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Original Article

Preliminary study of ruminal bio-hydrogenation and fermentation in response to linseed oil and fish oil addition to fistulated animals

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Abstract

Two experiments were conducted to evaluate the inhibitory bio-hydrogenation effects of fish oil (FO) and doses in combination with linseed oil (LSO). Experiment 1 was a 4×4 Latin square design and the treatments were comprised of: 1) no oil (control); 2) LSO; 3) 1:1 w/w LSO+FO; and 4) Ca-salt of LSO. The results found that feeding LSO+FO significantly increased *t11*-C18:1 and C22:6n-3, whereas C18:0 decreased. The ruminal acetic acid content was reduced at 4 and 6 h after feeding ($P<0.05$). In Experiment 2, three fistulated dry cows were assigned in a 3×3 Latin square design and the treatments were: 1) 2:1 w/w LSO+FO; 2) 1:1 LSO+FO; and 3) 1:2 w/w LSO+FO. The treatments were fed at 3% of total feed dry matter. The results revealed that the addition of 1:2 w/w LSO+FO significantly increased ruminal C20:5n-3 and C22:6n-3 ($P<0.05$). Additionally, 1:1 w/w LSO+FO significantly increased the concentration of *t11*-C18:1.

Keywords: ruminal bio-hydrogenation, linseed oil, fish oil, ruminal fermentation, fistulated dry cows

1. Introduction

The dietary recommendation for humans of highly unsaturated n-3 fatty acids, specifically C20:5 and C22:6, has increased from 0.15 to 0.65 g/d (Kris-Etherton *et al.*, 2000). Several authors have demonstrated that intestinal supply (Scholljegerdes *et al.*, 2001) and muscle tissue composition (Scollan *et al.*, 2001) of fatty acids in beef cattle were affected by fatty acid composition of dietary full-fat safflower seeds. Feeding lipids high in polyunsaturated fatty acids (PUFA) can enhance the fatty acid concentrations in beef cattle (Scollan *et al.*, 2001) and milk from dairy cattle (Whitlock *et al.*, 2002).

The concentrations of C18 PUFA, in particular C18:2n-6 and C18:3n-3, decreased as they are hydrogenated completely to C18:0 with formation of intermediates like conjugated linoleic acid (*c9,t11*-C18:2) and vaccenic acid (*t11*-C18:1) as the most important known ones (Harfoot & Hazlewood, 1997). Dohme, Fievez, Raes and Demeyer (2003) found that fish oil had lower lipolysis and bio-hydrogenation of C20:5n-3 and C22:6n-3 compared to C18:2n-6. This effect was also observed *in vivo* resulting in an enhanced duodenal flow of eicosapentaenoic acid and docosahexaenoic acid (Wachira *et al.*, 2000). The addition of fish oil in dairy cows showed a significant increase in the milk content of *c9,t11*-C18:2 and *t11*-C18:1 (Donovan *et al.*, 2000). These fatty acids (FAs) are the main intermediates in the rumen bio-hydrogenation of C18:3n-3 or C18:2n-6 or both. Since only small amounts of C18:2n-6 and C18:3n-3 are present in fish oil, it was hypothesized that supplementation of fish oil inhibited the complete bio-hydrogenation of C18:2n-6 and C18:3n-3 derived from sources other than fish oil (AbuGhazaleh, Schingoethe, Hippen, Kalscheur, & Whitlock, 2002; Bauman, Baumgard, Corl, & Griinar, 2000; Whitlock *et al.*, 2002). Feeding fish oil with oils high in linoleic or linolenic acid have been shown to be the most efficient dietary regimen to enhance the *c9,t11*-C18:2 level in milk (AbuGhazaleh, Schingoethe, Hippen, & Kalscheur, 2003; Donovan *et al.*, 2000).

Therefore the aim of this study was to evaluate the effects of different ratios of linseed oil in combination with fish oil supplementation in fistulated cattle on ruminal bio-hydrogenation and fermentation.

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2. Materials and Methods

The study was comprised of two experiments. Experiment 1 and Experiment 2 were conducted *in vivo*. All procedures performed in the study involving animals were in accordance with the ethical standards of the National Research Council of Thailand's guidelines for the care and use of animals at which the study was conducted.

2.1 Experimental design and animal management

Experiment 1 was conducted as a 4×4 Latin square design with four 21-day periods (7 days to adapt to the diets and 14 days for measurements) and four dietary treatments. Four crossbred Holstein Friesian dry cows that were previously fistulated were housed in individual pens and assigned to one of four treatments in a 4×4 Latin square design. Dietary treatments were: 1) no oil (control); 2) linseed oil (LSO); 3) 1:1 w/w LSO+fish oil (FO); and 4) calcium salt of linseed oil (Ca-LSO). Each supplemental oil was fed at 3% of total feed dry matter (DM).

