Abomasally infused SFA with varying chain length differently affect milk production and composition and alter hepatic and mammary gene expression in lactating cows

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Abstract

The aim of the present study was to compare the effects of post-ruminally infused fat supplements, varying in fatty acid (FA) chain length, on animal performance, metabolism and milk FA. Eleven multiparous Holstein dairy cows were used in a replicated incomplete 3 × 3 Latin square design with 7-d periods, separated by 7-d washouts. Treatments were administered as abomasal infusions of enrichments providing 280 g/d of FA: (1) palmitic acid (98·4 % 16 : 0; PA), (2) caprylic and capric acids (56·2 % 8 : 0, 43·8 % 10 : 0; medium-chain TAG (MCT)) and (3) stearic acid (99·0 % 18 : 0; SA). Relative to PA, SA decreased the efficiency of fat-corrected milk production, which was associated with a tendency for higher DM intake and lower FA absorption with SA, whereas MCT was not different from PA for these variables. Milk fat concentration and yield were increased by PA relative to SA, but only fat yield tended to be greater relative to MCT. Relative to PA, MCT increased milk fat concentration of FA < 16 C, whereas SA increased FA > 16 C. Expression of mammary stearoyl-coA desaturase 1 was lower with SA than with PA. Relative to PA, liver expression of adenosine monophosphate-activated protein kinase-1 and pyruvate kinase was increased with MCT, whereas expression of these genes tended to be increased by SA. The mechanism of increased fat secretion with PA does not seem to be related to a modulation of the expression of lipogenesis-related genes, but rather to increased substrate availability as reflected by milk FA profile.

Key words: Lactating ruminants: Dietary fat: Medium-chain TAG: Palmitic acid: Stearic acid: Liver: Mammary gland: Milk fat

Medium-chain fatty acids (MCFA) are those with an aliphatic tail of 7 to 12 C atoms[1]. Given the general antimicrobial effects of MCFA[2], and their negative impact on rumen fermentation[3] and DM intake (DMI)[4,5], lipid supplements rich in these fatty acids (FA) are not commonly used in diets for ruminants. The effects of MCFA on animal performance have not been extensively investigated, outside of their potential to reduce ruminal methanogenesis[6,7]. Furthermore, studies investigating the post-ruminal effects of caprylic (8 : 0) and capric (10 : 0) acids are scarce. Other SFA, such as palmitic (16 : 0) and stearic (18 : 0) acids, are considered to be ruminally inert and are fed to dairy cows to increase dietary energy density and support the demand for lactation. Relative to 18 : 0 or to no added fat diets, 16 : 0 has been shown to increase milk fat output in dairy cows[8–10]. Such differences in milk fat output could be related to lower digestibility[9,11] and/or inferior mammary uptake of 18 : 0 relative to 16 : 0[10].

The chain length of dietary lipids has important effects on their absorption and subsequent tissue-level metabolism. Although saturated MCFA can be incorporated into chylomicrons to be transported in lymph after intestinal absorption, in humans, this process has been shown to be highly inefficient compared with long-chain FA (LCFA) such as 16 : 0 and 18 : 0[12]. Rather, the majority of MCFA are attached to albumin and sent directly to the liver through the portal vein[12,13], where they are more readily oxidised relative to longer FA, resulting in reduced body weight and body fat accumulation in rats[14,15] and in humans[16,17]. Similar effects could potentially aid in regulation of body fat and help prevent metabolic disease of lactating dairy cows, but they remain largely unknown.

We hypothesised that 16 : 0 would increase milk fat synthesis relative to both 18 : 0 and MCFA TAG and that chain length would affect FA digestibility and, consequently, the transfer

efficiency of dietary FA to milk fat. In addition, liver oxidation of MCFA was expected to reduce their availability for milk fat synthesis.

Characterising the effect of FA chain length on animal metabolism could provide valuable insight into the functional impact of dietary fat supplements and their effects on animal performance and milk composition. The potential metabolic effects of MCFA could be of interest to the dairy industry as a method to prevent metabolic diseases such as fatty liver through increased oxidation of lipids as shown in other mammals[14–17].

Methods

Experimental design and treatments

All experimental procedures were approved by the Institutional Animal Care Committee at Université Laval. Eleven lactating multiparous Holstein cows (2·5 (SD 1·9) lactations, 150 (SD 52) d postpartum; 36·9 (SD 7·3) kg/d milk yield) were randomly assigned to 1 of 6 sequences in an incomplete replicated Latin square design, resulting in eleven observations per treatment. Periods were 7 d in length separated by 7-d washout intervals. Cows were housed in a tie-stall barn located at the Centre Recherche en Sciences Animales de Deschambault. All animals had continuous access to fresh water and were offered the same diet (Table 1; 09.00 hours) at 110 % of expected intake for the duration of the experiment. Treatments were administered as abomasal infusions of three different enrichments providing 280 g/d of FA: (1) free 16 : 0 (98·4 % 16 : 0; PA), (2) a mixture of 8 : 0 and 10 : 0 (56·2 % 8 : 0, 43·8 % 10 : 0; medium-chain TAG (MCT)) as

Table 1. Ingredient and nutrient composition of the experimental diet

	g/kg DM
Ingredients	
Alfalfa haylage	317
Maize silage	300
Ground maize	250
Soyabean meal	51
Maize gluten meal	51
Minerals and vitamins*	31
Nutrient	
Organic matter	926
Neutral-detergent fibre	267
Acid-detergent fibre	201
Crude protein	156
Fatty acids	
8 : 0	0·02
10 : 0	0·27
16 : 0	4·18
16 : 1c9	0·08
18 : 0	0·64
18 : 1c9	4·40
18 : 1c11	0·27
18 : 2c9,c12	10·3
18 : 3c9,c12,c15	2·93
Total	23·4

* Contained 16·0 % Ca, 3·4 % P, 6·5 % Na, 4·1 % Mg, 30·9 mg/kg iodine, 2488 mg/kg Fe, 412 mg/kg Cu, 1375 mg/kg Mn, 2026 mg/kg Zn, 13·8 mg/kg Co, 330 mg/kg F, 17·2 mg/kg Se, 209·4 kIU vitamin A, 68·8 kIU vitamin D, 1187 IU vitamin E, on a DM basis (La Coop Fédérée).

Table 2. Fatty acid composition of experimental fat supplements*

% of fatty acids	PA†	MCT†	SA‡
8 : 0	–	56·2	–
10 : 0	–	43·8	–
14 : 0	0·8	–	–
16 : 0	98·4	–	0·7
18 : 0	0·5	–	99·0
18 : 1c9	0·2	–	0·2

PA, palmitic acid; MCT, medium-chain TAG; SA, stearic acid.
* PA = free 16 : 0, MCT = a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG, SA = free 18 : 0.
† From Jefo Nutrition, Inc.
‡ From Sigma-Aldrich.

