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Original Research Article

Adding polyethylene glycol to steer ration containing sorghum tannins increases crude protein digestibility and shifts nitrogen excretion from feces to urine

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ABSTRACT

The objectives of the experiment were to study the effects of adding polyethylene glycol (PEG) to steer ration containing high sorghum tannins on rumen fermentation, nutrient digestion, nitrogen (N) balance and plasma biochemical parameters. Eight growing steers at 16 months of age were allotted to a replicated 4 × 4 Latin square design with 4 treatments and 4 periods (19 d each). Polyethylene glycol at 0, 1.75, 3.50 and 7.00 g/kg dry matter (DM) were added to a basal ration containing 27.82% DM of sorghum grain (total tannins 3.3 g/kg DM) as the treatments. The results indicated that adding PEG quadratically increased the ruminal pH ($P = 0.049$), tended to linearly increase the ruminal concentration of total volatile fatty acids ($P = 0.070$), increased the molar proportion of acetate ($P = 0.016$), linearly decreased the molar proportion of butyrate ($P = 0.015$), and tended to increase the molar proportion of iso-valerate ($P = 0.061$) and the ruminal concentration of ammonia N ($P = 0.092$). Adding PEG tended to quadratically decrease the relative abundance of methanogenic archaea ($P = 0.082$), linearly decreased the relative abundance of *Fibrobacter succinogenes* ($P = 0.008$) and decreased the relative abundance of *Butyrivibrio fibrisolvens* ($P = 0.048$) at 7.00 g/kg DM. Dietary addition with PEG increased the crude protein (CP) digestibility ($P < 0.001$) and tended to increase the neutral detergent fiber digestibility ($P = 0.066$) in a linear manner. Adding PEG to basal ration also increased the plasma globulin concentration ($P = 0.029$) and tended to linearly increase the plasma total protein concentration ($P = 0.069$). Adding PEG linearly decreased the fecal N excretion ($P < 0.001$) and the fecal N-to-total N excretion ratio ($P = 0.004$) and increased the urinary N-to-total N excretion ratio ($P = 0.004$) and urinary urea excretion ($P = 0.010$) without affecting the urinary N and total N excretions ($P > 0.05$). It was concluded that adding PEG effectively improved the CP digestibility of the ration containing high sorghum tannins but increased the urinary urea excretion without improving the N retention and N retention rate in steers.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop in the world after wheat, rice, maize and barley and has been widely grown in semi-arid and tropical regions of Africa and Asia because of its advantages in tolerating drought, flood and salt (Olukoya et al., 2015). Sorghum grain has been widely used as the feed for animals (Wang et al., 2019) and the food for humans (Irondi et al., 2019). The crude protein (CP) content of sorghum grain is 71 to 118 g/kg dry matter (DM) which is close to corn, and the content of essential amino acids is higher than that of corn (Pan et al., 2017). Replacing 25% to 100% of corn in lamb ration by

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sorghum grain improved meat quality but it did not affect daily liveweight gain (Zhong et al., 2016). However, the feeding value of sorghum grain for livestock is generally 85% to 95% of corn (Waniska et al., 2016) because of its high content of condensed tannins (CT) (Nyachoti et al., 1997) which was usually more than 10 g/kg DM (Dykes and Rooney, 2006). Many studies have shown that tannins can bind with feed protein to form tannin-protein complexes in rumen to prevent microbial degradation of protein and increase undegradable protein supply to the hindgut (Getachew et al., 2008; Min et al., 2002). However, some studies also indicated that dietary CT decreased CP digestibility in beef cattle (Stewart et al., 2019). The reason for the results could be that tannin-protein complexes were not fully decomposed in the lower digestive tract, resulting in low CP digestibility.

Polyethylene glycol (PEG) is an inert and synthetic polymer with a high molecular weight which is poorly digested and absorbed in the intestine of animals (Grosell and Genz, 2006). Polyethylene glycol has been widely used as a raw material in food and medicine industries (He et al., 2019; Knop et al., 2010). Because of its high affinity with tannins, PEG can bind with tannins to form tannin-PEG complex (Makkar et al., 1995). Therefore, adding PEG to rations containing high tannins improved feed intake and CP digestibility in sheep (Peng et al., 2016).

Many studies have shown that dietary inclusion of tannins shifts nitrogen (N) excretion from urine to feces in cattle without affecting total N excretion. It is unclear whether adding PEG to ration containing sorghum tannins would improve N retention and N retention rate (NRR) in cattle. The objectives of the experiment were to investigate effects of dietary PEG addition on rumen fermentation, nutrient digestibility, N balance and plasma biochemical parameters in steers fed a ration containing high sorghum tannins.

