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Additive effects of *trans*-10, *cis*-12 conjugated linoleic acid and propionic acid on milk fat content and composition in dairy cows

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Diet is a relatively simple way to modify milk fat yield and composition in dairy cows as the end-products of digestion are precursors or inhibitors of milk fat synthesis. The individual effects of these end-products are well-known, but it is still not known whether these nutrients have an additive effect or an interaction effect on milk fat secretion. Thus our objective was to investigate the effects of two of these nutrients on milk fat secretion, *trans*-10, *cis*-12 conjugated linoleic acid (CLA) and propionic acid (C3) supplied alone or together, under the same experimental conditions. Four Holstein dairy cows were used in a 4 × 4 Latin square design with 14-d periods. Treatments were control, CLA (duodenal infusion of 1·85 g/d of *trans*-10, *cis*-12 CLA), C3 (ruminal infusion of 500 g/d of C3) and CLA+C3 (duodenal infusion of 1·85 g/d of *trans*-10, *cis*-12 CLA plus ruminal infusion 500 g/d of C3). Infusions of *trans*-10, *cis*-12 CLA reduced milk fat content and yield by 18% whereas C3 infusions had no significant effect on milk fat secretion. *Trans*-10, *cis*-12 CLA decreased the yields of all milk fatty acids (FA). This reduction was proportionally greater for FA synthesized de novo than for preformed long-chain FA. Infusions of C3 decreased the yields and percentages of 4 : 0 and 18 : 0 and increased the yields and percentages of all odd-chain FA. Interactions between *trans*-10, *cis*-12 CLA and C3 infusions on milk fat content, yield and FA composition were never significant. Overall, this study showed that *trans*-10, *cis*-12 CLA has different and greater effects on milk fat secretion than C3. Moreover, under our experimental conditions, their effects on milk FA yields, which reflect their effects on mammary lipogenesis, were additive, whatever their individual effect.

Keywords: Propionic acid, *trans*-10, *cis*-12 CLA, milk fatty acids, dairy cow

Nutrition is the main environmental factor modifying milk fat secretion in cows. Dietary modifications can alter milk fat content, yield and composition, sometimes even leading to milk fat depression. Indeed, several nutrients produced during digestive processes are known to modify milk fat content and yield, as these nutrients are precursors or inhibitors of milk fat synthesis in the mammary gland. Acetic acid (Rook et al. 1965) and butyric acid (Huhtanen et al. 1993) increase milk fat content whereas propionic acid (C3) (Rigout et al. 2003), glucose (Hurtaud et al. 1998a) and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) (Shingfield & Griinari, 2007) decrease milk fat content. Variations in all these nutrients explain the variations in milk fat following dietary modifications.

Infusion experiments have characterized and quantified the individual effects of these nutrients on milk fat synthesis (reviewed in Rulquin et al. 2007). However, these nutrients vary simultaneously following dietary modifications, and there are scarce reports on their effects on milk fat synthesis when they are supplied together to dairy cows. It is still not known whether these nutrients have an additive effect or an interaction effect on milk fat secretion. Moreover, experimental conditions (diet, breed or period duration) differ among previously reported infusion experiments, thus preventing a comparison of effects between nutrients. A better knowledge of the respective effects of these nutrients under similar conditions and their putative interactions could also contribute to the prediction of the effects of dietary modifications on milk fat content and yield, which is not taken into consideration by current models used to estimate dairy cow requirements and formulate diets (NRC, 2001; INRA, 2007).

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Table 1. Ingredients and chemical composition† (g/kg dry matter unless stated otherwise) of the total mixed ration (TMR) and feedstuffs used

Ingredient		OM	CP	NDF	ADF	EE	NE_L, MJ/kgDM§
Orchard grass hay	198	915	112	590	320	25	5·11
Dehydrated corn pellet	396	905	74	402	215	27	6·64
Dehydrated alfalfa	79	856	222	343	247	31	5·30
Soybean meal	139	929	493	103	59	21	7·68
Energy concentrate‡	178	953	117	255	108	42	7·85
Vitamins and minerals	10	—	—	—	—	—	—
Total diet	1000	915	160	365	195	29	6·53

† OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract
‡ Wheat 200 g/kg, corn 200 g/kg, barley 200 g/kg, beet pulp 200 g/kg, wheat bran 150 g/kg, cane molasses 30 g/kg, vegetable oil 10 g/kg and salt 10 g/kg
§ Net energy for lactation values of feedstuffs were calculated from predictive equations (INRA, 2007)

The objectives of this experiment were (i) to compare the individual effects on milk fat secretion of two of these nutrients when supplied alone under identical experimental conditions and (ii) to study their effects when supplied together, in order to test the possible interaction on milk fat secretion between the two nutrients. The two nutrients chosen were *trans*-10, *cis*-12 CLA and C3, both of which are known to reduce milk fat secretion, but by different mechanisms.

Table 2. Composition of the fatty acid (FA) mixture used for infusion into the duodenum

Fatty acids	g/100 g total FA
16 : 0	5·8
18 : 0	3·6
cis-9-18 : 1	24·1
cis-9, *trans*-11 CLA	30·8
trans-10, *cis*-12 CLA	30·5
Others	5·2

Materials and Methods

Animals and diet

This study used four Holstein cows fitted with cannulas in the rumen and duodenum (207 ± 65 days in milk). Procedures related to surgery, care and use of animals for the experiment were approved by a French Ministry of Agriculture animal care committee in accordance with French regulations (Decree-law 2001-464, 29 May 2001). Cows were housed in tie stalls and milked at 06.30 and 17.00. They were individually fed a controlled amount of a total mixed ration (TMR) formulated to meet their energy, protein, mineral and vitamin requirements (INRA, 2007). TMR consisted of 198 g/kg dry matter (DM) orchard grass hay, 396 g/kg DM dehydrated corn pellets and 406 g/kg DM concentrate feedstuffs (Table 1), and was offered in equal amounts at 08·00 and 18·00.

Treatments and experimental design

The experiment was conducted according to a 4 × 4 Latin square design with 14-d periods. The four experimental treatments were in a 2 × 2 factorial arrangement: CON: control; CLA: *trans*-10, *cis*-12 CLA infused into the duodenum; C3: propionic acid infused into the rumen; and CLA+C3: *trans*-10, *cis*-12 CLA infused into the duodenum and propionic acid infused into the rumen. The *trans*-10, *cis*-12 CLA was provided by a mixture of fatty acids (Lutalin®, BASF AG, Ludwigshafen, D-67056, Germany) in which *trans*-10, *cis*-12 CLA represented 30% of total fatty acids (FA) (Table 2). The FA mixture was emulsified in skimmed milk (Lactalis, Bourgbarré, F-35230, France) with glycerol (Sigma-Aldrich Chimie, Saint Quentin, F-38297, France) and lecithin (Acros Organics, Geel, B-2440, Belgium) in order to obtain an adequate volume (2 l/d) for uniform and continuous infusion of *trans*-10, *cis*-12 CLA (A Ferlay, personal communication). The FA mixture was infused at 6·1 g/d, corresponding to 1·85 g/d of *trans*-10, *cis*-12 CLA. During CON and C3 treatments, only skimmed milk was infused into the duodenum. The daily dose of propionic acid (VWR International, Strasbourg, F-67023, France) was 500 g/d diluted in 40 l of tap water. C3 and CLA+C3 treatments included buffers (250 g/d $NaHCO_3$ and 100 g/d $KHCO_3$ dissolved in 10 l of tap water) infused into the rumen to limit the decrease in ruminal pH following propionic acid infusion. CON and CLA treatments included a solution of NaCl and KCl (volume 50 l) infused into the rumen in order to equalize Na and K supply across treatments. All solutions were prepared every morning prior to infusions. Peristaltic pumps delivered the infusates continuously over 24 h into the duodenum and the rumen via polyvinyl chloride tubes (0·5 cm i.d).

Measurements and sampling

Daily intake was determined by weighing TMR offered and refused. TMR and refusals were sampled each day and oven-dried for DM determination. Hay and concentrates were sampled at the end of each period. The chemical composition of ingredients and TMR is in Table 1.

