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Original Research Article

Effects of feeding whole linseed on ruminal fatty acid composition and microbial population in goats

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ABSTRACT

The objective of the present study was to evaluate the effect of feeding different levels of whole linseed, as a source of n-3 polyunsaturated fatty acids (PUFA), on ruminal fatty acid composition and microbial population in the goat. Twenty-four crossbred Boer goats were assigned to 3 dietary treatments: L0 (control), L10 and L20 containing 0, 10%, or 20% whole linseed, respectively. The ruminal pH and concentration of total volatile fatty acids (VFA) were not affected by dietary treatments. The feeding of L10 and L20 diets produced higher ($P < 0.05$) molar proportions of acetate and lower ($P < 0.05$) molar proportions of butyrate and valerate than the L0 diet. Molar proportions of myristic acid (C14:0) and palmitic acid (C16:0) were lower ($P < 0.05$) in the rumen of goats offered L10 and L20 diets than the control diet. However, stearic acid (C18:0), vaccenic acid (C18:1 trans-11), conjugated linoleic acid (CLA, C18:2 trans-10, cis-12) and α-lenolenic acid (C18:3 n-3) were higher ($P < 0.05$) in the rumen of goats fed L10 and L20 than L0. Both inclusion levels of linseed in the diet (L10 and L20) reduced the ruminal total bacteria, methanogens, and protozoa compared with L0 ($P < 0.05$). The effect of the dietary treatments on cellulolytic bacteria, varied between the individual species. Both inclusion levels of linseed resulted in a significant decrease ($P < 0.05$) in the population of *Fibrobacter succinogenes*, and *Rumunococus flavefaciens* compared with L0, with no significant difference between the groups fed linseed diets. The population of *Rumunococus albus* was not affected by the different dietary treatments. It was concluded that inclusion of whole linseed in the diet of goats could increase the concentration of PUFA in the rumen, and decrease the population of *F. succinogenes*, *R. flavefaciens*, methanogens and protozoa in rumen liquid of goats.

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1. Introduction

Feeding animals with sources of polyunsaturated fatty acids (PUFA) has been of interest in animal nutrition to enhance these beneficial fatty acids (FA) in animal products, specifically n-3 PUFA, which has been associated with significant physiological and health benefits in human populations. Compared with monogastric animals, increasing PUFA in ruminant products is more challenging, since most of the PUFA in the animal diet are hydrogenated by the rumen microorganisms. Yet, the inclusion of PUFA sources in diets of ruminants has been shown to increase the concentration of n-3

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PUFA in their meat (Palmquist, 2009). Furthermore, incomplete biohydrogenation of linoleic acid (LA) and α-linolenic acid (ALA) results in developing conjugated linoleic acids (CLA) isomers (Lee and Jenkins, 2011). CLA is now well known as an anticarcinogenic, anti-atherosclerotic, antimutagenic, antioxidant, antibacteriogenic, anti-diabetogenic, immunomodulator, and anti-obesity (Waghmare, 2013). Similar to CLA, vaccenic acid is an intermediate product of the microbial biohydrogenation of LA and ALA (Harfoot and Hazlewood, 1997). The increase of vaccenic acid in animal products is desirable since it performs as a precursor in the biosynthesis of CLA (Griinari et al., 2000), and may exert benefits similar to those related to CLA in humans (Field et al., 2009).

However, the presence of excessive amounts of PUFA in the rumen has the potential to radically disturb ruminal pH, volatile fatty acids (VFA) and microorganisms survivability, which perform a principal role in the overall process of ruminal fermentation (Machmüller et al., 1998; Maia et al., 2010). However, the type and sources of PUFA fed to ruminants might have different impacts on rumen fermentation and microbial populations (Ivan et al., 2012; Liu et al., 2012). Also, feeding plant based PUFA in the form of whole seeds might have less adverse effects on rumen fermentation than feeding free oils (Palmquist, 1995). The effect of PUFA on ruminal microbes differs depending on the type of microorganisms. For example, protozoa are more sensitive to dietary PUFA than bacteria. Polyunsaturated fatty acids may cause either total defaunation or significant reduction in the rumen protozoa population (Ivan et al., 2001). Within the bacteria species, feeding fish oil (Liu et al., 2012) or plant based PUFA have had different effects on growth of various species (Zhang et al., 2008; Ivan et al., 2012).

Linseed (*Linum usitatissimum*) is considered as a leading source of plant based n-3 FA (Legrand et al., 2010), because it contains about 40% oil, with a high level of ALA (50% to 60% of total FA) (Legrand et al., 2010). Also, linseed contains a lower concentration of LA and saturated FA (SFA) compared with other oilseeds such as soybeans, cottonseed, corn, and sunflowers (Maddock et al., 2005). Numerous studies have been undertaken to enhance n-3 PUFA content in ruminant meat and milk by feeding linseed (Abuelfatah et al., 2014). However, reports documenting the effects of feeding whole linseed on ruminal microorganisms are rare. Therefore, the objective of the present study was to evaluate the influence of feeding different levels of whole linseed, as a source of ALA n-3 PUFA, on ruminal microbial population of goats, using real time polymerase chain reaction (RT-PCR). We also tested the effect on ruminal pH, FA and VFA.

