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Original Article

Ruminal bio-hydrogenation and fermentation in response to soybean oil and fish oil addition to fistulated cattle's diets

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Abstract

Two experiments were carried out to evaluate the bio-hydrogenation of fish oil **(**FO**)** and combination with soybean oil **(**SBO**)** on fistulated dry cows**.** The experiment 1, was assigned treatments as follows; control, SBO, FO and SBO+FO **(**1**:**1**)**; using 4×4 Latin square design**.** Results showed that FO and SBO+FO decreased C18**:**0 when compared to control and SBO**.** However, the SBO+FO group had the greater *t11*-C18**:**1 after feeding**.** Supplemented FO were reduced ruminal pH and acetic acid at 2 hrs, while the ammonia-N **(**NH_3-N**)** was increased all times after feeding**.** The experiment 2, was supplementation of FO + SBO at ratios 2**:**1, 1**:**1 and 1**:**2; using 3×3 Latin square design**.** Results showed that SBO+FO **(**2**:**1**)** had high proportion of *t11*-C18**:**1 than 1**:**1 and 1**:**2 ratios**.** Moreover, increasing the proportion of FO in the combination oils can increased NH_3-N at 2 hrs and propionic acid at 4 and 6 hrs**.**

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

1. Introduction

The purpose of addition oils into the diet is to supply dietary energy to ruminants; however, oils themselves can modify the fatty acid composition of the animal products particularly to improve quality of animal products. Plant oils have been reported to be a good strategy for increasing milk *c9*, *t11*-C18:2 (CLA) contents in goats (Marín *et al.*, 2011). Additionally, multiple studies have attempted to increase the concentration of 20:5n-3 (EPA) and 22:6n-3 (DHA) in ruminant milk by adding fish oil to the diet, but the apparent transfer rate of these FAs from diet to milk is relatively low (Toral *et al.*, 2010). However, as a rumen bio-hydrogenation modulator, fish oil yields large increases in milk CLA concentrations, particularly when combined with plant oils either in goats, cows, or sheep (Gagliostro *et al.*, 2006; Shingfield *et al.* 2006; Toral *et al.* 2010).

Earlier studies on the addition of lipids to ruminant diets as an energy source raised concerns about detrimental effects of fatty acids on ruminal fermentation (Jenkins, 1993). Rumen bacteria play the main role in lipid metabolism in the rumen (Jenkins, Wallace, Moate, & Mosley, 2008). Lipids are extensively hydrolyzed in the rumen, rendering fatty acids that have bacteriostatic and bactericidal effects. Among them, unsaturated fatty acids are more antimicrobial than saturated ones (Harfoot & Hazlewood, 1997), and a differential toxicity of different PUFA to rumen microorganisms has also been observed (Maia, Chaudhary, Figueres, & Wallace, 2007). Dietary supplementation with oils has given inconsistent results on ruminal fermentation, with detrimental consequences (Fievez, Dohme, Daneels, Raes, & Demeyer, 2003). Therefore, the aim of this study was to evaluate the effect of different ratio of oil rich in soybean oil (SBO) and fish oil (FO) on ruminal bio-hydrogenation and fermentation in fistulated cattle.

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2. Materials and Methods

The study comprised of two experiments; Experiment 1 and Experiment 2 both 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

In experiment 1 four fistulated dry cows were conducted by 4x4 Latin square design including no oil (control), supplemented SBO, FO and 1:1 w/w SBO+FO and each supplemental oil was fed at 3% of total feed DM. The experiment consisted of four periods, with 21 days in each period with the first 7 day as adaptation followed by 14 day for trial. Followed by Experiment 2 was using three fistulated dry cows and assign into 3x3 Latin square design with three dietary as follows supplemented 3% SBO+FO of total feed DM in different ratios, 2:1 w/w, 1:1 w/w and 1:2 w/w SBO+FO. The experimental periods were divided into three periods of 21 days each, which were preceded by a 7 day period for adaptation while the last 14 days for trial.

All the animals in experiment 1 and 2 were housed in individual pen. Rice straw was used as roughage source and feeding was restrocted at 2.4 kg/d and 4.0 kg/d for the concentrate. The feeding divided into two equal meals and offered at 08.00 and 16.00 hrs.

