# Feeding transition cows with oilseeds: Effects on fatty acid composition of adipose tissue, colostrum and milk

Leiber, F ; Hochstrasser, R ; Wettstein, H R ; Kreuzer, M

DOI: https://doi.org/10.1016/j.livsci.2010.11.016

*Livestock Science*

# Feeding transition cows with oilseeds: effects on fatty acid composition of adipose tissue, colostrum and milk

F. Leiber[a,*], R. Hochstrasser[b], H.-R. Wettstein[a], M. Kreuzer[a]

[a]ETH Zurich, Institute of Plant, Animal and Agroecosystem Sciences, Universitaetstrasse 2, CH-8092 Zurich, Switzerland

[b]University of Zurich, Vetsuisse Faculty, Institute of Animal Nutrition, CH-8057 Zurich, Switzerland

*Corresponding author. Phone +41 44 6328942; Fax +41 44 6321128

E-mail address: fleiber@ethz.ch (F. Leiber)

**ABSTRACT**

The present investigation was aimed to test whether α-linolenic acid (ALA) concentrations in the adipose tissue can be increased by linseed feeding of dry cows and whether ALA is preferentially mobilized during the catabolic phase after parturition. The second objective was the determination of the influence of mobilization on fatty acid composition of colostrum and mature milk. For this purpose, four groups of six cows were fed diets supplemented either with crushed linseed (rich in ALA; two groups) or sunflower seed (rich in linoleic acid (LA)) or a 1:1 mixture of both for 6 weeks. This supplementation was either kept post partum or switched shortly before calving from linseed or the mixture to sunflower seed. The post partum experimental period lasted for another 40 d. Samples of blood were obtained in weeks -6, -2, 3 and 6 pre/post calving, those of backfat adipose tissue in weeks -2 and 6 pre/post calving (by biopsy) and milk samples on days 1, 2, 10, 20, 30 and 40 of lactation. Clear changes over time occurred in blood plasma metabolites and hormones as well as in body weight, body condition scores and backfat thickness, especially during the transition before and after calving. Milk yield and composition showed the typical time trends as well. Proportions of long-chain polyunsaturated fatty acids in the colostrum compared to the mature milk were clearly higher. This appeared similarly in all groups and was not influenced by the oilseed treatment. Compared with the physiological time effects, the effects of the oilseed treatments were weak. It was possible to load the adipose backfat tissue with ALA by supplementing linseed in the dry period, but the magnitude of this increase was small. Milk fat profile was not affected accordingly, although the changes found in adipose tissue post partum suggested the preferential mobilization of ALA during early lactation. Supplementing sunflower seed instead of linseed in the dry period did not elevate LA proportion in adipose tissue nor affected linoleic acid in milk. By contrast, direct transfer of LA, and especially of ALA, from feed to milk happened when sunflower seed or linseed were fed in the lactation period of the experiment. Overall this suggests that strategies aiming at changing n-3 fatty acid concentrations in milk fat composition by loading the adipose tissue of dry cows with ALA are not effective.

**Keywords:**

linoleic acid,

linolenic acid,

adipose tissue,

mobilization

milk

## 1. Introduction

Elevated concentrations of α-linolenic acid (ALA; C18:3n-3) in milk fat are considered beneficial for human health (Barceló-Coblijn and Murphy, 2009) and appear to be positively related with predominant forage feeding (Dewhurst et al., 2006). Under natural conditions, the main source for n-3 fatty acids (FA) for ruminants is plant-intrinsic ALA, and its metabolic concentrations depend, apart from intake, on the rate of escape from ruminal biohydrogenation (Chilliard et al., 2007). The n-3 FA are essential for all mammals, particularly for the fetus and the neonate (Sinclair et al., 2002; Barceló-Coblijn and Murphy, 2009), which is why apparently mechanisms exist ensuring a sufficient n-3 FA supply to the offspring (Burdge, 2004). One such mechanism could be the preferential release of ALA from adipose tissue during catabolic phases (Raclot, 2003), as was observed by Soppela and Nieminen (2002) in reindeer. A subsequent transfer of the released ALA to the fetus or the milk is assumed to be possible. This is corroborated by the negative correlation between BCS changes and ALA concentrations in the milk fat of buffaloes reported by Qureshi et al. (2010). It has been hypothesized, that such a mechanism could be one of the reasons for increased ALA concentrations in milk fat of cows in energy deficient metabolic situations (Leiber et al., 2005). A possibly successful attempt to increase ALA in the milk could, therefore, consist in creating body stores of ALA when the animal's metabolism is still in an anabolic state. More concretely, this could be a strategy for the transition period from pregnancy to lactation. Recently,

Santschi et al. (2009) demonstrated that part of ALA fed to cows in the dry period via linseed can be recovered in the colostrum of cows. However, this period of elevated ALA had been unexpectedly short.

Another aspect is that the dietary proportions of ALA (n-3) and linoleic acid (LA; 18.2n-6) are decisive for the formation of corresponding long-chain polyunsaturated fatty acids (PUFA) as both compete for the same enzymes required for endogenous chain-elongation and desaturation (Barceló-Coblijn and Murphy, 2009). Since in previous studies with transition cows, linseed supplements were rarely compared with LA containing fats the question whether the dietary LA/ALA proportion during the dry period influences the long-chain PUFA in subsequent milk synthesis is not yet investigated.

The first objective of the present study was to investigate whether adipose tissue can be loaded with ALA or LA by feeding linseed or sunflower seed during the anabolic phase ante partum. Secondly, the hypothesis that during the catabolic phase post partum ALA is preferentially released from adipose tissue had to be tested. Thirdly, we determined whether the character of the lipids fed ante partum contributes to the PUFA profile in colostrum and mature milk. The overall aim of this study was to determine the extent of ALA loading and mobilization more directly than had been possible previously (Santschi et al., 2009).

## 2. Materials and Methods

### *2.1 Experimental Design*

In a completely randomized block design, four groups of six cows in their dry period were allocated to four dietary treatments. Concentrates containing oilseeds were offered at 1.8 kg DM/d per cow during the dry period and early lactation. One concentrate was characterized by high proportions of linseed (L concentrate; Table 1), the other one was based on sunflower seed (S concentrate). Two control groups received either always L or S (further on denoted as L/L and S/S; Table 2). The third group received a 1:1 mix of L and S in the dry period and S during the lactation

93 (LS/S). A fourth group was offered L ante partum and S within the lactation (L/S). The intended
94 experimental period was 7 wk before and 6 wk after parturition, where 6 wk of the dry period were
95 dedicated to pre-partum treatment feeding and the last wk served for adaption to the lactation diet.
96 As the realized calving dates were on average later than anticipated, this adaptation period actually
97 lasted for 12 ± 7 d. The multiparous experimental cows, 14 of Holstein Friesian and 10 of Brown
98 Swiss breed, were allocated to the four treatment groups balancing for breed, lactation number, milk
99 yield and milk fat content in previous lactation. Their average BW prior to the experiment was 772
100 ± 32 kg and average lactation number was 3.0 ±1.8. The experiment was approved by the cantonal
101 veterinary office of Zug, Switzerland (approval no. ZG 42/05).

102 *2.2. Feeds and Feeding*

103 The L and S concentrates consisted of 50.8% linseed and 57.4% sunflower seed, respectively
104 (Table 1). The remaining part of these pelleted concentrates was composed of barley, straw meal
105 and molasses in order to balance concentrates for net energy. The oilseeds were steamed and ground
106 before being added to the other concentrate ingredients. The two concentrates were designed to
107 contain 19% FA DM from the oilseeds and few extra FA from the other ingredients. The realized
108 total FA contents for L concentrate was 22 % and for the S concentrate it was 26%. The linseed had
109 been steamed before use in order to inactivate anti-nutritive secondary compounds. Beside the
110 oilseeds, the experimental concentrates L and S contained other ingredients to balance them for
111 energy and protein. After parturition, the calculated extra energy and protein demands of the cows
112 for milk formation were covered by corresponding amounts of barley and soybean meal (prepared
113 with small amounts of molasses). Before calving, when the cows were kept individually in tie stalls,
114 concentrates were fed manually in four portions per day offered at 6.30 a.m., 10.45 a.m., 2.00 p.m.,
115 and 5.15 p.m. After calving, cows were kept in groups and received concentrates individually from
116 computerized concentrate feeders (Westfalia Landtechnik, Oelde, Germany) in portions of ≤ 1.5 kg
117 per feeding bout. The forage ration was changed with parturition as well (Table 1). In the dry
118 period, cows were fed a roughage mixture consisting of 49% grass silage, 13% hay (hay A, Table

1), and 38% barley straw. From adaptation through lactation cows received forages containing the same grass silage (60%) but additionally corn silage (30%) and ryegrass-white clover hay (10%; hay B, Table 1). The forage rations were always offered individually at *ad libitum* access using the same computerized feeding system equipped with Calan doors (Westfalia Landtechnik, Oelde, Germany) in both periods. Dry-period and lactation diets were supplemented (12 g/kg DM) with vitamin-mineral premixes (Table 1) put directly onto the forages, and extra NaCl at *ad libitum* access.

### *2.3. Data Recording and Sample Collection*

Forage and concentrate intakes as well as refusals were recorded daily. Samples were drawn weekly from all forage components. The samples were milled through a 1 mm sieve, with the silages being previously dried at 60°C for 48 h. The concentrates were sampled once per batch (two batches were used for the S and L concentrate, one batch for barley and soybean meal).

Body weight was determined by an electronic balance at the start of the experiment, 2 wk before anticipated calving, and 15 d as well as 36 d after calving. At the same occasion, the cows were subjectively scored for BCS by a trained person following Edmonson et al. (1989). At every second scoring date, backfat thickness was measured by ultrasound (Aloka echo camera ssd-630 equipped with a 5 MHz Aloka probe ust-586-5 transducer, Aloka, Tokyo, Japan). The measurement points were situated on the cow's left side. Point 1 (lumbar region) was in between the *procc. transversi* of the second to the fifth lumbar vertebrae, transducer in a right angle to the spine. Measurement point 2 (hip region) was located on a line between *tuber coxae* and *tuber ischiadicum*, about 5 cm nearer to the *tuber ischiadicum* than to the *tuber coxae*.

