Effects of Dietary Oat Hay Content on Rumen Fluid pH and Microbiota in Sheep: Postprint

Authors: Zhou Rui, Zhao Shengguo, Liu Lishan, Wu Jianping, Wang Chuan

Date: 2017-10-10T00:00:00+00:00

Abstract

To investigate the effects of dietary oat hay content on rumen fluid pH and microbiota in sheep. Nine German Merino × Mongolian crossbred rams with similar body condition and weight [(70.32$±2.14)*kg*], *fittedwithpermanentrumenfistulas*, *wereselectedandrandomly* 3*pergroup*)*usinga*3×$3 Latin square design. The three groups were fed three types of roughage: whole-plant corn silage, whole-plant corn silage + oat hay (1:1) (mixed group), and oat hay. The dietary concentrate-to-forage ratio was 34.50 65.50. Three feeding periods were conducted, each lasting 20 d (15 d preliminary period and 5 d sampling period). Rumen fluid was collected at 0 h (pre-feeding) and 1, 3, 5, and 7 h post-feeding to determine pH, and real-time quantitative PCR was used to determine the relative abundance of microorganisms. The results showed: 1) Rumen fluid pH in the whole-plant corn silage group was significantly lower than that in the oat hay group at 1 and 5 h ($P<0.05$), and extremely significantly lower than that in the mixed group at 3 h ($P<0.01$); 2) The relative abundance of fungi in rumen fluid in both the mixed group and oat hay group was extremely significantly higher than that in the whole-plant corn silage group at 0 h ($P<0.01$), and the oat hay group showed significantly higher fungal relative abundance than the whole-plant corn silage group at 5 h ($P<0.05$); 3) The relative abundance of protozoa in the mixed group was significantly lower than that in the whole-plant corn silage group at 1 and 5 h ($P<0.05$); 4) At 5 h post-feeding, the relative abundance of fiber-degrading bacteria was higher in both the mixed group and oat hay group, among which the relative abundance of Ruminococcus flavefaciens in the oat hay group was significantly higher than that in the whole-plant corn silage group ($P<0.05$), and the relative abundances of Ruminococcus albus and Fibrobacter succinogenes were extremely significantly higher than those in the whole-plant corn silage group ($P<0.01$). In conclusion, using whole-plant corn silage + oat hay (1:1) as roughage in diets with a concentrate-to-forage ratio of 34.50:65.50 is beneficial for maintaining rumen internal environment homeostasis and rumen microbial growth in sheep, with Ruminococcus albus

and Fibrobacter succinogenes being the dominant bacteria.

Full Text

Effects of Dried Oat Hay Content in Diet on Rumen Fluid pH and Microflora of Sheep

ZHOU Rui[1], **ZHAO Shengguo**[1], **LIU Lishan**[1], **WANG Chuan**[2], **WU Jianping**[1]*

(1. College of Animal Science & Technology, Gansu Agricultural University, Lanzhou 730070, China; 2. College of Veterinary Medicine, Gansu Agricultural University, Lanzhou 730070, China)

Abstract

To investigate the effects of dried oat hay content in diet on rumen fluid pH and microflora of sheep, nine German Merino × Mongolian crossbred rams with similar body condition and body weight [(70.32$±2.14)*kg*], *fittedwithpermanentrumenfistulas*, *wereusedinarepli*
Latin square design. The animals were randomly divided into three groups (n=3 per group) and fed three different roughage sources: whole corn silage, whole corn silage + dried oat hay (1:1 ratio; mixed group), and dried oat hay alone. The dietary concentrate-to-roughage ratio was 34.50:65.50. The experiment consisted of three periods, each lasting 20 days (15 days adaptation and 5 days sampling). Rumen fluid was collected before feeding (0 h) and at 1, 3, 5, and 7 h post-feeding to measure pH, and the relative abundance of microorganisms was determined using real-time quantitative PCR. The results showed: 1) Rumen pH in the whole corn silage group was significantly lower than that in the dried oat hay group at 1 and 5 h ($P<0.05$), and extremely significantly lower than that in the mixed group at 3 h ($P<0.01$). 2) The relative abundance of fungi in rumen fluid of the mixed and dried oat hay groups was extremely significantly higher than that of the whole corn silage group at 0 h ($P<0.01$), and the dried oat hay group remained significantly higher at 5 h ($P<0.05$). 3) The relative abundance of protozoa in the mixed group was significantly lower than that in the whole corn silage group at 1 and 5 h ($P<0.05$). 4) At 5 h post-feeding, the relative abundance of cellulolytic bacteria was higher in both the mixed and dried oat hay groups, with the dried oat hay group showing significantly higher *Ruminococcus flavefaciens* ($P<0.05$) and extremely significantly higher *Ruminococcus albus* and *Fibrobacter succinogenes* compared to the whole corn silage group ($P<0.01$). In conclusion, using whole corn silage + dried oat hay (1:1) as roughage in a diet with a concentrate-to-roughage ratio of 34.50:65.50 is beneficial for maintaining rumen homeostasis and microbial growth, with *Ruminococcus albus* and *Fibrobacter succinogenes* being the dominant bacterial species.

