Effects of Grape Seed Proanthocyanidins on In Vitro Rumen Fermentation Parameters and Microbiota in Dairy Cows (Postprint)

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Abstract

This experiment aimed to investigate the effects of grape seed proanthocyanidins on rumen fermentation parameters and microbial flora in dairy cows using an in vitro culture method. The experiment was divided into 6 groups, using 500 g of total mixed ration with a concentrate-to-forage ratio of 40:60 as the fermentation substrate, with each group supplemented with 0 (control), 0.1, 0.2, 0.3, 0.4, or 0.5 g/kg of grape seed proanthocyanidins. After 24 h of in vitro fermentation, gas production was recorded and rumen fermentation parameters and microbial contents were determined. The results showed that, compared with the control group: 1) Supplementation with 0.4 and 0.5 g/kg of grape seed proanthocyanidins significantly decreased the contents of butyric acid and isovaleric acid in the fermentation fluid ($P<0.05$), while supplementation with 0.2 g/kg of grape seed proanthocyanidins significantly increased the content of isobutyric acid in the fermentation fluid ($P<0.05$); 2) Supplementation with different levels of grape seed proanthocyanidins all significantly increased the pH of the fermentation fluid ($P<0.05$); 3) Supplementation with different levels of grape seed proanthocyanidins tended to reduce gas production from in vitro fermentation, with the 0.3 g/kg group showing a significant effect ($P<0.05$), while the 0.2 g/kg group significantly decreased methane production ($P<0.05$); 4) Grape seed proanthocyanidins significantly decreased the contents of protozoa (in the 0.2, 0.3, 0.4, and 0.5 g/kg groups), methanogens (in the 0.1, 0.2, 0.4, and 0.5 g/kg groups), Butyrivibrio fibrisolvens (in the 0.2, 0.3, 0.4, and 0.5 g/kg groups), and Fibrobacter succinogenes (in the 0.1, 0.3, 0.4, and 0.5 g/kg groups) in the fermentation fluid ($P<0.05$). Therefore, supplementation of grape seed proanthocyanidins in dairy cow rumen in vitro fermentation fluid improved the rumen fermentation pattern, significantly affected the rumen microbial flora, and significantly reduced methane production, with the supplementation level of 0.2 g/kg being relatively appropriate.

Full Text

Effects of Grape Seed Procyanidins on Rumen Fermentation Parameters and Microflora of Dairy Cows *In Vitro*

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Abstract: This trial was conducted to investigate the effects of grape seed procyanidins on rumen fermentation parameters and microflora in dairy cows using an *in vitro* culture method. The experiment consisted of six groups, with a total mixed ration (TMR) at a concentrate-to-forage ratio of 40:60 serving as the fermentation substrate. Grape seed procyanidins were added at levels of 0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5 g/kg. After 24 h of *in vitro* fermentation, gas production was recorded, and rumen fermentation parameters and microbial populations were measured. Compared with the control group, the results showed that: (1) supplementation with 0.4 and 0.5 g/kg grape seed procyanidins significantly decreased the contents of butyric acid and isovaleric acid in the fermentation fluid ($P<0.05$), while 0.2 g/kg supplementation significantly increased isobutyric acid content ($P<0.05$); (2) different levels of grape seed procyanidins significantly increased fermentation fluid pH ($P<0.05$); (3) grape seed procyanidins reduced *in vitro* gas production, with a significant effect observed in the 0.3 g/kg group ($P<0.05$), and the 0.2 g/kg group significantly decreased methane production ($P<0.05$); (4) grape seed procyanidins significantly reduced the populations of protozoa (0.2, 0.3, 0.4, and 0.5 g/kg groups), methanogens (0.1, 0.2, 0.4, and 0.5 g/kg groups), *Butyrivibrio fibrisolvens* (0.2, 0.3, 0.4, and 0.5 g/kg groups), and *Fibrobacter succinogenes* (0.1, 0.3, 0.4, and 0.5 g/kg groups) in the fermentation fluid ($P<0.05$). In conclusion, grape seed procyanidin supplementation improved rumen fermentation patterns, significantly affected rumen microflora, and substantially reduced methane production, with 0.2 g/kg being the optimal addition level.

