logistik, Schelldorf, Germany). The cell suspension was used for purification of MECs using an immunomagnetic separation technique according to the method first described by Alcorn *et al.* (2002), with some modifications. Briefly, dynabeads (Pan Mouse IgG, Dynal Biotech, Invitrogen, Karlsruhe, Germany) were first coated with a primary mouse monoclonal antibody directed against cytokeratin (clone 34 beta E12, DakoCytomation, Hamburg, Germany) by a 30-min incubation on a rotary mixer at 4°C, as described previously (Boutinaud *et al.*, 2008). The antibody-bead complex and milk cell suspension were incubated for 1 h on a rotary mixer at 4°C. Specifically bound cells were collected by placing the sample vials in a magnetic particle concentrator (1 min) after aspiration of the supernatant. Cells binding to the dynabeads were washed with 1 ml of 1% BSA-PBS and re-suspended in 1 ml of 1% BSA-PBS. A 10-μl aliquot was collected to be quantified by microscopy using a Kova Glasstic Slide 10 with grids (Hycor Biomedical, Kassel, Germany). The milk purified MECs were pelleted by a 5000 × **g** centrifugation at 4°C for 5 min. The cell pellets were re-suspended in 1 ml Trizol and stored at −80°C until RNA extraction.

RNA isolation and quantitative real-time reverse transcription-PCR

Total RNA was isolated from milk purified MECs using the RNeasy Mini Kit, according to the manufacturer's recommendations (Qiagen Inc., Courtaboeuf, France), which included a DNase treatment step to eliminate contaminating genomic DNA. Total RNA was isolated from ~80 mg of mammary tissue using TRIZOL reagent (Invitrogen Life Technologies, Cergy Pontoise, France) and further purified using the Invitrogen Total RNA Isolation system (Invitrogen Life Technologies, Cergy Pontoise, France), which included a DNase treatment step to eliminate contaminating genomic DNA. The total RNA concentration and purity were determined using a spectrophotometer (NanoDrop® ND-1000 UV-Vis) at 260 and 280 nm and a Bioanalyser 2100 system (Agilent Technologies, Massy, France).

The total RNA integrity was assessed by the RNA Integrity Number (RIN) generated by Agilent 2100 Expert software, version B.02 (Agilent Technologies, Massy, France).

Reverse transcription (RT) was performed using 4 μg of purified total RNA obtained from the mammary gland, 10 pmol of oligonucleotides (dT) and 200 U of Superscript II RNase H-reverse transcriptase (Invitrogen, Life Technologies, Cergy Pontoise, France) in a final volume of 20 μl. The reaction mix was then diluted with 30 μl of sterile water as previously described by Bernard *et al.* (2009a). cDNA was generated from the total RNA from the milk purified MECs in a Bio-Rad thermal cycler using a first-strand cDNA synthesis kit (Roche Diagnostics, Meylan, France). Five microlitres of RNA extracted from the milk purified MECs at a concentration of 40 ng/μl was incubated with 5 μl of the RT mix for 1 h at 42°C, according to the manufacturer's instructions. Reverse transcript products were stored at –80°C until use.

From samples of mammary tissues and milk cells, the mRNA levels of the following genes were measured due to their implication in the major metabolic pathways of lipogenesis (Ahnadi *et al.*, 2002; Baumgard *et al.*, 2002; Harvatine and Bauman, 2006): lipoprotein lipase (*LPL*), involved in the uptake of FAs from circulating triglycerides; fatty acid synthase (*FASN*), involved in *de novo* lipogenesis; stearoyl-CoA desaturase (*SCD1*), involved in the delta-9 desaturation of FA; glycerol-3-phosphate acyltransferase 1, mitochondrial (*GPAM*), whose transcribed protein catalyses the first step in the esterification of FAs to glycerol; and sterol regulatory element binding transcription factor 1 (*SREBF1*), involved in the regulation of lipogenic genes expression.

The mRNA abundances of the targeted genes (*SCD1*, *LPL*, *FASN*, *GPAM*, *SREBF1*) was determined by Real-time quantitative PCR (qPCR) using a StepOne™ Real-Time PCR system (Applied Biosystem, Foster City, CA, USA). The results are expressed as the mRNA copy number of each gene of interest (three repetitions/sample) relative to the geometric mean of three housekeeping genes (peptidyl-prolyl cis-trans isomerase A or cyclophilin A (*PPIA*), eukaryotic translation initiation factor 3 subunit K (*EIF3 K*), and ubiquitously expressed transcript (*UXT1*)) determined using GeNorm software (Vandesompele *et al.*, 2002), to account for variations in RNA integrity, RNA quantification and cDNA synthesis. These genes were previously described by Bonnet M., *et al.* (unpublished data) and Kadegowda *et al.* (2009) and were identified as suitable internal controls among several tested (Bonnet M., *et al.*, unpublished data).

The mRNA abundances of *SCD1*, *LPL*, *FASN*, and *PPIA* were quantified in triplicate using specific fluorescent Taqman probes (Applied Biosystem, Foster City, CA, USA) and specific primers (Supplemental Table) as previously described (Bernard *et al.*, 2005a), with the quantification determined from a calibration curve prepared by amplifying different copy numbers of a recombinant plasmid (Bernard *et al.*, 2005a). The abundances of *SREBF1*, *GPAM*, *UXT1* and *EIF3 K* mRNA were quantified in triplicate using a Power SYBR® Green PCR Master Mix Kit (Applied Biosystems, Foster City, CA, USA) and specific primers (Supplemental Table) according to the manufacturer's instructions. The cDNA quantification was performed from calibration curves generated from 5-point serial dilutions using bovine mammary cDNA. The development of the qPCR technology allow after the run of amplification to check the specificity of primer pairs using fusion curves of the nucleotide sequence using Sybergreen methodology, and recently systems were developed using this methodology (Bernard *et al.*, 2011).

For the eight genes, the calibration curves demonstrated similar amplification efficiencies calculated as $E = 10(^{-1/\text{slope}})$ whatever the methodology (Taqman or Sybergreen), varying from 1.85 to 1.95. A non-template negative control was incorporated into all PCR runs.

Statistical analyses

All of the data were analysed by the least-squares mean method using the GLM procedures in SAS(R) 2009. Treatment means were compared using the least-squares mean procedure (SAS Institute) with the significance set at $P \leqslant 0.05$. Values of P between 0.05 and 0.10 were considered to be tendencies. Relationships between different parameters were assessed after calculation of Pearson correlation coefficients. The following model for the analysis of variance with the fixed factor diet (D) was used:

$$Y_{ij} = \mu + D_i + E_{ij}$$

where μ is the overall mean, D_i is the diet effect ($i = 3$) and E_{ij} is the residual error.

