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Effects of oil and natural or synthetic vitamin E on ruminal and milk fatty acid profiles in cows receiving a high-starch diet

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ABSTRACT

Among *trans* fatty acids, *trans*-10,*cis*-12 CLA has negative effects on cow milk fat production and can affect human health. In high-yielding dairy cows, a shift from the *trans*-11 to the *trans*-10 pathway of biohydrogenation (BH) can occur in the rumen of cows receiving high-concentrate diets, especially when the diet is supplemented with unsaturated fat sources. In some but not all experiments, vitamin E has been shown to control this shift. To ascertain the effects of vitamin E on this shift of BH pathway, 2 studies were conducted. The first study explored in vitro the effects of addition of natural (RRR-α-tocopherol acetate) and synthetic (DL-α-tocopherol acetate) vitamin E. Compared with control and synthetic vitamin E, the natural form resulted in a greater *trans*-10/*trans*-11 ratio; however, the effect was very low, suggesting that vitamin E was neither a limiting factor for rumen BH nor a modulator of the BH pathway. An in vivo study investigated the effect of natural vitamin E (RRR-α-tocopherol) on this shift and subsequent milk fat depression. Six rumen-fistulated lactating Holstein cows were assigned to a 2 × 2 crossover design. Cows received 20-kg DM of a control diet based on corn silage with 22% of wheat, and after 2 wk of adaptation, the diet was supplemented with 600 g of sunflower oil for 2 more weeks. During the last week of this 4-wk experimental period, cows were divided into 2 groups: an unsupplemented control group and a group receiving 11 g of RRR-α-tocopherol acetate per day. A *trans*-10 shift of ruminal BH associated with milk fat depression due to oil supplementation of a high-wheat diet was observed, but vitamin E supplementation of dairy cows did not result in a reversal toward a *trans*-11 BH pathway, and did not restore milk fat content.

Key words: rumen biohydrogenation, *trans*-10 shift, milk fatty acid, vitamin E

INTRODUCTION

Due to partial and incomplete ruminal biohydrogenation (**BH**) of unsaturated FA, ruminant products, especially milk, contain BH intermediates, mainly *trans* FA including *trans* C18:1 and conjugated linoleic acids (**CLA**). A large number of positional and geometric isomers can be encountered. Among CLA, *trans*-10,*cis*-12 CLA has been shown to decrease the mammary synthesis of de novo FA and induces milk fat depression in dairy cows (Baumgard et al., 2000). Moreover, *trans*-10,*cis*-12 CLA has possible detrimental effects on human health (Ip et al., 2007). On the contrary, *cis*-9,*trans*-11 CLA is most desirable because of its anticarcinogenic and other health-promoting properties (Kritchevsky, 2000). Decreasing the *trans*-10/*trans*-11 ratio is, therefore, desirable. In most dietary conditions, *trans*-11 isomers are much more abundant than *trans*-10 isomers in milk fat. However, high-concentrate diets can result in a shift of BH from the *trans*-11 to *trans*-10 pathway, especially when based on corn silage or associated with an enrichment of the diet with an unsaturated FA source (Piperova et al., 2002; Loor et al., 2004; Nielsen et al., 2006). After addition of an unsaturated FA source, the isomeric profile progressively but strongly evolves, *trans*-10 C18:1 reaching a plateau after 10 d, and *trans*-11 C18:1 reaching a peak after 6 d and then decreasing (Roy et al., 2006; Shingfield et al., 2006).

Because such a shift is not encountered with grass-based diets, Kay et al. (2005) hypothesized that vitamin E, which is abundant in pasture, could prevent it. However, as supplementing a TMR based on corn and grass silages with a large amount of synthetic vitamin E resulted in a *trans*-10 C18:1 proportion in milk fat

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Table 1. Ingredient and chemical composition of cow diets and in vitro incubation substrate

Composition	In vivo experiment		In vitro substrate
	Control diet	Oil diet	
Ingredient, % of DM			
Corn silage	56.5	54.8	36.7
Wheat	22.2	21.5	44.1
Soybean meal	20.0	19.4	14.7
Sunflower oil	0.0	3.0	0.0
Pure linoleic acid	0.0	0.0	4.5
Minerals and vitamins[1]	1.3	1.3	0.0
Chemical composition, % of DM			
CP	17.0	16.5	15.5
NDF	31.0	30.1	24.6
Starch	31.9	31.0	41.5
Crude fat	2.5	5.4	6.6

[1]Contained 4% P, 26% Ca, 5% Mg, 2% Na, 5 g of Zn/kg, 4 g of Mn/kg, 1 g of Cu/kg, 40 mg of I/kg, 20 mg of Co/kg, 450,000 IU of vitamin A/kg, 100,000 IU of vitamin D/kg, and 1,500 IU of vitamin E/kg.

that was much over that encountered in pasture-fed cows, they concluded that vitamin E is not the primary reason explaining that *trans*-10 FA are not produced with grass diets. Pottier et al. (2006) partly succeeded in preventing the low milk fat syndrome due to *trans*-10 FA in cows receiving linseed-supplemented diets by adding supranutritional amounts of synthetic vitamin E to the diet, but when this vitamin addition started 3 wk after beginning linseed supplementation, no effect could be observed, suggesting an interaction between vitamin E and disturbance over time of ruminal microbiota by fat. This 3-wk interval is often considered as allowing a complete adaptation of the ruminal microbiota to a new diet, and the effects of vitamin E on the *trans*-10 shift sooner after oil addition (i.e., when the *trans*-10/*trans*-11 ratio strongly evolves; Roy et al., 2006) have not been investigated.

