

RUMINAL FATTY ACID BIO-HYDROGENATION IN RESPONSE TO LINSEED OIL AND SUNFLOWER OIL IN COMBINATION WITH FISH OIL ADDITION TO FISTULATED CATTLE'S DIETS

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Abstract

The effects of linseed oil or sunflower oil in combination with fish oil on ruminal bio-hydrogenation and rumen fermentation in fistulated cattle were studied. Four fistulated cattle were used in a 4×4 Latin square design; the experiment consisted of 4 periods of 21 d in each period, with the first 14 d for adaptation followed by 7 d of measurement period. The animals were fed 3 kg/d of 14% crude protein concentrate and 2 kg/d rice straw. The treatments were: 1) control no oil added, 2) supplemented with 70 g/d linseed oil (LO) plus 70 g/d fish oil (FO), 3) supplemented with 70 g/d sunflower oil (SO) plus 70 g/d FO, and 4) supplemented with 46.7 g/d LO, plus 46.7 g/d SO, plus 46.7 g/d FO. The results demonstrated that the ruminal C18: 1n-9t proportion was highest in the cattle fed SOFO followed by LOFO, while the ruminal proportion of C18: 2n-6 was highest in the cattle fed the SOFO diet. The ruminal C18: 3n-3 proportion was highest in the cattle fed the LOFO diet. No significant difference was found for ruminal pH, volatile fatty acids, and ammonia nitrogen (NH₃-N), except for NH₃-N at 4 h after feeding that was highest in the cattle fed the control diet. Based on the results from the current study, the FO addition to plant oils rich in C18: 2n-6 and C18: 3n-3 inhibited bio-hydrogenation of C18: 2n-6 and C18: 3n-3 to C18: 0 and yielded *trans*-11 - C18: 1 in the rumen. The increase in *trans*-11 - C18: 1 would increase the conjugated linoleic acid in the meat.

Keywords: *Trans-vaccenic acid*, bio-hydrogenation, oil supplementation, fatty acids

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Introduction

Supplementation of plant oils or animal fats to the ruminant diet is to supply energy to ruminants, particularly for those animals which are in a productive stage such as lactating dairy cows. In addition, the oils themselves can modify the fatty acid (FA) composition of the animal products, particularly to improve the quality of animal products. The addition of plant oils has been reported to be a good strategy for increasing the milk *c9*, *t11*-C18:2 (conjugated linoleic acid (CLA)) contents in goats (Martínez Marín *et al.* 2011). Additionally, multiple studies have attempted to increase the concentration of 20:5n-3 and 22:6n-3 in ruminant milk by adding fish oil to the diet, but the apparent transfer rate of these FAs from the diet to milk is relatively low (Toral *et al.* 2010a). However, as a rumen bio-hydrogenation modulator, fish oil yields large increases in milk CLA and *t11*-C18:1 concentrations, particularly when combined with plant oils either in goats, cows, or sheep (Gagliostro *et al.* 2006; Shingfield *et al.* 2006; Toral *et al.* 2010b).

Previous studies on the addition of oils to ruminant diets as an energy source raised concerns about the detrimental effects of FAs on ruminal fermentation (Jenkins, 1993). Rumen bacteria play a major role in fat metabolism in the rumen (Jenkins *et al.* 2008). Fats are extensively hydrolyzed in the rumen, rendering FAs that have bacteriostatic and bacteriocidal effects. Among them, unsaturated FAs have more antimicrobial activity than saturated ones (Harfoot and Hazlewood, 1997), and a differential toxicity of different poly-unsaturated fatty acids (PUFA) to rumen microorganisms has also been observed (Maia *et al.* 2007). Dietary supplementation with oils has given inconsistent results on ruminal fermentation, with some detrimental consequences (Fievez *et al.* 2003). Therefore, the aim of this study was to evaluate the effect of oils rich in omega-6 and omega-3 FAs and fish oil on ruminal bio-hydrogenation and fermentation in fistulated cattle.

