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Distribution of conjugated linoleic acid (CLA) isomers and other fatty acids in polar and neutral fractions of milk from cows fed different lipid supplements¤

Distribución de isómeros de ácido linoléico conjugado (CLA) y ácidos grasos en las fracciones neutra y polar de la leche de vacas alimentadas con diferentes suplementos lipídicos

Distribuição de isômeros do acido linoleico conjugado (CLA) e outros ácidos graxos nas frações polares e neutras no leite de vacas alimentadas com diferentes suplementos lipídicos

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Summary

Background: milk fatty acid composition has become very important for consumers due to the positive relationship that has been found between some fatty acids and human health. In recent years, content and fatty acid composition in the different fractions of milk lipids has also become important due to reported potential technological and sensory implications. **Objective:** the aim of this study was to evaluate the effect of dietary supplementation with several lipid supplements on the proportion of neutral (triglycerides) and polar lipids (phospholipids) of milk fat, alterations of the fatty acid composition and Conjugated Linolenic Acid isomers (CLA) of total milk lipids. **Methods**: 18 first lactation German Holstein cows were assigned to three dietary treatments for 10 weeks as follows: Rumen-stable fractionated palm fat, linseed oil plus algae, and sunflower oil plus algae. **Results:** dietary polyunsaturated fat supplements increased the proportion of phospholipids and decreased triglycerides in milk fat compared to Rumen-stable fractionated palm fat. Long chain polyunsaturated fatty acids were preferentially deposited into phospholipids. Diet effect was more pronounced in triglycerides than in phospholipids. Plant oil/algae supplemented diets induced lower proportions of total saturated fatty acids and higher proportions of total unsaturated fatty acids in triglycerides. **Conclusions:** linseed oil plus algae feeding generated the best results in reference to fatty acids related to human health. Sunflower oil plus algae caused accumulation of CLAtrans-10,cis-12, CLAtrans-7,cis-9, CLAtrans-7,trans-9

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and CLAtrans-10,trans-12 and decrease of CLAtrans-9,trans-11 in total milk fat, whereas linseed oil plus algae increased CLAtrans-12,trans-14, CLAtrans-11,trans-13, and CLAtrans-11,cis-13 deposition compared to rumen-stable fractionated palm fat.

Key words: *algae, linseed oil, phospholipids, sunflower oil, triglycerides.*

Resumen

Antecedentes: la composición de ácidos grasos de la leche ha tomado gran importancia para los consumidores a causa de la relación positiva que se ha encontrado entre algunos ácidos grasos y la salud humana. En los últimos años, el contenido y composición de ácidos grasos en las diferentes fracciones de los lípidos de la leche también ha tomado importancia debido a las posibles implicaciones tecnológicas y sensoriales que se han reportado. **Objetivo:** el objetivo del presente estudio fue evaluar los cambios en la proporción de lípidos neutros (triglicéridos) y lípidos polares (fosfolípidos) y alteraciones de la composición de ácidos grasos e isómeros del Ácido Linoléico Conjugado (CLA) del total de lípidos de la leche como resultado de la suplementación dietaria con diferentes suplementos lipídicos. **Métodos:** 18 vacas Holstein Alemán de primera lactancia fueron asignadas a tres tratamientos dietarios durante 10 semanas, así: grasa de palma fraccionada y estable al rumen, aceite de lino más alga, y aceite de girasol más alga. **Resultados:** la suplementación con ácidos grasos poliinsaturados incrementó la proporción de fosfolípidos y disminuyó la de triglicéridos en la grasa de la leche comparado con grasa de palma fraccionada y estable al rumen. Los ácidos grasos poliinsaturados de cadena larga fueron preferencialmente depositados en los fosfolípidos. El efecto de la dieta fue más pronunciado en los triglicéridos que en los fosfolípidos. Las dietas suplementadas con aceite vegetal y alga indujeron a menores proporciones de ácidos grasos saturados y mayores proporciones de ácidos grasos insaturados en los triglicéridos. **Conclusiones:** la suplementación con aceite de lino más alga generó los mejores resultados respecto a la composición de ácidos grasos relacionados con la salud humana. El aceite de girasol más alga causó una acumulación de isomeros CLAtrans-10,cis-12, CLAtrans-7,cis-9, CLAtrans-7,trans-9 y CLAtrans-10,trans-12 y disminuyó CLAtrans-9,trans-11 en la grasa total de la leche mientras que el aceite de lino más alga incrementó CLAtrans-12,trans-14, CLAtrans-11,trans-13, y CLAtrans-11,cis-13 comparado con la grasa de palma fraccionada y estable al rumen.