Pottier et al. (2006) measured the effects of vitamin E after 21 d of supplementation, and discussed the changes due to vitamin E as probably resulting from effects on ruminal BH, but did not directly study BH. Moreover, they used synthetic vitamin E (DL-α-tocopherol). The activity of synthetic vitamin E is known to be lower than that of natural vitamin E (RRR-α-tocopherol) in animals (Dersjant-Li and Peisker, 2010), but the difference has not been studied in microbes, and lower activity could be overcome by a higher dose. As a consequence, the objectives of our study were, with a diet inducing a *trans*-10 shift, to investigate in vitro the differences of effects between natural and synthetic vitamins E, and to investigate in vivo the effects of natural vitamin E supplementation beginning 1 wk after fat supplementation on ruminal and milk FA profiles.

MATERIALS AND METHODS

In Vitro Experiment

For the in vitro experiment, 2 ruminally fistulated lactating cows were used. They were adapted for 2 wk to the control diet described in Table 1. In vitro incubations were conducted during 3 consecutive weeks, and lasted 52 h. One liter of rumen fluid was collected from each donor cow before the morning meal, and strained through a metal sieve (1.6-mm mesh). The rumen fluids were transported quickly (30 min) to the laboratory in anaerobic conditions at 39°C. In the laboratory, the pH of the rumen fluids was measured and the 2 rumen fluids were mixed. The incubation substrate (Table 1) was based on the same ingredients as the cow diet, ground through a 0.5-mm screen, and supplemented with pure linoleic acid (Sigma Co., St. Louis, MO).

Three series of incubations were performed during 3 successive weeks. On d 1 of each week, 40 mL of each rumen fluid and 40 mL of a bicarbonate buffer solution (19.5 g of $Na_2HPO_4 \cdot 12H_2O$/L, 9.24 g of $NaHCO_3$/L, 0.705 g of NaCl/L, 0.675 g of KCl/L, 0.108 g of $CaCl_2 \cdot 2H_2O$/L, and 0.180 g of $MgSO_4 \cdot 7H_2O$/L) were placed into six 250-mL flasks containing 1.20 g of substrate, including 60 mg of pure linoleic acid. Two flasks were not supplemented with vitamin E, 2 flasks were supplemented with 30 mg of a 37% RRR-α-tocopherol acetate oil solution (Artimon Sarl, Plérin, France), and 2 flasks were supplemented with 24 mg of 50% DL-α-tocopherol acetate (DSM Nutritional Products France SAS, Courbevoie, France). The buffer solution was prewarmed to 39°C and saturated with CO_2. Its pH was then lowered to 6.0 with hydrochloric

acid (6 *N*) before being added to the 40 mL of rumen fluid. Next, the flasks were gassed with CO_2 and closed with a rubber cap with a plastic tube immersed in water to vent fermentation gas without allowing oxygen entry. Incubations were carried out at 39°C in a water bath rotary shaker (Aquatron; Infors AG, Bottmingen, Switzerland). Flasks were stirred at 130 rpm and kept out of the light during incubation. After 24 and 48 h of incubation, flasks were opened and 30 mL of buffer solution and the same substrate as at d 1, including linoleic acid and vitamins, was added. Flasks were filled with CO_2 before closing.

After 52 h of incubation, fermentations were stopped by placing the flasks into ice and the pH was measured. The contents of the flasks were immediately frozen at −20°C and freeze dried (Virtis Freezemobile 25; Virtis Co. Inc., Gardiner, NY), weighed, ground, and homogenized in a ball mill (Dangoumau; Prolabo SA, Nogent-sur-Marne, France), and then stored at −20°C until analysis.

In Vivo Experiment

For the in vivo experiment, 6 ruminally fistulated midlactation Holstein dairy cows were used in a 2 × 2 crossover design. Cows were housed in individual stalls. Each period lasted 4 wk and was divided into 2 subperiods: a 2-wk washout subperiod, followed by a 2-wk oil diet subperiod, with the supplementation of vitamin E beginning at d 8 of this oil diet subperiod. The washout was used to obtain a similar basal state of ruminal digestion before oil supplementation, allowing the study of FA profile kinetics without carryover effect of the diet used during the previous period.

During the washout subperiod, cows were allowed to consume 20-kg DM of a control diet based on corn silage (Table 1). During the 2-wk oil diet subperiod, cows received the control diet supplemented with 600 g of sunflower oil. The ingredients of the control diet were mixed before distribution, and oil was poured on the mixed ration. From d 8 of the oil diet subperiod, cows received daily either no supplemental vitamin E or 30 g of a 37% RRR-α-tocopherol acetate oil solution (Artimon Sarl). This dose was similar to that used by Pottier et al. (2006) and, assuming a 100-L rumen volume, was in the same range as our in vitro dose. Diets and vitamin E supplement were distributed in 2 equal meals at 0900 and 1700 h and water was available ad libitum.

