RUMINANT NUTRITION

Corn oil supplementation enhances hydrogen use for biohydrogenation, inhibits methanogenesis, and alters fermentation pathways and the microbial community in the rumen of goats

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Abstract

Enteric methane (CH_4) emissions are not only an important source of greenhouse gases but also a loss of dietary energy in livestock. Corn oil (**CO**) is rich in unsaturated fatty acid with >50% PUFA, which may enhance ruminal biohydrogenation of unsaturated fatty acids, leading to changes in ruminal H_2 metabolism and methanogenesis. The objective of this study was to investigate the effect of CO supplementation of a diet on CH_4 emissions, nutrient digestibility, ruminal dissolved gases, fermentation, and microbiota in goats. Six female goats were used in a crossover design with two dietary treatments, which included control and CO supplementation (30 g/kg DM basis). CO supplementation did not alter total-tract organic matter digestibility or populations of predominant ruminal fibrolytic microorganisms (protozoa, fungi, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*), but reduced enteric CH_4 emissions (g/kg DMI, –15.1%, $P = 0.003$). CO supplementation decreased ruminal dissolved hydrogen (dH_2, $P < 0.001$) and dissolved CH_4 ($P < 0.001$) concentrations, proportions of total unsaturated fatty acids ($P < 0.001$) and propionate ($P = 0.015$), and increased proportions of total SFAs ($P < 0.001$) and acetate ($P < 0.001$), and acetate to propionate ratio ($P = 0.038$) in rumen fluid. CO supplementation decreased relative abundance of family *Bacteroidales_BS11_gut_group* ($P = 0.032$), increased relative abundance of family *Rikenellaceae* ($P = 0.021$) and *Lachnospiraceae* ($P = 0.025$), and tended to increase relative abundance of genus *Butyrivibrio_2* ($P = 0.06$). Relative abundance ($P = 0.09$) and 16S rRNA gene copies ($P = 0.043$) of order *Methanomicrobiales*, and relative abundance of genus *Methanomicrobium* ($P = 0.09$) also decreased with CO supplementation, but relative abundance ($P = 0.012$) and 16S rRNA gene copies ($P = 0.08$) of genus *Methanobrevibacter* increased. In summary, CO supplementation increased rumen biohydrogenatation by facilitating growth of biohydrogenating bacteria of family *Lachnospiraceae* and genus *Butyrivibrio_2*

and may have enhanced reductive acetogenesis by facilitating growth of family *Lachnospiraceae*. In conclusion, dietary supplementation of CO led to a shift of fermentation pathways that enhanced acetate production and decreased rumen dH_2 concentration and CH_4 emissions.

Key words: corn oil, dissolved hydrogen, goat, methane, rumen fermentation, rumen microbes

Introduction

Enteric methane (CH_4) emissions from ruminant livestock contribute about 40% of the total greenhouse gases (**GHGs**) produced from livestock production (Gerber et al., 2013). Methane emissions also represent 2% to 12% of dietary energy waste in ruminants (Johnson and Johnson, 1995; Animut et al., 2008; Bhatta et al., 2012). Therefore, enteric CH_4 mitigation may help to decrease GHG concentrations in the atmosphere and improve feed efficiency and livestock productivity. Ruminal microbial fermentation releases metabolic hydrogen ([H]), which can be converted to molecular hydrogen (H_2) by hydrogenase and then utilized by methanogens to produce CH_4 (Hegarty and Gerdes, 1998). Enteric CH_4 mitigation can be achieved by diverting [H] in the rumen away from methanogenesis or by inhibiting rumen microbes involved in methanogenesis (Patra, 2012).

Many oils can effectively reduce methane emissions, while maintaining or improving the productivity of ruminants (Boadi et al., 2004; Beauchemin et al., 2009). Typically, inhibition of methanogenesis is expected to decrease the use of [H], leading to increased ruminal H_2 accumulation (Wang et al., 2013). Oil supplementation can decrease ruminal fiber degradation by inhibiting ruminal protozoa (Bhatt et al., 2011), causing a reduction in [H] release. Furthermore, oils may be rich in unsaturated fatty acid (**UFA**), and biohydrogenation of UFA to SFA can act as an alternative H_2 sink and thus may redirect [H] away from methanogenesis (Jenkins et al., 2008). However, the quantitative significance of biohydrogenation as an H_2 sink is uncertain (Woodward et al., 2006).

Corn oil (**CO**) is also rich in UFA with >50% PUFA, which may enhance ruminal biohydrogenation of UFA, leading to a change in ruminal H_2 metabolism and methanogenesis. The hypothesis of this study was that CO supplementation of a diet would decrease methanogenesis by altering H_2 production and consumption processes accompanied by changes in rumen fermentation, biohydrogenation and microbial community. To test this hypothesis, we used goat as experimental animal and then measured total-tract digestibility of nutrients, enteric CH_4 emissions, rumen dissolved gases, rumen fermentation, and microorganisms.

Materials and Methods

Animal and Diets

All animal procedures were approved by the Animal Care Committee (Approval number: ISA-Wang-201803), Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

Six healthy female Liuyang Black goats (a local breed in southern of China, 12 ± 0.5 months of age, nonpregnant) with a BW of 19 ± 1.2 (mean ± SD) kg were used in a study designed as a triplicated crossover with 2 consecutive periods. During each period, the goats were randomly allocated to 1 of 2 treatments (3 goats per treatment); control and CO supplementation. The control diet was formulated to supply 1.2 times the DE and CP requirements of nonpregnant female goats according to Zhang and Zhang (1998), and consisted of 50% of rice straw and 50% of concentrate (Table 1). CO (30 g/kg dietary DM) was supplemented to the diet at the expense of soybean meal (15 g/kg) and corn grain (15 g/kg). The ingredients, chemical composition, and long chain fatty acid (**LCFA**) content of diets are shown in Table 1.

