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Production of *trans* C18:1 and conjugated linoleic acid in continuous culture fermenters fed diets containing fish oil and sunflower oil with decreasing levels of forage

A. AbuGhazaleh[†] and B. N. Jacobson

Southern Illinois University Carbondale, IL 62901, USA

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Previously, feeding fish oil (FO) and sunflower seeds to dairy cows resulted in the greatest increases in the concentrations of vaccenic acid (VA, t11 C18:1) and conjugated linoleic acid (CLA) in milk fat. The objective of this study was to evaluate the effects of forage level in diets containing FO and sunflower oil (SFO) on the production of trans *C18:1 and CLA by mixed ruminal microbes. A dual-flow continuous culture system consisting of three fermenters was used in a 3 × 3 Latin-square design. Treatments consisted of (1) 75:25 forage:concentrate (HF); (2) 50:50 forage:concentrate (MF); and (3) 25:75 forage:concentrate (LF). FO and SFO were added to each diet at 1 and 2 g/100 g dry matter (DM), respectively. The forage source was alfalfa pellets. During 10-day incubations, fermenters were fed treatment diets three times daily (140 g/day, divided equally between three feedings) as TMR diet. Effluents from the last 3 days of incubation were collected and composited for analysis. The concentration of trans C18:1 (17.20, 26.60, and 36.08 mg/g DM overflow for HF, MF, and LF treatments, respectively) increased while CLA (2.53, 2.35, and 0.81 mg/g DM overflow) decreased in a linear manner (*$P < 0.05$*) as dietary forage level decreased. As dietary forage levels decreased, the concentrations of* t10 C18:1 *(0.0, 10.5, 33.5 mg/g DM) in effluent increased (*$P < 0.05$*) and* t10c12 CLA *(0.08, 0.12, 0.35 mg/g DM) tended to increases (*$P < 0.09$*) linearly. The concentrations of VA (14.7, 13.9, 0.0 mg/g DM) and c9t11 CLA (1.78, 1.52, 0.03 mg/g DM) in effluent decreased in a linear manner (*$P < 0.05$*) as dietary forage levels decreased. Decreasing dietary forage levels resulted in t10 C18:1 and* t10c12 CLA *replacing VA and* **c**9t11 CLA*, respectively, in fermenters fed FO and SFO.*

Keywords: conjugated linoleic acid, fish oil, forage level, sunflower oil, *trans* C18:1

Introduction

The different positional and geometric isomers of conjugated linoleic acid (CLA) confer different health effects on mammals (Belury, 2002). The *c*9*t*11 CLA isomer has been shown to be anti-carcinogenic (Ip *et al*., 1999), while the *t*10*c*12 CLA isomer has been shown capable of decreasing body fat and increasing lean body mass (Park *et al*., 1999). The *t*10*c*12 CLA isomer also decreases fat concentration in dairy cows' milk in a dose-dependent fashion (Peterson *et al*., 2002). These effects on mammals have encouraged research efforts to identify methods of increasing the *c*9*t*11 CLA isomer and to understand the mechanisms of *t*10*c*12 CLA production so that its synthesis would occur only when desired.

Conjugated linoleic acid and *trans* C18:1 fatty acids (FA) are produced during biohydrogenation of unsaturated FA in the rumen (AbuGhazaleh *et al*., 2005; Harfoot & Hazlewood, 1988) and are subsequently incorporated into milk and meat of ruminant animals. Formation of *trans* C18:1 and CLA in the rumen are influenced by dietary supplementation with unsaturated vegetable oils (AbuGhazaleh *et al*., 2003; Loor *et al*., 2004a and b) or changes in rumen pH as a result of alterations in dietary forage to concentrate ratios (F:C) (Piperova *et al*., 2002; Loor *et al*., 2003; Sackmann *et al*., 2003).