Milk yield was determined daily at each milking (0630 and 1730 h). Ruminal and milk samples were taken at morning and evening milkings on d 1, 3, 5, 7, 9, 11, and 13 of the second subperiod. One liter of ruminal content was taken 5 h after the morning meal, strained through a metal sieve (1.6-mm mesh), and the pH was measured. Two 8-mL aliquots were transferred into 10-mL vials containing 0.8 mL of 2% $HgCl_2$ and were stored at −20°C until VFA analysis, and a 100-mL sample was kept at −20°C for FA analysis. Milk samples were taken from morning and evening milking and were composited based on milk yield.

Chemical Analysis

Milk fat and true protein contents were determined by infrared analysis (MilkoScan 605; Foss Electric, Paris, France). The FA of milk and rumen in vivo and in vitro samples were analyzed as detailed by Zened et al. (2011). Briefly, FA were extracted and methylated using a procedure adapted from Park and Goins (1994). Thereafter, they were analyzed by GC (Agilent 6890N, equipped with a model 7683 autoinjector, Network GC System; Agilent Technologies Inc., Palo Alto, CA) using a fused silica capillary column (100 m × 0.25 mm i.d., 0.20-μm film thickness; CPSil88; Varian Inc., Middelburg, the Netherlands). Two temperature programs were used. The first temperature program started at 60°C for 2 min and the temperature then was increased by 8°C/min to 150°C, held at 150°C for 12 min, increased by 2°C/min to 175°C, held at 175°C for 20 min, increased by 5°C/min to 225°C, held at 225°C for 10 min, and finally increased by 10°C/min to 240°C and maintained at 240°C for 10 min. The second temperature program, which improved the separation of *trans*-13+*trans*-14 C18:1 from *cis*-9 C18:1, started at 60°C for 3 min and the temperature was then increased by 8°C/min to 190°C, held at 190°C for 13 min, increased by 5°C/min to 225°C, held at 225°C for 10 min, increased by 10°C/min to a final temperature of 230°C, and maintained at 230°C for 10 min.

Concentrations of VFA in the rumen were determined by the method of Playne (1985) using automated gas separation, modified as follows: the ruminal samples were first centrifuged at 4,000 × *g* for 20 min to separate the liquid phase. For protein removal, 1 mL of supernatant was mixed with 200 μL of 25% metaphosphoric acid and further centrifuged at 20,000 × *g* for 15 min. One milliliter of supernatant was added to 200 μL (1% vol/vol) of 4-methylvaleric acid as internal standard and 1 μL of the mixture was then injected into a gas chromatograph [model 5890 Series II, equipped with a flame ionization detector (FID); Hewlett-Packard Co., Avondale, PA].

Table 2. In vitro experiment: effects of natural or synthetic vitamin E on rumen FA profile (% of total FA methyl esters)

		Vitamin E			
FA[1]	No vitamin	Natural	Synthetic	SEM	*P*-value
C14:0	0.58	0.60	0.61	0.01	0.051
C16:0	7.35[b]	7.62[a]	7.58[ab]	0.06	0.022
C18:0	25.04	24.31	25.13	0.24	0.060
*c*9 C18:1	3.97[b]	4.90[a]	4.08[b]	0.08	<0.001
*t*4 to *t*8 *C*18:1	0.58[b]	0.75[a]	0.63[b]	0.02	<0.001
*t*9 C18:1	0.25[b]	0.33[a]	0.25[b]	0.01	<0.001
*t*10 C18:1	13.02	14.36	13.31	0.36	0.052
*t*11 C18:1	1.23	1.21	1.36	0.05	0.13
*t*12 to *t*16 *C*18:1	1.16[b]	1.31[a]	1.16[b]	0.07	0.003
*c*9,*c*12 C18:2	25.10[a]	22.25[b]	24.28[a]	0.45	0.002
*t*10,*c*12 CLA	1.76[c]	2.11[b]	2.46[a]	0.09	<0.001
*c*9,*t*11 CLA	0.06[b]	0.09[ab]	0.16[a]	0.02	0.023
*c*9,*c*12,*c*15 C18:3	0.65	0.65	0.68	0.01	0.14
Total *t* C18:1	16.24[b]	17.96[a]	16.72[ab]	0.41	0.030
Total CLA	1.82[c]	2.20[b]	2.62[a]	0.10	<0.001
Total *t*10 FA	14.78[b]	16.46[a]	15.78[ab]	0.43	0.046
Total *t*11 FA	1.29	1.30	1.52	0.07	0.047
*t*10/*t*11 ratio	12.56[b]	13.60[a]	11.64[c]	0.77	0.24

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]*c* = *cis*; *t* = *trans*.

Calculations and Statistical Analysis

Sums of CLA, *trans* C18:1, and *trans*-10 and *trans*-11 isomers were calculated by adding the assayed isomers. Data from the in vitro experiment were analyzed using the following model:

$$\text{Variable} = \text{mean} + \text{series effect} + \text{treatment effect} + \text{residual error},$$

where treatment levels were no vitamin, natural vitamin E, and synthetic vitamin E. When the treatment effect was significant, a Tukey test was used for pairwise comparisons.