Experiment 2 was carried out as a 3×3 Latin square design with four 21-day periods (7 days to adapt to the diets and 14 days for measurements) and three dietary treatments. Three crossbred Holstein Friesian dry cows that were previously fistulated were housed in individual pens and assigned to one of three treatments in a 3×3 Latin square design. Dietary treatments were: 1) 2:1 w/w LSO+FO; 2) 1:1 w/w LSO+FO; and 3) 1:2 w/w LSO +FO. Each supplemental oil was fed at 3% of total feed DM.

Diets consisted of 4 kg/day of concentrate and 2.4 kg of rice straw, divided into two equal meals and offered at 8:00 AM and 4:00 PM. Clean water was available at all times. Feed offered and feed refused were measured and recorded daily. The rice straw was sampled daily and the DM content (48 h at 60 °C) was determined daily to calculate dry matter intake (DMI) of each cow. The dried samples were pooled and then ground through a 1-mm screen for chemical analysis of analytical DM, crude protein (CP), ether extract (EE) and ash (Association of Official Analytical Chemists [AOAC], 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted based on the procedure described by Van Soest, Robertson and Lewis (1991).

Chemical compositions of the concentrate, rice straw, LSO, FO, and Ca-LSO used in the experiment are shown in Table 1. The concentrate used in this experiment contained 89.6% DM, 14.1% CP, and 3.7% EE. The DM, CP, and EE of rice straw were 88.7%, 2.1%, and 1.8%, respectively. LSO and FO in the current study contained 100% fat. The Ca-LSO contained 70.4% fat (Table 1).

Fatty acid compositions of the concentrate, rice straw, LSO, FO, and Ca-LSO used in the experiment are shown in Table 2. The C18:2n-3 proportion was the major fatty acid in the LSO (53.67% of total FAs) and Ca-LSO (35.94% of total FAs). FO had the highest proportion of C22:6n-3 and C20:5n-3 (30.42% and 8.03 of total FAs respectively).

Table 1. Chemical composition of the experimental diets.

Items	Concentrate[1]	Rice straw	LSO	FO	Ca-LSO
Dry matter	89.6	88.7	100	100	96.3
		…… % of DM……			
Ash	8.2	18.1			29.6
Crude protein	14.1	2.1			
Ether extract	3.7	1.8	100	100	70.4
Crude fiber	15.2	40.6			
NDF	40.1	76.1			
ADF	20.4	53.2			
ADL	4.9	17.1			

LSO = linseed oil, FO = fish oil, Ca-LSO = calcium salt of linseed oil, [1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg

Table 2. Fatty acid compositions (g/100 g fat) of concentrate, rice straw, and oils used in the experiment.

Fatty acids	Concentrate	Rice straw	LSO	FO	Ca-LSO
C12:0	22.72	6.31	2.90	2.15	ND
C14:0	7.80	8.25	0.35	4.40	ND
C16:0	16.54	45.70	22.75	28.01	31.32
C18:0	2.50	0.15	0.22	6.10	1.45
C18:1n-9	29.58	24.74	14.90	14.40	20.81
C18:2n-6	17.19	11.35	2.73	1.73	4.19
C18:3n-3	0.25	ND	53.67	0.93	35.94
C20:5n-3	ND	ND	ND	8.03	ND
C22:6n-3	ND	ND	ND	30.42	ND
Others[1]	3.42	3.50	2.48	3.73	6.29

LSO = linseed oil, FO = fish oil, Ca-LSO = calcium salt of linseed oil, [1]Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0, ND = Not detected

2.2 Fatty acid determination

The ruminal contents were collected on day 21 of each period at 0, 2, 4, and 6 h after the morning feeding to evaluate the fatty acid profile using a modified method by AbuGhazaleh *et al.* (2002). The ruminal content was stored at –20 ℃ until analysis.

Fatty acid compositions of the concentrate, rice straw, LSO, FO, Ca-LSO, and rumen content were extracted using a modification of the method used by Folch, Lees and Sloane-Stanley (1957) and Metcalfe, Schmitz and Pelka (1966) and then analyzed by gas chromatography (GC) (7890A GC System, Agilent Technology, USA).