Previous studies have demonstrated an increase in the vaccenic acid (VA) and *c*9*t*11 CLA content of bovine milk fat when fish oil (FO) and linoleic acid oil sources were added to dairy cattle rations containing 50% forage (AbuGhazaleh *et al*., 2000; Whitlock *et al*., 2002). Studies examining the effect of forage or concentrate feeding on the distribution of *trans* C18:1 and CLA isomers in the rumen (Loor *et al*., 2004a) or ruminant fat (Sackmann *et al*., 2003; Aharoni *et al*., 2004; Loor *et al*., 2005) have used only vegetable oils or FO. Feeding high concentrate diets (Piperova *et al*., 2002) or low

[†] E-mail: aabugha@siu.edu

forage diets supplemented with vegetable oils rich in linoleic acid or FO (Loor *et al*., 2004a) altered ruminal biohydrogenation resulting in *t*10 rather than VA being the predominant *trans* C18:1 intermediate. Alterations in the profile of *trans* C18:1 intermediates formed in the rumen have direct consequences on the supply of VA available for endogenous conversion in the mammary gland into *c*9*t*11 CLA (Bauman *et al*., 1999). Previously, AbuGhazaleh *et al.* (2000, 2003) and Whitlock *et al.* (2002) demonstrated that the greatest concentrations of VA and *c*9*t*11 CLA in milk fat and rumen contents can be obtained by adding FO along with linoleic acid fat source to ruminant animals' diet. The aim of this study was to investigate how changing dietary forage levels in diets containing FO and sunflower oil (SFO) affect *trans* C18:1 and CLA isomers distribution in the rumen.

Material and methods

Experiment protocol

Three 1700 ± 12 ml dual-flow continuous culture fermenters (Stern & Hoover, 1990) were used in a 3 × 3 Latin square over three periods of 10 days each. Each experimental period consisted of 7 days for adaptation and followed by 3 days for sample collection. Treatments used in this study were as follows: (1) 75:25 F:C (HF); (2) 50:50 F:C (MF); and (3) 25:75 F:C (LF). Menhaden FO (Omega Protein Inc., Hammond, LA) and SFO (purchased from a local store) were added to each diet at 1 and 2 g/100 g dry matter (DM) basis, respectively. Maize, soya-bean meal, limestone, vitamins and minerals made up the concentrate mix (Table 1). The forage source was alfalfa pellets. A total of 140 g of feed (DM basis) was placed in each fermenter daily in three equal portions at 0800, 1500, and 2100 h.

Continuous culture

Whole ruminal contents were taken from two ruminally fisulated Holstein cows fed a 50:50 F:C diet. At each collection time, approximately 4.5 kg of ruminal content were taken from the cow 4 h after feeding, strained through two layers of cheesecloth and transported to the laboratory in a sealed container and used within 20 min. Fermenter canisters (15 cm long and 12.5 cm wide) were filled with approximately 1300 ml of rumen fluid and 400 ml of prewarmed buffer with urea added (Weller & Pilgrim, 1974). Solids and liquid dilution rates were adjusted, twice daily, to values of 3 and 10% per h, respectively, by regulation of buffer input and filtrate removal rates. Fermenters were constantly mixed at 120 r.p.m. via a magnetic impeller stirrer unit, purged with N^2 gas (80 ml/min) and temperature was maintained at 39°C. The pH was measured daily at 0800, 1500, and 2100 h using a portable pH meter (Accumet* AP85 Portable, Fisher Scientific, Pittsburgh. PA).

Table 1 *Ingredient and chemical composition of treatment diets*

	Treatments[†]			
	HF	MF	LF	s.d.
Ingredients (g/100 g DM)				
Alfalfa	75.00	50.00	25.00	
Maize	19.70	38.70	57.70	
Soya-bean meal, 48%	2.00	8.00	14.00	
Sunflower oil	2.00	2.00	2.00	
Fish oil	1.00	1.00	1.00	
Vitamin and mineral mix	0.30	0.30	0.30	
Chemical composition (g/100 g DM)				
Crude protein	17.82	17.74	17.63	0.13
Neutral-detergent fibre	34.78	26.05	17.38	0.32
Acid-detergent fibre	22.77	16.33	9.88	0.29
Ether extract	6.04	6.43	6.82	0.16
Ash	6.29	5.40	4.70	0.14
Total fatty acids	3.77	3.93	4.25	0.10

[†] HF = 75:25 F:C, MF = 50:50 F:C, and LF = 25:75 F:C.