TAG and (3) free 18 : 0 (99·0 % 18 : 0; SA). The FA profile of each experimental enrichment is presented in Table 2. Emulsions were designed to provide equal amounts of FA and glycerol across treatments (online Supplementary Table S1), preparation was based on Dallaire et al.[18] and infusion was as described by Gressley et al.[19]. Briefly, emulsions were administered at the abomasum via a tube inserted through the rumen cannula. Given that the MCFA supplement was in the TAG form, glycerol was added to the PA and SA emulsions in order to equalise its supply across treatments and thus reduce the possibility of confounding effects. In addition, all emulsions contained equal proportions of whey protein concentrate (AMP 80, American Meat Protein Corporation) and Tween 80 (Fisher Scientific), as emulsifying agents.

DM intake and apparent total-tract digestibility

Refusals were weighed daily just before feeding. Samples of TMR (prior to feeding) and refusals (12·5 % of total, on an as-fed basis) were collected from day 5 to 7 of infusion, composited by period and stored at −20°C. Eight faecal samples were taken at 6-h intervals from day 5 to 7 and pooled by cow and period before storing at −20°C. All diet, refusal and faecal samples were lyophilised over 72 h (VirTual 50 L, VirTis SP Scientific) to determine DM content and then ground to pass a 1-mm screen using a Cyclotec mill (model 1093; Foss, Tecator AB, Hoganas). Analytical DM content was then determined at 98°C overnight using a vacuum oven. Indigestible neutral-detergent fibre was performed following a 288-h in situ incubation, as described by Rico et al.[20]. FA were directly trans-esterified using a two-step methylation (sodium methoxide, 0·5 M in methanol; HCl, 10 %), as described by Villeneuve et al.[21]. Concentrations of individual and total FA were quantified using tritridecanoin (13 : 0/13 : 0/13 : 0) and heneicosanoic acid (21 : 0; Nu-Chek Prep, Inc., Elysian) as internal standards. Indigestible neutral-detergent fibre was used as an internal marker for the calculation of apparent total-tract digestibility (ATTD) of DM and FA. Offered and refused amounts of feed, along with their DM and FA composition, were used to calculate intake. Daily flows of faecal DM and FA were calculated as described by Huhtanen et al.[22].

Milk sampling and analysis

Cows were milked twice daily at 07.00 and 17.00 hours. During the last 3 d of each infusion period, milk yield was

recorded and milk was sampled at both milkings. One milk subsample was stored at 4°C with preservative (Bronopol; 2-bromo-2-nitropropane-1,3-diol) until analysed for fat, protein, lactose and urea N by infrared spectroscopy (MilkoScan FT6000; Foss, Hillerød), then for somatic cell count by flow cytometry (Fossomatic FC; Foss) at Valacta (Dairy Production Center of Expertise, Québec-Atlantic, Ste-Anne-de-Bellevue, Canada). The production of 4 % fat-corrected milk (FCM) was calculated as described by Gaines[23]. A second subsample, without preservative, was stored at −20°C until used for the determination of FA profile. At the time of analysis, samples were thawed and lipids were extracted and methylated in the presence of sodium methoxide (0·5M in methanol) according to Chouinard *et al.*[24]. FA were quantified according to Boivin *et al.*[25] using a GC (7890 A; Agilent Technologies Canada, Inc.) equipped with a 100-m CP-Sil 88 capillary column (0·25-mm i.d., 0·20-μm film thickness; Agilent Technologies Canada, Inc.) and a flame ionisation detector. Glycerol in milk fat was calculated as described by Stamey *et al.*[26]. The milk FA desaturase indexes were calculated as an estimation of the activity of the stearoyl-coA desaturase 1 (SCD1) enzyme (product/(substrate + product)), as described by Kelsey *et al.*[27].

Blood sampling and analyses

Blood samples were collected from the coccygeal vessel using K_2-EDTA tubes (BD Vacutainer) at 14.00 hours on day 6 of each period. Tubes were placed on ice and then centrifuged at 2200 ***g*** for 15 min at 4°C. Using commercial kits, extracted plasma was analysed for concentrations of NEFA (Wako HR series NEFA-HR (2) Kit, Wako Chemicals USA), glucose (PGO assay, Sigma-Aldrich) and β-hydroxybutyric acid (Liquicolor Test, Stanbio Laboratory Inc., Stanbio Laboratory Inc.).

Tissue biopsies

Mammary and liver biopsies were performed on day 7 of each period 3 to 4 h after feeding. Animals were sedated by intravenous injection of 20 to 30 mg of xylazine, and 2 % lidocaine HCl was administered sub-dermally on each incision site (3 ml for mammary and 5 ml for liver biopsies). Mammary tissue was obtained at the mid-point of a rear quarter, which alternated between periods. Liver tissue was collected at the 11th to 12th intercostal space. All biopsies were harvested using a 14-gauge Tru-cut needle (Source Medical). Mammary and liver tissues were rinsed with Dulbecco's PBS checked for homogeneity and immediately frozen in liquid N_2. All samples were stored at −80°C until further analyses.

RNA isolation and real-time PCR

Total RNA was extracted from tissues using TRI Reagent (Molecular Research Center Inc.). Following extraction, RNA pellets were resuspended in RNase-free water and concentrations were determined using a ND-1000 spectrophotometer (NanoDrop Products). Total RNA was then reverse-transcribed to complementary DNA using the Omniscript RT kit (Qiagen) with Oligo-dT (Eurofins MWG/Operon) as the primer. Real-time quantitative PCR was performed using the GoTaq qPCR Master

Mix (Promega Corp.) in an Applied Biosystems 7300 real-time PCR machine (Applied Biosystems). Reactions were performed in duplicate wells as follows: one cycle at 95°C for 10 min, followed by forty cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. For each tissue, eight endogenous control genes were tested for stability, and, for each tissue, the three genes least affected by treatment were selected (B2 microglobulin, β-actin, and ribosomal protein S15 for mammary tissue; and mitochondrial ribosomal protein L39, eukaryotic translation initiation factor 3, subunit K, and ribosomal protein S15 for liver; online Supplementary Tables S2 and S3). Mammary genes evaluated were acetyl-CoA carboxylase (ACC), lipoprotein lipase, FA synthase, SCD1 and sterol regulatory element-binding protein 1 (online Supplementary Table S2). Hepatic genes evaluated were adenosine monophosphate-activated protein kinase 1 (AMPK1), apoB-100, CPT1, diacylglycerol acyltransferase 1 and liver pyruvate kinase (online Supplementary Table S3). The geometric mean of each set of three endogenous control genes was used as the endogenous control, and fold change was calculated using $2^{-\Delta\Delta Ct}$ method[28] with the PA treatment as the calibrator, given that all treatment comparisons were done relative to PA.