Biopsies of subcutaneous adipose tissue were taken between *tuber coxae* and *tuber ischiadicum* on the right body side 2 wk before the anticipated calving date, i.e., before changing to the lactation diet, and 6 wk .after parturition. The skin was first sheared, washed, disinfected, and anaesthetized. Subsequently, a stitch-incision was made with a scalpel. A sterile biopsy needle (Tru-Cut Precisa 14G × 100mm, HS Hospital Service, Pomezia, Italy) was inserted in a flat angle and the biopsy was

145 taken from the subcutaneous tissue at a depth of about 2.5 to 3 cm. The tissue sample
146 (approximately 0.5 g) was stored in heptane (containing butylhydroxitoluol) at – 20°C. The wound
147 was then treated with a disinfectant solution and left open to heal by secondary intention.

148 The blood samples were drawn in the same intervals as cows were weighed. Dual blood samples
149 (span between collections: 1 to 2 d) were taken from the jugular vein by a 50 ml syringe at 1.00
150 p.m. after cows had been fasted for 2 h. The blood was immediately transferred to three tubes
151 containing either EDTA, heparine or fluoride as anticoagulants. The tubes were stored on ice until
152 being centrifuged at 1500 × *g* and 4 °C. The resulting plasma then was stored at – 20°C.

153 Milking was performed daily at 5 a.m. and 5 p.m. by a milking system with an automatic milk
154 recording system (Metatron, Westfalia Landtechnik, Oelde, Germany). With this system, samples
155 were collected twice weekly, each from evening and morning milking. Colostrum samples were
156 drawn by hand from the first four milkings. One part of the samples was conserved with 2-bromo-2-
157 nitropropanone-1,3-diol (Bronopol, D&F Inc., Dublin, CA) and stored at + 5°C for ≤ 5 d. The other
158 part was pooled proportionately to milk amounts and then stored at – 20° for later FA analysis. The
159 latter analysis was performed for samples obtained on d 1, 2, 10, 20, 30 and 40 of lactation.

160 *2.4. Proximate analysis*

161 Proximate analysis of feeds was performed according to AOAC (1997), including DM and total
162 ash (AOAC index no. 942.05; using a TGA-500 furnace, Leco Corporation, St. Joseph, Michigan) and N
163 (AOAC index no. 977.02; using a C/N analyzer, type FP-2000, Leco Corporation, St.Joseph, MI).
164 Contents of NDF and ADF were analyzed with a Fibertec 1020 system (Tecator; Höganäs,
165 Sweden). Concerning NDF, α-amylase (Termamyl 120L, type S; Novo Nodirsk A/S, Bagsværd,
166 Denmark) was used but no sodium sulfite (Van Soest et al., 1991). Crude protein was calculated as
167 N × 6.25 and $NE_L$ was calculated based on tabulated values given in ALP (2008).

168 *2.5. Analysis of plasma metabolites and milk*

169 Plasma was analyzed with an auto-analyzer (Cobas Mira, Roche Diagnostics, Zürich,
170 Switzerland). Test-kits for ultraviolet spectrometry were used for glucose (Kit Nr 00116, 00072,
171 Axon Lab AG, Baden, Switzerland) and BHBA (Kit Nr. RB1007 from Randox Laboratories Ltd,
172 Ibach, Switzerland), photometric test kits for NEFA (WAKO Instrumentengesellschaft, Zürich,
173 Switzerland) and triglycerides (Kit Nr. 00069, Axon Lab AG, Baden, Switzerland). Insulin (Kit Nr
174 KAP1251, Biosource, Nivelles, Belgium) and insulin-like growth factor (IGF-1; Medidiagnost,
175 Reutlingen, Germany) were measured with ELISA. Leptin was determined with a multi species
176 Leptin RIA kit (Nr XL-85K, Linco Research, St. Charles MO).
177 Bronopol conserved milk was analyzed for contents of fat, protein and lactose by the infra-red
178 technique (Milkoscan 6000, Foss Electric, Hillerød, Denmark).

179 *2.6. Lipid extraction and analysis*

180 The lipids in the feeds were extracted with a hexane-isopropanol solution (3:2) (with addition of
181 butylhydroxitoluol) in an Accelerated Solvent Extractor (ASE200, Dionex Corp., Sunnyvale).
182 Heneicosanoic acid (21:0-Triglyceride, Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland)
183 was added as an internal standard. After saponification with methanolic sodium hydroxide,
184 methylation was catalyzed with $BF_3$ as described by Richter et al. (2010). Subsequently to
185 purification on a silica gel column and evaporating the solvent, FAME were dissolved in hexane
186 and injected into a GC (HP 6890, Hewlett-Packard, Wilmington Pennsylvania) equipped with a
187 Supelcowax-10 column (30 m × 0.32 mm × 0.25 µm, Supelco Inc., Bellefonte, PA). Amounts of 1
188 µl were applied by split injection (1:30, split temperature 260 °C). As carrier gas hydrogen was
189 used with a flow rate of 2.2 ml/min. Initial oven temperature was 160 °C for 0.5 min. Then, the
190 following temperature program was used: increase by 20 °C/min up to 190°C; increase by 7 C°/min
191 up to 230 °C; isotherm at 230 °C for 5.3 min; increase by 20 °C/min up to 250 °C; isotherm for 6
192 min. Detector temperature was 270 °C.
193 The frozen adipose tissue biopsies were defrosted in a water bath at 40°C allowing lipids from
194 the adipocytes to be better dissolved in n-heptane after homogenization. After evaporating the

heptane, the lipids were dissolved in hexane and centrifuged. The upper phase was transferred into a new vial. Subsequently, lipids were methylated with $BF_3$ according to IUPAC (1987). In order to prevent artifacts caused by cholesterol, the FA methyl esters (FAME) were sprayed onto a thin layer chromatography glass plate of a size of 20 × 20 cm coated with silica gel 60 $F_{254}$ (Merck KGaA, Darmstadt, Germany). The plates were developed vertically in a solvent system of hexane-diethyl ether-acetic acid 70:30:1 (v/v/v; according to Kaluzny et al., 1985) for approximately 30 min until the solvent had reached the upper border of the plate. The plates were sprayed with a fluorescent dye (2',7'-dichloro fluorescein, Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and the FAME were identified and marked in UV-light (440 nm). After separation, the area with the FAME was scratched into a tube and resolved in hexane. Subsequently, the same GC method as with the feeds was applied.

Colostrum and milk were thawed in a water bath at 37 °C and then gently mixed to achieve an even distribution of the milk fat. Fatty acids were extracted by the cold esterification method of Suter et al. (1997), using an internal standard solution consisting of triundecanin, tetradecen, pelargonic acid methyl esters and trivalerin. The FAME obtained were analyzed with the same column as used for the other FAME analyses, but with a modified method. Split temperature was 250 °C, flow of hydrogen 2.6 ml/min and initial oven temperature was 80 °C for 0.5 min, followed by and increase of 10 °C/min up to 230 °C; an isothermal phase with 230 °C for 4.5 min, an increase of 20 °C/min up to 250 °C and an other isothermal phase at 250 °C for 4 min. An amount of 1 µl was applied by split injection (1:30). A butter-fat standard (BCR 164, EC Reference Materials, Brussels, Belgium) was used for the determination of an overall response factor. Individual response factors for short chain FA were determined by using a reference milk powder (SRM 8435, National Institute of Standards and Technology, Gaithersburg, MD).

For separation and identification of the C18:1 *cis* and *trans* isomers in colostrum and milk, a second FA analysis run was performed with a Varian CP7421 column (200 m × 0.25 mm ×0.25 µm Varian BV, Middelburg, The Netherlands). An amount of 1 µl of the FAME was injected with a

split of 1:50. Initial oven temperature was 181°C for 60 min, followed by an increase of 5°C/min up to 230°C, an isothermal phase with 230°C for 32 min; then increase of 5°C/min up to 250°C and again an isothermal phase with 250°C for 12 min. Hydrogen was used as carrier gas with a flow of 1.7 ml/min for the first 59 min. Then the flow was decreased to 1.3 ml/min which was kept until the end of the analysis. Detector temperature was 300°C. The major FA were identified based on a mixed FAME standard (Supelco 37 Component, Supelco). Branched-chain fatty acids and other isomers, not included in the Supelco 37 standard, were identified by comparison with chromatograms published by Kramer et al. (2002) and Collomb and Bühler (2000).

### *2.7. Calculations and Statistical Analysis*

Milk data was used to calculate ECM (kg/d) as milk (kg/d) × [0.038 × fat (g/kg) + 0.024 × protein (g/kg) + 0.017 × lactose (g/kg)] / 3.14 (ALP 2008). Data was subjected to analysis using the Mixed procedure of SAS (Version 9.1, SAS Institute Inc., Cary, NC) applying two different models. Model 1 included only treatment as an effect and was separately applied for intake data before and after calving. Model 2 was applied for all other data and considered as effects treatment ($\alpha$), sampling date ($\tau$; represented either by week or, for milk data, by DIM), the interaction of $\alpha$ and $\tau$ as well as animal ($\chi$) as subject for repeated measurement. As error terms, animal within group and time ($\varepsilon$) and the residual error of the whole model ($\gamma$) were considered.

$$Y_{ijkl} = \mu + \alpha_i + \tau_j + \alpha \times \tau_{ij} + \chi_{jk} + \varepsilon_{ijk} + \gamma_{ijkl}$$

In the tables 3-7, LSM and the corresponding SE are given. Where treatment or sampling date were significant within the whole model, LSM were compared with Tukey's method both for treatment and sampling date (measurement wk or DIM). From the biopsy data (Table 5) one cow of group C/C and one of L/C had to be excluded due to unsufficient biopsy material. The same was done for one cow of group L/S for the BHBA concentrations in blood plasma, due to a damaged sample (Table 4). Breed effects had been tested before by a GLM procedure and no significances had been found. Therefore, breed was not included in the models applied here.