Goats were kept in individual pens and fed at 07:30 and 17:30 hours with equal portions of feed offered at each meal. Each period lasted 25 d, including 14 d of diet adaption, 5 d of feces and urine collection, 4 d of respiration chamber measurements, and 2 d of rumen sampling. Diets were offered ad libitum during the first 7 d of each period to allow the goats to adapt to diets and to measure individual feed intake. From days 8 to 25, DMI was restricted to 100% of the ad libitum DMI of the goats to avoid diet selection. Feed refusals, if present, were collected and measured to determine actual nutrient intake.

Nutrient Digestibility

Nutrient digestibility was measured from days 15 to 19. Total feces were collected by the separator which can separate the feces and urine in the pen and weighed before the morning and afternoon feedings. A subsample (~1%) of feces from each goat was obtained at each collection time and frozen immediately at −20 °C, and another subsample (~1%) was acidified using 10% (w/w) H_2SO_4 to prevent N loss and then frozen immediately at −20 °C. The subsamples were then individually combined by day and goat within period. Fecal samples were dried at 65 °C for 48 h in a forced-air oven, and ground through a 1-mm screen. The acidified samples were used for total N analysis, whereas non-acidified samples were used for other chemical analysis.

Enteric Methane Emissions

Methane emissions were measured from days 20 to 23 using three plexiglass respiration chambers that permitted the goats to see each other. The emissions were measured individually for each goat as described by Wang et al. (2017). The goats were restrained within the chambers and had free access to a feed bin and drinking water. Airflow through the chamber was controlled by a pump and maintained under negative pressure (flow rate = 35 m³/h). Outlet gas from the chambers and background gas were sampled by the multiport inlet to a gas analyzer (UGGA, Los Gatos Research) for measuring CH_4 concentration. The calibration of each chamber is described by Wang et al. (2017). The measurements were cycled every 30 min, with 6 min for analyzing background concentrations of CH_4 in incoming air and 8 min for analyzing gases from each of the 3 chambers. Daily CH_4 emission was calculated using net CH_4 concentration and flow rate of air through each chamber at 30-min intervals. The chambers were opened twice a day at 07:00 and 17:00 hours to deliver the diets, and chambers were cleaned before the morning feeding.

Table 1. Ingredients, and chemical and long chain fatty acid (**LCFA**) composition of the diets

Item[1]	Diet	
	Control	Corn oil
Ingredient, g/kg		
Rice straw	500	500
Concentrate		
Soybean meal	80.0	65.0
Corn grain	292	277
Wheat bran	98.0	98.0
$CaCO_3$	1.00	1.00
CaH_2PO_4	4.00	4.00
NaCl	5.00	5.00
Premix[2]	20.0	20.0
Corn oil[3]	0.00	30.0
Chemical composition, g/kg		
OM	910	913
CP	105	99.9
NDF	468	450
ADF	271	262
Starch	245	226
EE	10.2	40.0
GE, MJ/kg	16.1	16.3
Predominant LCFA composition, g/100 g fatty acids		
C16:0	20.2	15.0
C16:1	0.18	0.12
C17:0	0.39	0.20
C18:0	2.57	1.79
C18 UFA	73.4	81.1
C18:1n9t	0.11	0.13
C18:1n9c	21.8	25.9
C18:2n6c	48.7	53.7
C18:3n3	2.73	1.31
C20:0	0.54	0.37
C20:1	0.32	0.35
C22:0	0.36	0.17
Other	2.10	0.86
Total UFA	74.1	81.7
Total SFA	25.9	18.2

[1]OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; GE, gross energy; UFA, unsaturated fatty acids; SFA, saturated fatty acids.
[2]Premix was formulated to provide (per kg of premix): 400 g $NaHCO_3$, 2 g Fe, 1 g Cu, 0.01 g Co, 0.05 g I, 6.6 g Mn, 4.4 g Zn, 0.003 g Se, 333 mg retinol, 5 mg cholecalciferol, 838 mg α-tocopherol.
[3]The corn oil was supplied by Wilmar International Ltd., Wuhan, China, and was composed of 15% saturated fatty acids, 32% monounsaturated fatty acids, and 53% polyunsaturated fatty acids.

Rumen Sampling

Samples of rumen contents were collected at 0, 2.5, and 6 h after the morning feeding on days 24 and 25. Rumen contents were collected by oral stomach tubing according to the method described by Wang et al. (2016). More than 300 mL of rumen contents were collected, with the initial 100 mL discarded to avoid saliva contamination and the remaining sample used for subsequent measurements. The pH was measured immediately after sampling using a portable pH meter (Starter 300; Ohaus Instruments Co. Ltd, Shanghai, China). Two other subsamples (35 mL each) were immediately transferred into 50-mL plastic syringes for measuring dissolved H_2 (dH_2) and dissolved CH_4 (dCH_4) concentrations. Samples of 2 mL of rumen contents were collected and centrifuged at 15,000 × g for 10 min at 4 °C,

and then 1.5 mL of supernatant was transferred to 2-mL tubes and acidified with 0.15 mL of 25% (w/v) metaphosphoric acid. The acidified samples were stored at –20 °C for measuring VFA concentrations. Two 10 mL subsamples (not centrifuged) were immediately frozen in liquid N_2 and then stored at –80 °C for DNA extraction and microbial analysis. Another two 10 mL subsamples were immediately stored at –20 °C for LCFA measurement. The remaining sample of rumen content was stored at –20 °C for measuring ammonia (NH_4^+) concentration.