2. Materials and methods

2.1. Animals, experimental design and the basal ration

The experiment was carried out between April, 2019 and June, 2019 on a commercial beef cattle farm (N 37°53′; E 117°45′) in Shandong province, China. Eight castrated Simmental steers at 16 months of age with an initial liveweight of 357.4 ± 15.6 kg were used. The animals were assigned to a replicated 4 × 4 Latin square design. Four levels of PEG (molecular weight 4,000 kDa, purity ≥ 99.9%, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), i.e., 0, 1.75, 3.50 or 7.00 g/kg DM, were added to a basal ration containing high sorghum tannins (Table 1) as experimental treatments. The ration was formulated according to the Nutrient Requirements and Feeding Standards of Beef Cattle (Feng, 2000). Each experimental period lasted 19 d. The first 14 d were for adaptation and the last 5 d for sampling.

Each animal was supplied with 5.70 kg DM of total mixed ration (TMR) (Table 1) which was about 90% of ad libitum feed intake. The designed amount of PEG was completely mixed with the TMR before feeding. The daily allowance of TMR was divided into 2 equal meals which were provided to each steer at 07:00 and 17:00, respectively. No feed residuals were left during the experiment. The cattle were housed indoors in individual tie stalls (1.5 m × 2.5 m) and had free access to fresh drinking water.

2.2. Sampling

The liveweight of each steer was recorded before feeding in the morning on the first day and the last day of each experimental period. During each sampling period, the feces and urine were totally collected daily at 07:00. The feces were collected using plastic buckets, and the urine was collected using a rubber funnel connected by a polyvinyl chloride pipe to a plastic bucket surrounded with ice packs to keep low temperature. The daily output of feces from each steer was recorded and 2% of the total feces was sampled and a volume of 20 mL of H_2SO_4 (10%, vol/vol) was added to keep pH < 3.0 for preserving N. The daily output of urine from each animal was measured using a volumetric cylinder and 2% of the total urine was sampled and H_2SO_4 (10%, vol/vol) was added to keep pH < 3.0 for preserving N. The feeds were also sampled during each experimental period. On the second day of each sampling period, 2 h after morning feeding, 10 mL of blood was taken through the jugular vein of each steer using an evacuated K_2EDTA tube (Greiner Bio-one, Frickenhausen, Germany). The blood samples were centrifuged at 2,000 × *g* for 15 min at 4 °C to obtain plasma samples. On the third day of each sampling period, rumen fluid was taken using an esophageal stomach tube 2 h after feeding in the morning (Brito et al., 2014). The first tube of rumen fluid was discarded to avoid saliva contamination. Then, a volume of 100 mL of rumen fluid was taken and filtered through 4 layers of cheesecloth. The pH of rumen fluid was immediately measured using a portable pH meter (pH-HJ90; Aerospace Computer Company, Beijing, China). All samples were kept in a freezer at −20 °C for subsequent analysis.

Table 1
Ingredients and chemical composition of the basal ration (g/kg, DM basis).

Item	Content
Ingredients	
Corn silage	483.6
Sorghum grain	278.2
Wheat bran	5.8
Corn grain	15.1
Soybean meal	186.7
Sodium bicarbonate	10.2
Sodium chloride	10.2
Calcium carbonate	10.2
Chemical composition	
CP	143.1
OM	932.4
NDF	384.8
ADF	214.6
Tannins	3.3
NEmf[1], MJ/kg	5.9

DM = dry matter; CP = crude protein; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] NEmf refers to the net energy for maintenance and fattening of beef cattle, calculated based on gross energy, OM, and NDF according to the Nutrient Requirements and Feeding Standards of Beef Cattle (Feng, 2000).

2.3. Chemical analysis

The samples of corn silage and feces were lyophilized for 72 h using a freeze dryer (LGJ-12; Beijing Songyuan Huaxing Technology Development Co., Ltd, Beijing, China). The fecal samples were ground in a mortar and the feed samples were ground using a grinder (FW177, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) through a sieve with pore size of 1 mm. The DM and ash contents of feeds and feces were determined according to AOAC (2005) using methods No. 930.15 and 942.05, respectively. The organic matter (OM) content was calculated by DM content minus ash content. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were analyzed on a fiber analyzer (A200i, ANKOM Technology Co., New York, USA) using the methods of Van Soest et al. (1991) with sodium sulfite and heat-stable α-amylase for analyzing NDF.