## 3. Results

### *3.1.Intake*

During the dry period, DMI was 12.3 kg/d on average, thereof 1.8 kg/d concentrate (Table 3). Total DMI after parturition was 18.7 kg/d on average, whereof 5.7 kg/d was concentrate. Across the entire experimental period, no considerable differences between treatments occurred in either forage or concentrate intake or total DMI.

### *3.2.Blood metabolites*

In blood plasma, none of the variables was affected by treatment (Table 4). However, levels of all variables significantly changed with time; this mainly after calving and during early lactation. Triglycerides decreased to less than half after parturition, BHBA increased to more than 2-fold levels. NEFA increased in wk 3 after parturition and then decreased again. However, the latter change was significant only in group L/S. After parturition, leptin increased while IGF-1 sharply decreased. Glucose and insulin were both reduced in wk 3 post partum compared to the period before calving. Glucose levels slightly recovered from initially low post partum levels in wk 6, but insulin levels remained low.

### *3.3.Body condition, backfat thickness and backfat composition*

No effects of treatment on BW, BCS and backfat thickness occurred (Table 4). Parturition resulted in a loss of BW ($P < 0.05$). A decrease in BCS and backfat thickness after parturition was significant only for groups S/S and L/S, which led to a significant overall interaction of time and treatment for these parameters.

For most of the fatty acids, diet treatments did not significantly change the FA profile of the backfat both ante and post partum (Table 5). However, all groups fed with linseed ante partum had higher ALA proportions in the backfat obtained in week -2 ($P < 0.05$). For the backfat obtained in week 6 post partum, group L/L showed significantly higher ALA values than all other groups. Furthermore, time effects ($P < 0.05$) occurred in some FA. Across all diets, total BFA proportion in

backfat increased ($P < 0.05$) after parturition, which was also true for 18:0 and LA. The proportion of 18:3n-3 in backfat was lower across all groups ($P < 0.001$) post partum than ante partum. Concerning individual groups, this decline was significant only in the L/S treatment. These changes led to an increase ($P < 0.001$) of the n-6/n-3 ratio after parturition across all groups and, specifically, in group L/S.

*3.4.Milk yield and composition*

Energy corrected milk yield was not influenced by treatment but varied with DIM and reached a maximum around the d 20 of lactation (38.8 kg/d on average; Table 6). Fat and protein contents of the milk decreased with time, but were not affected by treatment. Lactose content increased with time in group L/L ($P < 0.001$), leading to a difference ($P < 0.05$) between groups S/S and L/L when cows were 30 and 40 DIM.

The proportions of total short- and medium-chain saturated FA (4:0-14:0) in milk fat were lowest in all groups when cows were 10 DIM and were highest between 30 and 40 DIM (Table 7). There was a treatment effect ($P < 0.05$), with an increased proportion of the short- and medium-chain saturated FA in the L/L group. Proportions of total long chain (16:0-22:0) saturated FA were highest in the colostrum and continuously decreased during early lactation. The proportion of total branched-chain FA increased from d 1 of lactation to d 40 of lactation in all groups. There was a trend ($P < 0.06$) for a treatment effect towards lower total mono-unsaturated FA (MUFA) in group L/L. The MUFA proportion peaked ($P < 0.001$) at d 10 and 20 of lactation. The highest values were found around the d 10 of lactation, the lowest values right at the start. Across all treatment groups the proportion of PUFA was similar but decreased ($P < 0.05$) from the first colostrum to the end of the experiment. Proportions of 20:4n-6 were higher by up to 2.5 times and those of 22:5n-3 were higher by up to 3 to 4 times in colostrum than in mature milk (Fig. 1). However, no effect of treatment and no treatment × DIM interaction on the proportions of these FA were found. In the n-6/n-3 ratio a clear treatment effect ($P < 0.001$) occurred, with the ratio being lower ($P < 0.05$) in group L/L than in all other groups from 20 DIM onwards. The interaction of treatment × DIM was

298 significant, too ($P < 0.001$), which was explained by the increasing difference between L/L and the
299 other groups and the shift in the position of the highest level from initially S/S to the L/S treatment.
300 Proportions in total fatty acids of stearic acid (18:0) and oleic acid (18:1*c*9) increased whereas LA
301 and ALA decreased ($P < 0.05$) from the colostrum to the mature milk (Fig. 1). The 18:1 *trans*10 and
302 *trans*11 (vaccenic acid) monoenes and, somewhat less clear, 18:2*c*9*t*11 (rumenic acid) were highest
303 concentrated between 10 and 20 DIM (Fig. 2). In the colostrum of the first day in milk, the different
304 ALA concentrations of the diets ante partum were reflected, but disappeared already at the second
305 day (Fig. 1). Treatment effects of the diet post partum ($P < 0.05$) on octadeca acids were found for
306 group L/L, where proportions in milk fat of 18:1*t*10 and LA were lower and of ALA were higher ($P$
307 $< 0.05$) compared to the other groups. Proportions of 20- and 22-PUFA were always higher ($P$
308 $< 0.05$) in the colostrum than in the mature milk.

309
310
311 ## 4. Discussion

312 ### *4.1.Metabolic effects of lipids rich in α-linolenic acid and linoleic acid around parturition*

313 The overall results for the metabolic parameters investigated in the present study agree well
314 with a recent broader study on transition cows (Graber et al., 2010), indicating that the metabolic
315 response was normal. Only the increase of leptin after parturition was clearly different from the
316 results of Graber et al. (2010), which might be a consequence of the high lipid content of the
317 concentrates (Bonnet et al., 2009).

318 Several studies have been carried out so far which included the influence of lipids fed ante
319 partum on the post partal metabolic status, body condition and performance traits. Andersen et al.
320 (2008) and Santschi et al. (2009), who both compared a highly unsaturated lipid source with a
321 saturated source, found only weak or no influences on blood metabolites indicative for energy
322 metabolism post partum. In the present study, where the comparison was done between two
323 different sources of mainly unsaturated fats, again the diet ante partum had no significant influence
324 on the metabolite concentrations post partum. Like in the studies of Andersen et al. (2008) and

325 Santschi et al. (2009), independently of pre partal fat supplementation, NEFA increased already
326 before parturition, indicating the start of the mobilization, and BHB clearly increased after
327 parturition. By contrast, Petit et al. (2007) had demonstrated a very clear reduction of NEFA and
328 BHB levels post partum when supplementing unsaturated fats. The lack of differences in blood
329 metabolites corresponds with the finding that also body condition traits and performance of the
330 cows did not differ between the feeding groups in the current study. This is consistent with the
331 results of Petit and Benchaar (2007), Andersen et al. (2008) and Santschi et al. (2009). Therefore,
332 the two oilseeds used as supplements in the current study seem to be widely equivalent in their
333 effects on energy metabolism of cows pre- and post partum.

334 *4.2.Changes from colostrum to mature milk in α-linolenic acid and linoleic acid*

335 Similar to findings by Santschi et al. (2009) in cows and by Martin et al. (1993) in humans, the
336 long chain PUFA proportions peaked in the colostrum in the present study, independently of dietary
337 treatment, and peaks disappeared until d 10 of lactation. This suggests that a certain level of long
338 chain PUFA in the colostrum is maintained by the animal, regardless of the actual diet and level of
339 adipose tissue stores. In contrast, the C18-PUFA proportions in colostrum lipids were, apart from
340 few exceptions, not higher than in mature milk which is again consistent with Santschi et al. (2009)
341 and Martin et al. (1993). This underlines the particular importance of the long chain PUFA to the
342 neonate, which are endogenously synthesized from LA and ALA (Barceló-Coblijn and Murphy,
343 2009). Even in ruminants a certain part of long-chain n-3 PUFA can be directly transferred from the
344 diet. This has been shown with fish oil supplemented diets both for colostrum (Cattaneo et al.,
345 2006; Annett et al., 2009) and milk (AbuGhazaleh and Holmes, 2009). The observation that extra
346 ALA from linseed had an effect only on ALA but not on long-chain PUFA in colostrum in the
347 present study, might reflect a bottleneck of endogenous chain elongation (Pawlosky et al., 2001).
348 But it could probably also indicate that physiologically necessary concentrations of n-3 PUFA in the
349 colostrum were maintained also in the sunflower groups and these were not ALA deficient in that
350 respect.

### *4.3.Loading of adipose tissue with α-linolenic acid and linoleic acid in the dry period*

It has been demonstrated in growing cattle (Fincham et al., 2009) and sheep (Palmquist et al., 2004) that the diet may influence the FA profile of subcutaneous adipose tissue on the long term. One question in the present study was, whether such a loading is also possible in adult dairy cows during the comparably short dry period. However, this selective preloading, as measured in the subcutaneous backfat, succeeded only for ALA, which might be due to the generally limited backfat gains in this period, as obvious from the lack of clear changes in backfat thickness. Although clear effects of LA feeding on backfat composition were demonstrated for sheep (Palmquist et al., 2004), in the current study group differences were noted only for ALA in the preparturient adipose biopsies. The data for the biopsies post partum show that this effect was not long lasting, probably due to the high adipose tissue turnover in early lactation (McNamara, 1989; Martin and Sauvant, 2007).