Data from the in vivo experiment collected from d 1 to d 7 (i.e., before vitamin E supplementation) were analyzed using a repeated measures procedure with SYSTAT (version 9; SPSS Inc., Chicago, IL), determining linear, quadratic, and cubic effects of time and interaction of sampling day by vitamin E supplementation (time × vitamin E). The model used was

$$\text{Variable} = \text{mean} + \text{sampling day effect} + \text{cow effect} + \text{residual error}.$$

Data from the in vivo experiment collected from d 7 to 13 (i.e., the last sampling day before the beginning of vitamin E supplementation and the samples following vitamin E supplementation) were analyzed using a repeated measures procedure with the following model:

$$\text{Variable} = \text{mean} + \text{sampling day effect} + \text{cow effect} + \text{vitamin E effect} + \text{residual error}.$$

Effects were declared significant at $P < 0.05$.

RESULTS

In Vitro Experiment

Compared with the control, natural vitamin E increased the percentages of C16:0, *cis*-9 C18:1, total *trans* C18:1, and some minor individual *trans* C18:1, total CLA, and *trans*-10,*cis*-12 CLA, but decreased the percentages of *cis*-9,*cis*-12 C18:2 (Table 2). Compared with the control, synthetic vitamin E increased both *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA. Compared with natural vitamin E, synthetic vitamin E resulted in lower *cis*-9 C18:1 and higher *cis*-9,*cis*-12 C18:2 and CLA proportions.

In Vivo Experiment

During the first week of the oil diet subperiod, milk yield was not affected over time (Table 3), but milk fat content decreased from 33.4 to 25.0 g/kg. Milk protein content varied quadratically but within a narrow range.

Table 3. In vivo experiment: effects of time from the beginning of oil incorporation to the diet and vitamin E supplementation on milk yield and composition

	wk 1						wk 2								
	d 1[1]	d 3	d 5	d 7		Effect[2]	d 9		d 11		d 13			Effect[2,3]	
Item	No vit. E[4]	No vit. E	No vit. E	No vit. E	SEM	Time	No vit. E	Vit. E	No vit. E	Vit. E	No vit. E	Vit. E	SEM	Time	Time × vit. E
Milk yield, kg/d	33.9	33.9	33.1	32.8	0.7		30.8	32.9	30.9	32.6	30.4	32.1	0.8		
Milk fat content, g/kg	33.4	28.2	24.2	25.0	2.3	L, Q	18.3	25.0	21.3	26.3	23.7	26.8	3.3	Q, C	Q
Milk protein content, g/kg	34.1	34.0	34.0	34.5	0.6	Q	35.5	35.5	37.0	35.1	37.4	35.4	0.6	L	

[1]First day = switch from control to oil diet.
[2]L = linear effect; Q = quadratic effect; C = cubic effect.
[3]Repeated measures analysis from d 7 to 13; no significant effect of vitamin E was observed.
[4]No vitamin (vit.) E addition. Vitamin E was added from d 8 (30 g of 37% RRR-α-tocopherol acetate).

During the second week of the oil diet subperiod, milk yield was not affected by time and no time × vitamin E interaction was detected. Milk fat content increased with quadratic and cubic components, and a quadratic time × vitamin E interaction was linked to a decrease in milk fat content at d 9 in cows that did not receive supplemental vitamin E. Milk protein content slightly increased over time and no time × vitamin E interaction was observed.

During wk 1 of the oil diet subperiod, the proportions of FA containing less than 18 carbons decreased in milk fat (Table 4). For C18 FA, the C18:0 proportion slightly increased and the proportion of *cis*-9,*cis*-12 C18:2, which is the main FA of sunflower oil, quadratically decreased over time, as did the proportion of total CLA, mainly due to a decrease in *trans*-10,*cis*-12 CLA. On the contrary, the proportion of total *trans* C18:1 FA increased over time, due to a more than 2-fold increase in *trans*-10 C18:1.

During wk 2 of the oil diet subperiod, time effects on FA with less than 18 carbons were much more limited: decreases in proportions were in a narrower range than during wk 1, and were not observed for C14:0 and C16:0. On the contrary, the proportion of this latter FA increased over time, but remained far lower than that observed at the beginning of the oil diet subperiod. Quadratic time × vitamin E interaction for even-chain saturated C4:0 to C12:0 FA reflected values that increased over time with vitamin E supplementation and were almost steady in the unsupplemented cows. The proportion of *cis*-9,*cis*-12 C18:2 was fairly constant with vitamin E addition and followed a quadratic pattern, with a maximal value at d 9 in unsupplemented cows. Total CLA were not affected by time or time × vitamin E interaction, but the proportion of *trans*-10,*cis*-12 CLA was affected by a time × vitamin E interaction, resulting in a 40% lower proportion in supplemented than in unsupplemented cows from d 9. *Trans*-10 C18:1 was also affected by a time × vitamin E interaction, but average values during the supplementation period were similar in supplemented and unsupplemented cows. During this second week, C18:0 proportion was not affected over time. The time × vitamin E interaction had a significant effect with a trend ($P = 0.064$) toward a quadratic effect.

In the rumen, pH and VFA concentrations were not affected by time during the 2 wk of oil supplementation, and were not affected by time × vitamin E interaction (Table 5). The proportions of acetate and isovalerate decreased during wk 1 and increased during wk 2, independently of vitamin E supplementation, whereas the proportion of valerate followed an opposite pattern.