The N contents of feeds and feces were analyzed using the Kjeldahl method of AOAC (2005) No. 981.13 and the CP content was calculated as N × 6.25. The N concentration of urine was analyzed using the Kjeldahl method of AOAC (2005) No.973.48. The gross energy (GE) of feed samples was determined by complete combustion on a calorimeter (Parr 6300, Parr Instrument Company, Moline, IL, USA). The tannins of sorghum grain were analyzed according to the procedures described by Price et al. (1978).

The volatile fatty acids (VFA) of rumen fluid were determined by gas chromatography (TP-2060F; Beijing Beifen Tianpu Instrument Technology Co. Ltd, Beijing, China) equipped with a flame ionization detector (FID) and a glass column filled with PEG 20 M + H_3PO_4. The ammonia N (NH_3—N) of rumen fluid was determined on a UV—Vis spectrophotometer (UV-1801; Beijing Beifen Ruili Analytical Instrument Co. Ltd, Beijing, China) using the colorimetric method described by Broderick and Kang (1980). The ruminal microbial crude protein (MCP) was determined using the method of Makkar et al. (1982). The genomic DNA of ruminal microorganisms was extracted by the QIAamp DNA Stool Mini Kit (No.51604; Hilden, Germany) according to the manufacturer's instructions except that the samples were incubated at 95 °C for 5 min to ensure the lysis of microbial cells. The final elution volume was 100 μL. The concentration and the purity of DNA were detected using a spectrophotometry (Nanodrop ND-1000; Thermo Scientific, Wilmington, DE, USA).

The two-step real-time PCR was performed using an FTC-3000 Real-time PCR system (Funglyn Biotech, Toronto, Canada) with fluorescence detection of SYBR green dye for initial denaturation at 95 °C for 30 s, followed by 45 cycles of 5 s at 95 °C and 30 s at 60 °C. The total volume of PCR mixture was 25 μL consisting of 12.5-μL SYBR Premix Ex Taq (No. RR420A; Takara, Dalian, China), 0.5-μL forward primer (10.0 μmol/L; Sangon, Shanghai, China), 0.5-μL reverse primer (10.0 μmol/L; Sangon, Shanghai, China), 2.0 μL of DNA template and 9.5 μL of sterile distilled water. The specific real-time PCR primers are listed in Table 2. After the PCR reaction, the dissociation curve analysis was performed to reveal the purity of the amplicon produced.

The plasma concentrations of total protein (TP) (the biuret method), albumin (ALB) (the bromocresol green method), urea (the urease-berthelot method), uric acid (the uricase-peroxidase method) and creatinine (Jaffe's method) were analyzed using an automatic biochemical analyzer (BS-420; 615 Shenzhen Mindray Biomedical Electronic Co., Ltd, Shenzhen, China). The plasma concentration of globulin was calculated by ALB subtracted from TP. The plasma concentrations of growth hormone and insulin-like growth factor-1 (IGF-1) were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (BioSino Bio-Technology and Science Inc., Beijing, China) on a microplate reader (DR-200BS, Wuxi Hiwell-Diatek Instruments Co., Ltd, Jiangsu, China). The plasma concentration of total amino acids, allantoin and hippuric acid were analyzed using colorimetric methods on a semi-automatic biochemical analyzer (A6; 621 Beijing Shining Sun Technology Co., Ltd, Beijing, China).

The urinary concentrations of urea and creatinine were analyzed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on the diacetyl monoxime method and the Jaffe's assay using a spectrophotometer (UV-1801; Beijing Beifen Ruili Analytical Instrument Co. Ltd, Beijing, China), respectively. The urinary concentrations of uric acid and allantoin were analyzed using a spectrophotometer (UV-1801; Beijing Beifen Ruili Analytical Instrument Co. Ltd, Beijing, China) according to the methods of Chen and Gomes (1992). The urinary concentration of hippuric acid was analyzed according to China Hygienic Standard (WS/T 52-1996) using a spectrophotometer (UV-1801; Beijing Beifen Ruili Analytical Instrument Co. Ltd, Beijing, China).

