Original Article

Influence of oil or fat supplementation on Rumen Fermentation Characteristics and Ruminal Fluid Fatty Acid Profile in Brahman Crossbred Fattening Steers

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Abstract

The aim of this experiment was to study the influence of oil or fat supplementation on rumen fermentation and ruminal fluid fatty acid profiles in Brahman crossbred steers. Twelve steers (87.5% Brahman crossbred) with average live weight (LW) of 337±54 kg and approximate age of 2 years old were stratified by their LW into 4 groups and each group was randomly assigned to four dietary treatments. All steers were fed 14% CP concentrate. The treatments included: 1) 7 kg/d concentrate, 2) 4 kg/d concentrate supplemented with 200 g/d palm oil (PO), 3) 4 kg/d concentrate supplemented with 100 g/d PO and 100 g/d linseed oil (LSO), and 4) 4 kg/d concentrate supplemented with 200 g/d LSO. The animals in treatment 1 were fed ad libitum rice straw (RS), whereas the animals in the other treatments were fed ad libitum fresh grass (FG). Results showed that the dietary treatments had no effect on nutrient intake while the oil supplements decreased dry matter intake (DMI). Feeding LSO at 2 h increased C18:3n3 and decreased C18:2n6 in ruminal fluid. Feeding LSO inhibited BH of C18:2 to C18:0, as indicated by the increased rumen flows and proportions of BH intermediates in ruminal fluid. Furthermore, LSO did not negatively influence rumen fermentation and did not change ruminal pH, NH_3-N, protozoa and VFA concentration.

Keywords: fatty acid, linseed oil, rumen fermentation, ruminal fluid

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Introduction

Lipids presented in most feeds used in animal feeding contain high proportions of unsaturated fatty acids (Van Soest, 1994), which affects the permeability of the microbial membrane; in particular, they inhibit the activity of gram-positive bacteria and protozoa and modify rumen fermentation (Nagaraja et al., 1997). The effects of lipids on the rumen and total digestion are difficult to predict and are highly variable because they depend on the nature and concentration of lipids in the diet, the types of chemicals and/or physical treatments added to feeds, and the nature and amounts of forages, concentrates, and minerals (especially calcium) in the diet (Jenkins and McGuire, 2006). Due to these complex interactions, the metabolic effects of lipid supplementation in the diet cannot be analyzed as a simply result of increase in the absorption of intact fatty acids (or transformation by the rumen) from the diet (Oliveira et al., 2007). Linseed is not frequently used in ruminant feeding, especially because several experiments in which more than 5% linseed oil was supplied to sheep at maintenance have shown a strong negative effect on ruminal digestion (Ikwuegbu and Sutton, 1982). However, recent data have demonstrated that adding 3% linseed oil to dairy cow diets does not depress ruminal digestion (Ueda et al., 2003). Until now, no experiment has been conducted with dairy cows fed diets containing linseeds at levels above 3% (Martin et al., 2008). Furthermore, maximum feeding levels of natural fat sources must be followed to minimize problems with ruminal fermentation. One approach is to establish feeding levels based on iodine value (IV) or use total unsaturates within a supplemental range of 2 to 3% of dietary DM (Eastridge, 2002). Thus, when one wants to supply lipids in the diet of ruminants, it is important to evaluate their effects on ingestion and digestion of nutrients so as not to impair the necessary uptake for the desired production (Jenkins and McGuire, 2006). Furthermore, linseed oil (LSO) supplementation in cattle feed can increase *trans*-11C18:1, *cis*-9, *trans*-11 CLA, and 18:3n-3 at the duodenum (Loor et al., 2004; Doreau et al., 2009), which accumulate in tissue lipids and milk fat (Destaillats et al., 2005; Akraim et al., 2007). Up to now, however, no study of the effects of oil or fat supplement in Thai beef cattle on rumen fermentation and ruminal fluid fatty acid profiles has been done. Therefore, the objective of this study was to examine the effects of linseed oil supplemented concentrate fed to Brahman crossbred fattening steers on rumen fermentation and ruminal fluid fatty acid profiles.

