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Distribution of *trans*-vaccenic acid and *cis*9,*trans*11-conjugated linoleic acid (rumenic acid) in blood plasma lipid fractions and secretion in milk fat of Jersey cows fed canola or soybean oil

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Abstract — To determine fatty acid distribution in lipid fractions of bovine blood plasma, 24 multiparous Jersey cows at peak lactation were fed a control diet (no supplemental oil, NOS) or the control diet supplemented at 35 g·kg^{-1} dry matter with canola oil (CAN), soybean oil (SOY), or a mixture of equal amounts of canola and soybean oil (MIX) for 4 wk. Plasma lipid fractions were isolated with aminopropyl columns. Fatty acid concentration in the phospholipid (PL), cholesterol ester (CE), and triglyceride (TG) fractions of plasma from cows fed supplemental oil was 142 (+47%), 144 (+57%), and 26 (+72%) μg·mL^{-1} greater than those of the control group. Oleic acid increased from 153 to 195, 100 to 151, 35 to 53, and 103 to 161 mg·g^{-1} of total fatty acids in the free fatty acid (FFA), PL, CE, and TG fractions, respectively, when CAN-fed cows were compared with NOS. In contrast, SOY intake increased 18:2n-6 in FFA, PL, CE, and TG fractions from 37 to 55, 327 to 366, 684 to 744, and 42 to 72 mg·g^{-1}, respectively. In the TG fraction, feeding SOY also increased *trans*11-18:1 from 40 to 105 mg·g^{-1} and *cis*9,*trans*11-18:2 from 1 to 12 mg·g^{-1}. Concentration and yield of *trans*11-18:1 in milk fat were 21 mg·g^{-1} and 13.7 g·d^{-1} when feeding NOS, and increased to 45 mg·g^{-1} and 43.1 g·d^{-1} in response to SOY. Similarly, concentration and yield of *cis*9,*trans*11-18:2 were 5 mg·g^{-1} and 7.3 g·d^{-1}, and increased to 13 mg·g^{-1} and 11.2 g·d^{-1} in response to SOY. Concentrations of *trans*-isomers in blood plasma and milk fat ranked by treatment reflected linoleic acid intakes, and were greater in SOY-fed cows compared with NOS or CAN-fed cows. Plasma lipid fractions can be used to appraise incomplete biohydrogenation of unsaturated fatty acids in the rumen.

rumenic acid / *trans*-vaccenic acid / bovine plasma / milk fat

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Résumé — Distribution de l'acide *trans*-vaccénique et de l'acide linoléique conjugué *cis*9, *trans*11 (acide ruménique) dans les fractions lipidiques du plasma sanguin et leur sécrétion dans la matière grasse du lait de vaches de race Jersiaise nourries à l'huile de canola et de soja. Afin de déterminer la distribution des acides gras dans les fractions lipidiques du plasma sanguin de bovins, 24 vaches multipares de race Jersiaise au pic de lactation ont reçu soit un régime témoin (sans addition d'huile, NOS) soit un régime témoin supplémenté de 35 g·kg^{-1} de matière sèche d'huile de canola (CAN) ou d'huile de soja (SOY), soit un mélange en quantité égale d'huile de canola et de soja (MIX) sur une période de 4 semaines. Les fractions lipidiques du plasma ont été isolées sur colonnes aminopropyle. La concentration en acides gras dans les fractions phospholipides (PL), esters de cholestérol (CE) et triglycérides (TG) du plasma a été, respectivement, plus élevée de 142 (+47 %), 144 (+57 %), et 26 (+72 %) µg·mL^{-1} chez les vaches recevant un régime enrichi en huile. Par rapport au témoin, l'acide oléique a augmenté de 153 à 195, de 100 à 151, de 35 à 53, et de 103 à 161 mg·g^{-1} d'acides gras totaux dans les fractions acides gras libres (FFA), PL, CE, et TG, respectivement, lorsque les vaches étaient alimentées avec le régime CAN. L'ingestion du régime SOY a augmenté la concentration du 18:2 n-6 dans les fractions FFA, PL, CE, et TG de 37 à 55, de 327 à 366, de 684 à 744, et de 42 à 72 mg·g^{-1}, respectivement, alors que dans la fraction TG, le régime SOY a augmenté les concentrations du *trans*11-18:1 de 40 à 105 mg·g^{-1} et du *cis*9, *trans*11-18:2 de 1 à 12 mg·g^{-1}. La concentration et la sécrétion du *trans*11-18:1 dans la matière grasse du lait ont été de 21 mg·g^{-1} et 13,7 g·j^{-1} avec le régime NOS, et ont atteint 45 mg·g^{-1} et 43,1 g·j^{-1} avec le régime SOY. De même, la concentration et la sécrétion du *cis*9, *trans*11-18:2 ont été de 5 mg·g^{-1} et 7,3 g·j^{-1}, et ont culminé jusqu'à 13,0 mg·g^{-1} et 11,2 g·j^{-1} en réponse au régime SOY. Entre traitements, les concentrations des isomères *trans* dans le plasma sanguin et la matière grasse du lait ont reflété les ingestions d'acide linoléique, et ont donc été plus importantes chez les vaches recevant le régime SOY comparées à celles du régime NOS ou CAN. Les fractions lipidiques du plasma peuvent être utilisées pour apprécier la composition en produits de la bio-hydrogénation des acides gras insaturés dans le rumen.

acide ruménique/ acide trans-vaccénique / plasma / matière grasse du lait / bovin

1. INTRODUCTION

The concentration of blood plasma lipid fractions in ruminants fed supplemental fat is elevated [5, 28]. However, plasma fatty acid profiles of cattle and sheep do not necessarily reflect dietary fatty acid content. During hydrogenation in the rumen, isomerization of 18: 2n-6 results in production of *cis*9,*trans*11-18:2 (CLA) [15]. *Trans*11-18:1 and 18:0, however, are the primary products from 18:2n-6 or 18:3n-3 hydrogenation [15, 33]. Oleic acid was either not hydrogenated [14], isomerized to *trans*11-18:1 (5% of total oleic acid substrate), or hydrogenated directly to 18:0 (80–90% of total oleic acid substrate) [23].

Under normal conditions, polyunsaturated fatty acids in blood plasma of sheep appear to be selectively incorporated into the plasma cholesterol ester (CE) and phospholipid (PL) fractions [26]. When soybean oil was fed to dairy cows [19] the proportion of 18:2n-6 in the PL and CE fractions increased. Stearic acid concentration, however, was higher in the triglyceride (TG) fraction. Similarly, proportions of 18:0 and 18:1 (*cis* and *trans* isomers were not separated) fatty acids in milk fat also were increased. More recently, ruminal infusion of canola oil (200 to 400 g·d^{-1} for 14 d) resulted in higher concentrations of 18:0 and *trans*11-18: 1 in blood plasma and milk TG [6].

Unsaturated oil feed ingredients are widely used in contemporary diets for lactating cows, and they can have a profound effect on the manufacturing and nutritional properties of milk fat [2, 29]. A significant portion of esterified fatty acids in milk fat are derived from plasma TG and non-esterified fatty acids FFA [30], and to a

lesser extent phospholipids PL [38], extracted by the mammary gland. Although several studies have previously evaluated fatty acid composition in selected plasma lipid fractions due to feeding lipid supplements to lactating dairy cows none, to our knowledge, reported responses in *trans*11-18:1 and *cis*9,*trans*11-18:2 systematically. Lipid fractions in ruminant plasma have traditionally been isolated using thin-layer chromatography [17, 19, 21, 26, 28]. This procedure, however, is relatively slow, may result in oxidation of polyunsaturated fatty acids due to extensive exposure to air, and produces low lipid recoveries [13, 22].