2.4. Calculations and statistical analysis

The PCR efficiency (E) for each primer pair was determined from the slope of the external calibration curve using the equation: $E = [10^{(-1/s)} - 1] \times 100$, where s is the slope of standard curve. The relative abundance of the microbial 16S rDNA gene copy number was expressed relative to the copy number of total rumen bacterial 16S rDNA and calculated as:

$$\text{Relative abundance} = 2^{-(Ct\ \text{target}-Ct\ \text{total bacteria})} \times 100$$

where Ct is the threshold cycle.

The total urinary excretion of purine derivatives (PD) was calculated and the rumen microbial N supply to steers was predicted using the methods of Chen and Gomes (1992):

$$\text{PD (mmol/d)} = \text{Allantoin (mmol/d)} + \text{Uric acid (mmol/d)}$$

Table 2
Real-time PCR primers used in the quantification of rumen micro-organisms.

Target microflora	Primer sequences (5′ to 3′)	References
Total bacteria	F: CGGCAACGAGCGCAACCC R: CCATTGTAGCACGTGTGTAGCC	Denman and McSweeney (2006)
Protozoa	F: GCTTTCGWTGGTAGTGTATT R: CTTGCCCTCYAATCGTWCT	Sylvester et al. (2004)
Methanogenic archaea	F: TTCGGTGGATCDCARAGRGC R: GBARGTCGWAWCCGTAGAATCC	Denman et al. (2007)
Fungi	F: AGGAAGTAAAAGTCGTAACAAGGTTTC R: CAAATTCACAAAGGGTAGGATGATT	Denman and McSweeney (2006)
Ruminococcus albus	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA	Stevenson and Weimer (2009)
Ruminococcus flavefaciens	F: CGAACGGAGATAATTTGAGTTTACTTAGG R: CGGTCTCTGTATGTTATGAGGTATTACC	Denman and McSweeney (2006)
Fibrobacter succinogenes	F: GTTCGGAATTACTGGGCGTAAA R: CGCCTGCCCCTGAACTATC	Denman and McSweeney (2006)
Butyrivibrio fibrisolvens	F: ACCGCATAAGCGCACGGA R: CGGGTCCATCTTGTACCGATAAAT	Stevenson and Weimer (2009)
Prevotella	F: GGTTCTGAGAGGAAGGTCCCC R: TCCTGCACGCTACTTGGCTG	Stevenson and Weimer (2009)

F = forward; R = reverse.

$$Y = 0.85X + 0.385BW^{0.75}$$

where Y refers to the total urinary PD; X, the absorbed PD excretion; $BW^{0.75}$, the metabolic body weight of steers (kg); 0.85, the recovery rate of absorbed purines as PD in urine; 0.385, the endogenous excretion of PD (mmol/kg $BW^{0.75}$ per d).

$$\text{Microbial } N \text{ (mg/d)} = (X \times 70)/(0.116 \times 0.83 \times 1{,}000) = 0.727X,$$

where X refers to the absorbed PD excretion (mmol/d); 70, the N content of purines (mg N/mmol); 0.116, the ratio of purine N to total N in mixed rumen microbes; 0.83, the digestibility of microbial purines.

Statistical analysis was performed using the general linear model procedures of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA) for a replicated Latin square design using the model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + \varepsilon_{ijk},$$

where Y_{ijk} refers to the dependent variable; μ, overall mean; T_i, PEG effect ($i = 1, 2, 3$ and 4); P_j, period effect ($j = 1, 2, 3$ and 4); C_k, steer effect ($k = 1, 2, 3, 4, 5, 6, 7$ and 8); ε_{ijk}, residual error. All differences among experimental diets were compared by Duncan's multiple range test. Polynomial contrasts were conducted to determine the linear and quadratic effects of PEG levels. Differences among treatments were considered significant at $P < 0.05$ and a trend towards significance at $0.05 < P < 0.10$.

3. Results

3.1. Rumen fermentation

The results in Table 3 showed that dietary addition with PEG quadratically increased the ruminal pH ($P = 0.049$), tended to linearly increase the ruminal concentrations of total VFA ($P = 0.070$) and NH_3–N ($P = 0.092$). Adding PEG up to 7.00 g/kg DM increased the molar proportion of acetate ($P = 0.016$), linearly decreased the molar proportion of butyrate ($P = 0.015$), and tended to increase the molar proportion of iso-valerate ($P = 0.061$) whereas it did not affect the ruminal concentrations of MCP and other VFA ($P > 0.05$).