Keywords: grape seed procyanidins; dairy cow; rumen fluid; *in vitro* fermentation; microflora

Introduction

Grape seed procyanidins are phenolic compounds found in grape fruits, with particularly high concentrations present in the seeds [1-3]. They possess various biological activities, including antioxidant [4-5], free radical scavenging [6], anti-radiation, and anti-stress effects [7-8]. Studies on their supplementation in ruminant and pig feeding have been reported both domestically and internationally [9-18]. In China, research on the effects of grape seed procyanidins on dairy cow production performance and on rumen fermentation in sheep has

been relatively extensive [9-14]; however, systematic studies on their impact on rumen fermentation and microflora in dairy cows remain limited, necessitating further in-depth investigation.

Currently, the addition of plant saponin substances to improve livestock performance shows broad application prospects, particularly in regulating rumen fermentation in dairy cows, making it a prominent research focus [19-21]. Wu et al. [9] reported that replacing portions of corn, wheat bran, and soybean meal with grape pomace, or supplementing dairy cow diets with appropriate proportions of grape seed meal [10], had no adverse effects on milk production performance, milk quality, or daily economic benefits, while improving milk fat percentage and production efficiency. Gessner et al. [11] found that substituting alfalfa hay with grape seed meal increased milk yield and milk fat percentage in dairy cows and reduced somatic cell counts. Sun et al. [12] observed significant improvements in production performance and feed conversion efficiency in adult sheep fed grape pomace. Zhao et al. [13] demonstrated that grape pomace supplementation enhanced nitrogen apparent digestibility and retention in sheep, increased propionic acid content in rumen fluid, and decreased butyric acid content and the acetate/propionate ratio' s effect on rumen pH. Li et al. [14] reported that adding different levels of grape pomace and grape seeds to diets significantly improved average daily gain, apparent digestibility of crude protein, and energy utilization in adult Small-tailed Han sheep. Meanwhile, numerous studies have shown that grape seed procyanidins also improve various aspects in weaned piglets, including production performance, intestinal digestive enzyme activity [15], immunity [16], antioxidant capacity [17], and reproductive performance [18].

Overall, current reports on grape seed procyanidins regulating *in vitro* fermentation patterns in dairy cows are scarce, particularly regarding their effects on rumen fermentation parameters and microflora. Therefore, this study aimed to investigate the effects of grape seed procyanidins on rumen fermentation parameters and microflora in dairy cows using *in vitro* methods, providing a theoretical basis for the application of grape seed procyanidins as rumen fermentation regulators in production practice.

1.1 Experimental Materials

Fermentation substrate: The total mixed ration (TMR) with a concentrate-to-forage ratio of 40:60 was obtained from a dairy farm in Beijing. The substrate contained 54.80% dry matter (DM), 15.68% crude protein (CP), and 39.80% neutral detergent fiber (NDF). After drying and grinding, the material was passed through a 40-mesh sieve. The composition and nutrient levels are presented in .

Grape seed procyanidins: Purchased from Tianjin Jianfeng Natural Product R&D Co., Ltd., containing 18.4% monomers, 20.4% dimers, 15.2% trimers, 14.1% tetramers, 28.5% oligomers (5-13 units), and 4.3% phenolic aldehydes.

Buffer solution: Prepared according to the method of Menke et al. [19], continuously infused with CO_2 and maintained at 39°C in a water bath until use.

Rumen fluid: Rumen contents were collected before morning feeding from four healthy Holstein cows fitted with rumen cannulas, fed a diet with a concentrate-to-forage ratio of 40:60 (700 g/kg Leymus chinensis and 300 g/kg concentrate supplement). The contents were mixed, filtered through four layers of cheesecloth at 39°C, placed in a thermos flask, and quickly transported to the laboratory, with all operations completed within the shortest possible time [21].