Palabras clave: *aceite de girasol, aceite de linaza, algas, fosfolípidos, triglicéridos.*

Resumo

Antecedentes: a composição de ácidos graxos do leite tem-se tornado muito importante para os consumidores devido à relação positiva encontrada entre alguns ácidos graxos e a saúde humana. Nos últimos anos, o conteúdo e composição dos ácidos graxos nas diferentes frações lipídicas no leite também se tornaram importantes devido às consequências tecnológicas e sensoriais que tem sido reportada recentemente. **Objetivo**: realizou-se um estudo para avaliar as mudanças na proporção de lipídios neutros (triglicerídeos) e polares (fosfolipídios) na gordura do leite e as alterações da composição dos ácidos graxos e os isômeros do Ácido Linoleico Conjugado (CLA) no total dos lipídeos do leite como resultado da suplementação na dieta com diferentes suplementos lipídeos. **Métodos:** 18 vacas Holandesas na Alemanha, todas de primeira lactação foram avaliadas em três tratamentos com regimes alimentares diferentes durante 10 semanas, assim: óleo de palma fracionada e estável no rúmen, óleo de linhaça mais algas e óleo de girassol mais algas. **Resultados:** a suplementação com ácidos graxos poli-insaturados incrementou a proporção de fosfolipídios e diminuiu a proporção de triglicerídeos na gordura do leite, comparado com o óleo de palma fracionada e estável no rúmen. Os ácidos graxos poliinsaturados de cadeia longa foram preferencialmente depositados nos fosfolipídios. O efeito da dieta foi mais pronunciado nos triglicerídeos que nos fosfolipídios. As dietas que foram suplementadas com óleo vegetal mais algas reduziram a proporção de ácidos graxos saturados, e aumentaram a proporção de ácidos graxos insaturados nos triglicerídeos. **Conclusões:** a suplementação com óleo de linhaça mais alga gerou os melhores resultados respeito à composição dos ácidos graxos relacionados com a saúde humana. O óleo de girassol mais alga causaram uma acumulação de CLAtrans-10, cis-12, CLAtrans-7, cis-9, CLAtrans-7, trans-9 e CLAtrans-10, trans-12 e diminuiu CLAtrans-9, trans-11 na gordura total do leite, enquanto que óleo de linhaça mais alga aumentou a deposição de CLAtrans-12, trans-14, CLAtrans-11, trans-13, e CLAtrans-11, cis-13, em comparação com o óleo de palma fracionada e estável no rúmen.

Palavras chave: *algas, fosfolipídios, óleo de girassol, óleo de linhaça, triglicerídeos.*

Introduction

There is a recent trend toward increased consumption of milk products with a higher content of healthier fatty acids (FA), such as linolenic and docosahexaenoic acid, associated with reduced cardiovascular problems and mortality (Palmquist, 2009); rumenic acid (*cis*-9, *trans*-11, CLA), shown to have anticancerogenic properties in animal models (Wahle *et al*., 2004); and *trans*-vaccenic acid (C18:1*trans*-11, TVA), the main precursor of rumenic acid, which is synthesized from TVA in humans (Adolf *et al.,* 2000). Most research has focused on two isomers, rumenic acid and CLA*trans*-10, *cis*-12 (Khanal and Dhiman, 2004). Studies on pure single isomers showed that the biological activities of such isomers are different (Banni *et al*., 2001; Martin and Valeille, 2002). Therefore, it is important to separate the individual CLA isomers (Dannenberger *et al*., 2004). Likewise, the decrease in total saturated fatty acids (SFA) has also been considered important because of its negative effects on cholesterol levels (Parodi, 2005). Dietary plant oil supplementation has an enhancing effect on the levels of milk rumenic acid and TVA (Ollier *et al*., 2009) being the highest levels accumulated when vegetable oils were used together with fish oil (Abu-Ghazaleh and Holmes, 2007; Cruz-Hernandez *et al.,* 2007).

