Effects of Different Molecular Weights and Concentrations of Chitosan on In Vitro Rumen Fermentation Parameters and Methane Emissions in Dairy Cows (Postprint)

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Abstract

This study aimed to investigate the effects of chitosan with different molecular weights and concentrations on in vitro rumen fermentation parameters and methane emissions in dairy cows. Three Holstein dairy cows with similar body condition, good health status, and equipped with permanent rumen fistulas were selected as experimental animals for rumen fluid collection. Chitosan with molecular weights of 1,000, 3,000, and 50,000 u was selected, with each molecular weight added to the substrate at concentrations of 0.4%, 0.8%, and 1.6% (substrate basis), establishing nine experimental groups. An additional control group (without chitosan) was included, with four replicates per group and three experimental batches. After 24 h of in vitro fermentation, gas production, methane yield, and rumen fermentation parameters were measured. The results showed that, compared with the control group, chitosan supplementation significantly reduced ruminal ammonia nitrogen concentration ($P<0.05$), significantly increased propionate concentration ($P<0.05$), and significantly decreased the acetate/propionate ratio ($P<0.05$), thereby promoting a shift in rumen fermentation pattern. Chitosan at concentrations of 1.6% and 0.8% with a molecular weight of 50,000 u exhibited a trend toward reducing methane production in fermentation fluid ($P<0.10$) without affecting dry matter digestibility. In conclusion, under in vitro conditions, chitosan supplementation can effectively regulate rumen microbial fermentation status. Taking all factors into consideration, chitosan at a concentration of 1.6% with a molecular weight of 50,000 u was deemed most appropriate.

Full Text

Effects of Different Molecular Weights and Concentrations of Chitosan on Rumen in Vitro Fermentation Parameters and Methane Emission in Dairy Cows

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Abstract

This study investigated the effects of chitosan with different molecular weights and concentrations on ruminal fermentation parameters and methane emission in dairy cows. Three healthy Holstein cows with similar body condition, good health status, and permanent rumen fistulas were used as experimental animals for rumen fluid collection. Chitosan with molecular weights of 1,000, 3,000, and 50,000 u was selected, with each molecular weight added to the substrate at concentrations of 0.4%, 0.8%, and 1.6% (substrate basis). A total of nine experimental groups and one control group (without chitosan) were established, with four replicates per group, and the experiment was repeated in three batches. After 24 h of in vitro fermentation, gas production, methane production, and rumen fermentation parameters were measured. The results showed that, compared with the control group, chitosan addition significantly reduced ammonia nitrogen concentration in rumen fermentation fluid ($P<0.05$), significantly increased propionic acid concentration ($P<0.05$), and significantly decreased the acetate/propionate ratio ($P<0.05$), thereby promoting changes in rumen fermentation patterns. Chitosan at a concentration of 1.6% with a molecular weight of 50,000 u and at a concentration of 0.8% with a molecular weight of 50,000 u tended to reduce methane production in fermentation fluid without affecting dry matter digestibility ($P<0.10$). In summary, chitosan addition can effectively regulate rumen microbial fermentation status under in vitro conditions. Considering all factors, chitosan at a concentration of 0.6% with a molecular weight of 50,000 u is most suitable.

Key words: chitosan; rumen fermentation in vitro; dairy cow

Methane (CH) produced by microbial fermentation in the gastrointestinal tract

of ruminants is the primary pathway of methane generation in the livestock industry. It is reported that annual global CH emissions from ruminant gastrointestinal tracts account for approximately 58% of total agricultural CH emissions [1] and up to 28% of total global CH emissions [2], with beef and dairy cattle contributing 74% of these emissions [3]. As global greenhouse effects intensify, reducing ruminal CH emissions from ruminants has become a critical research focus. Numerous studies have shown that chemical feed additives [4], antibiotics [5], CH inhibitors [6], and plant extracts [7] can improve ruminant production performance and reduce CH emissions. However, issues such as chemical residues in animal products, bacterial resistance to antibiotics, and the excessive toxicity and cost of some additives have limited their application in animal nutrition [8]. Therefore, the scientific community continues to actively seek alternative feed additives that can improve rumen function while promoting ecological sustainability.

