Gastrointestinal Segments Influenced Fermentation End-Products, Microbiota and Microbial Abundances in Goats

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Abstract

Purpose: Carbohydrate diets altered fermentation end-products and microbial community in the gastrointestinal tracts (GIT) of goats. Gastrointestinal contents used to determine the impact of carbohydrate feeds on fermentation end-products and microbial community in goats.

Methodology: in the study goats were assigned to one of the two treatments corn meal (CM) or Corn gluten (CG) in a randomized block design (400 g/kg DM each). Goats were slaughtered, GIT liquids were used to determine dissolved gasses, fatty acids and microbial community.

Results: Goats fed CG increased molar acetate ($P < 0.05$), lowered butyrate and propionate in the fore and hindgut comparing to those goats received CM. Goats received CM had higher ($P < 0.05$) dH_2 while lowered dH_2S in the fore and hindgut than those goats fed with CG treatment. The fore and hindgut had higher ($P < 0.01$) 16S rRNA gene copies of bacteria, protozoa, methanogens and 18S rRNA gene copies fungi than in the ileum and cecum. Goats fed CG diet had higher ($P < 0.05$) 16S rRNA gene copies of bacteria, protozoa, methanogens, and 18S rRNA gene copies of fungi than those goats fed with CM diet.

Conclusion fore and hindguts improved dissolved gasses, fatty acids and microbial community comparing with in the ileum and cecum. Goats fed CM had improved the *Methanobacterials* order and *Methanobrevibacter* genus as compared with those goats fed CG. The study suggested that hindgut segments have a reasonable contribution as foregut to methane emissions from goats.

Introduction

Fore and hindgut segments play a significant role for microbial digestion in ruminants. Ruminal carbohydrate fermentation produces VFA, H_2 , and CO_2 as end of fermentation products (Hungate 1967). In addition to foregut fermentation, microbial digestion of feeds is also takes place in the hindgut. Our study revealed that hindgut fermentation has significant contribution as that of foregut to fermentation products which is in line with the reports of Ulyatt et al (1975). Dihydrogen (H_2) is produced during feed fermentation in the fore and hindgut and eliminated by methanogens bacteria from the host animal in the form of methane (CH_4) for the normal physiological function and continuity of digestion in ruminants. Dietary interventions which stimulate production of alternative H_2 sink or inhibition of H_2 utilization is an option to ensure redirecting of H_2 away from methanogens to other beneficial pathways for animal productivity and of reduction of CH_4 emissions from ruminants. Unlike to fibrous carbohydrate, starchy carbohydrate can improve H_2 production, which stimulates reducing equivalents like propionate to sink H_2 than methane forming substrates (Bougouin et al. 2018). We hypothesized that Using a feedstuff with dietary high sulfur content could be used as H_2 reducing equivalent, since sulfate involves metabolic hydrogen in the reaction for the production of H_2S ($\Delta G_0 = -84.4 \text{ kJ}$), which can also favor alternative reducing equivalents than methanogenesis ($\Delta G_0 = -67.9 \text{ kJ}$) (Ungerfeld EM 2006). Corn is predominantly utilized in ruminant diet and recently as an input for bioenergy production in various countries, thus its cost intensive to use as animal feed ingredient. Therefore, using alternative corn by-product is a feasible and can replace corn in ruminant diet (Kerr et al. 2008). Using corn by-products in ruminant diet is underexplored, thus the study hypothesized that Carbohydrate diets could alter dissolved gas production and microbial community in the gastrointestinal tracts of goats. Therefore, the study investigated fermentation, dissolved hydrogen and microbial community in the gastrointestinal tract of goats fed corn meal or corn gluten (CG).

Materials And Methods

Experimental Design and Diets

Twenty-four male intact Xiangdong black goats (10 ± 0.2 months, 17.5 ± 2.67 kg (mean $\pm$ SD) were used for the study. The experiment was conducted using a randomized block design with two dietary treatments (corn meal (CM) or corn gluten (CG); 400 g/kg DM each). The ration formulated to meet 1.3 times maintenance requirements of animals (Table 1). Goats were assigned to 12 blocks based on initial weight. Each block consisted of two animals and each animal within a block was randomly allotted to one of the two dietary treatments. Animals were held in individual pens equipped with feeding and watering trough. Animals in each pens had free access to water and fed individually equal meals at 0800 and 1700 h. Animals had 28 d of acclimatization to the diets and 2 d for GIT fluid samples collection.