Materials and Methods

Experimental design and treatments: Twelve steers (87.5% Brahman crossbred) with average live weight (LW) of 337±54 kg and approximate age of 2 years old were stratified by their LW and assigned to four dietary treatments. All steers were fed 14% CP concentrate and had free access to clean water. The animals were individually housed in a free-stall unit. The treatments included: 1) 7 kg/d concentrate (HC), 2) 4 kg/d concentrate supplemented with 200 g/d palm oil (PO), 3) 4 kg/d concentrate supplemented with 100 g/d PO and 100 g/d linseed oil (LSO), and 4) 4 kg/d concentrate supplemented with 200 g/d LSO. The animals in treatment 1 were fed *ad libitum* rice straw, whereas the animals in the other treatments were fed *ad libitum* fresh grass (Hybrid napier; *Pennisetum purpureum x Pennisetum americanum*). The experiment lasted for 84 days including the first 14 days as adjustment period and the last 70 days (5 periods of 14 days) as measurement period.

Laboratory analyses: Feed offered and residues after eating of individual steer were weighed on 2 consecutive days weekly to calculate DM intakes. Feed samples were pooled to make representative samples for proximate (AOAC, 1995) and detergent analyses (Van Soest et al., 1991).

Fatty acids in the feed samples were extracted using a modified method used by Folch et al. (1957) and Metcalfe et al. (1966). Fatty acid methyl esters (FAME) were prepared by the procedure described by Ostrowska et al. (2000) for analyzing by gas chromatography (7890A GC System, Agilent Technology, USA) equipped with a 100 m × 0.25 mm × 0.2 µm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250 °C. Column temperature was kept at 70 °C for 4 min, then increased at 13 °C/min to 175 °C and held at 175 °C for 27 min, then increased at 4 °C/min to 215 °C and held at 215 °C for 17 min, then increased at 4 °C/min to 240 °C and held at 240 °C for 10 min.

Approximately 200 ml of ruminal fluid was collected by using a stomach tube with a strainer and a vacuum pump, and filtered through 4 layers of cheesecloth at 0 (pre feeding), 2, 4, and 6 h post feeding. One portion of rumen fluid was immediately analyzed for pH (pH meter model UB-5, Denver Instrument, Germany). Ruminal volatile fatty acids (VFA) and ammonia N of the rumen fluid samples were determined by taking 20 ml of the rumen fluid and combining it with 5 ml 6N HCl, then keeping it frozen for analysis of VFA and ammonia N. The samples were later thawed at 4 °C and centrifuged at 3,000 rpm for 15 min. The supernatant was analyzed for ammonia N by Kjeldahl and concentrations of VFA were determined by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m × 0.32 mm × 0.15 µm film fused silica capillary column (HP_Innowax, AB 002, Agient, USA). Injector and detector temperatures were 250 °C. Column temperature was kept at 80 °C for 5 min, then increased at 10 °C/min to 170 °C and then increased at 30 °C/min to 250 °C and held at 250 °C for 5 min. Protozoa populations in the rumen fluid samples, which were preserved with 10% formal saline solution, were counted by Hematocytometer.

Fatty acid in the rumen fluid sample was extracted using a modified method used by Romeu-Nadal et al. (2004). From a well-mixed aliquot of rumen fluid, 3 ml was placed in 50 ml centrifuge tubes. Then, 27 ml of a dichloromethane-methanol solution (2:1, v/v) was added to each tube. The mixture was shaken mechanically for 15 min and centrifuged at 2500 × *g* for 8 min at 4 °C. Approximately 8 ml of distilled water was pipette into each tube and, after shaking for 15 min

further, the sample was again centrifuged at 2500 × *g* for 8 min at 4 °C. The upper aqueous fraction was carefully removed as much as possible with a pipette. The organic layer was washed with 8 ml of a saturated solution of sodium chloride, and finally mixed mechanically for 15 min and then centrifuged for 8 min at 2500 × *g* at 4 °C. Again, the upper aqueous fraction was carefully removed with a pipette. The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium sulfate, and 3-5 ml of dichloromethane was passed through the filter. The fat solution was taken in a pre-weighed conical flask. Finally, the extract was concentrated by removing dichloromethane in a rotator evaporator and dried under a gentle stream of nitrogen. The weight difference of the conical flask before/after was assumed to be fat. The fat was stored at -20 °C and redissolved in dichloromethane (3%, w/v) intermediately before analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA).

Statistical analysis: All data were statistically analyzed as Completely Randomized Design using the ANOVA procedure of SAS (SAS, 2001).

Results

Feed Chemical and Fatty Acid Composition: The nutrient composition and FA composition of 14% CP concentrate, forage sources and oil supplements are summarized in Tables 1 and 2, respectively. The lipid from fresh grass provided high proportions of C18:3n-3 and PUFA and low proportions of C18:2n-6 and MUFA compared to the 14% CP concentrate and rice straw. Linseed oil had the highest proportion of PUFA while PO had the highest proportion of SFA. In all feeds, the main SFA was C16:0, whereas C18:1n-9 was the main MUFA in PO, C18:2n-6 was the main PUFA in the 14% CP concentrate, C18:3n-3 was the main PUFA in LSO, and MO, respectively (Table 2).