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Table 3. Feed intake, rumen pH and volatile fatty acids (VFA) in dairy cows infused with *trans*-10, *cis*-12 CLA into the duodenum or with propionic acid into the rumen, or both. Values are means with SEM for 4 animals.

	Treatments†					*P* values §		
	CON	CLA	C3	C3+CLA	SEM	CLA	C3	CLA × C3
Dry matter intake, kg/d	21·3	21·5	21·6	21·1	1·1	0·66	0·87	0·28
Intake NE_L, MJ/d	139·1	140·4	140·9	137·9	4·2	0·64	0·87	0·26
Total NE_L‡, MJ/d	139·1^{b}¶	142·1^{b}	148·3^{a}	145·4^{ab}	4·2	0·73	<0·01	0·26
pH	6·45	6·48	6·48	6·53	0·06	0·25	0·23	0·80
Total VFA, mmol/l	88·1	87·6	91·7	87·5	4·8	0·53	0·63	0·62
VFA, mol/100 mol								
Acetate	60·2^{ab}	62·5^{a}	58·2^{ab}	56·0^{b}	1·6	0·97	0·05	0·23
Propionate	21·9^{b}	21·3^{b}	25·1^{ab}	27·3^{b}	1·2	0·55	0·02	0·32
Isobutyrate	0·59	0·57	0·62	0·63	0·07	0·92	0·46	0·78
Butyrate	14·5	14·0	13·2	13·3	1·05	0·76	0·21	0·68
Isovalerate	0·90	0·87	0·96	0·89	0·11	0·38	0·55	0·76
Valerate	1·4	1·4	1·6	1·5	0·10	0·29	0·11	0·87
Caproate	0·48^{a}	0·45^{ab}	0·36^{ab}	0·35^{b}	0·05	0·71	0·03	0·76

† CON = control, CLA = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA, C3 = ruminal infusion of 500 g/d propionic acid, and CLA+C3 = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA plus ruminal infusion of 500 g/d propionic acid

‡ Net energy for lactation, Intake+infusion

§ CLA: main effect of CLA infusions, C3: main effect of C3 infusions, CLA × C3: interaction between CLA and C3 infusions

¶ Values within a row without a common superscript letter are significantly different ($P<0·05$)

At each milking, milk yield was recorded and fat and protein contents were determined by infrared analysis (Milkoscan, Foss Electric, Hillerød, Denmark). Lactose was measured by infrared analysis on day 12. On day 14, 100 ml of milk was taken from each cow at the morning and evening milkings. Morning and evening milk samples were pooled according to milk yields and stored at −20 °C until freeze-drying for analysis of FA composition. Fatty acids in freeze-dried milk were directly methylated and analysed according to Loor et al. (2005).

Ruminal fluid was assayed for pH, volatile fatty acids (VFA) and ammonia on day 13 of each period. Individual subsamples (50 ml) were collected from the ventral sac of the rumen via a polyvinyl chloride tube at −0·5, +3 and +6 h after the morning meal. Ruminal pH was measured immediately. Then, following filtration through six layers of cheesecloth, individual subsamples were pooled before measuring VFA as described by Rigout et al. (2003).

Statistical analysis

DM intake (DMI), milk yield and protein and fat contents were averaged over the last 5 d of each period. Data were analysed as a 4 × 4 Latin square using the MIXED procedure in SAS software (SAS Institute, Cary NC, USA). The statistical model included cow (random effect), period, CLA infusion, C3 infusion, interaction between CLA and C3 infusions (fixed effects) and residual error. Multiple comparisons of means were performed with the Tukey test, and differences ($P<0·05$) are indicated by different superscript letters in the tables. Milk Δ9-desaturase ratios were calculated according to Kelsey et al. (2003). Total milk FA yields were computed from milk fat yields × 0·933 (Glasser et al. 2007).