Chemical Analysis

All samples of feeds, refusals, and feces were dried at 65 °C and ground to pass through a 1-mm screen. The DM content was determined by oven drying at 105 °C for 24 h. The OM (method 942.05), CP (method 970.22), and ether extract (EE; method 963.15) were determined according to methods of AOAC (2005). The NDF and ADF were measured by a Fibretherm analyzer (C. Gerhardt Ins., Königswinter, Germany) following the procedures of Van Soest et al. (1991) and expressed as inclusive of residual ash with inclusion of α-amylase (Sigma, Shanghai, China) for NDF. GE was determined using an isothermal automatic calorimeter (5E-AC8018; Changsha Kaiyuan Instruments Co., Changsha, Hunan, China). Starch content was determined after pre-extraction with ethanol (80%), and glucose released from starch by enzyme hydrolysis was measured using amyloglucosidase (Kartchner and Theurer, 1981).

The technique used to extract dCH_4 and dH_2 from liquid to gas phase is described by Wang et al. (2014). Extracted gaseous CH_4 and H_2 were analyzed using gas chromatography (**GC**; Agilent 7890A, Agilent Inc., Palo Alto, CA). Concentrations of dH_2 and dCH_4 in the original liquid fraction were calculated using equations described by Wang et al. (2016).

Individual VFA concentrations were analyzed using GC according to the procedure of Wang et al. (2016). Ammonia concentration was measured using the procedure of Shahinian and Reinhold (1971). For LCFA composition determination, feedstuffs were processed in the state obtained, while rumen contents were freeze-dried (CHRIST RVC2-25 CDPIUS, Marin Christ CO., Ltd, Osterode, Germany). About 200 mg of sample was placed into 15.5 mL Pyrex screw-cap culture tubes that contained 0.7 mL of 10 N KOH in water and 5.3 mL of methanol. The tubes were incubated in a water bath at 55 °C for 1.5 h and hand-shaking vigorously for 5 s every 20 min, cooled in a water bath to below room temperature, 0.58 mL of 24 N H_2SO_4 in water was added, and samples were mixed by inversion to precipitate the K_2SO_4 present. Samples were incubated again in a water bath at 55 °C for 1.5 h, hand-shaking vigorously for 5 s every 20 min, then cooled in a water bath to below room temperature, 3 mL of hexane was added and tubes were vortex mixed for 5 min. Tubes were centrifuged for 5 min at 1,500 × g, and the hexane layer containing fatty acid methyl esters was transferred into a vial and stored at –20 °C until analysis. Individual LCFA composition of the fatty acid methyl esters was analyzed according to the direct fatty acid methyl esters synthesis procedure described by O'Fallon et al. (2007).

Microbial Analyses

Rumen samples collected 2.5 h after the morning feeding were freeze-dried (CHRIST RVC2-25 CDPIUS, Marin Christ CO., Ltd, Osterode, Germany) for microbial analysis. The DNA was extracted with repeated bead beating plus column purification as described by Yu and Morrison (2004), and eluted by 300 μL TE buffer (Tris 10 mmol/L, EDTA 1 mmol/L, pH = 8.0). The quality

and quantity of DNA was measured based on absorbance at 260 and 280 nm using a NanoDrop ND-2000 (NanoDrop Technologies Inc., Wilmington).

The 16S rRNA V3–V4 hypervariable regions of the bacteria genomic DNA were used for PCR amplification with the primers 5′-ACTCCTACGGGAGGCAGCAG-3″ (338F) and 5′-GGACTACHVGGGTWTCTAAT-3′ (806R) (Wang et al., 2017), while the 16S rRNA V4–V5 hypervariable regions of the methanogen genomic DNA were used for PCR amplification with the primers 5′-TGYCAGCCGCCGCGGTAA-3′ (524F) and 5′-YCCGGCGTTGAVTCCAATT-3′ (Arch958R) (Ma et al., 2018). The PCR reactions were performed in triplicate using a 20-µL mixture containing 0.8 µL of each primer (10 µmol/L), 10 ng of template DNA, 2 µL 2.5 mM dNTPs, 0.4 µL of FastPfu polymerase (Transgen, Beijing, China) and 4 µL 5 × FastPfu Buffer (Transgen). The thermal cycling programing was performed as follows: initial denaturation step, 95 °C, 3 min; denaturation, 27 cycles, 95 °C, 30 s; annealing, 55 °C, 30 s; elongation, 72 °C, 45 s; and final extension, 72 °C, 10 min. The PCR products were excised from 2% agarose gels and purified using a QIAquick Gel extraction kit (Qiagen, Hilden, Germany). The 16S rRNA library was prepared according to the instructions provided with Nextera XT Index kits (Illumina, San Diego, CA). A unique 10-base barcode was added at the 5′ end of each amplicon as well as to Illumina adapter sequences. Amplicons from each reaction mixture were quantified using QuantiFluorTM–ST system (Promega, Madison, WI), and pooled at equimolar ratios based on the concentration of each amplicon. Amplicons were sequenced with the Illumina MiSeq platform (PE300) at the Majorbio Bio-Pham Technology, Shanghai, China. MOTHUR v.1.39.5 (Schloss et al., 2009) was used for quality control of the sequence reads following the protocol described by Kozich et al. (2013). Quality filters were applied to trim raw sequences according to the following criteria: (i) reads with average quality score < 20 over a 10-bp sliding window were removed, and truncated reads shorter than 150 bp were discarded. (ii) Truncated reads containing homopolymers longer than 8 nucleotides, more than 0 base in barcode matching, or more than 2 different bases to the primer were removed from the dataset. The possible chimeras were checked and removed via USEARCH using the ChimeraSlayer "gold" database as described by Edgar et al. (2011). The average reads per sample used for operational taxonomic unit (**OTU**) determination of bacteria and Archaea was 25,184 ± 3,490.0 (Supplementary Fig. S1) and 29,991 ± 6,098.9 (Supplementary Fig. S2), respectively. The high-quality reads were clustered into OTU at 97% similarity using USEARCH (Edgar, 2013), and representative sequences defined by abundance from each OTU were identified using PyNAST (Caporaso et al., 2010) against SILVA database v.128 for bacteria and archaea (Quast et al., 2013). Taxonomy analysis was performed using RDP classifier v.11.1 with a minimum support threshold of 80% (Wang et al., 2007). α diversity analyses were generated by MOTHUR v.1.39.5 (Schloss et al., 2009), including observed species, Chao, Ace, Shannon-Weiner, and Simpson's indices. All 16S rRNA gene sequences were deposited into the NCBI Sequence Read Archive under accession number PRJNA494452 for bacteria and PRJNA494459 for methanogen.