Statistical analysis

An *a priori* sample size calculation for Latin squares design experiments was performed as described by Kononoff & Hanford[29]. This analysis predicted that differences in main variables of interest could be detected with a power above 80 % and an α of 5 % by using nine cows. For instance, detection of milk fat yield differences of 90 g/d and above was expected. At the start of the experiment, two additional cows were enrolled as spares, in the case of animal loss due to disease or other reasons, and their data were included in the analyses, for a total of eleven cows. Data were analysed using the MIXED procedure of SAS (version 9.3, SAS Institute). The statistical model included the random effect of treatment sequence, period and cow nested in treatment sequence, and the fixed effect of treatment. Preplanned contrasts were PA *v.* MCT and PA *v.* SA. Significance and tendencies were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

Results

DM intake, milk yield and milk composition

DMI was not different between MCT and PA, but tended to be higher (+1·3 kg/d) in SA relative to PA ($P = 0.06$; Table 3). No differences were observed for milk yield in any of the tested contrasts (Table 4). The yield of 4 % FCM was 2·2 kg/d greater with PA than with MCT ($P = 0.05$), but was not different between PA and SA. Moreover, the gross efficiency of FCM production was not different between PA and MCT, but was lower with SA relative to PA (−9·3 %; $P = 0.01$). The preplanned contrasts did not reveal any significant difference in the concentrations and yields of milk protein and lactose. Fat yield tended to be lower with MCT relative to PA (−5 %; $P = 0.06$), and fat concentration was not affected, whereas fat concentration and yield were lower with SA than with PA (−6·1 and −6·9 %, respectively; $P < 0.05$).

Table 3. Intake and apparent total-tract digestibility of DM and fatty acids in dairy cows abomasally infused with fatty acids varying in chain length (n 11)*
(Mean values and standard errors)

	Treatment†				P	
Items	PA	MCT	SA	SEM	PA v. MCT	PA v. SA
DM						
Intake (kg/d)	22·4	22·4	23·7	1·3	0·96	0·06
Absorption‡ (kg/d)	13·7	14·3	15·1	1·0	0·47	0·07
Digestibility (%)	61·4	63·4	63·0	3·6	0·31	0·36
Total fatty acids						
Intake (g/d)	797	800	827	30·4	0·86	0·05
Absorption (g/d)	559	579	406	38·5	0·55	<0·01
Digestibility (%)	70·1	71·5	48·3	4·4	<0·01	<0·01
Caprylic acid (8 : 0)						
Intake (g/d)	0·9	160	1·0	0·2	<0·01	0·24
Absorption (g/d)	0·7	159	0·7	0·1	<0·01	0·57
Digestibility (%)	77·9	99·9	76·7	4·5	<0·01	0·65
Capric acid (10 : 0)						
Intake (g/d)	5·8	128	6·4	1·0	<0·01	0·41
Absorption (g/d)	5·3	125	5·8	1·0	<0·01	0·65
Digestibility (%)	88·3	98·4	89·4	2·5	<0·01	0·61
16-C fatty acids						
Intake (g/d)	372	94·0	101	5·6	<0·01	<0·01
Absorption (g/d)	312	56·8	60·7	7·2	<0·01	<0·01
Digestibility (%)	83·8	57·5	58·8	3·2	<0·01	<0·01
18-C fatty acids						
Intake (g/d)	412	412	711	24·6	0·99	<0·01
Absorption (g/d)	247	242	345	30·9	0·87	<0·01
Digestibility (%)	59·7	58·2	47·9	5·4	0·72	0·01

PA, palmitic acid; MCT, medium-chain TAG; SA, stearic acid.
* From the basal diet and infused lipid emulsions.
† Experimental fat supplements were provided as abomasal infusions: PA = free 16 : 0, MCT = a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG, SA = free 18 : 0.
‡ Absorption was calculated as the amount collected daily in faeces minus daily intake.

Table 4. Milk production, milk composition and feed efficiency in dairy cows abomasally infused with fatty acids varying in chain length (n 11)
(Mean values and standard errors)

	Treatment*				P	
Items	PA	MCT	SA	SEM	PA v. MCT	PA v. SA
Production (kg/d)						
Milk	34·9	34·1	35·1	2·1	0·44	0·80
Fat-corrected milk†	37·3	35·1	35·7	2·0	0·05	0·12
Fat	1·56	1·48	1·46	0·08	0·06	0·01
Protein	1·18	1·14	1·19	0·07	0·34	0·70
Lactose	1·60	1·56	1·61	0·09	0·48	0·78
Composition (%)						
Fat	4·49	4·38	4·18	0·09	0·17	<0·01
Protein	3·41	3·41	3·42	0·09	0·97	0·86
Lactose	4·59	4·59	4·60	0·04	0·90	0·70
Feed efficiency‡ (kg/kg)	1·67	1·56	1·51	0·05	0·15	0·01

PA, palmitic acid; MCT, medium-chain TAG; SA, stearic acid.
* Experimental fat supplements were provided as abomasal infusions: PA = free 16 : 0, MCT = a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG, SA = free 18 : 0.
† 4 % Fat-corrected milk (kg/d) = (0·4 × milk production (kg/d)) + (15·0 × fat production (kg/d)), as described by Gaines[23].
‡ Fat-corrected milk (kg/d)/DM intake (kg/d).

Apparent digestibility

No differences were observed for apparent DM digestibility in any of the tested contrasts (Table 3). Similar to DMI, total

Table 5. Four-hour postprandial plasma metabolic profile in dairy cows abomasally infused with fatty acids varying in chain length (n 11)
(Mean values and standard errors)

	Treatment*				P	
Items	PA	MCT	SA	SEM	PA v. MCT	PA v. SA
NEFA (μEq/l)	99·0	91·2	77·7	12·18	0·41	0·02
Glucose (g/dl)	58·8	57·9	58·6	1·30	0·58	0·90
β-Hydroxybutyric acid (mM)	0·83	0·98	0·91	0·10	0·17	0·42

PA, palmitic acid; MCT, medium-chain TAG; SA, stearic acid.
* Experimental fat supplements were provided as abomasal infusions: PA = free 16 : 0, MCT = a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG, SA = free 18 : 0.

absorbed DM did not differ between PA and MCT, but tended to be higher in SA than in PA (+1·3 kg/d; $P = 0.07$). The ATTD of total FA was not different between PA and MCT, but was reduced by 31 % with SA relative to PA ($P < 0.01$; Table 3). The ATTD of 8 : 0 and 10 : 0 were greater (+28 and +11 %, respectively) with MCT as compared with PA ($P < 0.01$), but was not different between SA and PA. Both MCT and SA treatments exhibited lower ATTD of 16 C FA (−30 %; $P < 0.01$) relative to PA. Finally, ATTD of 18-C FA did not differ between MCT and PA treatments, but was reduced by SA relative to PA (−20 %; $P = 0.01$).

Plasma metabolic profile

The concentrations of NEFA were not different between MCT and PA, but were 21·5 % lower with SA relative to PA ($P = 0.02$; Table 5). Contrasts did not reveal any significant differences in plasma concentrations of glucose and β-hydroxybutyric acid.

