Effect of dietary vegetable oils on the fatty acid profile of plasma lipoproteins in dairy cows

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ABSTRACT

The aim of this study was to elucidate the effect of dietary soybean oil (SO) and hydrogenated palm oil (HPO) on transport of fatty acids (FA) within plasma lipoproteins in lactating and non-lactating cows. Three lactating and three non-lactating Holstein cows were used in two different 3×3 Latin squares that included three periods of 21 d. Dietary treatments for lactating cows consisted of a basal diet (Control; no fat supplement), and fat-supplemented diets containing SO (500 g/d per cow) and HPO (500 g/d per cow). For non-lactating cows, dietary treatments consisted of a basal diet (Control; no fat supplement), and fat-supplemented diets containing SO (170 g/d per cow) and HPO (170 g/d per cow). In plasma of lactating cows, compared with control and SO, HPO increased ($P<0.05$) concentrations (mg/dl) of C16:0, C18:0, C18:2 cis-9, 12, C18:3 cis-9, 12, 15, and total saturated and polyunsaturated FA. In non-lactating cows: compared with control and HPO, SO increased C18:1 trans-11 in plasma. In lactating cows, concentrations of C16:0, C18:0 and total saturated FA were increased ($p<0.05$) by HPO compared with control and SO in the high density lipoprotein. Total saturated FA were increased ($p<0.05$) by HPO in the very low density lipoprotein. In non-lactating cows, concentrations of C18:0 were increased ($p<0.05$) by HPO compared with control and SO in the high density lipoprotein whereas, C18:1 trans-11 was increased ($p<0.05$) by SO in the low density lipoprotein. Overall, we found that distribution and transport of FA within bovine plasma lipoproteins may be influenced by chain length and degree of unsaturation of dietary lipids. Also, distribution of individual FA isomers such as C18:1 trans-11 and C18:2 cis-9, trans-11 may vary depending in the physiological state (lactating or non-lactating) of the cow and are increased in plasma (lactating cows) and the high density lipoprotein (non-lactating cows) when cows are fed SO.

KEYWORDS Soybean oil; palm oil; plasma; lipoproteins; dairy cows

1. Introduction

Supplementing dairy cow diets with soybean oil (SO) can increase milk yield with no detrimental effect on milk fat content (Bu et al. 2007). Milk bioactive fatty acids (FA) such as vaccenic acid (C18:1 trans-11) can be increased by inclusion of SO into dairy cow diets (Allred et al. 2006; Vargas-Bello-Pérez et al. 2015). On the other hand, hydrogenated vegetable oils have been used to increase the energy content of dairy cow diets in housed (Kargar et al. 2012) and pasture systems (Schroeder *et al.,* 2002) without effect on milk composition (Vargas-Bello-Pérez et al. 2015).

It is difficult to increase polyunsaturated FA (PUFA) concentrations in milk, since large amounts of dietary lipid supplements are needed to achieve a meaningful rise in milk concentration of PUFA (Offer et al. 2001). In bovines, the effects of dietary PUFA on lipoprotein metabolism are difficult to define since they depend on the degree of protection against biohydrogenation (Scislowski et al. 2004a) and the location, orientation, and number of double bonds of dietary lipids (Tyburczy et al. 2008). Dietary PUFA can alter lipoproteins, especially the high density lipoprotein (HDL) fraction which is the major plasma lipoprotein fraction in bovines (Bauchart, 1993). For example, the HDL lipid profile can be modified when dietary PUFA are directly infused into the proximal duodenum (Scislowski et al. 2004a) or when preruminant calves are supplemented with soybean oil (Leplaix-Charlat et al. 1996) or when dairy cows are fed protected sunflower oil-seed (Ashes et al. 1982) or protected soybean (Storry et al. 1980).

Understanding how dietary FA affect lipoprotein metabolism in dairy cows is important because this knowledge could be used to modulate the effect of nutrition on milk fat yield and milk FA quality. Therefore, the aim of this study was to elucidate the effect of dietary FA on the FA profile of plasma lipoproteins when lactating and non-lactating cows are supplemented with polyunsaturated (SO) or saturated (hydrogenated palm oil) lipid sources.