1.2 Experimental Design

This experiment employed a single-factor design with six levels. Five hundred milligrams of fermentation substrate were placed in 150 mL anaerobic fermentation bottles, with grape seed procyanidins added at levels of 0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5 g/kg. Each group had six replicates. During inoculation, 50 mL of pre-warmed buffer solution and 25 mL of fresh rumen fluid filtered through four layers of cheesecloth were quickly added to each bottle. After continuous CO_2 infusion for 5 seconds, bottles were immediately sealed and connected to gas production sensors [22], followed by continuous incubation at 39°C for 24 h. The entire experiment was repeated three times.

1.3 Sample Collection and Analysis

1.3.1 Fermentation Fluid Sample Collection and Processing After 24 h of *in vitro* incubation, fermentation tubes were removed and placed in an ice-water bath to terminate fermentation. Fermentation fluid was transferred to pre-weighed 50 mL plastic centrifuge tubes, and pH was measured immediately. The fluid was then centrifuged at 5,000 × g for 10 min, and the supernatant was collected for determination of ammonia nitrogen (NH_3-N), microbial crude protein (MCP), and volatile fatty acid (VFA) contents according to the method of Yan et al. [24].

1.3.2 Gas Production and Methane Measurement The 24 h gas production was measured using the AGRS-III automatic microbial fermentation gas production recorder developed by Shen et al. [24]. Gas samples collected after 24 h fermentation were analyzed for methane content using an Agilent 7890B gas chromatograph with the following conditions: TCD detector, hydrogen carrier gas at 28 mL/min, PorapakQ packed column, detector temperature 100°C, inlet temperature 150°C, column temperature 38°C, and injection volume 1 mL.

1.3.3 Total DNA Extraction and Bacterial Quantification Total DNA was extracted from fermentation fluid using the bead-beating cetyltrimethylammonium bromide (CTAB) method [25]. Primers for real-time quantitative PCR were designed according to literature reports [23], with sequences listed in . Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. A 20 L

reaction system and conditions were established using SYBR Premix Ex Taq™ reagent [26], with three replicates per sample.

Target bacterial populations were expressed as a percentage of total rumen bacterial 16S rDNA based on the threshold cycle (Ct) values obtained from qRT-PCR using the following formula:

Target bacteria content (%) = 100 × 2^-(Ct target bacteria - Ct total bacteria) [25].

1.4 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 17.0 software, with Duncan' s multiple comparison test used for mean separation. Significance was declared at $P < 0.05$.

Results

2.1 Effects of Grape Seed Procyanidins on Rumen Fermentation Parameters

As shown in , compared with the control group, supplementation with different levels of grape seed procyanidins significantly increased fermentation fluid pH ($P < 0.05$), with the highest value observed in the 0.3 g/kg group (pH = 6.31). Ammonia nitrogen content ranged from 37.29 to 47.43 mg/dL, with the 0.2 g/kg group showing the lowest value, which was significantly lower than the control ($P < 0.05$), while the 0.4 g/kg group showed the highest value, significantly higher than the control ($P < 0.05$). The 0.2 g/kg group significantly decreased methane production ($P < 0.05$), whereas the 0.3 g/kg group significantly reduced total gas production ($P < 0.05$). No significant differences were observed in microbial crude protein content among groups ($P > 0.05$).

2.2 Effects of Grape Seed Procyanidins on Volatile Fatty Acid Contents

As presented in , grape seed procyanidin supplementation at different levels had no significant effects on total volatile fatty acids (TVFA), acetic acid, propionic acid, valeric acid contents, or the acetate/propionate ratio ($P > 0.05$). However, supplementation at 0.4 and 0.5 g/kg significantly decreased butyric acid and isovaleric acid contents ($P < 0.05$), while 0.2 g/kg supplementation significantly increased isobutyric acid content ($P < 0.05$).