Mammary and hepatic gene expression

Except for a 24 % reduction in the abundance of mammary SCD1 with SA relative to PA ($P = 0.04$; Fig. 1), expression of mammary genes related to lipid metabolism, including lipoprotein lipase, sterol regulatory element-binding protein 1, ACC and FA synthase, was not different between treatments. Relative abundances of hepatic CPT1, apoB-100 and diacylglycerol acyltransferase 1 mRNA were not affected by treatment (Fig. 2), whereas those of both AMPK1 and liver pyruvate kinase were 30 % greater with MCT than with PA ($P < 0.05$) and tended to be greater (+20 %) with SA than with PA ($P < 0.10$).

Milk fatty acid profile and desaturase indexes

With few exceptions (i.e. 4 : 0, 6 : 0 and 14 : 1c9), the concentrations of the majority of even-chain milk FA of less than 16 C were significantly increased by MCT and SA relative to PA (Table 6; Fig. 3). Notably, the concentrations of 8 : 0 and 10 : 0 in milk fat were 27 and 83 % greater with MCT than with PA, respectively ($P < 0.01$), and approximately 18 % greater for both FA with infusions of SA as compared with PA ($P < 0.01$).

When compared with PA infusion, milk fat concentrations of PA and palmitoleic acid (16 : 1c9) were 13 and 25 % lower, respectively, with MCT infusion, and 18 and 24 % lower, respectively, with SA infusion ($P < 0.01$). The concentration of SA in
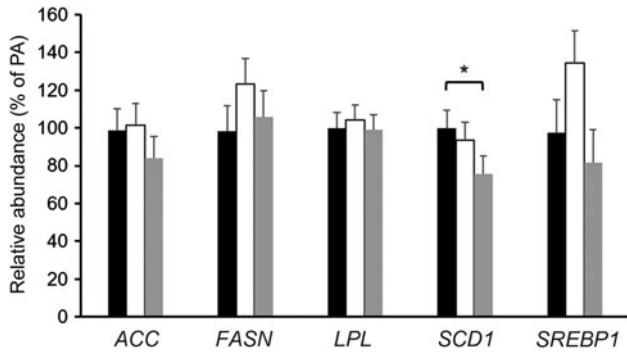
Fig. 1. Relative mRNA abundance of genes related to lipid metabolism in the mammary gland of dairy cows abomasally infused with fatty acids varying in chain length (*n* 11). Values are means with their standard errors. * Significant difference (*P* < 0·05). PA, palmitic acid (16 : 0); *ACC*, acetyl-CoA carboxylase; *FASN*, fatty acid synthase; *LPL*, lipoprotein lipase; *SCD1*, stearoyl-coA desaturase 1; *SREBP1*, sterol regulatory element-binding protein 1. ■, PA, free 16 : 0; □, medium-chain TAG, a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG; ▨, free 18 : 0 (stearic acid).
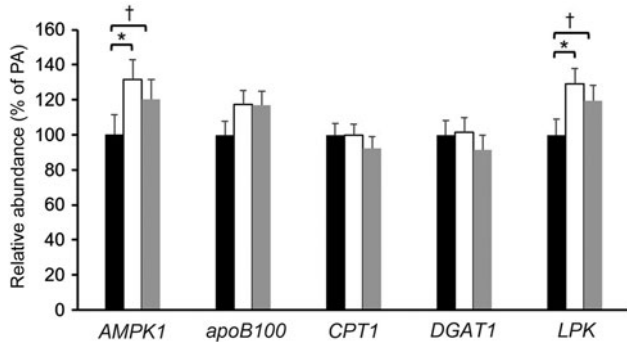
Fig. 2. Relative mRNA abundance of genes related to lipid metabolism and energy status in liver of dairy cows abomasally infused with fatty acids varying in chain length (*n* 11). Values are means with their standard errors. * Significant difference (*P* < 0·05); † tendency (*P* < 0·10). PA, palmitic acid (16 : 0); *AMPK1*, adenosine monophosphate-activated protein kinase 1; *CPT1*, carnitine palmitoyltransferase 1; *DGAT1*, diacylglycerol acyltransferase 1; *LPK*, liver pyruvate kinase. ■, PA, free 16 : 0; □, medium-chain TAG, a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG; ▨, free 18 : 0 (stearic acid).

Table 6. Composition of milk fat in dairy cows abomasally infused with fatty acids varying in chain length (*n* 11)*
(Mean values and standard errors)

Fatty acid, % of milk fat	Treatment†				P	
	PA	MCT	SA	SEM	PA *v.* MCT	PA *v.* SA
Butyric acid (4 : 0)	2·23	2·12	2·24	0·201	0·56	0·92
Caproic acid (6 : 0)	1·46	1·56	1·60	0·067	0·21	0·06
Caprylic acid (8 : 0)	0·81	1·02	0·97	0·048	<0·01	<0·01
Capric acid (10 : 0)	2·10	3·87	2·48	0·153	<0·01	<0·01
Lauric acid (12 : 0)	2·74	3·85	3·19	0·222	<0·01	<0·01
Myristic acid (14 : 0)	9·98	11·98	11·09	0·343	<0·01	<0·01
Myristoleic acid (14 : 1c9)	0·92	0·85	0·94	0·070	0·35	0·76
Palmitic acid (16 : 0)	37·1	32·5	30·4	0·67	<0·01	<0·01
Palmitoleic acid (16 : 1c9)	1·78	1·33	1·36	0·103	<0·01	<0·01
Stearic acid (18 : 0)	4·88	5·65	6·96	0·352	0·04	<0·01
Oleic acid (18 : 1c9)	13·22	12·58	16·05	0·622	0·27	<0·01
Linoleic acid (18 : 2c9,c12)	1·42	1·42	1·50	0·080	0·96	0·28
Linolenic acid (18 : 3c9,c12,c15)	0·35	0·37	0·39	0·018	0·15	0·01
Sum of fatty acids						
Fatty acids < 16 C	20·6	25·7	22·9	0·726	<0·01	<0·01
16-C fatty acids	38·9	33·8	31·7	0·707	<0·01	<0·01
Fatty acids > 16 C	25·0	24·3	29·5	0·746	0·46	<0·01
18-C fatty acids	24·0	23·4	28·5	0·740	0·44	<0·01
SFA	65·0	66·4	62·8	0·598	0·04	<0·01
Unsaturated fatty acids	22·9	21·2	24·9	0·616	0·02	<0·01
Desaturase index						
14 : 1c9/(14 : 0 + 14 : 1c9)	0·085	0·065	0·078	0·005	<0·01	0·18
16 : 1c9/(16 : 0 + 16 : 1c9)	0·046	0·039	0·043	0·003	<0·01	0·08
18 : 1c9/(18 : 0 + 18 : 1c9)	0·73	0·69	0·70	0·016	0·03	0·07

PA, palmitic acid; MCT, medium-chain TAG; SA, stearic acid.
* Complete fatty acid profile is presented in online Supplementary Table S4.
† Experimental fat supplements were provided as abomasal infusions: PA = free 16 : 0, MCT = a mixture of 8 : 0 and 10 : 0 (caprylic and capric acids) as TAG, SA = free 18 : 0.

milk fat was 16 % greater with MCT and 43 % greater with SA, relative to PA infusion (*P* < 0·01). The concentration of oleic acid (18 : 1c9) was not different between MCT and PA, but was 21 % greater with SA than with PA (*P* < 0·01).