Results

Intake

By design, 18:2n-6 was the major FA supplied by the SUNA diet (236, 262 and 491 g/d for SAT, LINA and SUNA, respectively), and 18:3n-3 was the major FA supplied by the LINA diet (86, 307 and 95 g/d for SAT, LINA and SUNA, respectively). Animals fed with the SUNA or LINA diet ingested a higher amount of DHA compared to animals that were fed the SAT diet without DHA (0.0, 5.6 and 5.9 g/d for SAT, LINA and SUNA, respectively). Experimental diets allowed for a higher intake of total C18:0 FAs (564, 773 and 858 g/d, for SAT, LINA and SUNA, respectively).

Animal performance

Treatments with plant oils and algae had no effect on the DMI, energy and protein intake (Table 2). Relative to the SAT diet, the LINA and SUNA treatments decreased the milk fat yield (−33 and −34 g/d, respectively; $P < 0.0001$) and fat concentration (−1.4 and −1.5 percentage unit, respectively; $P < 0.0001$). The LINA diet increased the milk lactose percentage ($P = 0.016$), and the SUNA diet had a tendency to increase it ($P = 0.10$; Table 2).

Milk FA secretions

Data on milk FA and CLA secretion (g/d and mg/d, respectively) are shown in Tables 3 and 4. Plant oils plus algae decreased ($P < 0.0001$) milk secretion of SFA from C10:0 to C16:0 and the total secretion of SFA and increased milk secretion of DHA ($P < .0001$), *trans*-7,*cis*-9-CLA ($P = 0.02$) and the total secretion of *trans*-C18:1 isomers ($P = 0.0002$). Milk secretions of *trans*-11-18:1 and *trans*-10,*cis*-12-CLA increased with the LINA and SUNA diets compared to the SAT diet and were higher for the SUNA diet compared to the LINA diet. The output of C18:3n-3, the total amount of n-3 FA, *trans*-12,*trans*-14-CLA, *trans*-11,*trans*-13-CLA, *trans*-11,*cis*-13-CLA and *trans*-9,*trans*-11-CLA increased ($P \leqslant 0.05$) with the LINA diet only. The output of C18:2n-6, *trans*-10-C18:1, *trans*-10, *trans*-12-CLA and *trans*-7,*trans*-9-CLA increased ($P \leqslant 0.05$) with the SUNA diet only (Tables 3 and 4).

The SUNA diet resulted in higher *cis*-9-14:1/14:0 and *cis*-9-16:1/16:0 ratios compared to the SAT diet ($P = 0.01$). The *cis*-9,*trans*-11-CLA/*trans*-11-18:1 ratio was significantly lower for the LINA and SUNA diets compared to the SAT diet ($P < 0.001$). The C18:2n-6 and C18:3n-3, from the diet were apparently transferred into milk with lower efficiencies for the LINA and SUNA diets compared to the SAT diet, whereas the converse was observed for DHA (Table 3).

Table 2 *Effect of experimental treatments on DMI, milk yield and composition and energy and protein balance in cows (average last week)*

	Treatment[1]				
	SAT	LINA	SUNA	s.e.m.[2]	*P*-value
DMI (kg/day)	20.0	19.2	21.7	0.77	0.25
Energy balance (NEL MJ/cow/day)	2.97	1.77	2.41	0.71	0.51
Protein balance (kg/day)	0.33	0.32	0.36	0.14	0.12
Yield (kg/day)					
Milk	29.1	32.9	35.0	1.97	0.13
Fat	1.07[a]	0.74[b]	0.75[b]	0.05	0.0008
Protein	0.96	1.02	1.09	0.05	0.23
Lactose	1.40	1.61	1.67	0.09	0.12
Concentration (%)					
Fat	3.67[a]	2.31[b]	2.17[b]	0.14	<.0001
Protein	3.26	3.15	3.18	0.09	0.70
Lactose	4.74	4.93	4.85	0.06	0.09

DMI = dry matter intake; NEL = net energy of lactation

Different letters indicate significant differences between treatments at $P \leqslant 0.05$.

[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA).

[2]Standard error of the mean for $n = 18$.

Table 3 *Effect of experimental treatment on the secretion of the major FAs in milk, including specific trans isomers of C18:1, milk fat Δ-9 desaturase ratios, and apparent transfer rate of C18 FAs and DHA in cows*

	Treatment[1]				
FAs (g/day)	SAT	LINA	SUNA	s.e.m.[2]	*P*-value
C10:0	29.47[a]	11.32[b]	9.84[b]	2.49	<0.0001
C12:0	38.84[a]	17.57[b]	16.14[b]	3.06	0.0001
C14:0	126.1[a]	76.38[b]	70.70[b]	9.21	0.0012
C14:1	10.86	10.05	11.96	1.61	0.71
C16:0	377.5[a]	180.7[b]	172.8[b]	21.81	<0.0001
∑C10–C16	571.9[a]	285.9[b]	269.5[b]	35.77	<0.0001
C16:1	18.00	11.88	14.30	1.76	0.08
C18:0	79.58	70.38	70.44	6.95	0.57
trans 6_8-C18:1	1.88	1.30	2.05	0.48	0.52
trans-9-C18:1	4.64	6.21	7.68	0.83	0.06
trans-10-C18:1	6.12[a]	8.73[a]	13.38[b]	0.98	0.004
trans-11-C18:1	14.15[a]	32.73[b]	48.35[c]	4.12	0.0001
trans-12-C18:1	9.33	15.48	15.13	2.92	0.28
∑*trans*-18:1	36.20[a]	62.60[b]	87.18[c]	6.51	0.0002
cis-9-C18:1	190.9	184.6	175.9	12.44	0.70
C18:2n-6	20.06[a]	14.79[ab]	27.14[c]	1.68	0.0004
C18:3n-3	4.39[a]	8.26[b]	3.34[a]	0.57	<0.0001
∑C18	373.6	413.2	448.0	27.26	0.19
C22:6n-3 (DHA)	0.17[a]	1.02[b]	1.08[b]	0.10	<0.0001
∑n-3	6.75[a]	11.33[b]	6.21[a]	0.78	0.0005
∑n-6	23.54[a]	16.42[b]	28.80[a]	1.80	0.0008
∑MUFA	277.4	299.4	313.8	19.76	0.44
∑SFA	675.7[a]	372.2[b]	355.8[b]	41.20	<0.0001
∑PUFA	34.33	34.66	39.64	2.70	0.32
Δ9-desaturase ratios					
cis-9 14:1/14:0	0.09[a]	0.13[ab]	0.17[b]	0.02	0.01
cis-9 16:1/16:0	0.05[a]	0.07[ab]	0.08[b]	0.01	0.01
cis-9 18:1/18:0	2.43	2.75	2.51	0.17	0.40
cis-9, trans-11-CLA/*trans*-11 18:1	0.41[a]	0.31[b]	0.16[b]	0.04	0.003
Apparent transfer rate[3] (%)					
18:2n-6	8.62[a]	5.65[b]	5.64[b]	0.63	0.006
18:3n-3	5.17[a]	2.69[b]	3.58[b]	0.58	<0.0001
∑C18	67.58	53.36	53.11	4.95	0.09
DHA	0.00[a]	18.03[b]	18.61[b]	1.67	<0.0001