Animal Nutrient Intake: Feed intake variables are presented in Table 3. Compared with diet HC, diet PO, MO and LSO in the basal diet had no effect on the total CP intake ($p>0.05$), but the oil supplements decreased total DMI (-1.70, -1.78 and -1.46 kg/d, respectively; $P < 0.05$). The oil supplemented group had increased intake of ether extract and net energy for growth (NE_g), mainly through the increase in oil intake. The addition of oil in the basal diet increased the intake of energy as compared with the HC treatment ($p<0.05$).

Rumen fermentation characteristics: Ruminal pH, ammonia nitrogen concentration (NH_3-N) and protozoa concentration were not affected by the oil supplementation ($p>0.05$) (Table 4). The increasing level of LSO supplementation decreased acetate molar concentration ($p<0.05$) at 4 h (post feeding), while the molar proportion of propionate was increased ($p<0.05$) at 2 h (post feeding), resulting in a decreased acetate: propionate ratio ($p<0.05$; Table 5). In this study, high molar proportion of propionate was found in the cows fed on 200 g/d MO and 200 g/d LSO, resulting in low acetate: propionate ratio.

Rumen fluid fatty acid profiles: Ruminal fluid fatty acid concentrations are shown in Tables 6, 7, 8 and 9. The C16:0 percentage was similar between HC, MO and LSO at 0 h (pre feeding), 2 and 4 h (post feeding) ($p>0.01$), however the oil supplement decreased C16:0 at 6 h (post feeding) ($p<0.01$). Dietary LSO and MO resulted in markedly lower C18:0 at 2 h (post feeding) ($p<0.01$), and increased percentages of C18:0 at 6 h (post feeding) ($p<0.05$). The percentage of C18:1 was higher in the HC treatment than in PO, MO, and LSO at 0 h (pre feeding), 4 h and 6 h (post feeding) ($p<0.01$). Both HC and LSO resulted in greater 18:2n6 at 4 h (post feeding) than PO and MO. Feeding LSO also resulted in greater percentages of C18:3n3 at 2, 4, and 6 h (post feeding) ($p<0.01$). Feeding MO and LSO increased percentages of SFA at 0 h (pre feeding), 2 and 6 h (post feeding) ($p<0.01$), and LSO increased percentages of PUFA at 2 and 4 h (post feeding) ($p<0.01$). In addition, the LSO supplement resulted in lower n-6/n-3 ratio than MO, PO and HC, respectively.

Discussion

The ingestion of DM, EE and NE_g except for CP was not affected ($p>0.05$) by the lipid content (Table 3). The decline in DMI that occurred when LSO was fed cannot be fully explained by disturbances in rumen function because digestibility was not different among the 3 oil supplemented groups. It is possible that the FA intake had a direct inhibitory effect on voluntary intake via inhibition of ruminoreticular motility (Chilliard, 1993). According to Jenkins and McGuire (2006), the main effects of the addition of lipids on intake reduction are related to modifications in rumen fermentation. Specifically, a reduction in the digestibility of fiber in the rumen leads to an increase in the retention time of the NDF, which results in greater rumen fill.

Ruminal pH, Ammonia nitrogen concentration (NH_3-N) and protozoa concentration were not affected by the oil supplementation (Table 4). Doreau et al. (2009) demonstrated that linseed oil did not affect the rumen fermentation pattern. Neveu et al. (2014) reported that the inconsistent response of ruminal fermentation to grain source could be due to various factors such as grain variety, extent of grain processing, and forage level and source. Furthermore, Messana et al. (2013) suggested that the rumen fermentation depended on the feed intake, feeding frequency and composition of the diet. Similar to the present experiment, Harvatine and Allen (2006) suggested that the use of saturated and unsaturated lipids had a minor or insignificant effect on ruminal fermentation parameters.

The average ruminal pH values were not affected by the treatments (Table 4). 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. However, in all treatments of the present study, the ruminal pH remained above 6.5; thus, the pH did not have a significant effect on ruminal

fermentation. Russell and Wilson (1996) and Mertens (1997) reported that pH levels greater than 6.2 did not affect ruminal fermentation.

