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Effects of rapeseed and soybean oil dietary supplementation on bovine fat metabolism, fatty acid composition and cholesterol levels in milk

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The main goal of this experiment was to study the effect of milk fat depression, induced by supplementing diet with plant oils, on the bovine fat metabolism, with special interest in cholesterol levels. For this purpose 39 cows were divided in three groups and fed different rations: a control group (C) without any oil supplementation and two groups with soybean oil (SO) or rapeseed oil (RO) added to the partial mixed ration (PMR). A decrease in milk fat percentage was observed in both oil feedings with a higher decrease of − 1·14 % with SO than RO with − 0·98 % compared with the physiological (− 0·15 %) decline in the C group. There was no significant change in protein and lactose yield. The daily milk cholesterol yield was lower in both oil rations than in control ration, while the blood cholesterol level showed an opposite variation. The milk fatty acid pattern showed a highly significant decrease of over 10 % in the amount of saturated fatty acids (SFA) in both oil feedings and a highly significant increase in mono (MUFA) and poly (PUFA) unsaturated fatty acids, conjugated linoleic acids (CLA) included. The results of this experiment suggest that the feeding of oil supplements has a high impact on milk fat composition and its significance for human health, by decreasing fats with a potentially negative effect (SFA and cholesterol) while simultaneously increasing others with positive (MUFA, PUFA, CLA).

Keywords: Milk fat depression, cholesterol, fatty acid, soy oil, rapeseed oil.

Milk and milk products are main components of modern human nutrition and are deeply implanted into culture, especially in western countries. The relatively high level of saturated fats and the frequency of their consumption have attracted the attention of the scientific community in the last decades (Menotti et al. 1999; Malpuech-Brugere et al. 2010). Although milk products are considered among the aliments with a neutral or positive effect on cardiovascular health, the frequency of their consumption causes the amount of cholesterol ingested from this group of aliments to account for almost the half of the recommended amount of 300 mg cholesterol/d (milk contains a mean value of 15 mg/dl cholesterol) (Royo-Bordonada et al. 2003; Viturro et al. 2006a). Cholesterol is a central molecule in life as a substrate for steroid hormones and cellular membranes, but when present in high concentrations in blood is also a mediator in arteriosclerosis and cardiovascular diseases (Ren et al. 2010; Weingartner et al. 2011).

In the last years, significant advances have been made in understanding the bovine cholesterol metabolism and its transport into milk (Farke et al. 2006; Viturro et al. 2006b). Together with animal breeding, a suitable first attempt of alteration of milk cholesterol levels might come from feeding management. Diverse effects of different oils in the feeding ration on milk composition have already been outlined by many studies and reviews (Glasser et al. 2008; Huang et al. 2008; Rego et al. 2009), but little is known about the impact of these supplements on bovine cholesterol metabolism. Some efforts in this direction have already been made by Reklewska et al. (2002) by feeding linseed combined with a mineral mixture, but more research with other plant supplements is needed. The supplements usually employed in these experiments are rich in polyunsaturated fatty acids, molecules well known to exert cholesterol-lowering properties in other

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species (Connor, 2000; Haug et al. 2007), so their potential to positively alter milk cholesterol concentration is very high.

Simultaneously to reducing cholesterol levels, oil supplements may favourably change the fatty acid composition of milk by lowering the amount of saturated fatty acids and increasing the proportion of mono- and polyunsaturated fatty acids (Jacobs et al. 2011). Special attention is given in the last years to the unsaturated fatty acids present in milk: while various trans fatty acids (**TFA**) consumed in high doses may have adverse effects on plasma lipids and other cardiovascular risk factors (Sun et al. 2007; Motard-Bélanger et al. 2008), conjugated linoleic acids (**CLA**) have antiatherogenic and anticancerogenic impacts (Lock & Bauman, 2004).

The present work analyses the potential of soybean oil and rapeseed oil supplementation for altering bovine cholesterol metabolism and reducing the concentration of this metabolite in milk based on the hypothesis that diet-induced milk fat depression may also have an influence on the cholesterol level due to a changed blood and milk composition. Results presented in this study may serve for further research in production of 'low fat, low cholesterol milk' as an alternative to conventional milk products.