2.3 Ruminal fermentation

To evaluate ruminal fermentation, on the last day of each period (day 21), ruminal fluid samples were collected from each cow at 0, 2, 4, and 6 h after the morning feeding. The pH of the rumen fluid was immediately determined at the time of sampling. For the volatile fatty acid (VFA) and NH_3-N determinations, 36 mL of rumen fluid from individual cows at each sampling time was put into 50 mL centrifuge tubes containing 4 mL of 1 M H_2SO_4. The tubes were centrifuged at8000*g* for 20 min at 4 ℃; supernatants were collected into 25 mL test tubes, capped, and then stored at –20℃ until analysis. The analysis of VFA was performed by GC (Hewlett Packard GC system HP6890, USA, 19091N-113 INNOWAX, length 30 m, I.D. 0.32 mm, WIDEBORE, film 0.25 µm). The NH_3-N concentration was determined by Kjeldahl analysis (AOAC, 2005).

2.4 Statistical analysis

Measurements of ruminal fatty acids and rumen fermentation in each period were analyzed by Proc GLM using the Statistical Analysis System (SAS Inst., Cary, NC, USA). When the overall treatment effect was significant ($P<0.05$), differences between treatment means were compared using Duncan's new multiple range test. A value of $P<0.05$ was used to declare significant differences among the treatments.

3. Results and Discussion

3.1 Experiment 1

3.1.1 Fatty acid profile in ruminal content

At all time points after feeding, the ruminal content from the control, LSO, and Ca-LSO cattle contained higher concentrations of ruminal C18:0 than from the LSO+FO cattle (Table 3). Increases in C18:0 reflected ruminal biohydrogenation of C18:1n-9, C18:2n-6, and C18:3n-3 in the control, LSO, and Ca-LSO diets. At all hours after feeding, the ruminal content from the LSO+FO cattle had higher C16:0, *t11*-C18:1, C20:5n-3, and C22:6n-3 than from the other cattle (Table 3). Since the FO contained higher C22:6n-3 (Table 2), the cattle on the LSO+FO diet consumed more C22:6n-3 than other cattle. The addition of FO resulted in decreased C18:0, meanwhile there was an increased amount of *trans*-C18:1 in the rumen (Jenkins, Wallace, Moate, & Mosley, 2008). Feeding C22 fatty acids sharply increased the proportion of *t11*-C18:1 in the rumen. AbuGhazaleh *et al.* (2002) previously reported that *t11*-C18:1 accumulated in all cultures over time with higher accumulations associated with higher levels of C22:6n-3 supplementation. In addition, AbuGhazaleh and Jenkins (2004) also reported a positive correlation between C22:6n-3 supplementation and *t11*-C18:1. The *t11*-C18:1 was the major source to synthesize *c9*,*t11*-C18:2 (CLA) in animal tissue. Doreau and Chilliard (1997) previously reported total C18:1 fatty acids increased while C18:0 decreased in the duodenal contents when FO was added to the rumen. In cattle, LSO in the diet increased *t11*-C18:1, *c9*,*t11*-CLA, and C18:3n-3 at the duodenum (Doreau, Laverroux, Normand, Chesneau, & Glasser, 2009b), whereas FO resulted in greater flows of *t11*-C18:1, C20:5n- 3, and C22:6n-3 (Kim *et al.*, 2008; Lee *et al.*, 2008). Loor, Ueda, Ferlay, Chilliard, & Doreau (2005) also observed an increase in C16:0 when FO was added to the diet compared with sunflower oil and LSO. Similarly, Kitessa *et al.* (2001) supplemented with protected tuna oil and tuna oil found an increase in C16:0 concentration in the rumen. Since FO contains C20:5n-3 and C22:6n-3**,** adding C22:6n-3 to the rumen alters a variety of fatty acids. Shingfield *et al.* (2011) also reported that the inclusion of LSO in the diet increased C16:0, C18:0, *trans* C18:1, conjugated linoleic acid (CLA), and C18:3n-3 at the duodenum, whereas FO increased the flow of C14:0, C16:0, total C16:1, *trans* C18:1, but decreased C18:0 at the duodenum.

3.1.2 Ruminal fermentation

Ruminal pH was not affected by treatments (Table 4). Similar results were reported (Beauchemin, McGinn, & Petit, 2007; Fievez, Dohme, Daneels, Raes, & Demeyer, 2003). Doreau, Aurousseau and Martin (2009a) demonstrated that linseed oil did not affect the rumen fermentation pattern. Harvatine and Allen (2006) suggested that the use of saturated and unsaturated lipids had a minor or insignificant effect on ruminal fermentation parameters. However, Messana *et al.* (2013) reported that in animals receiving the highest dietary lipid content (60 g/kg), rumen pH decreased quadratically ($P<0.001$) with an increase in the lipid content. Shingfield *et al.* (2003) found a significant decrease in pH when FO was supplemented because of the reduction in DMI related to decreased pH.