3.2. Rumen microflora

Adding PEG to the basal ration did not affect the relative abundances of protozoa and fungi but it quadratically decreased the relative abundance of methanogenic archaea ($P = 0.082$). Adding PEG also linearly decreased the relative abundance of *Fibrobacter succinogenes* ($P = 0.008$) and decreased the relative abundance of *Butyrivibrio fibrisolvens* at 7.00 g/kg DM ($P = 0.048$) whereas it did not affect *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Prevotella* ($P > 0.05$) (Table 4).

3.3. Nutrient digestibility

The results in Table 5 indicated that adding PEG linearly increased the CP digestibility ($P < 0.001$) and tended to increase the NDF digestibility ($P = 0.066$) whereas it did not affect the digestibility of DM, OM or ADF ($P > 0.05$).

3.4. N metabolism

Addition with PEG to the basal ration linearly decreased the fecal N excretion ($P < 0.001$) whereas it did not affect the urinary N and total N excretions ($P > 0.05$). Adding PEG linearly decreased the fecal N-to-total N excretion ratio ($P = 0.004$) and the fecal N-to-N intake ratio ($P < 0.001$), and linearly increased the urinary N-to-total N excretion ratio ($P = 0.004$) whereas it did not affect the urinary N-to-N intake ratio, N retention, NRR and the average daily gain (ADG) ($P > 0.05$) (Table 6).

3.5. Urinary nitrogenous components

Dietary addition with PEG linearly increased the urinary urea excretion ($P = 0.010$) and the urea-N-to-urinary N ratio ($P = 0.028$) whereas it did not affect the urinary excretions of uric acid, allantoin, creatinine and hippuric acid, and the ratios of hippuric acid N to urinary N, creatinine N to urinary N, allantoin N to urinary N, uric acid N to urinary N ($P > 0.05$), the urinary PD excretion and the estimated rumen microbial N ($P > 0.05$) (Table 7).

3.6. Plasma biochemical indices

Adding PEG to the basal ration tended to linearly increase the plasma concentration of TP ($P = 0.069$), linearly increased the plasma concentration of globulin ($P = 0.029$), tended to quadratically increase the plasma concentration of allantoin ($P = 0.079$) whereas it did not affect the plasma concentrations of ALB, urea, total amino acids, growth hormone, IGF-1, uric acid, creatinine and hippuric acid ($P > 0.05$) (Table 8).

Table 3
Effects of adding polyethylene glycol (PEG) to a steer ration containing high sorghum tannins on rumen fermentation.

Item	PEG, g/kg DM				SEM	*P*-value		
	0.00	1.75	3.50	7.00		T	L	Q
pH	6.64[b]	6.80[a]	6.81[a]	6.76[ab]	0.045	0.038	0.114	0.049
NH_3–N, mmol/L	9.97	8.48	10.34	11.02	0.721	0.092	0.166	0.178
TVFA, mmol/L	75.18	81.22	87.12	86.26	4.542	0.248	0.070	0.465
VFA, mmol/100 mmol								
Acetate	56.79[b]	58.59[a]	59.69[a]	59.40[a]	0.666	0.016	0.144	0.436
Propionate	22.81	23.06	22.79	22.79	0.264	0.890	0.957	0.871
Butyrate	15.11[a]	13.46[b]	12.79[b]	12.92[b]	0.529	0.005	0.015	0.164
Iso-butyrate	2.42	1.46	1.43	1.48	0.333	0.109	0.121	0.215
Valerate	1.02	1.20	1.03	1.22	0.107	0.333	0.360	0.973
Iso-valerate	1.85	2.25	2.19	2.20	0.107	0.061	0.989	0.149
Acetate-to-propionate ratio	2.56	2.57	2.67	2.64	0.049	0.378	0.661	0.917
MCP, μg/mL	134.7	133.0	137.6	129.8	3.69	0.517	0.600	0.498

SEM = standard error of the mean; NH_3–N = ammonia nitrogen; TVFA = total volatile fatty acids; VFA = volatile fatty acids; MCP = microbial crude protein; T = treatment; L = linear; Q = quadratic.
[a, b] Within a row, means without a common superscript differ significantly ($P < 0.05$).

Table 4
Effects of adding polyethylene glycol (PEG) to a steer ration containing high sorghum tannins on the relative abundance of rumen microorganisms (% total bacterial 16S rDNA).