Relative to PA, MCT infusion increased the concentration and yield of total even-chain FA with less than 16 C in milk fat by 25 and 19 %, respectively (*P* < 0·01; Fig. 4; online Supplementary Tables S4 and S5), whereas with SA, the increases were 11·4 and 4·5 %, respectively (*P* < 0·10). When compared with PA infusion, the concentration and yield of 16 C FA were lower (*P* < 0·01) with both MCT (−13 and −17 %, respectively) and SA (−19 and −23 %, respectively) treatments.

The C14, C16 and C18 desaturase indexes were lower (−23, −14 and −5 %, respectively; *P* < 0·05; Table 6) with MCT than with PA. The C16 and C18 indexes tended to be lower with SA as compared with PA (−6 and −4 %, respectively; *P* < 0·10), whereas the C14 index was not different between these two

treatments. Lastly, the concentration of SFA in milk fat was increased by 2·2 % with MCT but reduced by 3·3 % with SA, relative to PA infusion (*P* < 0·05). In contrast, the proportion of unsaturated FA was reduced by 7 % with MCT but increased by 9 % with SA, relative to PA (*P* < 0·05).

Discussion

The present experiment evaluated the post-ruminal effects of 16 : 0, 18 : 0, and a mixture of 8 : 0 and 10 : 0. Comparisons were performed relative to PA, as a source of 16 : 0, given that high-purity supplements of this FA have become widely used on dairy farms and have been well characterised in a number of studies comparing their effects to other LCFA[8,30] or to unsupplemented controls[20,31]. Abomasal infusions of FA emulsions were used to bypass the rumen and thus prevent the potentially negative effects of MCFA on ruminal micro-organisms and fermentation[24]. Taking this information into consideration, the length of experimental periods (7 d) was considered sufficient to
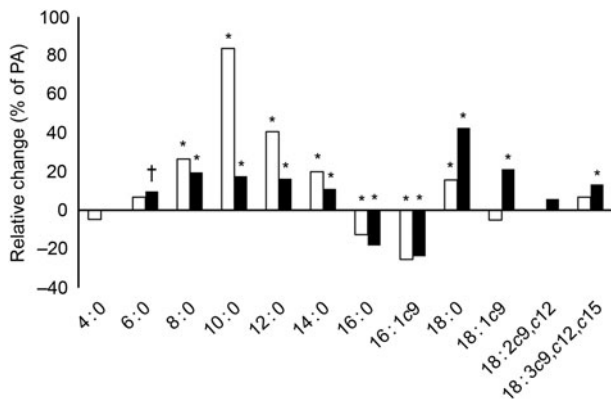
Fig. 3. Relative change in the concentration of selected fatty acids in milk from dairy cows abomasally infused with fatty acids varying in chain length (*n* 11). * Significant difference (*P* < 0.05); † tendency (*P* < 0.10). □, MCT *v.* PA; ■, SA *v.* PA. PA, free 16:0 (palmitic acid); MCT, medium-chain TAG, a mixture of 8:0 and 10:0 (caprylic and capric acids) as TAG; SA, free 18:0 (stearic acid).
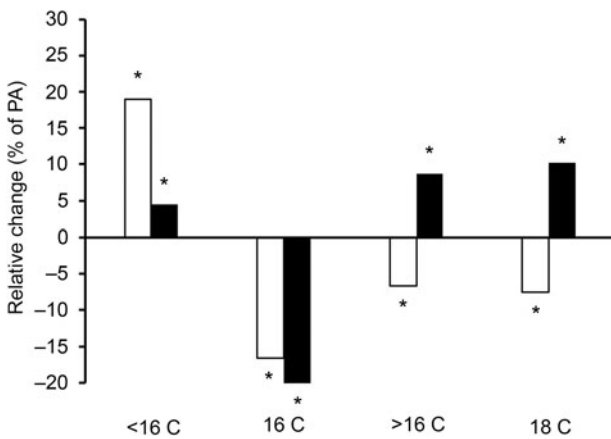
Fig. 4. Relative change in the yield of selected fatty acids in milk from dairy cows abomasally infused with fatty acids varying in chain length (*n* 11). * Significant difference (*P* < 0.05). < 16 C = sum of fatty acids with a chain length of less than 16 carbons; 16 C = sum of fatty acids with a chain length of 16 carbons; > 16 C = sum of fatty acids with a chain length of more than 16 carbons; 18 C = sum of fatty acids with a chain length of 18 carbons. Individual fatty acid yields are reported in online Supplementary Table S5. ■, MCT *v.* PA; □, SA *v.* PA. PA, free 16:0 (palmitic acid); MCT, medium-chain TAG, a mixture of 8:0 and 10:0 (caprylic and capric acids) as TAG; SA, free 18:0 (stearic acid).

represent adaptation to post-ruminal effects of FA supplements, including transfer into milk fat, which has been shown to be maximal within the course of 6–10 h for abomasally infused LCFA[32]. This approach contrasts with feeding experiments where microbial adaptation in the rumen is an important rate-limiting factor to attain steady-state conditions in response to changes in dietary nutrients[33]. Moreover, in the present study, a 7-d washout phase was used between experimental periods to allow for the restoration of pre-infusion conditions minimising residual effects of FA supplements. Given that supplements were administered abomasally, no effects on ruminal fermentation were expected, which agrees with the lack of effects of treatments on ATTD of DM. Lastly, the selected supply of 280 g/d of FA was equivalent to approximately 1·2 % of DMI, similar to the optimal concentration of dietary 16:0 (1·5 % of DM)

for maximal yield of FCM and milk fat, as reported by Rico *et al.*[34]. In addition, emulsions were formulated to provide equal amounts of FA, glycerol, whey protein and tween 80; thus, variation between treatments was only expected to arise from differences in FA chain length.

Although the post-ruminal effects of FA chain length on DMI have not been previously evaluated in dairy cows, a study by Rico *et al.*[8] reported no differences in feed consumption between diets containing high-purity 16:0 or 18:0 supplements at 2 % of diet DM. This observation contrasts with our results, where SA tended to increase DMI relative to PA while having no effects on milk or FCM yield, resulting in reduced feed efficiency. Considering the lower ATTD, and consequently the reduced absorption of total FA with the SA treatment (Table 3), it is possible that secretion of hypophagic gut peptides, such as glucagon-like peptide 1 and cholecystokinin[35,36], was lower with SA and allowed for greater DMI; however, these hormones were not determined in the present experiment. In contrast, circulating NEFA could be considered as an indicator of absorbed dietary FA, as their concentrations increase because of the addition of SFA to diets[37,38] and particularly when adding 16:0[31]. It would be expected that the lower plasma NEFA concentrations observed with SA could result in reduced hypophagic signalling[38], explaining the tendency for greater DMI by SA-supplemented cows. The observed reduction in NEFA is consistent with a previous report comparing 16:0 and 18:0 supplementation in dairy cows, in which insulin was not affected by treatment, but NEFA were reduced in 16:0 relative to 18:0 supplementation[8].