The ruminal proportions of FA with less than 18 carbons were only slightly affected by time (Table 6): even-

Table 4. In vivo experiment: effects of time from the beginning of oil incorporation to the diet and vitamin E supplementation on milk FA profile (% of total FA methyl esters)

	wk 1						wk 2								
	d 1[2]	d 3	d 5	d 7		Effect[3]	d 9		d 11		d 13			Effect[3,4]	
FA[1]	No vit. E[5]	No vit. E	No vit. E	No vit. E	SEM	Time	No vit. E	Vit. E	No vit. E	Vit. E	No vit. E	Vit. E	SEM	Time	Time × vit. E
C4:0 to C12:0 even FA	9.91	7.56	6.95	6.32	0.41	L, Q	5.08	5.66	4.68	6.03	4.80	6.12	0.32	Q	
C9 to C17 odd FA	3.71	3.12	2.78	2.72	0.07	L, Q	2.70	2.61	2.61	2.62	2.54	2.75	0.06		
C14:0	10.50	9.05	8.18	7.89	0.34	L, Q	7.24	7.78	7.48	7.78	7.23	8.07	0.22		
C16:0	25.43	21.51	19.71	18.53	0.53	L, Q, C	19.29	20.10	20.49	20.34	20.55	20.42	0.78	L	
C18:0	6.67	8.04	8.48	8.28	0.50	L, Q	7.39	8.38	8.22	8.11	9.85	7.98	0.57		G
*c*9 C18:1[5]	16.70	18.23	18.82	17.83	0.52	L, Q	18.17	19.51	19.41	19.14	22.07	18.66	1.06	G	Q
*t*4 to *t*8 *C*18:1[5]	0.60	0.69	0.68	0.65	0.06		0.66	0.59	0.74	0.54	0.74	0.59	0.10		
*t*9 C18:1	0.32	0.40	0.46	0.49	0.06		0.38	0.48	0.43	0.34	0.40	0.40	0.09		
*t*10 C18:1	9.50	14.99	17.22	20.58	0.69	L, C	20.61	17.88	17.98	17.74	14.77	17.92	1.17	L	Q, C
*t*11 C18:1	2.43	2.25	2.13	1.96	0.25		1.91	1.95	1.80	2.05	1.76	2.00	0.17		
*t*12 to *t*16 C18:1	1.13	1.22	1.14	1.22	0.08		1.32	1.11	1.29	1.14	1.35	1.07	0.16		
*c*9,*c*12 C18:2	2.79	2.39	2.41	2.44	0.16	Q	2.80	2.54	2.57	2.42	2.32	2.32	0.09	Q, C	Q
*t*10,*c*12 CLA	0.26	0.09	0.07	0.07	0.03	L, Q, C	0.10	0.05	0.08	0.05	0.08	0.05	0.01		Q, C
*c*9,*t*11 CLA	0.88	0.94	0.92	0.76	0.08		0.89	0.88	0.87	0.82	0.82	0.80	0.05		
*c*9,*c*12,*c*15 C18:3	0.23	0.19	0.19	0.21	0.01	Q	0.23	0.22	0.22	0.20	0.19	0.19	0.01	Q	
Total *t C*18:1	13.98	19.55	21.59	24.89	0.88	L, C	24.88	22.01	22.23	21.79	19.02	21.98	1.34	L	Q, C
Total CLA	1.23	1.11	1.07	0.91	0.09	L	1.08	1.01	1.04	0.95	0.97	0.93	0.06	G	
Total *t*10 FA	9.76	15.08	17.30	20.64	0.70	L, C	20.71	17.93	18.06	17.79	14.85	17.96	1.18	L	Q, C
Total *t*11 FA	3.40	3.27	3.13	2.80	0.31		2.89	2.91	2.75	2.95	2.66	2.88	0.20		
*t*10/*t*11 ratio	3.86	5.03	5.78	7.36	0.39	L	7.41	6.63	6.58	5.91	5.41	5.96	0.31	L, Q, C	

[1]*c* = *cis*; *t* = *trans*.

[2]First day = switch from control to oil diet.

[3]G = global effect without significance of linear; quadratic and cubic effects; L = linear effect; Q = quadratic effect; C = cubic effect.

[4]Repeated measures analysis from d 7 to 13; the effect of vitamin E was significant only for the *trans*10/*trans*11 ratio.

[5]No vitamin (vit.) E addition. Vitamin E was added from d 8 (30 g of 37% RRR-α-tocopherol acetate).