Results

Intake and rumen parameters

Infusions had no effect on dietary DM or energy intakes (Table 3). Total net energy for lactation (NE_L) (diet+infusions) was higher for C3 and CLA+C3 treatments than for CON and CLA treatments (+7·5 MJ/d on average). Infusions of C3 increased ruminal C3 percentage [from 21·6% of total VFA with CON to 25·1% and 27·2% with C3 and CLA+C3 treatments, respectively] at the expense of acetic acid. Following C3 infusions, there were no significant variations in the percentages of butyric, isobutyric, valeric and isovaleric acids, whereas caproic acid decreased. Compared with CON treatment, *trans*-10, *cis*-12 CLA infusions had no effect on rumen VFA percentages, confirming that there was no major modification of rumen fermentation. There was no significant between-treatment variation in ruminal pH and total VFA concentration.

Milk yield and composition

There were no significant treatment effects on milk yield (Table 4). *Trans*-10, *cis*-12 CLA infusions decreased milk fat yield and content by on average 18% but did not affect milk protein or lactose. C3 infusions did not modify milk fat or protein, but tended to increase the percentage of lactose in milk ($P\leqslant0·1$). Interactions between CLA and C3 infusions were never significant.

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Table 4. Milk yield and composition in dairy cows infused with *trans*-10, *cis*-12 CLA into the duodenum, or with propionic acid into the rumen, or both. Values are means with SEM for 4 animals

	Treatments†					*P* values§		
	CON	CLA	C3	CLA+C3	SEM	CLA	C3	CLA × C3
Milk yield, kg/d	30·1	31·7	30·9	31·0	3·6	0·31	0·95	0·33
Fat, g/kg	33·9[a] ¶	27·6[b]	32·8[a]	27·4[b]	3·2	<0·01	0·58	0·68
Fat, g/d	1006[a]	850[b]	998[a]	826[b]	99	<0·01	0·59	0·79
Protein, g/kg	31·7	31·6	31·5	32·3	1·2	0·18	0·24	0·11
Protein, g/d	938	990	978	993	81	0·12	0·28	0·36
Lactose, g/kg	48·7[ab]	47·8[b]	49·3[a]	48·7[ab]	0·40	0·10	0·09	0·66
Lactose, g/d	1472	1477	1539	1503	168	0·65	0·20	0·55

†CON = control, CLA = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA, C3 = ruminal infusion of 500 g/d propionic acid, and CLA+C3 = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA plus ruminal infusion of 500 g/d propionic acid

§CLA: main effect of CLA infusions, C3: main effect of C3 infusions, CLA × C3: interaction between CLA and C3 infusions

¶ Values within a row without a common superscript letter are significantly different ($P<0·05$)

Milk fatty acid composition

Interactions between *trans*-10, *cis*-12 CLA and C3 infusions were never significant for milk FA composition (Table 5). Infusions of *trans*-10, *cis*-12 CLA decreased the percentage of short- and medium-chain FA (6 : 0–12 : 0 and 16 : 0) and increased the percentage of total C_{18} (Table 5). Among the C_{18} FA, only *cis*-9 18 : 1, *cis*-11 18 : 1 and *trans*-10, *cis*-12 CLA were significantly increased with CLA infusions. Milk Δ9-desaturase ratios were not altered by CLA infusions. C3 infusions decreased the percentage of 4 : 0 but had no effect on the other even-chain FA synthesized de novo (6 : 0–16 : 0). C3 infusions decreased the percentage of 18 : 0 and increased the percentages of all the odd-chain FA (except 5 : 0 and 19 : 0). Milk Δ9-desaturase ratios tended to increase with C3 infusions ($P\leqslant 0·1$): the increases in 14 : 1/14+14 : 1 and 16 : 1/16+16 : 1 were the consequence of 14 : 1 and 16 : 1 percentage increases, whereas the increase in 18 : 1, cis9/18 : 0+18 : 1 was related to a reduction in 18 : 0 percentage.