Keywords: sheep; roughage; rumen; microflora; RT-qPCR

Introduction

Ruminants can digest and utilize roughage due to the large population of microorganisms inhabiting their rumen, including bacteria, protozoa, archaea, and fungi. These microbes convert plant fiber into digestible compounds that can be further absorbed and utilized by the host. Through long-term selection and evolution, a mutually restrictive and interdependent relationship has formed between microorganisms and the host, which plays a crucial role in maintaining ruminant health and improving production performance. Rumen bacteria are particularly important, being the most diverse (over 50 genera) and abundant (10^{10}-10^{11} cells/mL) microorganisms in the rumen. Currently, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* are the most studied and recognized as the three dominant fiber-degrading bacteria in rumen fiber degradation. Most rumen anaerobic fungi can decompose cellulose and possess highly active cellulase, xylanase, pectinase, and esterase for degrading plant cell walls. Protozoa account for approximately half of the total microbial biomass in rumen contents, and some ciliate protozoa can increase methane production while digesting fiber. Numerous studies have shown that rumen microbial composition and abundance are influenced by many factors, including individual animal differences, concentrate-to-roughage ratio, and dietary composition, with dietary factors being the most critical. In China, corn silage and oat hay are widely used as the main sources of dietary fiber for ruminants, but few studies have reported on their digestion mechanisms in the rumen and effects on microflora. This study investigated the effects of feeding different oat hay contents on the relative abundance of rumen fungi, protozoa, and three fiber-degrading bacterial species using real-time quantitative PCR, providing a theoretical basis for further research on dietary structure effects on rumen microflora and improving roughage utilization in sheep.

1.1 Experimental Design and Animal Management

Nine German Merino × Mongolian crossbred rams with similar body condition and body weight [(70.32$\pm$2.14)kg], $fitted with permanent rumen fistulas, were selected for this study. Roughage$
Latin square design was employed, with the nine sheep randomly divided into three groups (n=3 per group) receiving three different roughage treatments: whole corn silage, whole corn silage + dried oat hay (1:1 ratio; mixed group), and dried oat hay alone. The dietary concentrate-to-roughage ratio was 34.50:65.50. The composition and nutrient levels of experimental diets are shown in Table 1 . The experiment consisted of three periods, each lasting 20 days (15 days pre-test and 5 days sampling). Sheep were housed individually and fed equal amounts twice daily at 08:00 and 18:00, with free access to water.

1.2 Sample Collection

On the final day of each experimental period, rumen fluid was collected before feeding (0 h) and at 1, 3, 5, and 7 h post-feeding. Rumen pH was immediately measured using an HI98103 pen-type pH meter (Beijing Taiyasaifu Co., Ltd.).

The fluid was then filtered through four layers of sterile gauze, and the filtrate was placed in autoclaved sampling tubes and stored at -70 °C.

1.3 Primer Design and Synthesis

Specific primers were designed using Primer Premier 3.0 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) based on 16S rRNA sequences of *Ruminococcus albus* and *Fibrobacter succinogenes*, and 18S rRNA sequences of fungi and protozoa from GenBank. The primer sequences are listed in Table 2 . All primers were synthesized by Shanghai Bioengineering Co., Ltd.