Materials and Methods

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 under which the study was conducted.

Animals and Feeding

Four fistulated non-lactating dairy cows, averaging 478 ± 23 kg and 46 ± 4 mo old, housed in individual pens were each assigned to 1 of 4 treatments in a 4×4 Latin squares design. The trial consisted of 4 periods, with 21 d in each period, 14 d for adaptation to diets and 7 d for the measurement period. The treatments were: 1) control no oil added (CON), 2) supplemented with 70 g/d linseed oil (LO) plus 70 g/d fish oil (FO), 3) supplemented with 70 g/d sunflower oil (SO) plus 70 g/d FO, and 4) supplemented with 46.7 g/d LO, plus 46.7 g/d SO, plus 46.7 g/d FO. The supplemental oils were offered twice daily, divided into 2 equal meals at 08.00 and 16.00 h, by top-dressing onto concentrate and mixing thoroughly. The hypothesis was that the last step (converting *trans*-vaccenic acid to stearic acid) of long chain fatty acids bio-hydrogenation (C18:2n-6 and C18:3n-3 in SO and LO, respectively) would be inhibited by the FO inclusion.

Measurements and Chemical Analysis

Feed Intake

Diets were offered as 3 kg/d of concentrate, divided into 2 equal meals at 08.00 and 16.00 h together with 2 kg/d of rice straw and clean water. This was intended to feed fistulated cows based on their maintenance requirement. The feed offered and feed refused were measured and recorded daily during the experimental periods. The dry matter (DM) content (65°C, 48 h) of the concentrate and rice straw for individual fistulated cows were determined daily to calculate the dry matter intake (DMI).

The samples were ground through a 1 mm screen for chemical analysis. The DM of

Table 1. Chemical composition of concentrate and rice straw used in the experiment

Composition	Concentrate ¹	Rice straw
Dry matter (%)	93.33±0.02	92.08±0.01
Crude protein	14.21±0.07	1.32±0.02
Ether extract	4.11±0.13	1.74±0.10
Ash	8.14±0.50	14.05±0.01
Crude fiber	17.89±0.35	38.74±0.2
Non-fiber carbohydrate	38.65±0.39	7.15±0.14
Neutral detergent fiber	34.89±0.38	75.74±0.9
Acid detergent fiber	19.56±0.34	43.79±1.0
Acid detergent lignin	6.21±0.06	11.14±0.11

¹Concentrate contained (as DM basis) 24.0% cassava pulp, 24.0% palm meal, 19.0% soybean meal, 8.0% rice bran, 8.0% molasses, 7.0% cassava dried distillers meal, 6.0% cassava chip, 2.0% urea, and 2.0% mineral-vitamin mix.

the rice straw and concentrate were determined by oven-drying at 65°C to a constant weight. The samples were analyzed for crude protein (CP), ether extract (EE), crude fiber, and ash (AOAC International, 1995), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest *et al.*, 1991). The non-fiber carbohydrate was calculated according to the National Research Council (2000).

Ruminal Fermentation

To evaluate the ruminal fermentation, on the last day of each period (d 21), ruminal fluid samples were collected from each fistulated cow at 0, 2, 4, and 6 h after the morning feed. The pH of the rumen fluid was immediately determined at the time of sampling. For the volatile fatty acid (VFA) and NH₃-N determination, 36 mL of rumen fluid from each individual cow at each sampling time was put into a 50 mL centrifuge tube containing 4 mL of 1 M H₂SO₄ for VFA and NH₃-N analysis. All the tubes were centrifuged at 8,000 × g for 20 min at 4°C; the supernatants were collected into 25 mL test tubes, capped, and stored at -20°C until analysis. Analyses of VFAs were performed by gas chromatography (GC system HP6890, Hewlett-Packard Co., Palo Alto, CA, USA; 19091N-113 INNOWax, length 30 m, I.D. 0.32 mm, widebore, and film 0.25 µm, Agilent Technologies, Inc., Santa Clara, CA, USA). The NH₃-N concentration was determined by Kjeldahl analysis (AOAC International, 1995).