Most studies have focused on dietary effects upon FA composition of total bovine milk fat (Shingfield *et al.,* 2006; Mahecha *et al.,* 2008). However, few studies have evaluated these effects in different milk fat lipid fractions (Smith *et al.,* 1977; López *et al.,* 2008). Bovine milk lipids are largely composed of triglycerides (TG) and minor amounts of diacylglycerols (DG), monoacylglycerols, free FA (FFA), phospholipids (PL), and sterols (Jensen, 2002). Altering the proportion of the milk lipid fractions (Russell *et al*., 2010) as well as their FA composition (Vanderghem *et al*., 2010) is of great importance because of health, technology, and sensory consequences. The FA characteristics and distribution in the lipid fractions influence physical properties of milk fat, thereby altering its melting point (Chilliard and Ferlay, 2004).

The present study evaluated the effects of feeding different lipid supplements on the proportion of milk TG and PL, on the FA composition of both fractions, and on CLA isomer distribution of total milk fat.

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 mid-lactation primiparous German Holstein dairy cows, averaging 91.6 days in milk (DIM) (standard error of the mean: 7.8 days), were divided into three groups and offered one of the following experimental diets for 10 weeks: rumen-stable fractionated palm fat (SAT), linseed oil plus algae (LINA), and sunflower oil plus algae (SUNA). SAT 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 (SAT; Bergafat T-300, Dr. Pieper Technologie, 16818, Wuthenow, Germany; 3.1% basal diet dry matter [DM]).

The differentiator of SAT among the other two dietary treatments was the type of lipid supplementation used for the concentrate mixture. LINA included a combination of linseed oil (Dörnthaler Öhlmühle, Dörnthal, Germany; 2.7% basal diet DM) and alga (Docosahexaenoic acid, DHA; Gold, Novus Europe, Brussels, Belgium; 0.4% basal diet DM) while SUNA included a combination of sunflower oil (Teutoburger Ölmühle, Ibbenbüren, Germany; 2.7% basal diet DM) and algae (DHA Gold, Novus Europe, Brussels, Belgium; 0.4% basal diet DM). Data on ingredients and nutritional composition of diets were previously published by Angulo *et al.* (2012a, b) and are shown in table 1. Animals (first lactation; 92 days in milk) were randomly assigned to three groups of six cows. Milk samples were collected from morning milking on the last day of the experimental period to determine TG and PL for analysis of fatty acid composition and the CLA isomer HPLC. Samples were analyzed in triplicate.

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

	Treatment [1]		
Ingredients, % of DM	**SAT**	**LINA**	**SUNA**
Grass silage	17.50	17.30	17.50
Maize silage	35.30	35.40	35.30
Hay	2.00	2.00	2.00
Concentrate[2]	41.50	41.50	41.50
Bergafat T-300[3]	3.10	-	-
Linseed oil[4]+algae DHA gold[5]	-	3.10	-
Sunflower oil[6]+algae DHA gold	-	-	3.10
Minerals[7]	0.70	0.70	0.70
Nutrient composition (% of DM)			
Crude protein	16.60	16.60	16.60
Crude fiber	13.50	13.50	13.50
Crude fat	6.24	6.24	6.26
Net energy (MJ/kg DM)	7.38	7.37	7.37
Fatty acid intake (g/d)			
C14:0	162.7^a	122.0^b	136.9^b
C16:0	6510.8^a	2348.6^b	2678.4^b
C18:0	1025.6^a	455.9^b	533.6^b
C18:2n-6	5535.3^b	5909.0^b	9756.6^a
C18:3n-3	2265.6^b	5525.0^a	2494.4^b
C22:6n-3	0.0^b	84.0^a	90.6^a

[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]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.

[3]Bergafat T-300 – rumen-stable fractionated palm fat (TG) contained (g/100 g of fatty acids) C14:0 (1.4), C16:0 (71.2), C18:0 (11.0), cis-9-C18:1 (12.8), C18:2n-6 (3.2).

[4]Linseed oil contained (g/100 g of fatty acids) 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).

[5]DHA-rich algae contained (g/100 g of fatty acids) C14:0 (12.0), C16:0 (27.7), C18:0 (0.6), C18:1cis-9 (0.1), C18:2n-6 (0.04), C20:5n-3 (1.4), C22:5n-3 (0.3), and C22:6n-3 (53.1).

[6]Sunflower-seed oil contained (g/100 g of fatty acids) C14:0 (0.07), C16:0 (6.0), C18:0 (1.8), C18:1cis-9 (25.2), C18:2n-6 (64.1), and C18:3n-3 (0.09).

[7]Mineral feed mixture (Salvana 9522 declared as containing 92% ashes, 20% Calcium, 5% Phosphorous, 8% Sodium, 6% Magnesium).