### *4.4.Mobilization of linoleic acid and α-linolenic acid from adipose tissue and its influence on milk fat composition*

One hypothesis has been that, along with the mobilization of body reserves, ALA and LA are preferentially released and thus their occurrence in milk should be increased in early lactation of cows, especially when the adipose tissue had been loaded with these FA before. This hypothesis was based on the findings of Soppela and Nieminen (2002) in reindeer and Raclot (2003) in rats. Raclot (2003) stated that the relative release of FA from mobilized adipose tissue is decreasing with carbon chain length but increasing with the number of double bonds. Based on this, a particular high release of ALA, compared to the other 18-FA, in catabolic phases was expected. Blood metabolites, backfat thickness and BCS indicated the presence of a clear and common catabolic phase after parturition in all treatment groups. The total PUFA content of the backfat did not decrease from before to after parturition, indicating that, similar to humans (Martin et al., 1993), there was no general preference for the release of PUFA during mobilization. However, different from the human study of Martin et al. (1993) but consistent with the findings in the reindeer

(Soppela and Nieminen, 2002), a clear decrease in ALA proportion of the backfat occurred in the present study across all animals and especially in group L/S. This group had received the highest ALA supply ante partum and then switched to the low-ALA sunflower concentrate around parturition.

This preferential mobilization of ALA, would have supported the hypothesis that a negative energy balance of metabolically stressed cows could trigger increased concentrations of ALA in milk (Leiber et al., 2004 and 2005). However, an effect of this ALA release on milk fat composition was almost completely lacking. It was only possible to influence the colostrum in the case of ALA, but not as clear as in the study of Santschi et al. (2009). However, no longer lasting effect was found in either of the studies. As reported by Andersen et al. (2008), a high ALA diet fed pre partum was reflected in the plasma of the dry cows, but already 3 wk after parturition this effect had disappeared completely. Together with the present results this underlines the absence of a longer lasting effect of ante partum ALA supplementation on the ALA available for the synthesis of mature milk. A long lasting effect of the ALA and LA proportions in the backfat on milk fat may have been limited by the fact that turnover rate of adipose tissues during early lactation is too high (McNamara, 1989) for the conservation of proportions that have been created ante partum.

Enhancing ALA and LA in milk fat directly by feeding linseed or sunflower-seed was effective as had been expected from the literature (e.g., Glasser et al., 2008; Bernard et al., 2009). The lack of a treatment effect on vaccenic acid and rumenic acid indicates that not primarily the dietary ratio of LA to ALA but rather the sum of both is decisive for the concentrations of the former two FA in milk (Lock and Garnsworthy, 2002; Khiaosa-Ard et al., 2010).

## 5. Conclusions

Considering the low statistical power due to low numbers of animals, from the results of the present study can be carefully concluded, that (i) supplements for transition cows containing either α-linolenic acid or linoleic acid as the predominant polyunsaturated fatty acid do not differently

404 influence fat storage as well as mobilization and performance of cows after calving, (ii) a
405 preloading of adipose tissue with α-linolenic acid by dietary linseed supplements is possible, (iii) α-
406 linolenic acid is preferentially released from adipose tissues during mobilization and (iv) the fatty
407 acid composition of mature milk is independent of the lipid concentrate types fed ante partum. Only
408 in colostral fat the α-linolenic acid proportion of the diets ante partum was clearly reflected
409 indicating a transient effect of the mobilization of this fatty acid on its presence in milk.
410

411 **References**


412 AbuGhazaleh, A. A., Holmes, L., 2009. Altering dairy cows milk fatty acid composition through
413 grazing and oil supplementation. Milchwissenschaft 64, 241-244.

414 Andersen, J. B., Ridder, C., Larsen, T., 2008. Priming the cow for mobilization in the periparturient
415 period: effects of supplementing the dry cow with saturated fat or linseed. J. Dairy Sci. 91,
416 1029–1043.

417 Annett, R. W., Dawson, L. E. R., Edgar, H., Carson, A.F., 2009. Effects of source and level of fish
418 oil supplementation in late pregnancy on feed intake, colostrum production and lamb output of
419 ewes. Anim. Feed Sci. Technol. 154, 169-182.

420 ALP (Agroscope Liebefeld-Posieux), 2008. Fütterungsempfehlungen und Nährwerttabellen für
421 Wiederkäuer. Online version. Ed ALP, Posieux. http://www.agroscope.admin.ch/praxis/00215/
422 00801/01587/index.html?lang=de. Accessed on May 10, 2010.

423 AOAC, 1997. Official Methods of Analysis. Assoc. Off. Anal. Chem., Arlington, VA.

424 Barceló-Coblijn, G., Murphy, E.J., 2009. Alpha-linolenic acid and its conversion to longer chain n-
425 3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels.
426 Prog. Lipid Res. 48, 355-374.

427 Bernard, L., Bonnet, M., Leroux, C., Shingfield, K.J., Chilliard, Y., 2009. Effect of sunflower-seed
428 oil and linseed oil on tissue lipid metabolism, gene expression, and milk fatty acid secretion in
429 Alpine goats fed maize silage-based diets. J. Dairy Sci. 92, 6083-6094.

Bonnet, M., Delavaud, C., Bernard, L., Rouel, J., Chilliard, Y., 2009. Sunflower-seed oil, rapidly degradable starch, and adiposity up-regulate leptin gene expression in lactating goats. Domest. Anim. Endocrinol. 37, 93-103.

Burdge, G., 2004. α-Linolenic acid metabolism in men and women: nutritional and biological implications. Curr. Opin. Clin. Nutr. Metab. Care 7, 137–144.

Cattaneo, D., Dell'Orto, V., Varisco, G., Agazzi, A., Savoini, G., 2006. Enrichment in n-3 fatty acids of goat's colostrum and milk by maternal fish oil supplementation. Small Rumin. Res. 64, 22–29.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., Doreau, M., 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109, 828–855.

Collomb, M., Bühler, T., 2000. Analyse de la composition en acides gras de la graisse de lait. Mitt. Lebensm. Hyg. 91, 306-332.

Dewhurst, R. J., Shingfield, K.J., Lee, M. R. F., Scollan, N.D., 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim. Feed Sci. Technol. 131, 168–206.

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

Fincham, J. R., Fontenot, J.P., Swecker, W.S., Herbein, J.H., Neel, J. P. S., Scaglia, G., Clapham, W. M., Notter, D.R., 2009. Fatty acid metabolism and deposition in subcutaneous adipose tissue of pasture- and feedlot-finished cattle. J. Anim. Sci. 87, 3259–3277.

Glasser, F., Ferlay, A., Chilliard, Y., 2008. Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J. Dairy Sci. 91, 4687–4703.

Graber, M., Kohler, S., Kaufmann, T., Doherr, M.G., Bruckmaier, R.M., van Dorland, H.A., 2010. A field study on characteristics and diversity of gene expression in the liver of dairy cows during the transition period. J. Dairy Sci. 93, 5200-5215.

456 IUPAC (International Union of Pure and Applied Chemistry), 1987. Standard Methods for the
457 Analysis of Oils, Fats and Derivates. 7th ed., Blackwell Scientific Publications, Oxford, UK, pp.
458 123–125.
459 Kaluzny, M A., Duncan, L.A., Merritt, M. V., Epps, D.E., 1985. Rapid separation of lipid classes in
460 high yield and purity using bonded phase columns. J. Lipid Res. 26, 135–140.
461 Khiaosa-ard, R., Klevenhusen, F., Soliva, C. R., Kreuzer, M., Leiber, F., 2010. Transfer of linoleic
462 and linolenic acid from feed to milk in cows fed isoenergetic diets differing in proportion and
463 origin of concentrates and roughages. J. Dairy Res. 77, 331–336.
464 Kramer, J.K.G., Blackadar, C.B., Zhou, J., 2002. Evaluation of two GC columns (60-m
465 SUPELCOWAX 10 and 100-m CP Sil 88) for analysis of milkfat with emphasis on CLA, 18:1,
466 18:2 and 18:3 isomers, and short- and long-chain FA. Lipids 37, 823-835.
467 Leiber, F., Scheeder, M. R. L., Wettstein, H.-R., Kreuzer, M., 2004. Milk fatty acid profile of cows
468 under the influence of alpine hypoxia and high mountainous forage quality. J. Anim. Feed Sci.
469 13 (Suppl 1), 693–696.
470 Leiber, F., Kreuzer, M., Nigg, D., Wettstein, H.-R., Scheeder, M. R. L., 2005. A study on the causes
471 for the elevated n-3 fatty acids in cows' milk of alpine origin. Lipids 40, 191–202.
472 Lock, A. L., Garnsworthy, P.C., 2002. Independent effects of dietary linoleic and linolenic fatty
473 acids on the conjugated linoleic acid content of cows' milk. Anim. Sci. 74, 163–176.
474 Martin, J.-C., Bougnoux, P., Fignon, A., Theret, V., Antoine, J.-M., Lamisse, F., Couet, C., 1993.
475 Dependence of human milk essential fatty acids on adipose stores during lactation. Am. J. Clin.
476 Nutr. 58, 653-659.
477 Martin, O., Sauvant, D., 2007. Dynamic model of the lactating dairy cow metabolism. Animal 1,
478 1143-1166.
479 McNamara J.P., 1989. Regulation of bovine adipose tissue metabolism during lactation. 5.
480 Relationships of lipid synthesis and lipolysis with energy intake and utilization. J. Dairy Sci. 72,
481 407-418.