2. Materials and methods

2.1. Experimental animals, housing, feeds and feeding

Ruminal samples in the present experiment were collected at the end of feeding trial conducted in small ruminant research unit, University Putra Malaysia. The experimental procedures have been described in detail by Abuelfatah et al. (2013). Briefly, twenty four 5-month-old crossed Boer bucks with initial body weight (means and SE) of 14.23 ± 0.33 kg, were housed in individual pens. After 3 weeks of adaptation, goats were randomly divided into 3 equal groups of 8 animals each, and assigned to one of the 3 dietary treatments. The dietary treatments contained either 0 (L0), 10% (L10) or 20% (L20) whole linseed. The diets, ingredients and chemical and FA composition are presented in Table 1. At the end of the feeding experiment, which lasted for 110 days, all animals were slaughtered after overnight fasting. Animal care, handling techniques, and slaughter procedures were approved by the University Putra Malaysia Animal Care and Use Committee.

Table 1
Ingredients and composition of diets fed to goats.

Ingredient, % DM	Experimental diets		
	L0	L10	L20
Whole linseed	–	10	20
PKC	40	30	20
Soybean meal	11	9	6
Corn	20	20	20
Rice straw	20	20	20
Molasses	4	9	5
Palm oil	3	–	–
$CaCo_3$	1	1	1
NaCl	0.5	0.5	0.5
Mineral and vitamin mix	0.5	0.5	0.5
Chemical composition, % of DM			
Dry matter	89.79	89.22	90.17
Crude protein	14.25	14.45	14.69
Ether extract	4.86	5.09	7.38
NDF	48.58	46.63	48.30
ADF	30.10	27.34	27.09
Ash	10.19	9.32	9.14
Metabolizable energy, MJ/kg	11.30	11.00	11.00
Fatty acid composition, g/100 g fatty acids			
C12:0, lauric	5.28	3.17	1.97
C14:0, myristic	2.46	1.03	0.31
C16:0, palmitic	28.07	9.61	7.89
C16:1, palmitoleic	0.25	0.21	0.17
C17:0, heptadecanoic	0.72	0.39	0.38
C18:0, stearic	5.92	3.23	4.93
C18:1 n-9, oleic	33.48	27.67	27.06
C18:2 n-6, linoleic	21.88	21.25	18.00
C18:3 n-3, linolenic	1.92	33.42	39.27

L0 = control diet, containing 0 whole linseed; L10 = diet containing 10% whole linseed; L20 = diet containing 20% whole linseed; PKC = palm kernel cake.

2.2. Proximate analysis of feed

The proximate analysis of the experimental feed was performed following the standard methods of the Association of Official Analytical Chemists (AOAC, 2007). Briefly, feed samples were dried in a forced-air oven for 24 h at 105°C to determine dry matter (DM). Nitrogen was determined by Kjeltec Auto Analyzer and then converted to crude protein (CP = N × 6.25). Ether extract (EE) was determined by extracting the sample with petroleum ether (40 to 60°C) using a Soxtec Auto Analyzer. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the methods outlined by Van Soest et al. (1991) without adding alpha amylase and sodium sulfite. Values for NDF and ADF were expressed inclusive of residual ash. Samples were ashed in a muffle furnace at 550°C for 4 h to determine the ash content. Each analysis was performed in triplicate.

2.3. Rumen content sampling and pH measurement

Following animal slaughter, the esophagus was tied with nylon strings to conserve the ruminal environment until sampling time, which occurred directly upon evisceration. Rumen content was taken and squeezed through double layered gauze to remove the feed particles. About 100 mL of liquor was obtained from each animal. The pH of rumen liquid was measured instantly using a pH meter (Mettler-Toledo Ltd., England). The samples were stored at −80°C for FA and VFA analysis, and microbial quantification.