Table 1
Ingredients and chemical composition of the diets

Item	Diet	
	Corn meal	Corn gluten
Ingredient, g/kg DM		
Peanut vine ¹	400	400
Corn gluten ²	0	400
Corn meal ³	400	0
Soybean	130	130
Wheat bran	30	30
NaCl	5.0	5.0
CaCO ₃	2.0	2.0
CaH ₂ PO ₄	8.0	8.0
Corn oil	5.0	5.0
Premix ⁴	20	20
Chemical composition, g/kg DM		
DM	874	877
Ash	112	127
OM	888	873
CP	171.5	172
NDF	384	484
ADF	175	222
Sulfur	1.46	2.20
Starch	398	397

¹Peanut vine contained 882g/kg DM and 167g of Ash, 89.5g of CP, 540g of NDF, 380g of ADF, and 81.4g of starch and 1.01g of S per kg of DM. ⁴Premix was formulated to provide (per kg of dietary DM) 333 mg of retinol, 5 mg of cholecalciferol, 838 mg of alpha-tocopherol, 922 mg of Zn, 56 mg of Se, 2,247 mg of I, 1,377 mg of Fe, 227 mg of Co, 2,196 mg of Mn, and 889 mg of Cu.

² Corn gluten contained 900 g/kg DM and 41g of Ash, 191g of CP, 506g of NDF, 119g of ADF, 162g of starch and 2.3g of S per kg of DM, 6.98 Ammonia (mM)

³Corn meal vs. corn gluten were 91 vs. 191 g of CP; 196 vs. 506 g of NDF; 637 vs. 162 g of starch and 1.2 vs. 3.0 g of S per kg of DM and 5.18 Ammonia (mM).

⁴Premix was formulated to provide (per kg of dietary DM) 333 mg of retinol, 5 mg of cholecalciferol, 838 mg of alpha-tocopherol, 922 mg of Zn, 56 mg of Se, 2247 mg of I, 1377 mg of Fe, 227 mg of Co, 2196 mg of Mn, and 889 mg of Cu.

Item	Diet	
	Corn meal	Corn gluten
GE (MJ/kg)	14.8	14.6
¹ Peanut vine contained 882g/kg DM and 167g of Ash, 89.5g of CP, 540g of NDF, 380g of ADF, and 81.4g of starch and 1.01g of S per kg of DM. ⁴ Premix was formulated to provide (per kg of dietary DM) 333 mg of retinol, 5 mg of cholecalciferol, 838 mg of alpha-tocopherol, 922 mg of Zn, 56 mg of Se, 2,247 mg of I, 1,377 mg of Fe, 227 mg of Co, 2,196 mg of Mn, and 889 mg of Cu.		
² Corn gluten contained 900 g/kg DM and 41g of Ash, 191g of CP, 506g of NDF, 119g of ADF, 162g of starch and 2.3g of S per kg of DM, 6.98 Ammonia (mM)		
³ Corn meal vs. corn gluten were 91 vs. 191 g of CP; 196 vs. 506 g of NDF; 637 vs. 162 g of starch and 1.2 vs. 3.0 g of S per kg of DM and 5.18 Ammonia (mM).		
⁴ Premix was formulated to provide (per kg of dietary DM) 333 mg of retinol, 5 mg of cholecalciferol, 838 mg of alpha-tocopherol, 922 mg of Zn, 56 mg of Se, 2247 mg of I, 1377 mg of Fe, 227 mg of Co, 2196 mg of Mn, and 889 mg of Cu.		

GIT fluid Sampling

Goats were slaughtered, the abdomen was opened and each GIT segment (rumen, ileum, cecum, colon and rectum) were tied with a sterile thread at the start and end of each segment to avoid mixing of contents. Each GIT segment was longitudinally incised along the dorsal line using a sterile equipment. Samples of liquids from the rumen, ileum, cecum, colon and rectum were filtered through four layers of sterile cheesecloth and approximately 300 mL liquid from each segment was collected in air tight tubes according to Stevenson and Weimer (2007). One 100-mL liquid subsample from each GIT segment was directly chilled using liquid nitrogen and kept for one month at -80°C for DNA isolation and subsequent quantification of microbial groups. Another subsample ~ 20 mL from each GIT segment used for immediate measurement of rumen pH and dissolved gasses.