The concentration of NH_3-N was not affected by the treatments (Table 4). Van Soest (1994) suggested that a ruminal NH_3-N concentration below 13 mg/L of rumen fluid might affect the availability of nitrogen for microorganisms, which could compromise fiber ingestion and degradability. Furthermore, the CP intake was not affected (p>0.05) by the oil supplements (Table 3). Thus, the ruminal NH_3-N concentration obtained from the cows fed the 200 g/d LSO was below the suggested range. However, according to Messana et al. (2013), by feeding cows based on 20 g lipid /kg diet (1,080 g/d), no relationship between the concentration of NH_3-N and the ruminal availability of fiber could be established.

Table 1 Chemical composition (% DM) of experimental feeds

Items	14% CP	PO/LSO	Rice straw	Fresh grass
Dry matter	93.91	-	92.31	12.50
Ash	7.00	-	10.85	12.40
Crude protein	14.63	-	4.00	10.07
Ether extract	4.07	100	0.81	1.78
Crude fiber	17.13	-	39.79	36.04
Neutral detergent fiber	42.34	-	76.31	64.42
Acid detergent fiber	24.16	-	52.34	34.83
Acid detergent lignin	2.08	-	6.34	2.62
Neutral detergent insoluble N	1.09	-	0.51	0.32
Acid detergent insoluble N	0.89	-	0.41	0.35

PO: palm oil, LSO: linseed oil

Table 2 Fatty acid compositions of experimental feeds

Fatty acid (% of total FA)	14% CP	FG	RS	PO	MO	LSO
C8:0	0.74	ND	ND	0.05	0.03	0.05
C10:0	1.14	ND	ND	0.02	ND	ND
C12:0	17.96	1.42	ND	0.19	0.10	ND
C14:0	6.38	0.74	1.28	0.96	0.49	0.06
C16:0	17.85	19.66	47.49	38.29	21.11	4.91
C18:0	2.71	3.18	8.57	4.42	3.96	3.46
C18:1n-9*c*	31.90	6.55	16.76	40.61	29.26	17.88
C18:2n-6*c*	20.33	19.03	19.88	13.66	15.76	16.73
C20:0	0.00	0.54	0.00	0.04	0.14	ND
C18:3n-3	0.35	48.89	6.03	0.26	27.87	55.87
C18:3n-6	0.66	ND	ND	0.11	0.17	0.24
SFA[1]	46.77	25.53	57.34	44.05	25.94	8.70
MUFA[2]	31.90	6.55	16.76	41.07	29.61	17.96
PUFA[3]	21.34	67.92	25.91	14.89	44.45	73.34
total n-3[4]	0.35	48.89	6.03	0.43	28.09	56.20
total n-6[5]	20.99	19.03	19.88	14.46	16.30	17.04
PUFA:SFA	0.46	2.66	0.45	0.34	1.72	8.43
n-6/n-3	60.01	0.39	3.30	33.69	0.58	0.30

CP: crude protein, FG: fresh grass, RS: rice straw, PO: palm oil, MO: mixture of palm oil and linseed oil, LSO: linseed oil, ND: non-detectable

[1] SFA = Sum of saturated fatty acids from C4:0 – C20:0

[2] MUFA = Sum of monounsaturated fatty acids from C14:1 – C22:1

[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6

[4] Sum of n-6 fatty acids from C18:2n-6 – C22:4n-6

[5] Sum of n-3 fatty acids from C18:3n-3 – C22:6n-3

Table 3 Effect of linseed oil supplementation on nutrient intake of steers

Item	Treatments[1]				SEM	P-value
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
Dry matter intake, kg/d						
Concentrate	6.46	3.67	3.65	3.66	-	-
Roughage	4.76	5.64	5.59	5.91	0.02	0.279
Oil	-	0.2	0.2	0.2	-	-
Total	11.22[a]	9.52[b]	9.44[b]	9.76[b]	0.32	0.038
DMI, g/$BW^{0.75}$	131.19[a]	110.97[b]	109.19[b]	113.51[b]	2.81	0.011
Crude protein intake , g/d						
Concentrate	945[a]	537[b]	533[b]	534[b]	2.21	<0.01
Roughage	190[b]	568[a]	563[a]	594[a]	14.13	<0.01
Total	1,135	1,107	1,097	1,130	14.99	0.769
Ether extract intake, g/d						
Concentrate	263[a]	150[b]	148[b]	149[b]	0.61	<0.01
Roughage	39[b]	100[a]	100[a]	105[a]	2.57	<0.01
Oil	-	200	200	200	-	-
Total	301[b]	450[a]	448[a]	454[a]	0.29	<0.01
NE_g intake, Mcal/d						
Concentrate	5.56[a]	3.16[b]	3.15[b]	3.14[b]	0.01	<0.01
Roughage	1.19[b]	3.72[a]	3.69[a]	3.90[a]	0.09	<0.01
Oil	-	0.63	0.63	0.63	-	-
Total	6.65[b]	7.51[a]	7.45[a]	7.66[a]	0.09	<0.01