2.2 Feed sampling and analysis

The rice straw and concentrate were sampled daily and DM content (48 hrs at 60 °C) was determined daily to calculate DMI of each cow. Dried samples were pooled and then ground through a 1-mm screen for chemical analysis of analytical DM, CP, EE and ash (Association of Official Analytical Chemists, 1995). For NDF and ADF analyses were conducted based on the procedure described by Van Soest, Robertson, & Lewis (1991).

2.3 Ruminal fermentation

To evaluate ruminal fermentation, on the last day of each period (day 21), ruminal content samples were collected from each cow at 0, 2, 4 and 6 hrs after the morning feeding. The pH of rumen content was immediately determined at the time of sampling. For VFA and ammonia nitrogen (NH_3-N) determination, 36 mL of rumen content from individual cows at each sampling time was put into 50 mL centrifuge tubes containing 4 mL of 1 M H_2SO_4. Tubes were centrifuged at 8,000×g for 20 min at 4 °C; supernatants were collected into 25 mL test tubes, capped and stored at -20 °C until analysis. Analysis of acetic acid, propionic acid and butyric acid were performed by gas chromatography (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 (Association of Official Analytical Chemists, 1995).

2.4 Fatty acid determination

The ruminal content was collected on d 21 of each period at 0, 2, 4 and 6 hrs after the morning feeding described by AbuGhazaleh, Schingoethe, Hippen, Kalscheur, and Whitlock (2002) and stored at -20 °C until analysis. Fatty acids composition of concentrate, rice straw, oils 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 were analyzed by gas chromatography (GC) (7890A GC System, Agilent Technology, USA).

2.5 Statistical analysis

Measurements of mean DMI, pH, NH_3-N, and VFA 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 P-value of 0.05 was used to declare significant differences amongst treatments and tendencies were discussed at $0.05 < P < 0.10$.

3. Results and Discussion

3.1 Chemical composition and fatty acid profile of diets

The concentrate used in this study contained 14.1% CP and 3.4% EE. CP and EE of rice straw were 2.0% and 1.4% respectively. SBO and FO in the current study contained 100% EE (Table 1).

C18:2n-6 was the major fatty acid (FA) in the SBO approximately 44.74% of total FA. FO had the highest proportion of C22:6n-3 and C20:5n3 (30.47% and 7.98% of total FA, respectively). In the concentrate, C18:1n-9 (29.51% of total FA) and C12:0 (22.74% of total FA) were the main fatty acids. The main FA in rice straw was C16:0 (45.71% of total FA) shown in Table 2.

Table 1. Chemical composition of the experimental diets

Items	Concentrate[1]	Rice straw	SBO	FO
Dry matter	89.4	88.2	100	100
	…… % of DM……			
Ash	8.1	18.3		
Crude protein	14.1	2.0		
Ether extract	3.4	1.4	100	100
Crude fiber	14.8	40.1		
NDF	40.4	76.3		
ADF	22.8	53.8		
ADL	4.2	17.5		

SBO = Soybean oil, FO = Fish 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	SBO	FO
C12:0	22.74	6.37	0.43	2.16
C14:0	7.81	8.20	1.09	4.39
C16:0	16.63	45.71	13.74	28.02
C18:0	2.50	0.12	5.26	6.10
C18:1n-9	29.51	24.81	33.87	14.42
C18:2n-6	17.14	11.47	44.74	1.71
C18:3n-3	0.25	ND	0.35	0.93
C20:5n-3	ND	ND	ND	7.98
C22:6n-3	ND	ND	ND	30.47
Others[1]	3.42	3.28	0.52	3.83

[1]Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0. ND = Not detected

3.1.1 Experiment 1

1) Fatty acid profile in ruminal content

Supplementation of SBO, FO and SBO + FO resulted in higher ruminal concentration of *t11*-C18:1 after 2, 4 and 6 hrs feeding (Table 3). Similarly, Toral *et al.* (2010) added oil rich in C18:2n-6 into the diet and found significant increase in the amount of *t11*- C18:1. Increase in the concentration of *t11*-C18:1 with oil supplement resulted from the increase in inputs of dietary C18 unsaturated fatty acids, the precursors for *t11*-C18:1. The current study has confirmed the greater concentration of *t11*- C18:1 along with the lower concentrations of C18:0 with the FO addition relative to incomplete bio-hydrogenation from FO. FO addition will shift these processes by inhibition of bacterial conversion

