ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201812.00457

Effects of Niacin, Fructooligosaccharides and Rare Earth Citrate Combination on In Vitro Rumen Fermentation Characteristics and Microbial Community Structure in Beef Cattle: Postprint

Authors: Liang Huan, Zhao Xianghui, Xu Lanjiao, Wen Luhua, Chen Zuodong, Zhao Erlong, Qu Mingren

Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate the effects of combinations of niacin, fructooligosaccharides, and rare earth citrate on in vitro rumen fermentation characteristics and microbial community structure in beef cattle. Three healthy Jinjiang yellow cattle bulls fitted with permanent rumen fistulas [body weight (375±28) kg] were selected as rumen fluid donors. A 3-factor 3-level orthogonal experimental design was adopted to examine the effects of different levels of fructooligosaccharides (0.8%, 1.0%, 1.2%), niacin (400, 800, 1,200 mg/kg), and rare earth citrate (0.6%, 0.8%, 1.0%) on in vitro rumen fermentation characteristics in beef cattle using in vitro rumen fermentation culture technique, and high-throughput sequencing technology was employed to analyze the rumen microbial community structure. The in vitro incubation time was 48 h. The results showed: 1) Group G exhibited the highest pH in the in vitro culture fluid at 6.77, which was significantly higher than groups A, B, C, D, and E (P 0.05); Group G displayed the lowest ammonia nitrogen (NH3-N) content in the in vitro culture fluid at 9.77 mg/dL, which was significantly lower than all other groups (P 0.05); Different regulator combinations had no significant effect on microbial protein (MCP) content in the in vitro culture fluid (P>0.05). 2) Group I showed the highest propionate content in the in vitro culture fluid at 28.53%, which was significantly higher than groups A, B, D, and E (P 0.05), and the lowest acetate/propionate ratio, which was significantly lower than groups A, B, and D (P 0.05); No significant differences were observed among groups in total volatile fatty acids (TVFA) concentration, acetate, or butyrate contents in the in vitro culture fluid (P>0.05). 3) The dominant microbial communities in the in vitro culture fluid across different regulator combinations were Bacteroidetes and Firmicutes. At the phylum level, there were 4 significantly different micro-

bial taxa between groups A and I (P 0.05); at the genus level, there were 15 significantly different microbial taxa between groups A and I (P 0.05). Comprehensive analysis indicated that the optimal rumen regulator combinations under a corn-soybean meal-straw diet for beef cattle were group I (1.2% fructooligosaccharides + 1,200 mg/kg niacin + 0.8% rare earth citrate) and group G (1.2% fructooligosaccharides + 400 mg/kg niacin + 1.0% rare earth citrate).

Full Text

Effects of Nicotinic Acid, Fructooligosaccharides and Rare Earth Citrate Combinations on in Vitro Rumen Fermentation Characteristics and Bacterial Community of Beef Cattle

LIANG Huan, ZHAO Xianghui, XU Lanjiao, WEN Luhua, CHEN Zuodong, ZHAO Erlong, QU Mingren*

(Jiangxi Province Key Laboratory of Animal Nutrition/Engineering Research Center of Feed Development, Jiangxi Agricultural University, Nanchang 330045, China)

Abstract

This study investigated the effects of nicotinic acid (NA), fructooligosaccharides (FOS) and rare earth citrate (REC) combinations on in vitro rumen fermentation characteristics and bacterial community structure in beef cattle. Three healthy Jinjiang cattle bulls [body weight (375±28) kg] fitted with permanent rumen cannulas served as rumen fluid donors. A 3×3 factorial orthogonal experimental design was employed to examine nine combinations of FOS (0.8%, 1.0%, 1.2%), NA (400, 800, 1,200 mg/kg) and REC (0.6%, 0.8%, 1.0%) on rumen fermentation parameters using in vitro culture techniques, with bacterial community structure analyzed via high-throughput sequencing. The in vitro incubation period was 48 hours. The results showed: (1) Group G exhibited the highest pH (6.77), significantly higher than groups A, B, C, D and E (P 0.05), and the lowest NH -N content (9.77 mg/dL), significantly lower than all other groups (P 0.05). No significant differences were observed in microbial crude protein (MCP) content among groups (P>0.05). (2) Group I showed the highest propionate content (28.53%), significantly higher than groups A, B, D and E (P 0.05), and the lowest acetate/propionate ratio, significantly lower than groups A, B and D (P 0.05). No significant differences were detected in total volatile fatty acid (TVFA) concentration or acetate and butyrate contents among groups (P>0.05). (3) Bacteroidetes and Firmicutes dominated the bacterial communities across all treatment groups. At the phylum level, four bacterial taxa differed significantly between groups A and I (P 0.05), while at the genus level, fifteen taxa showed significant differences (P 0.05). Comprehensive analysis indicated that optimal rumen modulator combinations for beef cattle fed