2.4. FA and VFA determination

For FA analysis of rumen liquor, 2 mL of sample was used. Ruminal fatty acid composition was determined following the procedure described by Abuelfatah et al. (2014). The VFA

contents of the rumen liquor were measured using gas–liquid chromatography. The fixed rumen liquor (using metaphosphoric acid, 4:1, vol/vol) was centrifuged at 15,000 × *g* at 25°C for 20 min, and 0.5 mL of the supernatant was taken and added to an equal volume of internal standard (4-methyl-n-valeric acids, Sigma Chemical Co., St. Louis, Missouri, USA). The separation was conducted on a bonded phase fused silica capillary column 15 m, 0.32 mm ID, 0.25 μm film thickness (Quadrex 007 Series, Quadrex Corporation, New Haven, CT 06525 USA) in an Agilent 7890a Gas-Liquid Chromatography (Agilent Technologies, Palo Alto A, USA). The injector and detector temperature was programmed at 220 and 230°C, respectively. The column temperature was adjusted in the range of 70 to 150°C with temperature programming at the rate of 7°C/min increments to assist optimum separation. Peaks identification was achieved by comparison with accurate commercial standards of acetic, propionic, butyric, isobutyric, valeric, and isovaleric (Sigma Chemical Co., St. Louis, Missouri, USA).

2.5. DNA extraction

The DNA was extracted from rumen liquor using the QIAamp DNA mini stool kit (Qiagen, Hilden, GmbH, Germany) following the manufacturer's protocol with a few modifications as described by Abubakr et al. (2014). Real-time PCR was conducted using the BioRad CFX96 Touch (Bio-Rad laboratories, Inc., Hercules, CA, USA) with fluorescence detection of SYBR Green dye using MicroAmp tube strips and MicroAmp Optical Cap Strips. Primers used to quantify the population of various microbes groups are presented in Table 2. The PCR reaction was achieved on a total volume of 25 mL using the iQTMSYBR Green Supermix assay (BioRad, USA). Each reaction comprised 12.5 μL SYBR Green Supermix, 1 μL of each Primer, 2 μL of DNA samples and 8.5 μL H_2O. The reaction settings for DNA amplification were one cycle at 95°C for 5 min for initial denaturation followed by 40 cycles of 95°C for 30 s then by annealing temperatures for various primers as described in Table 2 for 30 s and then at 72°C for 30 s. For confirming the specificity of amplification, melting curve examination was performed after each last amplification cycle. Detection of the fluorescent product was adjusted at the last step of each cycle. Standards were prepared from Plasmid DNA from each microbial group. The concentration of the extracted DNA was measured using a UV spectrophotometer. The number of copies of a template DNA/mL of elution buffer was calculated online using the web site (http://scienceprimer.com/copy-number-calculator-for-realtime-pcr) based on the following formula:

$$\text{Number of copies} = \frac{\text{Amount of DNA}(\mu g/mL) \times 6.022 \times 10^{23}}{\text{length(bp)} \times 10^{9} \times 660}$$

Standard curves were created by serial dilution of plasmid DNA of each microbial group (Faseleh Jahromi et al., 2013).

2.6. Statistical analysis

Data of rumen fermentation parameters and microbial population were subjected to one-way analysis of variance using the GLM procedure of SAS (SAS, 2003). Microbial data which did not meet the normality requirement were subjected to log10-transform before analysis. Least-square means were computed and tested for differences by Duncan multiple range test. Differences between least squared means were considered to be significant at $P < 0.05$, and data were presented as means ± standard errors.

3. Results

3.1. Ruminal FA composition

The ruminal FA profile of goats fed diets containing different levels of whole linseed is presented in Table 3. The most abundant FA in the rumen for all experimental groups was stearic acid (C18:0). However, C18:0 was significantly ($P < 0.05$) higher in the animals fed linseed (L10 and L120) than those in the control group (L0). The palmitic (16:0) acid represented the second abundant FA in the rumen digesta in all experimental groups, and it was significantly ($P < 0.05$) higher in L0 than in L10 and L20. Feeding linseed at both inclusion levels (10% and 20%) significantly ($P < 0.05$) increased the proportions of vaccenic (C18:1 trans-11), CLA C18:2 trans-10, cis-12, and ALA (C18:3 n-3). However, no significant effect $P > 0.05$ has been observed in the proportion of oleic (C18:1 n-9), LA (C18:2 n-6), CLA isomer C18:2 cis-9, trans-11, and arachidonic (C20:4 n-6). Feeding linseed also resulted in a significant decrease in the n-6:n-3 ratio in rumen liquor compared with the control.

3.2. VFA and pH of rumen liquor

The VFA and the rumen pH of goats fed diets containing different levels of whole linseed are presented in Table 4. The concentration of total VFA in the rumen and pH was not affected by dietary treatments. However, whole linseed inclusion in the diet of goats significantly increased ($P < 0.05$) the molar proportion of acetate and decreased ($P < 0.05$) the molar proportion of butyrate and valerate with no effects on the other individual VFA.

Table 2
Rumen microbial primer sequences used for real-time PCR assay.