Chitosan, a product derived from the deacetylation of chitin, is a natural high-molecular-weight compound chemically known as -(1,4)-2-amino-2-deoxy-D-glucose. Since the 1970s, research on chitosan has advanced considerably, and it has been studied and developed as the "sixth life element for humans" [9]. In recent years, many reports have investigated chitosan as a feed additive to improve rumen fermentation. Li et al. [10] found that adding chitosan to goat diets with concentrate-to-forage ratios of 20:80 and 50:50 significantly increased the molar proportion of propionic acid and decreased the acetate/propionate ratio in goat rumen fluid. Henry et al. [11] reported that adding chitosan to beef cattle diets significantly reduced CH emissions. These results indicate that dietary chitosan supplementation affects rumen fermentation and CH production in ruminants, though reports on its effects on dairy cow rumen fermentation parameters are limited and inconsistent. Therefore, this study aimed to investigate the effects of chitosan with different molecular weights and concentrations on dairy cow rumen fermentation parameters in vitro, providing scientific basis and theoretical support for the practical application of chitosan as a ruminant feed additive.

1.1 Experimental Materials

The chitosan used in this experiment had molecular weights of 1,000, 3,000, and 50,000 u, all purchased from Zhejiang Golden-Shell Pharmaceutical Co., Ltd., with a degree of deacetylation above 85% and purity above 95%.

1.2 Experimental Animals and Management

Three Holstein cows with similar body condition, good health status, and permanent rumen fistulas were selected as rumen fluid donors. The cows were fed total mixed rations (TMR) at 08:00 and 18:00 daily. The composition and nutrient levels of the TMR are shown in Table 1 .

Table 1 Composition and nutrient levels of the TMR (DM basis)

Items	Content
Ingredients	
Alfalfa hay	
Chinese wildrye	
Corn	
Whole cottonseed	
Maize silage	
DDGS	
Steam-flaked corn	
Soybean meal	
Cottonseed meal	
Premix[1]	
NaCl	
Total	
Nutrient levels[2]	
NEL/(MJ/kg)	
EE	
CP	
NDF	
ADF	

[1]One kg of premix contained the following: Cu 1,230 mg, Zn 4,950 mg, Mn 1,760 mg, I 50 mg, Se 61 mg, VA 230,000 IU, VD 350,000 IU, VE 1,000 IU. The same as Table 2 .
[2]NEL was a calculated value [12], while the other nutrient levels were measured values. The same as Table 2.

1.3 Fermentation Substrate Preparation

The fermentation substrate consisted primarily of steam-flaked corn, soybean meal, Chinese wildrye, and alfalfa. Feed ingredients were dried at 65 °C for 48 h, ground thoroughly through a 1 mm sieve, weighed according to substrate proportions, and mixed evenly for later use. The composition and nutrient levels of the fermentation substrate are shown in Table 2 .

Table 2 Composition and nutrient levels of the substrate (DM basis)

Items	Content
Ingredients	
Steam-pressed corn	
Soybean meal	
Alfalfa	

Items	Content
Chinese wildrye	
CaHPO	
Premix	
Total	
Nutrient levels	
DM	
CP	
NDF	
NEL/(MJ/kg)	

1.4 Experimental Design and Methods

Rumen fluid was collected from the cows before morning feeding for in vitro fermentation. Approximately 0.5 L of rumen fluid was collected from each cow, mixed uniformly, and filtered through four layers of gauze.

Chitosan with molecular weights of 1,000, 3,000, and 50,000 u was selected. Each molecular weight was added to the substrate at concentrations of 0.4%, 0.8%, and 1.6% (substrate basis), establishing nine experimental groups plus one control group (without chitosan). Each group had four replicates, and the experiment was repeated in three batches.

The in vitro fermentation experiment was conducted according to the method of Menke et al. [13]. The artificial saliva salt solution composition is shown in Table 3 . Fresh rumen fluid was mixed with artificial saliva salt solution (rumen fluid:artificial saliva salt = 1:2) to a total of 70 mL, which was added to fermentation bottles. After continuous CO flushing for 5 s, bottles were immediately sealed and connected to gas production sensors, with gas bags attached to collect gas produced during rumen in vitro fermentation. Fermentation was carried out at 39 °C for 24 h continuously, and the experiment was repeated three times.