Fatty Acid Determination

To evaluate the FA profile, the ruminal contents were collected at the same time as the ruminal fluid sampling and stored at -20 °C for analysis using a modified method by AbuGhazaleh *et al.* (2002).

The FAs' composition of the concentrate, rice straw, LO, SO, FO, and rumen content were extracted using a modification of the method used by Folch *et al.* (1957) and Metcalfe *et al.* (1966) and then were analyzed by gas chromatography (GC) (7890A GC System, Agilent Technologies, Inc., Santa Clara, CA, USA).

Statistical Analysis

All data were analyzed using PROC GLM with repeated measurements for a 4×4 Latin squares design using SAS (SAS, 2002). Differences amongst means were considered statistically significant at P<0.05, and differences at 0.05<P<0.10 were accepted as showing tendencies toward significance.

Results and Discussion

Chemical and Fatty Acid Composition of Feeds and Oils

All cattle received the same basal concentrate containing 93.33% DM, 14.21% CP, 4.11% EE, and 17.89% crude fiber. The rice straw contained 92.08% DM, 1.32% CP, 1.74% EE, and 38.74% crude fiber (Table 1). The main FA in the concentrate was C18:1n-9 (31.90 g/100 g FA) and C18:2n-6 (20.34 g/100

Table 2. Fatty acid composition of feeds and oils used in the experiment

Fatty acid g/100g	Concentrate	Rice straw	SO	LO	FO
C8:0	0.74	ND	0.34	0.06	0
C10:0	1.34	ND	0.55	ND	0.75
C12:0	17.96	6.84	0.09	ND	0.18
C14:0	6.38	8.42	0.14	0.86	4.31
C16:0	17.85	40.06	8.18	4.91	24.25
C18:0	2.71	8.57	3.40	3.69	9.91
C18:1n-9	31.90	24.26	29.40	17.88	13.46
C18:2n-6	20.34	11.85	57.50	16.73	2.64
C18:3n-3	0.78	ND	0.40	55.87	1.83
C20:5n-3	ND	ND	ND	ND	10.91
C22:6n-3	ND	ND	ND	ND	31.76

SO = Sun flower oil; LO = Linseed oil, FO = Fish oil, ND = not detected

Table 3. DM, CP, and fat intakes of experimental fistulated cattle

Intake	CON	LOFO	SOFO	LOSOFO	SEM	P-value
DM (kg/d)						
Concentrate	2.80	2.70	2.70	2.60	0.03	0.601
Rice straw	1.90	1.90	1.90	1.90	—	—
Oils	0.00	0.14	0.14	0.14		
Total	4.70	4.74	4.74	4.64	0.03	0.601
CP (g/d)						
Concentrate	398	384	384	369	11.79	0.612
Rice straw	25	25	25	25	—	—
Total	423	409	409	395	11.79	0.612
Fat (g/d)						
Concentrate	115	111	111	107	0.08	0.221
Rice straw	19.8	19.8	19.8	19.8	—	—
Oils	—	140	140	140	—	—
Total	134.8 ^b	270.8 ^a	270.8 ^a	266.8 ^a	14.33	<0.01

^{a,b} Within a row, means without a common superscript letter differ at $P < 0.05$

CON = Control; LOFO = linseed oil + fish oil; SOFO = sunflower oil + fish oil; LOSOFO = linseed oil + sunflower oil + fish oil; SEM = standard error of the mean

g FA) whereas the main FA in rice straw was C16:0 (40.06 g/100 g FA) and C18:1n-9 (24.26 g/100 g FA). The LO oil contained mainly C18:3n-3 (55.87 g/100 g FA) while the main FA in the SO was C18:2n-6. The FO contained C22:6n-3 (26.76 g/100 g FA) and C16:0 (22.25 g/100 g FA) (Table 2).

Feed and Fatty Acid Intakes

All groups consumed similar ($P > 0.05$) DM and CP (Table 3); however, the oil-supplemented groups ate significantly more fat ($P < 0.01$) than the control group. No significant difference in fat consumption among the oil-supplemented group was observed.