The reduction in FA digestibility and FA absorption with the SA treatment agrees with a recent report by Boerman *et al.*[10], where increasing concentrations of dietary 18:0, from high-purity supplements, reduced the digestibilities of 16-C, 18-C and total FA. Furthermore, Boerman *et al.*[39], in a meta-analysis, observed that increasing the 18:0 flow through the duodenum reduced the digestibility of several individual FA, including 18:0 itself.

Fukumori *et al.*[40] reported lower DMI in lactating cows fed Ca salts of MCFA at 1·5 % of diet DM. In this case, reduced feed consumption might have been due to ruminal dissociation of Ca salts affecting fibre degradation, as free MCFA have been shown to negatively affect ruminal fermentation[3]. In the present trial, however, given that MCT was provided post-ruminally, no such effects on fermentation were expected, and in agreement with this, MCT had neither an effect on DMI, total DM absorbed, nor total FA absorbed. Fukumori *et al.*[40] also observed greater plasma concentrations of ghrelin and thus suggested that intake effects were probably related to high oxidation of MCFA in the liver.

Van Zijderveld *et al.*[6] observed a greater crude fat digestibility in cows receiving a diet supplemented with MCFA (8:0 + 10:0) as compared with a fat supplement rich in 16:0, or with PUFA from an extruded linseed product. Although digestion and absorption of MCFA have been reported to take place to some extent in the stomach of rats[41] and rabbits[42], such mechanism has not yet been described in the ruminant animal. However, rumen absorption of MCFA has been previously demonstrated[43]. The present experiment allowed only for the estimation of ATTD, but it is possible that the high digestibility value of 8:0 and 10:0 observed

here is a result of both gastric (abomasum) and intestinal absorption. However, the reason why ATTD of these FA was higher in the MCT treatment remains unclear.

Fat supplements rich in 16 : 0 have been shown to increase milk fat synthesis in several studies relative to unsupplemented controls[30,31,37] or relative to other LCFA supplements[8,20,30]. Like the results of Rico *et al.*[8], who directly compared 16 : 0 with 18 : 0 supplements, we observed greater milk fat yield for cows infused with PA than for cows fed SA. Lower absorption of FA, and reduced circulating NEFA in plasma, may have contributed to the lower milk fat synthesis observed with SA relative to PA. These results are similar to those reported by Enjalbert *et al.*[44] who showed lower digestibility for duodenally infused 18 : 0 as compared with 16 : 0. In addition, compared with 16 : 0, mammary uptake of 18 : 0 was also reported by the same group[10] to be lower, suggesting that part of the increase in milk fat secretion when feeding 16 : 0 supplements could be explained not only by the greater supply of preformed FA but also by a higher efficiency of mammary use.

In contrast, despite similar FA absorption and circulating NEFA with MCT, lower production of FCM and a tendency for reduced milk fat yield were observed in the present experiment as compared with PA infusion. Inconsistent results are reported in the literature regarding the effects of dietary MCFA on milk fat. On the one hand, van Zijderveld *et al.*[6] reported a greater milk fat concentration in cows receiving a diet complemented with a mixture of 8 : 0 and 10 : 0 as compared with a fat supplement rich in 16 : 0, or with PUFA from an extruded linseed product. On the other hand, Fukumori *et al.*[40] and Sugino *et al.*[45] observed no change on FCM or milk fat concentration and yield when diets were supplemented with Ca salts of 8 : 0 and 10 : 0 as compared with unsupplemented control.

As expected, FA profiles of milk fat followed the composition of infusion treatments, such that the concentrations of FA with less than 16 C were greater with MCT, concentrations of 16-C FA were greater with PA, and concentrations of 18-C FA were greater with SA. These findings are in accordance with Rico *et al.*[8], who compared high 18 : 0 and high 16 : 0 supplements. In our study, milk fat yield was approximately 100 g greater with PA than with MCT and SA, which was mostly explained by an increased milk fat output of 16-C FA with PA infusion as compared with MCT (−100 g/d) or SA (−140 g/d) infusions.

Although the effects of 16 : 0-enriched supplements on lactation performance have been extensively characterised in dairy cows, their potential effects on gene expression have not been examined. Interestingly, in the present trial, despite treatment differences in milk fat secretion, no change in mammary expression of genes related to FA uptake and lipid synthesis was detected. However, expression of SCD1 was reduced with SA relative to PA. The SCD1 is an enzyme that desaturates different SFA, including palmitoyl CoA and stearoyl CoA[46]. This mechanism is thought to help lower the melting point of milk fat in order to maintain its fluidity when the supplies of saturated LCFA are elevated[47,48]. In the present experiment, we observed a lower SCD1 expression with SA relative to PA, which was accompanied by tendencies for reduced 16-C and 18-C desaturase indexes. This could be related to the lower absorption of LCFA (estimated here as the sum of 16-C and 18-C FA), lower

plasma NEFA and lower secretion of LCFA in milk fat when infusing SA as compared with PA. All three calculated desaturase indexes were significantly reduced by MCT. The increased proportions of 8 : 0, 10 : 0 and 12 : 0, with relatively low melting points (16·7, 31·0 and 43·2°C, respectively) as compared with 16 : 0 (62·9°C) and 18 : 0 (69·3°C), may have reduced the need for FA desaturation in order to maintain milk fat plasticity. However, lower apparent desaturation was not accompanied with a reduction in mammary expression of SCD1. Uncoupling between the extent of apparent FA desaturation and mRNA abundance of SCD1 has been reported previously by Dallaire *et al.*[18] following abomasal infusion of varying fat supplements.

Expression of other genes related to *de novo* synthesis of milk FA, including sterol regulatory element-binding protein 1, ACC and FA synthase, has been shown to decrease in numerous experiments in which supply of bioactive FA such as 18 : 2*t*10, *c*12, a conjugated isomer of linoleic acid, is increased[49]. However, to our knowledge, despite the numerous studies investigating the effects of individual SFA such as 16 : 0 on milk fat synthesis in the dairy cow, the link with lipogenic gene expression has not been specifically investigated. Nonetheless, an *in vitro* study using dispersed goat mammary epithelial cells[50] reported that 16 : 0 stimulated *de novo* FA synthesis. In contrast, we observed lower secretion of *de novo* synthesised FA in milk from cows receiving PA as compared with MCT or SA treatments, similar to other *in vivo* studies[8,20]. The difference observed between PA and MCT agrees with data showing that LCFA can decrease the activity of ACC by inducing enzyme depolymerisation[51] and that 16 : 0 may inhibit FA synthesis by allosteric interaction with FA synthase or ACC[52]. On the other hand, the observed difference between PA and SA could be due to the lower digestibility of the SA supplement.