Separation of lipid classes and fatty acid analyses

TG and PL of the extracted milk fat were separated using thin layer chromatography (TLC). Fat extraction was previously described by Duske *et al.* (2009). TLC pre-coated silica gel SIL G-25 plates (20x20 cm, Macherey-Nagel, Düren, Germany) were pre-washed using the solvent mixture *n*-hexane/diethyl ether/acetic acid (70:30:2, v/v/v) and dried at 110 ºC for 90 min before use. Lipid extracts and a standard solution (25 mg TLC standard 18 - 5C [Nu-Check Prep Inc., Elysian, MN, USA] /10 mL chloroform/methanol 2:1 v/v) were automatically and rapidly deposited on the plates using the CAMAG Automatic TLC Sampler ATS 4 (CAMAG, Berlin, Germany). After sample application the plates were placed in a chromatography glass chamber containing about 50 mL of an *n*-hexane/diethyl ether/acetic acid (70:30:2, v/v/v) mixture. The migration time was close to 1 h until the solvent ascended to 1 cm below the top of the plate. After plate-drying all lipid classes were viewed under ultraviolet (UV) light after being sprayed with a solution of 0.1 % 2.7-dichlorofluorescein and the lipid fractions were scraped off. Samples were stored at -20 ºC for fatty acid analyses as described by Angulo *et al*. (2011).

Milk CLA isomer analyses

Identification and quantification analyses of the CLA isomers in milk fat extracts were performed by Ag^+-HPLC and involved an HPLC system (LC 10A, Shimadzu, Japan) equipped with a pump (LC-10AD VP), autosampler (SIL-10AF), 50 µL injection loop, a photodiode array detector (SPD-M 10Avp, Shimadzu, Japan), and a 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, Santa Clara, 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 the integrated area under the 233 nm peaks attributed to conjugated dienes. The identification and calibration of CLA isomers was made using the retention time of individual CLA methyl esters (CLA*cis*-9,*trans*-11, CLA*trans*-9,*trans*-11, CLA*trans*-10,*cis*-12, CLA*cis*-9,*cis*-11 and CLA*cis*-11,*trans*-13) at different levels.

Statistical analysis

The following model for the analysis of variance with the fixed factor diet (D) was used:

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

μ= overall mean

D_i= diet effect (i=3)

E_{ij}= residual error

All of the data were analyzed by the least-squares mean method using the GLM procedures of SAS® 2009. Treatment means were compared using the least-squares mean procedure (SAS Institute) with the significance level set at $p \leq 0.05$.

Results

In this study the effect of diet on milk PL and TG proportions was evaluated. It was demonstrated that supplementation of lactating cows with *n*-3 FA plus DHA-rich algae (LINA) tended to increase ($p=0.08$) the proportion of PL and decreased significantly the proportion of TG compared to SAT ($p \leq 0.05$). Supplementing lactating cows with a diet composed of *n*-6 FA plus DHA-rich algae (SUNA) significantly increased the proportion of PL ($p \leq 0.05$) and did not significantly affect the proportion of TG compared to SAT (Figure 1).

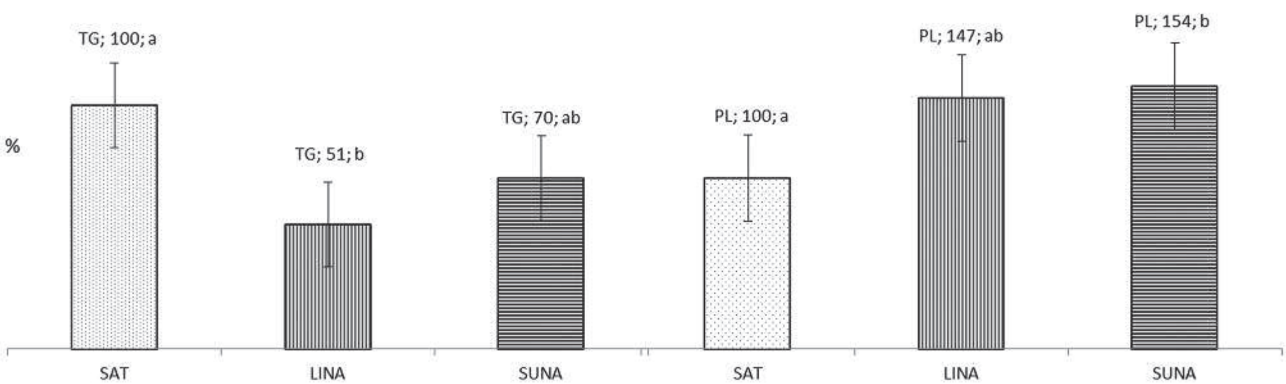

Figure 1. Percent change of triglycerides (TG) and phospholipids (PL) in milk fat from cows supplemented with rumen-stable fractionated palm fat (SAT), or a combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA). Different letters between columns for TG or PL indicate significant differences between treatments at $p \leq 0.05$.