FA = fatty acids; DHA = docosahexaenoic acid; MUFA = monounsaturated fatty acids; SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids; CLA = conjugated linoleic acid.
∑C10–C16 = C10:0 + C12:0 + C14:0 + C16:0.∑SFA = C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0.
∑MUFA = C14:1 + C15:1 + C16:1 + C17:1 + *trans*-9-C18:1 + *trans*-10-C18:1 + *trans*-11-C18:1 + *cis*-9-C18:1 + *cis*-11-C18:1 + C22:1 + C24:1.
∑n-3 FA = C20:3n-3 + C22:6n-3 + C22:5n-3 + C20:5n-3 + C18:4n-3 + C18:3n-3.
∑n-6 FA = C22:2n-6 + C20:2n-6 + C18:3n-6 + C22:4n-6 + C20:3n-6 + C18:2n-6 + C20:4n-6.
Different letters indicate significant differences between treatments at $P \leq 0.05$.
[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA).
[2]Standard error of the mean for $n = 18$.
[3]Apparent transfer rate calculated as the ratio between the milk FA secretion and its level provided by the diet.

Quantification of purified MECs and mRNA quality

The different diets did not have statistical effects on the concentration of milk purified MECs or the number of MECs exfoliated per day ($P = 0.18$). The total amount of RNA collected from milk purified MECs was similar between the diet groups ($P = 0.35$), averaging 2.3 μg (s.e. 0.3) (data not shown). The diet has no effect on total RNA quality even though a tendency was observed for a lower RIN with SUNA ($P = 0.08$) compared to SAT (Table 5).

Gene expression in mammary tissue and milk purified MECs

The addition of plant oils and algae to the diet decreased ($P \leq 0.05$) the abundance of *SCD1*, *FASN*, and *SREBF1* mRNAs in mammary tissue. Furthermore, plant oils and

algae had no effect ($P = 0.3$) on the abundance of the *GPAM* and *LPL* mRNAs in mammary tissue, except for a tendency ($P = 0.09$) of decreased *LPL* levels with the LINA diet compared to the SAT diet (Table 6).

In milk purified MECs, the treatments had no effect ($P > 0.10$) on the abundance of the *SCD1*, *FASN*, *SREBF1*, and *GPAM* mRNAs (Table 6).

Discussion

The present study investigated the effects of plant oils rich in either C18:3n-3 or C18:2n-6 plus DHA compared to a mostly saturated fat source from rumen-stable fractionated palm fat on milk fat composition and milk yield, particularly with respect to the expression of genes involved in the major pathways of lipogenesis in mammary tissue. These data are discussed in relation with other studies on the effect of nutrition on mammary gene expression all carried out using mammary tissue as source of mRNA. In addition, gene expression was also determined from milk purified MECs to evaluate this source of mRNA in lipid-supplemented animals for which so far only one study is available in ruminant (Murrieta *et al.*, 2006). The observations made in the present study (milk fat composition and gene expression) are related to the endpoint of a long-term adaptation period to the treatments (10 weeks) even though it is likely that a short-term adaptation occurred earlier.

Milk production and composition

Supplementing with a combination of linseed or sunflower oil with algae compared to saturated fat for 10 weeks did not

Table 4 *Effect of experimental treatment on the secretion of CLA isomers in milk in cows*

	Treatment[1]				
FAs (mg/day)	SAT	LINA	SUNA	s.e.m.[2]	*P*-value
cis-9,*trans*-11-CLA[3]	5720	8878	7613	815	0.07
cis-11,*trans*-13-CLA	20	40	30	7	0.15
cis-12,*trans*-14-CLA	20	70	30	20	0.09
trans-7,*cis*-9-CLA	1000^{a}	1520^{b}	1620^{b}	143	0.02
trans-8,*cis*-10-CLA	60	60	80	18	0.77
trans-10,*cis*-12-CLA	62^{a}	119^{b}	285^{c}	23	<.0001
trans-11,*cis*-13-CLA	90^{a}	250^{b}	60^{a}	20	<.0001
trans-7,*trans*-9-CLA	2^{a}	4^{ab}	8^{b}	1	0.02
trans-8,*trans*-10-CLA	20	20	30	3	0.32
trans-9,*trans*-11-CLA	50^{a}	80^{b}	50^{a}	7	0.008
trans-10,trans-12-CLA	40^{a}	40^{a}	80^{b}	5	<.0001
trans-11,*trans*-13-CLA	80^{a}	190^{b}	50^{a}	19	0.0003
trans-12,*trans*-14-CLA	40^{a}	120^{b}	30^{a}	9	<.0001

CLA = conjugated linoleic acid; FAs = fatty acids.
Different letters indicate significant differences between treatments at $P \leq 0.05$.
[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA).
[2]Standard error of the mean for $n = 18$.
[3]Coelutes with *trans*-9,*cis*-11-CLA.

Table 6 *Effect of experimental treatment on mRNA abundance of LPL, FASN, SCD1, GPAM, SREBF1 genes in mammary tissues and milk purified MEC of cows*

	Treatment[1]				
	SAT	LINA	SUNA	s.e.m.[2]	*P*-value
Mammary tissues					
LPL	85.44	47.14	56.87	11.83	0.09
FASN	631.52^{a}	421.34^{b}	436.78^{b}	61.28	0.05
SCD1	817.78^{a}	446.00^{b}	535.69^{b}	49.27	0.0002
GPAM	152.66	113.08	138.14	17.62	0.30
SREBF1	30.08^{a}	24.43^{b}	22.22^{b}	1.79	0.02
Milk purified MEC					
LPL	3.60	2.68	3.25	0.95	0.79
FASN	2.54	5.15	6.85	0.26	0.40
SCD1	9.26	16.42	26.38	9.58	0.47
GPAM	0.49	0.86	1.65	0.63	0.44
SREBF1	10.60	12.31	12.32	2.15	0.81

LPL = lipoprotein lipase; FASN = fatty acid synthase; SCD1 = stearoyl-CoA desaturase; GPAM = glycerol-3-phosphate acyltransferase 1, mitochondrial; SREBF1 = sterol regulatory element binding transcription factor 1; MEC = mammary epithelial cells.
Levels of mRNA expressed in arbitrary units determined as abundance relative to the geometric mean of three reference genes (*PPIA*, *EIF3 K* and *UXT*).
Different letters indicate significant differences between treatments at $P \leq 0.05$.
[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA).
[2]Standard error of the mean for $n = 18$.