corn-soybean meal-straw diets were group I (1.2% FOS + 1,200 mg/kg NA + 0.8% REC) and group G (1.2% FOS + 400 mg/kg NA + 1.0% REC).

Keywords: beef cattle; fructooligosaccharide; nicotinic acid; rare earth citrate; rumen; in vitro fermentation; bacterial community

Introduction

The rumen represents one of the most distinctive features differentiating ruminants from monogastric animals, with rumen fermentation function critically influencing ruminant health and production. Consequently, regulating rumen fermentation characteristics to enhance rumen function has remained a central focus in ruminant nutrition research.

Fructooligosaccharides (FOS), also known as oligofructose or fructans, constitute a group of low-molecular-weight carbohydrates formed by linking several D-fructose units to a sucrose molecule via -1,2-glycosidic bonds, and have demonstrated capacity to modulate rumen microbial populations [1]. Previous studies by Qu et al. [2] and Ling et al. [3] found that infusing 2.00% FOS significantly decreased rumen fluid pH and NH -N content while increasing volatile fatty acid (VFA) and microbial crude protein (MCP) concentrations in sheep, with an effective dietary inclusion level of 0.8%.

Nicotinic acid (NA), also known as vitamin PP or niacin, serves as a precursor for coenzyme I (NAD) synthesis [4]. Since NAD participates in numerous biochemical reactions involving biological electron transfer, alterations in intracellular NAD levels profoundly affect the entire metabolic network within cells, thereby influencing rumen microbial metabolism [5]. During carbohydrate metabolism, NAD regulates lactic acid metabolism in the rumen through participation in the reaction "pyruvate + NADH + lactate dehydrogenase → lactate + NAD", consequently promoting microbial glycolysis [6]. Zhang [7] reported that dietary NA supplementation enhanced rumen microbial proliferation, prevented drastic pH fluctuations, stabilized the rumen environment, and averted acidosis, with 800 mg/kg showing optimal effects.

Rare earth citrate (REC) represents a novel and promising feed additive, wherein rare earth elements exhibit strong physiological activity as metabolic activators that can modify nutrient digestion and absorption in animals and microorganisms, enhance protein and nucleic acid synthesis, and consequently promote growth. The effective dietary inclusion level for REC is 0.6% [8].

While these three additives individually demonstrate rumen-modulating effects through promoting rumen microbial metabolism and proliferation, current literature exclusively examines single-agent applications. No studies have investigated potential synergistic effects among these three modulators, whether combinations might enhance regulatory efficacy, or what constitutes optimal combinations. Therefore, this experiment aimed to evaluate the effects of NA, FOS

and REC combinations on in vitro rumen fermentation characteristics and bacterial community structure in beef cattle, providing scientific basis and technical support for developing effective composite rumen modulators.

1.1 Experimental Materials

Fructooligosaccharides were purchased from Hebei Weierkang Pharmaceutical Co., Ltd. with purity >90%. Nicotinic acid was obtained from Zhengzhou Jiahe Biological Products Co., Ltd. with active ingredient content 95%. Rare earth citrate was sourced from Jiangxi Kuanglu Technology Co., Ltd. as a white or light yellow free-flowing powder containing 99% REC, with rare earth elements (primarily lanthanides) 36%, and heavy metal limits of Cd 0.0001%, Pb 0.002%, As 0.0005%.