Quantitative real-time PCR (**qPCR**) was performed according to the procedures detailed by Jiao et al. (2014). In brief, the plasmid DNA containing exact 16S or 18S rRNA gene inserts were used to make a standard curve for each group and individual species. All standard curves met the following requirements: $R^2 > 0.99$ and 90% < E < 120%. Forward and reverse primers selected from the literature for qPCR groups are shown in Supplementary Table S1.

Statistical Analysis

Data were subjected to analysis using the SPSS 19.0 software. Fermentation and CH_4 emissions data were averaged to obtain a mean value for the 2 consecutive days before further analysis. The normal distribution of our data was checked with normality test (Kolmogorov-Smirnov). The data followed normal distribution were analyzed according to the following procedures. Data of nutrient digestibility, CH_4 emissions, qPCR, alpha diversity indexes, and relative abundance estimated from 16S rRNA gene library sequences were analyzed using a linear mixed model that included dietary treatment ($n = 2$) as fixed effect, and period ($n = 2$) and goat ($n = 6$) as random effects. Data of ruminal samples were analyzed using a linear mixed model with treatment ($n = 2$) and treatment by sampling time interaction as fixed effects, goat ($n = 6$) and period ($n = 2$) as random effects, and sampling time ($n = 3$) as a repeated measurement. For the data that not follow a normal distribution, the permutation test was run by R with linear mixed models (package minque, permutation number = 999; https://cran.r-project.org/web/packages/minque/index.html) as described by Wu (2012) according to MINQUE theory (Rao, 1971). Significance was considered when $P \leq 0.05$, and $0.05 < P \leq 0.1$ was accepted as a tendency to significance.

A principal coordinate analysis was performed in MOTHUR v.1.39.5 (Schloss et al., 2009) at OTU level according to 16S rRNA gene amplicon sequence data based on Bray–Curtis similarity distances (Bray and Curtis, 1957) to test the treatment and goat effects on bacteria and methanogens community structure. Data were then analyzed by nonparametric permutational multivariate analysis of variance (PERMANOVA) based on 9,999 permutations with treatment ($n = 2$) and goat ($n = 6$) as fixed effects (R statistics; Vegan package 2.4–4; Function adonis; Oksanen et al., 2017).

Results

Total-Tract Digestibility and Methane Emissions

CO supplementation had no effect on the DMI ($P = 0.29$), decreased ($P < 0.001$) total-tract digestibility of starch, tended to increase NDF digestibility ($P = 0.08$), and decreased CH_4 emission expressed as g/d (–15.0 %, $P = 0.003$), g/kg DMI (–15.1%, $P = 0.003$), g/kg OM digested (–10.8%, $P = 0.07$), g/kg NDF digested (–11.9%, $P = 0.043$), and MJ/MJ GE intake (–18.1%, $P < 0.001$; Table 2).

Rumen Fermentation

CO supplementation decreased ($P < 0.001$) UFA proportion and increased ($P < 0.001$) SFA proportion in rumen fluid (Table 3). For individual UFA and SFA, CO supplementation decreased proportion of C18:1n9c ($P = 0.007$), C18:2n6c ($P < 0.001$), C20:1 ($P = 0.001$), and C20:4n6 ($P < 0.001$), and increased ($P < 0.001$) proportion of C18:0. No interaction effect between treatment and sampling time on rumen LCFA profile was found.

CO supplementation decreased pH ($P < 0.001$), dH_2 (–33.3%, $P < 0.001$), and dCH_4 (–33.8%, $P < 0.001$) concentration, and increased (+17.2%, $P = 0.043$) ammonia-N concentration in rumen fluid (Table 4). There was an increase in total VFA concentration ($P = 0.027$), molar percentage of acetate ($P < 0.001$) and isobutyrate ($P < 0.001$), and acetate to propionate ratio ($P = 0.038$), and decreased molar percentage of propionate ($P = 0.015$), valerate ($P < 0.001$), and isovalerate ($P < 0.001$) with CO supplementation. No interaction effect between treatment and sampling time on rumen fermentation characteristics was found.