Sample Analysis

Subsamples of the feed offered and refusals were dried (105°C , 24 h), crashed to pass through a 1-mm sieve and determined for dry matter (DM). Ash was dried in the furnace at 550°C for 8 h. Organic matter (OM) was computed by the difference between DM and ash content. Crude protein (CP) ($\text{N} \times 6.25$) was determined using the Kjeldahl method (AOAC 1995). Gross energy (GE) was analyzed using an isothermal automatic calorimeter (5E-AC 8018, Changsha Kaiyuan Instruments Co. Ltd, Changsha, China). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) values were analyzed and expressed inclusive of residual ash according to Van Soest et al. (1991), and NDF was determined with the addition of amylase and sodium sulfite. The starch was analyzed according to Kartchner and Theurer (1981).

The pH of GIT samples was measured using a portable pH meter (Starter 300; Ohaus Instruments Co. Ltd, Shanghai, China). The dissolved hydrogen (dH_2) dissolved hydrogen sulfur (dH_2S) of GIT samples was determined using micro-sensor with H_2 and H_2S electrode, respectively, according to protocols of the manufacturer's manual (Unisense, Aarhus, Denmark). Concentrations of VFA were determined using 2 mL of GIT centrifuged at $12,000 \times g$ for 10 min at 4°C . Aliquots of the supernatants (1.5 mL) was transferred into plastic tubes each containing 0.15 mL of 25% (wt/vol) metaphosphoric acid. Then individual VFA concentrations were analyzed by gas chromatography (GC, Agilent 7890A, and Agilent Inc.). Ammonia concentrations were measured according to Weatherburn (1967).

Gastrointestinal fluid (GIT) samples were freeze-thawed prior to physical disruption using a bead beater. The total DNA were extracted from repeated beads beating plus column purification as described by Yu and Morrison (2004). The quality and quantity of DNA was determined by absorbance at 260 and 280 nm using a NanoDrop ND-2000 (NanoDrop Technologies Inc, Wilmington, USA). Total bacteria, protozoa, fungi, methanogens were quantified by qPCR according to Jiao et al. (2014; Table 2).

Ash was determined in a muffle furnace at 550°C for 8 h. Organic matter (OM) was calculated by the difference between DM and ash content. Crude protein (CP) (N x 6.25) was analyzed using the Kjeldahl method (AOAC 1995). Gross energy (GE) content was determined using an isothermal automatic calorimeter (5E-AC 8018, Changsha Kaiyuan Instruments Co. Ltd., Changsha, China). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined and expressed inclusive of residual ash according to Van Soest et al. (1991), and NDF was assayed with the addition of amylase and sodium sulfite. Starch content was analyzed according to Kartchner and Theurer (1981).

The pH of GIT samples was measured using pH meter (Starter 300; Ohaus Instruments Co. Ltd., Shanghai, China). The dissolved hydrogen (dH₂) and dissolved hydrogen sulfur (dH₂S) of GIT samples were measured using micro-sensor with H₂ and H₂S electrode, respectively, according to protocols of the manufacturer's manual (Unisense, Aarhus, Denmark). Concentrations of VFA were analyzed using samples of 2 mL from each GIT, centrifuged at 12,000 x g for 10 min at 4°C. Aliquots of the supernatants (1.5 mL) were transferred into plastic tubes each containing 0.15 mL of 25% (wt/vol) metaphosphoric acid. Then individual VFA concentrations were analyzed by gas chromatography (GC, Agilent 7890A, and Agilent Inc.). Ammonia concentrations were measured according to Weatherburn (1967).

The total DNA of GIT samples were isolated as described by Yu and Morrison (2004). Total bacteria, protozoa, fungi, methanogens were quantified by qPCR as described by Jiao et al. (2014; Table 2).

The 16S rDNA V4-V5 variable regions of the methanogen genomic DNA was used for PCR amplification with the primers 5'-TGYCAGCCGCCGCGTAA-3' (524F) and 5'-YCCGGCGTTGAVTCCAATT-3' (Arch958R). Amplicons were sequenced with the Illumina MiSeq platform (Illumina, San Diego, CA) at Majo Bio-Pham Technology (Shanghai, China). sequence reads were analyzed using QIME and processed according to Caporaso et al. 2010 and Edgar 2011.