In the present study, we utilized solid-phase extraction column chromatography as an alternative to thin-layer chromatography (TLC) for isolation of plasma lipid fractions. This procedure reportedly improved lipid recovery, sample throughput, and may reduce the risk of fatty acid oxidation compared with TLC [13]. Diets contained either canola oil, soybean oil, or equal amounts of canola plus soybean oil to vary the amount of oleic acid, linoleic acid, *cis*9,*trans*11-18:2, and *trans*11-18:1 flowing from the rumen to the small intestine for absorption and incorporation into plasma lipid fractions. The overall objective of the study was to determine changes in the profiles of lipid fractions due to dietary treatments imposed. Of particular interest was to examine relationships between intake of oleic and linoleic acid, profiles of *trans*11-18:1 and *cis*9,*trans*11-18:2 in blood plasma, and secretion of both biohydrogenation intermediates in milk fat.

2. MATERIALS AND METHODS

2.1. Animals and diets

Twenty-four Jersey cows between 60 and 90 d postpartum were housed in a free stall barn and individually fed a total mixed diet without supplemental oil for ad libitum intake using a Calan feeding system (American Calan, Inc., Northwood, NH, USA) for 7 d before the study. After the 7-d adjustment period, cows were randomly assigned to a diet (Tab. I) containing no supplemental oil (NOS, control) or diets in which corn grain in NOS was replaced (35 $g \cdot kg^{-1}$ dry matter) with canola oil (CAN), soybean oil (SOY), or an equal mixture of canola (17.5 $g \cdot kg^{-1}$ dry matter) and soybean oil (17.5 $g \cdot kg^{-1}$ dry matter) (MIX) for 4 wk. Diets were prepared daily and fed at 17:00 h. Feed allotment was calculated to allow 5 to 10% feed refusal. Diets were formulated using Dair4 [36] to meet nutrient requirements of lactating cows producing 23 kg milk and consuming 17 kg dry matter daily [27]. Water was available in the free stall barn at all times. Cows were milked each day at 13:00 and 01:00 h. Animal management and sampling procedures were approved by the Virginia Polytechnic Institute and State University Animal Care Committee.

2.2. Blood plasma collection, lipid extraction, and fatty acid analysis

Jugular blood samples (20 mL) were obtained by venipuncture on d 7 of the adjustment period and d 28 of the experimental period at 4 h after feeding. The concentrations of bovine plasma lipid fractions do not have a circadian rhythm when cows consume feed ad libitum [4]. The constant rate of passage of lipids from the rumen prevents abrupt increases in plasma fatty acids. Blood was transferred to tubes containing 286 IU heparin in 100 μL of sterile saline, and centrifuged at 3 000 × *g* for 15 min at 4 °C for harvesting plasma. Plasma was stored at –20 °C until lipid extraction and fatty acid analysis.

Lipids were extracted from plasma with chloroform/methanol (2:1, vol/vol). Subsequently, blood plasma lipid fractions were separated using Bond Elut® aminopropyl disposable columns (500 mg) with stainless

Table I. Ingredient and chemical composition of diets.

	NOS	CAN	MIX	SOY
Ingredient		g·kg^{-1} dry matter		
Alfalfa haylage	325	325	325	325
Corn silage	253	253	253	253
Corn grain	327	292	292	292
Soybean meal, 48% crude protein	59	59	59	59
Canola oil	0	35	17.5	0
Soybean oil	0	0	17.5	35
Prolak[1]	22	22	22	22
Mineral/vitamin mix[2]	14	14	14	14
Chemical composition				
NDF	293	288	288	288
ADF	195	193	193	193
Crude protein	168	163	163	163
Total fatty acids[3]	35	61	63	64
		mg·g^{-1} total fatty acids		
14:0	6	4	3	4
16:0	183	112	130	146
18:0	24	24	29	32
cis9-18:1	170	473	328	185
18:2n-6	481	303	411	520
18:3n-3	136	84	99	113

[1] Prolak (H.J. Baker & Bro., Inc., Atlanta, GA, USA): crude protein = 600 g·kg^{-1} dry matter, crude fat = 60 g·kg^{-1} dry matter, crude fiber = 20 g·kg^{-1} dry matter.
[2] Mineral/vitamin mix (Southern States Cooperative, Richmond, VA, USA): salt (38–48 g·kg^{-1}), $NaHCO_3$ (180 g·kg^{-1}), Ca (145–174 g·kg^{-1}), P (65 g·kg^{-1}), Cl (58 g·kg^{-1}), S (32 g·kg^{-1}), Mg (22 g·kg^{-1}), K (35 g·kg^{-1}), Mn (1 g·kg^{-1}), Zn (1 g·kg^{-1}), Fe (0.3 g·kg^{-1}), Cu (0.1 g·kg^{-1}), I (0.02 g·kg^{-1}), Co (0.003 g·kg^{-1}), Se (0.005 g·kg^{-1}), F (0.65 g·kg^{-1}), retinyl acetate (0.36 g·kg^{-1}), cholecalciferol (0.01 g·kg^{-1}), dl-α-tocopherol acetate (0.59 g·kg^{-1}).
[3] Sum of 14:0 to 18:3n-3.

steel frits in a Vac Elut® vacuum elution apparatus (Analytichem International, Harbor City, CA, USA) [13]. Samples were transmethylated with 0.5N methanolic NaOH at 50 °C for 30 min, followed by 14% boron trifluoride in methanol also at 50 °C for 30 min [32]. Methyl esters of fatty acids were separated by gas chromatography using a 30 m × 0.25 mm i.d. fused silica capillary column (SP-2380, Supelco, Inc., Bellefonte, PA, USA). Pure methyl ester standards (Nu-Check Prep, Elysian, MN, USA; Supelco, Inc., Bellefonte, PA, USA) were used to identify peaks, and determine correction factors for individual fatty acids. The injector temperature was maintained at 225 °C and the detector temperature at 275 °C. The initial column temperature was 205 °C (held for 12 min), and was programmed to increase 2 °C·min^{-1} to a final temperature of 220 °C (held for 2 min).

2.3. Sampling, measurements, and chemical analysis of diets

Milk production was recorded electronically at each milking throughout the study. At wk 0 and 4, two 30 mL aliquots of milk were collected at 01:00 and 13:00 h. A 30 mL aliquot was collected in a 50 mL vial

containing Bronopol (milk preservative; D & F Control Systems, San Ramon, CA, USA) immediately after milking. Milk was analyzed for milk fat, protein, lactose, and solids-not-fat by infrared analysis with a 4-channel spectrophotometer (Virginia Dairy Herd Improvement Association, VA, USA). An additional aliquot of milk without Bronopol also was collected, then frozen at –20 °C. Subsequently, samples were thawed at room temperature and centrifuged at 10 000 × *g* for 1 h to isolate milk fat. Methylation and chromatographic separation of fatty acids in milk fat were as described for blood plasma.