Table 5 *Effect of experimental treatment on milk purified MEC number and on the RNA quality of MEC and mammary tissue indicated by RIN*

	Treatment[1]				
	SAT	LINA	SUNA	s.e.m.[2]	*P*-value
MEC (cells/ml of milk)	4740	7060	4760	0.07	0.18
Loss of MEC in the milk (10^6 cells/day)	140,700	227,900	164,500	0.08	0.18
RIN of MEC RNA[3]	7.8	6.7	6.0	0.54	0.08
RIN of MG RNA	8.0	7.8	7.6	0.23	0.62

MEC = mammary epithelial cells; RIN = RNA integrity number; MG = mammary gland.
[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA).
[2]Standard error of the mean for $n = 18$.
[3]RIN generated by Agilent 2100 Expert software, version B.02 (Agilent Technologies, Massy, France).

affect the DMI. Similar results were found by Abughazaleh *et al.* (2004), in a study where lactating cows were supplemented with fish meal and extruded soybean (0.5% and 2% of FO and soybean oil, respectively) for 10 weeks. In contrast, Shingfield *et al.* (2005) found a significant decrease of DMI in cows fed a corn silage-based diet (forage : concentrate, 60 : 40, DM basis) supplemented with a mixture of FO (1.5% of DM) and sunflower oil (3.0% of DM) for 28 days compared to a control diet (without lipid supplement). Supplementing diets with oils rich in unprotected PUFA often results in a reduction in DMI, with the mechanisms of this hypophagic effect being attributed to effects on ruminal fermentation and gut motility, the palatability of diets containing added fat, the release of gut hormones and the oxidation of fat in the liver (Allen, 2000). The maintained DMI observed in the present study compared to other studies could be due to the nature of the control diet (rumen-stable fractionated palm fat), which presented the same amount of fat as SUNA and LINA diet, a possible difference in the effect of algae compared to FO on the diet palatability, the different level of algae compared to FO in the diets and/or the duration of the dietary treatment (10 weeks in the present study and in Abughazaleh *et al.*, 2004 *v.* 28 days in Shingfield *et al.*, 2005).

The decreases in milk fat yield (−30% and −31% for LINA and SUNA compared to SAT, respectively) and concentration (−38% and −41% for LINA and SUNA compared to SAT, respectively) are consistent with previous studies using FO in bovines (Whitlock *et al.*, 2002; AbuGhazaleh *et al.*, 2004). This effect is known as milk fat depression (MFD) and often occurs in dairy cows that are fed unprotected PUFAs (Shingfield *et al.*, 2006). MFD has been related to an alteration of ruminal biohydrogenation resulting from the production of different ruminal intermediates that have a negative effect on the gene expression of lipogenic enzymes (Bauman and Griinari, 2001). DHA-micro-algae alone have been used as a viable alternative to FO in cow's diets to modify rumen biohydrogenation (AbuGhazaleh *et al.*, 2009). Boeckaert *et al.* (2008) found that supplementing dairy cows with algae inhibited the total biohydrogenation of C18:2n-6 and C18:3n-3, resulting in increased concentrations of biohydrogenation intermediates in rumen, in particular the 18:1-*cis* and -*trans* isomers, whereas C18:0 decreased due to a decrease of the capacity of the butyrivibrio community to hydrogenate *trans*-C18:1 FAs. In the present experiment, both PUFA diets decreased the apparent transfer rate of C18:2n-6 and C18:3n-3 and increased the output of different *trans*-C18:1 isomers, particularly *trans*-11 compared to SAT. An estimation of the degree of ruminal *trans*-C18:1 reduction from the ratio of *trans*-C18:1/(C18:0 + *cis*-9-C18:1 + *trans*-11-C18:1; Palmquist and Griinari, 2006) in milk (i.e. 0.05, 0.11 and 0.17, for SAT, LINA and SUNA, respectively) is in line with FO acting as an inhibitor of the conversion of *trans*-18:1 to stearic acid in the rumen (Chilliard *et al.*, 2001).

Milk FA secretion and lipogenic enzyme gene expression from mammary tissue

In the present study, after 10 weeks feeding the diets supplemented with PUFAs, the decreased output of milk medium-chain FAs (C10–C16, −50% with LINA and −53% with SUNA) was related to a significant decrease in *FASN* mRNA levels (−33% with LINA and −31% with SUNA). These results are consistent with studies evaluating the nutritional regulation of lipogenic enzyme gene expression in lactating cows with high-concentrate diets (Peterson *et al.*, 2004), with rations containing soybean oil (Piperova *et al.*, 2000), FO (Ahnadi *et al.*, 2002), FO and soybean oil (Harvatine and Bauman, 2006) that induce MFD. Under these conditions, substantial reductions in milk FAs related to *de novo* synthesis (from 30% to 59% decreases in C10–C16:0) have been associated with a decrease in mammary *FASN* mRNA levels and/or activity. These effects were attributed to the synthesis of specific rumen biohydrogenation intermediates associated with the ruminal *trans*-10 pathway that exerts antilipogenic effects in bovines (Shingfield *et al.*, 2010). In particular, abomasal infusion of supraphysiological amounts of *trans*-10,*cis*-12-CLA has been shown to inhibit milk fat synthesis in ruminants (Baumgard *et al.*, 2002). Consistent with these findings, in the present study, the PUFA-rich SUNA and LINA diets led to a significant increase in milk *trans*-10, *cis*-12-CLA that may explain the observed decreases of both *FASN* gene expression and milk medium-chain FA secretion. However, these observations contrast with data from Invernizzi *et al.* (2010) obtained on dairy cows fed with a mixture of fish and soybean oils that lead to a decrease in milk *de novo* FA synthesis that was not associated neither to increase in milk *trans*-10,*cis*-12-CLA nor to significant effect on *FASN* mRNA abundance. These data, together with others combining plant oils with FO (Whitlock *et al.*, 2002; AbuGhazaleh *et al.*, 2004) underlined that MFD may occur with moderate increase (Whitlock *et al.*, 2002; AbuGhazaleh *et al.*, 2004) or without increasing (Invernizzi *et al.*, 2010) milk *trans*-10,*cis*-12-CLA. Thus, the implication of other biohydrogenation intermediates have been involved, such as *trans*-18:1 isomers and specifically the *trans*-10 18:1 isomer, which may exert potentially antilipogenic effects and contribute to the reduction in milk fat synthesis during diet-induced MFD in the lactating cow (Shingfield *et al.*, 2009). In addition, the *trans*-9,*cis*-11-CLA and the *cis*-10,*trans*-12-CLA isomers, have been also identified as inhibitors of milk fat synthesis (Shingfield *et al.*, 2010), but their outputs were very low in the present experiment. It cannot be ruled out that other isomers, such as *trans*-7,*cis*-9-CLA, which increased in milk for the LINA and SUNA diets, may exert an antilipogenic effect, as recently suggested by studies in lactating mice (Kadegowda *et al.*, 2010). In addition other mechanisms may be involved in the MFD (Loor and Herbein, 2003) such as an alteration in the melting point of fat, although not measured in our study, due to the large increase in the sum of *trans*-18:1 isomers without increase in *cis*-9-C18:1 (Table 3).