This study also analyzed the distribution of FA in milk TG and PL. PUFA were preferentially deposited into PL (17%) rather than into TG (5%). PUFA of PL were composed mainly of long chain PUFA (LCPUFA, 8.7%) whereas they comprised only 0.34% of the total PUFA in TG. Linoleic acid and DHA were some of the LCPUFA more incorporated into PL than into TG. Oleic acid was incorporated in similar proportions into TG and PL (on average 24.24% and 24.22%, respectively) being the most abundant fatty acid in PL and the second most abundant in TG. Linolenic acid and CLA*cis*-9,*trans*-11 were on average also incorporated in similar proportions into TG and PL (Linolenic acid 0.7% and 0.6%, respectively, while CLA*cis*-9, *trans*-11 1.1 and 1.0%, respectively) while TVA was incorporated on average as 4.29% of the total FA into TG and as 1.43% of the total FA into PL.

The relative amount of FA in TG and PL is shown in table 2. Linolenic acid and TVA were significantly increased by LINA and SUNA, respectively, compared to SAT in both TG and PL. The increase observed in TVA SUNA did not parallel changes in CLA*cis*-9,*trans*-11. There was a tendency for DHA to increase ($p=0.08$) in the milk of cows fed with LINA in TG but not in PL. Both PUFA diets decreased the proportion of SFA in TG but not in PL (Figure 2), and only LINA increased total unsaturated fatty acids (UFA) in TG.

Table 2. Selected fatty acids in milk lipid fractions from cows fed different lipid supplements.

	TRIGLYCERIDES (TG) g/100 g FA			PHOSPHOLIPIDS (PL) g/100 g FA		
	SAT	LINA	SUNA	SAT	LINA	SUNA
C18:0	9.00	10.34	10.11	9.78	10.18	13.02
C18:1*trans*-11	2.87[a]	4.23[a]	5.76[b]	1.08[a]	1.49[a]	1.72[b]
C18:1*cis*-9	21.14[a]	27.04[b]	24.54[ab]	23.98	24.92	23.75
C18:2*n*-6	2.60[ab]	2.11[a]	3.28[b]	5.99	5.45	6.58
C18:2CLA*cis*-9,*trans*-11	0.81	1.34	1.17	0.68	1.26	0.99
C18:3*n*-3	0.39[a]	1.08[b]	0.51[a]	0.46[a]	0.79[b]	0.47[a]
C22:6*n*-3	0.05[a]	0.13[ab]	0.10[a]	0.19	0.59	0.30
PUFA	4.34	5.35	5.49	18.41	17.40	15.53

Different letters in the same row indicate significant differences between treatments at p≤0.05. SAT refers to diets supplemented with rumen-stable fractionated palm fat. LINA refers to diets supplemented with a combination of linseed oil and algae. SUNA refers to diets supplemented with a combination of sunflower and algae.