Since the oils contained different FA profiles, the individual FA intakes varied according to the supplemented oil. The consumption of C8:0 and C10:0 were similar; however, the cattle that received the LOFO and SOFO diets ate significantly more C14:0, C16:0, C18:0, C18:1n-9, C20:5n-3, and C22:6n-3 than the cattle fed the control and LOSOFO diets, and the LOSOFO cattle significantly consumed more of these FAs than the non-supplemented control group. For other FAs, the control group ate significantly more C12:0 than the oil-supplemented group. The intake of C18:2n-6 was highest in the SOFO cattle, followed by the LOSOFO, LOFO, and the control group,

Table 4. Individual fatty acid intakes of experimental fistulated cattle

Intake (g/d)	CON	LOFO	SOFO	LOSOFO	SEM	P-Value
C8:0	0.84 ^c	0.85 ^c	1.05 ^a	0.97 ^b	0.03	0.001
C10:0	1.53 ^c	1.99 ^b	2.37 ^a	2.02 ^b	0.04	0.001
C12:0	20.45 ^a	19.86 ^b	19.92 ^b	19.15 ^c	0.12	0.001
C14:0	7.26 ^d	10.59 ^a	10.09 ^b	9.21 ^c	0.06	0.001
C16:0	20.32 ^d	39.82 ^b	42.09 ^a	36.16 ^c	0.32	0.001
C18:0	3.09 ^d	12.40 ^a	12.20 ^b	10.73 ^c	0.02	0.001
C18:1n-9	36.32 ^d	56.77 ^c	64.76 ^a	61.86 ^b	0.34	0.001
C18:2n-6	23.16 ^d	35.78 ^c	64.03 ^a	57.06 ^b	0.20	0.001
C18:3n-3	0.89 ^d	40.84 ^a	2.40 ^c	27.67 ^b	0.12	0.001
C20:5n-3	ND	7.56 ^a	7.56 ^a	5.04 ^b	0.49	0.001
C22:6n-3	ND	22.01 ^a	22.01 ^a	14.67 ^b	0.19	0.001
Total	113.85 ^c	248.49 ^a	248.49 ^a	244.54 ^b	14.33	0.001

^{a,b,c,d} Within a row, means without a common superscript letter differ at $P < 0.05$ CON = Control; LOFO = linseed oil + fish oil; SOFO = sunflower oil + fish oil; LOSOFO = linseed oil + sunflower oil + fish oil; SEM = standard error of the mean

respectively, whereas the consumption of C18:3n-3 was highest in the LOSO cattle, followed by the LOSOFO, SOFO, and the control group, respectively.

The current study limited the feed intake at the maintenance level to avoid different DM and CP consumptions; however, we focused on the ruminal bio-hydrogenation and fermentation in response to oil supplementation rather than feed intake. Thus, the DM and CP intakes were similar, although the oil-supplemented group consumed significantly more fat than the control group. The intakes of FAs varied depending on the type of supplemented oil. The cattle fed the LOFO and LOSOFO diets ate significantly more C18:3n-3 than the cattle fed the control and SOFO diets, whereas the cattle fed the SOFO and LOSOFO diets consumed significantly more C18:2n-6 than the cattle fed the control and LOFO diets. In addition, the cattle fed the LOFO and SOFO diets ate more C20:5n-3 and C22:6n-3 than the cattle fed the control and LOSOFO diets.