Table 3. Effect of SBO, FO and SBO+FO supplementation on fatty acid profile in ruminal content (g/100g fatty acids)

Fatty acids	Control	SBO	FO	SBO+FO	SEM	P-value
Pre - feeding						
C12:0	12.91	12.58	13.07	12.47	0.155	0.413
C14:0	9.18	8.80	8.83	8.50	0.262	0.884
C16:0	33.89	34.44	34.69	34.66	0.142	0.754
C18:0	38.76	37.51	37.15	38.73	0.363	0.367
t11-C18:1	1.45	2.19	2.00	1.73	0.102	0.365
C18:1n-9	2.49	2.88	2.65	2.28	0.389	0.833
C18:2n-6	1.31	1.71	1.60	1.63	0.243	0.984
2 hrs after feeding						
C12:0	7.42	6.62	7.13	7.85	0.275	0.382
C14:0	5.84	5.06	6.04	5.90	0.503	0.322
C16:0	34.21^{a}	18.37^{b}	33.09^{a}	26.73^{ab}	1.411	0.045
C18:0	48.04^{a}	28.70^{b}	6.47^{c}	8.28^{c}	1.194	<0.001
C18:1n-9	1.45^{b}	7.24^{a}	8.94^{a}	8.46^{a}	0.707	0.044
C18:2n-6	2.19^{b}	5.52^{a}	1.69^{b}	2.11^{b}	0.341	0.042
C18:3n-3	0.48	0.56	0.55	0.58	0.025	0.222
t11-C18:1	0.37^{c}	18.84^{b}	22.88^{ab}	26.72^{a}	0.670	<0.001
c9,t11-C18:2	ND^{b}	9.13^{a}	ND^{b}	9.25^{a}	0.125	<0.001
C20:5n-3	ND^{c}	ND^{c}	1.31^{b}	0.56^{a}	0.071	0.005
C22:6n-3	ND^{b}	ND^{b}	11.90^{a}	3.55^{b}	0.617	0.004
4 hrs after feeding						
C12:0	3.53^{b}	6.04^{a}	5.75^{ab}	7.30^{a}	0.338	0.046
C14:0	5.64	5.40	5.77	5.71	0.474	0.986
C16:0	41.33^{a}	20.53^{c}	34.15^{b}	28.47^{b}	0.957	0.003
C18:0	34.13^{a}	29.99^{b}	8.03^{c}	7.68^{c}	0.780	<0.001
C18:1n-9	1.21^{c}	6.41^{ab}	5.15^{b}	9.46^{a}	0.477	0.007
C18:2n-6	3.20^{ab}	4.58^{a}	1.07^{b}	1.31^{b}	0.362	0.044
C18:3n-3	0.11^{b}	0.57^{a}	0.66^{a}	0.56^{a}	0.044	0.025
t11-C18:1	10.85^{c}	21.59^{b}	25.72^{ab}	29.41^{a}	0.821	<0.001
c9,t11-C18:2	ND^{b}	4.88^{a}	ND^{b}	5.97^{a}	0.291	0.002
C20:5n-3	ND^{c}	ND^{c}	1.16^{a}	0.75^{b}	0.029	<0.001
C22:6n-3	ND^{c}	ND^{c}	12.53^{a}	3.36^{b}	0.201	<0.001
6 hrs after feeding						
C12:0	5.36^{b}	6.74^{ab}	8.81^{ab}	11.14^{a}	0.773	0.086
C14:0	5.40^{b}	4.45^{b}	8.05^{a}	6.98^{a}	0.309	0.017
C16:0	21.65^{b}	21.91^{b}	33.91^{a}	30.27^{a}	1.154	0.012
C18:0	48.17^{a}	37.97^{b}	7.40^{c}	8.30^{c}	1.920	0.013
C18:1n-9	2.03^{b}	4.82^{ab}	5.04^{ab}	6.99^{a}	0.454	0.051
C18:2n-6	2.23^{a}	1.80^{a}	0.96^{b}	0.97^{b}	0.116	0.025
C18:3n-3	0.06^{b}	0.61^{a}	0.65^{a}	0.78^{a}	0.032	0.002
t11-C18:1	15.10^{c}	16.96^{bc}	24.56^{ab}	29.43^{a}	1.146	0.020
c9,t11-C18:2	ND^{c}	4.73^{a}	ND^{c}	1.83^{b}	0.064	<0.001
C20:5n-3	ND^{c}	ND^{c}	0.57^{a}	0.26^{b}	0.016	<0.001
C22:6n-3	ND^{c}	ND^{c}	10.05^{a}	3.01^{b}	1.151	0.063