Table 3 Composition of artificial saliva salt solutions

Items	Reagent	Dosage
Microelement solution A	CaCl ·2H O, MnCl ·4H O, CoCl ·6H O, FeCl ·6H O	13.2 g, 10.0 g, 1.0 g, 8.0 g in 100 mL
Buffer bicarbonate B	NH HCO , NaHCO	4.0 g, 35.0 g in 1,000 mL
Phosphate buffer C	Na HPO , KH PO , MgSO ·7H O	5.7 g, 6.2 g, 0.6 g in 1,000 mL
Resazurin indicator	Resazurin	0.1 g in 100 mL
Reduction solution	Na S·9H O	160 mg, 625 mg in 100 mL

The pH of rumen culture fluid was measured using a Sartorius PB-20 pH meter. Gas production at 24 h was measured using an AGRS-III automatic micro-gas production recorder for microbial fermentation. Gas bags collected gas after 24 h of fermentation. Methane production in rumen culture fluid was measured using an Agilent 7890B gas chromatograph with a colorimetric method. Chromatographic conditions were: TCD detector, hydrogen carrier gas at 28 mL/min, Porapak Q packed column, detector temperature 100 °C, inlet temperature 150 °C, column temperature 38 °C, and injection volume 1 mL.

Dry matter digestibility was determined using the method of Tilley et al. [14]. Residues in fermentation bottles were filtered through gauze, rinsed twice with distilled water into 50 mL centrifuge tubes, centrifuged at 5,400×g for 15 min at 4 °C, and dried in an oven at 105 °C for 24 h. Dry matter digestibility was calculated as:

Dry matter digestibility (DM, %) = 100 × (sample DM weight - residue DM weight + blank tube DM weight) / sample DM weight

Volatile fatty acid (VFA) concentration was determined by the external standard method [15] using an Agilent 7890B gas chromatograph. Chromatographic conditions were: flame ionization detector temperature 220 °C, argon carrier gas at 30 mL/min, hydrogen provided by a hydrogen generator at 30 mL/min, air flow 300 mL/min, and injection volume 2 L.

Ammonia nitrogen (NH -N) concentration was determined by the indophenol colorimetric method [16]. The main steps were: add 0.05 mL sample to a test tube, mix while adding 2.5 mL phenol solution, add 2.0 mL sodium hypochlorite solution (5.25% concentration), shake well, heat in water bath at 95 °C for 5 min and 60 °C for 10 min, cool, and measure color at 630 nm using a Tiammei UV-2600 UV spectrophotometer.

1.5 Data Processing and Statistical Analysis

SPSS 20.0 software was used for multi-factor ANOVA with a full factorial model and Duncan' s multiple comparison test. Significance was defined as $P<0.05$. The t-test was used to estimate combination effects, with factors being chitosan molecular weight (three levels) and chitosan concentration (three levels). The statistical model was:

$$\mathbf{Yij} = \quad + \ \mathbf{i} + \ \mathbf{j} + \ \mathbf{ij}$$

Where: Yij represents the observed value; represents the overall mean; i represents the effect of the ith level of different molecular weight chitosan (i=1,2,3); j represents the effect of the jth level of different chitosan concentrations (j=1,2,3); and ij represents the random error.

2.1 Effects of Chitosan on Fermentation Fluid pH, NH -N Concentration, and Dry Matter Digestibility

As shown in Table 4 , the fermentation fluid pH in all chitosan groups was significantly lower than that in the control group (P<0.05), except for the group with 1.6% concentration and 1,000 u molecular weight. The NH -N concentration in all chitosan groups was significantly lower than that in the control group (P<0.05), and in the 0.4% concentration groups, NH -N concentration decreased significantly with increasing molecular weight of added chitosan (P<0.05). Dry matter digestibility in all chitosan groups showed no significant difference compared with the control group (P>0.05).