Rumen Microbial Community

CO supplementation had no effect on bacterial richness expressed as Chao and Ace scores (Table 5). Goats fed a diet supplemented with CO had a greater Shannon index ($P = 0.048$) and tended to have increased observed OTUs ($P = 0.10$). Goats had distinct separation of bacterial community ($P = 0.009$), and CO supplementation altered bacterial community of each individual goat (Fig. 1). CO supplementation decreased relative abundance of family *Bacteroidales_BS11_gut_group* ($P = 0.032$) and increased relative abundance of family *Rikenellaceae* ($P = 0.021$) and *Lachnospiraceae* ($P = 0.025$) (Table 5). CO supplementation tended to increase relative abundances of genus *Butyrivibrio_2* ($P = 0.06$) and *Prevotellaceae_UCG-001* ($P = 0.06$), and tended to decrease relative abundance of genus *Ruminococcus_2* ($P = 0.06$). The relative abundances of other major bacterial groups are supplied in Supplementary Table S2, with relative abundance >0.5% and $P > 0.10$ between treatments at genus level.

CO supplementation had no effect on methanogen richness expressed as Shannon index, Chao and Ace, decreased the observed OTUs ($P = 0.026$) and tended to increase Simpson index ($P = 0.06$) (Table 6). Goats had distinct separation of methanogen community ($P = 0.041$), and CO supplementation altered methanogen community of each individual goat (Fig. 2). CO supplementation increased ($P = 0.033$) relative abundance of order *Methanobacteriales* and tended to decrease ($P = 0.09$) relative abundance of order *Methanomicrobiales*. CO supplementation increased ($P = 0.012$) relative abundance of genus *Methanobrevibacter* and tended to decrease ($P = 0.09$) relative abundance of genus *Methanomicrobium*. The relative abundances of other major methanogen groups are supplied in Supplementary Table S3, with relative abundance >0.5% and $P > 0.10$ between treatments at genus level.

CO supplementation had no effect on the 16S/18S rRNA/ITS1 gene copies of total bacteria and methanogens, fungi, and protozoa, respectively, but it showed a tendency to decrease the 16S rRNA gene copies of *Ruminobacter amylophilus* ($P = 0.08$) and decreased 16S rRNA gene copies of *Methanomicrobiales* ($P = 0.043$), and tended to increase 16S rRNA gene copies of *Methanobacteriales* ($P = 0.09$) and *Methanobrevibacter* ($P = 0.08$; Table 7).

Discussion

Unsaturated fatty acid of oil is toxic to rumen microbes (Maia et al., 2007), and increased UFA intake may lead to an inhibition of fiber digestion (McGinn et al., 2004). Studies have reported that oil supplementation of diets can negatively affect nutrient digestibility (especially fiber digestibility) in dairy and beef cattle (50 g/kg DM sunflower oil; McGinn et al., 2004; 40 g/kg DM linseed oil; Benchaar et al., 2015). In our study, CO supplementation of diets fed to goats did not alter OM digestibility with a tendency for increased NDF digestibility and decreased starch digestibility. The dose of CO supplementation (30 g/kg DM) may not have been high enough to negatively affect fiber digestibility. Furthermore, the different LCFA composition among oils might be another possible explanation. For example, C18:3 fatty acids have greater toxicity than other fatty acids for the ruminal microbes (Maia et al., 2007). CO has lower C18:3 fatty acids (0.7%), which may have less effect on major fiber degraders and fiber digestibility.

Table 2. Effect of corn oil supplementation on nutrient intake, digestibility, and enteric methane emissions in goats ($n = 6$)

Item[1]	Treatment		SEM[2]	P-value
	Control	Corn oil		
DMI, g/d	472	478	4.4	0.29
Digestibility, %				
DM	67.2	67.7	0.94	0.57
OM	70.3	70.2	1.38	0.90
CP	63.9	63.6	2.30	0.93
NDF	56.6	58.9	2.17	0.07
Starch	97.8	97.0	0.25	<0.001
GE	66.2	67.0	0.92	0.43
Methane emission				
g/d	8.45	7.18	0.55	0.003
g/kg DMI	17.9	15.2	1.15	0.003
g/kg OM digested	27.9	24.9	1.77	0.07
g/kg NDF digested	74.2	65.4	5.65	0.08
MJ/MJ GE intake, %	6.14	5.03	0.39	<0.001

[1]DMI, dry matter intake; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; GE, gross energy.
[2]SEM is pooled standard error of means.

Table 3. Effect of corn oil supplementation on long chain fatty acid profile (g/100 g fatty acid) in the rumen content of goats ($n = 6$)

Item[1]	Treatment		SEM[2]	P-value		
	Control	Corn oil		Treatment	Time	Treatment × time
C14:0	5.78	2.65	0.39	<0.001	0.54	0.96
C16:0	30.1	24.6	0.43	<0.001	0.35	0.59
C16:1	1.48	0.81	0.07	<0.001	0.82	0.70
C17:0	1.94	1.08	0.11	<0.001	0.83	0.35
C18:0	33.3	47.2	1.07	<0.001	0.06	0.31
C18 UFA	25.0	22.5	0.80	0.011	<0.001	0.29
C18:1n9t	9.62	12.8	0.57	<0.001	<0.001	0.16
C18:1n9c	7.16	5.26	0.68	0.007	<0.001	0.42
C18:2n6c	7.68	4.24	0.54	<0.001	0.026	0.58
C18:3n3	0.58	0.22	0.05	<0.001	0.33	0.89
C20:0	0.53	0.54	0.03	0.48	0.022	0.73
C20:1	0.20	0.05	0.04	0.001	0.28	0.36
C20:4n6	1.69	0.61	0.18	<0.001	0.50	0.94
Others	0.21	0.05	0.03	<0.001	0.28	0.36
Total UFA	28.4	24.0	0.79	<0.001	0.003	0.29
Total SFA	71.6	76.0	0.79	<0.001	0.003	0.29

[1]UFA, unsaturated fatty acids; SFA, saturated fatty acids.
[2]SEM is pooled standard error of means.