Milk fatty acid yields

Infusions of *trans*-10, *cis*-12 CLA decreased the milk yield of all FA, i.e. short- and medium-chain FA (4 : 0–16 : 0), long-chain FA (total C_{18}) and odd-chain FA (Table 6). C3 infusions decreased the yields of 4 : 0 and 18 : 0 and increased the yield of odd-chain FA (sum 5 : 0 to 19 : 0). Concerning milk FA yield, there was no significant interaction between *trans*-10, *cis*-12 CLA and C3 infusions. Milk *trans*-10, *cis*-12 CLA was undetectable in the CON and C3 treatments. Milk yield of *trans*-10, *cis*-12 CLA in the CLA and CLA+C3 treatments increased to 0·38 g/d and 0·29 g/d.

Discussion

The first objective of this experiment was to compare individual effects on milk fat secretion of *trans*-10, *cis*-12 CLA and propionic acid in dairy cows. These two nutrients are produced during digestive processes and are known to decrease milk fat content and yield. The amounts of *trans*-10, *cis*-12 CLA and C3 infused were chosen so as to induce a similar and moderate (−3·0 to −5·0 g/kg) decrease in milk fat content, based on data from published experiments (Rulquin et al. 2007). These amounts chosen (1·85 g/d of *trans*-10, *cis*-12 CLA and 500 g/d of C3) were, however, lower than the quantities infused in most experiments, i.e. 4 g/d for *trans*-10, *cis*-12 CLA (reviewed in Shingfield & Griinari, 2007) and 1000 g/d for C3 (reviewed in Hurtaud et al. 1998b). The moderate amounts chosen in the present experiment were designed so that the sum of the decreases induced by the two products infused together (−6·0 to −10·0 g/kg) would still be physiological.

In the present study, *trans*-10, *cis*-12 CLA reduced milk fat yield and content by 168 g/d and 6·5 g/kg, respectively, which was slightly higher than the reduction predicted from other experiments using similar infused amounts (Peterson et al. 2002; de Veth et al. 2004). The other FA present in the FA mixture are neutral towards milk fat secretion (Christensen et al. 1994; Enjalbert et al. 1998; Baumgard et al. 2000) and cannot be responsible for the observed effects of *trans*-10, *cis*-12 CLA. The late lactation stage and the longer duration of experimental periods in the present experiment (14 d *v.* <5 d in most infusion experiments) could explain this higher than expected decrease: these two factors are suspected to increase the response to CLA infusions (Griinari & Bauman, 2006). *Trans*-10, *cis*-12 CLA infusion decreased the secretion of all FA, including the odd-chain FA. This decrease was proportionally greater for those FA synthesized de novo, resulting in a decrease in short and medium-chain FA percentages and an increase in preformed long-chain FA percentages. This result is consistent with the patterns generally observed with *trans*-10, *cis*-12 CLA infusions and the inhibiting role of this isomer on milk FA synthesis de novo (Shingfield & Griinari, 2007; Harvatine et al. 2009). As previously reported with small-amount infusions

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Table 5. Milk fatty acid (FA) profiles in dairy cows infused with *trans*-10, *cis*-12 CLA into the duodenum, or with propionic acid into the rumen, or both. Values are means with SEM for 4 animals