Table 4 Effects of chitosan on pH, NH -N concentration and dry matter digestibility of fermentation liquor

Items	Concentration/%	Molecular weight/u	pH	NH -N/(mg/dL)	Dry matter digestibility/%
			6.73a	21.96a	35a
			6.68b	20.48b	36a
			6.69b	18.38c	35a
			6.68b	18.01c	34a
			6.70b	20.22b	31ab
			6.69b	20.13b	34a
			6.73b	19.09bc	30ab
			6.71ab	17.50c	31a
			6.69b	17.15cd	32a
			6.69b	18.57c	
P-value			<0.0001	<0.0001	

In the same column, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different small letter superscripts mean significant difference (P<0.05). The same as below.

2.2 Effects of Chitosan on in Vitro Gas Production

As shown in Table 5 , total gas production in all chitosan groups was significantly higher than that in the control group (P<0.05), except for the group with 0.8% concentration and 50,000 u molecular weight. In the 0.8% and 1.6% concentration groups, total gas production decreased significantly with increasing molecular weight of added chitosan (P<0.05). Hydrogen production volume in all chitosan groups showed no significant difference compared with the control group (P>0.05). Methane production volume in all chitosan groups showed no significant difference compared with the control group (P>0.05), though the 50,000 u molecular weight groups showed a decreasing trend (P<0.10).

Table 5 Effects of chitosan on gas production, CH and H volume of in vitro fermentation

Items	Concentration/%	Molecular weight/u	Total gas production	H volume	CH volume
			82.39c	8.57ab	13.11c
			92.78a	9.51a	15.55b
			97.00a	9.06a	15.56b
			90.22b	7.77b	16.06a
			92.11a	8.05a	13.93b
			87.61b	8.37a	15.13a
			80.11c	6.78b	15.50a
			93.00a	8.55a	15.32a
			90.89a	8.56a	15.41a
			88.33b	7.76b	15.56a
P-value					

2.3 Effects of Chitosan on VFA Concentration in Fermentation Fluid

As shown in Table 6 , the total volatile fatty acid (TVFA) concentration in the 0.4% concentration and 1,000 u molecular weight group was significantly lower than that in the control group (P<0.05), while no significant differences were observed between other chitosan groups and the control group (P>0.05). The TVFA concentration in the 0.4% concentration groups increased significantly with increasing molecular weight of added chitosan (P<0.05). Concentrations of acetic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid in all chitosan groups showed no significant differences compared with the control group (P>0.05). However, propionic acid concentration in all chitosan groups was significantly higher than that in the control group (P<0.05), and in the 0.4% and 0.8% concentration groups, propionic acid concentration increased significantly with increasing molecular weight of added chitosan (P<0.05). The acetate/propionate ratio in all chitosan groups was significantly lower than that in the control group (P<0.05), with no significant differences among chitosan groups (P>0.05).

Table 6 Effects of chitosan on concentration of VFA of fermentation liquor

Items	Concentration/%	Molecular weight/u	Acetic acid/%	Propionic acid/%	Isobutyric acid/%	Butyric acid/%	Isovaleric acid/%	Valeric acid/%	Acetic acid/propionic acid	TVFA/(mmol/L)
			63.23a	13.11c	3.16a	2.75b	2.70b	2.61b	2.75b	52.98b
			67.15a	15.55b	2.75b	2.70b	2.61b	2.75b	2.71b	59.04ab
			64.74a	15.56b	2.70b	2.61b	2.75b	2.71b	2.88b	66.19a

Items	Concentration/%	Molecular weight/u	Acetic acid/%	Propionic acid/%	Isobutyric acid/%	Butyric acid/%	Isovaleric acid/%	Valeric acid/%	Acetic acid/propionic acid	TVFA/(mmol/L)
			62.46a	16.06a	2.61b	2.75b	2.71b	2.88b	2.74b	67.15a
			66.07a	13.93b	2.75b	2.71b	2.88b	2.74b	2.68b	64.74a
			65.19a	15.13a	2.71b	2.88b	2.74b	2.68b	2.70b	62.46a
			66.30a	15.50a	2.88b	2.74b	2.68b	2.70b		
P-value										

3.1 Effects of Chitosan on Fermentation Fluid pH, NH -N Concentration, and Dry Matter Digestibility

The results indicate that chitosan significantly reduced fermentation fluid pH, but all values remained within the normal range. pH is an important indicator for measuring rumen fermentation in ruminants, and maintaining pH within a normal range is a prerequisite for ensuring normal rumen fermentation [17]. In in vitro fermentation systems, the main factors affecting fermentation fluid pH are the production of alkaline substances (such as NH -N) and organic acids [18]. The pH reduction observed in this experiment may have been caused by the significant decrease in NH -N concentration.