SEM = standard error of the mean. ^{abc} Within a row means without a common superscript letter differ (P<0.05)

unsaturated fatty acids to saturated fatty acid (Jenkins *et al.*, 2008). The cattle received FO had greater ruminal concentration of C20:5 n-3 and C22:6n-3 when compared to those cattle received control and SBO. The main PUFAs in FO were C22:6n-3 and C20:5n-3. Loor *et al.* (2005) reported that FO supplementation in cows increased the concentration of C20:5n-3 and C22:6n-3 in the rumen of cattle.

2) Ruminal fermentation

In the current study addition of SBO and FO into the diet can decreased ruminal pH at 2 hrs after feeding when compare to control and SBO+FO group. Moreover, SBO and FO supplementation significantly reduced molar proportion of acetic acid while as increased molar proportion of propionic acid (Table 4). Amorocho, Jenkins, and Staples (2009) reported that when FO supplemented can decreased in ruminal pH and resulted in lower levels of lipolytic activity and bio-hydrogenation of unsaturated FA in ruminal fluid. Most rumen microbes are sensitive to low pH conditions as acidity in the rumen impact microbial growth and affected to VFAs production in the rumen (Jenkins *et al.*, 2008). The lower ruminal pH can reduce cellulolytic bacteria and decreased acetic acid production (Gudla, Ishlak, & AbuGhazaleh 2012). However, in the previous study it was reported that supplemented FO and sunflower oil and showed significant increase in molar proportion of propionic acid when compared to control group (Toral, Hervás, Suárez-Vega, Arranz, & Frutos, 2016).

FO added to the diet significantly increased ammonia nitrogen in rumen fluid at 2, 4 and 6 hrs after feeding, which similar resulted to Zhang *et al.* (2008) who observed significant decreases in NH_3-N when FO combine with linoleic acid sources were supplemented in sheep.

3.1.2 Experiment 2

1) Fatty acid profile in ruminal content

Adding high proportion of FO (1:2 w/w SBO+FO) into the diets increased the ruminal C20:5n-3 and C22:6n-3 (Table 5) from the greater intake of C20:5n-3 and C22:6n-3 similar to Kim *et al.* (2008) supplemented 2.3% and 6.9% FO and found that the concentration of C20:5n-3 and C22:6n-3 were linearly increased when compare to none supplemented FO. The ruminal *c9, t11*-C18:2 was significantly decreased in cattle fed 1:2 w/w SBO+FO. Jâlc, Certik, Kundrikova, and Namestkova (2007) showed that supplemented oil rich in C18:2n-6 mixed with FO at 1:1, 3:1 and 5:1, the concentration of *t11*-C18:1 and *c9,t11*-C18:2 in the rumen was linearly increased. Thus, high level of C18:2n-6 supplementation resulted in greater *t11*-C18:1 in the rumen. The *t11*-C18:1 was the product of incomplete bio-hydrogenation of C18:2n-6 in the rumen (Kepler, Tucker, & Tove, 1970). Beam, Jenkins, Moate, Kohn, and Palmquist (2000) reported that the overall rate of bio-hydrogenation of C18:2n-6 was 14.3%/hr but declined by 1.2% /hr for each percentage unit increase in C18:2n-6 added to the substrate.