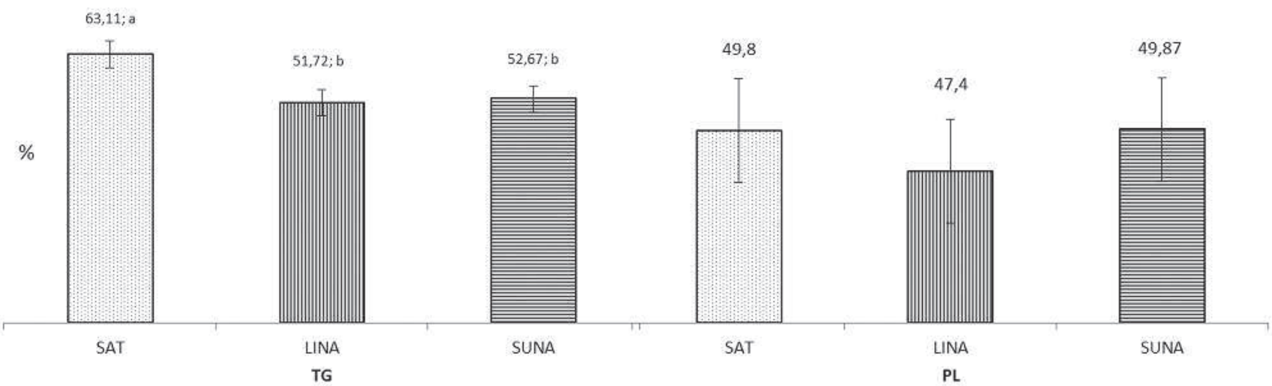

Figure 2. Relative proportion of total saturated fatty acids (SFA) in TG and PL of milk fat from cows supplemented with rumen-stable fractionated palm fat (SAT), combination of linseed oil and algae (LINA), or a combination of sunflower oil and algae (SUNA). Different letters between columns for TG or PL indicate significant differences between treatments at p≤0.05.

The distribution of single CLA isomers (% of total CLA isomers) analyzed by Silver-ion-HPLC in milk fat was also affected by fat supplementation (Table 3). CLA*trans*-11,*cis*-13 isomer significantly increased with LINA compared to SAT and SUNA. Proportions of CLA*trans*-10, *cis*-12 in milk fat were enhanced in response to SUNA supplementation compared to SAT. The relative proportion of CLA*trans*-7,*cis*-9 was also increased in total milk fat of the SUNA group. In the present study, a relationship was calculated between milk fat (%) and CLA*trans*-10,*cis*-12 and CLA*trans*-7,*cis-9* with r = -0.5 and r = -0.25, respectively.

Table 3. CLA isomer as a percentage of total CLA (%) in milk fat of cows fed different fat supplements.

	SAT[1] LSM[3]	LINA[1] LSM[3]	SUNA[1] LSM[3]	SE[2]
CLA*trans*-12,*trans*-14	0.5[a]	1.1[b]	0.30[a]	0.06
CLA*trans*-11,*trans*-13	1.0[a]	1.7[b]	0.5[c]	0.1
CLA*trans*-10,*trans*-12	0.5[a]	0.3[b]	0.8[c]	0.05
CLA*trans*-9,*trans*-11	0.8[a]	0.7[a]	0.5[b]	0.04
CLA*trans*-8,*trans*-10	0.3	0.2	0.3	0.05
CLA*trans*-7,*trans*-9	0.02[a]	0.03[a]	0.08[b]	0.01
CLA*cis*-12,*trans*-14	0.2	0.6	0.2	0.1
CLA*trans*-11,*cis*-13	1.1[a]	2.2[b]	0.6[a]	0.2
CLA*cis*-11,*trans*-13	0.3	0.4	0.3	0.1
CLA*trans*-10,*cis*-12	0.9[a]	1.0[a]	2.9[b]	0.2
CLA*cis*-9,*trans*-11	79.3	77.8	76.4	1.1
CLA*trans*-8,*cis*-10	0.9	0.5	0.8	0.2
CLA*trans*-7,*cis*-9	14.1[a]	13.3[a]	16.3[b]	0.6

[1]Refers to diets supplemented with rumen-stable fractionated palm fat (SAT), 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] LSM = least square means. Different letters in the same row indicate significant differences between treatments at p≤0.05.

Discussion

Considering that 98% of milk fat is TG (Jensen, 2002), the decrease of milk fat in the LINA (2.3%) and SUNA (2.2%) groups compared to the SAT (3.7%) group (Angulo *et al.,* 2012b) mainly reflects the decrease of TG ($p<0.05$). The decrease of milk fat could have a negative impact because the fat content of milk can represent up to 50% of the total value of milk in some markets and, therefore, a decrease in milk fat may have negative economic consequences for farmers. However, this is important for specific newly emerging markets in search of light milk fat. New Zealand scientists are breeding a herd of cows that produce lower-fat milk following the discovery of a natural gene mutation in one animal (Michigan Dairy Review, 2007). Feeding strategies to induce milk fat depression (MFD) could be used as a practical tool by the dairy industry in situations that favor the production of low fat milk. It is important to seek alternatives that lead to an increasing milk yield without altering milk protein when MFD is caused. In the present study, as reported Angulo *et al.* (2012b), plant oil/algae diets decreased milk fat (SAT 3.67%; LINA 2.31%; SUNA 2.17%), did not alter milk protein (SAT 3.26%; LINA 3.15%; SUNA 3.18%), and did not significantly alter mild yield (SAT 29.11; LINA 32.92; SUNA 35.0, kg/cow/d). Further research is needed to improve milk yield and/or to obtain a higher proportion of milk fat in order to maintain milk fat yield.