Sample collection and analysis

Effluent from each fermenter was collected into 5-l plastic jugs submerged approximately three-quarters into a 4°C water bath. The solid and liquid effluent volumes were cataloged daily before the morning feeding and discarded until the final 3 days of each period. On the last 3 days, the solid and liquid portions were combined, homogenised on a stir plate and a 10% volume subsample was collected and stored at −5°C. Subsamples from each fermenter were composited for days 8, 9 and 10 resulting in one sample per fermenter per period. Samples were thawed in a 50°C waterbath, transferred into 250 ml plastic bottles (10 cm long and 5 cm wide) and centrifuged (Beckman J2-21) at 15 000 r.p.m. for 15 min, after which the supernatant was removed. This process was repeated until all liquid in the thawed sample was removed. The bottle and fiber pellet was stored at −80°C for 48 h, freeze dried and then ground to 1 mm using a Willey mill (Arthur Thomas Company, Philadelphia, PA).

Samples of alfalfa pellets and concentrate mixes were collected twice each period (days 5 and 10) and stored at −20 °C until analysis. Samples were freeze dried for at least 48 h, then ground through a 2-mm screen of a standard Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA) and composited by period. Composites were analysed for crude protein (CP), ether extract, and ash according to Association of Official Analytical Chemists methods (1997). Samples were reground (Brinkman ultracentrifuge mill) through a 1-mm screen and analysed for neutral-detergent fibre (NDF, procedure B of Van Soest *et al.* (1991)) and acid detergent fibre (ADF, Robertson and Van Soest, 1981) using an ANKOM fiber analyser and filter bag technique (ANKOM Technology Corp., Fairport, NY).

Feed and effluent samples were methylated using $NaOCH_3$ and HCl two steps procedure as outlined by Kramer *et al.* (1997) and analysed in triplicate for FA on a Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, Maryland, USA) equipped with a flame ionisation detector and a Supelco 100-m SP-2560 fused silica capillary column (0.25 mm i.d. × 0.2 μm film thickness; Supelco Inc., Bellefonte, PA). The helium carrier

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gas was maintained at a linear velocity of 23 cm/s. The oven temperature was programmed at 170°C for 50 min, then increased at 5°C/min to 249°C and held for 10 min. The injector and detector temperatures were set at 255°C. One milligram of nonadecanoic acid (C19:0; 1 mg/ml benzene) was added to all samples before methylation as an internal standard. Peaks were identified by comparing the retention times with those of corresponding standards (Nu-Chek-Prep., Elysian, MN; Supelco, Bellefonte, PA; and Larodan Fine Chemicals, Malmo, Sweden). The *trans* C18:1 isomers that were not available commercially (*trans*-6/8, *trans*-10, *trans*-12, *trans*-13/14) were identified according to the elution sequence reported by Loor *et al.* (2004b). Conjugated linoleic acid isomers were identified according to the elution sequence reported by Roach *et al.* (2002) using Nu-Check-Prep #UC59mx (Elysian, MN; Supelco). Retention times for *c*9*t*11, *t*10*c*12, and *t*9*t*11 were confirmed with pure standards (Matreya LLC, Pleasant Gap, PA).

A summary of dietary ingredients and chemical composition of the experimental diets is shown in Table 1. Dietary CP was similar across diets averaging 17.7 g/100 g DM. Dietary ADF and NDF decreased as dietary forage level decreased (Table 1). Daily input of total FA (g/day) was also affected by dietary treatments, increasing as dietary forage decreased. Supplies of oleic and linoleic acids increased while linolenic acid decreased as dietary forage level decreased (Table 2). All treatments had an equal supply of C18:0, C20:5, and C22:6.

Statistical analysis

Data were analysed using the general linear model procedure of (Statistical Analysis Systems Institue, Inc., Cary, NC) According to the following statistical model:

$$Y_{ijk} = \mu + F_i + D_j + P_k + e_{ijk}$$

where Y_{ijk} = the observation; μ = overall mean; F_i = fermenter effect; D_j = diet effect; P_k = period effect and e_{ij} = residual error associated with Y_{ijk}.