2. Materials and methods

2.1 Animals and diets

All animals were handled following approved guidelines of the Animal Care and Use Committee of the Pontificia Universidad Católica de Chile. The study was conducted at the Estación Experimental Pirque (33°38'28"S, 70°34'27"W) of the Pontificia Universidad Católica de Chile. Three lactating cows averaging 169 ± 24 DIM at the beginning of the study [641.3±111.3 kg BW (average ± SD)] and 3 non-lactating non-pregnant Holstein cows (684.7±84.7 kg BW) were used in two different 3×3 Latin square designs with 3 periods consisting of 21 d. Cows were individually fed a TMR (total mixed ration) at a fixed rate once daily (0930 h).

All cows received a basal diet formulated with a 56:44 forage:concentrate ratio, which was fed at rates determined by NRC (2001). Lactating cows received 19.5 kg DM per day of the basal diet to meet requirements of cows producing 30 L milk per day; non-lactating cows received 10 kg DM per day of the basal diet. Dietary treatments for lactating cows consisted of the basal diet (Control; no fat supplement), and fat-supplemented diets containing soybean oil (SO; unrefined oil; 500 g/d per cow) and hydrogenated palm oil (HPO; 500 g/d per cow). For non-lactating cows dietary treatments were: basal diet (Control; no fat supplement), SO (basal diet + 170 g/d per cow) and HPO (basal diet + 170 g/d per cow). Amounts of oil fed to animals were based on Vargas-Bello-Perez et al. (2015). Experimental diets were calculated to be isonitrogenous. Standard procedures (AOAC, 2006) were used to determine the DM (934.01), Kjeldahl N (984.13), and ether extract (920.39). Neutral detergent fibre and ADF and lignin were determined by methods described by Van Soest et al. (1991). Chemical composition of the diets is shown in Table 1. A mixer wagon was used to mix forage and concentrates. Oils were administrated separately and mixed manually. The most important FA from dietary oils were the following: SO contained 25 g/100g of C18:1 cis-9 and 51 g/100g of C18:2 cis-9, 12; whereas HPO contained 47 g/100g of C16:0 and 43 g/100g of C18:0. Animals were housed in individual stalls (2.4 × 6 m) and had continuous access to water.

123

124 *2.2 Blood and lipoprotein samples*

125 Blood samples (50 ml/cow) were obtained at 10:00 h via jugular puncture on d 21 of each
126 period before the morning meal. Blood was transferred to tubes containing lithium
127 heparin (BD Vacutainer; Franklin Lakes NJ, USA) and immediately centrifuged for 15 min at
128 3,000 × *g* (C-28A, BOECO, Germany) for harvesting plasma. Plasma samples (10 ml per
129 cow) were kept at 4°C for 5h maximum until lipoprotein fractionation. Based on their
130 density, plasma lipoprotein fractions were separated sequentially by preparative
131 ultracentrifugation in a Sorvall WX Ultra 90 (Thermo Scientific) equipped with a T-865
132 rotor. Potassium bromide (KBr) was added to plasma to obtain the required density
133 calculated by use of the Radding-Steinberg (1960) formula. Plasma was ultracentrifuged at
134 291,400 × *g* at 4°C for 21.2 h at a density of 1.006 g/ml to remove the very low density
135 lipoprotein fraction, at 291,400 × *g* at 4°C for 26.6 h at a density of 1.063 g/ml to separate
136 the low density lipoprotein and at 365,900 × *g* at 4°C for 26 h to obtain the HDL fraction.

137

138 *2.3 Fatty acid analysis*

139 Lipids from oils, diets, plasma and lipoproteins were extracted by adaptation of the
140 method by Bligh and Dyer (1959) and methylated according to Chouinard et al. (1999). A
141 gas chromatograph (GC-2010) system (Shimadzu Scientific Instruments AOC-20s,
142 Columbia, MD, USA) equipped with a 100-m column (Rt-2560 column 100 m × 0.32 mm ×
143 0.20 um column; Restek, Bellefonte, PA) was used. The column had a highly polar phase
144 and it was biscyanopropyl polysiloxane - not bonded. The GC conditions were as follows:
145 the oven temperature was initially set at 110°C for 4 min after injection, and then ramped
146 to 170°C at 5°C/min for 10 min. The temperature was then ramped to 225°C at 3°C/min
147 and held for 10 min and finally ramped to 240°C at 3°C/min. The inlet and flame-ionization
148 detector temperatures were 260°C, the split ratio was 15:1 and a 2 µl injection volume
149 was used. The hydrogen carrier gas flow to the detector was 25 ml/min, airflow was 400
150 ml/min, and the flow of nitrogen makeup gas was 40 ml/min. Fatty acid GC peaks were
151 identified by using a FA methyl ester standard (FAME; Supelco 37 Component FAME mix,

Bellefonte, PA, USA), and reference standards for C18:1 trans-11 and C18:1 cis-9, trans-11 (Nu-Chek-Prep Inc., Elysian, MN, USA).