The increased concentration of SFA in milk from cows receiving MCT relative to PA was mostly explained by greater proportion of FA with less than 16 C, among which, the most important relative change was observed for 10 : 0. Thus, the lower concentration of FA with a chain length greater than 16 C with PA compared with MCT could be due to a combination of lower substrate supply and reduced ACC activity.

The enzyme AMPK1 is considered a sensor of cellular energy status and a main regulator of cell metabolism. Its activation inhibits cellular processes that utilise ATP and stimulates those that produce ATP[53,54]. The conversion of FA into acyl-CoA upon entry to the cytoplasm (i.e. FA activation) uses ATP and results in an increased AMP:ATP ratio, which activates AMPK1[55,56]. In contrast to LCFA, which are transported in lipoproteins upon absorption, MCFA are bound to albumin and directly transported to the liver, where they are readily catabolised through mitochondrial β-oxidation without the need of CPT1-mediated transport[57]. In the present experiment, infusion of PA resulted in reduced liver AMPK1 expression relative to MCT and SA. The enzyme AMPK1 is known to reduce lipogenesis and to increase β-oxidation through up-regulation of CPT1 activity[58]. However, despite differences in AMPK1 expression in the liver, no changes in expression of CPT1 were observed in the present experiment; however, this should be interpreted

carefully as only AMPK1 expression and not actual enzyme activity was measured, the latter being the predominant level for its regulation. Hepatic oxidation of MCFA seemed to be incomplete, based on the greater milk fat concentration and yield of 8 : 0 and 10 : 0 into milk fat. Storry *et al.*[59] also reported greater secretion of 8 : 0 and 10 : 0 following their individual intravenous infusion, along with increased yield of longer chain FA, up to 16 C, as a result of *in vivo* elongation. Accordingly, milk secretions of 12 : 0 and 14 : 0 were increased in the present experiment. In addition, the lack of treatment effects on liver diacylglycerol acyltransferase 1 and apo-B100 transcripts suggests neither difference in liver lipogenesis nor on lipid export.

Kawaguchi *et al.*[55] demonstrated that FA activation results in a net sparing of glucose in rat hepatocytes, through increased activity of AMPK1, reduced activity of carbohydrate-responsive element-binding protein, and down-regulation of liver pyruvate kinase, the latter being responsible for the last regulated step of glycolysis. In contrast, the increased hepatic AMPK1 expression observed with MCT and SA was accompanied by similar increases in liver pyruvate kinase, suggesting no involvement of carbohydrate-responsive element-binding protein-mediated regulation, although this was not determined in the present experiment. As previously stated, only gene expression is reported and actual regulation at the protein level is yet to be determined in future studies. Given that glucose concentrations at 4 h after initiation of feeding, as well as the daily lactose yield, were not affected, we speculate that FA chain length had no sparing effect on glucose. However, this hypothesis would need to be verified in studies investigating daily glucose kinetics and use. Proper assessment of the effects of FA chain length on glucose metabolism would require frequent measurements over the course of the day. Given the observed differences in DMI and absorption, longer-term studies would be necessary in order to evaluate if additional adaptations in metabolism and gene expression are observed.

In conclusion, differences in the absorption and metabolism of SFA varying in chain length may explain the effects on milk fat synthesis and milk production efficiency of lactating dairy cows. The mechanism of increased milk fat secretion with 16 : 0 relative to other saturated fat supplements does not seem to be related to the modulation of the expression of lipogenesis-related genes in mammary gland, but rather to increased substrate supply.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000379

References

1. Christie WW. The LipidWeb – Fatty Acids: Straight-Chain Saturated. http://www.lipidhome.co.uk/lipids/fa-eic/fa-sat/index.htm (accessed November 2017).
2. Desbois AP & Smith VJ (2010) Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol* **85**, 1629–1642.
3. Dohme F, Machmüller A, Wasserfallen A, *et al.* (2001) Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. *Lett Applied Microbiol* **32**, 47–51.
4. Hristov AN, Lee C, Cassidy T, *et al.* (2011) Effects of lauric and myristic acids on ruminal fermentation, production, and milk fatty acid composition in lactating dairy cows. *J Dairy Sci* **94**, 382–395.
5. Grummer RR & Socha MT (1989) Milk fatty acid composition and plasma energy metabolite concentrations in lactating cows fed medium-chain triglycerides. *J Dairy Sci* **72**, 1996–2001.
6. van Zijderveld SM, Dijkstra J, Perdok HB, *et al.* (2011) Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. *J Dairy Sci* **94**, 3094–3104.
7. Goel G, Arvidsson K, Vlaeminck B, *et al.* (2009) Effects of capric acid on rumen methanogenesis and biohydrogenation of linoleic and α-linolenic acid. *Animal* **3**, 810–816.
8. Rico JE, Allen MS & Lock AL (2014) Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows. *J Dairy Sci* **97**, 1057–1066.
9. Steele W & Moore J (1968) The digestibility coefficients of myristic, palmitic and stearic acids in the diet of sheep. *J Dairy Res* **35**, 371–376.
10. Enjalbert F, Nicot MC, Bayourthe C, *et al.* (1998) Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. *J Nutr* **128**, 1525–1532.
11. Boerman JP, de Souza J & Lock AL (2017) Milk production and nutrient digestibility responses to increasing levels of stearic acid supplementation of dairy cows. *J Dairy Sci* **100**, 2729–2738.
12. Swift LL, Hill JO, Peters JC, *et al.* (1990) Medium-chain fatty acids: evidence for incorporation into chylomicron triglycerides in humans. *Am J Clin Nutr* **52**, 834–836.
13. Schonfeld P & Wojtczak L (2016) Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. *J Lipid Res* **57**, 943–954.

British Journal of Nutrition

14. Noguchi O, Takeuchi H, Kubota F, *et al.* (2002) Larger diet-induced thermogenesis and less body fat accumulation in rats fed medium-chain triacylglycerols than in those fed long-chain triacylglycerols. *J Nutr Sci Vitaminol* **48**, 524–529.

15. Ooyama K, Wu J, Nosaka N, *et al.* (2008) Combined intervention of medium-chain triacylglycerol diet and exercise reduces body fat mass and enhances energy expenditure in rats. *J Nutr Sci Vitaminol* **54**, 136–141.

16. Tsuji H, Kasai M, Takeuchi H, *et al.* (2001) Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind, controlled trial in healthy men and women. *J Nutr* **131**, 2853–2859.

17. Kasai M, Nosaka N, Maki H, *et al.* (2003) Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. *Asia Pac J Clin Nutr* **12**, 151–160.

18. Dallaire MP, Taga H, Ma L, *et al.* (2014) Effects of abomasal infusion of conjugated linoleic acids, Sterculia foetida oil, and fish oil on production performance and the extent of fatty acid Delta(9)-desaturation in dairy cows. *J Dairy Sci* **97**, 6411–6425.