2.3 Effects of Grape Seed Procyanidins on Microbial Populations

As shown in , compared with the control, the 0.2, 0.3, 0.4, and 0.5 g/kg groups significantly reduced protozoa and *Butyrivibrio fibrisolvens* populations ($P < 0.05$). The 0.1, 0.2, 0.4, and 0.5 g/kg groups significantly decreased methanogen

populations ($P < 0.05$), while the 0.3, 0.4, and 0.5 g/kg groups significantly increased fungal populations ($P < 0.05$). The 0.1, 0.3, 0.4, and 0.5 g/kg groups significantly reduced *Fibrobacter succinogenes* populations ($P < 0.05$). No significant differences were observed in *Ruminococcus flavefaciens* or *Ruminococcus albus* populations among groups ($P > 0.05$).

Discussion

3.1 Effects on Rumen Fermentation Parameters

Ammonia nitrogen is an important product of rumen digestive metabolism in ruminants and serves as a primary substrate for microbial protein synthesis, with its concentration directly reflecting rumen fermentation conditions. In this study, appropriate supplementation with grape seed procyanidins significantly reduced fermentation fluid ammonia nitrogen content. Previous reports indicate that excessively high rumen ammonia nitrogen content can be absorbed into the bloodstream through the rumen wall, increasing the body' s nitrogen metabolic burden [27], wasting nitrogen resources, and potentially causing ammonia toxicity. In our trial, the 0.2 g/kg supplementation level significantly decreased fermentation fluid ammonia nitrogen content. If nitrogenous substances are insufficient, microbial growth is hindered, reducing animal performance; however, at this level, microbial protein content also reached its minimum value. Ammonia nitrogen content is closely correlated with microbial protein content [28]. Mireguli et al. [29] reported that grape seed essential oil reduced fermentation fluid ammonia nitrogen content through *in vitro* culture. Li et al. [30] found that feeding grape pomace significantly decreased rumen fluid ammonia nitrogen content in lambs. Li et al. [31-32] reported that condensed tannin-rich substances reduced microbial protein content in fermentation fluid in *in vitro* trials. Wang et al. [33] demonstrated that dietary supplementation with condensed tannin-rich substances significantly reduced protein degradation in the rumen. Pan et al. [34] showed that higher dietary tannin content more strongly inhibited microbial protein synthesis. Previous studies have reported a high positive correlation between *in vitro* fermentation fluid NH_3-N content and gas production ($r > 0.99$) [35], consistent with our findings that different levels of grape seed procyanidins not only reduced ammonia nitrogen content but also inhibited gas production. Di et al. [36] found that tannin content was negatively correlated with gas production. Li [37] reported that condensed tannins disrupted the cell membranes of some rumen bacteria, preventing normal bacterial reproduction and blocking enzyme-substrate binding, ultimately affecting rumen fermentation and reducing gas production.

3.2 Effects on Volatile Fatty Acid Contents

Volatile fatty acids serve as the primary energy source in ruminants, with their concentrations and composition ratios directly reflecting rumen metabolic activity. The VFAs produced during rumen fermentation are mainly acetic acid, propionic acid, and butyric acid, accounting for approximately 95% of TVFA.

Energy released during VFA fermentation generates ATP, which microorganisms utilize as an energy source for microbial protein synthesis [38]. In this study, TVFA content and the acetate/propionate ratio showed no significant differences among groups; however, increasing grape seed procyanidin supplementation levels significantly inhibited butyric acid production, consistent with findings by Lü [39], Mireguli et al. [29], and Zhao et al. [13]. Additionally, grape seed procyanidins significantly affected isobutyric acid content. Fermentation fluid pH ranged from 6.22 to 6.31 in this study, all within the normal physiological range [38] without adverse effects on rumen microbial growth. These results are similar to those reported by Li [37] and Li et al. [32], who found that condensed tannin-rich substances increased fermentation fluid pH in *in vitro* trials. These findings demonstrate that grape seed procyanidins can alter *in vitro* rumen fermentation patterns.