Table 2 *Fatty acids intake (g/day)*

	Treatments[†]		
	HF	MF	LF
Fatty acid			
C14:0	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
C16:0	0.63 ± 0.01	0.64 ± 0.02	0.66 ± 0.01
C18:0	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
C18:1 c9	0.76 ± 0.03	0.90 ± 0.04	1.14 ± 0.07
C18:2 n6	1.99 ± 0.05	2.26 ± 0.09	2.70 ± 0.13
C18:3 n3	0.48 ± 0.02	0.34 ± 0.02	0.21 ± 0.01
C20:5 n3	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
C22:5 n3	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
C22:6 n3	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
Total	5.29 ± 0.12	5.50 ± .14	5.95 ± 0.12

[†] HF = 75:25 F:C, MF = 50:50 F:C, and LF = 25:75 F:C.

The linear and quadratic effects of treatments were analysed by orthogonal contrasts. Least-square means are reported throughout and significance threshold was set at $P \leq 0.05$ and the trend at $P \leq 0.10$.

Results

As expected, fermenter pH was affected by dietary forage level (Figure 1), averaging 6.55, 6.15, and 5.65 for diet HF, MF and LF, respectively.

Effect of diet on effluent FA concentrations (mg/g of DM) is presented in Table 3. The concentration of *trans* C18:1 increased ($P < 0.03$) in a linear manner as dietary forage level decreased. Compared with HF, the concentration of *trans* C18:1 increased by 55 and 110% as dietary forage level decreased to 50 and 25%, respectively. Dietary treatments also affected the distributions of *trans* C18:1 isomers (Table 3). The concentration of VA decreased whereas the concentration of *t*10 C18:1 increased ($P < 0.01$) in a linear manner as dietary forage level decreased. Vaccenic acid and *t*10 C18:1 were the predominant *trans* C18:1 isomers in the HF and LF diets, respectively, accounting for 85 and 93% of total *trans* C18:1 isomers, respectively. The concentrations of *trans*-6/8, *trans*-9, *and trans*-12 was not affected ($P > 0.15$) by treatment diets (Table 3). *Trans-13/14* was detected only in the LF fermenter and was the second major *trans* C18:1 isomer.

Dietary treatments also affected effluent CLA concentrations and isomers distribution (Table 3). The concentration of CLA decreased linearly ($P = 0.03$) as dietary forage level decreased. The concentrations of *c*9*t*11 CLA decreased ($P < 0.02$), whereas the concentration of *t*10*c*12 CLA tended to increase ($P < 0.09$) in a linear manner as dietary forage decreased. *Cis*9 *t*11 and *tt* CLA were the major isomers in the HF diet accounting for 70 and 20% of total CLA, respectively, whereas *t*10*c*12 and *t*11*t*13 CLA were the predominate isomers in the LF diet accounting for 43 and 30% of total CLA (Table 3).

The effect of diet on C18 unsaturated FA biohydrogenation is presented in Table 4. As dietary forage decreased, biohydrogenation of linoleic and linolenic acids decreased

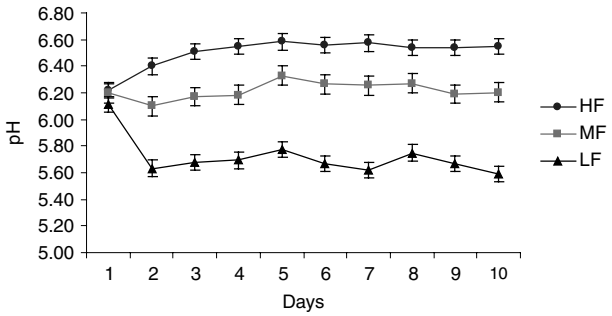

Figure 1 pH in continuous culture fermenters for HF = 75:25 F:C; MF = 50:50 F:C; and 25:75 F:C. Standard errors of the means were 0.06, 0.07, and 0.06, respectively.