NEg: net energy for growth
SEM: standard error of mean
[1]HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil
[a, b] Mean which different superscripts are significant difference (p<0.05)

Table 4 Effect of linseed oil supplementation on ruminal pH, NH_3-N, protozoa population in rumen fluid grass

Item	Treatments[1]					
	HC	200 g/d PO	200 g/d MO	200 g/d LSO	SEM	Pr<F
pH						
0 hr	7.51	7.46	7.15	7.31	0.048	0.084
2 hr	7.06	6.80	6.90	6.78	0.034	0.123
4 hr	7.25	7.19	7.13	6.87	0.119	0.078
6 hr	7.16	7.35	7.17	7.09	0.057	0.497
NH_3-N (mg/L)						
0 hr	8.87	12.31	12.71	12.81	0.376	0.054
2 hr	16.15	18.32	19.21	20.68	0.425	0.078
4 hr	11.45	12.32	16.06	17.63	0.690	0.090
6 hr	7.78	9.16	9.26	13.69	0.464	0.088
Protozoa ($x10^6$ cells/ml)						
0 hr	8.75	4.50	5.00	7.25	0.805	0.340
2 hr	3.00	3.00	3.25	3.00	0.763	0.257
4 hr	4.25	4.50	4.25	4.00	0.916	0.668
6 hr	6.25	5.50	6.00	4.75	1.365	0.863

SEM: standard error of mean
[1] HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil

Table 5 Effect of linseed oil supplementation on Volatile fatty acid (VFA) in ruminal fluid

Item	Treatments[1]					
	HC	200 g/d PO	200 g/d MO	200g/d LSO	SEM	Pr<F
VFA (mol/100 mol)						
Acetate, C2						
0 hr	73.85	73.83	73.30	71.99	0.306	0.251
2 hr	73.01	73.33	69.83	70.57	0.664	0.298
4 hr	73.43[a]	72.90[a]	70.26[b]	70.66[b]	0.276	0.034
6 hr	73.26	73.97	70.90	71.46	0.394	0.133
Propionate, C3						
0 hr	14.92	13.95	14.89	15.89	0.280	0.257
2 hr	16.35[b]	15.82[b]	17.93[a]	17.43[a]	0.081	0.028
4 hr	15.26	14.62	17.20	16.89	0.349	0.145
6 hr	14.70	14.97	16.12	15.59	0.467	0.724
Butyrate, C4						
0 hr	11.24	12.23	11.82	12.13	0.134	0.174
2 hr	10.64	10.85	12.25	12.01	0.619	0.745
4 hr	11.31	12.48	12.55	12.45	0.172	0.162
6 hr	12.05	11.06	12.99	12.95	0.709	0.752
Acetate: Propionate						
0 hr	4.94	5.30	4.93	4.54	0.112	0.267
2 hr	4.47[a]	4.64[a]	3.90[b]	4.05[b]	0.057	0.028
4 hr	4.82	4.99	4.11	4.19	0.338	0.095
6 hr	4.98	5.00	4.42	4.59	0.143	0.470

SEM: standard error of mean

[1] HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil

[a, b] Mean which different superscripts are significant difference (p<0.05)

These results indicated that the populations of protozoa were not affected by the dietary LSO (Table 4). Beauchemin and colleagues (2009) reported linseed (seeds or oil) supplementation that rich in linolenic acid did not affect the numbers of ruminal protozoa. Doreau et al. (2009) observed no changes in protozoa numbers in ruminal fluid of cows supplemented with 2.6% of LO. Based on these results, it appears that 200 g/d LSO is required to not affect the numbers of ruminal protozoa.

The oil supplementation led to lower VFA concentration, especially a decrease in acetate:propionate ratio in the rumen. It is suggested that unsaturated fatty acid from oil could interfere with ruminal fermentation resulting in greater gut fill and reduction in residual DM digestion (Yang et al., 2009). Onetti et al. (2001) reported that when feeding supplemental lipid the molar proportion of ruminal acetate was decreased and of propionate was increased; resulting in decreased acetate:propionate ratio. Furthermore, a high supply of linseed oil was shown in a literature to increase propionic acid at the expense of acetic and butyric acid (Sutton et al., 1983). Ruminal digestibility was not reduced by the supply of linseed oil, while propionate was increased at the expense of either butyrate (Machmüller et al., 2000) or acetate (Gonthier et al., 2004). The reduction in the acetate to propionate ratio often improves the efficiency of feed utilization, since relatively higher propionate production is associated with less of energy in form of gas (Machmüller et al., 2000).