Table 4. Effect of SBO, FO and SBO+FO supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in ruminal content

Item	Control	SBO	FO	SBO_FO	SEM	P-value
Pre-feeding						
pH	6.94	6.87	6.89	6.93	0.019	0.238
NH_3N	8.92	8.99	8.81	8.87	0.093	0.747
Acetic acid	67.63	67.77	67.97	67.64	0.771	0.273
Propionic acid	16.64	16.57	16.86	16.87	0.576	0.181
Butyric acid	15.73	15.66	15.17	15.49	0.772	0.391
A:P ratio	4.06	4.09	4.03	4.01	0.094	0.119
2 hrs after feeding						
pH	6.92^{a}	6.76^{b}	6.76^{b}	6.88^{a}	0.016	0.027
NH_3N	14.52^{b}	15.18^{b}	19.23^{a}	15.74^{b}	0.380	0.024
Acetic acid	64.93^{a}	57.06^{b}	55.94^{b}	62.58^{a}	0.528	0.006
Propionic acid	20.96^{b}	27.84^{a}	28.69^{a}	23.05^{b}	0.376	0.002
Butyric acid	14.10	15.10	15.38	14.37	0.262	0.294
A:P ratio	3.10^{a}	2.06^{c}	1.96^{c}	2.72^{b}	0.038	<0.001
4 hrs after feeding						
pH	6.66	6.58	6.78	6.51	0.054	0.345
NH_3N	5.68^{b}	5.64^{b}	8.27^{a}	6.93^{ab}	0.334	0.048
Acetic acid	62.64	64.79	63.75	64.27	0.290	0.136
Propionic acid	23.62	22.18	24.07	22.07	0.558	0.448
Butyric acid	13.74	13.07	12.18	13.66	0.561	0.674
A:P ratio	2.66	2.92	2.69	2.92	0.070	0.355
6 hrs after feeding						
pH	6.72	6.52	6.54	6.42	0.053	0.299
NH_3N	6.09^{b}	6.50^{b}	8.74^{a}	7.68^{ab}	0.247	0.048
Acetic acid	66.94	68.48	63.67	64.03	1.336	0.476
Propionic acid	23.63	20.13	24.35	22.13	0.681	0.198
Butyric acid	9.43	11.39	11.98	13.84	0.735	0.251
A:P ratio	3.05	3.40	2.63	3.02	0.092	0.111

SEM = standard error of the mean. A:P ratio = Acetic acid:Propionic acid. ^{abc} Within a row means without a common superscript letter differ ($P<0.05$).

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

Items	SBO+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	12.81	12.23	12.41	0.177	0.516
C14:0	9.10	9.06	8.54	0.292	0.782
C16:0	34.41	33.97	34.40	0.282	0.145
C18:0	37.92	39.29	38.84	0.383	0.235
C18:1n-9	2.44	2.36	2.33	0.350	0.990
C18:2n-6	1.32	1.18	1.44	0.036	0.189
t11-C18:1	1.99	1.89	2.03	0.132	0.917
2 hrs after feeding					
C12:0	4.72	5.05	5.00	0.426	0.515
C14:0	4.46	5.76	5.34	0.539	0.174
C16:0	24.46	31.04	27.45	3.225	0.240
C18:0	7.28	8.03	7.53	0.631	0.858
C18:1n-9	4.60	3.78	5.63	0.576	0.194
C18:2n-6	2.90^{a}	2.58^{ab}	2.41^{b}	0.046	0.047
C18:3n-3	0.59	0.66	0.61	0.194	0.487
t11-C18:1	39.16^{a}	28.60^{b}	29.69^{b}	1.782	0.032
c9,t11-C18:2	5.38^{a}	6.69^{a}	2.56^{b}	1.059	0.022
t10,c12-C18:2	2.53	1.19	1.19	0.460	0.775
C20:5n-3	0.69	0.71	0.68	0.371	0.446
C22:6n-3	2.20^{c}	5.05^{b}	7.61^{a}	0.677	0.048
4 hrs after feeding					
C12:0	4.43	4.73	4.34	0.588	0.734
C14:0	4.50	5.84	4.29	0.673	0.175
C16:0	25.57	24.87	28.93	1.701	0.142
C18:0	6.70^{c}	7.92^{b}	8.14^{a}	0.126	0.017
C18:1n-9	5.78	4.91	4.62	0.560	0.222
C18:2n-6	6.17^{a}	1.55^{b}	1.89^{b}	0.808	0.043
C18:3n-3	0.37	0.76	0.33	0.185	0.141
t11-C18:1	36.5^{6}	40.15	39.60	2.437	0.523
c9,t11-C18:2	6.86^{a}	3.90^{b}	0.94^{c}	1.581	0.434
C20:5n-3	0.09^{b}	0.38^{ab}	0.59^{a}	0.187	0.564
C22:6n-3	2.97^{c}	4.99^{b}	6.33^{a}	0.662	0.043
6 hrs after feeding					
C12:0	4.90	4.68	3.48	1.486	0.555
C14:0	4.93	5.25	5.31	0.770	0.824
C16:0	27.77^{b}	31.20^{ab}	34.43^{a}	1.392	0.045
C18:0	7.96	7.32	8.73	0.821	0.312
C18:1n-9	3.80	4.07	4.94	0.659	0.290
C18:2n-6	0.88	0.98	1.04	0.068	0.166
t11-C18:1	46.56^{a}	41.04^{b}	34.06^{c}	0.575	0.002
C20:5n-3	1.23^{b}	1.42^{ab}	1.65^{a}	0.083	0.044
C22:6n-3	1.97^{c}	4.04^{b}	6.36^{a}	0.087	0.037