2.4 Statistical analysis

Data from each group of cows (lactating and non-pregnant) were analysed as a 3 × 3 Latin Square using the GenStat (12th Edition) statistical package (VSN International Ltd, Oxford, UK). Fixed effects were experimental period and treatment and the random effect was the individual cow. When significant treatment effects were detected, means were separated using Tukey test. Probability of $p < 0.05$ was used to determine significant differences among means.

3. Results and discussion

Details of milk production and performance have been reported elsewhere (Vargas-Bello-Pérez et al. 2015). Fatty acid composition of the diets is shown in Table 1. As expected, compared with control and SO diets, HPO was characterized by higher concentrations of saturated FA: 46 g/100g of C16:0 and 36 g/100g of C18:0, whereas SO was characterized by higher concentrations of PUFA: C18:2 cis-9, 12 (50 g/100g of total FA). It has been shown that enriched diets with PUFA increase lipoprotein fluidity compared with saturated fatty acid (SFA) -rich diets, this is relevant because changes in the physical state of lipoproteins can interfere with the physiological roles of lipoproteins and can cause hypercholesterolemia and decreased fluidity (Scislowski et al. 2004b). Additionally, changes in the FA profile of lipoproteins can contribute to chronic disorders, for example, in humans these can cause inflammatory and immune disorders, neurological dysfunction and deterioration in the coagulation functions (Williams, 2000). Supplementing cows with either PUFA or SFA dietary lipids may improve performance; however, research is needed to study the consequences on bovine health of long-term modifications in the lipoprotein FA profile.

The concentrations (mg/l) of total lipids in the plasma from lactating cows fed control, SO and HPO diets at 4240, 4279 and 5073 respectively were not significantly different; and from non-lactating cows fed control, SO and HPO diets at 4000, 4100 and 4252 respectively, were not significantly different. Because both groups had different dietary treatments, a comparison was not possible, however, it has been shown (Herdt and Smith, 1996) that plasma lipoprotein concentrations are influenced by lactation cycle and dietary fat supplementation in dairy cows. In this study it appears that physiological state related to lactation may be influencing the FA profile of plasma and lipoproteins.

In general, our results agree with previous studies where the most abundant FA were C16:0, C18:0 and C18:2 cis-9, 12 which showed how dietary lipid composition can be reflected in the FA concentrations of plasma (Loor et al. 2002; Jacobs et al. 2011) and lipoproteins (McCarthy et al. 1968). In plasma of lactating cows, compared with control and SO, HPO increased concentrations (mg/dl) of C16:0, C18:0, C18:2 cis-9, 12, C18:3 cis-9, 12, 15, and total saturated and polyunsaturated FA. In non-lactating cows, compared with control and HPO, SO increased C18:1 trans-11 in plasma (Table 2).

In this study, increases in saturated FA in plasma and some lipoproteins are consistent with the HPO FA profile which indicated that saturated FA are the major products leaving the rumen and thus taken up and packed into lipoproteins. In lactating cows, concentrations (mg/dl) of C16:0, C18:0 and total saturated FA were increased by HPO compared with control and SO in the high density lipoprotein. Total saturated FA were increased by HPO in the high density lipoproteins and the very low density lipoproteins (Table 3). In non-lactating cows, concentrations (mg/dl) of C18:0 were increased HPO compared with control and SO in the high density lipoprotein whereas, C18:1 trans-11 was increased by SO in the low density lipoprotein (Table 4).

Because in this study we focused on characterization the FA profile of plasma lipoproteins from lactating and non-lactating cows supplemented with different lipid sources only the

total lipid fraction in blood plasma and lipoproteins were analysed, which includes cholesterol esters (CE), phospholipids (PL), triacylglycerols (TG) and non-esterified FA. The low transfer efficiency of PUFA from diet to milk in cows is explained by the fact that the bovine mammary gland primarily extracts FA from the TG and non-esterified FA fractions in blood plasma (Loor et al. 2002), whereas PUFA are specifically incorporated into plasma CE and PL (Tyburczy et al. 2008). In order to elucidate this transport mechanism, future research will need to separate plasma and lipoproteins into lipid subgroups.