Forages and concentrate were sampled during the last day of each experimental wk. Samples were dried in a forced-air oven at 60 °C, then stored in sealed plastic containers. Equal amounts of samples from each wk were combined to determine chemical composition. In preparation for analyses, dried forages and concentrate were ground first through a 2 mm screen (Thomas-Wiley Laboratory Mill), then through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Forages and concentrates were analyzed for acid detergent fiber and neutral detergent fiber [40] and total nitrogen [1]. Lipid extraction, methylation, and chromatographic separation of fatty acids in forages and concentrates were as described for blood plasma.

2.4. Statistical analysis

Data were analyzed by analysis of covariance using the MIXED procedure of SAS [34], with observations at the end of the adjustment period serving as covariate for observations at 28 d. Data for fatty acid concentrations in plasma lipid fractions, dry matter intake, and fatty acid intake are presented as Least squares means with pooled SEM. Data for milk production and composition, and fatty acid profiles of milk fat were reported previously [2]. The model for statistical analysis of all data included: covariate adjustment, treatment (NOS, CAN, SOY, or MIX), cow within treatment, and residual error. Relationships between the intake of oleic or linoleic acid and concentrations of *cis*9-18:1, 18:2n-6, *trans*11-18:1, or *cis*9,*trans*11-18:2 in blood plasma phos- pholipids, triglycerides, and free fatty acids were obtained using the REG procedure of SAS [34]. Relationships obtained from regression analysis were deemed linear or curvilinear based on coefficient of determinations. Differences between treatment means were determined by Tukey's studentized procedure, and were accepted as significantly different at $P < 0.05$. One cow in the group fed soybean oil was omitted from the statistical analysis, because she inadvertently had access to the control diet for 2 d before sampling at 28 d.

3. RESULTS AND DISCUSSION

A recent review indicated that as the percentage of unsaturated oil supplementation in the diet of lactating cows increases above 35 g·kg^{-1} dry matter, feed intake is depressed [7]. The level of oil supplementation in the present study was adequate to increase daily intake of total fatty acids by 426 g·d^{-1} without affecting dry matter intake (Tab. II). On average, cows fed supplemental oil consumed greater amounts of 16:0 (22 g·d^{-1}), 18:0 (15 g·d^{-1}), *cis*9-18:1 (239 g·d^{-1}), 18:2n-6 (131 g·d^{-1}), and 18:3n-3 (19 g·d^{-1}) compared with controls. In addition, cows fed CAN consumed 386 g more *cis*9-18:1, whereas cows fed SOY consumed 222 g·d^{-1} more 18:2n-6 compared with NOS-fed cows. Feeding MIX also increased *cis*9-18:1 and 18:2n-6 intake by 253 and 152 g·d^{-1} compared with NOS. Fatty acid intakes in this study were comparable with those indicated in previous reports concerning high-oleic or high-linoleic oil fed to lactating cows [19, 35, 37].

Milk production and milk fat percentage (data not shown) were not affected by

Table II. Intake of dry matter and fatty acids by Jersey cows fed a control (NOS) diet or the control diet supplemented with 35 g·kg^{-1} dry matter canola oil (CAN), soybean oil (SOY), or equal amounts of canola and soybean oils (MIX).

	NOS	CAN	MIX	SOY	SEM
Dry matter intake, kg·d^{-1}	17.1	17.5	17.3	15.5	1.1
Fatty acid intake, g·d^{-1}					
14:0	3.7	4.2	3.3	4.0	0.2
16:0	112.7^{b}	116.2^{a,b}	141.8^{a}	145.5^{a}	8.0
18:0	14.8^{c}	24.9^{b}	31.7^{a}	31.9^{a}	1.5
*cis*9-18:1	104.7^{d}	490.8^{a}	357.7^{b}	184.4^{c}	19.2
18:2n-6	296.3^{c}	314.4^{c}	448.3^{b}	518.2^{a}	23.1
18:3n-3	83.8^{b}	87.2^{b}	107.9^{a}	112.6^{a}	5.9
Total	616.1^{b}	1 037.6^{a}	1 090.6^{a}	996.7^{a}	56.5

^{a,b,c,d} Least squares means within row and treatment category with different superscripts differ ($P < 0.05$).

treatments and averaged 22.1 vs. 23.6 ± 1.7 kg milk·d^{-1}, and 4.7 vs. 4.9 ± 0.4% fat when NOS or oil-supplemented diets were fed, respectively. However, the numerically higher milk yield and fat percentage in oil-fed cows resulted in higher milk fat yield compared with NOS (1.0 vs. 1.2 ± 0.1 kg·d^{-1}).

Total fatty acid concentration in plasma lipid fractions from oil-fed cows increased by 306 μg·mL^{-1} (48%) compared with NOS, and reflected higher intake of fatty acids (Fig. 1). The supplemental fatty acids were distributed primarily in the PL, CE, and TG fractions, which increased by 142 (47%), 144 (57%), and 26 (72%) μg·mL^{-1}, respectively. Higher amounts of PL, CE, and TG in blood plasma were reported previously when soybean oil, or oleic acid were fed to lactating cows [19, 31, 35]. Presumably an increase in the concentration of the major plasma lipid fractions is an obligatory response to accommodate transport of greater amounts of unsaturated and total fatty acids absorbed from the small intestine [5].

Overall, the average amount of fatty acids in the FFA fraction from oil-fed cows was similar to NOS, but this was due to the lower amount (48%) of FFA in cows fed MIX compared with those fed CAN or SOY. Other reports indicated no changes [19, 35] or increases [31, 37] in the concentration of FFA in plasma when oils or hydrolyzed fat were fed to lactating cows. In the latter case, it was suggested that higher levels of FFA were a reflection of increased TG concentration and hydrolysis when supplemental fat was fed [31].

Intake of *cis*9-18:1 and 18:2n-6 due to oil supplementation was the major factor leading to changes in the distribution of *cis*9-18:1, 18:2n-6, *trans*11-18:1, and *cis*9,*trans*11-18:2 in lipid fractions. Relative to basal (NOS), increases in the concentration of *cis*9-18:1 (100 to 155 mg·g^{-1} of total fatty acids) and 18:2n-6 (327 to 369 mg·g^{-1}) in PL (Tab. III) corresponded with additional intake of these fatty acids when CAN or SOY were fed (Tab. II). The increase in *trans*11-18:1 (12 to 31 mg·g^{-1}) and *cis*9,*trans*11-18:2 (0.3 to 3 mg·g^{-1}), however, was related with higher 18:2n-6 intake when SOY was fed. Linoleic acid accounted for 684 mg·g^{-1} in the CE fraction

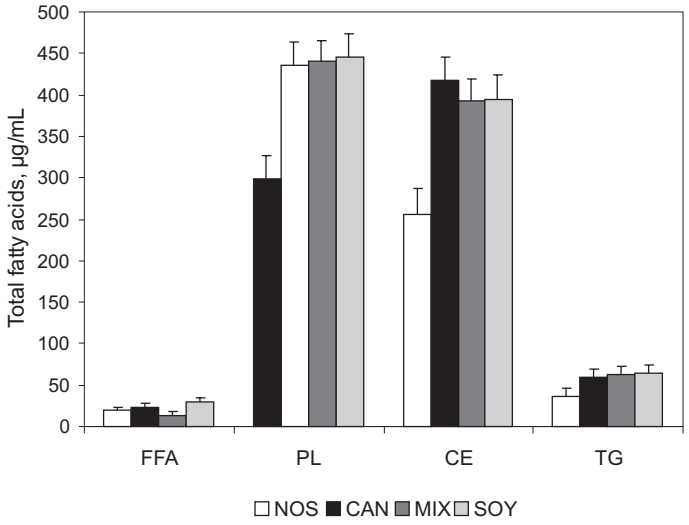

Figure 1. Total fatty acid concentration in blood plasma free fatty acids (FFA), phospholipids (PL), cholesterol esters (CE), and triglycerides (TG) from Jersey cows fed a control diet (NOS) or the control diet supplemented at 35 g·kg^{-1} dry matter with canola oil (CAN), soybean oil (SOY), or an equal mixture of canola and soybean oil (MIX). Feeding supplemental oils increased ($P < 0.05$) total fatty acid content in blood plasma PL, CE, and TG compared with the control diet.