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Table 3 *Effect of treatment diets on the outflow fatty acids (mg/g of dry matter)*

	Treatments[†]				Contrast[‡]	
	HF	MF	LF	s.e.	Lin	Quad
Fatty acid						
C14:0	1.27	1.39	1.31	0.12	0.80	0.53
C16:0	11.06	11.72	10.92	0.36	0.81	0.25
C18:0	12.53	6.98	4.95	0.57	0.05	0.14
C18:1 *trans*	17.20	26.60	36.08	2.47	0.03	0.90
*t*6/8	0.91	0.86	0.50	0.09	0.12	0.54
*t*9	0.66	0.37	0.00	0.16	0.11	0.90
*t*10	0.00	10.54	33.54	0.97	0.01	0.23
*t*11	14.65	13.89	0.00	0.84	0.01	0.17
*t*12	0.98	0.94	0.60	0.18	0.26	0.56
*t*13/14	0.00	0.00	1.44	0.06	0.01	0.01
C18:1 *c*9	8.08	8.99	13.30	0.53	0.02	0.12
C18:1 *c*11	0.89	1.24	1.43	0.13	0.10	0.69
C18:2 n6	7.14	9.48	18.81	1.05	0.02	0.15
C18:3 n3	1.77	1.21	1.64	0.13	0.62	0.14
CLA[§]	2.53	2.35	0.81	0.21	0.03	0.12
*c*9*t*11	1.78	1.52	0.03	0.19	0.02	0.12
*t*10*c*12	0.08	0.12	0.35	0.03	0.09	0.48
*c*9*c*11	0.03	0.07	0.00	0.02	0.34	0.09
*t*11*t*13	0.13	0.14	0.24	0.01	0.02	0.09
tt	0.51	0.50	0.20	0.06	0.06	0.19
C20:3 n3	0.07	0.09	0.19	0.19	0.26	0.61
C20:5 n3	0.73	0.87	1.01	0.10	0.21	0.98
C22:5 n3	0.43	0.54	0.59	0.01	0.01	0.19
C22:6 n3	0.85	1.10	1.28	0.14	0.18	0.85
Total fatty acids	81.05	89.69	110.58	2.13	0.01	0.14

[†] HF = 75:25 F:C, MF = 50:50 F:C, and LF = 25:75 F:C.
[‡] Linear and quadratic effects.
[§] CLA = conjugated linoleic acid.

linearly ($P < 0.03$). Biohydrogenation of oleic acid also tended to decrease linearly ($P = 0.06$) with decreasing forage proportion in the diet.

Discussion

Trans C18:1 in effluents accounted for 21, 30, and 33% of total FA for diet HF, MF, and LF, respectively (Table 3). The increase in concentration of *trans* C18:1 in LF (36.1 mg/g of effluent) compared with HF (17.20 mg/g) indicated that low forage diets promote *trans* C18:1 accumulation. The linear increase in *trans* C18:1 concentration as dietary forage level decreased may have resulted, in part from the higher unsaturated C18 FA input for MF and LF diets compared with HF diet (Table 2). Daily input for unsaturated C18 FA was higher by 8 and 25% for MF and LF diets compared with HF diet. Incomplete biohydrogenation of unsaturated C18 FA in the rumen results in *trans* C18:1 accumulation (Harfoot & Hazlewood, 1988). Additionally, high *trans* C18:1 accumulation observed in the LF diet may have resulted from inhibiting the reductase enzyme in ruminal micro-organisms responsible for the terminal hydrogenation of *trans* C18:1 to C18:0. The low concentration of C18:0 observed in LF effluent supports the suggestion that inhibition occurred at the final reductase step. The linear increase in *trans* C18:1 concentration indicates that low ruminal pH favours *trans* C18:1 accumulation. Low ruminal pH, caused by a high-concentrate diet, increased the accumulation of *trans* C18:1 in other studies (Karlscheur et al., 1997; Piperova *et al*., 2002; Loor *et al*., 2004b). Although this study and others (Karlscheur et al., 1997; Piperova *et al*., 2002; Loor *et al*., 2004b) clearly show that low rumen pH promotes *trans* C18:1 accumulation, the mechanism is still unknown. The accumulation of *trans* C18:1 under low rumen pH conditions may be caused by altering the rumen ecosystem and/or inhibiting the reductase activity of ruminal microorganisms, causing the accumulation of *trans* C18:1. Low rumen pH has been shown to have a negative effect on microbial growth (Russell & Dombrowski, 1980; Martin *et al*., 2002), particularly on the growth of cellulolytic bacteria, the main rumen biohydrogenating bacteria, (Harfoot & Hazlewood, 1988).