[abc] Within a row means without a common superscript letter differ ($P<0.05$)

2) Ruminal fermentation

There were no significant differences in ruminal pH at all hours post-feeding (Table 6). Toral, Belenguer, Frutos, and Hervás (2009) who supplemented different ratios of oil rich in C18:2n-6 to FO and found no difference between treatments in ruminal pH. Similar results had also been reported (Beauchemin, McGinn, & Petit, 2007) which they suggested that the pH was not affected by oil supplementation and in agreement with previous *in vivo* studies using different lipid sources. Moreover, in the current study supplemented oils lower than 6% of total feed intake will not affect to ruminal (Messana *et al.*, 2013). NH_3-N concentration was significantly increased in cattle fed 1:2 w/w SBO+FO at 2 hrs post-feeding with the same result to Keady and Mayne (1999) supplemented FO up to 450 g/d and found an increase in ruminal ammonia nitrogen concentration. However, Ferreira *et al.* (2016) concluded that animals receiving diets with 40 g/kg DM of SBO exhibited lower ruminal ammonia concentrations in comparison to the control treatment. It can also be said that an increased ruminal ammonia concentration was due to lower utilization of ammonia available in the rumen for microbial growth.

At 4 hrs post-feeding, molar proportion of propionate was significantly increased in cattle fed 1:2 SBO+FO resulting in significant degreased acetate: propionate ratio. The molar proportion of propionate was significantly increased in cattle fed 1:2 SBO+FO at 6 hrs after feeding. Decreasing of ruminal acetate concentration is a common response to the addition of FO by PUFA may exert an

Table 6. Effect of SBO+FO in difference ration supplementation on pH, ammonia nitrogen (mg/100ml) and volatile fatty acids (mol/100mol) in ruminal content

Items	SBO+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	6.73	6.72	6.74		
NH_3N	9.95	10.78	9.36	2.035	0.961
Acetic acid	65.57	64.39	66.93	0.682	0.463
Propionic acid	20.53	22.07	21.27	0.355	0.392
Butyric acid	13.94	13.54	11.73	0.804	0.583
A:P ratio	3.22	2.94	3.15	0.056	0.303
2 hrs after feeding					
pH	6.42	6.33	6.35	0.039	0.677
NH_3N	20.95^{b}	24.47^{b}	30.28^{a}	0.483	0.031
Acetic acid	61.57	65.50	62.15	0.322	0.115
Propionic acid	27.56	25.86	28.23	0.221	0.134
Butyric acid	10.87	9.64	9.62	0.491	0.583
A:P ratio	2.23^{b}	2.49^{a}	2.20^{b}	0.012	0.044
4 hrs after feeding					
pH	6.00	6.07	6.07	0.040	0.724
NH_3N	10.99	11.20	9.96	0.384	0.497
Acetic acid	67.53^{a}	67.74^{a}	61.70^{b}	0.373	0.039
Propionic acid	23.19^{b}	23.26^{b}	27.08^{a}	0.357	0.072
Butyric acid	9.28	9.00	11.22	0.349	0.200
A:P ratio	2.92^{a}	2.93^{a}	2.29^{b}	0.038	0.032
6 hrs after feeding					
pH	5.99	6.08	5.94	0.105	0.867
NH_3N	7.47	8.09	7.05	1.137	0.934
Acetic acid	67.16	68.74	64.27	0.781	0.247
Propionic acid	22.35^{b}	22.09^{b}	25.41^{a}	0.198	0.045
Butyric acid	10.49	10.31	9.17	0.663	0.721
A:P ratio	2.69	3.13	2.56	0.054	0.092