Material and methods

Animals and experimental design

The present study was performed at the research farm Hirschau of the Technische Universitaet Muenchen (TUM, 85354 Freising, Germany) from January 2011 to April 2012. 39 multiparous Holstein cows (3·39 ± 0·205, mean lactation number ± SEM) with a mean lactation yield of 30·69 ± 9·77 kg were examined. The study aimed at the comparison between three diets: soybean oil-supplemented ration (**SO**), rapeseed oil-supplemented ration (**RO**) and a control ration (**C**). The length of the feeding phases was four weeks and all rations were isoenergetic in order to avoid misinterpretation of results (Table 1). A washout period of two weeks, in which the animals received control ration, was included between all experimental feeding phases. The duration of two weeks for the washout was fixed taking the literature on the topic into account, which quotes 10 to 14 d as sufficient (Collomb et al. 2004; Zened et al. 2012). Due to practical reasons (i.e. insufficient farm personnel), a plan with alternate four-weeks feeding periods was designed in which all animals in the farm rotated simultaneously through the same feeding plan. Individuals were afterwards classified into a concrete experimental group depending on their lactation stage and the ration given at each moment. Three time periods were considered: early (days in milk (**DiM**) 1–100), mid (DiM 101–200), and late (DiM 201–300) phase (Table 1). Only the individuals which could be clearly assigned to a specific time group were considered for the analysis; those whose start and end day spanned between two different time groups were excluded to avoid misclassification of data. The result was therefore a different but comparable number of individuals for each of the nine feed-lactation stage combinations (Table 1). The department responsible for animal welfare affairs approved all experiments.

Table 1. Ingredients and chemical composition of control diet (C) or treatment diets with supplemented soybean oil (SO) or rapeseed oil (RO) for a 20 kg milk yield

Item	C	SO	RO
Cows assigned to study, *N*	37	39	34
Early (0–100 DiM)	12	14	10
Mid (101–200 DiM)	12	15	16
Late (201–300 DiM)	13	10	8
Parity	3·39	3·05	3·18
Mean days in milk†	134·2 ± 14·2	126·0 ± 12·9	125·7 ± 13·2
Ingredient, kg DM			
Grass silage	6·12	6·12	6·12
Corn silage	5·96	5·96	5·96
Barley straw	0·26	0·26	0·26
Hay	0·86	0·86	0·86
Salt	0·04	0·04	0·04
Soy meal	1·58	1·76	1·76
Wheat meal	2·29		
Soybean oil		0·83	
Rapeseed oil			0·83
Feed lime	0·07	0·07	0·07
MILKINAL‡	0·10	0·13	0·13
SINCROPAC‡	0·10	0·15	0·15
NEL MJ/kg§	6·7	7·0	7·0

† Mean ± SEM
‡ Product of Trouw Nutrition Deutschland GmbH (Burgheim, Germany)
§ 1 Mega Joule (MJ) = 238·845896627 kcal

Housing and feeding

Cows were kept in a free stall barn with rubber mat bedding and the exercise areas had a solid floor surface and a scrapper system for waste removal. Cows were milked twice daily at 5 am and 4 pm in a double six herringbone milking parlour (DeLaval GmbH, 21509 Glinde, Germany). Six weeks before expected calving, cows were dried off using an antibiotic drying supplement (Orbenin, Pfizer, 10785 Berlin, Germany) and a non-antibiotic intramammary seal (OrbeSeal, Pfizer, 10785 Berlin, Germany). Cows were fed a partial mixed ration (**PMR**) calculated on a minimal milk yield of 22 kg (Table 1) with additional concentrate feeding according to individual milk yield, with a maximum of 8 kg per d at the beginning of lactation. Concentrate was given through an automatic dispenser with an integrated body weighing system and the animals had ad libitum access to fresh water. Cows were fed daily at 9 am with continuous access to food and automatic recording of the amounts consumed at each time by an electronic weighing system integrated to the scale pans.

Sampling

PMR Samples were collected weekly for DM and composition analysis. Milk samples were collected twice a week

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during the whole experiment. At the sampling days, aliquots of both morning and evening milking were obtained and mixed according to the milk yield to get a representative sample for the complete day and avoid composition differences between morning and evening milkings. Samples were sent to MPR Bayern e.V. (accredited laboratory after DIN EN ISO/IEC 17025 and DIN EN ISO 9001 certified, 85283 Wolnzach, Germany) for analysis of major milk components and several aliquots were kept at −20 °C for posterior analysis of cholesterol level and fatty acid composition.

Blood samples were taken in the morning of days 1 and 28 of each feeding period representing the 'before' and 'after' feeding situation. Samples were gained by puncturing the jugular vein, collected in K2EDTA tubes (Vacuette, Greiner Bio-One, 4550 Kremsmünster, Austria) and immediately centrifuged at 4500 rpm for 15 min. Plasma aliquots were frozen in Eppendorf tubes at −80 °C until further analysis.

Milk yield, feed intake and body weight were automatically measured and recorded daily until the end of the project for statistical evaluation. Daily milk yield data was transformed into energy corrected milk yield (**ECM**) according to the formula: ECM (kg/d) = [(0·38 × fat% + 0·21 × protein% + 1·05)/3·28] × milk yield (kg/d) (Schlamberger et al. 2010).

Analysis

Analysis of PMR composition was performed at LKS-Landwirtschaftliche Kommunikations- und Service-GmbH (accredited laboratory after DIN EN ISO/IEC 17025 and DIN ISO 9001 certified, 09577 Lichtenwalde, Germany) using Weender analysis (Table 1).