Target microorganism	Primer sequence (5′-3′)	Annealing temperature, °C	Reference
Total bacteria F	CGGCAACGAGCGCAACCC	60	(Denman and McSweeney, 2006)
Total bacteria R	CCATTGTAGCACGTGTAGCC		
Rumunococus albus F	CCCTAAAAGCAGTCTTAGTTGG	55	(Koike and Kobayashi, 2001)
Rumunococus albus R	CCTCCTTGCGGTTAGAACA		
Rumunococus flavefaciens F	CGAACGGAGATAATTTGAGTTTACTTAGG	60	(Koike and Kobayashi, 2001)
Rumunococus flavefaciens R	CGGTCTCTGTATGTTATGAGGTATTACC		
Fibrobacter succinogenes F	GTTCGGAATTACTGGGCGTAAA	55	(Koike and Kobayashi, 2001)
Fibrobacter succinogenes R	CGCCTGCCCCTGAACTATC		
Methanogens F	TTCGGTGGATCDCARAGRGC	58	(Zhang et al., 2008)
Methanogens R	GBARG TCGWA WCCGT AGAAT CC		
Total protozoa F	CTTGCCCTCYAATCGTWCT	55	(Sylvester et al., 2004)
Total protozoa R	GCTTTCGWTGGTAGTGTATT		

F = forward; R = reverse.

Table 3
Effect of feeding different levels of whole linseed on goat's rumen fatty acid profiles.[1]

Fatty acids	Experimental diets			P-value
	L0	L10	L20	
C12:0, lauric	3.02 ± 0.25	ND	ND	–
C14:0, myristic	3.99 ± 0.40[a]	0.67 ± 0.40[b]	0.95 ± 0.34[b]	<0.01
C14:1, myristoleic	0.70 ± 0.04	0.27 ± 0.04	0.66 ± 0.30	0.17
C15:0, pentadecanoic	0.61 ± 0.06[a]	0.44 ± 0.06[b]	ND	0.01
C15:1, pentadecanoic	0.63 ± 0.10	ND	ND	–
C16:0, palmitic	25.12 ± 0.36[a]	11.95 ± 0.36[b]	9.58 ± 1.47[b]	0.00
C16:1, palmitoleic	1.52 ± 0.85	1.05 ± 0.85	0.57 ± 0.22	0.45
C17:0, heptadecanoic	1.36 ± 0.54	0.78 ± 0.54	0.63 ± 0.12	0.27
C17.1, heptadecenoic	ND	ND	0.10 ± 0.03	–
C18:0, stearic	48.69 ± 2.28[b]	66.34 ± 2.28[a]	67.32 ± 3.72[a]	<0.01
C18:1 n-9 cis, oleic	7.75 ± 3.28	8.09 ± 3.28	10.60 ± 2.59	0.70
C18:1 trans-11, vaccenic	2.53 ± 0.42[b]	3.91 ± 0.42[a]	3.49 ± 0.78[a]	0.04
C18:2 n-6, linoleic	1.56 ± 0.42	2.26 ± 0.42	2.35 ± 0.60	0.39
C18:2 cis-9, trans-11. CLA	0.50 ± 0.24	0.96 ± 0.34	0.82 ± 0.26	0.39
C18:2 trans-10, cis-12, CLA	0.29 ± 0.15[b]	0.65 ± 0.15[a]	0.88 ± 0.30[a]	0.07
C18:3 n-3, α-linolenic	0.75 ± 0.14[b]	1.44 ± 0.14[a]	1.59 ± 0.16[a]	0.04
C20:4 n-6, arachidonic	0.78 ± 0.10	0.98 ± 0.10	0.46 ± 0.17	0.47
SFA[2]	82.80 ± 2.92	80.41 ± 2.92	78.49 ± 2.63	0.48
UFA[3]	17.20 ± 2.92	19.59 ± 2.92	21.51 ± 2.63	0.48
MUFA[4]	12.53 ± 3.29	13.71 ± 3.29	15.42 ± 2.74	0.73
PUFA n-3[5]	0.75 ± 0.14[b]	1.44 ± 0.14[a]	1.59 ± 0.16[a]	0.06
PUFA n-6[6]	2.34 ± 0.34	3.24 ± 0.34	2.80 ± 0.50	0.32
Total CLA[7]	0.79 ± 0.30[b]	1.61 ± 0.30[a]	1.70 ± 0.55[a]	0.06
PUFA n-6/n-3 ratio	3.63 ± 0.14[a]	2.44 ± 0.19[b]	1.75 ± 0.16[b]	0.01
UFA/SFA	0.21 ± 0.04	0.24 ± 0.02	0.28 ± 0.04	0.49
PUFA/SFA	0.04 ± 0.001	0.06 ± 0.001	0.06 ± 0.01	0.17

L0 = control diet, containing 0% whole linseed; L10 = diet containing 10% whole linseed; L20 = diet containing 20% whole linseed; ND = not detected; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CLA = conjugated linoleic acids.
[a,b,c]Values with different superscripts within a row differ significantly at $P < 0.05$.
[1] Data are presented as means ± SEM of total fatty acids (g/100 g).
[2] SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0.
[3] UFA = C14:1 + C16:1 + C17:1 + C18:1n-9 trans + C18:2 + C18:3 + C20:4, C22:6, C20:5n-3 + C22:5-3 + C22:6n-3.
[4] MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1 n-9 trans + C18:1 n-9 cis + C18:1 n-7 + C20:1 n-9.
[5] PUFA n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[6] PUFA n-6 = C18:2 n-6 + C20:2 n-6 + C20:3 n-6.
[7] Total CLA = C18:2 cis-9, trans-11 + C18:2 trans-10, cis-12, CLA.