Item	PEG, g/kg DM				SEM	*P*-value		
	0.00	1.75	3.50	7.00		T	L	Q
Protozoa	6.78	4.39	2.47	3.52	0.023	0.629	0.322	0.511
Methanogenic archaea, $\times 10^{-3}$	7.95	5.70	4.12	6.68	0.001	0.193	0.370	0.082
Fungi, $\times 10^{-2}$	8.64	6.96	5.34	6.65	0.002	0.713	0.417	0.565
R. albus, $\times 10^{-2}$	2.56	2.15	2.91	1.75	0.004	0.319	0.531	0.528
R. flavefaciens, $\times 10^{-2}$	2.70	2.49	3.68	2.98	0.001	0.621	0.565	0.758
F. succinogenes, $\times 10^{-1}$	7.45^{a}	7.98^{a}	3.56^{b}	3.43^{b}	0.104	0.002	0.008	0.802
B. fibrisolvens, $\times 10^{-2}$	6.46^{a}	4.82^{ab}	4.03^{ab}	2.70^{b}	0.104	0.048	0.142	0.865
Prevotella	5.20	5.25	5.08	5.24	3.130	0.977	0.979	0.892

SEM = standard error of the mean; T = treatment; L = linear; Q = quadratic.
[a, b] Within a row, means without a common superscript differ significantly ($P < 0.05$).

Table 5
Effects of adding polyethylene glycol (PEG) to a steer ration containing high sorghum tannins on nutrient digestibility (%).

Item	PEG, g/kg DM				SEM	*P*-value		
	0.00	1.75	3.50	7.00		T	L	Q
DM	67.3	67.7	67.9	68.8	0.48	0.179	0.140	0.735
OM	69.3	69.4	69.8	70.7	0.45	0.111	0.107	0.528
CP	66.2^{c}	68.2^{b}	70.1^{a}	71.1^{a}	0.62	<0.001	<0.001	0.555
NDF	63.5	64.2	64.1	65.8	0.59	0.070	0.066	0.533
ADF	54.0	55.0	55.3	56.5	1.14	0.890	0.191	0.945

SEM = standard error of the mean; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; T = treatment; L = linear; Q = quadratic.
[a, b, c] Within a row, means without a common superscript differ significantly ($P < 0.05$).

4. Discussion

Tannins can combine with dietary protein through hydrogen bonds to form protein–tannin complexes which can prevent protein degradation by rumen microorganisms (Patra and Saxena, 2011). Tannins are also able to inhibit dietary protein degradation in rumen by reducing the activity of endogenous enzymes and rumen microorganisms (Alonso-Díaz et al., 2010). Polyethylene glycol that has high affinity for tannins can form PEG-tannin complexes to deactivate tannins. Thus, PEG can improve the ruminal CP degradation (Mkhize et al., 2018) and the CP digestibility (Alipanahi et al., 2019) of rations containing tannins.

In the present experiment, dietary addition with PEG tended to increase ruminal concentration of NH_3–N and increased CP digestibility, suggesting that PEG inhibited the condensed tannins (CT) activity in rumen. Similar results were also reported by Alipanahi et al. (2019) that adding PEG improved CP digestibility in goats fed with an oak acorn ration containing hydrolysable tannins and by Huang et al. (2015) that adding PEG increased CP digestibility in lambs fed with purple prairie clover containing CT. The increased plasma concentration of TP and globulin in the present experiment could be attributed to the increased CP digestibility.

Tannins can inhibit the activities of ruminal bacteria and anaerobic fungi (Alonso-Díaz et al., 2010) and bind to fiber (Makkar et al., 1995). Thus intake of plants containing CT reduced the ruminal digestibility of fiber (Hassan et al., 2020). In the present experiment, dietary addition with PEG increased the ruminal concentration of acetate and tended to improve NDF digestibility, suggesting that PEG neutralized the negative effects of sorghum CT on fiber digestion. Dietary addition with PEG also tended to increase the molar proportion of ruminal isovaleric acid. Because isovaleric acid is mainly derived from the deamination of amino acids in rumen (Patra and Saxena, 2011), sorghum tannins should inhibit the ruminal fermentation of both carbohydrates and protein.

Previous studies indicated that dietary addition of CT or tannic acid decreased the relative abundances of ruminal protozoa and methanogenic bacteria (Jayanegara et al., 2015; Yang et al., 2016). However, the results of the present experiment showed that adding PEG to the ration containing high sorghum tannins did not

Table 6
Effects of adding polyethylene glycol (PEG) to steer ration containing high sorghum tannins on N metabolism.