(Tab. III) of cows fed NOS, but the proportion increased to 748 mg·g^{-1} in cows fed SOY. *Trans*11-18:1 (0 to 2.2 mg·g^{-1}) and *cis*9,*trans*11-18:2 (0 to 1.5 mg·g^{-1}) in CE also were increased by feeding SOY. A marked increase in the concentration of *trans*11-18:1 (40 to 125 mg·g^{-1}) and *cis*9-18:1 (103 to 165 mg·g^{-1}) in TG (Tab. IV) also was evident when SOY or CAN were fed compared with NOS. *Cis*9,*trans*11-18:2 in plasma TG of cows fed NOS was 1.4 mg·g^{-1}, but it increased to 7.4 or 12.4 mg·g^{-1} in response to feeding CAN and MIX or SOY. In FFA, feeding SOY increased 18:2n-6 (37 to 55 mg·g^{-1}), *trans*11-18:1 (51 to 84 mg·g^{-1}), and *cis*9,*trans*11-18:2 (0 to 21 mg·g^{-1}). In contrast, feeding CAN increased *cis*9-18:1 (153 to 195 mg·g^{-1}) (Tab. IV). Only feeding SOY altered 18:0 concentration by causing its decrease in PL (223 to 206 mg·g^{-1}) and TG (469 to 380 mg·g^{-1}).

Results suggest that some selectivity exists regarding the distribution of absorbed unsaturated fatty acids in the major plasma lipid fractions of cows fed oil. This selectivity becomes important when appraising the fatty acid contribution of lipid fractions to the mammary gland for milk fat synthesis. For example, fractional removal rate of fatty acids from plasma TG (of VLDL and chylomicra) in lactating cows is very rapid compared with the remaining LDL fraction [30] such that, during passage of blood from through the udder, it was estimated that 70% of plasma TG were hydrolyzed and their fatty acids made available for milk fat synthesis [9]. At high arterial concentrations, PL also provided fatty acids for milk fat synthesis [24, 38]. Therefore, it seems reasonable that the mammary gland could utilize non-esterified fatty acids derived from TG and PL when their concentrations in plasma are increased in response to oil feeding.

Greater concentrations of *cis*9-18:1 and 18:2n-6 were found in all major lipid fractions when CAN or SOY was fed in this

Table III. Concentration of fatty acids in the phospholipid and cholesterol ester fractions of blood plasma from Jersey cows fed a control (NOS) diet or the control diet supplemented with 35 g·kg^{-1} dry matter canola oil (CAN), soybean oil (SOY), or equal amounts of canola and soybean oils (MIX).

	NOS	CAN	MIX	SOY	SEM
Fatty acid			mg·g^{-1} total fatty acids		
Phospholipid					
14:0	2.5^{a}	2.1^{b}	1.8^{b}	2.0^{b}	0.1
*cis*9-14:1	3.1^{a}	2.3^{b}	2.4^{b}	2.1^{b}	0.2
15:0	7.9^{a}	6.7^{b}	6.8^{b}	6.8^{b}	0.2
16:0	140.5^{a}	128.2^{b}	134.4^{a}	130.5^{a}	3.4
*cis*9-16:1	7.5^{a}	5.4^{b}	5.5^{b}	5.2^{b}	0.4
17:0	17.1^{a}	11.8^{b}	12.0^{b}	11.4^{b}	0.4
18:0	222.9^{a}	226.5^{a}	215.3^{a}	206.3^{b}	4.1
*trans*11-18:1	12.0^{c}	12.3^{c}	17.2^{b}	26.9^{a}	1.1
*cis*9-18:1	100.3^{b}	151.1^{a}	105.2^{b}	97.7^{b}	3.8
18:2n-6	327.4^{c}	303.5^{d}	353.1^{b}	366.3^{a}	6.2
*cis*9,*trans*11-18:2	0.3^{d}	1.2^{c}	2.0^{b}	3.1^{a}	0.2
18:3n-3	27.9^{a}	25.3^{a}	23.8^{a}	20.1^{b}	1.0
20:3n-3	48.5^{a}	43.0^{b}	41.1^{b}	41.1^{b}	2.0
Other	81.9	80.6	79.4	80.5	. . .
Cholesterol ester					
14:0	9.1^{a}	7.7^{b}	7.7^{b}	7.2^{b}	0.4
*cis*9-14:1	16.9^{a}	13.8^{b}	12.1^{c}	11.0^{c}	0.6
15:0	11.1^{a}	9.6^{b}	8.3^{b}	8.5^{b}	0.3
16:0	44.9^{a}	37.5^{b}	35.9^{b}	36.0^{b}	1.1
*cis*9-16:1	15.0^{a}	15.0^{a}	11.7^{b}	11.9^{b}	1.0
17:0	2.7^{a}	1.6^{b}	1.6^{b}	1.1^{b}	0.2
18:0	7.9	7.0	7.3	6.8	0.5
*trans*11-18:1	ND[1]	1.1^{b}	1.4^{b}	2.2^{a}	0.3
*cis*9-18:1	35.4^{b}	53.1^{a}	34.7^{b}	30.2^{c}	1.4
18:2n-6	683.5^{b}	682.6^{b}	735.5^{a}	744.3^{a}	7.0
*cis*9,*trans*11-18:2	ND	0.1^{b}	0.4^{b}	1.5^{a}	0.2
18:3n-3	99.9	105.4	76.9	77.3	8.5
20:3n-3	6.9^{a}	6.3^{a}	5.7^{b}	5.4^{b}	0.3
Other	23.4	23.6	22.1	22.6	. . .

[1] Not detected.
[a,b,c,d] Least squares means within row and treatment category with different superscripts differ ($P < 0.05$).

study compared with NOS. Linoleic acid was preferentially incorporated into PL and CE [5], but under conditions where large amounts of unsaturates are absorbed from the small intestine the rate of synthesis of PL may be insufficient to accommodate the increased supply of these fatty acids and extensive incorporation into TG also can occur. In vitro data indicated that 18:2n-6 and 18:3n-3, but not *cis*9-18:1, are the preferred substrates for the synthesis of CE in bovine plasma via the lecithin: cholesterol acyl transferase enzyme system [25]. This was consistent with the data of Moore et al. [21] which reported increased concentrations of 18:2n-6 and 18:3n-3 in plasma PL and CE of sheep following ruminal infusion of primarily linoleic or linolenic acid. We

Table IV. Concentration of fatty acids in the triglyceride and free fatty acid fractions of blood plasma from Jersey cows fed a control (NOS) diet or the control diet supplemented with 35 g·kg^{-1} dry matter canola oil (CAN), soybean oil (SOY), or equal amounts of canola and soybean oils (MIX).