Table 4
Effect of feeding different levels of whole linseed on ruminal volatile fatty acid concentration and pH in goats.

Item	Treatment diets			P-value
	L0	L10	L20	
Total VFA, mmol/L	69.72 ± 3.02	65.09 ± 4.03	70.37 ± 2.54	0.47
Acetate, mol/100 mol	36.98 ± 1.02[b]	38.57 ± 071[ab]	39.99 ± 0.45[a]	0.04
Propionate, mol/100 mol	28.94 ± 0.89	30.22 ± 0.92	30.55 ± 1.02	0.64
Butyrate, mol/100 mol	23.89 ± 0.68[a]	21.87 ± 0.65[b]	20.31 ± 0.55[b]	0.05
Isobutyrate, mol/100 mol	3.83 ± 0.44	3.01 ± 0.19	3.12 ± 0.17	0.79
Valerate, mol/100 mol	3.82 ± 0.26[a]	3.06 ± 0.12[b]	3.12 ± 0.08[b]	0.02
Isovalerate, mol/100 mol	2.41 ± 0.21	2.20 ± 0.38	2.26 ± 0.08	0.83
Acetate: propionate	1.28 ± 0.06	1.28 ± 0.06	1.32 ± 0.03	0.89
pH	6.16 ± 0.04	6.28 ± 0.06	6.26 ± 0.04	0.13

L0 = control diet, containing 0% whole linseed; L10 = diet containing 10% whole linseed; L20 = diet containing 20% whole linseed.
[a,b]Values with different superscripts within a row differ significantly at $P < 0.05$.

3.3. Rumen microbial populations

The effects of feeding different levels of whole linseed as a source of ALA n-3 PUFA on rumen microbial populations of goats are presented in Table 5. In the present study, the total bacteria in the rumen were significantly affected by the dietary treatments. The concentration of total bacteria was lower ($P < 0.05$) in the rumen of goats fed linseed diets (L10 and L20 diets) than in those fed control diet (L0). However, no significant difference ($P > 0.05$) was observed between L10 and L20. Among the individual cellulolytic species, *Rumunococus albus* was not affected by dietary treatments, whereas the concentration of *Fibrobacter succinogenes* and *Rumunococus flavefaciens* were lower ($P < 0.05$) in goats that received L10 and L20 than in those fed L0. Similar to total bacteria and cellulolytic bacteria species, the population of total methanogens and protozoa were reduced ($P < 0.05$) in both inclusion levels (L10 and L20) compared with L0 with no differences between L10 and L20.

4. Discussion

Inclusion of sources of PUFA in animal diets comes mainly to increase these beneficial FA in animal products. In our previous studies, it has been reported that inclusion of whole linseed in diets

Table 5
Effect of feeding different levels of whole linseed on rumen microbial population (copies/mL) in goats.

Microorganism	Treatment diets			P-value
	L0	L10	L20	
Total bacteria ($\times 10^{10}$)	3.80 ± 0.82[a]	0.84 ± 0.25[b]	0.67 ± 0.13[b]	<0.001
Rumunococus albus ($\times 10^{6}$)	2.20 ± 0.56	2.18 ± 0.68	2.14 ± 0.85	0.68
Rumunococus flavefaciens ($\times 10^{6}$)	1.42 ± 0.56[a]	0.17 ± 0.06[b]	0.15 ± 0.07[b]	0.016
Fibrobacter succinogenes ($\times 10^{4}$)	9.36 ± 1.50[a]	1.27 ± 0.57[b]	1.62 ± 0.49[b]	0.003
Total methanogens ($\times 10^{6}$)	5.86 ± 1.02[a]	0.92 ± 0.15[b]	0.87 ± 0.23[b]	0.003
Total protozoa ($\times 10^{5}$)	2.81 ± 0.94[a]	0.34 ± 0.03[b]	0.23 ± 0.03[b]	<0.001

L0 = control diet, containing 0% whole linseed; L10 = diet containing 10% whole linseed; L20 = diet containing 20% whole linseed.
[a,b]Values with different superscripts within a row differ significantly at $P < 0.05$.

resulted in increasing the proportion of ALA and total n-3 PUFA in goat muscles and adipose tissues as the inclusion level of linseed increased (Abuelfatah et al., 2014). The growth performance and apparent digestibility were not affected by inclusion of linseed at level of 10% or 20%; however, at the level of 20%, the feed intake was negatively affected (Abuelfatah et al., 2013). The objective of this study was to examine the effects of feeding different levels (0%, 10% or 20%) of whole linseed, as a source of n-3 PUFA, FA composition of ruminal digesta and some microbial population.