When unprotected oils, particularly with high content of unsaturated FA such as soybean oil, are included in dairy cow diets, an increase in ruminal biohydrogenation intermediates is usually observed (Shingfield et al. 2013). Some of these intermediates (e.g., C18:1 trans-10 and C18:2 trans-10, cis-12) can affect expression of several genes involved in lipid metabolism in the mammary gland (Bauman et al. 2011). In this regard, C18:1 trans-10 was found in plasma and high density lipoprotein from lactating cows, although dietary treatment did not affect its concentration; this C18:1 isomer is important because it is related to milk fat depression in lactating ruminants (Bauman et al. 2006) and is an intermediate of ruminal biohydrogenation which affects milk yield and milk fat yield, possibly by altering the average melting point of milk FA (Gama et al. 2008). Tyburczy et al. (2008) reported no difference in C18:1 trans-10 concentrations of plasma cholesterol esters (CE), TG and PL when cows were abomasally infused with free FA of C18:1 cis-9 (45.5 g/d), C18:1 trans-9 (41.7 g/d) and C18:1 trans-11 (41.4 g/d) acids; however, in that study no fractionation of plasma into lipoprotein fractions was performed.

In lactating cows, compared with control and HPO, SO increased C18:2 cis-9, trans-11 in plasma and in non-lactating cows increased C18:1 trans-11. In terms of plasma transport of octadecenoic acids, Loor et al. (2002) showed that as a proportion of FA within a lipid fraction, C18:1 trans-11 was greatest in plasma TG, although Mosley et al. (2006) found the greatest C18:1 trans-11 concentration in plasma PL. Those differences were likely related to the absolute concentration of plasma FA in the PL and TG fractions as shown by

238 Tyburczy et al. (2008) who found twice the amount of C18:1 trans-11 in both PL and TG
239 lipid groups.
240
241 Lactation stage has an important effect on FA utilization to satisfy specific requirements
242 for energy and milk fat synthesis (Palmquist, 1976). This may be reflected in the different
243 FA profile of lipoprotein lipids found in the current experiment (mid-lactation and non-
244 lactating cows) compared with those reported by Offer et al. (2001) (mid-lactation cows)
245 and Tyburczy et al. (2008; mid-lactation cows). In general, concentrations of saturated FA
246 in lactating cows were slightly higher than those from non-lactating cows; however,
247 physiological stage was confounded with amounts of diet fed. Further research will need
248 to compare both responses in order to confirm that physiological stage has a direct impact
249 on use of FA and FA transportation within plasma lipoproteins.
250
251 The stearoyl-CoA desaturase (SCD) activity on plasma lipids is a factor that may explain the
252 differences found in the FA profile of plasma lipoproteins in lactating and non-lactating
253 dairy cows. The SCD converts SFA into monounsaturated fatty acids (MUFA) by
254 introducing a double bond between carbon atoms 9 and 10 in the saturated carbon chain,
255 but it can also catalyse the desaturation of different monounsaturated fatty acyl-CoA
256 substrates, including C18:1 trans-11 to generate C18:2 cis-9, trans-11 (Jacobs et al., 2011).
257 The consequences of regulating the stearoyl-CoA desaturase by PUFA and cholesterol may
258 be relevant to lipoprotein metabolism since liver and adipose cell metabolic homeostasis
259 depend on SCD; for example, the hepatic packaging and secretion of the very low density
260 lipoprotein requires synthesis of apolipoprotein B-100 as well as sufficient amount of
261 C18:1 cis-9 which would either come from the diet or from synthesis by SCD (Ntambi,
262 1999).
263
264 In the current study, we performed transesterification of FA with sodium methoxide at
265 low temperature which quickly methylates FA of triglycerides and phospholipids (Christie,
266 1982). It is possible that FA of cholesterol esters were not completely methylated, which is

important because more than 90% of plasma FA are carried by the HDL fraction, mainly in cholesterol esters and phospholipids groups (Offer et al. 2001). In the current study, however, the objective was to compare dietary effects on FA profiles of plasma and lipoprotein fractions, not to compare lipid structures. Future studies should use an acid-catalysed transesterification coupled with thin layer chromatography to confirm complete methylation of cholesterol esters.

4. Conclusions

Overall, we found that distribution and transport of fatty acids within bovine plasma lipoproteins may be influenced by chain length and degree of unsaturation of dietary lipids. Also, distribution of individual FA isomers such as C18:1 trans-11 and C18:2 cis-9, trans-11 may vary depending in the physiological state (lactating or non-lactating) of the cow and are increased in plasma (lactating cows) and the high density lipoprotein (ono-lactating cows) when cows are fed SO.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Allred SL, Dhiman TR, Brennand CP, Khanal RC, McMahon DJ, Luchini ND. 2006. Milk and cheese from cows fed calcium salts of palm and fish oil alone or in combination with soybean products. J. Dairy Sci. 89: 234-248.