	NOS	CAN	MIX	SOY	SEM
Fatty acid			mg·g^{-1} total fatty acids		
Triglyceride					
14:0	34.1	32.7	28.5	30.5	4.5
15:0	6.9^{a}	3.7^{b}	3.3^{b}	4.8^{b}	0.7
16:0	263.1^{a}	219.3^{b}	226.1^{b}	240.3^{b}	6.6
18:0	468.8^{a}	423.1^{b}	430.8^{b}	379.5^{c}	19.5
*trans*11-18:1	40.4^{d}	58.9^{c}	71.5^{b}	105.2^{a}	4.1
*cis*9-18:1	102.8^{c}	161.2^{a}	132.3^{b}	100.7^{c}	5.2
18:2n-6	41.9^{c}	58.7^{b}	59.3^{b}	71.5^{a}	3.9
*cis*9,*trans*11-18:2	1.4^{c}	6.7^{b}	8.0^{b}	12.4^{a}	0.9
18:3n-3	9.9	7.7	5.8	8.8	1.8
Other	30.7	28.0	28.4	28.3	. . .
Free fatty acid					
14:0	42.0	36.6	35.8	28.6	4.2
*cis*9-14:1	9.7	6.6	8.7	10.0	1.4
16:0	274.0^{a}	248.1^{b}	240.9^{b}	242.9^{b}	8.3
*cis*9-16:1	7.2	12.9	10.7	9.1	2.4
17:0	11.4^{a}	8.5^{b}	8.3^{b}	9.1^{b}	0.6
18:0	356.2	331.7	339.7	342.2	11.3
*trans*11-18:1	45.3^{b}	61.4^{b}	84.0^{a}	83.8^{a}	8.4
*cis*9-18:1	152.5^{b}	194.5^{a}	174.5^{b}	132.1^{b}	12.7
18:2n-6	37.1^{b}	33.8^{b}	35.0^{b}	55.1^{a}	5.7
*cis*9,*trans*11-18:2	ND[1]	4.1^{b}	5.5^{b}	20.5^{a}	2.4
18:3n-3	13.1	10.6	9.8	8.4	1.6
20:3n-3	9.3	8.9	5.9	17.1	4.3
Other	42.2	42.3	41.2	41.1	. . .

[1] Not detected.
[a,b,c] Least squares means within row and treatment category with different superscripts differ ($P < 0.05$).

found that as the level of supplemental 18:2n-6 intake increased (Fig. 2A), the incorporation of linoleic acid into all lipid fractions also increased linearly ($R^2 = 0.69$, $P < 0.001$). Intake of *cis*9-18:1 also was a good predictor ($R^2 = 0.52$, $P < 0.001$) of oleic acid incorporation into plasma FFA, TG, and PL (Fig. 2B). Greater absorption of *cis*9-18:1 when CAN was fed may have increased the amount of this fatty acid esterified to plasma lecithin, thus elevating its proportion in PL.

Daily abomasal infusion of 0 to 400 g high-oleic sunflower oil (86% *cis*9-18:1) linearly increased blood plasma TG and oleic acid percentage in TG [17]. The concentration of FFA tended to increase ($P = 0.08$) as the rate of *cis*9-18: 1 infusion increased, but no clear pattern could be observed. As with PL, our data indicated that *cis*9-18:1 content of TG was a reflection of dietary oleic acid intake. It appears that significant amounts of dietary *cis*9-18:1 escaped biohydrogenation in the rumen

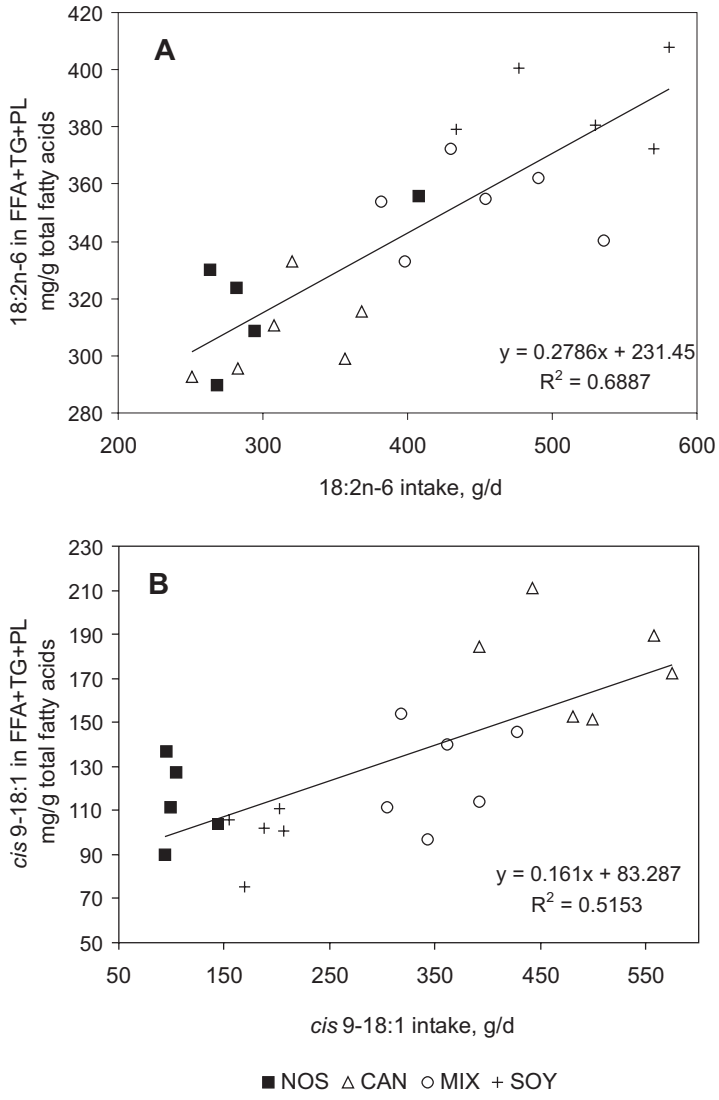

Figure 2. Relationships between linoleic acid intake and linoleic acid concentration (Panel A), or between oleic acid intake and oleic acid concentration (Panel B) in plasma free fatty acids (FFA), phospholipids (PL) and triglycerides (TG) (as a proportion of total plasma fatty acids) from Jersey cows fed a control diet (NOS) or the control diet supplemented at 35 g·kg^{-1} dry matter with canola oil (CAN), soybean oil (SOY), or an equal mixture of canola and soybean oil (MIX).

resulting in a higher percentage of this fatty acid in plasma PL, TG, and FFA. We did not find a relationship between unsaturated fatty acid intake and 18:0 concentration in lipid fractions (data not shown) which may indicate that, biohydrogenation of unsaturated fatty

acids was not complete or that there was significant desaturation of stearic acid in peripheral tissues and small intestine.