The proportions of palmitic acid (C16:0) in the rumen digesta of experimental groups mirror that of their diets (Tables 1 and 3). The greatest concentration of C16:0 in the rumen coming from animals fed the diet with the highest C16:0 concentrations was also reported by (Kim et al., 2007). However, stearic (C18:0) was offered in the diets at a low proportion (3.23% to 5.92% of total FA), but it represented the major FA in the rumen digesta (48.69% to 67.32% of total FA) (Table 2). However, the 18-carbon UFA (C18:3 n-3, C18:2 n-6, and C18:1 n-9), which represent the major FA in experimental diets, were detected in low proportion in the rumen digesta. The increase in C18:0 and decrease in 18-carbon unsaturated fatty acids (UFA) indicated that a considerable amount of 18-carbon UFA was subjected to biohydrogenation since C18:0 is the end product of biohydrogenation of these FA (Harfoot and Hazlewood, 1997; McKain et al., 2010). However, the significantly higher proportion of ALA in the rumen digesta of goats fed linseed compared with L0 indicates that feeding whole linseed as a source of ALA was provided partial protection from biohydrogenation, even though the digesta were collected after 24 h of animals feeding. The significant increment in the proportion of vaccenic (C18:1 trans-11), and C18:2 trans-10, cis-12 CLA in animals fed linseed diets was expected since these trans-FA are intermediate products in biohydrogenation of unsaturated 18-carbon FA (Harfoot and Hazlewood, 1997; Kim et al., 2007; Lee and Jenkins, 2011). However, the cis-9 *trans*11 CLA was not significantly affected by level of linseed, because the *cis*-9 *trans*-11 CLA is the main CLA isomer during the biohydrogenation of LA rather than ALA (Lee and Jenkins, 2011). We noted that the pattern of FA composition of ruminal digesta of the experimental animals in this study resembles the pattern of FA composition of muscles taken from the same animals. The data related to FA composition of muscles has been published (Abuelfatah et al., 2013, 2016).

It is well known that ruminal pH is an important characteristic for assessing fermentation in the rumen (Liu et al., 2012). In the present study, the absence of any influence of feeding whole linseed on ruminal pH and total concentration of VFA in goats (Table 4) agrees with previous studies in lactating dairy cows fed diets containing about 10% (wt/wt) of crushed sunflower, flax, or canola seeds (Beauchemin et al., 2009), and in sheep fed diets containing linseed oil (Ueda et al., 2003; Kim et al., 2007). In contrast Czerkawski et al. (1975) reported decreased total VFA concentration when the diet supplemented with 90 g linseed oil/d. The different effect of PUFA on the proportions of individual VFA is also reported by (Machmüller et al., 2000; Soder et al., 2012). The reduction in molar proportion of butyrate in this current study agrees with Beauchemin et al. (2009) that the molar proportion of butyrate decreases with oilseed supplementation.

Ruminal microorganisms (bacteria, protozoa, and fungi) establish the key link between the diets and the ruminant animal (Weimer et al., 1999). The VFA resulting from the fermentation activity of these microorganisms as well as the microbial protein are digested and absorbed by the host for growth and production. Therefore, the study of rumen microbiology is fundamental for a greater understanding of the feed utilization and metabolic disorders of ruminants. Studies on the effects of lipids, especially PUFA, on rumen microbes have attracted considerable interest not only due to the human health aspects, but also for environmental issues. In this current study, the reduction in population of the total bacteria in L10 and L20 compared with L0 can be attributed to their high lipid content. Dietary lipids could inhibit the growth of bacteria in the rumen (Harfoot and Hazlewood, 1997; Maia et al., 2010). The total rumen bacteria were not affected by feeding oilseeds containing high concentrations of LA, ALA in cattle (Ivan et al., 2012), or linseed oil in goats (Ebrahimi, 2012) when the lipid content of the diet was similar to or less than the control, but the effect of PUFA on bacterial populations is not the same. Bacterial populations that are relevant for fiber digestion and biohydrogenation have been found to be sensitive to PUFA. Therefore, the impact of PUFA supplementation on ruminal bacteria should be made by examining specific bacterial species rather than the total number of bacteria (Liu et al., 2012). The results of the present experiment indicated different effects of feeding whole linseed on the selected strains of rumen bacteria. *F. succinogenes*, *R. flavefaciens*, and methanogen are strongly inhibited by inclusion of whole linseed, whereas the population of *R. albus* was not affected negatively by the treatment diets. The reduction in the population of *F. succinogenes* has been reported previously in cattle fed dietary PUFA (Ivan et al., 2012; Liu et al., 2012). The effect of PUFA on *R. flavefaciens* varied among comparable studies. Ebrahimi (2012) reported a reduction in *R. flavefaciens* in goat fed linseed oil. In contrast, Ivan et al. (2012) reported an increase in *R. flavefaciens* population in dairy cattle. The different findings can be attributed to level concentration of PUFA in the rumen. The growth of *R. flavefaciens* increases when PUFA in the rumen were at a low level, but decreases when these acids were fed higher levels (Zhang et al., 2008). The *R. albus*, which is the most important cellulolytic bacteria, was not affected negatively by the treatment diets in this current study. This finding is in agreement with Zhang et al. (2008) and Liu et al. (2012). However, Ivan et al. (2012) and Ebrahimi (2012) reported increases in population of *R. albus* when cattle and goats were fed PUFA, respectively.