Table 2
Primers used for quantitative PCR (qPCR)

Target species	Primer	Primer sequence (5'-3')	Size (bp)	References	Efficiency (%)
Bacteria	Forward	CGGCAACGAGCGCAACCC	146	Denman and McSweeney (2006)	100.4
	Reverse	CCATTGTAGCACGTGTGTAGCC			
Protozoa	Forward	GCTTTGWTGGTAGTGTATT	223	Sylvester et al. (2004)	96.3
	Reverse	CTTGCCCTCYAATCGTWCT			
Methanogens	Forward	GGATTAGATACCCSGGTAGT	192	Hook et al. (2009)	101.9
	Reverse	GTTGARTCCAATTAAACCGCA			
Fungi	Forward	GAGGAAGTAAAAGTCGTAACAAGGTTTC	120	Denman and McSweeney (2006)	97.2
	Reverse	CAAATTCACAAAGGGTAGGATGATT			
<i>Prevotella ruminicola</i>	Forward	GAAAGTCGGATTAATGCTCTATGTTG	74	Stevenson and Weimer (2007)	99.8
	Reverse	CATCCTATAGCGGTAAACCTTTGG			
<i>Selenomonas ruminantium</i>	Forward	CAATAAGCATTCCGCCTGGG	138	Stevenson and Weimer (2007)	102.2
	Reverse	TTCACCTCAATGTCAAGCCCTGG			
<i>Ruminococcus albus</i>	Forward	CCCTAAAAGCAGTCTTAGTTCG	176	Koike and Kobayashi (2001)	101.3
	Reverse	CCTCCTTGCGGTTAGAACA			
<i>Ruminococcus flavefaciens</i>	Forward	CGAACGGAGATAATTTGAGTTTACTTAGG	132	Denman and McSweeney (2006)	102.2
	Reverse	CGGTCTCTGTATGTTATGAGGTATTACC			
<i>Fibrobacter succinogenes</i>	Forward	GTTCGGAATTACTGGGCGTAAA	121	Denman and McSweeney (2006)	100.7
	Reverse	CGCCTGCCCCTGAACTATC			

Statistical analysis

Statistical analysis was analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL). The experimental model was a randomized blocks arranged in a factorial arrangement (diet by GIT). Data were analysed using a mixed linear model including diet and GIT segments as fixed effect, and, block and interaction as a random effect. Tukey's adjustment was used to test mean separation. Differences of $P \leq 0.05$ were considered as a significant and $0.05 < P \leq 0.1$ was considered as a trend.

Results

Gastrointestinal tract and diet influenced the molar concentrations of volatile fatty acids and ammonia. Rumen (foregut), cecum, colon and rectum (hindgut) had higher ($P < 0.05$) ammonia than in the ileum (Table 3). However, total volatile fatty acids (VFA), molar acetate, propionate and butyrate were higher in the rumen, cecum and colon than in the ileum and cecum. Goats fed corn gluten (CG) had higher molar concentration of ammonia than those goats fed with CM. Goats fed corn gluten (CG) had higher concentration of acetate in the fore and hindgut than those

fed with corn meal (CM; Fig. 1b; $P < 0.05$), while goats fed with CM improved the concentration of butyrate and propionate in the fore and hindgut than those fed with CG. (Fig. 1c and d; $P < 0.05$).

Table 3
Molar Concentration of VFA in the GIT of goats fed CM or CG

Item	Gastrointestinal tract (GIT)					Diet			<i>P</i> -value		
	IL	RU	CE	CO	RE	SEM	CM	CG	GIT	Diet	G*D
Concentration (mM)											
pH	7.7	6.7	7.7	7.7	7.3	0.43	6.4	6.7	0.91	0.09	0.12
Ammonia	2.3 ^b	6.9 ^a	6.8 ^a	7.1 ^a	7.2 ^a	0.02	5.18 ^b	6.98 ^a	0.02	0.03	0.31
VFA	25.7 ^c	67.4 ^a	65.1 ^b	69.0 ^a	68.7 ^a	9.67	60.8	58.1	0.02	0.33	0.11
Ace/Pro	1.8 ^b	3.2 ^a	3.6 ^a	3.3 ^a	3.4 ^a	0.02	2.4	3.7	0.03	0.17	0.21
Acetate	12.2 ^c	42.5 ^a	41.6 ^b	43.1 ^a	42.7 ^a	5.96	34.6	38.6	0.02	0.32	0.02
Propionate	7.6 ^c	13.7 ^a	12.4 ^b	13.5 ^a	13.2 ^a	0.56	14.1	9.9	0.04	0.26	0.04
Butyrate	5.0 ^c	10.4 ^a	10.2 ^b	11.2 ^a	11.7 ^a	3.13	11.1	8.3	0.03	0.25	0.03
valerate	0.1	0.2	0.3	0.5	0.2	0.05	0.2	0.3	0.30	0.21	0.21
Iso-butyrate	0.1	0.3	0.3	0.3	0.5	0.04	0.3	0.3	0.29	0.14	0.16
Iso-valerate	0.2	0.3	0.3	0.4	0.4	0.08	0.3	0.3	0.67	0.95	0.13
Ace/Pro = acetate to propionate ratio; CE = cecum; CG = corn gluten; CM = corn meal; CO = colon; G*D = interaction (GIT x diet); IL = ileum; P = probability; RE = rectum; RU = rumen; VFA, volatile fatty acids											