It is well established that a portion of dietary 18:2n-6 and 18: 3n-3 is hydrogenated in the rumen [15, 16, 33], mainly to *trans*11-18:1 and(or) 18:0. Incomplete biohydrogenation of 18:2n-6 also results in the production of conjugated fatty acids, of which *cis*9,*trans*11-18:2 is the predominant isomer [15]. The major Gram-positive ruminal bacterium, *Butyrivibrio fibrisolvens*, has the capacity to hydrogenate linoleic acid only to *trans*11-18:1. Apparently, two distinct microbial populations in the rumen are involved in the complete biohydrogenation of 18: 2n-6 to 18:0, with the hydrogenation of *trans*11-18:1 to 18:0 being the rate-limiting step [14]. Thus, diets containing supplemental unsaturated fatty acids increase flow and intestinal absorption of fatty acid intermediates derived from the ruminal biohydrogenation process.

Feeding SOY in the present study increased *trans*11-18:1 concentration in plasma to the greatest extent. O'Kelly and Spiers [28] observed increased proportions of *trans*11-18:1 in PL and TG when steers were fed safflower oil. An exponential relationship ($R^2 = 0.69$, $P < 0.001$) between 18:2n-6 intake and concentrations of *trans*11-18:1 in FFA, TG, and PL fractions provided the best fit for our data (Fig. 3A). This response indicates that the extent of hydrogenation was less complete, more *trans*11-18:1 accumulation, as dietary 18:2n-6 intake increased. *Trans*11-18:1 is preferentially incorporated into the sn-1 position of PL [5], but substantial enrichment in the CE and TG fractions also occurred when human diets were supplemented with a mixture of *trans*-fatty acids [39].

Absorption of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 through the lymphatic pathway of rats averaged 96% and was similar to 18:2n-6 [20]. This may explain why transfer of CLA isomers to the mammary gland for its incorporation into milk fat triglycerides is relatively rapid [18]. Abomasal infusion of CLA linearly increased the percentage of CLA in plasma TG, PL, and milk fat within 24 h [18]. During CLA infusion, there was a positive relationship between the percentage of CLA in plasma TG and PL with CLA percentage in milk fat.

Similar to *trans*11-18:1, feeding SOY increased concentrations of *cis*9,*trans*11-18:2 to the greatest extent in the plasma fractions studied (Tabs. III and IV). Milk fat *cis*9,*trans*11-18: 2 concentration (data not shown) also was higher when oils (10 $mg \cdot g^{-1}$ of total fatty acids) were fed compared with NOS (5 $mg \cdot g^{-1}$), but was highest in response to SOY (14 $mg \cdot g^{-1}$). Feeding a mixture of *cis,trans*-conjugated dienoic fatty acids (5% of dietary energy) to humans doubled their concentrations in PL [39]. As with *trans*11-18:1, higher availability of *cis*9,*trans*11-18:2 in the enterocyte could have increased its binding to lecithin for synthesis of PL via the LCAT enzyme system. Intake of 18:2n-6 was positively related ($R^2 = 0.57$, $P < 0.001$) with CLA concentrations in plasma fractions (Fig. 3B), but this relationship was not as strong as that between linoleic acid intake and *trans*11-18:1 in blood plasma (Fig. 3A). It could be possible that a portion of *trans*11-18:1 was desaturated to *cis*9,*trans*11-18: 2 in the intestine [3]. The stronger relationship ($R^2 = 0.65$, $P < 0.001$) between *trans*11-18:1 and *cis*9,*trans*11-18:2 in blood plasma (Fig. 4A) suggests that desaturation of ruminally derived *trans*11-18:1 could be a source of endogenous *cis*9,*trans*11-18:2. Morales et al. [22] found a higher ratio of *trans*11-18:1 to *cis*9,*trans*11-18:2 in plasma TG of cows fed roasted soybeans, which was taken as an indication that some desaturation of rumen-derived *trans*11-18:1 had occurred. The contribution of intestinal Δ^9-desaturase to *cis*9,*trans*11-18:2 synthesis, however, has not been evaluated. Alternatively, the above relationships could simply

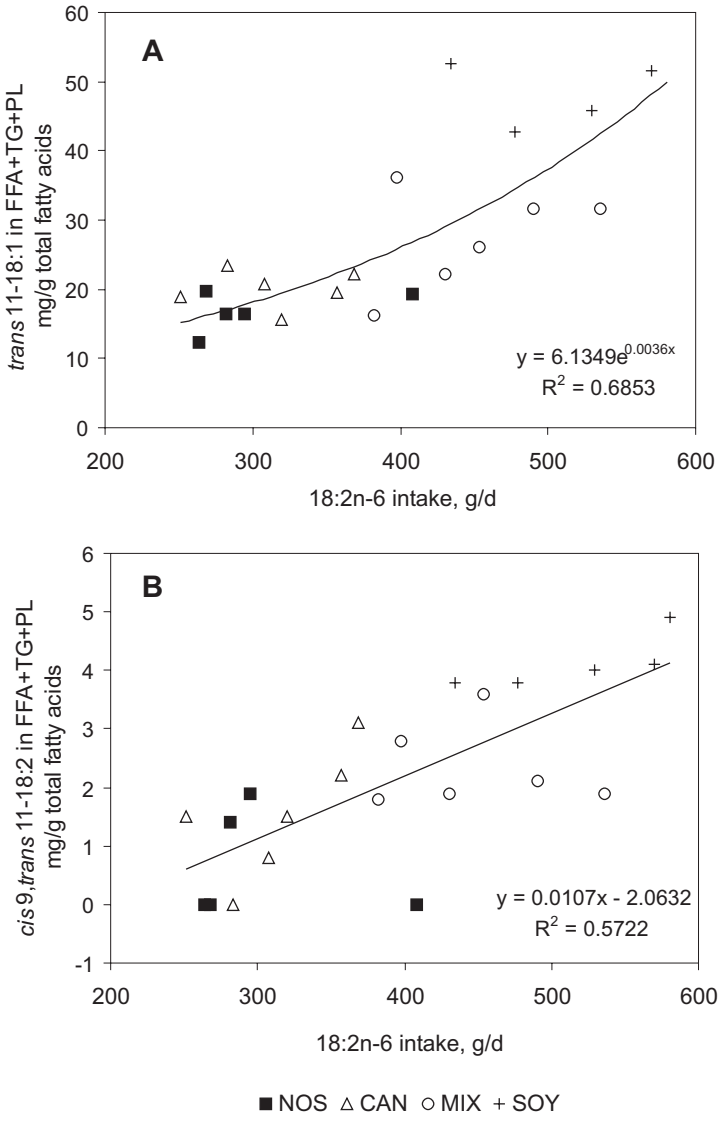

Figure 3. Relationships between linoleic acid intake and *trans*-vaccenic acid (Panel A) or *cis*9,*trans*11-CLA (Panel B) concentration in plasma free fatty acids (FFA), phospholipids (PL) and triglycerides (TG) (as a proportion of total plasma fatty acids) from Jersey cows fed a control diet (NOS) or the control diet supplemented at 35 g·kg^{-1} dry matter with canola oil (CAN), soybean oil (SOY), or an equal mixture of canola and soybean oil (MIX).