Table 4. Effect of corn oil supplementation on metabolites in the rumen of goats ($n = 6$)

Item	Treatment			P-value		
	Control	Corn oil	SEM[1]	Treatment	Time	Treatment × time
pH	6.56	6.38	0.02	< 0.001	< 0.001	0.22
Dissolved gas						
Hydrogen, μmol/L	0.47	0.32	0.03	< 0.001	0.035	0.11
Methane, mmol/L	1.33	0.87	0.04	< 0.001	0.20	0.26
Ammonia-N, mmol/L	6.38	7.48	1.72	0.043	< 0.001	0.23
VFA[2], mmol/L	77.6	85.0	3.52	0.027	0.09	0.60
Acetate to propionate ratio	3.45	3.82	0.33	0.038	0.31	0.88
Molar percentage of individual VFA, mol/100 mol						
Acetate	69.5	72.2	1.89	< 0.001	0.73	0.83
Propionate	20.2	18.9	1.51	0.015	0.07	0.86
Butyrate	7.22	6.39	0.73	0.07	0.40	0.77
Isobutyrate	0.81	1.10	0.09	< 0.001	< 0.001	0.12
Valerate	0.60	0.50	0.08	< 0.001	< 0.001	0.75
Isovalerate	1.39	1.18	0.11	< 0.001	< 0.001	0.14

[1]SEM is pooled standard error of means.
[2]VFA, volatile fatty acid.

Table 5. Effect of corn oil supplementation on α diversity and bacterial groups (relative abundance >0.5%, $P \leq 0.10$ between treatments at genus level) in the rumen of goats ($n = 6$)

Item	Treatment		SEM[2]	P-value
	Control	Corn oil		
α diversity index				
Observed OTUs	792	837	65.8	0.10
Shannon	4.17	4.59	0.11	0.048
Simpson	0.06	0.04	0.011	0.12
Chao	716	729	16.5	0.34
Ace	707	726	15.8	0.21
Relative abundances assessed by amplicon sequences[1], %				
f__Prevotellaceae	33.1	29.9	6.37	0.66
g__Prevotellaceae_UCG-001	0.69	1.72	0.081	0.06
f__Bacteroidales_BS11_gut_group	16.3	8.49	1.701	0.032
g__norank_f__Bacteroidales_BS11_gut _group	16.3	8.49	1.701	0.032
f__Ruminococcaceae	13.2	15.3	2.43	0.47
g__Ruminococcus_2	0.79	0.44	0.111	0.06
g__Ruminococcaceae_UCG-002	0.47	1.14	0.299	0.09
f__Rikenellaceae	4.45	8.05	1.478	0.021
g__unclassified_f__Rikenellaceae	1.09	2.38	0.315	0.07
f__Lachnospiraceae	6.11	7.99	1.263	0.025
g__Butyrivibrio_2	0.21	0.73	0.155	0.06

[1]f_, family; g_, genus.
[2]SEM is pooled standard error of means.

The rumen has a wide-range of fibrolytic microbial groups that degrade fiber. Fungi, protozoa, and fibrolytic bacteria (i.e., *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*) are active fiber degraders (Wang and McAllister, 2002). Yang et al. (2009) reported that linseed oil (40 g/kg DM) supplementation inhibited *R. flavefaciens* in dairy cows. Patra and Yu (2013) reported that coconut oil supplementation (6.2 mL/L culture fluid) decreased NDF degradation by inhibiting *R. flavefaciens* and *F. succinogenes* during in vitro ruminal fermentation. In the present study, CO supplementation did not alter population density of the major fiber degraders, which is consistent with the lack of negative effects on digestibility.

Oil supplementation can inhibit rumen methanogenesis. Puchala et al. (2018) found that coconut oil and soybean oil supplementation (40 g/kg DM) can reduce 34% and 32% of CH_4

emissions in goat, respectively. Machmüller et al. (2003) reported that coconut oil (50 g/kg DM) could reduce 14% of CH_4 emissions in sheep. We observed that 30 g CO/kg DM supplementation reduced CH_4 emissions by 10.8% (g/kg OM digested) to 18.1% (MJ/MJ GE intake), depending upon how CH_4 was expressed. Moreover, the observed reduction in methanogenesis is further supported by the decreased ruminal dCH_4 concentration. Oil supplementation can inhibit ruminal fermentation, decrease protozoa and provide an alternative H_2 sink redirecting [H] away from methanogenesis (Johnson and Johnson, 1995). In the present study, CO supplementation did not alter OM digestibility or 18S rRNA gene copies of protozoa, but increased VFA concentration, indicating that the reduction in CH_4 emissions was unlikely caused by decreased ruminal substrate fermentation or by the inhibition of protozoa growth. Machmüller et al. (2003) have also
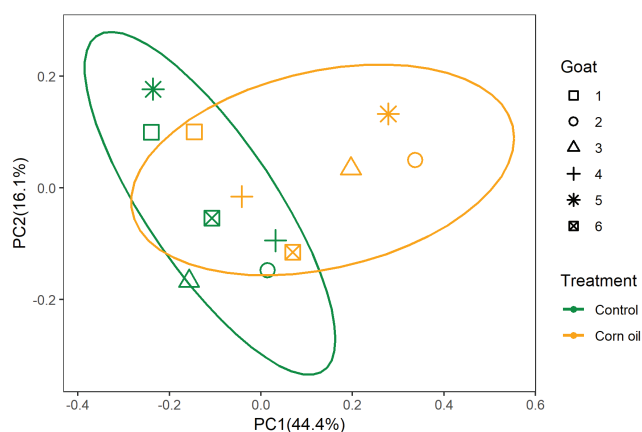
Figure 1. Principal coordinate analysis (**PCoA**) plots of the Bray–Curtis dissimilarities for the bacterial community of rumen samples (treatment, $P = 0.18$; goat, $P = 0.009$; $n = 12$). Percentages of data variation explained by the analysis are shown on the axes. Green ellipse represents the control group, orange ellipse represents the corn oil treatment, and the confidence interval is 95%.
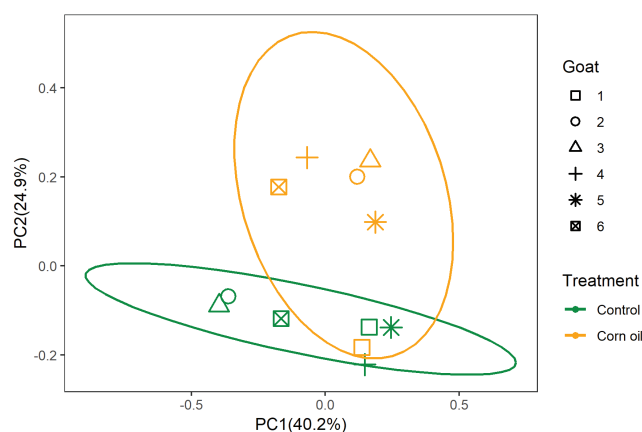