The total secretion of C18, representing the major part of the milk long-chain FAs, was maintained in response to the addition for 10 weeks of PUFAs to the diet, whereas the secretion of DHA was increased (Table 3). These small variations in the milk long-chain FA were not related to variations in mammary *LPL* mRNA levels measured after 10 weeks

feeding period, even though a tendency for a decrease with the LINA compared to the SAT treatment was observed. This result concurs with studies in cows showing that mammary *LPL* mRNA levels were not significantly affected during MFD with high-concentrate diets (Peterson *et al.*, 2004) or in response to intravenous infusions of *trans*-10,*cis*-12-CLA as free FA (Gervais *et al.*, 2009); these treatments decreased the total secretion of C18. Conversely, other studies in cows have shown that the abundance of *LPL* mRNA in mammary tissue is decreased during MFD with diets containing FO (Ahnadi *et al.*, 2002) or a combination of FO and soybean oil (source of n-6 FA; Harvatine and Bauman, 2006) or in response to intravenous (Harvatine and Bauman, 2006) or abomasal infusion (Baumgard *et al.*, 2002) of supraphysiological doses of *trans*-10,*cis*-12-CLA; these treatments had either no effect or decreased secretion of long-chain FAs in milk. Altogether, these results showing variable effects of diet on the mammary secretion of long-chain FA and *LPL* expression suggest that factors such as substrate availability are important in the regulation of mammary long-chain FA metabolism in the bovine in line with previous studies in ruminant (Bernard *et al.*, 2008).

The decrease in *SCD1* mRNA levels in mammary tissue following the inclusion of plant oils and algae in the diet for 10 weeks is in line with previous studies with FO (Ahnadi *et al.*, 2002) or soybean oil (Jacobs *et al.*, 2011) in cows and formaldehyde-treated linseeds in goats (Bernard *et al.*, 2005a). Conversely, other studies in cows have shown that feeding them high-concentrate diets (Peterson *et al.*, 2004) or FO and soybean oil (Harvatine and Bauman, 2006, Invernizzi *et al.*, 2010) causing MFD are mostly not associated with altered *SCD1* mRNA levels or associated with an increase in *SCD1* mRNA (Invernizzi *et al.*, 2010). In these studies, both the absence of variation or the increase of *SCD1* mRNA expression were attributed to the role of *SCD1* in regulating the synthesis of milk triglycerides and maintaining milk fat fluidity via the endogenous synthesis of *cis*-9-C18:1 from C18:0 in the mammary gland (Loor and Herbein, 2003). The discrepancy between our results on SCD expression and those of Invernizzi *et al.* (2010) could be due to differences in the origin (algae *v.* FO) and doses (0.4% *v.* 1% DM) of oils used as well as to differences in the adaptation period to the treatments (10 *v.* 3 weeks). In the present experiment, the down-regulation of *SCD1* gene expression by the LINA and SUNA diets could be related to the observed increase of *trans*-10,*cis*-12-CLA in milk. Indeed, *in vivo* (Baumgard *et al.*, 2002; Gervais *et al.*, 2009) and *in vitro* (Peterson *et al.*, 2004; Kadegowda *et al.*, 2009) studies have demonstrated that *trans*-10,*cis*-12-CLA decreases *SCD1* transcription in the bovine although in most of them supraphysiological doses of this CLA isomer were used. The LINA and SUNA treatments also increased DHA levels, which as a member of the n-3 FAs, could be involved in the down-regulation of *SCD1* gene regulation in mammary (Ahnadi *et al.*, 2002; Bernard *et al.*, 2005a), muscle (Waters *et al.*, 2009) and adipose (Daniel *et al.*, 2004) tissues in ruminants. In addition, the combination of sources of n-3 (linseed oil) or n-6 (sunflower oil) FAs with a source of DHA (algae) altered rumen biohydrogenation, causing the production of specific intermediates that may affect *SCD1* expression. Thus, post-ruminal infusion studies in cows using *trans*-10,*trans*-12-CLA (Sæbø *et al.*, 2005) or *trans*-9,*trans*-11-CLA (Perfield et al., 2007) reduced milk fat delta-9 desaturase ratios, and therefore, these biohydrogenation intermediates would be expected to be potential down-regulators of *SCD1* gene expression and/or activity. In the present study, *trans*-10,*trans*-12-CLA and *trans*-9,*trans*-11-CLA yields were increased in milk for the SUNA and LINA treatments, respectively. Thus, the increase of these CLA intermediates in addition to *trans*-10,*cis*-12-CLA and DHA may contribute to mediate the down-regulation of *SCD1* gene expression from the LINA and SUNA treatments.

Additionally, the observed decrease in the *SCD1* mRNA level could be related to the decrease in the milk delta9-desaturase ratio of *cis*-9,*trans*-11-CLA (Table 3). Indeed, the strong increase of *trans*-11-18:1 with the LINA (by 131%) and SUNA (by 242%) diets compared to the SAT diet was not related to a significant increase in *cis*-9,*trans*-11-CLA, which may indicate a lower level of SCD activity for these treatments. However, variable effects were observed on the other milk delta9-desaturase ratios, with either no effect of the dietary treatment or an increase only for the SUNA compared to the SAT treatment for the C14 and C16 milk delta9-desaturase ratios. These data are in line with others in cows (Bionaz and Loor, 2008; Gervais *et al.*, 2009; Invernizzi *et al.*, 2010) supporting a doubt in the accuracy of desaturase indexes to infer activity and/or expression of SCD. Such discrepancies could be due to differences in other factors that may regulate milk fat desaturase ratios, such as differential uptake, turnover and utilisation of the pairs of FA in these ratios in mammary tissue in addition to variation in the accuracy of *cis*-9 containing FA determinations in milk. In addition, differences between mammary *SCD1* mRNA levels and the calculated milk desaturase ratios could be due to effects of the treatments at the protein level of SCD as described in goats fed with supplements rich in rich in 18:1n-9, 18:2n-6 and 18:3n-3 (Bernard *et al.*, 2005b and 2009b) in addition to effects at the transcription step.