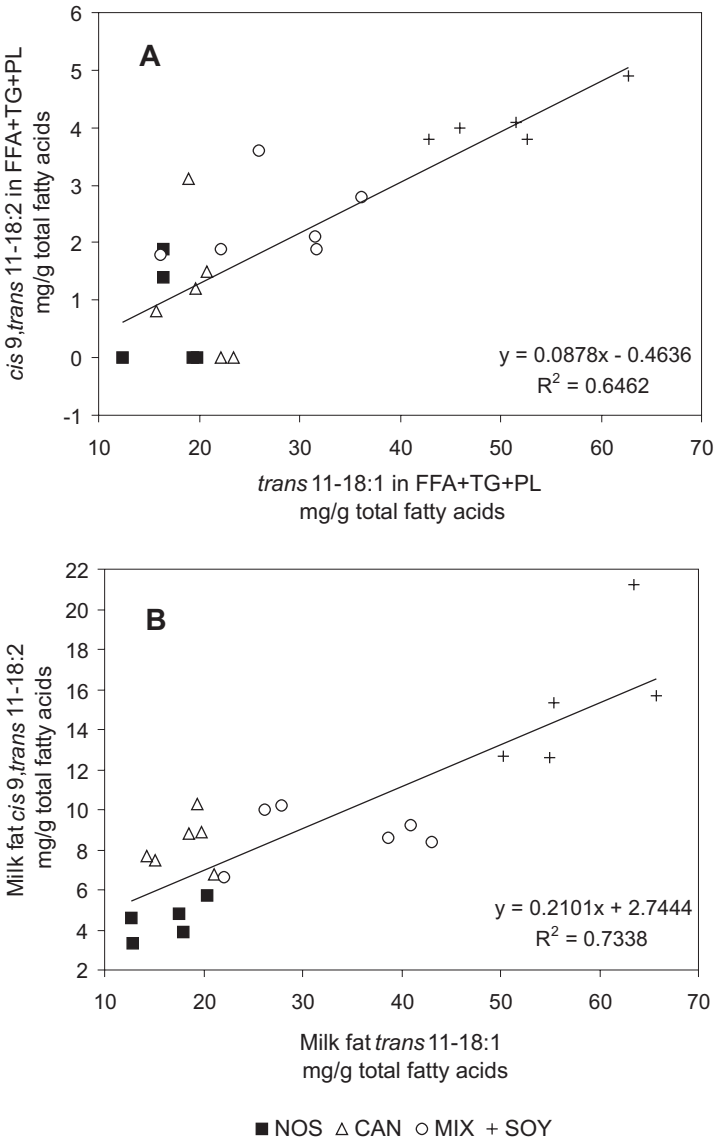

Figure 4. Relationships between *trans*-vaccenic acid and *cis*9,*trans*11-CLA concentrations in plasma free fatty acids (FFA), phospholipids (PL) and triglycerides (TG) (as a proportion of total plasma fatty acids) (Panel A), or in milk fat (Panel B) from Jersey cows fed a control diet (NOS) or the control diet supplemented at 35 g·kg^{-1} dry matter with canola oil (CAN), soybean oil (SOY), or an equal mixture of canola and soybean oil (MIX).

indicate that partial biohydrogenation of *cis*9,*trans*11-18:2 to *trans*11-18:1 in the rumen caused greater intestinal flow, absorption, and availability of both fatty acids in blood for uptake by tissues.

Mammary gland absorption of *cis*9, *trans*11-18:2 and *trans*11-18:1 from plasma and incorporation into milk fat appeared to be substantial. The yield (data not shown) of *trans*11-18:1 increased from 13.7 $g \cdot d^{-1}$ when feeding NOS to 16.8, 37.2, or 43.1 $g \cdot d^{-1}$ in response to feeding CAN, MIX, or SOY, respectively. Similarly, *cis*9,*trans*11-18:2 yield in milk fat was 3.8 $g \cdot d^{-1}$ when NOS was fed compared with 7.3, 7.7, or 11.2 $g \cdot d^{-1}$ when CAN, MIX, or SOY were fed, respectively. A positive correlation ($R^2 = 0.73$, $P < 0.001$) between *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat (Fig. 4B) may indicate that the mammary gland could readily take up both fatty acids from blood and(or) synthesize CLA from *trans*11-18:1. When bovine mammary cells were incubated with *trans*11-18:1 compared with *cis*9-18:1, 18:2n-6, or *cis*9,*trans*11-18:2, there was an increase in Δ^9-desaturase activity and mRNA abundance [10]. Indeed, a 55% increase in milk *cis*9,*trans*11-18:2 concentration in response to abomasal infusion of *trans*11-18:1 suggests that desaturation of *trans*11-18:1 is feasible [8].

Stearic acid is one of the predominant fatty acids in blood plasma PL and TG of ruminants under normal dietary conditions [5], and its concentration may increase when plant oils with high *cis*9-18:1 content are fed [6, 35]. Morris [23] indicated that oleic and elaidic acid in the rumen are biohydrogenated directly to stearic acid without prior isomerization. Intake of *cis*9-18:1 from whole soybeans resulted in greater 18:0 concentration in plasma TG [22]. In our study, however, feeding SOY decreased 18:0 in PL and TG when compared with other treatments. A decrease in 18:0 concentration coupled with an increase in *cis*9,*trans*11-18:2 and *trans*11-18:1 are further indications that biohydrogenation was less complete as dietary 18:2n-6 intake increased. This also was reflected in the poor relationships (data not shown) between dietary *cis*9-18:1 and 18:2n-6 intake with plasma 18:0 concentrations.

In general, feeding supplemental oils reduced the concentrations of tetradecanoic, tetradecenoic, pentadecanoic, hexadecanoic, heptadecanoic, and eicosatrienoic acids. O'Kelly and Spiers [28], Jenkins et al. [12] and Jenkins [11] also found reduced concentrations of eicosatrienoic, pentadecanoic, and heptadecanoic acid when safflower or soybean oil were fed to ruminants, and suggested that oil supplementation reduced de novo lipid synthesis by ruminal microbes.

4. CONCLUSIONS

Feeding high-oleic or high-linoleic oils to ruminants enhances the amount of dietary oleic or linoleic acid that escapes biohydrogenation in the rumen. Thus, incorporation of these fatty acids into plasma triglycerides, cholesterol esters, and phospholipids increases. A portion of supplemental linoleic acid, however, is hydrogenated and enhances *trans*11-18:1 and *cis*9,*trans*11-18:2 content of plasma lipid fractions and milk fat. Production of these isomers in the rumen is enhanced as linoleic acid intake increases, suggesting the capacity of microbes to hydrogenate may be overcome by high levels of unsaturated fatty acids. The overall result is greater concentration of *trans*11-18:1 and *cis*9,*trans*11-18:2 but lower 18:0 in plasma. Although indirectly, our results concur with previous data indicating that the mammary gland extracts *cis*9,*trans*11-18:2 and *trans*11-18:1 bound to triglycerides and(or) phospholipids. However, more detailed studies of mammary gland extraction and uptake of *trans*-fatty acids from plasma lipid fractions are needed to confirm the contribution

of blood phospholipids to the *trans*11-18:1 and *cis*9,*trans*11-18:2 content of milk fat.

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REFERENCES

[1] AOAC, Official Methods of Analysis, 15th ed., AOAC, Arlington, VA, 1990.

[2] Bandara A.B.P.A., Modifying fatty acid composition of bovine milk by abomasal infusion or dietary supplementation of seed oils or fish oil, Ph.D. Dissertation, Virginia Tech (1997).