NH -N concentration in rumen fluid is an important parameter of the rumen internal environment, reflecting the supply status of microbial nitrogen in the rumen. This study found that chitosan significantly reduced NH -N concentration in rumen fermentation fluid, which differs from the results of Ren [19] and Tian et al. [20]. This discrepancy may be due to differences in the concentration and molecular weight of chitosan used. The results of Li [21] are consistent with this study, showing that chitosan addition significantly reduced rumen fluid NH -N concentration. The reduction in NH -N concentration may indicate changes in rumen microbial community structure.

3.2 Effects of Chitosan on Gas Production, CH and H Volume

In this experiment, total gas production in all chitosan groups was significantly higher than that in the control group, except for the 0.8% concentration and 50,000 u molecular weight group, without affecting dry matter digestibility. Previous reports indicate that higher gas production during in vitro culture suggests more intense fermentation activity of feed in the rumen [22]. This study found that chitosan addition facilitated feed fermentation in the rumen, which differs from the results of Goiri et al. [23]. This discrepancy may be due to differences in chitosan selection; the low molecular weight chitosan and three concentrations used in this experiment may be more conducive to feed fermentation in the rumen.

In this study, the 1.6% concentration and 50,000 u molecular weight group and the 0.8% concentration and 50,000 u molecular weight group showed a decreasing trend in CH production. According to the mechanism of CH and VFA formation in the rumen [24], propionic acid production can competitively consume H , thereby effectively inhibiting CH formation. Although this study showed no significant change in H production volume, H likely existed in a dynamic equilibrium state. The results indicate that chitosan addition significantly increased propionic acid concentration, so the decreasing trend in CH volume in the 1.6% concentration and 50,000 u molecular weight group and the 0.8% concentration and 50,000 u molecular weight group was likely due to propionic acid production competitively binding H .

3.3 Effects of Chitosan on VFA Concentration in Fermentation Fluid

The primary functions of VFA are to provide energy for animal production and maintain the rumen environment [25]. According to this study' s results, chitosan addition significantly increased propionic acid concentration and decreased the acetate/propionate ratio in rumen fermentation fluid. Although acetic acid concentration did not change significantly, chitosan can be considered to change the rumen fermentation pattern, shifting from acetate-type fermentation to propionate-type fermentation, which is currently one of the main mechanisms by which feed additives inhibit CH production in ruminants [26].

The results show that chitosan increased propionic acid concentration, thereby reducing the acetate/propionate ratio and changing the rumen fermentation pattern, which is speculated to be closely related to the antibacterial action of chitosan. Reports indicate that the main antibacterial mechanism of chitosan is that, as a polycation, it attracts negative charges on microbial surfaces, causing changes in cell permeability. These electrostatic interactions promote hydrolysis of peptidoglycan in microbial cell walls, ultimately leading to cell lysis [27]. Since the peptidoglycan layer is more abundant in Gram-positive bacteria than in Gram-negative bacteria, the antibacterial effect of chitosan is more significant against Gram-positive bacteria [28]. The antibacterial effect of chitosan is more pronounced against Gram-positive bacteria. In the rumen, the phylum Firmicutes, which primarily produces acetic acid, consists of Gram-positive bacteria, while the phyla Bacteroidetes and Proteobacteria, which mainly produce propionic acid, are Gram-negative bacteria [29]. From this perspective, the antibacterial effect of chitosan on Gram-positive bacteria leads to decreased abundance of acetic acid-producing bacteria and increased abundance of propionic acid-producing bacteria [30], resulting in changes in VFA concentrations and ultimately altering the rumen fermentation pattern. However, how chitosan specifically acts on rumen microbial communities requires further experimental verification. The results of this study are basically consistent with the in vitro fermentation results of Belanche et al. [30] and Ren et al. [31].