The percentages of the individual volatile fatty acids were affected by the GIT segments, the percentages of acetate were higher ($P < 0.05$) in the fore and hindgut than in the ileum, while a lowered ($P < 0.05$) propionate was detected in the ileum than those in the fore and hindgut segments. A lowered trend ($P = 0.07$) of butyrate was also observed in the fore and hindgut than in the ileum (Table 4). Dietary treatments influenced propionate, goats fed with CM increased ($P < 0.05$) propionate than those goats fed with CG treatment. However, no interaction effects GIT and diet noted for the proportion of individual fatty acids.

Table 4
Molar proportion of VFA in the GIT of goats fed CM or CG

Item	Gastrointestinal tract (GIT)					Diet			P-value		
	IL	RU	CE	CO	RE	SEM	CM	CG	GIT	Diet	G*D
Molar proportion of individual VFA (mmol/100 mol VFA)											
Acetate	48.5 ^b	62.7 ^a	61.3 ^a	62.0 ^a	63.3 ^a	0.67	55.6	63.8	0.03	0.14	0.21
Propionate	29.0 ^a	20.4 ^b	19.5 ^b	19.7 ^b	19.2 ^b	3.51	24.0	18.8	0.02	0.04	0.32
Butyrate	19.9 ^a	15.5 ^b	17.4 ^b	16.3 ^b	16.2 ^b	3.36	18.8	15.3	0.07	0.23	0.18
Valerate	0.7	0.3	0.3	0.7	0.4	0.20	0.3	0.6	0.17	0.33	0.22
Isobutyrate	0.6	0.4	0.7	0.4	0.3	0.19	0.4	0.6	0.30	0.78	0.31
Isovalerate	1.1	0.4	0.5	0.5	0.3	0.08	0.5	0.6	0.18	0.01	0.27
Dissolved gasses											
dH ₂	1.3 ^c	16.7 ^a	16.3 ^a	15.8 ^{ab}	16.3 ^a	3.5	18.1	8.8	0.01	0.01	0.02
dH ₂ S	40.9 ^c	224.1 ^{ab}	226.7 ^a	227.0 ^a	225.6 ^a	3.4	155.6	233.6	0.01	0.03	0.03
CE = cecum; CG = corn gluten; CM = corn meal; CO = colon; dH ₂ (μM); dH ₂ S (mM); G*D = interaction (GIT x diet); IL = ileum; P = probability; RE = rectum; RU = rumen; VFA = volatile fatty acids											

The concentrations of dissolved gasses, dissolved hydrogen (dH₂) and dissolved hydrogen sulfur (dH₂S) were affected by the GIT segments and dietary treatments (Table 4) and, interaction of GIT and diet (Fig. 2). Fore and hindgut segments had higher ($P < 0.01$) dH₂ and dH₂S than in the ileum. Goats fed CM had higher ($P < 0.01$) dH₂ than those goats fed with CG treatment, while goats fed CG had higher ($P < 0.05$) dH₂S comparing with goats fed with CM treatment (Table 4). Goats fed CM had higher dH₂ in the fore and hindgut than those fed with CG dietary treatment (Fig. 2a; $P < 0.05$). However, goats fed with CG had higher ($P < 0.05$) dH₂S in the fore and hindgut than those fed with CG dietary treatment (Fig. 2b; $P < 0.05$).

The positive associations were detected between dH₂ and molar propionate in the gastrointestinal tract of goats. The strong positive associations between dH₂ and molar propionate or proportion, respectively occurred in the rumen ($P < 0.05$; $r^2 = 0.61, 0.70$), cecum ($P < 0.05$; $r^2 = 0.88, 0.61$), colon ($P < 0.05$; $r^2 = 0.81, 0.52$), and rectum ($P < 0.05$; $r^2 = 0.73, 0.58$) (Fig. 3).