Similar to our results, López *et al.* (2008) also found an increase of PL (+18%) in milk from cows fed a diet rich in polyunsaturated fatty acids (PUFA). Technological, sensorial, and nutritional consequences of the increase of PL in the lipid composition of milk induced by dietary manipulation have gained importance in recent years. Emulsifying properties and acid tolerance are two of the most important characteristics of cream. The effects of the buttermilk component, especially its PL, on the emulsifying properties and acid tolerance of cream were investigated by Ihara *et al.* (2011). Buttermilk with a higher PL content improved the emulsifying properties and acid tolerance of cream. Unexpectedly, the addition of PL or lysophospholipids did not improve the acid tolerance of cream, a finding that was attributable to the formation of PL complexes and protein in milk fat. Implementing these findings may result in high-quality cooking cream. Likewise, current research on bioactive molecules in milk has demonstrated the health advantages of bovine milk and its components for pharmaceutical purposes. Russell *et al.* (2010) monitored changes in skin morphology upon skin exposure to UV light and evaluated the potential of milk PL in preventing photodamage to skin-equivalent models. The results suggest that milk PL act on skin cells in a protective manner against the effect of UV radiation. According to this research, the potential of increasing PL in milk for pharmaceutical use is beginning to emerge.

The average distribution of PUFA between TG and PL corresponds to that reported by Jensen (2002), being higher in PL (17.1%) than in TG (5.1%). LCPUFA are the structural and functional components of membranes that play a major role in maintaining viable cell membranes (Martonosi, 1975). Interestingly, CLA*cis*-9,*trans*-11 was distributed in a similar way between TG and PL. Considering that approximately 98% of milk fat is TG (Jensen, 2002), the obtained CLA*cis*-9,*trans*-11 contents show higher incorporation into TG. Contarini *et al.* (2009) found that 95 to 97% of CLA*cis*-9,*trans*-11 in bovine milk was incorporated in the TG fraction. Banni *et al.* (2001) reported incorporation of CLA*cis*-9,*trans*-11 into various lipid fractions in the livers of rats fed either a high or low CLA diet. Apparently, the presence of CLA*cis*-9,*trans*-11 in PL membranes is related to the chemical and biological properties of cellular membranes (Soveral *et al*., 2009; Amarú and Field, 2009). *In vitro* studies with mammary carcinoma cells have demonstrated that CLA in PL can decrease other FA oxidation and eicosanoids synthesis, and inhibit cell proliferation (Kelley *et al.,* 2007). Similarly, Ip *et al.* (1991) reported that synthetically prepared CLA is an effective agent for inhibiting mammary tumor development in rats. CLA incorporation into PL increased with dietary intake; CLA feeding resulted in a decrease in the extent of lipid peroxidation in the mammary gland. However, there is no final conclusion about the effect of CLA on human cancer (Kelley *et al.,* 2007).

The diets had a significant effect on the proportion of some fatty acids in TG and PL, which are important for human health. The down-regulation of stearoyl-CoA desaturase (SCD) mRNA by PUFA treatments compared to SAT, as reported by Angulo *et al.* (2012b) in the same study, has been suggested as a possible explanation for the increase of TVA without a clear increase of CLA*cis*-9,*trans*-11. In general, PL was more stable than TG to changes by PUFA supplementation and only minor changes were detected. Barbano and Sherbon (1981) and Sleigh *et al.* (1976) reported little change in fatty acid composition of bovine milk PL in response to the protected unsaturated diet in contrast to milk diglycerides and TG. However, another study (Smith *et al.,* 1977) found that phospholipids obtained from cows fed protected sunflower/soy bean oil supplements contained a higher proportion of linoleic acid than the phospholipids from regular milk diets. Production of TG with a higher proportion of unsaturated FA found in the present study is important for technological applications. Double bonds produce a bend in the fatty acid molecule. Molecules with many such bends cannot be packed as closely together as straight molecules; therefore, these fats are less dense. As a result, TG containing more unsaturated fatty acids are softer and melt more readily at lower temperatures (Webb *et al.,* 2008). Hence, LINA might be used to produce a softer milk fat that spreads as easily as margarine.