Quantification of milk fat, protein, lactose and urea by infrared spectrophotometry was performed at MPR Bayern e.V. laboratories (85283 Wolnzach, Germany) using a MilkoScan-FT-6000 spectrometer (Foss, 3400 Hillerod, Denmark). Cholesterol measurement in milk was performed after a previously approved colorimetric method (Viturro et al. 2010). Plasma cholesterol analysis was performed by synlab.vet GmbH (86156 Augsburg, Germany) using photometry. Blood beta-hydroxy-butyric-acid (**BHBA**), non-esterified-fatty-acids (**NEFA**), glucose and triglyceride levels were measured at the Clinic for Ruminants of the Ludwig-Maximilians-University Munich (80539 Munich, Germany). A Roche C311 auto analyser system (Roche Deutschland Holding GmbH, 79639 Grenzach-Wyhlen, Germany) was used with a Randox NEFA Kit for measuring NEFAs and a RANBUT D-3-Hydroxybutyrate Kit for BHBA measurement (Randox Laboratories Ltd, BT294QY Crumlin, UK), a GPO-PAP Kit (LT-SYS, Eberhard Lehmann GmbH, 14167 Berlin, Germany) for triglycerides, and a COBAS GLUH2 Kit (Roche Deutschland Holding GmbH, 79639 Grenzach-Wyhlen, Germany) for glucose level determination. Fatty acid profiling was performed by gas-chromatography (GC) at the Institute of Bioanalytics (Technische Universität München, 85354 Freising, Germany) using a Hewlett Packard GC Flame Ionisation Detector (FID) (HP/Agilent 6890 Series, Agilent Technologies, 95051 Santa Clara California, USA), equipped with a Hewlett Packard Injector (HP 7683 series), an Agilent Auto Sampler (7683 series) and a WCOT Fused Silica capillary column (select fame Varian cp 7420) (100 m × 0·25 mm ID), with hydrogen (40·0 ml/min) as carrier gas and nitrogen (30·0 ml/min) as make-up gas (Agilent Technologies, 95051 Santa Clara California, USA). Chromatograms were evaluated with Chromeleon 6.80 software with a 1.5 percentage of unidentified peaks.

Statistics

Statistical analysis was performed with SPSS 19 software (IBM Deutschland GmbH, 71139 Ehningen, Germany). Dry matter intake data is presented as the difference between the first two and the last 2 d of the feeding period. ECM, fat content, milk cholesterol, fatty acid content and blood composition is reported as difference between days 1 and 28 of the feeding period, respectively. Body weight data was pooled to weekly means (week 1 compared with week 4) before statistical analysis. Data were analysed using *t*-tests and Levene's test for equality of variances.

Results

Feeding and body weight

C, SO, and RO had comparable protein and energy levels. Starch level of C was higher compared with SO and RO due to calculated energy compensation of fat supplement in SO and RO (Table 1). The different rations offered had no significant effect on the daily feed intake and body weight during the 4 week feeding periods (Table 2).

Plasma composition

NEFA, BHBA and glucose contents are presented in Table 2. Blood plasma showed a significant increase in BHBA level in SO ($P < 0·05$). All other components measured had no significant change between beginning and end of feeding period compared with each other.

Milk yield and milk composition

Feeding of oil supplemented rations resulted on a highly significant reduction in milk fat percentage and milk fat yield compared with C (Table 2). This difference was maintained when analysing mid and late lactating animals separately ($P < 0·05$, Fig. 1b, c) but not for early lactating cows (Fig. 1a) with a significantly higher reduction in mid lactating cows fed SO than RO ($P < 0·05$). There was no significant difference in ECM, protein and lactose yield of oil feedings and control feeding, except late lactating cows fed SO had a significant reduction in milk yield compared with C ($P < 0·05$) (Table 2, Fig. 1c).

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Table 2. Feed intake, body weight, milk and blood composition(mean ± SEM) changes (value at end – value at beginning of feeding period) compared among treatment groups (Control (CO), soybean oil (SO), rapeseed oil (RO)). Groups with different letter superscripts are significantly different ($P<0·05$)

Treatment group	C	SO	RO
PMR intake (kg DM)	−0·5 ± 0·87	0·75 ± 0·84	−1·29 ± 0·49
Body weight (kg)	−2·76 ± 3·62	−4·30 ± 2·15	1·90 ± 3·63
ECM (kg)	−1·71 ± 0·99	−3·06 ± 0·98	−3·06 ± 0·69
Fat (%)	−0·15 ± 0·15^{a}	−1·14 ± 0·13^{b}	−0·88 ± 0·1^{b}
Fat (g/d)	−72·32 ± 36·69^{a}	−303·75 ± 57·36^{b}	−257·35 ± 35·98^{b}
Protein (%)	−0·2 ± 0·14	−0·16 ± 0·06	−0·15 ± 0·07
Protein (g/d)	−49·36 ± 48·11	−17·61 ± 25·02	−37·74 ± 23·71
Lactose (%)	0·09 ± 0·08	−0·02 ± 0·02	0·04 ± 0·02
Milk cholesterol (mmol/l)	−0·02 ± 0·04^{a}	−0·17 ± 0·03^{b}	−0·11 ± 0·03^{ab}
Blood cholesterol (mmol/l)	0·1 ± 0·17^{a}	1·48 ± 0·2^{b}	0·68 ± 0·07^{c}
Blood NEFA	−0·04 ± 0·02	−0·03 ± 0·04	−0·06 ± 0·03
Blood BHBA	−0·07 ± 0·05^{a}	0·2 ± 0·12^{b}	−0·03 ± 0·07^{ab}
Blood glucose	0·16 ± 0·07	0·03 ± 0·06	0·16 ± 0·05
Blood triglyceride	0·00 ± 0·01	0·02 ± 0·01	0·00 ± 0·01