This study showed that chitosan had no effect on acetic acid concentration in in vitro fermentation fluid. However, in the study by Belanche et al. [30], chitosan

significantly reduced acetic acid concentration. This difference may be due to the different molecular weights of chitosan selected; the three different molecular weights of chitosan used in this experiment all belong to low molecular weight chitosan, and molecular weights that are too small may be insufficient to reduce acetic acid concentration. The specific reasons require further verification.

Furthermore, in this study, the 1.6% concentration and 50,000 u molecular weight group and the 0.8% concentration and 50,000 u molecular weight group showed the best effects. These two groups not only significantly increased propionic acid concentration and decreased the acetate/propionate ratio like other groups, but also tended to reduce CH production volume. However, the 0.8% concentration and 50,000 u molecular weight group showed a decreasing trend in dry matter digestibility. Therefore, chitosan at a concentration of 1.6% with a molecular weight of 50,000 u is most suitable.

Conclusions

1. Compared with the control group, chitosan addition significantly reduced NH -N concentration, significantly increased propionic acid concentration, and significantly decreased the acetate/propionate ratio in rumen fermentation fluid, promoting changes in rumen fermentation patterns.
2. Compared with the control group, the 1.6% concentration and 50,000 u molecular weight group and the 0.8% concentration and 50,000 u molecular weight group showed a decreasing trend in CH production volume.
3. Adding chitosan at a concentration of 1.6% with a molecular weight of 50,000 u had the most significant effects on rumen fermentation parameters and methane inhibition.

References

[1] KNAPP J R, LAUR G L, VADAS P A, et al. Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions[J]. Journal of Dairy Science, 2014, 97(6): 3231–3261.

[2] YUSUF R O, NOOR Z Z, ABBA A H, et al. Methane emission by sectors: a comprehensive review emission sources and mitigation methods[J]. Renewable and Sustainable Energy Reviews, 2012, 16(7): 5059–5070.

[3] SOLIVA C R, AMELCHANKA S L, DUVAL S M, et al. Ruminal methane inhibition potential of various pure compounds in comparison with garlic oil as determined with a rumen simulation technique (Rusitec)[J]. British Journal of Nutrition, 2011, 106(1): 114–122.

[4] GERBER P J, HRISTOV A N, HENDERSON B, et al. Technical options for the mitigation of direct methane nitrous oxide emissions livestock: a review[J]. Animal, 2013, 7(Suppl. 2): 220-234.

[5] MUSTAPHA N A, SAKAI K, SHIRAI Y, et al. Impact of different antibiotics on methane production using waste-activated sludge: mechanisms and microbial community dynamics[J]. Applied Microbiology and Biotechnology, 2016, 100(21): 9355-9364.

[6] WALL E, SIMM G, MORAN D. Developing breeding schemes to assist mitigation of greenhouse gas emissions[J]. Animal, 2010, 4(3): 366-376.

[7] KIM E T, KIM C H, MIN K S, et al. Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in in vitro[J]. Asian-Australasian Journal of Animal Sciences, 2012, 25(6): 806-811.

[8] WINA E, MUETZEL S, BECKER K. The impact of saponins or saponin-containing plant materials ruminant production-a review[J]. Journal Agricultural Chemistry, 2005, 53(21): 8093-8105.

[9] 韩德承. 人体的第六生命要素——壳寡糖 [J]. 祝您健康, 2013(11): 36-37.

[10] 李朝云, 杨昕涧, 孙菲菲, 等. 不同精粗比日粮中添加壳聚糖对奶山羊瘤胃发酵、甲烷产生及微生物区系的影响 [C]//中国畜牧兽医学会动物营养学分会第七届中国饲料营养学术研讨会论文集. 郑州: 中国畜牧兽医学会动物营养学分会, 2014.

[11] HENRY, DARREN D. Effects of feeding a natural biopolymer (Chitosan) on methane emissions and performance in beef cattle[J]. 2013.