[3] Bickerstaffe R., Annison E., Glycerokinase and desaturase activity in pig, chicken, and goat intestinal epithelium, Comp. Biochem. Physiol. 31 (1969) 47–54.

[4] Bitman J., Wood D., Lefcourt A., Rhythms in cholesterol, cholesteryl esters, free fatty acids, and triglycerides in blood of lactating dairy cows, J. Dairy Sci. 73 (1990) 948–955.

[5] Christie W., The effect of diet and other factors on the lipid composition of ruminant tissues and milk, in: Christie W. (Ed.), Lipid Metabolism in Ruminant Animals, Pergamon Press, England, 1981, pp. 193–226.

[6] Echelmeier S., DePeters E., Perez-Monti H., Taylor S., Effects of continuous infusions of canola oil into either the rumen or the abomasum of lactating dairy cows on milk composition and nutrient digestion, J. Anim. Sci. 76 (1998) (Suppl. 1) 301.

[7] Firkins J., Eastridge M., Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production, J. Dairy Sci. 77 (1994) 2357–2366.

[8] Griinari J., Corl B., Lacy S., Chouinard P., Nurmela K., Bauman D., Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9 desaturase, J. Nutr. 130 (2000) 2285–2291.

[9] Glascock R., Welch V., Contribution of the fatty acids of three low density serum lipoproteins to bovine milk fat, J. Dairy Sci. 57 (1974) 1364–1370.

[10] Jayan G., Herbein J., "Healthier" dairy fat using *trans*-vaccenic acid, Nutr. Food Sci. 30 (2000) 304–309.

[11] Jenkins T., Butylsoyamide protects soybean oil from ruminal biohydrogenation: effects of butylsoyamide on plasma fatty acids and nutrient digestion in sheep, J. Anim. Sci. 73 (1995) 818–823.

[12] Jenkins T., Thies E., Fotouhi N., Dietary soybean oil changes lipolytic rate and composition of fatty acids in plasma membranes of ovine adipocytes, J. Nutr. 124 (1994) 566–570.

[13] Kaluzny M., Duncan L., Merritt M., Epps D., Rapid separation of lipid classes in high yield and purity using bonded phase columns, J. Lipid Res. 26 (1985) 135–140.

[14] Kemp P., White R., Lander D., The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species, J. Gen. Microbiol. 90 (1975) 100–114.

[15] Kepler C., Hirons K., McNeill J., Tove S., Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*, J. Biol. Chem. 241 (1966) 1350–1354.

[16] Kepler C., Tove S., Biohydrogenation of unsaturated fatty acids, III. Purification and properties of a linoleate delta-12-*cis*,delta11-*trans*-isomerase from *Butyrivibrio fibrisolvens*, J. Biol. Chem. 242 (1967) 5686–5692.

[17] La Count D., Drackley J., Laesch S., Clark J., Secretion of oleic acid in milk fat in response to abomasal infusions of canola or high oleic sunflower fatty acids, J. Dairy Sci. 77 (1994) 11372–1385.

[18] Loor J., Herbein J., Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acids synthesis, J. Nutr. 128 (1998) 2411–2419.

[19] Macleod G., Wood A., Yao Y., Influence of dietary fat on rumen fatty acids, plasma lipid and milk fat composition in the cow, J. Dairy Sci. 55 (1972) 446–453.

[20] Martin J., Sebedio J., Caselli C., Pimont C., Martine L., Bernard A., Lymphatic delivery and in vitro pancreatic lipase hydrolysis of glycerol esters of conjugated linoleic acids in rats, J. Nutr. 130 (2000) 1108–1114.

[21] Moore J., Noble R., Steele W., Factors affecting the polyunsaturated fatty acid content of the plasma lipids of sheep, Brit. J. Nutr. 22 (1968) 681–688.

[22] Morales M., Palmquist D., Weiss W., Effects of fat source and copper on unsaturation of blood and milk triacylglycerol fatty acids in Holstein and Jersey cows, J. Dairy Sci. 83 (2000) 2105–2111.

[23] Morris L., Mechanisms and stereochemistry in fatty acid metabolism, Biochem. J. 118 (1970) 681–693.

[24] Nielsen M., Jakobsen K., Changes in mammary uptake of free fatty acids, triglyceride, cholesterol, and phospholipid in relation to milk

synthesis during lactation in goats, Comp. Biochem. Physiol. A109 (1994) 857–967.

[25] Noble R., O'Kelly J., Moore J., Observations on the lecithin: cholesterol acyltransferase in bovine plasma, Biochim. Biophys. Acta 270 (1972) 519–528.

[26] Noble R., Steele W., Moore J., The incorporation of linoleic acid into the plasma lipids of sheep given intraruminal infusions of maize oil or free linoleic acid, Br. J. Nutr. 23 (1969) 709–714.

[27] NRC, Nutrient Requirements of Dairy Cattle, 6th rev. ed., Natl. Acad. Sci., Washington, DC, 1989.

[28] O'Kelly J., Spiers W., Effects of supplementation with safflower oil on voluntary feed intake and tissue fatty acid composition of steers fed low quality hay ad libitum, Aust. J. Exp. Agric. 33 (1993) 693–697.

[29] O'Shea M., Devery R., Lawless F., Murphy J., Stanton C., Milk fat conjugated linoleic acid inhibits growth of human mammary MCF-7 cancer cells, Antican. Res. 20 (2000) 3591–3602.

[30] Palmquist D., A kinetic concept of lipid transport in ruminants. A review, J. Dairy Sci. 59 (1976) 355–363.

[31] Palmquist D., Conrad H., High fat rations for dairy cows effects on feed intake, milk and milk fat production, and plasma metabolism, J. Dairy Sci. 61 (1978) 890–901.

[32] Park P., Goins R., In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods, J. Food Sci. 59 (1994) 1262–1266.

[33] Polan C., McNeill J., Tove S., Biohydrogenation of unsaturated fatty acids by rumen bacteria, J. Bacteriol. 88 (1964) 1056–1064.

[34] SAS®, Version 8 Edition, SAS Inst., Inc., Cary, NC, 2000.

[35] Selner D., Schultz L., Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows, J. Dairy Sci. 63 (1980) 1235–1241.

[36] Stallings C., Kroll G., Kelley J., McGilliard M., A computer ration evaluation program for heifers, dry cows, and lactating cows, J. Dairy Sci. 68 (1985) 1015-1019.

[37] Tesfa A., Touri M., Syrjala-Quist L., The effects of increasing levels of rapeseed oil in the diets of lactating milking cows on milk yield, milk composition, blood and rumen metabolites, World Rev. Anim. Prod. 27 (1992) 34–40.

[38] Thivierge M., Chouinard P., Levesque J., Girard V., Seoane J., Brisson G., Effects of buffer on milk fatty acids and mammary arteriovenous differences in dairy cows fed Ca salts of fatty acids, J. Dairy Sci. 81 (1998) 2001–2010.

[39] Vidgren H., Louheranta A., Agren J., Schwab U., Uusitupa M., Divergent incorporation of dietary *trans* fatty acids in different serum lipid fractions, Lipids 33 (1998) 955–962.

[40] Van Soest P., Robertson J., Lewis B., Methods of dietary fiber, neutral detergent fiber, and nonstarch polysacharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597.