In reference to CLA distribution and contrary to other studies (Cruz-Hernandez *et al.*, 2007; Bernard *et al.,* 2009) no significant dietary effect was found for the most abundant CLA*cis*-9, *trans*-11 (%), although there was an increase of TVA in milk TG. The increased level of CLA*cis*-9, *trans*-11 in milk with sunflower, fish, or plant oil supplement decreased with the duration of the feeding period (Cruz-Hernandez *et al.,* 2007; Roy *et al.,* 2006). In the present trial, cows were fed longer (70 d) when compared to the trials by Cruz-Hernadez *et al.* (2007) (38 d) and Roy *et al.* (2006) (20 d). Therefore, a direct comparison of the results seems to be difficult. The type of forage and the proportion of concentrate in the basal diets (Shingfield *et al.,* 2005, Abu-Ghazaleh and Holmes, 2007) as well as the variation among animals (Kelly *et al.,* 1998) have also been reported to affect the distribution of single CLA isomers in milk fat.

In agreement with Kraft *et al.* (2003), CLA*trans*-11,*cis*-13 was the second most abundant CLA isomer found in milk. These authors hypothesized that linolenic acid is an indirect precursor of CLA*trans*-11,*cis*-13. Indeed, in the present study, this CLA isomer increased significantly with LINA compared to SAT and SUNA, and was identified in milk by Kraft *et al.* (2003) as an indicator of pasture feeding. The highest relative proportion of CLA*trans*-11,*trans*-13 was also measured in milk in the LINA group. This CLA isomer was the most abundant isomer among the *trans, trans*-CLA. The higher proportion of CLA*trans*-10,*cis*-12 in milk fat in response to SUNA compared to SAT is concordant with goat milk (Bernard *et al.,* 2009), cow milk (Roy *et al.,* 2006), and ewe milk (Hervas *et al.,* 2008) studies. On the contrary, Cruz-Hernandez *et al.* (2007) found a reduction of this CLA*trans*-10,*cis*-12 in milk fat from cows fed sunflower and fish oil. CLA*trans*-7,*cis*-9 is another FA related to MFD and was increased in SUNA group. CLA*cis*-9, *trans*-11 and CLA*trans*-7,*cis*-9 are the most prevalent CLA isomers in milk. CLA*trans*-7,*cis*-9 in milk fat originates almost exclusively from endogenous synthesis via SCD (Corl *et al.,* 2002). Kadegowda *et al.* (2008) reported that in addition to CLA*trans*-10,*cis*-12, CLA*trans*-7,*cis-9* was also negatively correlated with percentage of milk fat when evaluated in three experiments using the principal component analysis. Dietary C18:1*trans*-7 induced conversion to CLA*trans*-7,*cis-9* and MFD in lactating mice (Kadegowda *et al*., 2010).

The effect of diet on milk CLA distribution (proportion) was similar to the results obtained by Angulo *et al*. (2012b), with the exception of CLA*trans*-10,*cis*-12 and CLA*trans*-7,*cis*-9. The output of these fatty acids increased with LINA and SUNA compared to SAT (0.119, 0.285, 0.062; 1.520, 1.620, 1.000; g/d, respectively). Conversely, the proportion of these fatty acids only increased with SUNA. Fatty acid output, expressed as amount of fatty acids in milk (g/d) is considered more appropriate to explain gene expression and to understand MFD than their proportions in milk

(Glasser *et al.,* 2007). Accordingly, the authors speculate that milk fat synthesis might be mediated not only by CLA*trans*-10,*cis*-12, but also by CLA*trans*-7,*cis-9*. The effect of PUFA diets on CLA*trans*-10,*cis*-12 and CLA*trans*-7,*cis-9* and their relationship with milk fat were concordant with the negative correlation found between these FA and milk fat.

In summary, linseed/algae feeding tended to increase the proportion of PL while significantly decreasing the proportion of TG in bovine milk, producing TG with high proportion of TVA, oleic acid, linolenic acid, DHA, and UFA, and a low proportion of SFA. Sunflower/algae feeding significantly increased PL. The commercial and technological applications of this finding are to be explored. Sunflower/algae feeding caused accumulation of CLA*trans*-10,*cis*-12, CLA*trans*-7, *cis*-9, CLA*trans*-7,*trans*-9, CLA*trans*-10,*trans*-12, and a decrease of CLA*trans*-9,*trans*-11 in total milk fat, whereas LINA increased the CLA*trans*-12,*trans*-14, CLA*trans*-11,*trans*-13, and the CLA*trans*-11,*cis*-13 deposition compared to SAT. Further studies should be conducted in order to understand the relationship between these isomers and gene expression of mammary lipogenic enzymes under specific conditions.

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