Ruminal Fatty Acid Concentration

At 0 h before feeding, there were no significant differences in ruminal FA content among the treatments (Table 5). At 2 h post-feeding, ruminal concentrations of C12:0, C14:0, and C18:1n-9c were similar; however, the ruminal proportion of C16:0 was significantly higher ($P < 0.01$) in the control

group than in the oil-supplemented groups (Table 5). There was no significant difference in the ruminal proportion of C16:0 among the oil-supplemented groups. The ruminal proportion of C18:0 was highest in the control group, followed by the LOSOFO cattle and was lowest in the LOFO and SOFO cattle. The ruminal concentration of C18:1n-9t was lowest in the control group but was highest in the SOFO cattle. The cattle receiving the SOFO diet showed significantly higher ($P < 0.01$) ruminal C18:2n-6c content than the other cattle, while the cattle receiving the LOFO diet had a significantly higher ruminal C18:3n-3 proportion than the other cattle. The ruminal concentration of *c9, t11*-C18:2 was significantly higher ($P < 0.01$) in the LOFO and SOFO groups than in the control and LOSOFO groups. The cattle receiving the LOFO and SOFO diets had significantly higher ruminal C20:5n-3 and C22:6n-3 than the cattle receiving the LOSOFO diet while these FAs were not found in the cattle receiving the control diet.

At 4 h post-feeding (Table 5), the ruminal proportions of C12:0 and C14:0 were similar in all treatments; however, the ruminal concentrations of C16:0 and C18:0 were significantly higher ($P < 0.01$) in the cattle fed the control diet than those on oil-supplemented diets. The ruminal contents of C18:1n-9t and C18:2n-6c were significantly higher ($P < 0.01$) in the cattle fed the SOFO and LOSOFO diets

Table 5. Ruminal fatty acid content of experimental fistulated cattle

Fatty acid (g/100g)	CON	LOFO	SOFO	LOSOFO	SEM	P-Value
0 h pre-feeding						
C12:0	7.36	7.41	7.07	7.45	0.09	0.462
C14:0	6.42	6.26	6.05	6.00	0.07	0.501
C16:0	36.69	37.04	36.95	37.08	0.09	0.631
C18:0	33.95	33.69	34.53	34.20	0.13	0.264
C18:1n-9t	10.39	10.27	10.15	10.13	0.04	0.152
C18:1n-9c	5.19	5.34	5.26	5.15	0.09	0.876
2 h post-feeding						
C12:0	7.13	7.64	6.95	7.63	0.29	0.758
C14:0	9.23	7.71	7.34	8.80	0.65	0.504
C16:0	32.36 ^a	17.88 ^b	17.04 ^b	17.87 ^b	0.30	0.001
C18:0	32.17 ^a	7.73 ^c	7.71 ^c	15.07 ^b	0.36	0.001
C18:1n-9t	9.05 ^c	14.72 ^b	18.50 ^a	15.92 ^{ab}	0.38	0.001
C18:1n-9c	5.19	6.23	5.26	5.80	0.18	0.196
C18:2n-6c	1.48 ^c	1.97 ^c	18.39 ^a	7.05 ^b	0.36	0.001
C18:3n-3	1.29 ^d	20.06 ^a	4.50 ^c	11.68 ^b	0.30	0.001
c9,t11- C18:2	2.10 ^b	3.40 ^a	3.04 ^a	2.09 ^b	0.15	0.041
C20:5n-3	ND	4.63 ^a	3.72 ^a	2.28 ^b	0.15	0.001
C22:6n-3	ND	8.03 ^a	7.55 ^a	5.82 ^b	0.24	0.001
4 h post-feeding						
C12:0	7.19	8.46	8.14	7.59	0.31	0.491
C14:0	8.39	8.43	8.54	7.73	0.10	0.157
C16:0	32.63 ^a	14.40 ^c	14.75 ^c	18.14 ^b	0.28	0.001
C18:0	38.28 ^a	8.06 ^c	8.83 ^c	10.99 ^b	0.23	0.003
C18:1n-9t	4.70 ^c	10.99 ^b	16.17 ^a	15.85 ^a	0.45	0.001
C18:1n-9c	5.22 ^b	6.90 ^a	6.13 ^a	5.77 ^{ab}	0.14	0.001
C18:2n-6c	1.17 ^b	2.17 ^b	11.39 ^a	11.24 ^a	0.26	0.009
C18:3n-3	0.31 ^d	22.24 ^a	5.26 ^c	11.61 ^b	0.33	0.003
c9,t11- C18:2	2.11 ^c	3.77 ^b	7.58 ^a	3.05 ^b	0.17	0.008
C20:5n-3	ND	5.44 ^a	4.36 ^a	2.26 ^b	0.19	0.001
C22:6n-3	ND	9.14 ^a	8.82 ^a	5.78 ^b	0.25	0.001
6 h post-feeding						
C12:0	8.32 ^a	5.95 ^b	5.78 ^b	6.88 ^b	0.16	0.001
C14:0	21.25 ^a	16.89 ^b	16.10 ^b	14.48 ^b	0.08	0.001
C16:0	41.93	40.40	40.76	47.08	0.88	0.277
C18:0	11.51 ^a	0.41 ^b	0.29 ^b	2.44 ^b	0.28	0.001
C18:1n-9t	8.69 ^b	13.06 ^a	14.14 ^a	10.26 ^a	0.47	0.001
C18:1n-9c	4.91 ^c	7.36 ^a	6.55 ^a	5.81 ^b	0.16	0.001
C18:2n-6c	2.44	2.08	1.99	2.19	0.12	0.577
C18:3n-3	0.95	0.77	0.67	0.72	0.07	0.586
C20:5n-3	ND	2.07	2.37	2.56	0.33	0.098
C22:6n-3	ND	11.02 ^a	11.36 ^a	7.57 ^b	0.36	0.001