This study is the first to characterise in cows fed a combination of plant and algae lipids the mammary expression of genes involved in different pathways of lipogenesis: the uptake of circulating FAs (*LPL*), *de novo* FA synthesis (*FASN*), delta9-desaturation of FAs (*SCD1*) and triglycerides synthesis (*GPAM*) and of a transcription factor (*SREBF1)* expressed in mammary tissue and reported to control the transcription of lipogenic genes in the bovine (Harvatine and Bauman, 2006; Bionaz and Loor, 2008). The results of the study also demonstrated significant positive associations between the expression of the following pairs of genes: *LPL* and *SCD1* ($r = +0.64$, $P = 0.01$; $Y = 0.1025X + 1.6585$), *FASN* and *SCD1* ($r = +0.64$, $P = 0.01$; $Y = 0.5527X + 165.05$) and *GPAM* and *SCD1* ($r = +0.66$, $P = 0.01$; $Y = 0.1454X + 47.444$) suggesting similar regulation for these pair genes. A joint decrease in the mammary expression of *SREBF1*, *FASN* and *SCD1* was observed for the LINA and SUNA treatments that could be related to MFD. These results are in line with others

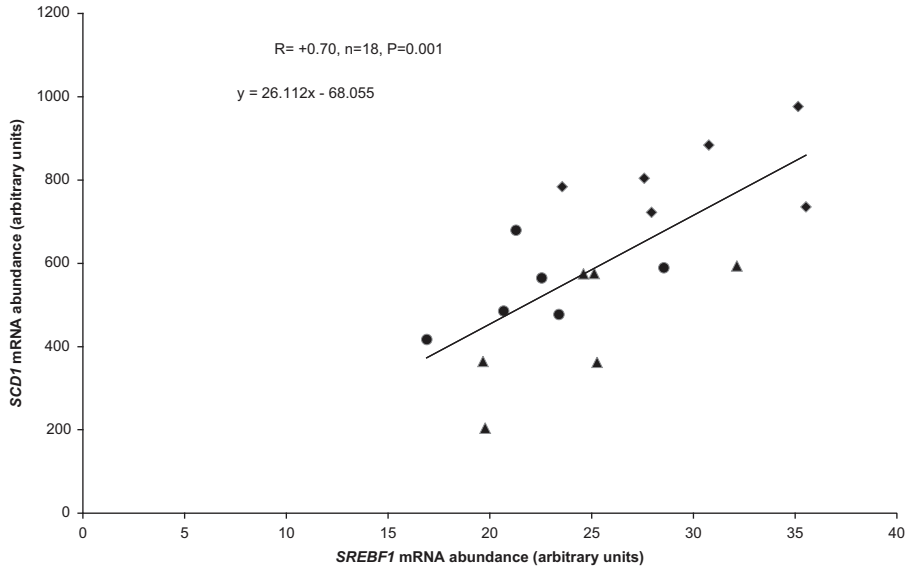

Figure 1 Relationship between mRNA abundances of stearoyl-CoA desaturase (SCD1) and sterol regulatory element binding transcription factor 1 (SREBF1) in mammary tissue of cows fed diets supplemented with rumen-stable fractionated palm fat (SAT, ■), or a combination of linseed oil and algae (LINA, ▲), or a combination of sunflower oil and algae (SUNA, ●). The mRNA abundances are expressed in arbitrary units (AU) determined as abundance relative to the geometric mean of 3 reference genes (PPIA, EIF3K and UXT).

(Harvatine and Bauman, 2006) demonstrating a joint decrease in the mammary expression of *SREBF1* and lipogenic gene expression together with MFD in lactating cows that were fed a low-forage/high-oil diet or one infused with *trans*-10,*cis*-12-CLA.

A positive inter-individual relationship between *SCD1* and one of its putative transcription factors, *SREBF1* ($r = +0.70$, $P = 0.01$; Figure 1), was observed and suggests that, in our dietary conditions, either the regulation of these two genes may be similar or that *SREBF1* is transcriptionally regulated and in turn regulates *SCD1* gene expression. In line with the first hypothesis, it was recently reported that the bovine *SREBF1* gene contains in its promoter responsive elements for both *SREBF1* (SRE) that could allow for its positive feedback regulation, and for liver X receptor (LXR-RE; Lengi and Corl, 2010). These two responsive elements (SRE, LXR-RE) have been also characterised in *SCD1* gene promoter in human, mouse or chicken (Mauvoisin and Mounier, 2011) and are conserved between species suggesting that they may be present in bovine *SCD1* gene promoter.

Elsewhere, a similar positive relationship between *SCD1* and *SREBF1* gene expression was observed in the muscle tissue of beef cattle supplemented with dietary n-3 PUFAs (Waters *et al.*, 2009). We suggest that in the bovine mammary gland, as observed in muscle, the negative effect of dietary PUFAs on *SCD1* mRNA expression could be mediated at least in part by reduced *SREBF1* gene expression. In addition, we hypothesise that the *SREBF1* down-regulation observed in the LINA and SUNA treatments is mediated by the observed increase in milk *trans*-10,*cis*-12-CLA and long-chain FA of the n-3 family. Because the SREBF1 protein is regulated at both the levels of transcription and protein maturation (Eberlé *et al.*, 2004), different effects of PUFAs and their rumen intermediates on *SREBF1* gene transcription or on the proteolytic activation process of the SREBF1 protein may be observed (Peterson *et al.*, 2004; Harvatine and Bauman, 2006).

In addition to *SREBF1*, other proteins such as sterol regulatory element-binding protein cleavage-activating protein (SCAP) or transcription factors such as peroxisome proliferative activated receptor, gamma (PPRG, as proposed by Bionaz and Loor, 2008), and LXR (as discussed above), which are also expressed in mammary tissues (Sundvold *et al.*, 1997; Kadegowda *et al.*, 2009; Invernizzi *et al.*, 2010; McFadden and Corl., 2010), may contribute to the observed regulation of lipogenic gene expression.

Concordance between mRNAs from milk purified MEC and mammary tissue

There was a lack of an effect of polyunsaturated lipid supplementation on the mRNA levels of lipogenic enzymes using milk purified MECs. In addition, our study did not uncover any relationship between the response to the dietary treatments of the mRNA levels of lipogenic enzymes from mammary tissue and from milk purified MECs, whereas previous studies demonstrated that gene expression in milk cells reflects what occurs in the mammary gland (Boutinaud *et al.*, 2002; Murrieta *et al.*, 2006). From these results it is tempting to conclude that milk purified MECs are not a valuable source of mRNA for the analysis of lipogenic gene expression in response to lipid supplements. In our study, supplementation with PUFAs and DHA resulted in a tendency for reducing RNA quality in milk cells compared to rumen-stable fractionated palm fat. According to Fleige and Pfaffl (2006), qRT-PCR performance is affected by the RNA integrity. Thus, we believe that the type of lipid supplementation used in our study impact the milk purified MEC RNA quality that may be responsible for the discrepancy between gene expression results in mammary tissue *v.* milk epithelial cells. Additionally, the number of

obtained milk purified MECs per millilitre had high variability in all treatments (CV = 20–48%), and the numbers in some samples were very low resulting in very low extracted RNA. The high variation of the milk purified MEC population and its RNA quality could also partly explain the absence of concordance between gene expression in the mammary tissue and in milk purified MECs. Moreover, we hypothesise that milk purified MEC population is modified by the nature of the lipid supplements, as suggested by a study showing that linseed oil decreases mammary cell proliferation and increases apoptosis in breast cancer (Saggar *et al*., 2010). In order to test this hypothesis we analysed the gene expression of a pro apoptotic gene Bax. However, we did not observe any modification of Bax mRNA abundance neither in milk purified MEC nor in mammary tissue (data not shown). A better indicator of changes in cell proliferation and apoptosis would have been immunohistochemical analyses (Boutinaud *et al*., 2012), but mammary tissue sections could not be prepared in the current study. In our study, linseed oil plus algae increased the loss of MEC in the milk, but the differences were not significant (49% higher than SAT and 48% higher than SUNA). Thus, we suggest that it is important to consider the population of epithelial cells recovered from milk and its RNA quality before using these cells as a source of mammary transcripts. Further studies should be carried out to improve the collection conditions and the preparation of cell extracts for the use of these cells as a non-invasive tool under all treatment conditions.