The present study found that LSO+FO significantly increased ruminal ammonia nitrogen content at the early hours after feeding (4 hours after feeding) (Table 4). Similar results also reported that FO supplementation increased the NH_3-N (Keady & Mayne, 1999). In addition, another study found inconsistent results with significant increases in the NH_3-N when linolenic acid sources were supplemented in sheep (Zhang *et al.*, 2008)

The current study observed that LSO or Ca-LSO had no effect on ruminal VFA concentrations at 2 hours after feeding (Table 4); however, LSO+FO tended to reduce the molar proportion of acetic acid at 4 and 6 hours post-feeding ($P=0.055$ and $P=0.052$, respectively). The molar proportions of propionic acid significantly increased at 4 and 6 hours after feeding ($P=0.018$ and $P=0.017$, respectively) and tended to increase at 1 hour post-feeding ($P=0.071$) by LSO+FO addition (Table 4). LSO+FO tended to increase the molar

Table 3. Effect of LSO, FO, and Ca-LSO supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids).

Fatty acids	Control	LSO	LSO+FO	Ca-LSO	SEM	P-value
Pre-feeding						
C12:0	6.94	7.86	7.58	6.82	0.534	0.745
C14:0	4.88	5.74	5.94	3.95	0.292	0.175
C16:0	24.89	23.33	24.02	24.18	0.629	0.200
C18:0	57.83	57.75	57.42	59.94	0.654	0.298
C18:1n-9	3.85	3.62	3.66	3.80	0.066	0.586
C18:2n-6	1.61	1.69	1.37	1.30	0.146	0.590
2 hours after feeding						
C12:0	7.57	10.35	12.63	9.65	0.628	0.120
C14:0	3.62^{c}	5.96^{b}	10.33^{a}	5.75^{b}	0.282	0.002
C16:0	31.08^{a}	20.04^{b}	38.89^{a}	21.52^{b}	1.303	0.011
C18:0	52.14^{a}	48.66^{a}	9.83^{b}	47.21^{a}	2.231	0.004
C18:1n-9	1.84	4.53	2.35	2.38	0.611	0.394
C18:2n-6	2.17	2.37	1.57	2.86	0.258	0.357
C18:3n-3	0.43^{c}	2.01^{a}	1.24^{b}	1.51^{ab}	0.088	0.008
t11-C18:1	0.55^{d}	5.72^{c}	13.88^{a}	8.88^{b}	0.460	0.010
c9,t11-C18:2	0.58	0.10	0.48	0.17	0.081	0.176
t10,c12-C18:2	ND	0.15	ND	ND	0.044	0.847
C20:5n-3	ND^{b}	ND^{b}	0.83^{a}	ND^{b}	0.076	0.001
C22:6n-3	ND^{b}	ND^{b}	7.64^{a}	ND^{b}	0.376	0.001
4 hours after feeding						
C12:0	3.58^{c}	7.02^{bc}	11.33^{b}	16.25^{a}	0.717	0.007
C14:0	5.74^{bc}	4.75^{c}	10.19^{a}	6.60^{b}	0.203	0.001
C16:0	41.55^{a}	19.17^{b}	41.13^{a}	19.04^{b}	0.732	0.001
C18:0	44.23^{b}	53.32^{b}	11.47^{c}	44.08^{a}	0.861	0.001
C18:1n-9	0.67^{b}	6.07^{a}	6.06^{a}	7.78^{a}	0.302	0.002
C18:2n-6	3.24^{a}	2.64^{ab}	1.47^{b}	2.88^{a}	0.176	0.053
C18:3n-3	0.12^{b}	1.76^{a}	2.22^{a}	1.75^{a}	0.085	0.002
t11-C18:1	0.84^{c}	4.78^{b}	10.75^{a}	1.59^{c}	0.240	0.001
c9,t11-C18:2	ND	0.07	0.09	ND	0.031	0.517
t10,c12-C18:2	ND^{b}	0.39^{a}	ND^{b}	ND^{b}	0.012	0.001
C20:5n-3	ND^{b}	ND^{b}	0.54^{a}	ND^{b}	0.013	0.001
C22:6n-3	ND^{b}	ND^{b}	4.51^{a}	ND^{b}	0.267	0.001
6 hours after feeding						
C12:0	4.36^{b}	9.01^{a}	7.23^{ab}	7.98^{a}	0.445	0.050
C14:0	4.29	4.93	6.95	4.35	0.407	0.149
C16:0	16.65^{d}	18.68^{c}	28.14^{a}	22.02^{b}	0.340	0.001
C18:0	15.16^{c}	54.85^{a}	8.53^{d}	45.65^{b}	0.613	0.001
C18:1n-9	2.13^{b}	4.85^{ab}	6.81^{ab}	11.07^{a}	0.943	0.070
C18:2n-6	2.23^{ab}	ND^{b}	0.93^{b}	4.30^{a}	0.463	0.067
C18:3n-3	ND^{b}	0.42^{ab}	0.28^{ab}	1.03^{a}	0.133	0.122
t11-C18:1	55.17^{a}	7.26^{c}	39.06^{b}	3.51^{c}	0.724	0.001
c9,t11-C18:2	ND	ND	0.12	0.07	0.021	0.148
t10,c12-C18:2	ND	ND	0.11	0.09	0.039	0.558
C22:6n-3	ND^{b}	ND^{b}	1.69^{a}	ND^{b}	0.040	0.018