^{a,b,c} Within a row, means without a common superscript letter differ at $P < 0.05$ CON = Control; LOFO = linseed oil + fish oil; SOFO = sunflower oil + fish oil; LOSOFO = linseed oil + sunflower oil + fish oil; SEM = standard error of the mean

than those on the control and LOFO diets, whereas the ruminal proportion of C18:1n-9c was highest in the cattle fed the LOFO and SOFO diets when compared with the cattle fed the control diet. The cattle supplemented with LOFO had a significantly higher ($P < 0.01$) ruminal content of C18:3n-3 than the other cattle. The highest ruminal concentration of c9, t11-C18:2 was found in the cattle fed the SOFO diet, followed by the cattle fed the LOFO and LOSOFO diets, whereas the cattle fed the control diet had the lowest concentration of this FA. The highest ruminal concentrations of C20:5n-3 and C22:6n-3 were

found in the LOFO and SOFO cattle, followed by the LOSOFO and the lowest concentration was found in the control group.

At 6 h post-feeding (Table 5), there were no significant differences in the ruminal contents of C16:0, C18:2n-6c, C18:3n-3, and C20:5n-3; however, the ruminal proportions of C12:0, C14:0, and C18:0 were significantly higher ($P < 0.01$) in the control group than in the other groups. The cattle receiving the oil-supplemented diets had significantly higher ($P < 0.01$) contents of C18:1n-9t and C18:1n-9c than the cattle receiving the control diet, whereas the cattle receiving the SOFO and

Table 6. Ruminal pH, ammonia N concentration, total VFA concentration, and individual VFA proportion of fistulated cattle