1.2 Experimental Animals, Diets and Management

Three healthy Jinjiang cattle bulls weighing (375±28) kg and fitted with permanent rumen cannulas served as rumen fluid donors. All animals received oral ivermectin for deworming and were housed individually. The basal diet consisted of a corn-soybean meal-rice straw formulation prepared according to *Feeding Standard of Beef Cattle* (NY/T 815-2004) with a concentrate-to-forage ratio of 20:80. Diet composition and nutrient levels are presented in Table 1 . Animals were fed equal amounts twice daily at 08:00 and 18:00 with free access to water.

1.3 In Vitro Fermentation Method

Buffer solution was prepared according to Menke et al. [10], comprising trace element solution, carbonate buffer, phosphate buffer, sodium sulfide (Na S) reducing solution, and resazurin indicator. The mixed buffer was continuously flushed with CO for over 30 minutes until pH reached 6.8, then maintained in a 39°C water bath. Rumen fluid was collected before morning feeding from four locations (upper, lower, anterior, posterior) within the rumen using long-arm gloves, mixed thoroughly, and immediately transferred into pre-warmed (39°C) sterilized beakers under CO atmosphere. Beakers were placed in thermos flasks containing 39°C distilled water, sealed immediately, and transported rapidly to the laboratory. The fluid was filtered through four layers of cheesecloth, flushed with CO for 5 minutes, then quickly dispensed into pre-warmed culture bottles containing substrate (500 mg basal diet + corresponding modulator combination) and 40 mL buffer solution, with 20 mL rumen fluid added to each bottle. Bottles were connected to syringes and incubated with shaking for 48 hours. The in vitro batch culture system was designed following methods described by Lu [11] and Cheng [12], consisting primarily of a temperature-controlled shaking water bath with adjustable temperature and oscillation speed. Culture

vessels were 150 mL yogurt bottles sealed with rubber stoppers and connected to syringes via rubber tubing with needles.

1.4 Experimental Design

A 3×3 orthogonal experimental design was employed with nine modulator combinations, each replicated in three culture bottles. Additive levels in the basal diet are shown in Table 2 .

1.5 Sample Pretreatment

After 48-hour incubation, culture fluid pH was measured immediately. Residues were transferred quantitatively into pre-weighed nylon bags (200 mesh). Filtrate samples were collected; portions were frozen at -20°C for rumen fermentation parameter analysis (NH -N, VFA, MCP), while other portions were stored at -80°C for high-throughput sequencing.

1.6 Analytical Methods

Dietary crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca) and phosphorus (P) were determined according to *Feed Analysis and Feed Quality Detection Technology* [13]. Culture fluid pH was measured using a PHS-25 laboratory pH meter (Shanghai Jinmai Instrument Co., Ltd.). NH -N content was determined by the phenol-hypochlorite colorimetric method [14]. VFA content was analyzed using a Waters-Baseline520 HPLC system at 214 nm with a C18 column maintained at 30°C, mobile phase 0.02 mol/L KH PO /H PO (pH 2.37), flow rate 1 mL/min, and injection volume 10 L [15]. MCP content was measured spectrophotometrically [16].

1.7 High-Throughput Sequencing and Bioinformatics Analysis

Based on preliminary results, groups A and I (showing maximal differences) were selected for high-throughput sequencing analysis. Five mL of in vitro rumen culture fluid from each sample was sent to Shanghai Xiangyin Biotechnology Co. for DNA extraction. The bacterial 16S rDNA V4-V5 region (515F-907R) was sequenced using the Illumina MiSeq PE250 platform. Raw data underwent quality control by discarding low-quality sequences (tail base quality <20, post-QC read length <50 bp). High-quality sequences were clustered using Usearch 7.1 software with 97% similarity threshold for operational taxonomic unit (OTU) delineation. OTUs were aligned against the RDP database for taxonomic classification via RDP Classifier. Richness indices (Chao, Ace) and diversity indices (Shannon, Simpson) were calculated using Mothur 1.30.1 software. Metastats analysis was employed for comparative analysis of differential bacterial communities between groups.