[AOAC] Association of Official Analytical Chemist. 2006. Official Methods of Analysis. 18th ed. Gaithersburg, MD.

Ashes JR, Burley RW, Davenport JB, Sidhu GS. 1982: Effects of dietary supplements of protected lipids on the concentration and transport of ß-carotene and cholesterol in bovine blood and milk: unusual chromatographic behavior of the high-density lipoprotein with high levels of b-carotene. J. Dairy Res. 49: 39–49.

Bauchart D. 1993. Lipid absorption and transport in ruminants. J. Dairy Sci. 76: 3864–3881.

Bauman DE, Harvatine KJ, Lock AL. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Bauman DE, Mather IH, Wall RJ, Lock AL. 2006. Major advances associated with the biosynthesis of milk. J. Dairy Sci. 89:1235–43

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Bu DP, Wang JQ, Dhiman TR, Liu SJ. 2007. Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. J. Dairy Sci. 90:998-1007.

Chouinard PY, Corneau L, Saebo A, Bauman DE. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82: 2737-2745.

Christie WW. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. Journal of Lipid Research 23: 1072-1075.

Gama MA, Garnsworthy PC, Griinari JM, Leme PR, Rodrigues PH, Souza LW, Lanna DP. 2008. Diet-induced milk fat depression: Association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115: 319-331.

Herdt TH, Smith JC. 1996. Blood-lipid and lactation-stage factors affecting serum vitamin e concentrations and vitamin e cholesterol ratios in dairy cattle. J. Vet. Diagn. Invest. 8: 228–232.

Jacobs AA, van Baal J, Smits MA, Taweel HZ, Hendriks WH, van Vuuren AM, Dijkstra J. 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. J. Dairy Sci. 94:874-887.

Kargar S, Ghorbani GR, Alikhani M, Khorvash M, Rashidi L, Schingoethe DJ. 2012. Lactational performance and milk fatty acid profile of Holstein cows in response to dietary fat supplements and forage: Concentrate ratio. Livest. Sci. 150: 274-283.

Leplaix-Charlat L, Bauchart D, Durand D, Laplaud PM, Chapman MJ. 1996. Plasma lipoproteins in preruminant calves fed diets containing tallow or soybean oil with and without cholesterol. J. Dairy Sci. 79: 1267–1277.

Loor JJ, Herbein JH, Polan CE. 2002. Trans 18:1 and 18:2 isomers in blood plasma and milk fat of grazing cows fed a grain supplement containing solvent-extracted or mechanically extracted soybean meal. J. Dairy Sci. 85:1197–1207.

McCarthy RD, Chandler PT, Oriel LC, Porter GA. 1968. Fatty acid composition of blood serum lipoproteins from normal and ketonic cows. J. Dairy Sci. 51: 392.

Mosley EE, Shafi B, Moate PJ, McGuire MA. 2006. Cis-9, trans-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136: 570–575.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Ntambi JM. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res. 40: 1549-1558.

Offer NW, Speake BK, Dixon J, Marsden M. 2001. Effect of fish-oil supplementation on levels of (n-3) poly-unsaturated fatty acids in the lipoprotein fractions of bovine plasma. J. Anim. Sci. 73: 523-531.

Palmquist DL. 1976. A kinetic concept of lipid transport in ruminants. J. Dairy Sci. 59: 355–63.

Radding CM, Steinberg D. 1960. Studies on the synthesis and secretion of serum lipoproteins by rat liver slices. J. Clin. Invest. 39: 1560-1569.

Schroeder GF, Gagliostro GA, Becu-Villalobos D, Lacau-Mengido I. 2002. Supplementation with partially hydrogenated oil in grazing dairy cows in early lactation. J. Dairy Sci. 85: 580-594.

Scislowski V, Durand D, Gruffat D, Bauchart D. 2004a. Dietary linoleic acid-induced hypercholesterolemia and accumulation of very light HDL in steers. Lipids 39:125-133.

Scislowski V, Durand D, Gruffat-Mouty D, Motta C, Bauchart D. 2004b. Linoleate supplementation in steers modifies lipid composition of plasma lipoproteins but does not alter their fluidity. Br. J. Nutr. 91:575-584.

Shingfield KJ, Bonnet M, Scollan ND. 2013. Recent developments in altering the fatty acid composition of ruminant derived foods. Animal 7:132–162.