482 Palmquist, D.L., St-Pierre, N., McClure, K.E., 2004. Tissue fatty acid profiles can be used to
483 quantify endogenous rumenic acid synthesis in lambs. J. Nutr. 134, 2407-2414.
484 Pawlosky, R. J., Hibbeln, J. R., Novotny, J. A., Salem, N., 2001. Physiological compartmental
485 analysis of α-linolenic acid metabolism in humans. J. Lipid Res. 42, 1257–1265.
486 Petit, H. V., Benchaar, C., 2007. Milk production, milk composition, blood composition, and
487 conception rate of transition dairy cows fed different profiles of fatty acids. Can. J. Anim. Sci.
488 87, 591–600.
489 Petit, H. V., Palin, M.F., Doepel, L., 2007. Hepatic lipid metabolism in transition dairy cows fed
490 flaxseed. J. Dairy Sci. 90, 4780–4792.
491 Qureshi, M. S., Mushtaq, A., Khan, S., Habib, G., Swati, Z.A., 2010. Variation in milk fatty acid
492 composition with body condition in dairy buffaloes (*Bubalus bubalis*). Asian-Austral. J. Anim.
493 Sci. 23, 340–346.
494 Raclot, T., 2003. Selective mobilization of fatty acids from adipose tissue triacyglycerols. Prog.
495 Lipid Res. 42, 257–288.
496 Richter, E.K., Spangenberg, J.E., Kreuzer, M., Leiber, F., 2010. Characterization of rapeseed
497 (Brassica napus) oils by bulk C, O, H, and fatty acid C stable isotope analyses. J. Agric. Food
498 Chem. 58, 8048-8055.
499 Santschi, D. E., Wettstein, H.-R., Leiber, F., Witschi, A.-K. M., Kreuzer, M., 2009. Colostrum and
500 milk fatty acids of dairy cows as influenced by extruded linseed supplementation during the
501 transition period. Can. J. Anim. Sci. 89, 383–392.
502 Sinclair, A. J., Attar-Bashi, N. M., Li, D., 2002. What is the role of α-linolenic acid for mammals?
503 Lipids 37, 1113–1123.
504 Soppela, P., Nieminen, M., 2002. Effect of moderate wintertime undernutrition on fatty acid
505 composition of adipose tissues of reindeer (*Rangifer tarandus tarandus* L.). Comp. Biochem.
506 Physiol. A 132, 403–409.

507 Suter, B., Grob, K., Pacciarelli, B., 1997. Determination of fat content and fatty acid composition
508 through 1-min transesterification in the food sample; principles. Z. Lebensm. Unters. Forsch. A
509 204, 252–258.
510 Van Soest, P. J., Robertson, J. B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent
511 fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583−3597.
512

***Figure captions***

**Fig. 1.** Evolution with time of the proportions of various long-chain fatty acids in milk fat after calving as affected by oilseed treatment. S/S, sunflower supplement before and after parturition; LS/S, sunflower and linseed supplement (1:1) before and only sunflower supplement after parturition; L/S, linseed supplement before and sunflower supplement after parturition; L/L, linseed supplement before and after parturition. Data points carrying different letters within the same DIM are significantly different at $P < 0.05$.

**Fig. 2.** Evolution with time of the proportions of 18 *trans* fatty acids in milk fat after calving as affected by oilseed treatment. S/S, sunflower supplement before and after parturition; LS/S, sunflower and linseed supplement (1:1) before and only sunflower supplement after parturition; L/S, linseed supplement before and sunflower supplement after parturition; L/L, + linseed supplement before and after parturition.

**Table 1**

Analyzed nutrient composition and calculated $NE_L$ contents of the experimental feeds

| | Concentrates | | | | Forages | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | L (Linseed)[1] | S (Sunflower)[2] | Soybean meal[3,5] | Barley[3,5] | Grass silage | Corn silage[5] | Barley straw[4] | Hay A[4] | Hay B[5] |
| Num ber of determinations | 2 | 2 | 1 | 1 | 13 | 7 | 6 | 6 | 7 |
| DM, % of original substance | 90.5 | 90.3 | 87.9 | 88.3 | 61.1 | 32.2 | 89.9 | 89.3 | 88.1 |
| OM, % of DM | 95.6 | 95.9 | 93.4 | 96.9 | 86.9 | 96.1 | 93.6 | 93.8 | 90.0 |
| CP, % of DM | 15.9 | 14.8 | 52.3 | 14.2 | 16.9 | 7.5 | 2.8 | 7.1 | 16.2 |
| NDF, % of DM | 29.9 | 27.7 | 11.7 | 19.2 | 50.5 | 40.4 | 84.8 | 64.3 | 52.1 |
| ADF, % of DM | 18.6 | 15.7 | 8.3 | 7.2 | 29.0 | 24.2 | 55.3 | 38.9 | 29.4 |
| Total fatty acids, % of DM | 21.6 | 25.5 | 2.3 | 2.6 | 2.4 | 2.7 | 1.1 | 1.2 | 2.1 |
| Fatty acid profile, % of total fatty acid methyl esters | | | | | | | | | |
| C16:0 | 6.5 | 11.4 | 15.4 | 18.4 | 14.4 | 15.1 | 1.43 | 20.8 | 16.7 |
| C18:0 | 3.6 | 5.9 | 4.4 | 2.3 | 1.5 | 2.1 | 0.7 | 2.0 | 1.7 |
| C18:1n9 | 17.4 | 30.1 | 18.1 | 16.2 | 2.5 | 17.7 | 0.9 | 3.9 | 2.4 |
| C18:2n6 | 18.5 | 39.7 | 51.9 | 53.8 | 16.5 | 46.3 | 1.6 | 19.2 | 15.1 |
| C18:3n3 | 51.9 | 1.0 | 6.1 | 5.6 | 52.8 | 6.6 | 1.9 | 38.0 | 51.7 |
| $NE_L$, MJ/kg DM | 8.64 | 8.76 | 8.02 | 7.87 | 6.23 | 6.30 | 3.14 | 4.20 | 5.62 |

[1]Linseed, 50.8%, wheat straw meal, 32.4%, barley, 14.1%, molasses, 2.7% (as fed).

[2]Sunflower seed, 57.4%, barley, 39.9%, molasses, 2.7% (as fed).

[3]Supplemented with molasses (2.7%).

[4]Fed only during the dry period. Forage composed as grass silage, 49%, straw, 38% and hay A, 13% (on a DM basis) together with a mineral-vitamin mix consisting (per kg) of: Ca, 193 g; P, 56.8 g; Mg, 90.9 g, Na, 45.5 g, Zn, 4.5 g; Mn, 2.3 g; Cu, 0.6 g, Se, 34 mg; I, 23 mg; Co, 17 mg; vitamin A, 909,000 IU, vitamin D3, 136,000 IU; vitamin E, 1875 mg.

[5]Fed only from start of adaptation through early lactation. Forage composed as grass silage, 60%, corn silage, 30% and hay B, 10% (on a DM basis) together with 12g/kg of a mineral-vitamin mix consisting (per kg) of: Ca, 193 g; P, 56.8 g; Mg, 45.5 g, Na, 45.5 g, Zn, 3.4 g; Mn, 3.5 g; Cu, 0.9 g, Se, 17 mg; I, 23 mg; Co, 23 mg; vitamin A, 1,140,000 IU, vitamin D3, 227,000 IU; vitamin E, 1875 mg.

.

**Table 2**

Composition (g/100g DM) of the experimental diets as consumed (means ± SD). n=2 per treatment

| | Treatment[1] | | | |
|---|---|---|---|---|
| | S/S | LS/S | L/S | L/L |
| Dry period | | | | |
| OM | 91.4 ± 0.38 | 91.3 ± 0.44 | 91.4 ± 0.44 | 91.3 ± 0.30 |
| CP | 10.9 ± 1.04 | 11.0 ± 0.94 | 11.5 ± 0.69 | 11.5 ± 0.54 |
| Total fatty acids | 5.29 ± 0.282 | 4.82 ± 0.353 | 4.73 ± 0.471 | 4.72 ± 0.262 |
| C16:0 | 0.74 ± 0.189 | 0.60 ± 0.122 | 0.50 ± 0.028 | 0.49 ± 0.033 |
| C18:0 | 0.28 ± 0.048 | 0.21 ± 0.035 | 0.16 ± 0.016 | 0.16 ± 0.012 |
| C18:1n9 | 1.30 ± 0.327 | 0.93 ± 0.201 | 0.64 ± 0.095 | 0.64 ± 0.048 |
| C18:2n6 | 1.63 ± 0.968 | 1.16 ± 0.464 | 0.84 ± 0.094 | 0.84 ± 0.045 |
| C18:3n3 | 0.58 ± 0.049 | 1.38 ± 0.078 | 2.28 ± 0.249 | 2.28 ± 0.139 |
| $NE_L$, MJ/kg DM | 5.38 ± 0.261 | 5.36 ± 0.218 | 5.49 ± 0.125 | 5.50 ± 0.140 |
| Lactation period | | | | |
| OM | 91.0 ± 0.84 | 91.0 ± 0.98 | 91.0 ± 0.11 | 90.0 ± 1.36 |
| CP | 25.6 ± 1.84 | 25.0 ± 1.70 | 25.8 ± 0.16 | 25.2 ± 1.11 |
| Total fatty acids | 4.71 ± 0.421 | 4.51 ± 0.289 | 4.73 ± 0.065 | 4.11 ± 0.163 |
| C16:0 | 5.21 ± 0.055 | 0.52 ± 0.081 | 0.54 ± 0.073 | 0.46 ± 0.012 |
| C18:0 | 0.17 ± 0.010 | 0.16 ± 0.023 | 0.17 ± 0.029 | 0.11 ± 0.008 |
| C18:1n9 | 0.82 ± 0.047 | 0.7.7 ± 0.112 | 0.85 ± 0.143 | 0.58 ± 0.046 |
| C18:2n6 | 2.18 ± 0.626 | 2.00 ± 0.527 | 2.21 ± 0.702 | 1.07 ± 0.028 |
| C18:3n3 | 0.70 ± 0.077 | 0.73 ± 0.087 | 0.64 ± 0.046 | 1.67 ± 0.093 |
| $NE_L$, MJ/kg DM | 6.75 ± 0.014 | 6.80 ± 0.110 | 6.75 ± 0.102 | 6.73 ± 0.078 |

[1]S = sunflower seed concentrate, L = linseed concentrate; before/after calving.