1.4 Total DNA Extraction and Quantitative Analysis

Total rumen microbial DNA was extracted using the Tiangen Biochemical Technology (Beijing) Co., Ltd. fecal DNA extraction kit (DP328). Rumen microbial quantification was performed using the real-time quantitative PCR comparative threshold method, and the relative abundance of target bacteria was calculated using the $2^{(-\Delta\Delta Ct)}$ method. The PCR reaction system and parameters were performed according to the Roche kit (Cat. No. 06402712001) instructions. The reaction system (20 L) contained: Master Mix 10 L, template 2 L (20 ng), forward and reverse primers 0.6 L each, and ddH_2O 6.8 L. Reaction parameters were: pre-denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, and extension at 72 °C for 15 s. Fluorescence signals were collected during the extension phase.

1.5 Data Processing

Experimental data were compiled using Excel 2010 and analyzed using one-way ANOVA in SPSS 19.0 software for statistical analysis and significance testing. LSD method was used for multiple comparisons. Results are expressed as mean ± standard error, with $P<0.05$ considered significant and $P<0.01$ considered extremely significant.

Results

2.1 Effects of Dietary Oat Hay Content on Rumen Fluid pH in Sheep

As shown in Table 3 , rumen fluid pH in all three groups exhibited a trend of decreasing then increasing over time, with the highest values observed before morning feeding (0 h). After feeding, pH began to decline, reaching its lowest point at 3 h before gradually rising again. Before feeding (0 h), the whole corn silage group had the highest rumen pH, which was significantly higher than that of the dried oat hay group ($P<0.05$). At 1 and 5 h post-feeding, the whole corn silage group was significantly lower than the dried oat hay group ($P<0.05$), and at 3 h was extremely significantly lower than the mixed group ($P<0.01$).

2.2 Effects of Dietary Oat Hay Content on Relative Abundance of Fungi, Protozoa, and Fiber-Degrading Bacteria in Sheep Rumen Fluid

Using the whole corn silage group as the control, the effects of oat hay content on relative fungal abundance showed different temporal patterns between the mixed and dried oat hay groups. Both groups exhibited highest fungal abundance at 0 h, which decreased to the lowest level at 3 h post-feeding. At 0 h, fungal abundance in the mixed and dried oat hay groups was extremely significantly higher than in the whole corn silage group ($P<0.01$). At 1 h post-feeding, fungal abundance in these two groups decreased, with the mixed group being significantly lower than the whole corn silage group ($P<0.05$). At 3 h post-feeding, the mixed and dried oat hay groups were extremely significantly ($P<0.01$) and significantly ($P<0.05$) lower than the whole corn silage group, respectively. At 5 h post-feeding, the dried oat hay group showed significantly higher fungal abundance than the whole corn silage group ($P<0.05$). At 7 h post-feeding, no significant differences were observed among the three groups (Table 4).

Using the whole corn silage group as control, the relative abundance of protozoa showed different temporal trends, but both the mixed and dried oat hay groups reached peak abundance at 3 h post-feeding. The mixed group was significantly lower than the whole corn silage group at 1 and 5 h ($P<0.05$). Except for equal abundance at 0 h and higher abundance at 3 h compared to the whole corn silage group, the dried oat hay group showed lower protozoal abundance at all other time points, reaching its lowest value at 7 h (Table 5).

Using the whole corn silage group as control, the relative abundance of *Ruminococcus flavefaciens* showed a trend of decreasing, then increasing, then decreasing again over time. The dried oat hay group was extremely significantly lower than the whole corn silage group at 1 h ($P<0.01$), and significantly or extremely significantly lower than the other two groups at 3 and 7 h ($P<0.01$). At 5 h post-feeding, both the mixed and dried oat hay groups showed higher *R. flavefaciens* abundance than the whole corn silage group, with the dried oat hay group being significantly higher than the other two groups ($P<0.05$) (Table 6).

Using the whole corn silage group as control, the relative abundance of *Ruminococcus albus* in the mixed and dried oat hay groups was higher than that in the whole corn silage group throughout the 0–7 h period, showing a trend of decreasing then increasing then decreasing again. Except at 1 h post-feeding when the mixed and dried oat hay groups were significantly higher than the whole corn silage group ($P<0.05$), all other time points showed extremely significant differences ($P<0.01$). Furthermore, at 5 and 7 h post-feeding, the dried oat hay group was significantly ($P<0.05$) and extremely significantly ($P<0.01$) higher than the mixed group, respectively (Table 7).