Storry JE, Brumby PE, Tuckley B, Welch VA, Stead D, Fulford RJ. 1980. Effect of feeding protected lipid to dairy cows in early lactation on the composition of blood lipoproteins and secretion of fatty acids in milk. J. Agri. Sci. 94:503–516.

Tyburczy C, Lock AL, Dwyer DA, Destaillats F, Mouloungui Z, Candy L, Bauman DE. 2008. Uptake and utilization of trans octadecenoic acids in lactating dairy cows. J. Dairy Sci. 91:3850-3861.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vargas-Bello-Pérez E, Fehrmann-Cartes K, Íñiguez-González G, Toro-Mujica P, Garnsworthy PC. 2015. Short communication: Chemical composition, fatty acid composition, and sensory characteristics of Chanco cheese from dairy cows supplemented with soybean and hydrogenated vegetable oils J. Dairy Sci. 98:111-117.

Williams CM. 2000. Dietary fatty acids and human health. Ann. Zootechnol. 49: 165–180.

Table 1 *Ingredient and chemical composition of control, soybean oil (SO), or partially hydrogenated palm oil (HPO) diets*

	Diet		
	Control	SO	HPO
Ingredient composition [% of DM]			
Alfalfa hay	17	17	17
Corn silage	18	18	18
High-moisture corn	10	10	10
Soybean hulls	34	34	34
Wheat bran	19	19	19
Vitamin and mineral premix*	2	2	2
Soybean oil	0	2.6† (1.7‡)	0
Hydrogenated palm oil	0	0	2.6† (1.7‡)
Chemical composition [%]			
Dry matter	51.0	53.7	53.6
Crude protein	16.7	15.9	16.6
Ether extract	2.3	5.1	6.3
Neutral detergent fibre	39.2	39.2	38.9
Acid detergent fibre	21.0	20.1	19.5
Lignin	3.2	3.8	3.6
Fatty acid composition [g/100g of total fatty acids]			
C4:0	0.03	0.09	0.73
C6:0	0.05	0.04	0.01
C8:0	0.03	0.03	0.07
C10:0	1.63	0.15	0.10
C12:0	0.16	0.13	2.08
C14:0	0.26	0.15	1.70
C16:0	15.6	13.7	45.9
C18:0	18.8	18.8	36.3
C18:1 cis-9	0.42	1.78	0.04
C18:2 cis-9, 12	46.9	49.5	5.03
C18:3 cis-6, 9, 12	0.17	0.10	0.19
C18:3 cis-9, 12, 15	7.44	6.38	6.55
C18:2 cis-9, trans-11	0.05	0.09	-
Other#	8.49	9.06	1.3

Notes: *Contained per kg of diet: 500 mg of P; 1,600 mg of Ca; 500 mg of Mg; 32.24 mg of S; 6,000 IU of vitamin A; 1,000 IU of vitamin D_3 and 32 IU of vitamin E; †Lactating cows: C (control) = basal diet without fat supplement; SO = basal diet supplemented with 500 g/d per cow of SO; HPO = basal diet supplemented with 500 g/d per cow of HPO; ‡Non-lactating cows: C (control) = basal diet without fat supplement; SO = basal diet supplemented with 170 g/d per cow of SO; HPO = basal diet supplemented with 170 g/d per cow of HPO; #Other = FA unidentified or present at <0.3 g/100g; $'-' = Not detected or detected at <0.01 g/100g.

Table 2 *Fatty acid composition of plasma from lactating and non-lactating cows fed control, soybean oil (SO) or hydrogenated palm oil (HPO) dietary treatments (mg / dl of plasma)*