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil; SEM = standard error of the mean
[abc] Within a row means without a common superscript letter differ.

proportion of butyric acid at 2 hours after feeding (P=0.072). The acetate:propionate ratios (A:P) significantly decreased by the LSO+FO at 2, 4, and 6 hours post-feeding. Wachira *et al.* (2000) and Fievez *et al.* (2003) demonstrated a reduction in molar proportion of acetate. Shingfield *et al.* (2011) supplemented LSO and FO alone or as an equal mixture and reported that supplements of FO had no effect on rumen pH, but shifted rumen fermentation toward propionate at the expense of acetate with no change in molar proportions of butyrate. FO modified rumen fermentation, causing a decrease in the molar A:P ratio. Earlier studies reported that FO had no major effect on fermentation characteristics in growing cattle (Lee *et al.,* 2008; Kim *et al.,* 2008), but increased propionic acid in steers (Shingfield *et al.*, 2010). Inclusion of LSO in the diet had no effect on rumen fermentation patterns compared with the control which was consistent with previous reports in cattle Doreau *et al.* (2009a). In other experiments, LSO (Ueda *et al.*, 2003) or linseed (Gonthier *et al.*, 2004) that supplied 3.0 to 4.0% of additional lipids in the diet were shown to increase the molar proportions of propionate at the expense of acetate. Given that FO altered ruminal VFA, whereas LSO had no effect, it appears that the changes in rumen fermentation to LSO+FO were due to FO.

3.2 Experiment 2

3.2.1 Fatty acid profile in ruminal content

At 2 hours after feeding, cattle on 1:1 LSO+FO at 3% of feed DM had a significantly higher ruminal proportion

Table 4. Effect of LSO, FO, and Ca-LSO supplementation on pH, ammonia nitrogen (mg/100 mL), and volatile fatty acids (mol/100 mol) in fistulated cattle.

Item	Control	LSO	LSO+FO	Ca-LSO	SEM	P-value
Pre-feeding						
pH	6.35	6.36	6.35	6.34	0.032	0.833
NH_3N	8.12	7.48	8.92	7.63	0.673	0.321
Acetic acid	72.43	73.48	71.35	72.68	1.512	0.723
Propionic acid	17.53	16.63	17.51	17.17	0.863	0.704
Butyric acid	10.04	9.89	11.14	10.15	0.732	0.783
A:P ratio	4.13	4.41	4.07	4.23	0.482	0.642
2 hours after feeding						
pH	6.48	6.43	6.40	6.41	0.023	0.251
NH_3N	12.61	11.49	11.13	13.11	0.634	0.512
Acetic acid	70.24	71.60	68.99	71.99	0.488	0.212
Propionic acid	17.97	18.52	19.54	17.84	0.414	0.412
Butyric acid	11.79	9.87	11.46	10.17	0.135	0.072
A:P ratio	3.91	3.92	3.59	4.09	0.098	0.306
4 hours after feeding						
pH	6.03	6.03	6.06	6.04	0.032	0.293
NH_3N	4.67^{b}	4.52^{b}	8.01^{a}	4.80^{b}	0.091	0.001
Acetic acid	72.83	72.33	67.86	72.56	0.372	0.055
Propionic acid	16.28^{a}	17.77^{b}	19.53^{a}	16.96^{b}	0.101	0.018
Butyric acid	10.89	9.89	12.61	10.49	0.359	0.159
A:P ratio	4.47^{a}	4.14^{b}	3.48^{c}	4.34^{a}	0.013	0.002
6 hours after feeding						
pH	6.31	6.33	6.32	6.30	0.064	0.363
NH_3N	4.98	3.61	4.69	5.02	0.402	0.473
Acetic acid	72.94	73.52	68.93	73.61	0.363	0.052
Propionic acid	17.01^{b}	16.71^{b}	18.72^{a}	16.25^{b}	0.102	0.017
Butyric acid	10.05	9.78	12.35	10.14	0.364	0.170
A:P ratio	4.28^{a}	4.48^{a}	3.69^{b}	4.62^{a}	0.030	0.012