[12] 冯仰廉, 周建民, 张晓明, 等. 我国奶牛饲料产奶净能值测算方法的研究 [C]//第三届全国反刍动物营养学术研讨会论文集. 北京: 中国畜牧兽医学会, 2011.

[13] MENKE K H, RAAB L, SALEWSKI A, et al. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro[J]. Journal of Agricultural Science, 1979, 93(1): 217-222.

[14] TILLEY J M A, TERRY R A. A two-stage technique for the in vitro digestion of forage crops[J]. Grass and Forage Science, 1963, 18(2): 104-111.

[15] 戈婷婷. 不同组合的功能性寡糖对锦江黄牛瘤胃体外发酵的影响 [D]. 硕士学位论文. 南昌: 江西农业大学, 2011.

[16] CZERKAWSKI J W. Chemical composition of microbial matter in the rumen[J]. Journal of the Science of Food and Agriculture, 1976, 27(7): 621-632.

[17] 韩昊奇, 刘大程, 高民, 等. 不同 NFC/NDF 比对奶山羊瘤胃微生物及瘤胃 pH 值变化的影响 [J]. 畜牧与饲料科学, 2011, 32(9/10): 54-58.

[18] 唐志文, 蒋林树, 杨亮, 等. 金银花提取物对瘤胃体外发酵参数及产气量的影响 [J]. 动物营养学报, 2018, 30(2): 790-796.

[19] 任海军. 壳聚糖对奶牛产奶性能和免疫功能影响的研究 [D]. 硕士学位论文. 呼和浩特: 内蒙古农业大学, 2008.

[20] 田雨佳, 闫素梅, 任海军, 等. 壳聚糖在奶牛瘤胃中的降解率及其对瘤胃发酵的影响 [J]. 中国畜牧杂志, 2011, 47(19): 53-56.

[21] 李朝云. 壳聚糖与纤维素酶对瘤胃发酵、甲烷产生及微生物区系的影响 [D]. 博士学位论文. 咸阳: 西北农林科技大学, 2014.

[22] MCGINN S M, BEAUCHEMIN K A, COATES T, et al. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid[J]. Journal of Animal Science, 2004, 82(11): 3346-3356.

[23] GOIRI I, GARCIA-RODRIGUEZ A, OREGUI L M. Effect of chitosan on mixed ruminal microorganism fermentation using the rumen simulation technique (Rusitec)[J]. Animal Feed Science and Technology, 2009, 152(1/2): 92-102.

[24] WHITELAW F G, EADIE J M, BRUCE L A, et al. Methane formation in faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid proportions[J]. British Journal of Nutrition, 1984, 52(2): 261-275.

[25] 冯仰廉. 反刍动物营养学 [M]. 北京: 科学出版社, 2004.

[26] 苑忠央, 杨维仁. 反刍动物瘤胃甲烷排放、测定及减排技术的研究进展 [J]. 饲料与畜牧, 2016(10): 40-45.

[27] KONG M, CHEN X G, XING K, et al. Antimicrobial properties of chitosan and mode of action: a state of the art review[J]. International Journal of Food Microbiology, 2010, 144(1): 51-63.

[28] 陈娟. 壳聚糖抗菌性的研究进展 [J]. 烟台南山学院学报, 2017(1): 56-58.

[29] HENDERSON G, COX F, GANESH S, et al. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range[J]. Scientific Reports, 2015, 5: 14567.

[30] BELANCHE A, PINLOCHE E, PRESKETT D, et al. Effects and mode of action of chitosan and ivy fruit saponins on the microbiome, fermentation and methanogenesis in the rumen simulation technique[J]. FEMS Microbiology Ecology, 2016, 92(1): fiv160.

[31] 任海军, 闫素梅, 邬亚青, 等. 壳聚糖在奶牛瘤胃内的降解及其对奶牛瘤胃体外发酵特性和产奶性能的影响 [C]//中国畜牧兽医学会动物营养学分会第十次学术研讨会论文集. 杭州: 中国畜牧兽医学会动物营养学分会, 2008.

Note: Figure translations are in progress. See original paper for figures.