Using the whole corn silage group as control, the relative abundance of *Fibrobacter succinogenes* showed inconsistent temporal patterns. The mixed group was extremely significantly higher than both the whole corn silage and dried oat hay groups at 1 h ($P<0.01$). The whole corn silage group was extremely significantly lower than both the mixed and dried oat hay groups at 5 h ($P<0.01$). The mixed group was significantly higher than the dried oat hay group at 5 h ($P<0.05$), while the dried oat hay group was significantly higher than the mixed group at 7 h ($P<0.05$) (Table 8).

Discussion

3.1 Effects of Dietary Oat Hay Content on Rumen Fluid pH

Rumen fluid pH is a comprehensive indicator reflecting rumen fermentation level and internal environment in ruminants, and can be used to evaluate rumen fermentation. Rumen pH is typically maintained within the range of 6.0–7.0. In this study, rumen pH ranged from 5.8 to 6.7, and all three groups showed a decreasing then increasing trend over time, with highest pH at 0 h and lowest pH at 3 h post-feeding. These results are consistent with other studies. Wang et al. reported that rumen pH decreased after feeding different roughages to beef cattle, reaching its lowest point at 2–4 h and returning to pre-feeding levels at 8–10 h, though no significant differences were observed among groups. Guo et al. indicated that optimal pH ranges differ for various rumen fermentation processes: 6.0–6.8 for cellulose digestion, 5.8–7.4 for protein synthesis, 4.2–6.6 for volatile fatty acid (VFA) production, and 6.2 for ammonia production. Rumen pH results from the comprehensive effects of VFA interaction with salivary buffers in digesta, VFA absorption by rumen epithelium, and digesta outflow. In this study, the whole corn silage group showed rumen pH slightly below 6.0 at 1–3 h, likely due to large amounts of VFA produced from microbial digestion of dietary carbohydrates. As feed was digested and salivary buffers increased, rumen pH gradually recovered. No significant differences in rumen pH among the three groups suggest that concentrate levels of 30%–50% have minimal impact on rumen pH. However, the mixed and dried oat hay groups showed higher rumen pH than the whole corn silage group, possibly because oat hay has higher structural carbohydrate content and lower palatability than whole corn silage, resulting in slower intake rate, lower degradation rate, slower outflow, and longer retention time in the rumen.

3.2 Effects of Dietary Oat Hay Content on Rumen Fungi, Protozoa, and Fiber-Degrading Bacteria

In this study, the relative abundance of fungi in the mixed and dried oat hay groups was extremely significantly higher than in the whole corn silage group at 0 h, then decreased sharply from 0 to 3 h as the abundance of the three bacterial species increased. This may occur because fungi preferentially utilize lignin rather than cellulose-like substances in feed. Additionally, due to their low proportion in total rumen microorganisms, their overall contribution to fiber degradation is smaller than that of rumen bacteria. Early studies demonstrated that *R. albus* and *R. flavefaciens* can inhibit the ability of anaerobic fungi to degrade corn stalks and cellulose. In this study, the dried oat hay group showed relatively high abundance of both fungi and the three fiber-degrading bacterial species at 5 h post-feeding, but bacterial abundance was higher than fungal abundance, indicating antagonistic interactions between rumen fungi and bacteria. Atasoglu et al. reported that dietary type significantly affects rumen fungal populations, with animals fed high-cellulose diets having greater fungal numbers than those fed low-cellulose diets, consistent with our findings of higher fungal abundance at 0 h in the mixed and dried oat hay groups compared to the whole corn silage group.

Jounay et al. reported that the primary fermentation substrates for protozoa are starch and soluble sugars. In this study, except for higher abundance at 3 h, the relative abundance of protozoa in the mixed and dried oat hay groups was similar to or lower than that in the whole corn silage group at all time points, and lower than the abundance of the three fiber-degrading bacteria and fungi at the same levels. The NRC (2001) reported that the non-structural carbohydrate (NSC) content of oat hay is 13.6% (DM basis), lower than that of whole corn silage (34.7%). This suggests that fungi and fiber-degrading bacteria play major roles when sheep are fed oat hay.