The gene copies of major microbial groups and bacterial species were influenced by the GIT segments and dietary treatments (Table 5), with no interaction effects of GIT and diet detected for the gene copies of microbial groups. The fore and hindgut had higher ($P < 0.01$) 16S rRNA gene copies of bacteria, methanogens and, 18S rRNA gene copies of protozoa and fungi than in the ileum and cecum. Goats fed CG had higher ($P < 0.05$) 16S rRNA gene copies of bacteria, methanogens and 18S rRNA gene copies of protozoa and fungi than those goats fed CM (Table 5). Likewise, fore and hindgut had higher ($P < 0.05$) 16S rRNA gene copies of *Prevotella ruminicola*, *Selenomonas ruminantion*, *Ruminococcus amylophilus*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* than in the ileum. However, lowered gene copies of *Selenomonas ruminantion*, *Ruminococcus amylophilus*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, recorded in the hindgut than in the rumen. Goats fed CM had higher ($P < 0.05$)

Ruminococcus amylophilus, and lowered *Prevotella ruminicola*, *Selenomonas ruminantium*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* than those goats fed CG.

Table 5
Major microbial groups (log₁₀ copies/mL) in the GIT contents of goats fed with CM or CG diets

Item	Gastrointestinal tract (GIT)						Diet		P-value	
	IL	RU	CE	CO	RE	SEM	CM	CG	GIT	Diet
<i>Bacteria</i>	6.7 ^c	12.1 ^a	8.0 ^b	11.6 ^a	12.4 ^a	0.2	9.3	10.1	0.01	0.03
<i>Fungi</i>	4.6 ^c	8.5 ^a	6.3 ^b	8.4 ^a	8.5 ^a	0.1	8.0	8.5	0.01	0.02
<i>Protozoa</i>	5.1 ^c	10.1 ^a	7.8 ^b	9.6 ^a	9.8 ^a	0.1	9.1	9.8	0.01	0.02
<i>Methanogens</i>	3.7 ^c	9.9 ^a	8.4 ^b	10.2 ^a	10.4 ^a	0.2	9.0	10.0	0.01	0.03
Selected bacterial species										
<i>Prevotella ruminicola</i>	4.7 ^c	11.1 ^a	8.9 ^b	11.3 ^a	11.9 ^a	0.2	8.4	9.3	0.02	0.04
<i>Selenomonas ruminantium</i>	3.6 ^c	10.1 ^a	8.3 ^b	8.4 ^b	8.7 ^b	0.3	7.8	10.7	0.03	0.02
<i>Ruminococcus amylophilus</i>	3.1 ^d	12.1 ^a	8.8 ^{bc}	9.1 ^b	8.9 ^{bc}	0.1	10.2	8.9	0.03	0.03
<i>Ruminococcus albus</i>	4.1 ^d	10.5 ^a	8.4 ^b	8.6 ^b	7.9 ^{bc}	0.2	9.2	10.5	0.04	0.04
<i>Ruminococcus flavefaciens</i>	3.6 ^d	11.9 ^a	7.3 ^{bc}	8.7 ^b	9.0 ^b	0.2	8.3	9.2	0.02	0.02
<i>Fibrobacter succinogenes</i>	4.1 ^d	11.6 ^a	6.8 ^b	10.9 ^a	11.2 ^a	0.1	8.3	10.4	0.03	0.03
abc means superscripts within a row are significantly different (P < 0.05); CE = cecum; CG = corn gluten; CM = corn meal; CO = colon; IL = ileum; P = probability; RE = rectum; RU = rumen										

Methanogens diversity indices of Chao1 (P = 0.06) and ace (P < 0.04) were influenced by the dietary groups, goats fed CG tended to have lower chao1 and ace diversities than those goats fed with CM diet. The species diversity was also higher (P < 0.04) for goats fed CM diet than those goats fed CG. However, the rest of alpha diversity metrics were not affected by the dietary treatments (Table 6).

Table 6
Alpha diversity indexes of goats fed with CM or CG

Item	Diet		SEM	P-value
	Corn meal	Corn gluten		
Chao1	38.1	23.3	6.46	0.06
Coverage	0.99	0.99	0.00	0.12
Observed species	33.3	21.9	0.03	0.03
PD_whole_tree	5.83	3.65	1.34	0.27
Shanon	1.89	2.07	0.12	0.32
Ace	47.0	25.7	7.05	0.04
Simpson	0.41	0.36	0.04	0.44

Goats fed CG had higher abundances of the orders *Methanosarcinales* ($P < 0.05$), *Methanomicrobiales* ($P = 0.09$) and *Thermoplasmalates* recently called *Methanomassiliicoccales* ($P = 0.08$) than those fed with CM, while, less ($P < 0.05$) abundance of *Methanobacteriales* than those fed with CM diet. Regardless of the dietary regimens. Goats fed CG has tended to have higher abundances of the genera of *Methanimicrococcus* ($P = 0.06$) and *Methanomicrobium* ($P = 0.09$), and lowered abundance of *Methanobrevibacter* ($P < 0.05$) than those goats fed with CM diet (Table 7).