Table 5. In vivo experiment: effects of time from the beginning of oil incorporation to the diet and vitamin E supplementation on rumen fermentation parameters

	wk 1						wk 2							
	d 1[1]	d 3	d 5	d 7		Effect[2]	d 9		d 11		d 13			Effect[2,3]
Item	No vit. E[4]	No vit. E	No vit. E	No vit. E	SEM	Time	No vit. E	Vit. E	No vit. E	Vit. E	No vit. E	Vit. E	SEM	Time
pH	5.79	5.85	5.66	5.82	0.11		5.72	5.77	5.79	5.76	5.77	5.74	0.11	
Total VFA, m*M*	116	107	118	116	6		117	108	115	95	102	99	7	
Individual VFA, % of total VFA														
Acetate	52.9	51.9	50.1	48.5	1.4	L	48.3	48.9	47.6	50.3	55.4	54.2	1.6	L
Propionate	32.0	33.5	34.9	34.0	1.8		33.3	34.0	36.7	32.1	28.2	29.4	2.1	
Isobutyrate	0.69	0.64	0.62	0.65	0.05		0.64	0.63	0.70	0.84	1.18	1.00	0.09	L
Butyrate	9.54	8.92	9.38	10.82	0.65	Q	12.10	10.59	9.59	11.27	10.58	10.63	0.72	
Isovalerate	1.38	1.36	1.22	1.30	0.17	L	1.27	1.14	1.24	1.27	1.99	1.68	0.17	Q
Valerate	3.41	3.65	3.80	4.72	0.31	L	4.43	4.70	4.12	4.20	2.60	3.10	0.42	L, Q

[1]First day = switch from control to oil diet.
[2]L = linear effect; Q = quadratic effect; C = cubic effect.
[3]Repeated measures analysis from d 7 to 13; no significant effects of vitamin E or time × vitamin E interaction were observed.
[4]No vitamin (vit.) E addition. Vitamin E was added from d 8 (30 g of 37% RRR-α-tocopherol acetate).

and odd-chain FA with less than 18 carbons decreased at the beginning of the first week and then remained stable. During the second week, C14:0 increased over time, and vitamin E supplementation resulted in an increase of odd-chain FA over time. The proportions of *cis*-9 C18:1, *cis*-9,*cis*-12 C18:2, and *cis*-9,*cis*-12,*cis*-15 C18:3 in the rumen content were not affected by time or time × vitamin interaction. Total CLA decreased during wk 2, but with high variability. Among CLA isomers, *trans*-10,*cis*-12 CLA remained fairly constant over time and did not depend on vitamin E supplementation, and *cis*-9,*trans*-11 CLA strongly increased from 0.14 to 0.51% of total FA during wk 1, and decreased to 0.03% during wk 2, independently of vitamin E supplementation. *Trans*-10 C18:1 was the major *trans* isomer and increased during wk 1 and decreased during wk 2. A time × vitamin E interaction with a trend toward a linear effect ($P = 0.057$) was observed during wk 2, the proportions of *trans*-10 C18:1 being fairly constant in vitamin-supplemented cows, but decreasing in unsupplemented cows. The proportion of C18:0 also was stable at around 27% during wk 2 in vitamin-supplemented cows, but increased from 22 to 31% between d 9 and 13 in unsupplemented cows.

DISCUSSION

Effects of Oil Addition

Our dietary management resulted in a very rapid and important *trans*-10 shift, as ruminal proportions of *trans*-10,*cis*-12 CLA and *trans*-10 C18:1 were 2.2 and 5.8 times greater than those of *cis*-9,*trans*-11 CLA and *trans*-11 C18:1, respectively, as early as d 1 of the oil diet subperiod. The *trans*-10/*trans*-11 ratio reached around 10 in the rumen and 6 in milk from d 3. Our *trans*-10,*cis* 12-CLA proportions were very high compared with the literature data. In a recent meta-analysis, Glasser et al. (2008) reported that this isomer ranged from 0.0 to 6.6% of total duodenal C18:2 in their data set, whereas it represented 6.1 to 12.5% of total ruminal C18:2 in our experiment. Our kinetics data of milk FA also contrast from those of Roy et al. (2006) who, using a 72% concentrate diet based on corn silage and adding sunflower oil, observed *trans*-10/*trans*-11 ratios around 0.6 before oil supplementation and during wk 1 of the oil diet subperiod, and around 5 after 10 d of oil feeding. The difference between studies could be due to the nature of concentrate: we used wheat, a highly fermentable starch, whereas Roy et al. (2006) used corn grain, a slowly fermentable starch. Accordingly, Jurjanz et al. (2004) observed higher milk *trans*-10 FA content when cows received wheat than when they received potatoes (another slowly ferment-

Table 6. In vivo experiment: effects of time from the beginning of oil incorporation to the diet and vitamin E supplementation on rumen FA profile (% of total FA methyl esters)