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil; SEM = standard error of the mean; A:P ratio = Acetic acid:Propionic acid
[abc] Within a row means without a common superscript letter differ.

of C12:0 ($P<0.05$) than other cattle while cattle on 1:2 LSO+FO at 3% of feed DM contained the highest ruminal proportion of C22:6n-3 followed by cattle on 1:1 LSO+FO at 3% of feed DM and cattle on 2:1 LSO+FO at 3% of feed DM (Table 5). At 4 hours post-feeding, the proportion of ruminal *t11*-C18:1 was the highest in cattle fed 1:1 LSO+FO at 3% of feed DM followed by cattle that received 1:2 and 2:1 LSO+FO at 3% of feed DM, whereas the proportion of ruminal C20:5n-3 and C22:6n-3 was significantly higher ($P<0.05$) in cattle fed 1:2 LSO+FO at 3% of feed DM than the cattle on 1:1 and 2:1 LSO+FO at 3% of feed DM. However, at 6 hours after feeding, there were no significant differences in the proportions of all ruminal fatty acids measured. Linear increases in the ruminal proportion of C20:5n-3 and C22:6n-3 of cattle that received FO at 4 hours post-feeding reflected the higher intake of C20:5n-3 and C22:6n-3 from FO since FO contained a high proportion of these two fatty acids. A similar response was previously reported (Kim *et al.*, 2008) and observed whereby supplementation of 2.3% and 6.9% FO linearly increased C20:5n-3 and C22:6n-3. Similarly, Palmquist & Griinari (2006) added 0, 0.33, 0.67, and 1.00% FO to the diets of dairy cows and observed a linear increase in the concentration of C20:5n-3 and C22:6n-3 in milk as the FO increased. Chow *et al.* (2004) reported that the lipolysis rates of C20:5n3 and C22:6n3 were always lower than the average lipolysis of C18:2n6 and C18:3n3. In the current study, supplementation of 2:1, 1:1, and 1:2 LSO+FO at 3% of feed DM did not affect the ruminal proportion of C18:2n-6 and C18:3n-3 at all times after feeding. In an *in vitro* study, Chow *et al.* (2004) showed that the apparent bio-hydrogenation of C18:2n-6 and C18:3n-3 was not affected by FO addition. Hydrogenation was dose dependent, with the lower level of FO inclusion generally subject to more extensive bio-hydrogenation. Similar *in vitro* observations were also reported by Gulati *et al.* (1999) when incubating cottonseed supplemented with or without FO. *In vivo* experiments of AbuGhazaleh *et al.* (2002) showed no significant difference in ruminal C18:2n-6 content of animals on a diet containing extruded soybean or FO+extruded soybean. Similarly, Wachira *et al.* (2000) reported no difference in duodenal flow of C18:3n-3 when offering LSO or LSO+FO supplement. The concentration of C18:0 at all times after feeding in this study was similar among treatments. It was clear that FO inhibited complete bio-hydrogenation to C18:0 and this effect was dose dependent. This is in line with *in vivo* observations by Wachira *et al.* (2000) who reported a significantly higher C18:0 duodenal flow in sheep fed a LSO diet compared to LSO+FO. The current study found an increase in *t11*-C18:1 in the rumen at 4 hours post-feeding when supplemented 1:1 LSO+FO at 3% of feed DM compared with 1:2 LSO+FO and 2:1 LSO+FO. FO is a potent inhibitor of the conversion of *t11*-C18:1 to C18:0, and LSO+FO would complementarily maximize *t11*-C18:1 production which is the primary source of CLA in milk fat (Palmquist, Lock, Shingfield, & Bauman, 2005). Chow *et al.* (2004) showed that an increase in the FO proportion in combination oil found a highly significant

Table 5. Effect of LSO+FO in different ratio supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids).