SEM = standard error of the mean. A:P ratio = Acetic acid:Propionic acid. ^{abc} Within a row means without a common superscript letter differ ($P<0.05$).

inhibitory effect on acetate-producing bacteria (Toral *et al.* 2009). Toral *et al.* (2016) supplemented FO and SFO and showed significant increased molar proportion of propionic acid when compare to the control group. This suggests that acetate-producing bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, which are predominant cellulolytic bacteria in the rumen, may have been more inhibited by PUFA (Zhang *et al.*, 2008). From a physiological point of view, a shift in the rumen microbial communities may result in changes in bio-hydrogenation. Furthermore, a decrease in acetate concentration might contribute to a reduction in mammary or tissue de novo fatty acid synthesis, which requires acetate as a precursor (Doreau & Chilliard, 1997).

4. Conclusions

The series of these studies commence from the first experiment that was conducted to determine whether ruminal concentrations of *t11*-C18:1, *c9, t11*- C18:2 and C18:2n-6 were increased by SBO, FO and SBO+FO supplementation. The result clearly demonstrated that the ruminal concentrations of *t11*-C18:1 and *c9, t11*- C18:2 were significantly increased by SBO and SBO+FO addition while the concentration of C18:0 was reduced by FO and SBO+FO supplementation. The result from second experiment showed that ruminal concentration of *t11*-C18:1 was positively enhanced by different ratios of SBO+FO supplementation. The result clearly showed that feeding 2:1 w/w SBO + FO compromised the ruminal concentrations of *t11*-C18:1. These can be used as guide to improve CLA content in ruminant's product by *t11*-C18:1 was the main precursor for CLA synthesized. Further studies should focus on using these oils to investigate production trials.

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References

AbuGhazaleh, A. A., Schingoethe, D. J., Hippen, A. R., Kalscheur, K. F., & Whitlock, L. A. (2002). Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. *Journal of Dairy Science*, *85*(9), 2266-2276.

Amorocho, A. K., Jenkins, T. C., & Staples, C. R. (2009). Evaluation of catfish oil as a feedstuff for lactating

Holstein cows. *Journal of Dairy Science*, *92*(10), 5178-5188.

Association of Official Analytical Chemists. (1995). *Official methods of analysis* (16th ed.). Washington, DC: Author.

Beam, T. M., Jenkins, T. C., Moate, P. J., Kohn, R. A., & Palmquist, D. L. (2000). Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. *Journal of Dairy Science*, *83*(11), 2564-2573.

Beauchemin, K. A., McGinn, S. M., & Petit, H. V. (2007). Methane abatement strategies for cattle: lipid supplementation of diets. *Canadian Journal of Animal Science*, *87*(3), 431-440.

Doreau, M., & Chilliard, Y. (1997). Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. *Reproduction Nutrition Development*, *37*(1), 113-124.

Ferreira, E. M., Pires, A. V., Susin, I., Biehl, M. V., Gentil, R. S., Parente, M. D. O. M., & de Almeida, E. (2016). Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. *Animal Feed Science and Technology*, *216*, 30-39.

Fievez, V., Dohme, F., Danneels, M., Raes, K., & Demeyer, D. (2003). Fish oils as potent rumen methane inhibitors and associated effects on rumen fermentation *in vitro* and *in vivo*. *Animal Feed Science and Technology*, *104*(1-4), 41-58.

Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of biological chemistry*, *226*(1), 497-509.

Gagliostro, G. A., Rodriguez, A., Pellegrini, P. A., Gatti, P., Muset, G., Castañeda, R. A., & Chilliard, Y. (2006). Effects of fish oil or sunflower plus fish oil supplementation on conjugated linoleic acid (CLA) and omega 3 fatty acids in goat milk. *Revista Argentina de Produccion Animal*, *26*(2), 71-87.