1.8 Statistical Analysis

Data were initially processed using Excel 2003, then subjected to one-way ANOVA using SPSS 17.0 software. When significant differences were detected, Duncan’ s multiple range test was applied. Results are expressed as means ± standard error, with P 0.05 considered statistically significant.

2.1 Analysis of pH, NH -N and MCP in Culture Fluid

As shown in Table 3 , different modulator combinations significantly affected culture fluid pH and NH -N content (P 0.05). Group G exhibited the highest pH (6.77), significantly higher than groups A, B, C, D and E (P 0.05), while group A showed the lowest pH (6.66), significantly lower than groups E, F, G, H and I (P 0.05). Group C had the highest NH -N content (12.04 mg/dL), significantly higher than groups G and H (P 0.05), whereas group G had the lowest NH -N content (9.77 mg/dL), significantly lower than all other groups (P 0.05). No significant differences in MCP content were observed among groups (P>0.05), though group I showed the highest value (37.55%) and groups B and C the lowest (34.97% each).

2.2 Analysis of VFA Content in Culture Fluid

Table 4 shows that no significant differences existed among groups in TVFA concentration or acetate and butyrate contents (P>0.05), with group A showing highest TVFA and acetate levels and group C highest butyrate content. However, modulator combinations significantly affected propionate content and acetate/propionate ratio (P 0.05). Group I demonstrated the highest propionate content (28.53%), significantly exceeding groups A, B, D and E (P 0.05), and the lowest acetate/propionate ratio, significantly lower than groups A, B and D (P 0.05).

2.3 OTU Clustering Analysis

Groups A and I (six samples: A1, A2, A3, I1, I2, I3) were selected for high-throughput sequencing, yielding 426,895 total sequences (average 71,149 per sample). At 97% similarity, 12,531 OTUs were obtained (average 2,089 per sample), with OTU counts of 2,012, 1,522 and 2,050 for A1, A2, A3, and 2,482, 1,662 and 2,803 for I1, I2, I3, respectively. As shown in Figure 1 [Figure 1: see original paper], samples from the same group clustered together. Principal component analysis (PCA) revealed PC1 and PC2 contributions of 77% and 16%, respectively. Phylum-level abundance heatmaps indicated that dominant bacterial communities originated from Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia, Planctomycetes and Spirochaetes.

2.4 Bacterial Community -Diversity Analysis

-Diversity reflects species diversity within individual samples, where Chao and Ace indices indicate community richness, while Shannon and Simpson indices reflect community diversity. Table 5 shows that compared with group A, group I exhibited higher Chao, Ace and Shannon indices but lower Simpson index, indicating greater bacterial community diversity in group I.

2.5 Bacterial Community Structure Analysis

Differential bacterial communities between groups A and I are presented in Table 7 . At the phylum level, four taxa differed significantly (P 0.05): Spirochaetes, Lentisphaerae, Synergistetes and Tenericutes. At the genus level, fifteen taxa showed significant differences (P 0.05): *Prevotella*, *Paraprevotella*, subdivision 5 (genera incertae sedis), *Schwartzia*, *Pseudobutyrivibrio*, *Treponema*, *Clostridium* XIVa, *Victivallis*, unclassified subdivision 3 (genera incertae sedis), *Fretibacterium*, *Oligosphaera*, *Clostridium* XIVb, *Selenomonas*, *Hydrogenibacillus* and *Defluviitalea*.

3.1 Effects of NA, FOS and REC Combinations on pH, NH -N and MCP

Rumen fluid pH serves as a comprehensive indicator of rumen fermentation, with appropriate pH essential for normal microbial activity. The normal pH range is 5.5–7.5, and all treatment groups in this study fell within this range. While dietary oligosaccharides typically decrease rumen pH, our results showed pH increased significantly with FOS supplementation level, possibly due to NA's effect on reducing lactic acid content and elevating pH, suggesting an interaction between FOS and NA.