**Table 3**

Realized intake (kg DM/head/d) of the experimental diets.

n=6 per group

| | Treatment (T)[1] | | | | |
|---|---|---|---|---|---|
| | S/S | LS/S | L/S | L/L | SE |
| Dry period | | | | | |
| Total | 12.0 | 12.6 | 12.0 | 11.8 | 0.06 |
| L concentrate | – | 0.91 | 1.82 | 1.81 | |
| S concentrate | 1.81 | 0.90 | – | – | |
| Lactation period | | | | | |
| Total | 17.0 | 19.5 | 18.8 | 20.7 | 1.44 |
| Forage | 11.7 | 13.7 | 12.8 | 14.5 | 1.03 |
| Concentrate | 5.31 | 5.87 | 6.01 | 6.21 | 0.531 |
| L concentrate | – | – | – | 1.71 | 0.151 |
| S concentrate | 1.59 | 1.64 | 1.71 | – | 0.092 |
| Soybean meal | 2.35 | 2.50 | 2.60 | 2.69 | 0.203 |
| Barley | 1.37 | 1.71 | 1.72 | 1.79 | 0.269 |

[1]S = sunflower seed concentrate, L = linseed concentrate; before/after calving.

**Table 4**

Blood plasma variables, body weight, body condition score and backfat thickness. n=6 per group

| | Week (W)[1] | Treatment (T)[2] S/S | LS/S | L/S | L/L | SE | Significance (*P*-value) T | W | T × W |
|---|---|---|---|---|---|---|---|---|---|
| Blood plasma variables | | | | | | | | | |
| Triglycerides, µmol/l | -6 | 248$^{z}$ | 235$^{z}$ | 300$^{z}$ | 292$^{z}$ | 16.1 | 0.113 | <0.001 | 0.052 |
| | -2 | 282$^{z}$ | 243$^{z}$ | 257$^{z}$ | 311$^{z}$ | | | | |
| | 3 | 139$^{y}$ | 116$^{y}$ | 137$^{y}$ | 137$^{y}$ | | | | |
| | 6 | 126$^{y}$ | 148$^{y}$ | 138$^{y}$ | 155$^{y}$ | | | | |
| NEFA, µmol/l | -6 | 129$^{y}$ | 117$^{y}$ | 161$^{y}$ | 133 | 88.4 | 0.147 | <0.001 | 0.509 |
| | -2 | 371$^{z}$ | 288$^{z}$ | 464$^{yz}$ | 284 | | | | |
| | 3 | 450$^{z}$ | 294$^{z}$ | 671$^{z}$ | 310 | | | | |
| | 6 | 334$^{yz}$ | 199$^{yz}$ | 302$^{yz}$ | 172 | | | | |
| BHBA, mmol/l | -6 | 0.40$^{y}$ | 0.36$^{y}$ | 0.34 | 0.34 | 0.312 | 0.347 | <0.001 | 0.676 |
| | -2 | 0.45$^{y}$ | 0.44$^{y}$ | 0.47 | 0.36 | | | | |
| | 3 | 2.03$^{z}$ | 1.77$^{z}$ | 1.33 | 1.12 | | | | |
| | 6 | 1.23$^{yz}$ | 0.72$^{yz}$ | 1.14 | 0.73 | | | | |
| Leptin, ng/ml | -6 | 3.99 | 3.09$^{z}$ | 3.26 | 2.64$^{y}$ | 0.622 | 0.608 | 0.046 | 0.173 |
| | -2 | 3.58 | 1.98$^{y}$ | 3.34 | 2.98$^{yz}$ | | | | |
| | 3 | 3.74 | 2.74$^{z}$ | 3.76 | 3.52$^{z}$ | | | | |
| | 6 | 3.76 | 3.10$^{z}$ | 3.67 | 3.23$^{yz}$ | | | | |
| Glucose, mmol/l | -6 | 3.68$^{z}$ | 3.55 | 3.58$^{yz}$ | 3.67 | 0.163 | 0.080 | <0.001 | 0.521 |
| | -2 | 3.70$^{z}$ | 3.66 | 3.77$^{z}$ | 3.87 | | | | |
| | 3 | 2.74$^{y}$ | 2.88 | 2.98$^{x}$ | 3.43 | | | | |
| | 6 | 3.01$^{yz}$ | 3.45 | 3.21$^{xy}$ | 3.44 | | | | |
| Insulin, pg/ml | -6 | 16.6$^{z}$ | 17.5 | 13.9$^{z}$ | 15.9 | 2.07 | 0.286 | <0.001 | 0.402 |
| | -2 | 14.8$^{z}$ | 15.0 | 18.4$^{z}$ | 17.2 | | | | |
| | 3 | 9.6$^{y}$ | 12.5 | 9.2$^{y}$ | 12.2 | | | | |
| | 6 | 7.5$^{y}$ | 15.3 | 9.1$^{y}$ | 12.0 | | | | |
| IGF-I, ng/ml | -6 | 167$^{z}$ | 157$^{z}$ | 168$^{z}$ | 180$^{z}$ | 18.9 | 0.591 | <0.001 | 0.768 |
| | -2 | 140$^{z}$ | 105$^{yz}$ | 124$^{yz}$ | 160$^{yz}$ | | | | |
| | 3 | 56$^{y}$ | 67$^{y}$ | 55$^{y}$ | 73$^{x}$ | | | | |
| | 6 | 61$^{y}$ | 63$^{y}$ | 82$^{y}$ | 91$^{xy}$ | | | | |
| Body weight,kg | -6 | 777$^{z}$ | 770$^{z}$ | 779$^{z}$ | 762$^{z}$ | 31.3 | 0.998 | <0.001 | 0.530 |
| | -2 | 779$^{z}$ | 776$^{z}$ | 765$^{z}$ | 768$^{z}$ | | | | |
| | 3 | 664$^{y}$ | 666$^{y}$ | 656$^{y}$ | 682$^{y}$ | | | | |
| | 6 | 652$^{y}$ | 672$^{y}$ | 661$^{y}$ | 680$^{y}$ | | | | |
| Body condition score | -6 | 3.22$^{z}$ | 2.96 | 3.16$^{z}$ | 3.33 | 0.189 | 0.245 | 0.057 | <0.001 |
| | -2 | 3.24$^{z}$ | 2.99 | 2.98$^{yz}$ | 3.37 | | | | |
| | 3 | 2.87$^{yz}$ | 2.85 | 2.50$^{xy}$ | 3.24 | | | | |
| | 6 | 2.67$^{y}$ | 2.80 | 2.48$^{x}$ | 3.14 | | | | |
| Backfat thickness, cm | | | | | | | | | |
| Hip region | -6 | 3.89$^{z}$ | 3.16 | 3.72$^{z}$ | 3.69 | 0.254 | 0.194 | 0.261 | <0.001 |
| | -2 | 3.92$^{z}$ | 3.09 | 3.41$^{yz}$ | 3.46 | | | | |
| | 3 | 3.45$^{yz}$ | 2.88 | 2.92$^{xy}$ | 3.41 | | | | |
| | 6 | 3.10$^{y}$ | 2.72 | 2.68$^{x}$ | 3.19 | | | | |
| Lumbar region | -6 | 4.08 | 3.44 | 3.61 | 4.36 | 0.382 | 0.437 | 0.193 | 0.024 |
| | -2 | 4.04 | 3.67 | 3.85 | 3.91 | | | | |
| | 3 | 3.84 | 3.45 | 3.45 | 4.47 | | | | |
| | 6 | 3.55 | 3.42 | 3.17 | 4.01 | | | | |

[y–z]Mean values within the same column and variable sharing no common superscript are significantly different ($P < 0.05$).

[1]Week ante- and post-partum.

[2]S = sunflower seed concentrate, L = linseed concentrate; before/after calving.

**Table 5**

Fatty acid profile[1] in adipose tissue biopsies (% of total fatty acid methyl esters)

| | Week (W)[2] | Treatment (T)[3] S/S n=5 | LS/S n=6 | L/S n=5 | L/L n=6 | SE | Significance (*P*-value) T | W | T × W |
|---|---|---|---|---|---|---|---|---|---|
| Total SFA | -2 | 39.9 | 39.5 | 41.6 | 37.9 | 1.90 | 0.522 | 0.256 | 0.983 |
| | 6 | 40.6 | 40.3 | 42.6 | 39.4 | | | | |
| Total BFA | -2 | 1.56 | 1.83 | 1.42 | 1.74 | 0.123 | 0.095 | 0.032 | 0.808 |
| | 6 | 1.72 | 1.88 | 1.59 | 1.83 | | | | |
| Total MUFA | -2 | 53.9 | 53.7 | 52.5 | 55.3 | 1.84 | 0.700 | 0.066 | 0.907 |
| | 6 | 53.2 | 52.6 | 50.7 | 53.3 | | | | |
| Total PUFA | -2 | 2.45 | 2.73 | 2.41 | 2.75 | 0.443 | 0.729 | 0.143 | 0.593 |
| | 6 | 2.37 | 3.08 | 3.27 | 3.10 | | | | |
| C18:0 | -2 | 9.93 | $10.81^{y}$ | $10.12^{y}$ | $7.87^{y}$ | 1.396 | 0.271 | <0.001 | 0.041 |
| | 6 | 10.48 | $14.06^{z}$ | $13.86^{z}$ | $10.59^{z}$ | | | | |
| C18:1*c*9 | -2 | 40.9 | 41.8 | 40.9 | 41.0 | 1.51 | 0.961 | 0.122 | 0.867 |
| | 6 | 41.4 | 42.4 | 42.6 | 42.5 | | | | |
| C18:1*t*11 | -2 | 2.78 | 2.38 | 1.84 | 2.25 | 0.311 | 0.264 | 0.229 | 0.817 |
| | 6 | 2.49 | 2.27 | 1.86 | 2.03 | | | | |
| C18:2n-6 | -2 | 1.16 | 1.28 | $1.18^{z}$ | 1.17 | 0.332 | 0.752 | 0.034 | 0.476 |
| | 6 | 1.17 | 1.63 | $1.98^{y}$ | 1.65 | | | | |
| C18:2*c*9*t*11 | -2 | 0.548 | 0.669 | 0.453 | 0.668 | 0.0826 | 0.257 | 0.121 | 0.682 |
| | 6 | 0.541 | 0.537 | 0.423 | 0.552 | | | | |
| C18:3n-3 | -2 | $0.392^{b}$ | $0.536^{a}$ | $0.529^{a,z}$ | $0.573^{a}$ | 0.0446 | 0.010 | <0.001 | 0.023 |
| | 6 | $0.315^{c}$ | $0.450^{b}$ | $0.334^{c,y}$ | $0.521^{a}$ | | | | |
| C20:4n-6 | -2 | 0.019 | 0.032 | 0.016 | 0.022 | 0.0078 | 0.638 | 0.142 | 0.388 |
| | 6 | 0.020 | 0.030 | 0.031 | 0.032 | | | | |
| C20:5n-3 | -2 | 0.011 | 0.014 | 0.009 | 0.012 | 0.0081 | 0.510 | 0.455 | 0.180 |
| | 6 | 0.009 | 0.007 | 0.034 | 0.014 | | | | |
| C22:6n-3 | -2 | 0.026 | 0.015 | 0.023 | 0.019 | 0.0122 | 0.195 | 0.316 | 0.240 |
| | 6 | 0.019 | 0.014 | 0.059 | 0.024 | | | | |
| n-6/n-3 ratio | -2 | 3.01 | 2.49 | $2.21^{z}$ | 2.08 | 0.588 | 0.738 | 0.001 | 0.173 |
| | 6 | 3.51 | 3.25 | $4.27^{y}$ | 3.01 | | | | |