Fibrobacter succinogenes, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are the most important fiber-degrading bacteria in the rumen, with stronger cellulose digestion capacity than other fiber-degrading bacteria, accounting for 33.0%, 2.6%, and 46.0% of total fiber-degrading bacteria in cattle rumen, respectively. In this study, the mixed and dried oat hay groups showed significantly higher relative abundance of *F. succinogenes* and *R. albus* than the whole corn silage group, indicating that *R. albus* and *F. succinogenes* are the dominant bacterial species when sheep are fed oat hay. This may be because *R. albus* has greater affinity and fiber attachment capacity than *R. flavefaciens*, and they compete for the same attachment sites and barriers during adsorption, allowing *R. albus* to occupy sites that would otherwise be used by *R. flavefaciens*. In contrast, *R. flavefaciens* and *F. succinogenes* have different attachment sites and exhibit little competitive relationship. However, when *R. flavefaciens* and *F. succinogenes* are fully colonized, *R. albus* attachment can increase the detachment of *R. flavefaciens*.

Conclusions

1. Using whole corn silage + dried oat hay (1:1) as roughage in a diet with a concentrate-to-roughage ratio of 34.50:65.50 can maintain rumen fluid pH within the normal range, which is beneficial for maintaining rumen homeostasis and microbial growth.
2. Using whole corn silage + dried oat hay (1:1) as roughage in a diet with a concentrate-to-roughage ratio of 34.50:65.50 reduces protozoal numbers while increasing the abundance of the three major fiber-degrading bacteria, with *Ruminococcus albus* and *Fibrobacter succinogenes* being the dominant bacterial species.

References

[1] HESPELL R B, AKIN D E, DEHORITY B A. Bacteria, fungi and protozoa of the rumen[M]//MACKIE R I, WHITE B A, ISAACSON R E. Gastrointestinal microbiology. New York: Chapman and Hall Press, 1997: 59-186.

[2] MACKIE R I. Mutualistic fermentative digestion in the gastrointestinal tract: diversity and evolution[J]. Integrative and Comparative Biology, 2002, 42(2): 319-326.

[3] LEY R E, HAMADY M, LOZUPONE C, et al. Evolution of mammals and their gut microbes[J]. Science, 2008, 320(5883): 1647-1651.

[4] ZILBER-ROSENBERG I, ROSENBERG E. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution[J]. FEMS Microbiology Reviews, 2008, 32(5): 723-735.

[5] RAWLS J F, SAMUEL B S, GORDON J I. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota[J]. Proceedings of the National Academy of Sciences of the United States of America, 2004, 101(13): 4596-4601.

[6] QI Ru, LIN Yingting, CHENG Ming, et al. Research progress on rumen microflora and their interrelationships[J]. Feed Review, 2011(8): 9-13.

[7] XUE Feng, WANG Hongrong, LIU Dacheng, et al. Research progress on rumen microflora[J]. Animal Husbandry and Feed Science, 2007, 28(2): 31-33.

[8] FORSBERG C W, CHENG K J, WHITE B A. Polysaccharide degradation in the rumen and large intestine[M]//MACKIE R I, WHITE B A, ISAACSON R E. Gastrointestinal microbiology. New York: Chapman and Hall Press, 1997: 319-379.

[9] ORPIN C G, JOBLIN K N. The rumen anaerobic fungi[M]//HOBSON P N, STEWART C S. The Rumen microbial ecosystem. London: Blackie Academic & Professional, 1997: 140-196.

[10] JOUNAY P, USHIDA K. The protozoa digestion-review[J]. Asian-Australasian Journal of Animal Science, 1999, 12(1): 113-128.

[11] FU Qi, HOU Xianzhi, GAO Aiwu. Research progress on rumen microflora and their interrelationships[J]. China Dairy Cattle, 2009(5): 18-21.

[12] YANG S L, MA S C, CHEN J, et al. Bacterial diversity in the rumen of Gayals (Bos frontalis), Swamp buffaloes (Bubalus bubalis) and Holstein cow as revealed by cloned 16S rRNA gene sequences[J]. Molecular Biology Reports, 2010, 37(4): 2063-2073.

[13] SADET-BOURGETEAU S, MARTIN C, MORGAVI D P. Bacterial diversity dynamics in rumen epithelium of wethers fed forage and mixed concentrate forage diets[J]. Veterinary Microbiology, 2010, 146(1/2): 98-104.

[14] PITTA D W, PINCHAK W E, DOWD S E, et al. Rumen bacterial diversity dynamics associated with changing from Bermudagrass hay to grazed winter wheat diets[J]. Microbial Ecology, 2010, 59(3): 511-522.