Milk fatty acid composition

The effects of oil supplements in diet on fatty acid composition are presented in Table 3. The content of acids of the short chain fraction (C6:0 to C17:0) decreased more in SO and RO than in C. Especially C10:0, C12:0, C14:0, and C16:0 had the greatest diminution in both SO and RO respectively with the biggest decrease of myristic and palmitic acid. Total saturated fatty acids (**SFA**) increased in C and decreased highly significantly in both SO and RO except C18:0, which showed an increase compared with C. Monounsaturated fatty acids (**MUFA**), polyunsaturated fatty acids (**PUFA**), CLAs and TFAs showed a substantial increase in SO and RO. The decrease of saturated fatty acids and the increase of unsaturated fatty acids appeared in every lactation stage for oil supplemented diets with the greatest changes in mid lactation (Fig. 1a–c). For SFA the reduction was highly significant in early, mid, and late lactation ($P<0·01$ except SO late $P=0·057$). MUFAs had the opposite effect and showed a highly significant increase in all lactation stages of oil diets ($P<0·01$ except SO late $P<0·05$). Compared with C there was also a significant higher increase in PUFA, TFA and CLA in early and mid lactating cows ($P<0·01$). Late lactating cows with SO and RO showed smaller effects compared with C than the earlier lactation periods. PUFAs, TFAs and CLAs in SO Late showed no significant difference compared with C Late ($P>0·05$). The increase in CLA including C18:2t10c12 and transC18:1 is correlated with the observed reduction of milk fat in both oil rations (Tables 2 and 3). Compared with cows fed the C diet, those with SO and RO diet had a tremendous increase in C18:1 fatty acids. In the C18:1 fraction, C18:1 cis-9 was the most prominent isoform with the biggest increase compared with C.

Cholesterol in milk and plasma

Milk and plasma cholesterol levels are presented in Fig. 2. In the mean of all lactation stages there was a highly significant decrease in milk cholesterol level in SO and a tendency to decrease in RO compared with C (Table 2). The milk cholesterol level had a bigger decrease in all lactation stages of the two oil feedings compared with C, but was significantly proved just in late lactating cows fed the SO diet ($P<0·05$). In contrast to this decrease in milk cholesterol, a significant increase in plasma cholesterol level in SO and in RO compared with C was observed. Blood cholesterol had a significant increase in all lactation stages in SO group (early $P<0·05$, mid $P<0·01$, late $P<0·01$) and a significant increase in mid and late lactation in RO group ($P<0·05$) compared with C.

Discussion

Feed intake, milk yield and major milk components

The primary objective of the study was to test the hypothesis that diet-induced milk fat depression may also have an influence on the cholesterol levels in milk. A milk fat depression (**MFD**) was successfully achieved by adding 0·83 kg of oil to the diet, as demonstrated by the drastic reduction in fat percentage and fat yield on the SO and RO groups. The altering effects of certain fatty acids on milk fat content have already been described previously (Perfield II et al. 2004; Kadegowda et al. 2008). Gama et al. (2008) reduced milk fat percentage by more than 1 % by adding 1·6 % fish oil to the diet which is similar to the reduction achieved with this experiment adding SO and RO.

Other major milk parameters, such as milk yield and protein yield were not affected by oil supplementation