Table 7
Relative composition (% of total reads) of methanogen in goats fed CM or CG

Item	Diet		SEM	P-value
	Corn meal	Corn gluten		
Order				
<i>Methanosarcinales</i>	35.7	46.3	8.70	0.04
<i>Methanobacteriales</i>	62.7	49.3	8.39	0.02
<i>Methanomicrobiales</i>	1.07	2.66	0.01	0.09
<i>Thermoplasmalates</i>	0.74	1.50	0.28	0.08
<i>Unidentified</i>	1.04	1.37	0.36	0.53
<i>Others</i>	0.70	0.00	0.32	0.15
<i>Unidentified</i>	0.33	0.05	0.16	0.26
Genus				
<i>Methanimicrococcus</i>	35.3	46.3	8.77	0.06
<i>Methanobrevibactor</i>	61.4	48.2	8.13	0.04
<i>Methanomicrobium</i>	0.07	2.66	1.02	0.09
<i>Methanosphaera</i>	1.12	0.94	0.03	0.79
<i>Candidatus_methanoplasma</i>	0.23	0.26	0.01	0.85
<i>Candidatus_methanomethylophilus</i>	0.07	0.12	0.01	0.54
<i>Unidentified</i>	1.04	1.37	0.36	0.53
<i>Others</i>	0.70	0.00	0.32	0.15

Discussion

The increased volatile fatty acids (VFA) production in the fore and hindgut segments can be associated with inhabitation of abundant microbes in the aforementioned segments that possibly hydrolyze the diet to organic acids. In addition, the higher volatile fatty acids in the rumen, colon and rectum over cecum and ileum caused by relatively higher extent of microbes and fermentation products noticed in the fore and distal hindgut (colon and rectum). The improved molar acetate in the fore and hindgut of goats fed corn gluten is associated with higher fiber (NDF and ADF) contents of the diet, while decreased propionate and butyrate for those goats fed with CM caused by higher starch content of the diet.

Hydrogen regulates the production of volatile fatty acids (VFA), methane and other fermentation pathways in ruminant digestion. Enteric methane (CH₄) primarily produced using hydrogen (H₂) substrate in ruminants (Teklebrhan et al. 2018). Least fiber carbohydrate fermentation influenced volatile fatty acids production pathways, caused less efficiency of H₂ production per mol of glucose fermented than fibrous diet (Wang et al. 2016), consistent with improved propionate production pathway in goats fed with elevated starch in CM than those in CG treatment in the present study. In addition, digestible carbohydrate has faster rate and degree of fermentation than fiber and

protein feeds, resulting a fast accumulation of dH_2 in the rumen (Teklebrhan et al. 2020), agreed to this findings, goats fed higher starch in CM diet improved dH_2 in the fore and hindgut than those goats received CG diet. Thermodynamically, the higher dH_2 concentrations in the fore and hindgut segments of goats fed CM diet indicated less utilization of H_2 and thus facilitates fermentation pathways produced less H_2 over more H_2 per unit glucose fermented in the segments, which is supported by the positive correlations between dissolved dH_2 and molar percentage of propionate in the segments in the current study. The lower dH_2 in the fore and hindgut of goats fed CG treatment was resulted from lower starch, and higher protein content of the diet, increased ammonia concentration (+ 25 % vs. CM), indicating competence of amino acid consumption for microbial growth was lowered, causing to decrease H_2 . However, increased dH_2S in the fore and hindgut of goats fed CG was caused by higher sulfur in the CG than CM dietary treatment because sulfate sinks H_2 and directed the electron away from methanogens towards dH_2S production.