	Treatment†					*P values*‡		
	CON	CLA	C3	CLA+C3	SEM	CLA	C3	CLA × C3
FA, g/100g total FA								
4 : 0	2·76^{a}††	2·63^{a}	2·43^{ab}	2·22^{b}	0·15	0·15	0·01	0·70
5 : 0	0·018	0·024	0·032	0·011	0·008	0·38	0·96	0·15
6 : 0	2·09^{a}	1·81^{b}	1·95^{a}	1·63^{b}	0·11	0·02	0·14	0·86
7 : 0	0·027^{bc}	0·018^{c}	0·039^{a}	0·030^{b}	0·003	0·01	<0·01	0·85
8 : 0	1·45^{a}	1·26^{ab}	1·38^{ab}	1·19^{b}	0·09	0·02	0·30	0·97
9 : 0	0·035^{bc}	0·023^{c}	0·053^{a}	0·037^{b}	0·004	<0·01	<0·01	0·55
10 : 0	3·79	3·28	3·72	3·33	0·32	0·04	0·94	0·76
10 : 1	0·38^{a}	0·31^{b}	0·39^{a}	0·33^{ab}	0·04	0·01	0·35	0·72
11 : 0	0·068^{bc}	0·050^{c}	0·099^{a}	0·084^{b}	0·007	0·04	<0·01	0·85
12 : 0	4·65	4·23	4·69	4·48	0·36	0·09	0·39	0·55
13 : 0§	0·23^{bc}	0·20^{c}	0·28^{a}	0·26^{ab}	0·02	0·11	<0·01	0·89
14 : 0	15·04	14·88	14·80	14·86	0·48	0·79	0·50	0·56
14 : 1	1·35^{b}	1·43^{ab}	1·57^{ab}	1·69^{b}	0·20	0·36	0·04	0·83
15 : 0	1·12^{b}	1·11^{b}	1·37^{a}	1·35^{a}	0·06	0·56	<0·01	0·99
ante 15 : 0	0·56	0·59	0·55	0·59	0·03	0·19	0·94	0·94
iso 15 : 0	0·27	0·27	0·27	0·27	0·02	0·93	0·92	0·79
16 : 0	31·98^{ab}	30·15^{b}	32·61^{a}	30·67^{ab}	1·30	0·03	0·41	0·94
iso 16 : 0	0·19^{b}	0·24^{a}	0·20^{ab}	0·23^{a}	0·02	0·02	0·70	0·66
*cis*9 16 : 1	1·26	1·34	1·58	1·58	0·14	0·74	0·06	0·76
17 : 0	0·62^{b}	0·61^{b}	0·69^{a}	0·72^{a}	0·02	0·73	<0·01	0·52
ante 17 : 0	0·41	0·45	0·40	0·45	0·04	0·19	0·84	0·83
17 : 1	0·14^{b}	0·16^{ab}	0·18^{ab}	0·19^{a}	0·02	0·31	0·04	0·89
18 : 0	6·69^{ab}	7·21^{a}	5·97^{b}	6·02^{b}	0·30	0·19	0·01	0·59
iso 18 : 0	0·026	0·033	0·028	0·034	0·004	0·16	0·64	0·92
trans-4 18 : 1	0·016	0·020	0·021	0·018	0·003	0·90	0·63	0·23
trans-5 18 : 1	0·019	0·019	0·021	0·013	0·004	0·36	0·68	0·37
trans-6+8 18 : 1	0·22	0·27	0·22	0·25	0·03	0·09	0·51	0·61
trans-9 18 : 1	0·18	0·20	0·19	0·21	0·02	0·10	0·52	0·94
trans-10 18 : 1	0·40	0·44	0·37	0·41	0·05	0·22	0·27	0·92
trans-11 18 : 1	1·41	1·59	1·38	1·52	0·13	0·14	0·58	0·84
*trans*12 18 : 1	0·24	0·27	0·24	0·26	0·02	0·12	0·62	0·57
trans-13 18 : 1	0·49	0·49	0·40	0·40	0·04	0·99	0·04	0·90
cis-9 18 : 1	15·51^{b}	17·20^{a}	15·47^{b}	17·07^{a}	1·14	<0·01	0·82	0·91
cis-11 18 : 1	0·33^{b}	0·40^{ab}	0·37^{ab}	0·43^{a}	0·03	0·04	0·26	0·98
cis-12 18 : 1	0·17	0·22	0·19	0·22	0·03	0·16	0·57	0·67
cis-13 18 : 1	0·04	0·05	0·03	0·05	0·01	0·30	0·77	0·92
cis-15 18 : 1	0·082	0·089	0·087	0·091	0·006	0·36	0·58	0·76
cis-9,*trans*-11 CLA	0·68	0·86	0·76	0·90	0·09	0·06	0·45	0·77
trans-10, *cis*-12 CLA	<0·001^{b}	0·05^{a}	<0·001^{b}	0·03^{ab}	0·01	0·01	0·57	0·57
cis-9, *cis*-11 CLA	0·03	0·04	0·03	0·04	0·01	0·38	0·94	0·92
n-6 18 : 3	0·03	0·02	0·04	0·04	0·01	0·84	0·36	0·79
n-3 18 : 3	0·35	0·39	0·35	0·38	0·02	0·13	0·68	0·99
19 : 0	0·025	0·031	0·027	0·034	0·003	0·04	0·44	0·94
20 : 0	0·076	0·080	0·076	0·077	0·006	0·64	0·75	0·75
Others C_{20}	0·35^{b}	0·37^{ab}	0·36^{ab}	0·41^{a}	0·03	0·05	0·12	0·37
22 : 0	0·038	0·038	0·041	0·034	0·003	0·27	0·81	0·25
Others C_{22}	0·11	0·12	0·11	0·14	0·02	0·13	0·28	0·40
24 : 0	0·027	0·027	0·027	0·027	0·002	0·93	0·82	0·85
6 : 0+8 : 0+10 : 0+12 : 0	11·98	10·58	11·74	10·62	0·83	0·04	0·84	0·77
Sum odds 5 : 0 to 19 : 0	2·14^{b}	2·06^{b}	2·59^{a}	2·52^{a}	0·08	0·18	<0·01	0·99
Total C_{18}	29·30^{ab}	32·58^{a}	28·51^{b}	31·35^{ab}	1·87	0·02	0·34	0·83
Δ9-desaturase ratios¶								
14 : 1/(14 : 0+14 : 1)	0·082	0·088	0·095	0·102	0·011	0·32	0·07	0·93
16 : 1/(16 : 0+16 : 1)	0·051	0·057	0·059	0·065	0·005	0·14	0·11	0·91
*cis*9-18 : 1/(18 : 0+*cis*9-18 : 1)	0·697^{b}	0·704^{b}	0·721^{a}	0·731^{a}	0·008	0·10	<0·01	0·74