3.3 Effects on Microbial Populations

This study demonstrated that grape seed procyanidins significantly reduced protozoa and methanogen populations in fermentation fluid. Protozoa primarily prey on bacteria and fungi in the rumen, producing substantial methane during metabolism and maintaining a symbiotic relationship with methanogens [40]. Therefore, literature suggests that eliminating protozoa can reduce methanogen populations and subsequently decrease methane emissions [41]. Wang et al. [42] found that tannic acid significantly reduced rumen protozoa numbers in *in vitro* trials, and Anantasook et al. [43] observed similar effects with dietary condensed tannins, consistent with our results. This reduction may occur through disruption of protozoal living environments or direct action on protozoa. Reports indicate that protozoa provide growth substances for methanogens, which contain coenzymes related to methane production [44]. Our results showed that grape seed procyanidins significantly reduced methanogen populations, consistent with findings by Zhao [45]. Since most methane in the rumen is produced directly by methanogens, reducing their populations is crucial for methane mitigation.

Fungi and bacteria play major roles in cellulose degradation by rumen microorganisms. Wood et al. [46] demonstrated through *in vitro* studies that rumen fungi promote cellulose degradation. Yuan [47] found that tannic acid supplementation significantly increased fungal populations in the rumen, similar to our results showing that grape seed procyanidins significantly enhanced fungal populations in fermentation fluid. Although rumen fungi secrete highly active cellulolytic enzymes, their reproduction rate is slower than that of bacteria, which dominate the rumen environment [48]. Major rumen bacteria include *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes*. *Butyrivibrio fibrisolvens* is an anaerobic Gram-positive bacterium that plays an important role in protein degradation in the rumen. Due to its proteolytic enzyme activity, enzyme activity is inhibited when the diet contains resistant proteins. Our results showed that grape seed procyani-

dins significantly reduced *B. fibrisolvens* populations, consistent with Zhao [45]. This may occur because condensed tannins in grape seed procyanidins are plant-derived resistant proteins that bind to dietary proteins, forming complexes that reduce *B. fibrisolvens* populations.

Fibrobacter succinogenes is a Gram-negative bacterium with high cellulolytic activity [44]. Under pure culture conditions, *F. succinogenes* exhibits strong degradation capabilities, breaking down structurally tough substances and some cellulose types that *R. flavefaciens* cannot degrade, while also showing strong antibiotic tolerance [49]. This study found that grape seed procyanidins significantly reduced *F. succinogenes* populations. Wang [50] reported that condensed tannins strongly inhibited both endogenous and exogenous enzymes of *F. succinogenes*, thereby reducing its numbers, which aligns with our findings.

Ruminococcus flavefaciens and *R. albus* are the predominant rumen cocci involved in cellulose degradation. These two species exhibit both synergistic and inhibitory interactions, as *R. albus* produces various bacteriocins that inhibit *R. flavefaciens* growth [51]. Li et al. [52] found that dietary tannin supplementation significantly reduced both *R. flavefaciens* and *R. albus* populations in goat rumen fluid. However, our study showed no significant effects of grape seed procyanidins on these two bacterial species, possibly due to differences between the complex rumen environment *in vivo* and experimental conditions *in vitro*.

Conclusion

1. Grape seed procyanidins increased fermentation fluid pH, but all values remained within the normal physiological range. Appropriate supplementation levels reduced NH_3-N content, increased isobutyric acid content, and decreased gas and methane production; however, levels exceeding 0.2 g/kg promoted NH_3-N release and inhibited butyric acid and isovaleric acid production. Grape seed procyanidins had no significant effects on TVFA, acetic acid, propionic acid, valeric acid contents, or the acetate/propionate ratio.
2. Grape seed procyanidins reduced populations of protozoa, methanogens, *Butyrivibrio fibrisolvens*, and *Fibrobacter succinogenes* in fermentation fluid, while showing no significant effects on *Ruminococcus flavefaciens* and *Ruminococcus albus*.
3. Based on comprehensive evaluation, the optimal supplementation level of grape seed procyanidins for *in vitro* rumen fermentation in dairy cows is 0.2 g/kg.