	wk 1						wk 2								
	d 1[2]	d 3	d 5	d 7		Effect[3]	d 9		d 11		d 13			Effect[3,4]	
FA[1]	No vit. E[5]	No vit. E	No vit. E	No vit. E	SEM	Time	No vit. E	Vit. E	No vit. E	Vit. E	No vit. E	Vit. E	SEM	Time	Time × vit. E
C6:0 to C12:0 even FA	1.74	1.50	1.21	1.48	0.17	Q, C	1.44	1.20	1.44	1.48	1.48	1.49	0.16		
C7 to C17 odd FA	1.65	1.41	1.34	1.47	0.08	Q	1.34	1.31	1.31	1.51	1.32	1.43	0.06		L
C14:0	0.86	0.91	0.88	0.87	0.06		0.89	0.95	0.99	0.94	1.04	1.03	0.06	L	
C16:0	8.74	8.15	8.33	8.21	0.24	C	8.32	8.34	8.28	8.33	8.29	8.38	0.30		
C18:0	22.59	26.47	25.45	24.01	2.45		22.24	27.24	28.59	26.57	31.32	26.70	2.57		L
*c*9-C18:1	5.67	4.63	5.05	4.34	0.49	G	5.33	4.70	4.88	4.03	4.85	4.19	0.58		
*t*4 to *t*8 *C*18:1	1.10	0.82	0.79	0.71	0.13	L	0.88	0.72	0.91	0.65	0.95	0.70	0.18		
*t*9-C18:1	0.65	0.45	0.54	0.39	0.13	C	0.72	0.24	0.44	0.48	0.35	0.42	0.09		Q
*t*10 C18:1	23.37	23.43	27.23	27.16	2.18	L, C	29.03	25.05	23.31	24.29	21.79	24.04	2.75	L	G
*t*11 C18:1	4.04	3.07	3.11	2.66	0.50		2.79	2.83	2.44	2.65	2.11	2.81	0.45		
*t*12 to *t*16 *C*18:1	1.74	1.52	1.80	1.41	0.21	C	1.58	1.40	1.70	1.30	1.60	1.28	0.23		
*c*9,*c*12 C18:2	2.62	2.91	2.89	3.00	0.41		3.97	3.48	3.09	2.94	3.11	3.69	0.79		
*t*10,*c*12 CLA	0.31	0.31	0.47	0.34	0.11		0.29	0.26	0.34	0.26	0.27	0.28	0.10		
*c*9,*t*11 CLA	0.14	0.39	0.41	0.51	0.12	L	0.10	0.52	0.01	0.03	0.02	0.04	0.17	L	
*c*9,*c*12,*c*15 C18:3	0.23	0.22	0.20	0.22	0.02		0.21	0.20	0.22	0.23	0.18	0.22	0.02		
Total *t* C18:1	30.90	29.29	33.47	32.31	2.42	C	35.00	30.24	28.81	29.36	26.80	29.25	3.07	L	L
Total CLA	0.55	0.84	1.02	0.98	0.21		0.49	0.97	0.43	0.35	0.37	0.38	0.20	L	
Total *t*10 FA	23.68	23.74	27.69	27.50	2.22	L, C	29.32	25.31	23.65	24.55	22.05	24.32	2.73	L	L
Total *t*11 FA	4.28	3.59	3.66	3.30	0.51		2.99	3.54	2.53	2.73	2.22	2.91	0.57	L	
*t*10/*t*11 ratio	8.18	8.68	9.16	11.10	1.26		11.19	11.18	9.63	9.92	9.87	9.84	0.99	C	

[1]*c* = *cis*; *t* = *trans*.

[2]First day = switch from control to oil diet.

[3]G = global effect without significance of linear, quadratic, and cubic effects; L = linear effect; Q = quadratic effect; C = cubic effect.

[4]Repeated measures analysis from d 7 to 13; no significant effect of vitamin E was observed.

[5]No vitamin (vit.) E addition. Vitamin E was added from d 8 (30 g of 37% RRR-α-tocopherol acetate).

able starch). Indeed, the quick ruminal degradation of wheat starch both leads to a large amount of starch degraded in the rumen and a big pH decrease after meals, compared with corn or potato starches. This can explain that corn or potatoes result in a lower *trans*-10/*trans*-11 ratio than wheat, as both decreasing dietary starch (Kucuk et al., 2001) and adding ruminal buffers to the diet (Troegeler-Meynadier et al., 2007) decrease *trans*-10 FA in the rumen.

Milk fat content linearly and quadratically decreased during wk 1 of the oil diet subperiod, which is in line with present knowledge regarding the relationship between *trans*-10,*cis*-12 CLA and milk fat content (Baumgard et al., 2000). Nevertheless, in spite of high values of *trans*-10,*cis*-12 CLA in milk and rumen at d 1, milk fat content decrease was delayed. This is in agreement with the study of Perfield et al. (2004): using ruminal protected CLA, these authors observed a progressive decrease of milk fat content that became stable after 4 d of supplementation, but they published the FA composition of milk after 6 and 7 d of supplementation, preventing any kinetics approach of the relationship between milk *trans*-10,*cis*-12 CLA and milk fat content. In our experiment, at d 1, milk *trans*-10,*cis*-12 CLA exhibited a very high value compared with subsequent days, whereas rumen *trans*-10,*cis*-12 CLA was similar to values observed during the following days. This decrease in *trans*-10,*cis*-12 CLA in milk in spite of a constant ruminal proportion could suggest an evolution of the transfer of this FA from the digestive tract to milk fat. Consistent with our much lower proportion of *trans*-10,*cis*-12 CLA in milk fat than in the rumen from d 3, Chouinard et al. (1999) already demonstrated a low transfer of *trans*-10,*cis*-12 CLA from the digestive tract to milk, but their measures were performed after 5 d of abomasal infusion, which does not preclude a time evolution at the beginning of infusions. Such a decrease over time of *trans*-10,*cis*-12 CLA transfer from the digestive tract to milk could be explained by an effect of this isomer on milk transfer of FA with more than 16 carbons, as enzymes implicated in their mammary uptake are inhibited by *trans*-10,*cis*-12 CLA (Peterson et al., 2003). As for milk fat depression, reduction of FA uptake could have been delayed in the present study, underlying a time-dependent adaptation of mammary metabolism to a high ruminal production of *trans*-10,*cis*-12 CLA.