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
Pre - feeding					
C12:0	5.54	5.91	6.80	0.183	0.193
C14:0	5.37	6.92	5.96	0.150	0.329
C16:0	32.38	32.01	31.86	0.292	0.380
C18:0	50.16	48.94	49.09	0.529	0.599
C18:1n-9	3.66	3.12	3.43	0.093	0.265
C18:2n-6	2.89	3.08	2.85	0.216	0.875
2h after feeding					
C12:0	4.80^{b}	5.23^{a}	4.79^{b}	0.090	0.041
C14:0	4.82	5.66	5.93	0.473	0.181
C16:0	25.59	28.13	29.44	3.854	0.564
C18:0	7.68	8.07	7.17	2.044	0.871
C18:1n-9	6.69	6.06	6.57	0.954	0.773
C18:2n-6	1.28	1.13	0.84	0.143	0.120
C18:2n-6	7.41	5.72	5.30	1.139	0.260
C18:3n-3	4.43	4.29	4.71	0.115	0.399
t11-C18:1	29.27	28.78	22.04	4.419	0.285
c9,t11-C18:2	1.94	0.42	1.3	2.640	0.800
t10,c12-C18:2	0.86	0.43	2.28	0.718	0.155
C20:5n-3	1.31	1.57	1.11	0.328	0.410
C22:6n-3	3.92^{c}	4.51^{b}	8.52^{a}	0.323	0.045
4h after feeding					
C12:0	5.10	5.25	4.82	1.193	0.908
C14:0	5.97	5.13	5.63	0.844	0.573
C16:0	30.04	27.19	31.73	4.023	0.506
C18:0	8.62	6.80	8.23	2.435	0.683
C18:1n-9	5.38	5.33	4.67	0.469	0.316
C18:2n-6	4.53	2.75	1.00	1.552	0.204
C18:3n-3	4.11	3.56	3.71	0.170	0.181
t11-C18:1	26.95^{c}	36.43^{a}	31.04^{b}	2.441	0.039
c9,t11-C18:2	1.31	2.02	1.39	1.131	0.740
t10,c12-C18:2	2.53	0.00	0.00	2.530	0.500
C20:5n-3	0.26^{c}	0.55^{b}	0.83^{a}	0.114	0.049
C22:6n-3	4.00^{c}	4.99^{b}	6.33^{a}	0.185	0.040
6h after feeding					
C12:0	4.79	5.08	4.24	0.355	0.187
C14:0	6.05	6.22	4.47	1.413	0.416
C16:0	29.39	31.32	33.18	1.425	0.158
C18:0	8.91	7.45	7.92	1.661	0.624
C18:1n-9	3.35	3.95	3.96	0.286	0.186
C18:2n-6	4.50	3.34	2.75	0.747	0.189
C18:3n-3	0.59	0.47	0.36	0.480	0.856
t11-C18:1	37.13	35.92	37.26	4.074	0.967
C20:5n-3	1.58	1.57	1.54	0.231	0.969
C22:6n-3	3.71	4.68	4.24	1.181	0.661

LSO = linseed oil; FO = fish oil, ^{abc}Within a row means without a common superscript letter differ

accumulation of *t11*-C18:1. In addition, Wachira *et al.* (2000) reported a 63% increase of duodenal flow of *trans* C18:1 when supplementing FO in sheep diets containing linseed, which is in accordance with the 54.9% increase of *t11*-C18:1 in an *in vitro* study with 4% LSO+FO. Comparably, Donovan *et al.* (2000) reported a continuous and gradual increase in milk *t11*-C18:1 and *c9,t11*-CLA as the amount of fish oil increased.

3.2.2 Ruminal fermentation

The current study found no significant difference in ruminal pH at all times after feeding when different ratios of combination oils were fed (Table 6). Similar results were also observed by Toral *et al.* (2009) when supplemented combination oils containing FO were fed at different levels. They observed that the ruminal pH was not affected by oil supplementation which was in agreement with previous *in vivo* studies using different lipid sources, including FO and sunflower oils (Beauchemin *et al.*, 2007; Fievez *et al.*, 2003;). Ruminal ammonia nitrogen in this study was not significantly affected by oil supplements at all times post-feeding (Table 6) which was similar to the work of Gudla *et al.* (2012) who added FO in combination with other oils and reported no significant difference in ammonia nitrogen compared to a non-oil supplement. Similarly, Toral *et al.* (2009) fed FO at 3 g

Table 6. Effect of LSO+FO in different ratios of supplementation on pH, ammonia nitrogen (mg/100mL) and volatile fatty acids (mol/100 mol) in fistulated cattle.