References

[1] PRIEUR C, RIGAUD J, CHEYNIER V, et al. Oligomeric and polymeric procyanidins from grape seeds[J]. Phytochemistry, 1994, 36(3): 781–784.

[2] HUANG Yining. Study on grape procyanidins and cloning of its ANR gene[D]. Master' s thesis. Fuzhou: Fujian Agriculture and Forestry University, 2008.

[3] WAN Benyi, LI Hong, DONG Haizhou. Research progress on extraction and application of grape seed procyanidins[J]. Grain and Oil, 2002(2): 43-45.

[4] VITSEVA O, VARGHESE S, CHAKRABATI S, et al. Grape seed and skin extracts alter platelet function and release of reactive oxygen species[J]. Journal of the American College of Cardiology, 2004, 43(Suppl.2): A518.

[5] YIN Jin, HU Yixiu, HU Yuming, et al. Effects of grape seed procyanidin extract on MDA, SOD and GSH-Px in mice[J]. China Tropical Medicine, 2007, 7(8): 1285-1286.

[6] BAYATLI F, AKKUS D, KILIC E, et al. The protective effects of grape seed extract on MDA, AOPP, apoptosis and eNOS expression in testicular torsion: an experimental study[J]. World Journal of Urology, 2013, 31(3): 615-622.

[7] CHARRADI K, EIKAHOUI S, KARKOUCH I, et al. Grape seed and skin extract alleviates high-fat diet-induced renal lipotoxicity and prevents copper depletion in rat[J]. Applied Physiology, Nutrition, and Metabolism, 2013, 38(3): 259-267.

[8] LIU Xiangju, GAO Haiqing, QIU Jie, et al. Effects of grape seed procyanidins on oxidative stress in rabbit atherosclerosis[J]. Journal of Shandong University: Medical Edition, 2010, 48(8): 25-27.

[9] WU Jianmin, CHENG Yaoxing, XU Jun, et al. Study on feeding dairy cows with grape seed residues[J]. Chinese Journal of Animal Science, 2007, 43(7): 62-63.

[10] DU Daoquan, YANG Wenhua. Study on application of grape seed meal in dairy cow diets[J]. Henan Journal of Animal Husbandry and Veterinary Medicine: Comprehensive Edition, 2009, 30(8): 28-29.

[11] GESSNER D K, WINKLER A, KOCH C, et al. Analysis of hepatic transcript profile and plasma lipid profile in early lactating dairy cows fed grape seed and grape marc meal extract[J]. BMC Genomics, 2017, 18(1): 253.

[12] SUN Zhanpeng, WANG Xi, LI Huiju, et al. Effects of different doses of grape pomace on weight gain in adult sheep[J]. China Herbivore Science, 2010, 30(1): 46-48.

[13] ZHAO Dong, ZHENG Chen, LI Fadi, et al. Effects of grape pomace tannins on nutrient digestion, metabolism and rumen fermentation in sheep[J]. Acta Prataculturae Sinica, 2014, 23(4): 285-292.

[14] LI Huiju, SUN Zhanpeng. Effects of dietary grape pomace on body weight of adult Small-tailed Han ewes[J]. Feed Industry, 2010, 31(3): 33-36.

[15] XIE Lingna, MAO Tingting, LIU Chang, et al. Effects of grape seed procyanidins on digestive enzyme activities, relative visceral weights and blood cell parameters in weaned piglets[J]. Journal of Beijing University of Agriculture, 2012, 27(4): 13-15.

[16] ZHAO Jiaqi, HAO Ruirong, GAO Junjie, et al. Effects of grape seed procyanidins on immunity and antioxidant function in weaned piglets[J]. Journal of Shanxi Agricultural University: Natural Science Edition, 2016, 36(10): 735-739.