† CON = control, CLA = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA, C3 = ruminal infusion of 500 g/d propionic acid, and CLA+C3 = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA plus ruminal infusion of 500 g/d propionic acid

‡ CLA: main effect of CLA infusions, C3: main effect of C3 infusions, CLA × C3: interaction between CLA and C3 infusions

§ Possibly collected with some 12 : 1

¶ Δ9-desaturase ratios were defined as [product of Δ^9-desaturase]/[product of Δ^9-desaturase+substrate of Δ9-desaturase]

†† Values within a row without a common superscript letter are significantly different ($P<0·05$)

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Table 6. Milk fatty acid yields in dairy cows infused with *trans*-10, *cis*-12 CLA into the duodenum, or with propionic acid into the rumen, or both. Values are means with SEM for 4 animals

	Treatments†					*P* values‡		
Fatty acid, g/d	CON	CLA	C3	CLA+C3	SEM	CLA	C3	CLA × C3
4 : 0	26·3[a]§	21·2[bc]	23·1[ab]	17·3[c]	3·6	<0·01	0·05	0·83
6 : 0+8 : 0+10 : 0+12 : 0	112·3[a]	85·4[b]	109·2[a]	82·9[b]	13·0	<0·01	0·67	0·96
14 : 0	140·6[a]	118·5[b]	137·4[a]	114·8[b]	14·0	<0·01	0·50	0·96
16 : 0	305·6[a]	242·2[b]	308·3[a]	239·4[b]	42·6	<0·01	0·99	0·84
18 : 0	62·3[a]	56·4[ab]	55·4[b]	47·8[c]	47·8	0·02	<0·01	0·68
Total C_{18}	270·4[a]	253·8[ab]	261·7[ab]	237·8[b]	14·5	0·01	0·12	0·62
Odds (5 : 0 to 19 : 0)	21·2[b]	17·3[c]	25·7[a]	20·7[b]	1·7	<0·01	<0·01	0·57
trans-10, *cis*-12 CLA	<0·001[b]	0·38[a]	<0·001[b]	0·29[ab]	0·12	0·02	0·68	0·68

† CON = control, CLA = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA, C3 = ruminal infusion of 500 g/d propionic acid, and CLA+C3 = duodenal infusion of 1·85 g/d *trans*-10, *cis*-12 CLA plus ruminal infusion of 500 g/d propionic acid

‡ CLA: main effect of CLA infusions, C3: main effect of C3 infusions, CLA × C3: interaction between CLA and C3 infusions

§ Values within a row without a common superscript letter are significantly different ($P<0·05$)

(Peterson et al. 2002; Saebo et al. 2005), *trans*-10, *cis*-12 CLA did not alter Δ9-desaturase ratios, even if milk fat depression occurred.