19. Gressley TF, Reynal SM, Colmenero JJO, *et al.* (2006) Technical note: development of a tool to insert abomasal infusion lines into dairy cows. *J Dairy Sci* **89**, 3965–3967.

20. Rico DE, Ying Y & Harvatine KJ (2014) Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. *J Dairy Sci* **97**, 3739–3751.

21. Villeneuve MP, Lebeuf Y, Gervais R, *et al.* (2013) Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. *J Dairy Sci* **96**, 7181–7194.

22. Huhtanen P, Kaustell K & Jaakkola S (1994) The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim Feed Sci Technol* **48**, 211–227.

23. Gaines WL (1928) An efficiency formula for dairy cows. *Science* **67**, 353–354.

24. Chouinard PY, Levesque J, Girard V, *et al.* (1997) Dietary soybeans extruded at different temperatures: milk composition and in situ fatty acid reactions. *J Dairy Sci* **80**, 2913–2924.

25. Boivin M, Gervais R & Chouinard PY (2013) Effect of grain and forage fractions of corn silage on milk production and composition in dairy cows. *Animal* **7**, 245–254.

26. Stamey JA, Corl BA, Chouinard PY, *et al.* (2010) Letter to the editor: estimation of milk fatty acid yield: A comment on Glasser et al. (2007). *J Dairy Sci* **93**, 3405.

27. Kelsey JA, Corl BA, Collier RJ, *et al.* (2003) The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J Dairy Sci* **86**, 2588–2597.

28. Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods* **25**, 402–408.

29. Kononoff PJ & Hanford KJ (2006) Estimating statistical power of mixed models used in dairy nutrition experiments. *J Dairy Sci* **89**, 3968–3971.

30. Rico DE, Ying Y & Harvatine KJ (2014) Comparison of enriched palmitic acid and calcium salts of palm fatty acids distillate fat supplements on milk production and metabolic profiles of high-producing dairy cows. *J Dairy Sci* **97**, 5637–5644.

31. Piantoni P, Lock AL & Allen MS (2013) Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. *J Dairy Sci* **96**, 7143–7154

32. Harvatine KJ & Bauman DE (2011) Characterization of the acute lactational response to *trans*-10, *cis*-12 conjugated linoleic acid. *J Dairy Sci* **94**, 6047–6056.

33. Rico DE, Preston SH, Risser JM, *et al.* (2015) Rapid changes in key ruminal microbial populations during the induction of and recovery from diet-induced milk fat depression in dairy cows. *Br J Nutr* **114**, 358–367.

34. Rico JE, de Souza J, Allen MS, *et al.* (2017) Nutrient digestibility and milk production responses to increasing levels of palmitic acid supplementation vary in cows receiving diets with or without whole cottonseed. *J Anim Sci* **95**, 436–446.

35. Relling AE & Reynolds CK (2007) Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. *J Dairy Sci* **90**, 1506–1515.

36. Choi BR, Palmquist DL & Allen MS (2000) Cholecystokinin mediates depression of feed intake in dairy cattle fed high fat diets. *Domest Anim Endocrinol* **19**, 159–175.

37. Drackley JK, Klusmeyer TH, Trusk AM, *et al.* (1992) Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. *J Dairy Sci* **75**, 1517–1526.

38. Choi BR & Palmquist DL (1996) High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. *J Nutr* **126**, 2913–2919.

39. Boerman JP, Firkins JL, St-Pierre NR, *et al.* (2015) Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta-regression. *J Dairy Sci* **98**, 8889–8903.

40. Fukumori R, Sugino T, Shingu H, *et al.* (2013) Ingestion of medium chain fatty acids by lactating dairy cows increases concentrations of plasma ghrelin. *Domest Anim Endocrinol* **45**, 216–223.

41. Clark SB, Brause B & Holt PR (1969) Lipolysis and absorption of fat in the rat stomach. *Gastroenterology* **56**, 214–222.

42. Perret JP (1980) Gastric lipolysis of material milk triglycerides and gastric absorption of medium chain fatty acids in the young rabbit. *J Physiol* **76**, 159–166.

43. Hagemeister H, Kaufmann W & Wiechen A (1979) Messungen des resorptionsortes von fettsä uren in Verdauungstrakt der Milchkuh. *Kieler Milchw Forsch* **31**, 11–29.

44. Enjalbert F, Nicot MC, Bayourthe C, *et al.* (2000) Effects of duodenal infusions of palmitic, stearic, or oleic acids on milk composition and physical properties of butter. *J Dairy Sci* **83**, 1428–1433.

45. Sugino T, Tateno A, Ueno G, *et al.* (2014) Effects of calcium salts of medium-chain fatty acids on plasma metabolite and hormone concentrations in early lactating dairy cows. *Anim Prod Sci* **54**, 1699–1702.

46. Enoch HG, Catala A & Strittmatter P (1976) Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. *J Biol Chem* **251**, 5095–5103.

47. Chilliard Y, Ferlay A, Mansbridge RM, *et al.* (2000) Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Ann Zootech* **49**, 181–205.

48. Toral PG, Bernard L, Chilliard Y, *et al.* (2013) Short communication: diet-induced variations in milk fatty acid composition have minor effects on the estimated melting point of milk fat in cows, goats, and ewes: insights from a meta-analysis. *J Dairy Sci* **96**, 1232–1236.

49. Bauman DE, Perfield JW, II, Harvatine KJ, *et al.* (2008) Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. *J Nutr* **138**, 403–409.

50. Kinsella JE & Gross M (1973) Palmitic acid and initiation of mammary glyceride synthesis via phosphatidic acid. *Biochim Biophys Acta* **316**, 109–113.

51. Clarke BA & Clarke SD (1982) Polymer-protomer transition of acetyl-CoA carboxylase as a regulator of lipogenesis in rat liver. *Arch Biochem Biophys* **218**, 92–100.

52. Wright TC, Cant JP & McBride BW (2002) Inhibition of fatty acid synthesis in bovine mammary homogenate by palmitic acid is not a detergent effect. *J Dairy Sci* **85**, 642–647.

53. Winder WW & Hardie DG (1999) AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol Endocrinol Metab* **277**, E1–E10.

54. Hardie DG & Pan DA (2002) Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochem Soc Trans* **30**, 1064–1070.

55. Kawaguchi T, Osatomi K, Yamashita H, *et al.* (2002) Mechanism for fatty acid 'sparing' effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. *J Biol Chem* **277**, 3829–3835.

56. Hardie DG (2003) Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology* **144**, 5179–5183.

57. Papamandjaris AA, MacDougall DE & Jones PJ (1998) Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. *Life Sci* **62**, 1203–1215.

58. Browning JD & Horton JD (2004) Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* **114**, 147–152.

59. Storry JE, Tuckley B & Hall AJ (1969) The effects of intravenous infusions of triglycerides on the secretion of milk fat in the cow. *Br J Nutr* **23**, 157–172.