Fatty acid	Lactating*					Non-lactating†				
	C	SO	HPO	SED‡	*p*	C	SO	HPO	SED	*p*
C10:0	14.3	14.9	15.4	9.57	0.99	16.9	23.1	30.7	11.7	0.53
C14:0	1.61	1.30	1.71	0.55	0.75	0.50	1.07	1.21	0.33	0.16
C16:0	53.0^{b}	55.2^{b}	70.2^{a}	3.61	<0.01	38.4	59.3	65.3	16.0	0.28
C18:0	99.1^{b}	101.3^{b}	117.8^{a}	3.98	<0.01	68.0	114	112	27.9	0.25
C18:1 trans-10	0.33	0.05	0.85	0.44	2.26	ND	ND	ND	ND	ND-
C18:1 trans-11	0.90	1.65	3.06	1.58	0.43	1.31^{c}	6.89^{a}	3.66^{b}	1.87	0.05
C18:1 cis-9	27.5	32.4	39.4	5.08	0.13	22.4	31.0	30.3	9.70	0.63
C18:2 cis-9, 12	133.0^{b}	134.9^{b}	158.2^{a}	8.00	0.03	53.4	85.9	83.9	22.6	0.34
C18:3 cis-9, 12, 15	3.43^{b}	3.34^{b}	5.10^{a}	0.62	0.05	1.98	2.90	3.48	0.85	0.28
C18:2 cis-9, trans-11	0.13^{b}	0.56^{a}	0.31^{b}	0.19	0.03	0.17	0.49	0.45	0.22	0.36
Σ Saturated	170.8^{b}	175.5^{b}	209.4^{a}	11.3	0.02	125	199	213	51.7	0.26
Σ Monounsaturated	35.4	41.5	47.5	5.65	0.18	26.8	43.4	38.0	11.7	0.41
Σ Polyunsaturated	188.3^{b}	180.0^{b}	218.0^{a}	9.56	<0.01	97.0	145	149	40.2	0.41
Other$^{\$}$	29.5	31.0	32.5	2.68	0.58	17.6	22.5	26.1	7.40	0.54

Notes: *Lactating cows: C (control) = basal diet without fat supplement; SO = basal diet supplemented with 500 g/d per cow of SO; HPO = basal diet supplemented with 500 g/d per cow of HPO; †Non-lactating cows: C (control) = basal diet without fat supplement; SO = basal diet supplemented with 170 g/d per cow of SO; HPO = basal diet supplemented with 170 g/d per cow of HPO; ‡SED = Standard error of the difference; # '-' = Not detected; $Other = FA unidentified or present at <0.05 mg/dl; a,b,c Means in the same row with different superscripts differ significantly for treatment effect with the *p*-value shown.

Table 3 *Fatty acid composition of plasma lipoproteins from lactating cows fed control (C), soybean oil (SO) or hydrogenated palm oil (HPO) dietary treatments (mg / dl of plasma)*

Fatty acid	High density lipoprotein					Low density lipoprotein					Very Low density lipoprotein				
	C	SO	HPO	SED*	*p*	C	SO	HPO	SED	*p*	C	SO	HPO	SED	*p*
C10:0	12.1	17.6	24.6	6.98	0.27	-	-	-	-	-	-	-	-	-	-
C14:0	0.14	0.11	0.26	0.18	0.72	0.03	0.24	0.04	0.20	0.55	0.96	1.04	1.20	0.39	0.83
C16:0	40.5[b]	37.9[b]	51.7[a]	2.06	<0.01	9.9	10.4	9.7	4.42	0.98	7.39	8.13	10.2	1.25	0.14
C18:0	76.8[b]	72.8[b]	89.8[a]	3.93	0.01	17.4	15.6	13.4	5.77	0.79	7.76	7.94	8.74	1.58	0.80
C18:1 trans-10	0.24	0.16	0.08	0.23	0.81	-	-	-	-	-	-	-	-	-	-
C18:1 trans-11	0.44	3.00	2.10	1.66	0.36	0.22	1.54	0.67	0.54	0.12	-	-	-	-	-
C18:1 cis-9	23.6	27.4	30.0	2.93	0.16	4.78	5.81	3.67	1.64	0.47	0.8	0.3	0.0	0.42	0.20
C18:2 cis-9, 12	113	111.3	123.5	7.61	0.29	18.0	17.7	11.7	5.46	0.47	2.23	1.62	2.16	0.48	0.43
C18:3 cis-9, 12, 15	2.55	3.12	3.83	0.64	0.22	-	-	-	-	-	1.32	1.63	2.01	0.29	0.13
C18:2 cis-9, trans-11	0.10	0.13	0.06	0.11	0.79	-	-	-	-	-	-	-	-	-	-
C20:5 n-3	1.94	2.51	3.14	0.63	0.24	0.07	0.06	0.09	0.11	0.95	-	-	-	-	-
C20:4 n-6	12.9	11.2	13.7	0.86	0.06	1.94	1.61	1.26	0.66	0.62	-	-	-	-	-
C22:6 n-3	12.8	12.9	13.0	2.47	0.99	2.54	2.51	2.93	1.55	0.95	-	-	-	-	-
Σ Saturated	149.3[b]	147.1[b]	190.3[a]	7.15	<0.01	30.8	29.4	26.0	11.0	0.90	16.4[b]	17.4[b]	20.3[a]	0.45	<0.01
Σ Monounsaturated	33.0	36.9	40.5	4.31	0.29	5.86	8.22	5.22	2.25	0.43	0.84	0.29	0.0	0.42	0.20
Σ Polyunsaturated	143.3	141.2	157.3	6.58	0.09	23.2	22.5	16.4	7.08	0.59	3.55	3.25	4.17	0.33	0.07
Other‡	13.56	17.11	17.6	1.92	0.14	4.42	4.80	3.88	1.67	0.86	0.36	0.43	0.86	0.42	0.48