The production of methane during fermentation in the rumen is an energy loss to ruminants but also has a potential impact on the

environment (Moss et al., 2000). Therefore, decreasing methane production in ruminants has significance for efficient animal production and for global environmental protection (Zhang et al., 2008). Methane is produced by the metabolic activity of the methanogens in the rumen (Tan et al., 2011). Protozoa are also involved in methanogenesis, because protozoa produce H_2, which is utilized by methanogens to produce methane (Vogels et al., 1980). In this study, both inclusion levels of linseed in the diets significantly decreased the population of methanogens and protozoa in rumen liquid of goats. In general, adding lipids to the diets of ruminants is a promising strategy to decrease the methane emissions because of the toxic effects of free FA on both methanogens and protozoa (Van Nevel and Demeyer, 1996; Maia et al., 2010). The limited ability of methanogens and protozoa to absorb and transform lipids leads to swelling and consequent rupture of the protozoa cells (Girard and Hawke, 1978).

5. Conclusion

Inclusion of linseed in the diet of goats at either 10% or 20% increases the molar proportion of acetate and decreases molar proportion butyrate and valerate. Feeding linseed also promotes changes in rumen microbial populations, such that both inclusion levels significantly decreased the population of *F. succinogenes*, *R. flavefaciens*, methanogens and protozoa in rumen liquid of goats.

References

Abubakr A, Alimon AR, Yaakub H, Abdullah N, Ivan M. Effect of feeding palm oil by-products based diets on total bacteria, cellulolytic bacteria and methanogenic archaea in the rumen of goats. PLoS One 2014;9(4):e95713.

Abuelfatah K, Zakaria MZAB, Meng GY, Sazili AQ. Changes in fatty acid composition and distribution of N-3 fatty acids in goat tissues fed different levels of whole linseed. Sci World J 2014;2014:10. http://dx.doi.org/10.1155/2014/934154. Article ID 934154.

Abuelfatah K, Zuki A, Goh Y, Sazili A. Effects of enriching goat meat with n-3 polyunsaturated fatty acids on meat quality and stability. Small Rumin Res 2016;136:36–42.

Abuelfatah K, Zuki ABZ, Goh YM, Sazili AQ. Effects of dietary n-3 fatty acids on growth performance, apparent digestibility, and carcass characteristics of crossbred Boer goat under tropical conditions. Asian J Anim Vet Adv 2013;8(6): 775–85.

AOAC. In: Herlick K, editor. Official methods of analysis. 15th ed. Arlington, Va, USA: Association of Official Analytical Chemists; 2007.

Beauchemin K, McGinn S, Benchaar C, Holtshausen L. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. J Dairy Sci 2009;92(5):2118–27.

Czerkawski J, Christie W, Breckenridge G, Hunter ML. Changes in the rumen metabolism of sheep given increasing amounts of linseed oil in their diet. Br J Nutr 1975;34:25–44.

Denman SE, McSweeney CS. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microb Ecol 2006;58(3):572–82.

Ebrahimi M. Production of Omega-3 polyunsaturated fatty acids enriched choven using treated oil palm (*Elaeis guineensis jacq*) fronds [vol. Ph.D]. Universiti Putra Malaysia; 2012.

Faseleh Jahromi M, Liang JB, Mohamad R, Goh YM, Shokryazdan P, Ho YW. Lovastatin-enriched rice straw enhances biomass quality and suppresses ruminal methanogenesis. BioMed Res Int 2013;2013:13. http://dx.doi.org/10.1155/2013/397934. Article ID 397934.

Field CJ, Blewett HH, Proctor S, Vine D. Human health benefits of vaccenic acid. Appl Physiol Nutr Met 2009;34(5):979–91.