Conclusions

This study showed that the addition of plant oils and algae for 10 weeks to the diet of lactating cows induced a MFD related to a joint down-regulation in the mammary gland of lipogenic gene expression (SCD1, *FASN*) and of the *SREBF1* transcription factor expression. In addition, a positive relationship between the expression of *SCD1* and *SREBF1* was observed, suggesting a possible similarity in the regulation of these two genes. We propose that the effect of dietary PUFAs on *SCD1* mRNA expression could be mediated at least in part by the reduction of *SREBF1* gene expression, with the hypothesis that *SREBF1* down-regulation is mediated by *trans*-10,*cis*-12-CLA and long-chain FA of the n-3 family. In addition to the early adaptation of the transcriptome reported in response to lipid supplements (Invernizzi *et al*., 2010), our results demonstrate a long-term regulation of lipogenic gene expression and milk fat synthesis in the bovine mammary gland supporting the implication of SREBF1. The observed effects of dietary PUFAs on milk FA secretion and mammary lipogenic gene expression were associated to milk *trans*-10,*cis*-12-CLA and DHA. In addition, probably that other mechanisms or biohydrogenation intermediates could be involved as well, such as C18:1-*trans*-FA or *trans*-7,*cis*-9-CLA, but further experiments will be required. Moreover, this study provides information supporting the type of lipid supplements that may affect the milk purified MEC population and RNA quality, which are important factors to consider in the future for the analysis of gene expression using milk cells.

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References

AbuGhazaleh AA, Schingoethe DJ, Hippen AR and Kalscheur KF 2004. Conjugated linoleic acid increases in milk when cows fed fish meal and extruded soybeans for an extended period of time. Journal of Dairy Science 87, 1758–1766.

AbuGhazaleh AA, Potu RB and Ibrahim S 2009. Short communication: the effect of substituting fish oil in dairy cow diets with docosahexaenoic acid-micro algae on milk composition and fatty acids profile. Journal of Dairy Science 92, 6156–6159.

Ahnadi CE, Beswick N, Delbecchi L, Kennelly JJ and Lacasse P 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. Journal of Dairy Research 69, 521–531.

Alcorn J, Lu X, Moscow JA and McNamara PJ 2002. Transporter gene expression in lactating and nonlactating human mammary epithelial cells using real-time reverse transcriptionpolymerase chain reaction. Journal of Pharmacology and Experimental Therapeutics 303, 487–496.

Allen MS 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83, 1598–1624.

Association of Official Analytical Chemists (AOAC) 1997. Official methods of analysis, 16th edition. AOAC, Gaithersburg, MD, USA.

Bauman DE and Griinari JM 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Production Science 70, 15–29.

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA and Bauman DE 2002. trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. Journal of Dairy Science 85, 2155–2163.

Bernard L, Leroux C and Chilliard Y 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. In Bioactive components of milk, advances in experimental biology and medicine, vol. 606 (ed. Z Bösze), pp. 67–108. Springer, New York, USA.

Bernard L, Bonnet M, Leroux C, Shingfield KJ and Chilliard Y 2009a. Effect of sunflower-seed oil and linseed oil on tissue lipid metabolism, gene expression and milk fatty acid secretion in Alpine goats fed maize silage based diets. Journal of Dairy Science 92, 6083–6094.

Bernard L, Leroux C, Faulconnier Y, Durand D, Shingfield KJ and Chilliard Y 2009b. Effect of sunflower-seed oil or linseed oil on milk fatty acid secretion and lipogenic gene expression in goats fed hay-based diets. Journal of Dairy Research 76, 241–248.

Bernard L, Leroux C, Rouel J, Bonnet M and Chilliard Y 2011. Effect of the level and type of starchy concentrate on tissue lipid metabolism, gene expression and milk fatty acid secretion in Alpine goats receiving a diet rich in sunflower-seed oil. British Journal of Nutrition (In press), doi: 10.1017/S0007114511004181.

Bernard L, Rouel J, Leroux C, Ferlay A, Faulconnier Y, Legrand P and Chilliard Y 2005a. Mammary lipid metabolism and milk fatty acid secretion in alpine goats fed vegetable lipids. Journal of Dairy Science 88, 1478–1489.

Bernard L, Leroux C, Bonnet M, Rouel J, Martin P and Chilliard Y 2005b. Expression and nutritional regulation of lipogenic genes in mammary gland and adipose tissues of lactating goats. Journal of Dairy Research 72, 250–255.

Bionaz M and Loor JJ 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. Biomedical Central Genomics 9, 366–387.

Boeckaert C, Vlaeminck B, Dijkstra J, Issa-Zacharia A, Van Nespen T, Van Straalen W and Fievez V 2008. Effect of dietary starch or micro alga supplementation on rumen fermentation and milk fatty acid composition of dairy cows. Journal of Dairy Science 91, 4714–4727.

Boutinaud M, Ben Chedly MH, Delamaire E and Guinard-Flament J 2008. Milking and feed restriction regulate transcripts of mammary epithelial cells purified from milk. Journal of Dairy Science 91, 988–998.

Boutinaud M, Rulquin H, Keisler DH, Djiane J and Jammes H 2002. Use of somatic cells from goat milk for dynamic studies of gene expression in the mammary gland. Journal of Dairy Science 80, 1258–1269.

Boutinaud M, Lollivier V, Finot L, Bruckmaier RM and Lacasse P 2012. Mammary cell activity and turnover in dairy cows treated with the prolactin-release inhibitor quinagolide and milked once daily. Journal of Dairy Science 95 (1), 177–187.

Chilliard Y, Ferlay A and Doreau M 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livestock Production Science 70, 31–48.

Cruz-Hernandez C, Kramer KG, Kennelly JJ, Glimm DR, Sorensen BM, Okine EK, Goonewardene LA and and Weselake RJ 2007. Evaluating the conjugated linoleic acid and trans 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. Journal of Dairy Science 90, 3786–3801.

Daniel ZC, Wynn RJ, Salter AM and Buttery PJ 2004. Differing effects of forage and concentrate diets on the oleic acid and conjugated linoleic acid content of sheep tissues: the role of stearoyl-CoA desaturase. Journal of Animal Science 82, 747–758.

Duske K, Hammon HM, Langhof AK, Bellmann O and Losand B 2009. Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. Journal of Dairy Science 92, 1670–1684.