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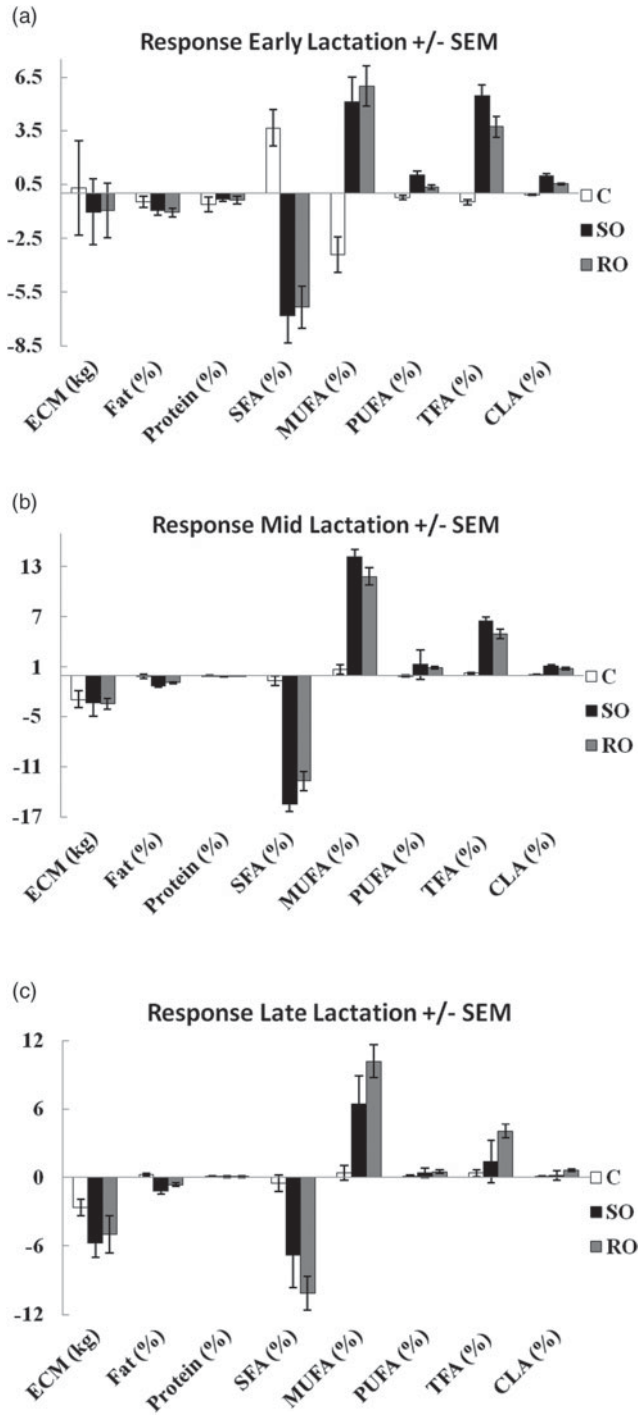

Fig. 1. (a) Percent change in milk composition parameters after the 28 d feed supplementation in individuals at early lactation stage (DiM 0 to 100). (b) Percent change in milk composition parameters after the 28 d feed supplementation in individuals at mid lactation stage (DiM 101 to 200). (c) Milk composition response (value at end – value at beginning of feeding period) of early (a) mid (b) and late (c) lactating cows fed control PMR (C) or soybean oil supplement (SO) or rapeseed oil supplement (RO) on energy corrected milk (ECM kg), fat %, protein %, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), trans fatty acids (TFA), conjugated linoleic acid (CLA).

compared with the control diet. The influence of MFD on protein yield is not clear in the available literature and the results from similar feeding experiments are contradictory. For example, in the experiments by He et al. (2012) cows were fed diets with up to 4 % supplemental fat, observing no effect in milk yield and protein yield, but resulting on an increased milk protein concentration. However, previous research also reports unaffected milk yield, protein yield and lactose yield but a decrease in milk protein concentration by 2·5 % oil supplementation (Abdelqader et al. 2009).

Body weight and dry matter intake (**DMI**) were not affected in our study, probably due to the short feeding periods of four weeks. AlZahal et al. (2008), Huang et al. (2008) and Jacobs et al. (2011) also failed to observe changes in DMI by feeding SO or RO. He et al. (2012) described a slightly reduced DMI when cows were fed an oil diet rich in C18:1 or C18:2 whereas body weight remained unchanged.

Blood plasma

The unaffected blood plasma parameters glucose, triglycerides, NEFA and BHBA in RO (except the increase in BHBA in SO) are in good agreement with previous studies which also reported that oil feedings did not change these plasma metabolites (Abdelqader et al. 2009; Dai et al. 2011). In the study of Abdelqader et al. (2009) though a corn oil diet was fed and the plasma levels showed no significant difference compared with a control ration without oil supplement. In Dai et al. (2011) NEFAs showed a numerical increase but did not reach significance, whereas all other plasma metabolites stayed unchanged when fed RO.

Fatty acid composition

Feeding vegetable oils, rich in C18:1 and C18:2 fatty acids, comes naturally associated with a lowering of the amount of milk saturated fat and an increase of unsaturated fat, as widely described before (Dai et al. 2011; Jacobs et al. 2011; He et al. 2012). Stearic acid (C18:0) is the only SFA increasing in the oil feeding groups, due to the abundance of C18 unsaturated fatty acids in SO and RO diet. This effect is in agreement with Loor & Herbein (2003a) and Huang et al. (2008) who presented an increase of C18:0 in a SO diet compared with a diet with no supplemented oil and is also present in other ruminant species such as goats (Chilliard & Ferlay, 2004).