Fatty acid concentrations in the rumen fluid varied depending on the time after feeding and oil supplements in the diet. Harvatine and Allen (2006) completed an *in vivo* experiment with lactating dairy cows to determine rates of fatty acid (FA) biohydrogenation (BH) of fat supplements with different grades of unsaturation, and developed a kinetic model of ruminal BH. Based on their results, they showed that passage rates of C16:0, C18:0 and total C18 carbon FA linearly decreased as UFA increased. The increasing UFA increased the extent of C18:2 and C18:3 biohydrogenation, and decreased the extent of C18:1 BH. Gulati et al. (2000) reported that the concentration of C18:0 indicated a shift of the BH of UFA to the accumulation of C18:1 in the rumen. In contrast to sunflower oil and rapeseed oil, the higher concentration of C18:0 with LSO indicated a shift of the BH of UFA to a lower mean concentration of C18:1. The accumulation of C18:1 is probably due to an excess of free fatty acids that inhibited the final hydrogenation of C18:1 to C18:0. According to Lock and Garnsworthy

(2003), possible reasons for the increases in concentration of C18:1 include an increased intake of substrates (C18:2n6 and C18:3n3) and/or a decrease in the final hydrogenation step from C18:1 to C18:0 in the rumen. The higher concentration of c9,t11 CLA in the rumen fluid in this experiment was observed with PO to a maximum of 0.26% of total FA (2 h after feeding) whilst with HC, MO, and LSO the maximum c9,t11 CLA concentration was 0.18, 0.16, and 0.14% of total FA, respectively (2 h after feeding).

Table 6 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 0 h (pre feeding)

Item	Treatments[1]					
	HC	200 g/d PO	200 g/d MO	200g/d LSO	SEM	Pr<F
C12:0	1.66	1.27	1.41	1.61	0.1	0.572
C14:0	2.98	2.59	2.35	2.65	0.28	0.888
C16:0	33.05	34.1	35.23	31.86	0.42	0.166
C18:0	52.21	52.73	54.22	56.52	0.54	0.149
C18:1	7.39[a]	6.57[a]	4.91[b]	4.38[b]	0.18	0.012
C18:2	1.84	1.87	1.55	1.15	0.11	0.23
C18:3	0.01	0.05	0.35	0.2	0.02	0.078
C20:0	0.8	0.75	0.81	1.08	0.02	0.031
C9,T11	0.08[a]	0.09[a]	0.03[b]	0.04[b]	0.01	<0.01
SFA[2]	89.90[b]	90.68[b]	93.20[a]	92.64[a]	0.23	0.018
PUFA[3]	2.72	2.75	2.42	2.46	0.13	0.726
PUFA/SFA	0.03	0.03	0.03	0.03	0.001	0.479
n-6/n-3	271.00[a]	64.10[b]	76.20[b]	12.90[b]	8.16	<0.01

SEM: standard error of mean
[1] HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil
[2] SFA = Sum of saturated fatty acids from C4:0 – C20:0
[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6
[a, b] Mean which different superscripts are significant difference (p<0.05)

Table 7 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 2 h (post feeding)

Item	Treatments[1]					
	HC	200 g/d PO	200 g/d MO	200g/d LSO	SEM	Pr<F
C12:0	6.05	5.87	4.15	4.00	0.27	0.106
C14:0	9.6	11.03	11.24	10.77	0.27	0.272
C16:0	27.81	33.29	34.17	36.20	0.69	0.06
C18:0	34.83[a]	30.23[b]	25.82[c]	24.86[c]	0.92	0.049
C18:1	15.74	17.75	12.06	12.98	0.58	0.077
C18:2	2.72	2.69	2.52	2.49	0.04	0.259
C18:3	0.03[d]	1.55[c]	2.79[b]	4.71[a]	0.11	<0.01
C20:0	2.42	2.3	2.33	2.65	0.05	0.203
C9,T11	0.18[b]	0.26[a]	0.16[b]	0.14[b]	0.01	0.081
T10,C12	0.64	0.41	0.37	0.28	0.03	0.05
SFA[2]	78.93	75.45	80.15	77.05	0.73	0.252
PUFA[3]	5.34[c]	6.80[b]	7.80[b]	9.98[a]	0.15	<0.01
PUFA/SFA	0.07[c]	0.09[c]	0.10[b]	1.13[a]	0.01	<0.01
n-6/n-3	221.25[a]	3.42[b]	1.80[b]	1.13[b]	10.81	<0.01