NH -N represents the end product of peptide, amino acid, protein and non-protein nitrogen compound degradation, and serves as the primary substrate for MCP synthesis. Optimal rumen NH -N concentration ranges from 0.35–29.00 mg/dL [18]; our values (9.77–12.04 mg/dL) fell within this range. NH -N content decreased significantly with increasing FOS level, consistent with Qu et al. [2]. However, overall NH -N levels were lower than in previous studies, suggesting enhanced microbial utilization, likely because both NA and REC promote energy metabolism, providing energy for NH -N incorporation into MCP. This indicates positive combinational effects among the three modulators. Additionally, 0.8% REC supplementation significantly reduced NH -N content, consistent with Liu et al. [19], possibly due to enhanced NH -N utilization by rumen microbes.

MCP constitutes the primary nitrogen source for ruminants, meeting 40–80% of their nitrogen requirements. Although no significant differences in MCP content were observed among groups, group I showed the highest value (37.55%), with

overall MCP levels tending to increase with FOS supplementation. The lack of significant differences may reflect interactions among the three modulators.

3.2 Effects of NA, FOS and REC Combinations on VFA Content

Propionate is the main precursor for gluconeogenesis in ruminants and its fermentation utilizes hydrogen, reducing methane production and improving energy conversion efficiency [20]. The acetate/propionate ratio reflects fermentation pattern, with lower values indicating higher propionate proportion and feed energy utilization. Significant effects on propionate content and acetate/propionate ratio demonstrated that modulator combinations markedly influenced fermentation type. Group I showed highest propionate and lowest acetate/propionate ratio, likely attributable to its specific combination (highest FOS and NA levels, 0.8% REC). These findings align with Zhang et al. [21] showing oligosaccharides decrease acetate/propionate ratio, Schaetzel and Johnson [22] demonstrating NA reduces this ratio, and Liu et al. [19] reporting increased propionate with rare earth supplementation. Compared with single-agent studies, our higher propionate and lower acetate/propionate ratios indicate positive combinational effects, with NA playing a particularly significant role in promoting propionate fermentation. The lack of significant differences in acetate and butyrate contents may reflect NA' s predominant effect on carbohydrate fermentation, increasing propionate while decreasing acetate and butyrate [23], thereby counteracting FOS-induced increases in these VFAs.

3.3 Effects of NA, FOS and REC Combinations on Bacterial Diversity

Using Illumina MiSeq sequencing of 16S rDNA, we compared bacterial diversity between modulator combinations. Group I exhibited higher richness and diversity than group A. Analysis of the combinations revealed that group I contained the highest FOS (1.2%) and NA (1,200 mg/kg) levels, while group A had the lowest levels, indicating that increasing oligosaccharide and NA supplementation in low-concentrate diets promotes microbial growth and enhances bacterial diversity. These results concur with Min et al. [24], Pan et al. [25] and Ottou et al. [26]. However, Gao et al. [27] reported decreased bacterial diversity with NA supplementation under high-concentrate diets, a discrepancy possibly attributable to REC' s role as a physiological activator that promotes microbial growth and enhances diversity.

3.4 Effects of NA, FOS and REC Combinations on Bacterial Community Structure

Our results showed that *Bacteroidetes* and *Firmicutes* dominated across all treatments, consistent with other ruminant studies [28,29]. While *Ruminococcus flavefaciens*, *R. albus* and *Fibrobacter succinogenes* are primary fiber-degrading bacteria [30], non-fiber-degrading bacteria can synergistically enhance cellulose

degradation [31]. *Prevotella ruminicola* [32] and *Treponema* [33] are such synergistic bacteria, and our study found significantly increased abundances of *Prevotella* and *Treponema* in group I, potentially promoting fiber-degrading activity. *Succiniclasticum ruminis* [34] and *Schwartzia* [35] convert succinate to propionate, while *Selenomonas ruminantium* synergizes with fiber-degrading bacteria to enhance cellulose degradation and propionate production [36]. The significantly increased abundances of *Schwartzia* and *Selenomonas* in group I corresponded with its highest propionate proportion, confirming these mechanisms.

---

4 Conclusion

1. Different combinations of nicotinic acid, fructooligosaccharides and rare earth citrate significantly altered rumen fermentation patterns, increasing propionate content in vitro.
2. These combinations significantly affected the abundances of *Prevotella*, *Schwartzia*, *Treponema* and *Selenomonas* in the rumen bacterial community.
3. Under corn-soybean meal-rice straw dietary conditions, optimal modulator combinations were group I (1.2% FOS + 1,200 mg/kg NA + 0.8% REC) and group G (1.2% FOS + 400 mg/kg NA + 1.0% REC).