The decrease in C4:0 to C17:0 fraction, originating from de novo lipogenesis, is strongly correlated to the CLA content and follows the theory formulated by Bauman and co-workers (Bauman et al. 2008). Interestingly, the SO diet showed a bigger potential to reduce this group of fats than the RO diet, consistent with previous studies which reported a decrease in FA shorter than 16 carbons with a bigger decrease in C18:2 – rich soybean oil supplement than in C18:1-rich rapeseed oil supplemented diet because of an altered ruminal biohydrogenation and its intermediates

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Table 3. Milk fatty acid composition of treatment groups (control (C), soybean oil (SO), rapeseed oil (RO)), comparing secretion response (value at end – value at beginning of feeding period) (mean ± SEM). Groups with different letter superscripts are significantly different ($P<0{\cdot}05$)

	Treatment group		
Fatty acid. %†	C	SO	RO
4:0	−0·014 ± 0·07^{a}	−0·351 ± 0·13^{b}	−0·179 ± 0·07^{ab}
6:0	0·063 ± 0·05^{a}	−0·498 ± 0·09^{b}	−0·389 ± 0·06^{b}
8:0	0·052 ± 0·03^{a}	−0·373 ± 0·06^{b}	−0·297 ± 0·04^{b}
10:0	0·125 ± 0·08^{a}	−0·993 ± 0·14^{b}	−0·853 ± 0·09b
10:1	0·011 ± 0·08^{a}	−0·086 ± 0·02^{b}	−0·081 ± 0·01^{b}
11:0	0·004 ± 0·00^{a}	−0·025 ± 0·00^{b}	−0·015 ± 0·00^{c}
12:0	0·142 ± 0·10^{a}	−1·045 ± 0·15^{b}	−0·998 ± 0·10^{b}
12:1 *cis*-9	0·003 ± 0·00^{a}	−0·015 ± 0·00^{b}	−0·019 ± 0·00^{b}
13:0	0·011 ± 0·01^{a}	−0·036 ± 0·01^{b}	−0·019 ± 0·00^{c}
Anteiso 13:0	0·003 ± 0·00^{a}	−0·020 ± 0·00^{b}	−0·022 ± 0·00^{b}
14:0	0·336 ± 0·19^{a}	−1·903 ± 0·28^{b}	−1·523 ± 0·21^{b}
Iso-14:0	0·005 ± 0·00^{a}	−0·013 ± 0·00^{b}	−0·011 ± 0·00^{b}
14:1 *cis*-9	0·013 ± 0·03	0·113 ± 0·05	0·035 ± 0·04
15:0	0·089 ± 0·04^{a}	−0·231 ± 0·04^{b}	−0·131 ± 0·03^{c}
Iso-15:0	0·019 ± 0·01^{a}	−0·019 ± 0·01^{b}	−0·029 ± 0·00^{b}
Anteiso-15:0	0·036 ± 0·01^{a}	−0·001 ± 0·01^{b}	−0·020 ± 0·01^{b}
16:0	−0·045 ± 0·52^{a}	−6·427 ± 0·72^{b}	−8·007 ± 0·60^{b}
Iso-16:0	0·010 ± 0·01^{a}	0·000 ± 0·01^{ab}	−0·019 ± 0·01^{b}
16:1 *trans*-9	−0·000 ± 0·00^{a}	0·112 ± 0·01^{b}	0·061 ± 0·01^{c}
16:1 *cis*-9	−0·115 ± 0·04	−0·020 ± 0·11	−0·214 ± 0·07
17:0	0·002 ± 0·01^{a}	−0·099 ± 0·01^{b}	−0·075 ± 0·01^{b}
Iso-17:0	0·013 ± 0·00^{a}	0·036 ± 0·01^{b}	0·006 ± 0·00^{a}
Anteiso-17:0	0·010 ± 0·01	0·007 ± 0·01	0·004 ± 0·01
17:1 *cis*-9	−0·011 ± 0·01	−0·037 ± 0·01	−0·028 ± 0·01
18:0	−0·083 ± 0·33^{a}	1·827 ± 0·25^{b}	2·146 ± 0·41^{b}
Iso-18:0	−0·002 ± 0·00^{a}	−0·011 ± 0·00^{b}	−0·006 ± 0·00^{ab}
18:1 ∑*trans*-6/*trans*-9/*trans*-10	0·012 ± 0·02	2·277 ± 0·31	2·241 ± 0·27
18:1 *trans*-11	0·016 ± 0·08	1·604 ± 0·37	1·390 ± 0·17
18:1 *cis*-9	−0·622 ± 0·47	5·083 ± 0·69	6·006 ± 0·63
18:1 *cis*-11	−0·040 ± 0·02^{a}	−0·003 ± 0·03^{a}	0·184 ± 0·03^{b}
18:1 *cis*-12	0·005 ± 0·01^{a}	0·139 ± 0·05^{b}	0·058 ± 0·02^{b}
18:2 *cis*-9, *cis*-12	−0·066 ± 0·04^{a}	0·214 ± 0·05^{b}	0·004 ± 0·04^{a}
18:2 *cis*-9, *trans*-11 (CLA)	0·023 ± 0·03^{a}	0·797 ± 0·15^{b}	0·653 ± 0·06^{b}
18:2 *tans*-10, *cis*-12 (CLA)	0·000 ± 0·00^{a}	0·013 ± 0·00^{b}	0·018 ± 0·01^{b}
18:3 *cis*-9, *cis*-12, *cis*-15	−0·005 ± 0·01	0·020 ± 0·03	0·006 ± 0·02
20:0	0·004 ± 0·00^{a}	0·030 ± 0·03^{ab}	0·086 ± 0·03^{b}
20:1 *cis*-11	−0·002 ± 0·00^{a}	0·010 ± 0·00^{b}	0·079 ± 0·01^{c}
20:2 *cis*-11, *cis*-14	0·000 ± 0·00	0·000 ± 0·00	0·001 ± 0·00
20:3 *cis*-8, *cis*-11, *cis*-14	−0·001 ± 0·01^{a}	−0·017 ± 0·00^{b}	−0·005 ± 0·00^{a}
20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14	−0·005 ± 0·01^{a}	−0·036 ± 0·00^{b}	−0·017 ± 0·01^{a}
20:5 *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17	−0·004 ± 0·00^{a}	−0·010 ± 0·00^{b}	−0·015 ± 0·00^{b}
21:0	0·002 ± 0·00^{a}	−0·001 ± 0·00^{b}	−0·001 ± 0·00^{b}
22:0	0·004 ± 0·00	0·002 ± 0·00	0·001 ± 0·00
22:1 *cis*-13	−0·001 ± 0·00^{a}	−0·002 ± 0·00^{a}	0·004 ± 0·00^{b}
22:5 *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19	−0·003 ± 0·01^{a}	−0·020 ± 0·00^{b}	−0·014 ± 0·00^{b}
22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19	0·002 ± 0·00	0·005 ± 0·00	0·002 ± 0·00
23:0	0·002 ± 0·00	0·002 ± 0·00	0·003 ± 0·00
24:0	0·004 ± 0·00^{a}	0·003 ± 0·00^{a}	−0·003 ± 0·00^{b}
Summation			
SFA‡	0·791 ± 0·55^{a}	−10·139 ± 1·17^{b}	−10·350 ± 0·85^{b}
MUFA§	−0·733 ± 0·53^{a}	9·174 ± 1·06^{b}	9·717 ± 0·8^{b}
PUFA¶	−0·058 ± 0·06^{a}	0·965 ± 0·15^{b}	0·633 ± 0·08^{b}
CLA††	0·023 ± 0·03^{a}	0·809 ± 0·15^{b}	0·671 ± 0·07^{b}
TFA‡‡	0·051 ± 0·13^{a}	4·801 ± 0·64^{b}	4·363 ± 0·35^{b}