SEM: standard error of mean
[1] HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil
[2] SFA = Sum of saturated fatty acids from C4:0 – C20:0
[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6
[a, b, c] Mean which different superscripts are significant difference (p<0.05)

Table 8 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 4 h (post feeding)

Item	Treatments[1]					
	HC	200 g/d PO	200 g/d MO	200g/d LSO	SEM	Pr<F
C12:0	5.01	5.58	4.38	5.74	0.24	0.034
C14:0	12.02[b]	12.54[b]	13.80[a]	14.97[a]	0.15	<0.01
C16:0	32.94	32.83	34.1	35.07	0.66	0.625
C18:0	31.02	30.5	33.08	28.38	0.93	0.45
C18:1	12.07[a]	12.96[a]	8.19[b]	8.18[b]	0.16	<0.01
C18:2	2.81[a]	2.48[b]	2.50[b]	2.73[a]	0.02	0.021
C18:3	ND	0.1[b]	0.36[b]	1.13[a]	0.04	<0.01
C20:0	3.47[a]	2.67[b]	3.37[a]	3.52[a]	0.04	<0.01
C9,T11	0.08[a]	0.07[ab]	0.03[c]	0.02[bc]	0.01	0.046
T10,C12	0.61	0.3	0.21	0.28	0.03	0.023
SFA[2]	81.58[b]	81.73[b]	85.56[a]	84.43[a]	0.18	<0.01
PUFA[3]	6.35[b]	5.31[c]	6.25[b]	7.41[a]	0.09	<0.01
PUFA/SFA	0.08[ab]	0.07[c]	0.08[bc]	0.09[a]	0.002	0.032
n-6/n-3	ND	52.83[a]	17.93[b]	5.58[b]	2.23	<0.01

SEM: standard error of mean
[1] HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil
[2] SFA = Sum of saturated fatty acids from C4:0 – C20:0
[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6
[a, b, c] Mean which different superscripts are significant difference (p<0.05)

Table 9 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 6 h (post feeding)

Item	Treatments[1]					
	HC	200 g/d PO	200 g/d MO	200g/d LSO	SEM	Pr<F
C12:0	6.51	6.8	4.69	5.52	0.08	0.121
C14:0	11.37	11.57	11.34	12.42	0.24	0.436
C16:0	39.78[a]	34.20[b]	33.94[b]	36.43[b]	0.33	0.01
C18:0	30.29[b]	34.05[b]	40.62[a]	34.59[b]	0.7	0.028
C18:1	7.73[b]	9.22[a]	5.29[d]	6.71[c]	0.07	<0.01
C18:2	1.69	1.84	1.82	1.73	0.04	0.56
C18:3	ND	0.03[b]	0.06[b]	0.13[a]	0.01	<0.01
C20:0	2.11[b]	2.15[b]	2.17[b]	2.45[a]	0.02	0.022
C9,T11	0.03[b]	0.35[a]	0.04[b]	0.35[a]	0.01	<0.01
T10,C12	0.51[a]	0.12[b]	0.04[b]	ND	0.02	<0.01
SFA	88.45[b]	86.73[c]	90.63[a]	88.96[b]	0.08	<0.01
PUFA	3.83	4.06	4.08	4.34	0.06	0.127
PUFA/SFA	0.04	0.04	0.04	0.05	0.01	0.381
n-6/n-3	ND	134.17[a]	78.47[b]	33.18[c]	5.12	<0.01

SEM: standard error of mean
[1] HC: 7 kg/d concentrate, PO: 200 g/d palm oil, MO: 100 g/d PO and 100 g/d linseed oil, LSO: 200 g/d linseed oil
[2] SFA = Sum of saturated fatty acids from C4:0 – C20:0
[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6
[a, b, c, d] Mean which different superscripts are significant difference (p<0.05)

Loor et al. (2004) reported the enhancement of c9,t11 CLA in the rumen fluid of cows fed a high concentrate diet. The lower concentration of these isomers with LSO was accompanied by the higher concentration of C18:3n3 in the rumen fluid compared to PO, MO and HC. The input of C18:3n3, when hydrogenation is incomplete, may result in an enhanced ruminal outflow of C18:2 and C18:1 (Loor et al., 2004). According to Loor et al. (2002), LSO may increase the endogenous synthesis of c9,t11 CLA in

tissues by enhancing the post absorptive availability of C18:1. It is evident that the differences in c9,t11 CLA concentration between oil supplements are influenced by the level of C18:2n6 in the original oils used to produce CLA (Szolloskei et al., 2005). Greater C18:3n3 hydrogenation with LSO and greater C18:2n6 BH with HC was documented by Loor et al. (2004). Therefore, the high concentration of linoleic acid in the diet would reduce biohydrogenation and increase the postruminal flow of this unsaturated fatty acid (Beam et al., 2000).