Eberlé D, Hegarty B, Bossard P, Ferre P and Foufelle F 2004. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86, 839–848.

Feng S, Salter AM, Parr T and Garnsworthy PC 2007. Extraction and quantitative analysis of stearoyl-CoA desaturase mRNA from dairy cow milk somatic cells. Journal of Dairy Science 90, 4128–4136.

Fleige S and Pfaffl MW 2006. RNA integrity and the effect on the real-time qRT-PCR performance. Molecular Aspects of Medicine 27, 126–139.

Gervais R, McFadden JW, Lengi AJ, Corl BA and Chouniard PY 2009. Effects of intravenous infusion of trans-10, cis-12 18:2 on mammary lipid metabolism in lactating dairy cows. Journal of Dairy Science 92, 5167–5177.

Glasser F, Doreau M, Ferlay A and Chilliard Y 2007. Technical note: estimation of milk fatty acid yield from milk fat data. Journal of Dairy Science 90, 2302–2304.

Harvatine KJ and Bauman DE 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. Journal of Nutrition 136, 2468–2474.

Invernizzi G, Thering BJ, McGuire MA, Savoini G and Loor JJ 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Functional Integrative Genomics 10, 561–575.

Jacobs AAA, Van Baal J, Smits MA, Taweel HZH, Hendriks WH, Van Vuuren AM and Dijkstra J 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. Journal of Dairy Science 94, 874–887.

Kadegowda AKG, Bionaz M, Piperova LS, Erdman RA and Loor JJ 2009. Peroxisome proliferator-activated receptor-gamma activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. Journal of Dairy Science 92, 4276–4289.

Kadegowda AK, Connor EE, Teter BB, Sampugna J, Delmonte P, Piperova LS and Erdman RA 2010. Dietary trans fatty acid isomers differ in their effects on mammary lipid metabolism as well as lipogenic gene expression in lactating mice. Journal of Nutrition 140, 919–924.

Lawson RE, Moss AR and Givens DI 2001. The role of dairy products in supplying conjugated linoleic acid to man's diet: a review. Nutrition Research Reviews 14, 153–172.

Lengi AJ and Corl BA 2010. Short communication: identification of the bovine sterol regulatory element binding protein-1c promoter and its activation by liver X receptor. Journal of Dairy Science 93, 5831–5836.

Loor JJ and Herbein JH 2003. Reduced fatty acid synthesis and desaturation due to exogenous trans10,cis12-CLA in cows fed oleic or linoleic oil. Journal of Dairy Science 86, 1354–1369.

Mauvoisin D and Mounier C 2011. Hormonal and nutritional regulation of SCD1 gene expression. Biochimie 93, 78–86.

McFadden JW and Corl BA 2010. Activation of liver X receptor (LXR) enhances de novo fatty acid synthesis in bovine mammary epithelial cells. Journal of Dairy Science 93, 4651–4658.

Murrieta CM, Hess BW, Scholljegerdes EJ, Engle TE, Hossner KL, Moss GE and Rule DC 2006. Evaluation of milk somatic cells as a source of mRNA for study of lipogenesis in the mammary gland of lactating beef cows supplemented with dietary high-linoleate safflower seeds. Journal of Animal Science 84, 2399–2405.

Offer NW, Marsden M, Dixon J, Speake BK and Thacker FE 1999. Effect of dietary fat supplements on levels of n-3 poly-unsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk. Journal of Animal Science 69, 613–625.

Palmquist DL and Griinari JM 2006. Milk fatty acid composition in response to reciprocal combinations of sunflower and fish oils in the diet. Animal Feed Science and Technology 131, 358–369.

Parodi PW 2005. Dairy product consumption and the risk of breast cancer. Journal of American College of Nutrition 24, 556S–568S.

Perfield II JW, Lock AL, Griinari JM, Sæbø A, Delmonte P, Dwyer DA and Bauman DE 2007. Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. Journal of Dairy Science 90, 2211–2218.

Peterson DG, Matitashvili EA and Bauman DE 2004. The inhibitory effect of trans-10, cis-12 CLA on lipid synthesis in bovine mammary epithelial cells involves reduced proteolytic activation of the transcription factor SREBP-1. Journal of Nutrition 134, 2523–2527.

Piperova LS, Teter BB, Bruckental I, Sampugna J, Mills SE, Yurawecz MP, Fritsche J, Ku K and Erdman RA 2000. Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. Journal of Nutrition 130, 2568–2574.

Sæbø A, Sabo PC, Griinari JM and Shingfield KJ 2005. Effect of abomasal infusions of geometric isomers of 10,12 conjugated linoleic acid on milk fat synthesis in dairy cows. Lipids 40, 823–832.

Saggar JK, Chen J, Corey P and Thompson LU 2010. The effect of secoisolariciresinol diglucoside and flaxseed oil, alone and in combination, on MCF-7 tumor growth and signaling pathways. Nutrition and Cancer 62, 533–542.

SAS Institute Inc 2009. SAS/STAT_R 9.2, user's guide, 2nd edition. SAS Institute Inc., Cary, NC, USA.

Shen X, Dannenberger D, Nuernberg K, Nuernberg G and Zhao R 2011. Trans-18:1 and CLA isomers in rumen and duodenal digesta of bulls fed n-3 and n-6 PUFA-based diets. Lipids 46, 831–841.

Shingfield KJ, Bernard L, Leroux C and Chilliard Y 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4, 1140–1166.

Shingfield KJ, Sæbø A, Saeb PC, Toivonen V and Griinari JM 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. Journal of Dairy Science 92, 4317–4329.

Shingfield KJ, Reynolds CK, Hervas G, Griinari JM, Grandison AS and Beever DE 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. Journal of Dairy Science 89, 714–732.

Shingfield KJ, Reynolds CK, Lupoli B, Toivonen V, Yurawecz MP, Delmonte P, Griinari JM, Grandison AS and Beever DE 2005. Effect of forage type and proportion of concentrate in diet on milk fatty acid composition in cows fed sunflower oil and fish oil. Journal of Animal Science 80, 225–238.

Sundvold H, Brzozowska A and Lein S 1997. Characterization of bovine peroxisomse proliferator-activated receptors γ1 and γ2: genetic mapping differential expression of the two isoforms. Biochemical and Biophysical Research Communications 239, 857–861.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A and Speleman F 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3, 1–12.

Wahle KW, Heys SD and Rotondo D 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Progress in Lipid Research 43, 553–587.

Waters SM, Kelly JP, O'Boyle P, Moloney AP and Kenny DA 2009. Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Δ9-desaturase in muscle of beef cattle. Journal of Animal Science 87, 244–252.

Whitlock L, Schingoethe DJ, Hippen AR, Kalscheur KF, Baer RJ, Ramaswamy N and Kasperson KM 2002. Fish oil and extruded soybeans fed in combination increase CLA in milk of dairy cows more than when fed separately. Journal of Dairy Science 85, 234–243.