†Results are shown as delta values day 2 vs. day 26 of feeding period ± SEM

‡Saturated fatty acids: ∑ (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, anteiso-C13:0, C14:0, iso-C14:0, C15:0, iso-C15:0, anteiso-C15:0, C16:0, iso-C16:0, C17:0, iso-C17:0, anteiso-C17:0, C18:0, iso-C18:0, C20:0, C21:0, C22:0, C23:0, C24:0). Iso and anteiso Fatty Acids are not shown in the table

§Monounsaturated fatty acids: ∑ (C10:1, C12:1 c9, C14:1 c9, C16:1 t9, C16:1 c9, C17:1 c9, C18:1 t6+t9+t10, C18:1 t11, C18:1 c9, C18:1 c11, C18:1 c12, C20:1 c11, C22:1 c13)

¶ Polyunsaturated fatty acids: ∑ (C18:2 c9c12, C18:2 c9t11 CLA, C18:2 t10c12 CLA, C18:3 c9c12c15, C20:2 c11c14, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3)

††Conjugated linoleic acid: ∑ (C18:2 c9t11, C18:2 t10c12)

‡‡Trans fatty acid: ∑ (C16:1 t9, C18:1 t6+t9+t10, C18:1 t11, C18:2 c9t11 CLA, C18:2 t10c12 CLA)

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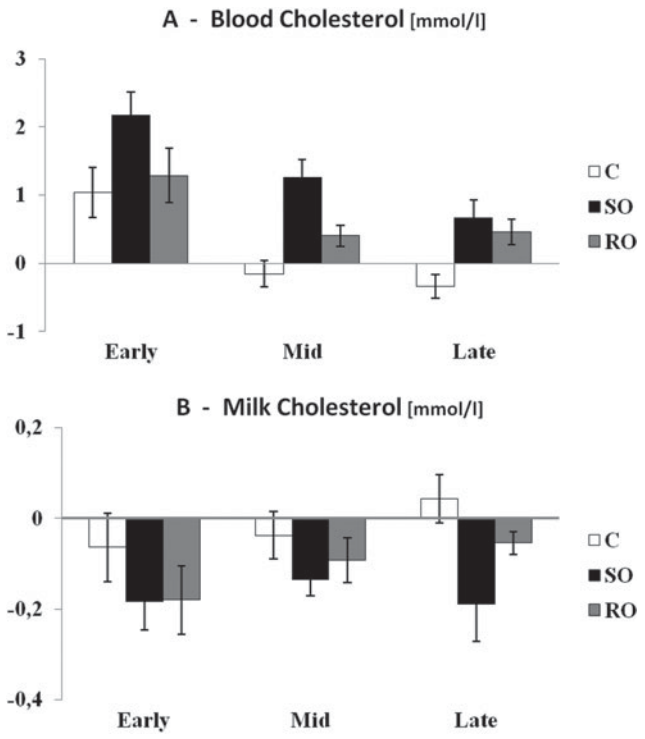

Fig. 2. Response (value at end – value at beginning of feeding period) after feeding soybean oil supplement (SO) or rapeseed oil supplement (RO) compared with control (C) on blood cholesterol [mmol/l] (panel A) and milk cholesterol [mmol/l](panel B).