[15] BELANCHE A, DOREAU M, EDWARDS J E, et al. Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation[J]. The Journal of Nutrition, 2012, 142(9): 1684-1692.

[16] XU J, HOU Y J, YANG H B, et al. Effects of forage sources on rumen fermentation characteristics, performance, and microbial protein synthesis in midlactation cows[J]. Asian-Australasian Journal of Animal Sciences, 2014, 27(5): 667-673.

[17] MUYZER G, DE WAAL E C, UITTERLINDEN A G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA[J]. Applied and Environmental Microbiology, 1993, 59(3): 695-700.

[18] WANG Hairong. Effects of different dietary concentrate-to-roughage ratios and nitrogen sources on rumen fiber-degrading bacterial populations and fiber degradation in sheep[D]. PhD Thesis. Hohhot: Inner Mongolia Agricultural University, 2006.

[19] KUMAR S, DAGAR S S, SIROHI S K, et al. Microbial profiles, in vitro gas production and dry matter digestibility based on various ratios of roughage to concentrate[J]. Annals of Microbiology, 2013, 63(2): 541-545.

[20] HAO Zhengli, LIU Shimin, MENG Xianzheng. Ruminant nutrition[M]. Lanzhou: Gansu Nationalities Publishing House, 1999.

[21] WANG Hongliang, SUN Xiaoyu, ZHAO Fuzhong. Study on effects of common roughages on rumen environment of beef cattle in Heilongjiang Province[J]. China Cattle Science, 2013, 39(2): 6-10.

[22] GUO Yongqing, ZHANG Yingjie. Rumen environment optimization technology and its application[C]//Proceedings of the 4th China Cattle Industry Development Conference. Nanyang: China Animal Agriculture Association, 2009: 274-277.

[23] FENG Yanglian. Ruminant nutrition[M]. Beijing: Science Press, 2004.

[24] ZHANG Ying, ZHOU Jianwei, LIU Hao, et al. Response of rumen fermentation parameters to oat hay restriction in Tibetan sheep and estimation of nitrogen maintenance requirement[J]. Chinese Journal of Animal Nutrition, 2014, 26(2): 371-379.

[25] MICHEL Wattiaux. Nutrition and feeding[M]. Translated by SHI Yan, SHI Fushun. Beijing: China Agricultural University Press, 2004.

[26] BARGO F, MULLER L D, DELAHOY J E, et al. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture

allowances[J]. Journal of Dairy Science, 2002, 85(7): 1777-1792.

[27] TUYEN V D, CONE J W, BAARS J J P, et al. Fungal strain and incubation period affect chemical composition and nutrient availability of wheat straw rumen fermentation[J]. Bioresource Technology, 2012, 111: 336-342.

[28] AKIN D E. Histological and physical factors affecting digestibility of forages[J]. Agronomy Journal, 1989, 81(1): 17-25.

[29] ROGER V, GRENET E, JAMOT J, et al. Degradation of maize stem by two rumen fungal species, *Piromyces communis* and *Caecomyces communis*, in pure cultures or in association with cellulolytic bacteria[J]. Reproduction Nutrition Development, 1992, 32(4): 321-329.

[30] BERNALIER A, FONTY G, BONNEMOY F, et al. Degradation and fermentation of cellulose by the rumen anaerobic fungi in axenic cultures or in association with cellulolytic bacteria[J]. Current Microbiology, 1992, 25(3): 143-148.

[31] DEHORITY B A, TIRABASSO P A. Antibiosis between ruminal bacteria and ruminal fungi[J]. Applied and Environmental Microbiology, 2000, 66(7): 2921-2927.

[32] ATASOGLU C, WALLACE R J. De novo synthesis of amino acids by the ruminal anaerobic fungi, *Piromyces communis* and *Neocallimastix frontalis*[J]. FEMS Microbiology Letters, 2002, 212(2): 243-247.

[33] KOIKE S, KOBAYASHI Y. Fibrolytic rumen bacteria: their ecology functions[J]. Asian-Australasian Journal of Animal Sciences, 2009, 22(1): 131-138.

[34] MOSONI P, FONTY G, GOUET P. Competition between ruminal cellulolytic bacteria for adhesion to cellulose[J]. Current Microbiology, 1997, 35(1): 44-47.

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

Source: ChinaXiv —Machine translation. Verify with original.