The increased 16S rRNA gene copies of bacteria, protozoa, methanogens and 18S rRNA gene copies of fungi in the fore and hindgut segments indicate the segments are primarily evolved for microbial fermentation. However, the increased gene copies of the microbial groups of bacteria, protozoa, methanogens and fungi in the distal hindgut may indicate its importance for microbial fermentation of the feeds escaped ruminal degradation than cecum. Moreover, the increased bacterial species 16S rRNA gene copies of *Prevotella ruminicola*, *Selenomonas ruminantion*, *Ruminococcus amylophilus*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* in the fore and hindgut associated with high rate of microbial fermentation in these segments than in the ileum. Whereas, the lowest gene copies of *Selenomonas ruminantion*, *Ruminococcus amylophilus*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, in the hindgut is primarily because these bacterial species are typically predominated in the rumen for cellulolytic functions. Dietary groups also influenced the microbial groups, goats fed CG treatment increased the gene copies of the 16S rRNA gene copies of bacteria, protozoa, methanogens and 18S rRNA gene copies of fungi. The increased gene copies of those microbial populations could be caused by elevated fiber and protein content of the CG vs. CM treatment. Which is in agreement with the previous findings, higher fiber, stimulates the growth of fiber utilizing microbes (Pan et al. 2017; Xue et al. 2018).

The study also evaluated the methanogen diversities between the dietary groups, goats fed CG had lower abundances of the *Methanobacteriales* order which is responsible for CH_4 production and it was consistent with higher methane production noticed in goats fed CM versus CG (Teklebrhan et al. 2020). Which is in agreement with Ozbayram et al. (2017) reported *Methanobacteriales* as the dominant order of methanogen for methane production in ruminant. *Methanobrevibacter* population was the dominant genus in the rumen fluid of 'Xiangdong black goats', which aligned with previous studies report *Methanobrevibacter* as the dominant genus in the rumen of goats (Hook et al. 2011; Li et al. 2014; Wang et al. 2017).

Conclusion

Fore and hindguts improved dissolved gasses, fatty acids and microbial community comparing with ileum and cecum segments. Goats fed CG improved the production of acetate, while decreased propionate and butyrate in the fore and hindgut, then those goats fed CM. Goats fed CG reduced the production of dH_2 , but increased dH_2S in the fore and hindgut segments than those goats fed CM. Fore and hindgut segments increased the populations of bacteria, protozoa, methanogens and fungi than in the ileum. Goats fed CG increased the 16S rRNA gene copies of bacteria, methanogens, and 18S rRNA gene copies of protozoa and fungi than those goats fed with CM treatment. Goats fed CM had improved the *Methanobacteriales* order and *Methanobrevibacter* genus as compared with those

goats fed CG. The study suggested that hindgut segments have a reasonable contribution as foregut in terms of dissolved gasses and methane emissions from goats.

Declarations

Ethical statement

Ethics approval and consent to participate

All authors agreed to the content and publish the paper.

Consent for publication

Not applicable

Availability of data and materials.

Available on reasonable request

Conflicts of interests

Authors declared that there is no conflict of interest

Funding

There was no specific fund received

Authors' contributions

TT designed study and wrote the manuscript. ZL revised and edited the manuscript. All authors read and approved the manuscript for the current submission.

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Figures

Figure 1

Concentration of VFA in the GIT of goats fed CM or CG Least square means and SEM for volatile fatty acids, acetate, propionate and butyrate productions of different gastrointestinal tracts of goats fed corn meal or corn gluten diet. (a) Volatile fatty acids (b) molar acetate (c) molar propionate and, (d) molar butyrate. Different letters for different dietary treatments at each of the gastrointestinal tract are different at P < 0.05.

Figure 2

dissolved gasses in the GIT of goats fed CM or CG The figure illustrates the least square means and standard error of means (SEM) for the concentrations dissolved hydrogen (dH₂) and dissolved hydrogen sulfur (dH₂S) of different gastrointestinal tracts of goats fed corn meal or corn gluten dietary treatments. (a) dH₂ and (b) dH₂S, different letters for different dietary treatments at each of gastrointestinal tract is significantly different at $P < 0.05$.

Figure 3

Relationships between dissolved hydrogen and propionate in the gastrointestinal tract of goats The associations between dissolved hydrogen (dH₂) and a concentration of propionate or molar propionate in different gastrointestinal tract (GIT), (a) rumen, (b) cecum (c) colon and (d) rectum. The closed circle represents concentration (μM) open circle represent proportion (mmol/100 mol) of total volatile fatty acids (VFA). Relations are significant at $P < 0.05$. The positive relations detected between dH₂ and propionate concentrations in the GIT of goats. The strong positive associations between dH₂ and propionate concentrations or proportion, respectively occurred in the rumen ($P < 0.05$; $r^2 = 0.61, 0.70$), cecum ($P < 0.05$; $r^2 = 0.88, 0.61$), colon ($P < 0.05$; $r^2 = 0.81, 0.52$), and rectum ($P < 0.05$; $r^2 = 0.73, 0.58$).