Figure 2. PCoA plots of the Bray–Curtis dissimilarities for methanogen community of rumen samples (treatment, $P = 0.21$; goat, $P = 0.041$, $n = 12$). Percentages of data variation explained by the analysis are shown on the axes. Green ellipse represents the control group, orange ellipse represents the corn oil treatment, and the confidence interval is 95%.

Table 6. Effect of corn oil supplementation on α diversity and methanogens groups (relative abundance >0.5%, $P \leq 0.10$ between treatments at genus level) in the rumen of goats ($n = 6$).

Item	Treatment			–
	Control	Corn oil	SEM[2]	P-value
α diversity index				
Observed OTUs	150	134	14.1	0.026
Shannon	1.70	1.57	0.141	0.13
Simpson	0.26	0.32	0.039	0.06
Chao	53.2	64.8	9.40	0.25
Ace	63.8	67.4	11.62	0.58
Relative abundances assessed by amplicon sequences[1], %				
o__Methanobacteriales	65.4	82.8	7.80	0.033
g__Methanobrevibacter	59.3	79.9	6.36	0.012
o__Methanomicrobiales	23.7	8.00	5.940	0.09
g__Methanomicrobium	23.7	8.00	5.940	0.09

[1]o_, order; g_, genus.
[2]SEM is pooled standard error of means.

reported that the inhibited methanogenesis with coconut oil supplementation is not caused by the depression of protozoa population in sheep.

Unsaturated fatty acids in oil utilize [H] during the ruminal biohydrogenation process and thus can compete H_2 away from methanogenesis (Jenkins et al., 2008). The UFA concentration in the CO diet was greater than that in the control diet. However, goats fed the CO diet had greater SFA proportion in rumen contents than those fed the control diet, indicating that CO consumed [H] by facilitating ruminal UFA biohydrogenation for SFA synthesis. Loor et al. (2004) found greater ruminal biohydrogenation in dairy cows fed a diet supplemented with linseed oil (30 g/kg DM). Furthermore, the decreased dH_2 concentration also indicated that CO supplementation enhanced H_2 consumption by incorporating [H] for UFA biohydrogenation. The predominant LCFA is C16 and C18 fatty acids, which accounted for more than 85% of total LCFA in this study. Goats fed CO had lower total or individual C18 UFA (such as C18:1n9c, C18:2n6c, and C18:3n3) than those fed the

control diet, indicating an enhancement of biohydrogenation. Studies have reported that beef steers fed a diet with greater oil content had greater biohydrogenation of total and individual C18 UFA than those fed a control diet (Bolte et al., 2001; Duckett et al., 2002).

Oil supplementation can affect rumen fermentation profile. Benchaar et al. (2015) reported that linseed oil supplementation (40 g/kg DM) increased propionate production at the expense of acetate in dairy cows. Gonthier et al. (2004) demonstrated that flaxseed supplementation (126 g/kg DM) decreased acetate and increased propionate percentage. However, such changes in VFA profiles are not always observed with oil feeding. For instance, Machmüller et al. (2003) observed that coconut oil (50 g/kg) supplementation of diets increased acetate-to-propionate ratio in sheep. In our study, CO supplementation increased acetate and decreased propionate molar percentage in goats, and such facilitation of acetate production was consistent with the observed low ruminal dH_2 concentration in the rumen of the goats. Janssen (2010) stated that H_2 forming pathways have more negative Gibbs free energy changes at low dH_2 concentration. Acetate generation is thermodynamically favorable under low ruminal H_2 partial pressure, which facilitates H_2 generation through acetate production (Martinez-Fernandez et al., 2017). Furthermore, acetate can be produced from H_2 and carbon dioxide ($4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$) through the acetyl-CoA pathway by acetogens, such as family *Lachnospiraceae* and *Ruminococcaceae* (Gagen et al., 2015). CO supplementation increased relative abundance of family *Lachnospiraceae* and acetate molar percentage, indicating that reductive acetogenesis might have played a role in H_2 consumption, and may have contributed to the decrease in dH_2 concentration and CH_4 emissions.