Girard V, Hawke J. The role of holotrichs in the metabolism of dietary linoleic acid in the rumen. BBA-Lipid Lipid Met 1978;528(1):17–27.

Griinari J, Corl B, Lacy S, Chouinard P, Nurmela K, Bauman D. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. J Nutr 2000;130(9):2285–91.

Harfoot C, Hazlewood G. Lipid metabolism in the rumen. In: Hobson PN, Stewart CS, editors. The rumen microbial ecosystem. London: Springer; 1997. p. 382–426.

Ivan M, Mir P, Koenig K, Rode L, Neill L, Entz T, et al. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Rumin Res 2001;41(3):215–27.

Ivan M, Petit H, Chiquette J, Wright A-D. Rumen fermentation and microbial population in lactating dairy cows receiving diets containing oilseeds rich in C-18 fatty acids. Br J Nutr 2012;1(1):1–8.

Kim S, Adesogan A, Badinga L, Staples C. Effects of dietary n-6: n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. J Anim Sci 2007;85(3):706–16.

Koike S, Kobayashi Y. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol Lett 2001;204(2):361–6.

Lee YJ, Jenkins TC. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. J Nutr 2011;141(8):1445–50.

Legrand P, Schmitt B, Mourot J, Catheline D, Chesneau G, Mireaux M, et al. The consumption of food products from linseed-fed animals maintains erythrocyte omega-3 fatty acids in obese humans. Lipids 2010;45(1):11–9.

Liu S, Bu D, Wang J, Liu L, Liang S, Wei H, et al. Effect of incremental levels of fish oil supplementation on specific bacterial populations in bovine ruminal fluid. J Anim Physiol Anim Nutr 2012;96(1):9–16.

Machmüller A, Ossowski D, Kreuzer M. Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. Anim Feed Sci Technol 2000;85(1):41–60.

Machmüller A, Ossowski D, Wanner M, Kreuzer M. Potential of various fatty feeds to reduce methane release from rumen fermentation in vitro (Rusitec). Anim Feed Sci Technol 1998;71(1):117–30.

Maddock TD, Anderson VL, Lardy GP. Using flax in livestock diets. 2005. NDSU Extension Service.

Maia MR, Chaudhary LC, Bestwick CS, Richardson AJ, McKain N, Larson TR, et al. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. BMC Microbiol 2010;10(1):52.

McKain N, Shingfield KJ, Wallace RJ. Metabolism of conjugated linoleic acids and 18: 1 fatty acids by ruminal bacteria: products and mechanisms. Microbiol 2010;156(2):579–88.

Moss AR, Jouany JP, Newbold J. Methane production by ruminants: its contribution to global warming. In: Annales de Zootechnie, vol. 49. Paris: Institut national de la recherche agronomique; 2000. p. 231–54. 1960–2000.

Palmquist D. Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Prof Anim Sci 2009;25(3):207–49.

Palmquist DL. Digestibility of cotton lint fiber and whole oilseeds by ruminal microorganisms. Anim Feed Sci Technol 1995;56(3):231–42.

SAS. User's guide: statistics. 9.1 ed. Cary, NC: SAS Institute Inc; 2003.

Soder K, Brito A, Rubano M, Dell C. Effect of incremental flaxseed supplementation of an herbage diet on methane output and ruminal fermentation in continuous culture. J Dairy Sci 2012;95(7):3961–9.

Sylvester JT, Karnati SK, Yu Z, Morrison M, Firkins JL. Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. J Nutr 2004;134(12):3378–84.

Tan HY, Sieo CC, Abdullah N, Liang JB, Huang XD, Ho YW. Effects of condensed tannins from Leucaena on methane production, rumen fermentation and populations of methanogens and protozoa in vitro. Anim Feed Sci Technol 2011;169(3–4):185–93.

Ueda K, Ferlay A, Chabrot J, Loor J, Chilliard Y, Doreau M. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage: concentrate ratios. J Dairy Sci 2003;86(12):3999–4007.

Van Nevel C, Demeyer D. Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. Reprod Nutr Dev 1996;36:53–63.

Van Soest PJ, Robertson J, Lewis B. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74(10):3583–97.

Vogels GD, Hoppe WF, Stumm CK. Association of methanogenic bacteria with rumen ciliates. Appl Environ Microbiol 1980;40(3):608–12.

Waghmare JS. Conjugated linoleic acid: a significant review. Ind J Sci 2013;2(3): 8–12.

Weimer PJ, Waghorn GC, Odt CL, Mertens DR. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. J Dairy Sci 1999;82(1):122–34.

Zhang CM, Guo YQ, Yuan ZP, Wu YM, Wang JK, Liu JX, et al. Effect of octadeca carbon fatty acids on microbial fermentation, methanogenesis and microbial flora in vitro. Anim Feed Sci Technol 2008;146(3–4):259–69.