[a-c] Mean values within the same row sharing no common superscript are significantly different ($P < 0.05$).

[y–z] Mean values within the same column and variable sharing no common superscript are significantly different ($P < 0.05$).

[1] Total means that all peaks were considered. SFA = saturated fatty acids; BFA = branched-chain fatty acids (contains C15aiso, C15iso, C16iso, C17aiso, C17iso, C18aiso, C18iso); MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.

[2] Week ante- and post-partum.

[3] S = sunflower seed concentrate, L = linseed concentrate; before/after calving.

**Table 6**

Milk yield and composition. n=6 per group.

| | | Treatment (T)[1] | | | | | Significance (*P*-value) | | |
|---|---|---|---|---|---|---|---|---|---|
| | DIM | S/S | LS/S | L/S | L/L | SE | T | DIM | T×DIM |
| ECM, kg/head/d | 10 | 38.4 | 34.6 | 38.2 | 37.6 | 2.99 | 0.847 | 0.008 | 0.511 |
| | 20 | 39.2 | 36.5 | 38.5 | 38.2 | | | | |
| | 30 | 33.0 | 35.5 | 41.8 | 36.5 | | | | |
| | 40 | 33.0 | 33.1 | 33.0 | 33.2 | | | | |
| Fat, % | 10 | 4.98 | 4.34 | $5.08^{z}$ | $4.81^{z}$ | 0.275 | 0.099 | <0.001 | 0.899 |
| | 20 | 4.65 | 3.90 | $4.27^{yz}$ | $4.20^{yz}$ | | | | |
| | 30 | 4.39 | 3.54 | $3.96^{y}$ | $3.78^{yz}$ | | | | |
| | 40 | 4.36 | 3.76 | $3.90^{y}$ | $3.71^{y}$ | | | | |
| Protein, % | 10 | $3.94^{z}$ | $3.71^{z}$ | $3.89^{z}$ | $3.94^{z}$ | 0.125 | 0.430 | <0.001 | 0.885 |
| | 20 | $3.27^{y}$ | $3.13^{y}$ | $3.13^{y}$ | $3.26^{y}$ | | | | |
| | 30 | $3.08^{y}$ | $2.97^{y}$ | $2.93^{y}$ | $3.14^{y}$ | | | | |
| | 40 | $2.93^{y}$ | $2.88^{y}$ | $2.89^{y}$ | $3.13^{y}$ | | | | |
| Lactose, % | 10 | 4.66 | 4.86 | 4.74 | $4.85^{y}$ | 0.070 | 0.008 | <0.001 | 0.121 |
| | 20 | 4.81 | 5.01 | 4.93 | $5.04^{yz}$ | | | | |
| | 30 | $4.72^{b}$ | $5.00^{ab}$ | $4.94^{ab}$ | $5.08^{a,yz}$ | | | | |
| | 40 | $4.76^{b}$ | $4.81^{ab}$ | $4.83^{ab}$ | $5.11^{a,z}$ | | | | |

$^{a–b}$Mean values within row and $^{y–z}$mean values within the same column and variable sharing no common superscript are significantly different ($P < 0.05$).

[1]S = sunflower seed concentrate, L = linseed concentrate; before/after calving.

**Table 7**

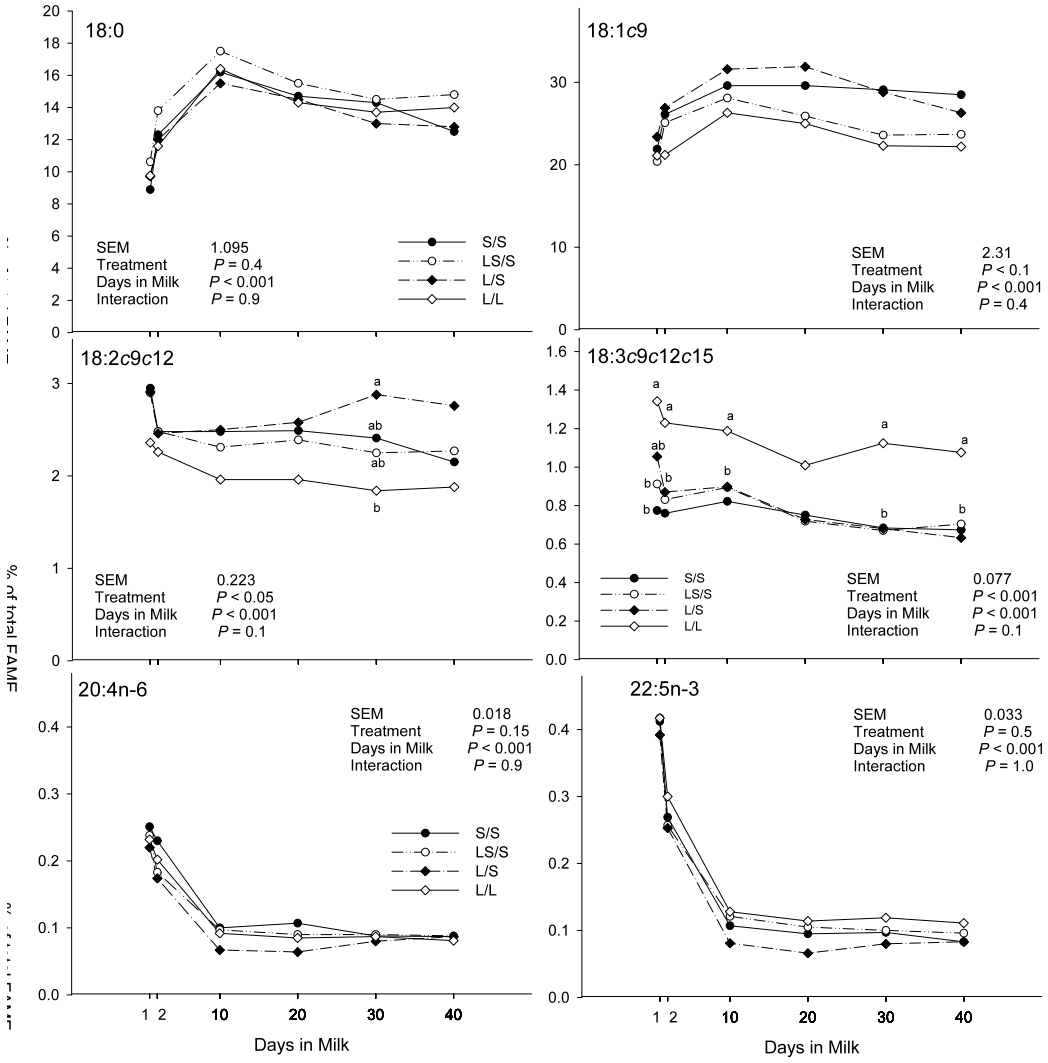
Main groups of fatty acids[1] in milk fat (% of total fatty acid methyl esters). n=6 per group