Six *trans* C18:1 isomers were identified in effluents (Table 3). As dietary forage level decreased, VA concentration decreased, but *t*10 C18:1 concentration increased linearly. This shift indicates that incremental increases in concentrate level have the potential to enhance ruminal production of *t*10 C18:1. To our knowledge, no *in vivo* or *in vitro* experiment has evaluated the ruminal distribution of *trans* C18:1 isomers, primarily VA and *t*10 C18:1, in response to a graded decrease of dietary forage level at a constant level of supplemental FO and SFO. When cows were switched from a control diet (60:40 F:C) to a high-concentrate diet (25:75 F:C), the proportion of *t*10 C18:1 increased, concomitant with decreases in VA (Piperova *et al*., 2002). Additionally, *t*10 C18:1 replaced VA as the predominant *trans* C18:1 isomer in the rumen when high concentrate-low fibre diets were fed to cows (Griinari *et al*., 1998) and steers (Sackmann *et al*., 2003). Under such conditions, Bauman *et al.* (1999) proposed a putative pathway for the production of *t*10 C18:1 where the *t*10*c*12 CLA-producing bacteria become predominant in the rumen resulting in formation of *t*10*c*12 CLA as the first intermediate during linoleic acid biohydrogenation. Hydrogenation of the *c*12 bond would then result in formation of *t*10, analogous to the production of VA from *c*9*t*11 CLA. High concentrate diets have been shown to promote the growth

Table 4 *Effect of treatment diets on the biohydrogenation (%) of unsaturated fatty acid*

	Treatments[†]				Contrast[‡]	
	HF	MF	LF	s.e.	Lin	Quad
Fatty acid						
C18:1 *c*9	55.6	45.7	44.4	4.22	0.06	0.19
C18:2 n6	88.6	87.5	71.3	3.15	0.02	0.12
C18:3 n3	87.4	83.5	66.1	4.21	0.02	0.15

[†] HF = 75:25 F:C, MF = 50:50 F:C, and LF = 25:75 F:C.
[‡] Linear and quadratic effects.

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of *Megasphera elsdenii* YJ-4 which can convert linoleic acid to *t*10*c*12 CLA (Kim *et al*., 2002). Furthermore, feeding high grain diets to steers has been shown to stimulate the growth of the YE34 strain of *Megasphera elsdenii* and cause a rapid decline in *Butyrivibrio fibrisolvens* YE44 (Klieve *et al*., 2003) and its is well established that VA is an intermediate of linoleic acid metabolism by isolates of *Butyrivibrio fibrisolvens* (Harfoot & Hazlewood, 1988).

The *t*10 C18:1 could also arise via the isomerisation of oleic acid (AbuGhazaleh *et al*., 2005). The fact that *t*10 accounted for 93% of total *trans* C18:1 isomers with the LF diet compared with the 37% and 60% reported by Griinari *et al.* (1998) and Sackmann *et al.* (2003), when high-concentrate diets supplemented with linoleic acid oil source were fed, supports our previous finding that docosahexaenoic acid (C22:6; DHA) in FO blocks the final step in the biohydrogenation of unsaturated C18 FA causing *trans* C18:1 accumulation (AbuGhazaleh and Jenkins, 2004). What seems evident from this experiment and others (Griinari *et al*., 1998; Sackmann *et al*., 2003) is that a supply of high linoleic acid oil along with FO under low pH condition has the greatest potential to enhance *t*10 C18:1 production in the rumen. These conditions simultaneously eliminated or reduced concentration of *t*6/8, *t*9, and *t*12 C18:1 in effluent, but increased concentration of *t*13/14 C18:1. Such changes in effluent *trans* C18:1 isomers profile may suggest a possible alteration in the microbial ecosystem and/or enzyme activities as a result of altering the proportion of forage in the diet. The total absence of *t*10 and VA in effluents of HF and LF, respectively, should be taken with precaution since the separation of these two isomers can be difficult when their concentrations are very different. Indeed, a small VA shoulder peak was observed in some LF *t*10 peaks, however, the ratio of VA to *t*10 was consistently less than 1:30.