Notes: Lactating cows: C (control) = basal diet without fat supplement; SO = basal diet supplemented with 500 g/d per cow of SO; HPO = basal diet supplemented with 500 g/d per cow of HPO; *SED = Standard error of the difference; †'-' = Not detected; ‡Other = FA unidentified or present at <0.05 mg/dl; [a,b] Means in the same row with different superscripts differ significantly for treatment effect with the *p*-value shown.

458 **Table 4** *Fatty acid composition of plasma lipoprotein from non-lactating cows fed control (C), hydrogenated palm oil (HPO) and*
459 *soybean oil (SO) dietary treatments (mg / dl of plasma)*

Fatty acid	High density lipoprotein					Low density lipoprotein					Very Low density lipoprotein				
	C	SO	HPO	SED*	*p*	C	SO	HPO	SED	*p*	C	SO	HPO	SED	*p*
C10:0	26.7	25.7	19.5	6.54	0.52	-	-	-	-	-	-	-	-	-	-
C14:0	-	-	-	-	-	0.04	0.01	0.03	0.02	0.56	0.82	0.76	0.88	0.24	0.89
C16:0	39.8	37.9	47.8	3.48	0.06	11.5	10.5	13.7	1.06	0.06	6.56	7.80	8.36	0.70	0.10
C18:0	76.6[b]	76.7[b]	85.8[a]	3.43	0.05	17.6	18.1	20.3	2.19	0.47	8.56	7.04	8.00	2.16	0.78
C18:1 trans-11	1.15[b]	4.33[a]	1.55[b]	0.57	<0.01	0.84	2.03	0.89	0.49	0.08	-	-	-	-	-
C18:1 cis-9	23.1	24.5	25.1	2.38	0.72	5.42	5.18	4.71	0.46	0.35	0.2	0.3	-	0.33	0.61
C18:2 cis-9, 12	71.4	77.8	77.0	7.36	0.66	10.4	10.9	10.7	1.37	0.93	1.46	2.38	1.56	1.02	0.63
C18:3 cis-9, 12, 15	2.30	2.53	3.10	0.39	0.18	-	-	-	-	-	1.28	1.89	1.59	0.80	0.76
C18:2 cis-9, trans-11	0.11	0.26	0.12	0.18	0.66	-	-	-	-	-	-	-	-	-	-
C20:5 n-3	4.18	3.70	3.83	0.68	0.77	0.08	0.14	0.12	0.10	0.87	-	-	-	-	-
C20:4 n-6	25.9	26.0	26.3	2.91	0.98	3.54	3.43	3.37	0.77	0.97	-	-	-	-	-
C22:6 n-3	14.5	13.5	15.0	1.17	0.47	2.32	2.52	1.43	0.87	0.46	-	-	-	-	-
Σ Saturated	163.1	160.3	173.1	9.76	0.43	32.5	31.9	37.5	3.06	0.21	16.2	15.9	17.9	1.90	0.56
Σ Monounsaturated	28.4	33.3	31.8	1.88	0.09	7.54[b]	8.11[a]	6.83[b]	0.40	0.05	0.23	0.33	-	0.32	0.61
Σ Polyunsaturated	118.5	123.8	125.4	7.86	0.67	16.7	17.4	16.1	2.82	0.90	2.74	4.26	3.14	1.79	0.69
Other	9.97	10.5	9.84	1.64	0.91	3.87	4.82	4.10	0.55	0.28	0.74	0.0	0.22	0.31	0.13

460 Notes: Dry non-pregnant cows: C (control) = basal diet without fat supplement; SO = basal diet supplemented with 170 g/d per cow
461 of SO; HPO = basal diet supplemented with 170 g/d per cow of HPO; *SED = Standard error of the difference; †'-' = Not detected;
462 ‡Other = FA unidentified or present at <0.05 mg/dl; [a,b] Means in the same row with different superscripts differ significantly for
463 treatment effect with the *p*-value shown.
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