(He et al. 2012). Especially the drastic reduction of the myristic acid amount is of great interest due to its potential benefits for human health (Müller et al. 2001; Dabadie et al. 2005).

As observed in our study, AlZahal et al. (2008) associated milk fat depression with an increase in total trans C18:1 and total CLA. The negative correlation of milk fat content and CLA C18:2 t10c12 was also well described by Peterson et al. (2003), Bauman et al. (2008), and Glasser et al. (2010). Zened et al. (2012) suggested that a shift from the trans – 11 to trans – 10 pathway of ruminal biohydrogenation associated with milk fat depression was due to oil supplementation of a high-wheat diet. Loor & Herbein (2003b) induced a reduced milk fat production by performing a CLA C18:2t10c12 infusion and proposed that its uptake by the mammary gland may decrease the de novo fatty acid synthesis and desaturation of long chain fatty acids. The decrease of desaturated long chain fatty acids is apparent in this experiment for the fatty acids C20:3n-6, C20:4n-6 and C22:5 for SO and C20:5n-3 in SO and RO. In our experiments, an increase in other unsaturated long chain fatty acids (C22:1 in RO or C20:1n-9 in SO and RO) was also observed, in agreement with Rego et al. (2009) who detected an increase in C20:1 and a decrease in C20:3, C20:4, C20:5 FAs by feeding SO or RO. An increased milk TFA level was also observed after oil supplementation, but the role of this group of fats in human diet is still a topic of discussion and might need a differentiation between fats of ruminant and industrial origin. The publications reporting a potential negative effects of trans fatty acids produced by ruminants refer to very high intake doses (Sun et al. 2007; Motard-Bélanger et al. 2008), whereas Jakobsen et al. (2008) and Gebauer et al. (2011) suggests that ruminal trans fatty acids are not associated with a higher risk of coronary diseases.

Milk and plasma cholesterol

Diet induced milk fat depression can be also considered as a powerful method for studying milk fat synthesis and general bovine fat metabolism. Many studies on this extreme metabolic situation have previously reported its effects on milk composition (Gama et al. 2008; Glasser et al. 2010; He et al. 2012), but to our knowledge none of them focused on milk cholesterol level and its variation in comparison with other fat components. An increase in milk cholesterol level and a decrease in the amount of this metabolite in blood during the lactating cycle is a natural process well known in the existing literature (Maynard et al. 1931; Puppione et al. 1980; Strzałkowska et al. 2009). In the case of fat supplementation of diet, the inverse relationship of these parameters is maintained and a decreased milk cholesterol level effect is always accompanied by an increased plasma cholesterol concentration. The increased plasma cholesterol levels observed in SO and RO are similar to effects described by Bremmer et al. (1998), Khorasani & Kennelly (1998), and Abdelqader et al. (2009). Previously, Nestel et al. (1978) also suggested that this hypercholesteremia might be due to an increased requirement of cholesterol for the transport of long chain fatty acids as chylomicrons from the intestine into blood, which leads to an enhanced cholesterol biosynthesis in the intestine and a decreased faecal excretion of bile acids. The high amount of fatty acids available from vegetable oils in our experimental feedings may therefore be the reason for the increased blood cholesterol levels observed in the SO and RO groups and confirm this theory. In addition, the induced milk fat depression results in a lower need for milk cholesterol as part of the milk fat and the resulting reduced transport and synthesis of this metabolite to and in the mammary gland. A possible explanation of this effect is the reduction of the expression of the cholesterol transport regulatory genes in the mammary epithelial cells and milk fat globules described by Mani et al. (2011). On the other hand, Singh et al. (2012) observed increased milk cholesterol content accompanied by a non-affected total plasma cholesterol level after feeding buffaloes with *Asparagus racemosus*. However, recent results from our group (unpublished data) suggest that physiological cholesterol levels highly differ between this species and *Bos taurus*.

Conclusions

In summary, a diet induced milk fat depression after supplementation with RO and SO positively affects milk fat

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composition by not only reducing the proportion of saturated fatty acids and increasing mono- and polyunsaturated fatty acids and CLAs, but also significantly reducing the milk cholesterol level. Whether this is a secondary effect due to the mechanisms of milk fat globule formation or the result of a specific gene expression regulation mechanism should be elucidated in future works.

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