Infusion of C3 did not modify milk fat content or yield. This result is in contrast with two published papers that reported a significant milk fat reduction with similar or smaller amounts infused (Rigout et al. 2003; Vanhatalo et al. 2003). Two hypotheses can be raised to explain this result: first, the basal percentage of C3 in the rumen was higher than in the two other studies (on average 22% *v.* 17%) which could explain why the increase in rumen C3 provoked by the infusions was lower. Second, comparison with other experiments suggests that the decrease in milk fat could be more pronounced when the initial milk fat content is high (i.e. above 40 g/kg) for a given amount infused and under similar basal diets (Rook et al. 1965; Ueyama et al. 1972). The initial milk fat content in the present experiment was lower than in the two experiments using similar amounts (Rigout et al. 2003; Vanhatalo et al. 2003), i.e. only 33 g/kg here *v.* more than 40 g/kg. With the C3 infusion, although there was no decrease in milk fat content or yield, milk FA profiles and yields were modified: 4 : 0 and 18 : 0 yields and percentages decreased while all the odd-chain FA from 7 : 0 to 17 : 0 increased. This result is consistent with several published reports (Hurtaud et al. 1998b; Rigout et al. 2003). In the present experiment, however, we did not observe the decrease in short- and medium-chain even-chain FA reported by these authors. The drop in even-chain FA is the consequence of a decrease in plasma concentrations of the following precursors: acetate and β-hydroxybutyrate for de-novo synthesized FA, non-esterified FA and total glycerides for preformed FA in all experiments with (Hurtaud et al. 1993; Huhtanen et al. 1998; Rigout et al. 2003) or without isoenergetic supplementation (this study: data not shown; Lemosquet et al. 2009). However, the precise mechanisms by which C3 acts on milk fat synthesis remain unknown. The increase in odd-chain FA can be explained by the increase in propionate available to the udder as a precursor of de-novo synthesis (Massart-Leen et al. 1983; Emmanuel & Kennelly, 1985). The increase in Δ9-desaturase ratios has already been observed with C3 infusions (Hurtaud et al. 1993; Miettinen & Huhtanen, 1996) suggesting increased desaturase activity by the mammary gland due to the C3 supply.

The second objective of this experiment was to study the combined effects of *trans*-10, *cis*-12 CLA and C3 on milk fat secretion when supplied together to dairy cows, and to determine whether their effects were additive. Under our conditions, interactions between CLA and C3 were never significant, meaning that the effects of the two nutrients on milk FA yields were additive, whatever their individual effects: (i) when both nutrients had a negative effect, these effects were additive when they were provided together (e.g. on 4 : 0 and 18 : 0 yields); (ii) when C3 had no significant effect and *trans*-10, *cis*-12 CLA had a negative effect, the effect of CLA+C3 was similar to that of CLA alone (e.g. 16 : 0 yield); (iii) when C3 had a positive effect and *trans*-10, *cis*-12 CLA had a negative effect, the result of CLA+C3 was similar to that of the control treatment (e.g. odd-chain FA yield). These three situations confirm the additive effects of these two nutrients on mammary lipogenesis. The observed additivity could result from the different mechanisms by which *trans*-10, *cis*-12 CLA and C3 act on milk fat synthesis.

In conclusion, this study describes the individual effects on milk fat secretion of *trans*-10, *cis*-12 CLA and C3 supplied alone or together, under the same experimental conditions. It shows that *trans*-10, *cis*-12 CLA infusions had a greater effect than C3 infusions, and induced different patterns of change in the milk FA profiles and yields. Moreover, under our conditions, their effects on milk FA yields were additive when they were supplied together. If these findings are validated for other nutrients, this could be used to estimate milk fat and FA changes based on variations of nutrient supplies.

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