CO supplementation altered some groups of bacteria related to biohydrogenation, H_2-production, and H_2-utilization. Genus *Butyrivibrio* and some unknown genera within the family *Lachnospiraceae* are the most important biohydrogenating bacteria (Boeckaert et al., 2008), especially for C18 biohydrogenation (Van de Vossenberg and Joblin, 2003; Paillard et al., 2007). Goats fed diet with CO supplementation had greater relative abundance of family *Lachnospiraceae* and a tendency for increased genus *Butyrivibrio_2*, which may have been responsible for utilizing H_2 for C18 biohydrogenation in our study. Decreased ruminal H_2 may

Table 7. Effect of corn oil supplementation on selected microbial groups (log$_{10}$ copies/g DM rumen contents) in the rumen of goats (n = 6)

Item[1]	Treatment			
	Control	Corn oil	SEM[2]	P-value
Bacteria	13.6	13.6	0.08	0.80
Fungi	10.3	10.2	0.17	0.44
Protozoa	10.2	10.2	0.23	0.83
Methanogens	11.9	11.9	0.09	0.92
Selected groups of bacteria				
Prevotella	13.1	13.1	0.08	0.77
Fibrobacter succinogenes	10.7	10.9	0.22	0.45
Ruminococcus albus	9.83	10.1	0.17	0.12
Ruminococcus flavefaciens	10.0	9.96	0.13	0.76
Ruminobacter amylophilus	11.1	10.8	0.14	0.08
Selected groups of methanogens				
Methanobacteriales	11.1	11.5	0.19	0.09
Methanobrevibacter	10.2	10.4	0.12	0.08
Methanomicrobiales	9.23	9.04	0.26	0.043

[1]16S rRNA gene copies were measured for bacteria and methanogen, 18S rRNA gene copies were measured for protozoa, and the multiple alignments of 18S rRNA and ITS1 gene copies were measured for fungi.
[2]SEM is pooled standard error of means.

facilitate H$_2$ production and inhibit H$_2$ utilization (Janssen, 2010; Wang et al., 2016). The family *Rikenellaceae* is described as rod-shaped bacteria and can ferment carbohydrates to produce H$_2$ (Su et al., 2014). CO supplementation increased relative abundance of family *Rikenellaceae*. The family *Bacteroidales_BS11_gut_group* belongs to order *Bacteroidales* and is an H$_2$ user (Gast et al., 2009), while *R. amylophilus* is a starch-using bacteria and is also an H$_2$-user (Anderson, 1995). CO supplementation decreased relative abundance of family *Bacteroidales_BS11_gut_group* and 16S rRNA gene copies of *R. amylophilus*. A shift in the bacterial community composition indicated that CO supplementation favored growth of family *Lachnospiraceae* and genus *Butyrivibrio_2* that use H$_2$ for UFA biohydrogenation. However, there was also a decline in the relative abundance of some other H$_2$-using bacterial groups as well as an increase in the relative abundance of some H$_2$-producing bacterial groups.

The C18 fatty acids of oil are toxic to protozoa (Machmüller et al., 2000). Fatty acids are considered to inhibit methanogens by binding to the cell membrane and interrupting membrane transport (Dohme et al., 2001). Oil supplementation has been shown to decrease the methanogen population (Mao et al., 2010), which was not the case in our study. This lack of effect on methanogen population is consistent with the unchanged protozoa population, as 10% to –20% of methanogens are associated with protozoa (Stumm et al., 1982). However, CO supplementation may also alter some groups of methanogens, as shifts of methanogen community were observed within each individual goat. CO supplementation decreased relative abundance and 16S rRNA gene copies of order *Methanomicrobiales*, and increased relative abundance and 16S rRNA gene copies of order *Methanobacteriales* and genus *Methanobrevibacter*. The reduction in dH$_2$ concentration due to CO supplementation decreased H$_2$ availability for methanogens, which may have changed the relative abundances of *Methanobrevibacter* and *Methanomicrobium*, as methanogens could have different advantages for using available rumen H$_2$ (Danielsson et al., 2017). Methanogens hold genes encoding

the key methanogenic enzyme methyl-coenzyme M reductase (**MCR**). MCRI and MCRII are two isoenzymes of MCR. MCRI is usually expressed at low H$_2$ concentrations, while the MCRII is usually expressed at high H$_2$ concentrations (Reeve et al., 1997). *Methanobrevibacter ruminantium* M1 can only code for MCRI (Leahy et al., 2010). Genus *Methanobrevibacter* may also code for MCRI, and thus was increased at lower H$_2$ concentrations for CO treatment.

Conclusions

CO supplementation decreased ruminal dH$_2$ and dCH$_4$ concentrations, CH$_4$ emissions, and increased ruminal SFA proportion. The unsaturated fatty acids in CO provided an additional H$_2$ sink for biohydrogenation that competed with methanogenesis for available rumen H$_2$. Reductive acetogenesis may have been another cause of enhanced H$_2$ consumption leading to increased acetate production. CO supplementation increased acetate molar percentage and favored growth of some H$_2$-producing bacterial groups, and decreased propionate molar percentage and inhibited growth of some H$_2$-using bacterial groups. CO supplementation did not alter methanogen population, indicating that decreased methanogensis can be caused by the decreased activity of methanogens under low rumen H$_2$. Thus, CO supplementation decreased the available H$_2$ for methanogens through enhanced biohydrogenation and reductive acetogenesis, leading to enhanced acetate production and decreased CH$_4$ emissions. Further researches are still need to investigate its effect on animal productivity and CH$_4$ emissions over a longer period.Figure S1. The multiple samples rarefaction curve of bacteria. SP1–12 are number of samples.Figure S2. The multiple samples rarefaction curve of methanogens. SP1–12 are number of samples.

Conflict of interest statement

The authors declare no conflict of interest.

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