Harfoot, C. G., & Hazlewood, G. P. (1997). Lipid metabolism in the rumen. In P. N. Hobson (Eds.), *The rumen microbial ecosystem*. (pp. 382–426). London, England: Stewart Published by Blackie Academic and Professional.

Jalc, D., Certik, M., Kundrikova, K., & Namestkova, P. (2007). Effect of unsaturated C18 fatty acids (Oleic, Linoleic and alfa-linolenic acid) on ruminal fermentation and production of fatty acid isomers in an artificial rumen. *Veterinary Medicine Journal*, *52*(3), 87-94.

Jenkins, T. C. (1993). Lipid metabolism in the rumen. *Journal of dairy science*, *76*(12), 3851-3863.

Jenkins, T. C., Wallace, R. J., Moate, P. J., & Mosley, E. E. (2008). Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *Journal of Animal Science*, *86*(2), 397-412.

Keady, T. W. J., & Mayne, C. S. (1999). The effects of level of fish oil inclusion in the diet on rumen digestion and fermentation parameters in cattle offered grass silage based diets. *Animal Feed Science and Technology*, *81*(1-2), 57-68.

Kepler, C. R., Tucker, W. P., & Tove, S. B. (1970). Biohydrogenation of unsaturated fatty acids IV. Substrate specificity and inhibition of linoleate Δ12-cis, Δ11-trans-isomerase from *Butyrivibrio fibrisolvens*. *Journal of Biological Chemistry*, *245*(14), 3612-3620.

Kim, E. J., Huws, S. A., Lee, M. R., Wood, J. D., Muetzel, S. M., Wallace, R. J., & Scollan, N. D. (2008). Fish oil increases the duodenal flow of long chain polyunsaturated fatty acids and trans-11 18:1 and decreases 18:0 in steers via changes in the rumen bacterial community. *The Journal of Nutrition*, *138*(5), 889-896.

Loor, J. J., Doreau, M., Chardigny, J. M., Ollier, A., Sebedio, J. L., & Chilliard, Y. (2005). Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of trans-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. *Animal Feed Science and Technology*, *119*(3-4), 227-246.

Maia, M. R., Chaudhary, L. C., Figueres, L., & Wallace, R. J. (2007). Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie Van Leeuwenhoek*, *91*(4), 303-314.

Marín, A. M., Gómez-Cortés, P., Castro, A. G., Juárez, M., Alba, L. P., Hernández, M. P., & De la Fuente, M. A. (2011). Animal performance and milk fatty acid profile of dairy goats fed diets with different unsaturated plant oils. *Journal of Dairy Science*, *94*(11), 5359-5368.

Messana, J. D., Berchielli, T. T., Arcuri, P. B., Reis, R. A., Canesin, R. C., Ribeiro, A. F., & Fernandes, J. J. D. R. (2013). Rumen fermentation and rumen microbes in Nellore steers receiving diets with different lipid contents. *Revista Brasileira de Zootecnia*, *42*(3), 204-212.

Metcalfe, L. D., Schmitz, A. A., & Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chemistry*, *38*(3), 514-515.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., & Beever, D. E. (2006). Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *Journal of Dairy Science*, *89*(2), 714-732.

Toral, P. G., Belenguer, A., Frutos, P., & Hervás, G. (2009). Effect of the supplementation of a high-concentrate diet with sunflower and fish oils on ruminal fermentation in sheep. *Small Ruminant Research*, *81*(2-3), 119-125.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., & De la Fuente, M. A. (2010). Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. *Journal of Dairy Science*, *93*(4), 1604-1615.

Toral, P. G., Hervás, G., Suárez-Vega, A., Arranz, J. J., & Frutos, P. (2016). Isolation of RNA from milk somatic cells as an alternative to biopsies of mammary tissue for nutrigenomic studies in dairy ewes. *Journal of Dairy Science*, *99*(10), 8461-8471.

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583-3597.

Zhang, C. M., Guo, Y. Q., Yuan, Z. P., Wu, Y. M., Wang, J. K., Liu, J. X., & Zhu, W. Y. (2008). Effect of octadeca carbon fatty acids on microbial fermentation, methanogenesis and microbial flora *in vitro*. *Animal Feed Science and Technology*, *146*(3-4), 259-269.