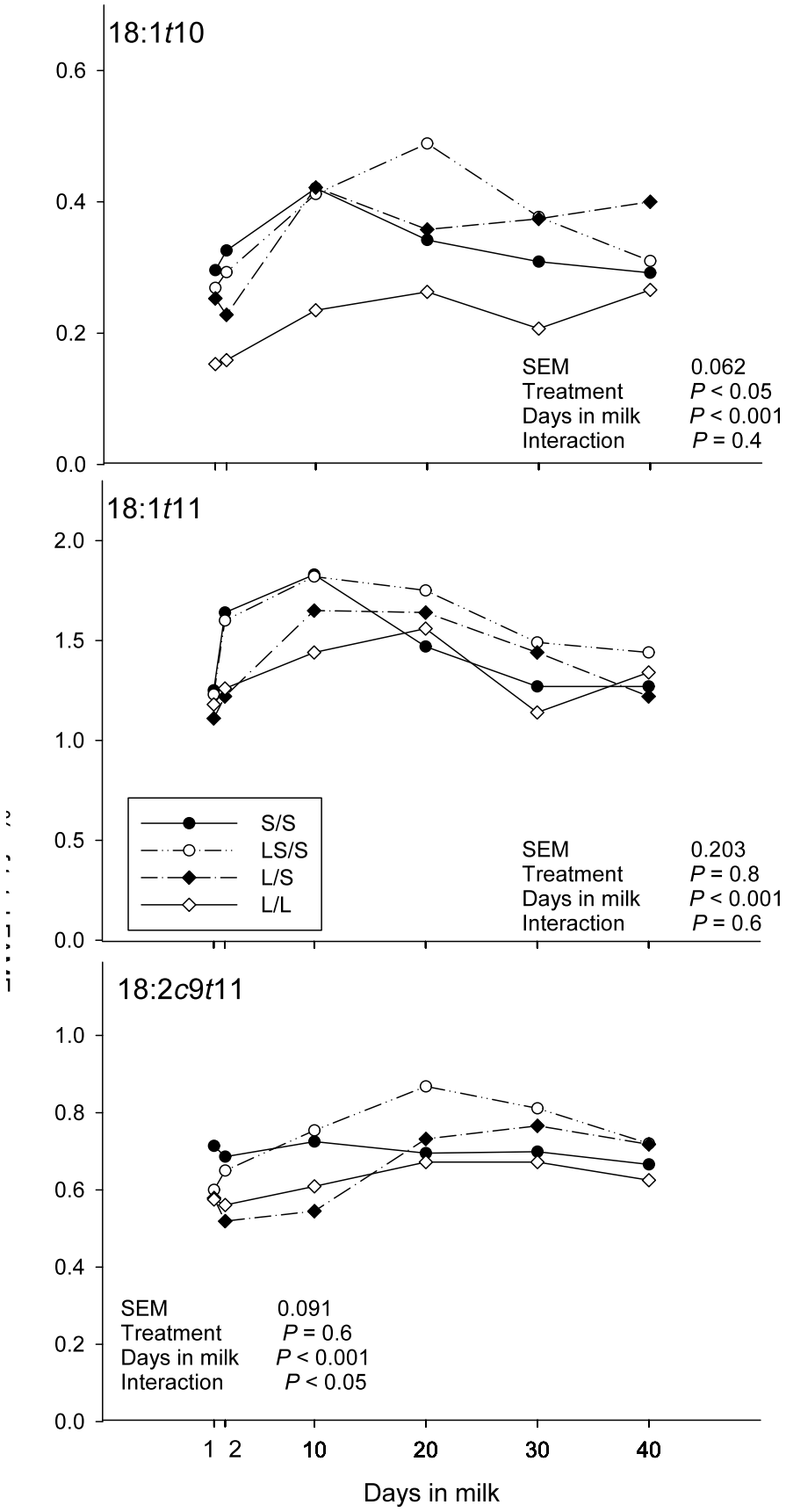
| | | Treatment (T)[2] | | | | | Significance (*P*-value) | | |
|---|---|---|---|---|---|---|---|---|---|
| | DIM | S/S | LS/S | L/S | L/L | SE | T | DIM | T×DIM |
| Total SMC-SFA | 1 | 19.3 | $19.7^{yz}$ | $18.1^{yz}$ | $19.5^{yz}$ | 1.71 | 0.027 | <0.001 | 0.102 |
| | 2 | 16.3 | $16.5^{y}$ | $15.1^{xyz}$ | $20.1^{yz}$ | | | | |
| | 10 | 14.1 | $14.6^{y}$ | $11.6^{x}$ | $17.6^{y}$ | | | | |
| | 20 | 16.1 | $18.7^{yz}$ | $13.5^{xy}$ | $19.9^{yz}$ | | | | |
| | 30 | 17.4 | $22.3^{z}$ | $17.6^{yz}$ | $23.9^{z}$ | | | | |
| | 40 | 18.6 | $21.9^{z}$ | $20.1^{z}$ | $22.5^{yz}$ | | | | |
| Total LC-SFA | 1 | $40.6^{z}$ | $42.6^{z}$ | $40.5^{z}$ | $41.6^{z}$ | 1.11 | 0.445 | <0.001 | 0.800 |
| | 2 | $39.4^{yz}$ | $41.2^{yz}$ | $41.2^{z}$ | $41.4^{z}$ | | | | |
| | 10 | $37.8^{yz}$ | $39.2^{xyz}$ | $39.2^{yz}$ | $38.5^{yz}$ | | | | |
| | 20 | $36.6^{y}$ | $37.1^{x}$ | $36.4^{xy}$ | $36.5^{y}$ | | | | |
| | 30 | $36.4^{y}$ | $36.9^{x}$ | $35.2^{x}$ | $36.5^{y}$ | | | | |
| | 40 | $35.8^{y}$ | $37.6^{xy}$ | $35.5^{xy}$ | $37.3^{y}$ | | | | |
| Total BFA | 1 | $1.13^{x}$ | $1.24^{x}$ | $1.12^{x}$ | $1.29^{x}$ | 0.106 | 0.082 | <0.001 | 0.991 |
| | 2 | $1.23^{xy}$ | $1.39^{xy}$ | $1.18^{xy}$ | $1.40^{xy}$ | | | | |
| | 10 | $1.43^{yz}$ | $1.57^{yz}$ | $1.32^{xyz}$ | $1.65^{yz}$ | | | | |
| | 20 | $1.50^{yz}$ | $1.63^{yz}$ | $1.37^{xyz}$ | $1.65^{yz}$ | | | | |
| | 30 | $1.55^{z}$ | $1.71^{z}$ | $1.44^{yz}$ | $1.78^{z}$ | | | | |
| | 40 | $1.60^{z}$ | $1.75^{z}$ | $1.57^{z}$ | $1.77^{z}$ | | | | |
| Total MUFA | 1 | $29.2^{y}$ | $27.3^{y}$ | $30.6^{y}$ | 27.9 | 2.45 | 0.054 | <0.001 | 0.391 |
| | 2 | $34.1^{yz}$ | $32.7^{yz}$ | $34.5^{yz}$ | 28.0 | | | | |
| | 10 | $38.4^{z}$ | $36.2^{z}$ | $40.3^{z}$ | 33.7 | | | | |
| | 20 | $37.5^{z}$ | $33.9^{yz}$ | $40.5^{z}$ | 32.9 | | | | |
| | 30 | $36.3^{z}$ | $30.4^{yz}$ | $36.9^{yz}$ | 28.6 | | | | |
| | 40 | $36.0^{yz}$ | $30.5^{yz}$ | $34.1^{yz}$ | 29.3 | | | | |
| Total PUFA | 1 | $5.66^{z}$ | $5.64^{z}$ | $5.73^{z}$ | $5.51^{z}$ | 0.342 | 0.913 | <0.001 | 0.646 |
| | 2 | $4.87^{yz}$ | $4.80^{yz}$ | $4.70^{yz}$ | $5.01^{yz}$ | | | | |
| | 10 | $4.44^{xyz}$ | $4.38^{y}$ | $4.24^{y}$ | $4.19^{y}$ | | | | |
| | 20 | $4.32^{xy}$ | $4.36^{y}$ | $4.32^{y}$ | $4.05^{y}$ | | | | |
| | 30 | $4.17^{xy}$ | $4.11^{y}$ | $4.66^{yz}$ | $4.05^{y}$ | | | | |
| | 40 | $3.83^{x}$ | $4.07^{y}$ | $4.47^{y}$ | $3.99^{y}$ | | | | |
| n-6/n-3 ratio | 1 | $2.27^{y}$ | $1.95^{y}$ | $1.85^{x}$ | 1.27 | 0.287 | <0.001 | <0.001 | <0.001 |
| | 2 | $2.28^{y}$ | $2.06^{y}$ | $1.97^{x}$ | 1.38 | | | | |
| | 10 | $2.82^{yz}$ | $2.62^{yz}$ | $2.61^{xy}$ | 1.64 | | | | |
| | 20 | $3.21^{a,z}$ | $3.24^{a,z}$ | $3.28^{a,yz}$ | $2.01^{b}$ | | | | |
| | 30 | $3.39^{a,z}$ | $3.34^{a,z}$ | $3.83^{a,z}$ | $1.83^{b}$ | | | | |
| | 40 | $3.15^{a,z}$ | $3.22^{a,z}$ | $3.85^{a,z}$ | $1.82^{b}$ | | | | |

[a–b]Mean values within row and [x–z]mean values within the same column and variable sharing no common superscript are significantly different ($P < 0.05$).

[1]Total means that all peaks were considered. SMC-SFA = short- and medium-chain saturated fatty acids (<C16); LC-SFA = long-chain saturated fatty acids (≥C16); BFA = branched-chain fatty acids (contains C15aiso, C15iso, C16iso, C17aiso, C17iso, C18aiso, C18iso); MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.

[2]S = sunflower seed concentrate, L = linseed concentrate; before/after calving.


18:0
SEM 1.095
Treatment P = 0.4
Days in Milk P < 0.001
Interaction P = 0.9
S/S
LS/S
L/S
L/L
18:1c9
SEM 2.31
Treatment P < 0.1
Days in Milk P < 0.001
Interaction P = 0.4
18:2c9c12
SEM 0.223
Treatment P < 0.05
Days in Milk P < 0.001
Interaction P = 0.1
18:3c9c12c15
SEM 0.077
Treatment P < 0.001
Days in Milk P < 0.001
Interaction P = 0.1
20:4n-6
SEM 0.018
Treatment P = 0.15
Days in Milk P < 0.001
Interaction P = 0.9
22:5n-3
SEM 0.033
Treatment P = 0.5
Days in Milk P < 0.001
Interaction P = 1.0
% of total FAME
Days in Milk


18:1t10
0.6
0.4
0.2
0.0
SEM 0.062
Treatment P < 0.05
Days in milk P < 0.001
Interaction P = 0.4
18:1t11
2.0
1.5
1.0
0.5
0.0
S/S
LS/S
L/S
L/L
SEM 0.203
Treatment P = 0.8
Days in milk P < 0.001
Interaction P = 0.6
18:2c9t11
1.0
0.8
0.6
0.4
0.2
0.0
SEM 0.091
Treatment P = 0.6
Days in milk P < 0.001
Interaction P < 0.05
1 2 10 20 30 40
Days in milk

581