Item	PEG, g/kg DM				SEM	*P*-value		
	0.00	1.75	3.50	7.00		T	L	Q
N intake, g/d	130.7	130.7	130.7	130.7	–	–	–	–
N excretion, g/d								
Fecal N	44.1^{a}	41.6^{b}	39.1^{c}	37.8^{c}	0.81	<0.001	<0.001	0.555
Urinary N	63.0	65.4	65.1	66.4	1.25	0.304	0.299	0.805
Total	107.2	107.0	104.1	104.2	1.21	0.159	0.1682	0.956
Total N excretion, %								
Fecal N	41.2^{a}	40.0^{b}	37.6^{bc}	36.3^{c}	0.78	0.002	0.004	0.677
Urinary N	58.8^{c}	61.1^{b}	62.4^{ab}	63.7^{a}	0.78	0.002	0.004	0.677
N intake, %								
Fecal N	33.8^{a}	31.8^{b}	29.9^{c}	28.9^{c}	0.62	<0.001	<0.001	0.555
Urinary N	48.2	50.0	49.8	50.8	0.95	0.304	0.299	0.805
N retention, g/d	23.5	23.7	26.6	26.5	1.21	0.159	0.168	0.956
NRR, %	18.0	18.2	20.3	20.3	0.93	0.159	0.168	0.956
ADG, kg/d	0.65	0.65	0.68	0.68	0.454	0.881	0.540	0.945

SEM = standard error of the mean; N = nitrogen; NRR = N retention rate; ADG = average daily gain; T = treatment; L = linear; Q = quadratic.
[a, b, c] Within a row, means without a common superscript differ significantly ($P < 0.05$).

Table 7
Effects of adding polyethylene glycol (PEG) to steer ration containing high sorghum tannins on urinary nitrogenous components.

Item	PEG, g/kg DM				SEM	*P*-value		
	0.00	1.75	3.50	7.00		T	L	Q
Urea, mmol/d	1,333.7[b]	1,399.5[ab]	1,620.4[a]	1,635.4[a]	84.35	0.044	0.010	0.783
Urea N:urinary N ratio, %	59.0	60.6	70.1	68.9	3.57	0.073	0.028	0.723
Uric acid, mmol/d	2.5	2.7	2.7	3.3	0.36	0.501	0.407	0.698
Uric acid N:urinary N ratio, %	0.2	0.2	0.2	0.3	0.03	0.664	0.514	0.751
Allantoin, mmol/d	66.3	73.2	65.1	78.5	4.54	0.163	0.249	0.557
Allantoin N/urinary N ratio, %	5.9	6.3	5.7	6.6	0.40	0.343	0.469	0.517
Creatinine, mmol/d	78.5	87.8	77.4	91.3	5.21	0.176	0.242	0.664
Creatinine N/urinary N ratio	5.2	5.7	5.0	5.8	0.35	0.353	0.540	0.684
Hippuric acid, mmol/d	144.3	145.8	147.7	146.7	20.46	0.999	0.927	0.955
Hippuric acid N:urinary N ratio, %	3.2	3.1	3.2	3.1	0.43	0.999	0.969	0.997
PD, mmol/d	68.8	75.9	67.7	81.8	4.70	0.158	0.217	0.531
Estimated rumen microbial N, g/d	29.6	35.7	28.5	36.9	3.68	0.299	0.446	0.786

SEM = standard error of the mean; N = nitrogen; PD = purine derivatives; T = treatment; L = linear; Q = quadratic.
[a, b] Within a row, means without a common superscript differ significantly ($P < 0.05$).

Table 8
Effects of adding polyethylene glycol (PEG) to a steer ration containing high sorghum tannins on plasma parameters.

Items	PEG, g/kg DM				SEM	*P*-value		
	0.00	1.75	3.50	7.00		T	L	Q
Nutrients								
TP, g/L	68.73[b]	70.29[a]	70.26[a]	70.86[a]	0.539	0.043	0.069	0.530
ALB, g/L	27.58	27.53	27.73	28.04	0.311	0.659	0.514	0.745
Globulin, g/L	41.15[b]	42.76[a]	42.52[a]	42.82[a]	0.243	0.050	0.029	0.169
Urea, μmol/L	3.96	3.89	3.99	3.79	0.083	0.351	0.487	0.632
TAA, μmol/L	4.28	4.19	3.96	4.29	0.197	0.620	0.917	0.606
Hormones								
GH, ng/mL	5.21	5.97	6.80	6.82	0.595	0.207	0.148	0.664
IGF-1, ng/mL	193.10	199.45	188.85	183.95	6.182	0.281	0.436	0.606
Purine derivatives								
Uric acid, mg/L	12.76	14.03	11.79	14.79	1.335	0.418	0.762	0.760
Allantoin, mmol/L	1.05	0.87	0.87	0.98	0.061	0.096	0.549	0.079
Other metabolites								
Creatinine, μmol/L	137.44	143.36	139.34	140.95	2.836	0.519	0.720	0.593
Hippuric acid, μmol/L	37.61	35.38	36.54	37.06	1.578	0.763	0.947	0.403