Beside this rapid change of *trans* FA, time effects were observed on other milk FA. The percentages of short- and medium-chain FA decreased during wk 1 of the oil diet subperiod, which is consistent with the literature relative to the effects of fat supplementation (Glasser et al., 2007). Some odd- and branched-chain VFA and FA in the rumen and milk fat also decreased during wk 1, which suggests modifications of microbial populations (Vlaeminck et al., 2006).

Effects of Vitamin E Addition

In vitro, both vitamin E forms increased the proportions of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, synthetic vitamin E resulting in larger increases than the natural form, but in a higher proportion of *cis*-9,*cis*-12 C18:2 than the natural vitamin E, which suggests that the differences in CLA percentages were not due to differences in isomerization efficiency. Similarly, the proportion of total *trans*-10 C18:1 was increased by natural vitamin E, but, whereas statistically significant, the change was within a narrow range. Although not completely similar, the effects of natural and synthetic vitamin E were very limited, suggesting that vitamin E was neither a limiting factor for rumen BH nor a modulator of BH pathway.

Vitamin E supplementation did not affect fermentation parameters in vivo, which contrasts with the results of Naziroğlu et al. (2002), who observed in vitro that vitamin E addition increased the concentrations of acetate and propionate, but decreased that of butyrate. Hino et al. (1993) demonstrated that vitamin E addition partly alleviates the depression of growth and fibrolytic activity of rumen bacteria in incubates supplemented with safflower oil. Such an effect could be expected to affect rumen VFA production. In our experiment, we only measured rumen VFA concentrations. They were not clearly depressed by oil addition, which could explain the lack of effect of vitamin E.

As a whole, effects of vitamin E on FA profiles were limited. Vitamin E did not affect the rumen proportions of unsaturated dietary FA and most BH intermediates, which is consistent with the results of Chikunya et al. (2004), who demonstrated that vitamin E supplementation of the sheep diet did not affect the efficiency of rumen BH and the proportion of *trans* C18:1 among FA. Some time × vitamin E interactions slightly affected FA profiles: during wk 2 of the oil diet subperiod in the in vivo experiment, vitamin E addition resulted in fairly steady percentages of rumen and milk C18:0, total *trans* C18:1 and *trans*-10 C18:1, whereas without vitamin E supplementation, the C18:0 percentage increased and the *trans* C18:1 percentage decreased over time. These different patterns could suggest that vitamin E resulted in a more rapid adaptation of the reduction of *trans* C18:1 FA to C18:0 with a high-oil diet. The implication of vitamin E in the reduction of *trans* C18:1 to C18:0 has not been studied, contrary to the known implication of vitamin E in the reduction of CLA to *trans* C18:1 (Hughes et al., 1982). In this latter reaction, α-tocopherolquinol acts as an electron donor

during the reduction, but, as far as we are aware, no quantitative recommendation of vitamin E supply for cows related to this function has been proposed. In our study, vitamin E supplementation did not affect the ratio of *trans* C18:1 to CLA, which could be due to the 300 mg of vitamin E supplied by the standard commercial premix to all cows being sufficient to sustain this reduction. The more stable proportion of *trans*-10 C18:1 observed in milk fat in vitamin E-supplemented cows was due to a decrease in this proportion at d 9 (i.e., 1 d after the beginning of vitamin E supplementation). However, the same decrease was observed in the rumen at d 7 in cows that began to receive vitamin E at d 8 (results not shown), which makes a true relationship between vitamin E supplementation and this stabilization questionable.

The *trans*-10,*cis*-12 CLA proportion was lowered by vitamin E supplementation in milk fat, but not in rumen content. This suggests that vitamin E could have modified the transfer efficiency of *trans*10,*cis*12 CLA isomer from the rumen to the mammary gland, which could create discrepancies between digestive and metabolic effects of vitamin E. Previous experiments regarding the effects of vitamin E on the *trans*-10 shift of BH only addressed FA composition of tissues or milk and milk fat content, and also showed limited effects of vitamin E supplementation. In steers receiving high-grain diets, Juárez et al. (2010) observed a reduction in the *trans*-10/*trans*-11 ratio in the backfat when steers fed a high-barley diet received supplemental vitamin E, but the same authors observed no effect of vitamin E on *trans*-10 C18:1 in intramuscular fat when steers received flax as a source of PUFA (Juárez et al., 2011). In dairy cows, vitamin E supplementation resulted in an increase in *trans*-10 FA (Bell et al., 2006) or no effect (Ferlay et al., 2010), but with a *trans*-10/*trans*-11 ratio that was far under 1, which strongly differs from our experimental conditions. In dairy cows, Pottier et al. (2006) observed a decrease in this ratio when vitamin E was supplemented from the beginning of linseed oil addition, but did not observe this effect when vitamin E was provided 3 wk after oil supplementation. Similarly, in our experiment, vitamin E was supplemented after the *trans*-10 shift, so our lack of effects is in line with the results of Pottier et al. (2006).

CONCLUSIONS

Vitamin E supplementation of dairy cows exhibiting a *trans*-10 shift of ruminal BH due to addition of oil supplementation to a high wheat diet did not result in a reversal of BH pathway toward *trans*-11 isomers production, which precluded any conclusion regarding differential effects of natural and synthetic vitamin E.

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