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
Pre - feeding					
pH	6.87	6.89	6.87	0.052	0.988
NH_3N	11.68	12.44	11.20	0.272	0.358
Acetic acid	64.70	64.60	64.80	0.486	0.986
Propionic acid	22.90	22.40	22.47	0.310	0.773
Butyric acid	12.40	12.90	12.73	0.173	0.529
A:P ratio	5.21	5.03	5.18	0.107	0.619
2 hours after feeding					
pH	6.54	6.51	6.62	0.017	0.223
NH_3N	22.82	23.64	21.98	1.042	0.826
Acetic acid	66.22	63.96	66.80	1.804	0.805
Propionic acid	23.05	24.87	22.79	1.458	0.831
Butyric acid	10.73	11.27	10.41	0.471	0.795
A:P ratio	2.97	2.58	2.99	0.294	0.776
4 hours after feeding					
pH	6.46	6.33	6.41	0.037	0.484
NH_3N	8.13	8.12	8.38	0.254	0.895
Acetic acid	64.53	66.90	65.18	1.169	0.732
Propionic acid	23.92	21.73	24.19	1.052	0.644
Butyric acid	11.55	11.37	10.63	0.298	0.525
A:P ratio	2.76	3.09	2.71	0.170	0.669
6 hours after feeding					
pH	6.50	6.44	6.31	0.063	0.572
NH_3N	6.71	5.46	6.23	0.413	0.522
Acetic acid	66.95	65.03	64.19	0.565	0.322
Propionic acid	21.15	23.20	24.09	0.285	0.096
Butyric acid	11.89	11.77	11.72	0.281	0.965
A:P ratio	3.17	2.81	2.70	0.051	0.114

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean; A:P ratio = Acetic acid:Propionic acid, [abc] Within a row means without a common superscript letter differ.

and 10 g per day in sheep and found no effect on ammonia nitrogen in the rumen compared to the control group. Keady and Mayne (1999) supplemented FO at 150g/day and 300 g/day and showed no significant difference in ruminal ammonia nitrogen concentration; however, when supplemented up to 450 g/day, the ruminal ammonia nitrogen increased. They suggested that the lack of a significant effect on the concentrations of either ammonia or the VFA originated from the deamination of some amino acids.

Different proportions of FO and LSO in the present study did not affect ruminal VFA concentration (Table 6). Similar results were also reported (Toral *et al.,* 2009). Previously, Doreau and Chilliard (1997) offered FO in one feed daily and concluded that the inclusion of 200 g FO had no effect on rumen fermentation patterns, whereas the inclusion of 400 g FO in one feed reduced the molar proportions of acetate and increased the molar proportion of propionate. At 6 hours after feeding in the present study, the molar proportion of propionate tended to increase (P=0.096) when cattle received a high proportion of FO. According to Keandy and Mayne (1999), FO supplementation at 150 and 300 g/day showed no effect on molar proportion of propionic acid. However, when supplemented at 450 g/day the molar proportion of propionate increased and the molar proportion of acetate reduced. A decreased ruminal acetate concentration is a common response to the addition of FO (Fivez *et al.,* 2003; Toral *et al.,* 2009) or linoleic acid-rich sources to the diet (Zhang *et al.,* 2008). This trend supports the hypothesis that polyunsaturated fatty acids may exert an inhibitory effect on acetate-producing bacteria (Toral *et al.,* 2009).

4. Conclusions

These series of studies aim to obtain healthy and beneficial fatty acids or their isomers in the ruminal content for absorption. The ruminant tissues can then uptake these fatty acids or their isomers for deposition or synthesis for subsequent retention in the milk or meat products. The studies commenced from the first experiment which determined whether ruminal concentrations of *t11*-C18:1, the CLA synthesized precursor, and omega-3 fatty acids increased due to different forms of LSO and in combination with FO. The results clearly revealed that the ruminal concentrations of *t11*-C18:1 and C22:6n-3 increased while C18:0 decreased by the addition of LSO+FO. None of the imposed oil treatments had any effect on ruminal pH, ammonia nitrogen or VFA proportion compare to the non-supplemented control. The second experiment was carried out in accordance with the results from experiment 2 to determine ruminal concentrations of *t11*-C18:1 and omega-3 fatty acids were favorably changed by adding different ratios of LSO+FO. The results clearly indicated that the ruminal concentration of *t11*-C18:1 had

increased by the 1:1 w/w LSO+FO, whereas the ruminal concentrations of C20:5n-3 and C22:6n-3 increased by the addition of 1:2 w/w LSO+FO. These findings can be used as guidelines to improve the quality of animal products. The optimum level of oil supplement is one of the many factors to improve animal performance, particularly growth rate, carcass quality, and the milk yield and composition. Therefore, to manipulate the feeding approach to improve the healthy and beneficial fatty acids without or fewer negative effects, further research in production trials is advisable.

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