Details	h	CON	LOFO	SOFO	LOSOFO	SEM	P-value
pH	0 h	7.16	7.20	7.32	7.29	0.05	0.214
	2 h	7.06	7.14	7.12	7.01	0.06	0.478
	4 h	6.71	6.81	6.84	6.75	0.09	0.791
	6 h	6.75	6.85	6.99	6.92	0.08	0.354
NH ₃ -N (mg/ 100 mL)	0 h	74.6	71.9	89.1	77.3	9.8	0.648
	2 h	151.4	144.9	165.5	168.4	10.9	0.486
	4 h	89.7 ^a	69.7 ^b	68.8 ^b	55.7 ^c	0.11	0.031
	6 h	63.3	45.1	43.2	39.9	0.45	0.764
Acetate (Ac) (mol/100 mol)	0	74.17	72.33	72.27	72.73	1.05	0.432
	2	72.46	72.27	75.14	72.62	0.79	0.148
	4	69.29	68.97	72.14	71.29	1.49	0.473
	6	66.57	71.45	67.30	70.18	1.29	0.774
Propionate (Pr) (mol/100 mol)	0	18.74	22.37	22.02	19.76	0.65	0.339
	2	19.70	19.19	19.28	22.19	0.50	0.127
	4	22.99	23.27	19.93	20.65	0.97	0.624
	6	24.89	22.12	24.99	22.19	1.08	0.651
Butyrate (Bu) (mol/ 100 mol)	0	7.09	5.30	5.71	7.51	0.52	0.354
	2	7.84	8.54	5.58	5.18	0.43	0.069
	4	7.72	7.75	7.93	8.06	0.67	0.132
	6	8.54	6.43	7.71	7.63	0.60	0.483
Ac:Pr	0	3.96	3.23	3.28	3.68	0.15	0.344
	2	3.68	3.77	3.90	3.27	0.12	0.157
	4	3.01	2.96	3.62	3.45	0.24	0.621
	6	2.67	3.23	2.69	3.16	0.22	0.868

CON = Control; LOFO = linseed oil + fish oil; SOFO = sunflower oil + fish oil; LOSOFO = linseed oil + sunflower oil + fish oil; SEM = standard error of the mean

LOFO diets had significantly higher ruminal C22:6n-3 than those cattle fed the control and LOSOFO diets.

Before feeding (h 0), the ruminal proportions of all the FAs were similar; however, at 2, 4, and 6 h after feeding the ruminal proportions of C16:0 and C18:0 were significantly decreased. In contrast, the ruminal proportion of *t11*-C18:1 was increased by oil supplementation. Similar results have also been reported (Lor et al., 2005). It is well known that in the bio-hydrogenation process, there are 2 groups of bacteria involved, namely Group A and Group B bacteria according to the metabolic pathway involved or the pattern of bio-hydrogenation (Kemp and Lander, 1984). Group A bacteria can hydrogenate PUFA to *t11*-C18:1 while Group B bacteria are involved in hydrogenation of the C18:1 and C18:1 isomers to C18:0. Thus the major intermediates of the bio-hydrogenation process are *t11*-C18:1 and C18:0 (AbuGhazaleh et al., 2002). However, FO inclusion can inhibit bacteria from altering unsaturated FA (UFA)

to saturated fatty acid (SFA) (Jenkins et al., 2008).

An increase in *t11*-C18:1 in the present study is a result of FO inclusion in combination with LO and/or SO compared to the control. Similarly, Toral et al. (2010a) supplemented SO in the diet and found a significant increase in *t11*-C18:1 but a reduction in C18:0 when FO was added. AbuGhazaleh et al. (2002) previously reported an increase accumulation in *t11*-C18:1 with increasing C22:6n-3 additions. Moreover, AbuGhazaleh and Jenkins (2004) reported a positive correlation between the C22:6n-3 and *t11*-C18:1 proportions. However, there is still an unidentified source of *t11*-C18:1, and it is only known that *t11*-C18:1 is a precursor for *c9*, *t11*-C18:2 syntheses in animal tissues. Supplementation of 1, 2, 3, or 4% C22:6n-3 can increase the *trans*-18:1 isomer but inhibit bio-hydrogenation of C18:1n-9 and C18:2n-6 resulting in a reduction of C18:0 (AbuGhazaleh and Jenkins, 2004). This indicates that the LO and FO blend resulted in increased C18:3n-3 and C22:6n-3

flows and may also increase the biohydrogenation intermediates of C18:1n-9, C18:2n-6, and CLA.

Loor *et al.* (2005) reported that supplementation of FO to cattle diets increased ruminal concentrations of C20:5n-3 and C22:6n-3 while those cattle with diets supplemented with LO and SO did not have these FAs. The present study observed an increase in ruminal *c9*, *t11*-C18:2 in cattle receiving the LOFO and SOFO diets compared with the cattle on the control diet. AbuGhazaleh *et al.* (2002) also reported that the addition of soybean oil, FO, and oil blended into the diets significantly increased ruminal *c9*, *t11*-C18:2. Similarly, Jalč *et al.* (2009) supplemented plant oils rich in C18:2n-6 in combination with FO in different proportions of C18:2n-6 and found linear increases in *c9*, *t11*-C18:2 with increasing C18:2n-6.