The effect of dietary treatments on CLA is presented in Table 3. Total concentrations of CLA were highest in the HF, intermediate in MF, and least in LF indicating that CLA formation is favoured by high ruminal pH. Troegeler-Meynadier *et al.* (2003) also reported more CLA accumulation in rumen cultures incubated at high than low pH. The predominate CLA isomer detected in the HF and LF were *c*9*t*11 CLA and *t*10*c*12 CLA, respectively. In ewes, duodenal flow of *t*10*c*12 CLA increased and *c*9*t*11 CLA decreased in response to graded increments of dietary concentrate with a constant level (7.4 g/100 g diet DM) of supplemental FA from soya-bean oil (Kucuk and Hess, 2004). Our results support the findings of others (Beaulieu *et al*., 2002; Duckett *et al*., 2002; Shingfield *et al*., 2005) that high concentrate diets support *t*10*c*12 CLA formation in the rumen. Replacing incremental portions of red clover with maize grain in dual-flow continuous cultures resulted in a linear increase in the output of *t*10*c*12 CLA into effluent (Latham *et al*., 1972). Shingfield *et al*., (2005) reported higher *c*9*t*11 CLA concentration in milk when cows were fed a high forage diet (65:35) than low forage diet (35:65) with FO and SFO as lipid supplements. These results indicates that incremental grain or concentrate have the potential to enhance ruminal production of *t*10*c*12 CLA. *Trans*10*c*12 CLA is a potent inhibitor of fat synthesis (Baumgard *et al*., 2002). What seems evident from this study and others (Beaulieu *et al*., 2002; Duckett *et al*., 2002; Kucuk and Hess, 2004) is that a high linoleic acid oil source along with low dietary F:C ratio has the greatest potential to increase *t*10*c*12 CLA. The *t*11*t*13 CLA was the second predominant isomer in the LF. A similar increase in *t*11*t*13 CLA concentration was also reported by Loor *et al*., (2004b) when linseed oil was added to a high-concentrate diet. The formation of these different CLA isomers in ruminal cultures provides comparative evidence for the existence of alternative pathways for the biohydrogenation of C18 polyunsaturated FA other than those established by Harfoot & Hazlewood (1988).

The biohydrogenation C18 unsaturated FA were affected by treatment diets (Table 4). The biohydrogenation of oleic, linoleic and linolenic acids was greater in HF and MF than LF diet. Wang *et al.* (2002) and Troegeler-Meynadier *et al.* (2003) also reported decreased biohydrogenation of linoleic and linolenic acids at low pH *in vitro.* Under *in vivo* conditions, Sackmann *et al.* (2003) and Kucuk and Hess (2004) have shown that higher dietary forage levels increase biohydrogenation of dietary C18 unsaturated FA. The decreased biohydrogenation of linoleic and linolenic acids with the LF diet suggested a lower biohydrogenation activity by cultures microbes. The low biohydrogenation values for oleic acid in this study compared with others (Duckett *et al*., 2002; Sackmann *et al*., 2003Kucuk and Hess, 2004) may have resulted from the DHA effect on the reduction step in the biohydrogenation (AbuGhazaleh and Jenkins, 2004).

Conclusion

Dietary forage level affected *trans* C18:1 and CLA concentrations in effluent with more *trans* C18:1 and less CLA accumulation seen in LF diet. Decreasing dietary forage levels resulted in *t*10 C18:1 and *t*10*c*12 CLA replacing VA and *c*9*t*11 CLA, respectively as predominate *trans* C18:1 and CLA isomers in the rumen. Our results showed that a supply of high linoleic acid oil along with FO under low pH condition has the greatest potential to enhance *t*10 C18:1 and *t*10*c*12 CLA production in the rumen.

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