SEM = standard error of the mean; TP = total protein; ALB = albumin; TAA = total amino acids; GH = growth hormone; IGF-1 = insulin-like growth factor-1; T = treatment; L = linear; Q = quadratic.
[a, b, c] Within a row, means without a common superscript differ significantly ($P < 0.05$).

affect the relative abundances of protozoa and methanogens. The results indicated that sorghum tannins did not affect ruminal protozoa and methanogenic bacteria. *F. succinogenes* are predominant cellulolytic bacteria in rumen and can digest fibrous plant particles (Cotta, 1988). It was reported that adding CT from ascophyllum nodosum to rumen bacterial cultures inhibited the growth of *F. succinogenes* (Wang et al., 2009). In the present experiment, dietary addition with PEG did not increase but decreased the relative abundance of *F. succinogenes*, suggesting that PEG also has an inhibitory effect on *F. succinogenes*. *B. fibrisolvens* are also involved in the ruminal degradation of cellulose and other polysaccharides with butyric acid as the major end-product (Kohno et al., 2009). The decreased molar proportion of butyric acid by adding PEG in the present experiment could be attributed to the decreased relative abundances of *F. succinogenes* and *B. fibrisolvens*. *Prevotella* are predominant ruminal bacteria which can utilize NH_3 for MCP synthesis (Stevenson and Weimer, 2009; Wallace et al., 1997). Adding PEG did not affect the relative abundance of *Prevotella* in the present experiment. This could explain the results that adding PEG did not affect ruminal concentration of MCP.

Previous studies have shown that inclusion of tannins in ruminant rations shift N excretions from urine to feces (Theodoridou et al., 2010) because tannins–protein complexes formed in rumen could not be completely decomposed in the hindgut (Wang et al., 1996). In the present experiment, adding PEG decreased fecal N excretion, but did not affect urinary N excretion, and decreased fecal N-to-total N excretion ratio and increased urinary N-to-total N excretion ratio. The results showed that adding PEG to the ration containing high sorghum tannins shifted N excretions from feces to urine.

The results of the present experiment also showed that adding PEG increased the urinary urea excretion in steers. The reason for the results could be that PEG tended to increase the ruminal concentration of NH_3 by inactivating sorghum CT, resulting in increasing NH_3 absorption and urea synthesis in liver. Because urinary urea accounts for the major part of urinary nitrogenous compounds and can be rapidly transformed to NH_4^+ in manure and utilized as the precursor to produce the potent greenhouse gas nitrous oxide (N_2O) (Oenema et al., 2005; Sakadevan and Nguyen, 2017), adding PEG to the rations containing high sorghum tannins had a potential to increase the N_2O emissions from steer excreta to the environment.

Previous studies indicated that adding CT to the rations of growing lambs and calves did not affect urinary PD excretions (Sharma et al., 2008). The results of the present experiment showed that adding PEG did not affect the urinary excretions of PD and the estimated rumen microbial N supply in steers, suggesting that

sorghum tannins had no effect on the ruminal microbial N synthesis. The results agreed with the unaffected ruminal concentration of MCP by adding PEG.

5. Conclusions

Adding PEG to the ration containing high sorghum tannins improved ruminal fermentation of carbohydrates, increased CP digestibility, and tended to increase NDF digestibility, but did not improve N retention and NRR in steers. It shifted N excretions from feces to urine and increased urinary urea excretion in steers. Hence it had a potential to increase the N_2O emissions from steer excreta. Adding PEG to the ration containing high sorghum tannins had only a minor effect on nutrient utilization in steers and a potentially negative effect on the environment. It is necessary to investigate new approaches to improve both nutrient digestibility and NRR in steers fed rations containing high sorghum tannins.

Author contributions

Biao Xie: Investigation, Data curation, Software, Writing – original draft. **Xiao Yang**: Investigation. **Ling Yang**: Investigation. **Xianjiang Wen**: Investigation. **Guangyong Zhao**: Conceptualization, Methodology, Supervision, Writing – review & editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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