In conclusion, the LSO supplementation in Brahman crossbred steers did not negatively influence the ruminal fermentation including ruminal pH, NH_3-N, protozoa and VFA concentration. The concentration of lipid supplements might be not enough to affect the ruminal fermentation characteristics. In addition, at 2 h post feeding, LSO causing greater percentages of C18:3n3, PUFA concentration and low n-6/n-3 fatty acid ratio in the ruminal fluid was observed. Therefore, the high concentration of linoleic acid in the diet could increase the postruminal flow of this unsaturated fatty acid concentration and accumulate in tissue lipids.

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บทคัดย่อ

ผลของการใช้น้ำมันหรือไขมันเสริมในอาหารโคขุนต่อประสิทธิภาพการหมักย่อยในกระเพาะหมัก และการเปลี่ยนแปลงองค์ประกอบของกรดไขมันของของเหลวในกระเพาะหมักของโคเนื้อ

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วัตถุประสงค์ของงานวิจัยเพื่อศึกษาผลของการใช้น้ำมันหรือไขมันเสริมในอาหารโคขุนต่อประสิทธิภาพการหมักย่อยในกระเพาะหมักและการเปลี่ยนแปลงองค์ประกอบของกรดไขมันของของเหลวในกระเพาะหมักของโคเนื้อ ทำการทดลองในโคเนื้อขุน (ระดับสายเลือดพันธุ์บรามันมากกว่า 87.5%) จำนวน 12 ตัว อายุเฉลี่ยประมาณ 2 ปี วางแผนการทดลองแบบสุ่มสมบูรณ์ โดยสุ่มสัตว์ทดลองแบบแบ่งชั้นจากน้ำหนักตัวเป็น 4 กลุ่ม และสุ่มอาหารทดลองให้แก่สัตว์ทดลองแบ่งเป็น 4 กลุ่มตามอาหารทดลอง โคทุกตัวได้รับอาหารข้นชนิดเม็ดที่มีโปรตีนไม่น้อยกว่า 14% มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) กลุ่มควบคุมได้รับอาหารข้น 7 กิโลกรัม/ตัว/วัน และฟางข้าวเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ 2) เสริมน้ำมันปาล์มปริมาณ 200 กรัม/ตัว/วันและอาหารข้น 4 กิโลกรัม/ตัว/วัน และหญ้าสดเป็นอาหารหยาบแบบไม่จำกัดปริมาณ 3) เสริมน้ำมันปาล์มปริมาณ 100 กรัม/ตัว/วัน ร่วมกับน้ำมันลินสีดปริมาณ 100 กรัม/วันและได้รับอาหารข้น 4 กิโลกรัม/ตัว/วัน และหญ้าสดเป็นแหล่งของอาหารหยาบแบบไม่จำกัดปริมาณ 4) เสริมน้ำมันลินสีดปริมาณ 200 กรัม/ตัว/วัน และอาหารข้น 4 กิโลกรัม/ตัว/วัน และหญ้าสดเป็นอาหารหยาบแบบไม่จำกัดปริมาณ การทดลองพบว่า อาหารทดลองไม่มีผลต่อการกินได้ของโภชนะในโคเนื้อขุน อย่างไรก็ตามการเสริมน้ำมันในอาหารโคเนื้อขุนมีผลทำให้ปริมาณการกินได้ของวัตถุแห้งลดลง ขณะที่ค่าความเป็นกรดด่าง แอมโมเนียไนโตรเจน โปรโตซัว และความเข้มข้นของกรดไขมันระเหยได้ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ นอกจากนี้การเสริมน้ำมันลินสีดที่ระดับ 200 กรัม/วันส่งผลให้มีการเปลี่ยนแปลงของกรดไขมันชนิด C18:3n3 ในของเหลวในกระเพาะหมักสูงที่สุดหลังจากการกินอาหารในชั่วโมงที่ 2 และมีปริมาณของกรดไขมันชนิด C18:2n6 ลดลง

คำสำคัญ: กรดไขมัน น้ำมันลินสีด การหมักย่อยในกระเพาะหมัก

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