Ruminal pH, NH₃-N and VFAs

The addition of 140 g/d LO or SO in combination with FO had no influence on ruminal pH at all the sampling times (Table 6). No significant differences in ruminal pH were reported when the cattle's diets were supplemented with FO or SO, respectively (Fievez *et al.*, 2003; Beauchemin *et al.*, 2007). Doreau *et al.* (2009) reported that LO had no effect on the ruminal fermentation pattern, including ruminal pH. Additionally, Harvatine and Allen (2006) suggested that the use of SFA and UFA in the diets had minor or no influence on the ruminal pH. However, Messana *et al.* (2013) reported that, in animals receiving fat up to 60 g/kg, the ruminal pH curvilinear reduced as the fat level increased. Shingfield *et al.* (2003) also observed a significant reduction in ruminal pH when FO was fed, which is because of a decrease in the DMI resulting in a reduced pH.

In the present study, the ruminal NH₃-N concentration was significantly reduced ($P < 0.05$) in the oil-supplemented group at 4 h after feeding when compared with the control group. Shingfield *et al.* (2013) reported a tendency toward a reduction in NH₃-N when oil was added. The source of linoleic acid supplementation also reduced ruminal NH₃-N in sheep (Shingfield

et al., 2006), whereas Zhang *et al.* (2008) supplied FO in combination with the source of C18:2n-6 in sheep's diet and reported a significant decrease in ruminal NH₃-N. Neveu *et al.* (2014) mentioned that ruminants use NH₃-N for amino acid synthesis and microbial growth.

In the current study, to avoid factors disturbing ruminal fermentation, oil supplements were limited at 30 g/kgDM intake; thus, at all h after feeding, the molar proportions of acetic, propionic, and butyric acids were similar as well as the acetic:propionic acid ratio. Lee *et al.* (2008) and Doreau *et al.* (2009) also previously reported no influence of the respective FO and various forms of linseed supplementation on the molar proportions of those VFAs; however, Shingfield *et al.* (2010) found an increase in the ruminal proportion of propionate:acetate in steers fed an incremental level of FO. It is possible that change in the ruminal fermentation pattern relates to the effect of FO on nutrient digestibility in the rumen and a change in the microbial population. Additionally, Shingfield *et al.* (2011) supplemented linseed oil and FO alone, or a blend of these oils, and reported that the FO manipulated ruminal fermentation toward an increase in the molar proportion of propionate rather than acetate with no change in the molar proportion of butyrate. In another study, Ueda *et al.* (2003) supplemented LO or whole linseed at 3 and 4% of added oil in the diet and showed higher increases in the molar proportion of propionate rather than acetate. Additionally, Jalč *et al.* (2009) supplemented a different level of oil rich in omega-6 FA in combination with FO and found an increase in propionic acid and a reduction in acetic acid when compared to the control. Similarly, Toral *et al.* (2010b) found a significant increase in the molar proportion of propionic acid when FO and SO were supplemented compared to the un-supplemented control.

Conclusions

The present study reveals that, after feeding, supplementation of LO or SO in combination with FO markedly altered the ruminal FA

concentration. The ruminal C18:1n-9t and CLA proportion were highest in cattle fed LOFO, SOFO, and LOSOFO, followed by the proportion of C18:0 which was decreased. The ruminal C18:3n-3 proportion was highest in the cattle fed the LOFO diet. Additional oils with FO in the diet significantly increased C20:5n-3 and C22:6n-3. However, no significant difference was found for ruminal pH, VFAs, and NH₃-N, except for NH₃-N at 4 h after feeding that was highest in the cattle fed the control diet.

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