[17] ZHAO Jiao, ZHOU Zhaohong, LIANG Xiaofang, et al. Effects of grape seed procyanidins and vitamin E on growth performance, serum redox status and liver oxidative damage in stressed piglets[J]. Scientia Agricultura Sinica, 2013, 46(19): 4157-4164.

[18] LIU Haiyan, YU Wei, SU Xiuxia, et al. Feeding trial of grape seed in growing-finishing pigs[J]. Heilongjiang Animal Science and Veterinary Medicine, 2008(11): 35-36.

[19] MENKE K H, STEINGASS H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid[J]. Animal Research Development, 1988, 28(1): 7-55.

[20] FENG Yanglian, LU Zhinian. Nutritional Requirements of Dairy Cows and Feed Composition[M]. 3rd ed. Beijing: China Agriculture Press, 2007.

[21] ZHOU Min, YE Zihong, JIANG Linshu. Factors affecting ruminal biohydrogenation of polyunsaturated fatty acids[J]. Chinese Agricultural Science Bulletin, 2010, 26(8): 38-44.

[22] PAN Long, NIU Junli, BU Dengpan, et al. Effects of saikosaponins on *in vitro* fermentation parameters and bacterial population changes[J]. Acta Pratacultural Sinica, 2015, 24(6): 85-91.

[23] YAN Shuhong, ZHAO Shiping, JIANG Qihui, et al. Effects of tea saponin on rumen fermentation and microflora in dairy cows[J]. Chinese Journal of Animal Nutrition, 2016, 28(8): 2485-2496.

[24] SHEN Ying, SONG Zhenghe, YANG Hongjian, et al. Development of an automatic recording system for feed *in vitro* fermentation gas production based on virtual instrument technology[J]. Transactions of the Chinese Society of Agricultural Engineering, 2006, 22(12): 159-163.

[25] BÜRGMANN H, PESARO M F, WIDMER F, et al. A strategy for optimizing quality and quantity extracted soil[J]. Journal of Microbiological Methods, 2001, 45(1): 7-20.

[26] LIU Wei, XIN Hangshu, LIU Caijuan, et al. Effects of hainanmycin on rumen fermentation pattern, methane production and microbial flora[J]. Acta Veterinaria et Zootechnica Sinica, 2012, 43(2): 242-249.

[27] COTTA M A, RUSSELL J B. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture[J]. Journal of Dairy Science, 1982, 65(2): 226-234.

[28] SRINIVAS B, GUPTA B N. Rumen fermentation, bacterial and total volatile fatty acid (TVFA) production rates in cattle fed on urea-molasses-mineral block licks supplement[J]. Animal Feed Science & Technology, 1997, 65(1-4): 275-286.

[29] MIREGULI·Yimamu, YU Xiong, WANG Gaiqin, et al. Effects of grape seed essential oil on *in vitro* rumen fermentation and methane production[J]. Animal Husbandry and Veterinary Medicine, 2012, 44(1): 4-7.

[30] LI Chong, WANG Hongbo, LI Fadi, et al. Effects of grape pomace on rumen microflora and fermentation function in lambs[J/OL]. Beijing: Sciencepaper Online (2017-04-14). http://www.paper.edu.cn/releasepaper/content/201704-2

[31] LI Chengyun, YUAN Yingliang, PIAO Guangyi. Effects of condensed tannins on rumen volatile fatty acids and microbial growth[J]. Feed Research, 2010(11): 5-7.

[32] LI Chengyun, YUAN Yingliang, LIU Caihong. *In vitro* study on effects of condensed tannins extracted from hazelnut leaves on protein digestion and rumen fermentation[J]. Heilongjiang Animal Science and Veterinary Medicine, 2010(17): 103-104.

[33] WANG Y, BARBIERI L R, BERG B P, et al. Effects of mixing sainfoin with alfalfa on ensiling, ruminal fermentation and total tract digestion of silage[J]. Animal Feed Science and Technology, 2007, 135(3-4): 296-314.

[34] PAN Faming, WANG Cailian, LIU Longsheng, et al. Application prospects of tannins in ruminant feed[J]. China Animal Husbandry and Veterinary Medicine, 2013, 40(5): 222-225.