References

[1] FIRKINS J L, OLDICK B S, PANTOJA J, et al. Efficacy of liquid feeds varying in concentration and composition of fat, nonprotein nitrogen, and nonfiber carbohydrates for lactating dairy cows [J]. Journal of Dairy Science, 2008, 91(5): 1969-1984.

[2] QU M R, LING B M, LU D X, et al. Effects of fructooligosaccharide infusion on rumen fermentation function in growing sheep [J]. Acta Veterinaria et Zootechnica Sinica, 2006, 37(8): 779-784.

[3] LING B M, YU X L, CHEN S J, et al. Functional oligosaccharides and their application in ruminant nutrition [J]. Guangdong Journal of Animal and Veterinary Science, 2006, 31(5): 6-9.

[4] LI X J, GAO T Y, WANG Y C. Research progress on nicotinic acid in ruminant nutrition [J]. Anhui Agricultural Science Bulletin, 2006, 12(3): 133-135.

[5] WANG G L, WANG Y N, MA J F, et al. Effects of different reductive carbon sources on anaerobic fermentation for succinic acid production by recombinant *Escherichia coli* [J]. China Brewing, 2009, 28(3): 16-19.

[6] FOSTER J W, PARK Y K, PENFOUND T, et al. Regulation of NAD metabolism in *Salmonella typhimurium*: molecular sequence analysis of the bifunctional nadR regulator and the nadA-pnuC operon [J]. Journal of Bacteriology, 1990, 172(8): 4187-4196.

[7] ZHANG Q. Effects of nicotinic acid and its antagonists on rumen fermentation parameters and lactic acid metabolism [D]. Master' s thesis. Nanchang: Jiangxi Agricultural University, 2014.

[8] YANG N D. Application research of rare earth feed additives in animal production [J]. Anhui Agricultural Science Bulletin, 2008, 14(24): 88-89.

[9] Ministry of Agriculture of the People' s Republic of China. NY/T 815-2004 Feeding standard of beef cattle [S]. Beijing: China Agriculture Press, 2004.

[10] 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 and Development, 1988, 28: 7-15.

[11] LU D X. Review and prospect of modernization process in ruminant nutrition [J]. Feed Industry, 2010(S1): 1-5.

[12] CHENG M J. Study on the production, degradation, absorption, flow and microbial uptake of oligopeptides in sheep rumen [D]. PhD thesis. Hohhot: Inner Mongolia Agricultural University, 2000: 20-22.

[13] ZHANG L Y. Feed Analysis and Feed Quality Detection Technology [M]. 2nd ed. Beijing: China Agricultural University Press, 2003: 45-100.

[14] FENG Z C, GAO M. Improved method for determination of rumen fluid ammonia nitrogen content by colorimetry [J]. Animal Husbandry and Feed Science, 1993(6): 40-41.

[15] ZHAO J J, ZHAO X C, WANG Z, et al. Determination of volatile fatty acids and lactic acid in rumen contents by high performance liquid chromatography [J]. Chinese Journal of Veterinary Science, 1993(4): 393-394.

[16] WANG F. Preliminary study on determination methods for rumen bacterial and protozoal protein [J]. Chinese Journal of Animal Science, 1990(2): 43.

[17] VIDOVÁ M, HRONSKÁ H, TOKOSOVÁ S, et al. Importance of prebiotic and probiotic: the role of galactooligosaccharides as prebiotic additives: a review [J]. Potravinarstvo Scientific Journal for Food Industry, 2013, 7(1): 28-35.

[18] OWENS F N, BERGEN W G. Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications [J]. Journal of Animal Science, 1983, 57 Suppl 2: 498-518.

[19] LIU Q, WANG C, HUANG Y X, et al. Effects of lanthanum on rumen fermentation, urinary excretion of purine derivatives and digestibility in steers [J]. Animal Feed Science and Technology, 2008, 142(1/2): 121-132.

[20] GUILOTEAU P, TOULLEC R, GRONGNET J F, et al. Digestion of milk, fish and soya-bean protein in the preruminant calf: flow of digesta, apparent digestibility at the end of the ileum, amino composition ileal digesta [J]. British Journal of Nutrition, 1986, 55(3): 571–592.

[21] ZHANG X F, QU M R, WANG L G, et al. Effects of soybean oligosaccharides on rumen fermentation and screening of optimal addition level [J]. Heilongjiang Animal Science and Veterinary Medicine, 2008(10): 46–47.

[22] SCHAETZEL W P, JOHNSON D E. Nicotinic acid and dilution rate effects on in vitro fermentation efficiency [J]. Journal of Animal Science, 1981, 53(4): 1104–1108.

[23] SOLIVA C R, KUNZ C. Preliminary study on the effects of ammonium nicotinate on in vitro ruminal fermentation as determined using rumen simulation technique (Rusitec) [J]. Animal Production Science, 2011, 51(3): 233-239.

[24] MIN L, QU M R, GE T T, et al. Effects of different functional oligosaccharide combinations on rumen solid-phase microbial diversity in Jinjiang cattle [J]. Acta Agriculturae Universitatis Jiangxiensis, 2012, 34(4): 769–774.

[25] PAN L, BU D P, CHENG J B, et al. Effects of nicotinic acid on rumen fermentation and heat stress alleviation in dairy cows [J]. China Feed, 2013(7): 25–27, 31.

[26] OTTOU J F, DOREAU M. Influence of niacin on in vitro ruminal fermentation and microbial synthesis depending on dietary factors [J]. Animal Science Technology, 1996, 58(3/4): 187–199.

[27] GAO Y F. Effects of nicotinic acid on rumen microbial community in cattle fed high-concentrate diets [D]. Master' s thesis. Nanchang: Jiangxi Agricultural University, 2016: 32–35.

[28] PINLOCHE E, MCEWAN N, MARDEN J P, et al. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle [J]. PLoS One, 2013, 8(7): e67824.

[29] LENG J, CHENG Y M, ZHANG C Y, et al. Molecular diversity of bacteria in Yunnan yellow cattle (*Bos taurus*) from Nujiang region, China [J]. Molecular Biology Reports, 2012, 39(2): 1181–1192.

[30] SATOSHI K, YASUO K. Fibrolytic rumen bacteria: their ecology and functions [J]. Asian-Australasian Journal of Animal Sciences, 2009, 22(1): 131-138.

[31] WOLIN M J, MILLER T L, STEWART C S. Microbe-microbe interactions [M]//HOBSON P N, STEWART C S, eds. The Rumen Microbial Ecosystem. Dordrecht: Springer Netherlands, 1997: 467-491.

[32] FONDEVILA M, DEHORITY B A. Interactions between *Fibrobacter succinogenes*, *Prevotella ruminicola*, and *Ruminococcus flavefaciens* in the digestion

of cellulose from forages [J]. Journal of Animal Science, 1996, 74(3): 678-684.

[33] KUDO H, CHENG K J, COSTERTON J W. Interactions between *Treponema bryantii* and cellulolytic bacteria in the in vitro degradation of straw cellulose [J]. Canadian Journal of Microbiology, 1987, 33(3): 244-248.

[34] VAN GYLSWYK N O. *Succiniclasticum ruminis* gen. nov., sp. nov., a ruminal bacterium converting succinate to propionate as the sole energy-yielding mechanism [J]. International Journal of Systematic Bacteriology, 1995, 45(2): 297–300.

[35] VAN GYLSWYK N O, HIPPE H, RAINEY F A. *Schwartzia succinivorans* gen. nov., sp. nov., another ruminal bacterium utilizing succinate as an energy source [J]. International Journal of Systematic Bacteriology, 1997, 47(1): 155–159.

[36] SAWANON S, KOIKE S, KOBAYASHI Y. Evidence for the possible involvement of *Selenomonas ruminantium* in rumen fiber digestion [J]. FEMS Microbiology Letters, 2011, 325(2): 170–179.

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

Source: ChinaXiv —Machine translation. Verify with original.