[35] MENG Qingxiang, ZHANG Hongjun, RONG Yi, et al. Study on a new *in vitro* method for estimating rumen degradability of feed protein[J]. Journal of China Agricultural University, 1991, 4(4): 95-101.

[36] DI Lingfeng, CAO Xue, QIN Weize, et al. Study on application value of two tannin-containing forages for ruminants[J]. Feed Industry, 2017, 38(7): 43-50.

[37] LI Xiaopeng. Effects of condensed tannins on *in vitro* fermentation characteristics[D]. Master' s thesis. Lanzhou: Gansu Agricultural University, 2009.

[38] FENG Yanglian. Ruminant Nutrition[M]. Beijing: Science Press, 2004.

[39] LÜ Zhonglei. Effects of different molecular weight condensed tannins on rumen fermentation and microflora in Yanbian yellow cattle[D]. Master' s thesis. Yanji: Yanbian University, 2014.

[40] RUSSI J P, WALLACE R J, NEWBOLD C J. Influence of the pattern of peptide supply on microbial activity in the rumen simulating fermenter (RUSITEC)[J]. British Journal of Nutrition, 2002, 88(1): 73-80.

[41] CHEN Dandan, DIAO Qiyu, JIANG Chenggang, et al. Research progress on methane production mechanism and mitigation technologies in ruminants[J]. China Herbivore Science, 2012, 32(4): 66-69.

[42] WANG Huiling, WANG Xiaoping, GAZANG Sangzhi, et al. Effects of tannic acid on *in vitro* rumen fermentation and methane production in sheep[J]. China Herbivore Science, 2013, 33(6): 46-48.

[43] ANANTASOOK, WANG Cong, JIN Dapeng. Effects of dietary supplementation with rain tree pods containing tannins and saponins on rumen fermentation and methane production in dairy cows[J]. China Animal Husbandry and Veterinary Medicine, 2014, 41(2): 152-152.

[44] GARCIA J L, PATEL B K C, OLLIVIER B. Taxonomic, phylogenetic, and ecological diversity of methanogenic archaea[J]. Anaerobe, 2000, 6(4): 205-226.

[45] ZHAO Wei. Effects of Chinese herbal medicine feed additives on production performance and methane emissions in Northern Shaanxi white cashmere goats[D]. Master' s thesis. Yangling: Northwest A&F University, 2014.

[46] WOOD T M, WILSON C A, MCCRAE S I, et al. A highly active extracellular cellulase from anaerobic rumen fungus *Neocallimastix frontalis*[J]. FEMS Microbiology Letters, 1986, 34(1): 37-40.

[47] YUAN Yingliang. Effects of dietary condensed tannins on rumen fermentation and feed digestibility[D]. Master' s thesis. Yanji: Yanbian University, 2010.

[48] CHEN Xiaolian, LIU Jianxin, WANG Jiakun, et al. Research progress on rumen cellulolytic bacterial cellulosomes and their analogs[J]. Chinese Journal of Animal Science, 2009, 45(3): 50-53.

[49] LIU Zhanying. Isolation and identification of major fiber-degrading bacteria in sheep rumen and effects of different nitrogen sources on their fiber degradation ability[D]. PhD thesis. Hohhot: Inner Mongolia Agricultural University, 2008.

[50] WANG Chuan. Study on antioxidant activity of grape seed tannins[J]. Food Science and Technology, 2009, 34(2): 184-187.

[51] JIAO Wanhong, LI Li. Investigation on structure and function of major bacteria in bovine rumen[J]. China Animal Husbandry and Veterinary Medicine, 2016, 12(2): 84.

[52] LI Dabiao, ZHANG Meimei, YU Yongqiang, et al. Effects of tannins and polyethylene glycol on rumen fiber-degrading bacteria in sheep and goats[J]. Chinese Journal of Animal Nutrition, 2015, 27(2): 596-605.

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