

Effects of *Bacillus licheniformis* on Methane Emission and Digestive Metabolism in Fattening Sheep (Postprint)

Authors: Xiao Yi, Tao Dayong, Zhao Mingming, Zhao Jiangbo, Ma Tao, Tu Yan, Diao Qiyu

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

Abstract

This study was conducted to investigate the effects of different dietary supplementation levels of *Bacillus licheniformis* on methane emission and digestion and metabolism in meat sheep, and to provide a basis for using microbial preparations to reduce methane emission and improve feed utilization efficiency in meat sheep. Twenty-four adult Dorper () × Small-tailed Han () F1 hybrid wethers with good body condition and body weight of $(45.00 \pm 1.96) \text{ kg}$ were selected and allocated to 4 groups using a single-factor randomized block design, with 6 sheep per group and each sheep serving as one replicate. The control group was $0 \text{ CFU}/(\text{sheep} \cdot \text{d})$, designated as low-, medium-, and high-dose groups, respectively. The trial lasted for 20 days, including an 8-day preliminary period and a 12-day formal experimental period. Methane emission was measured using the Sable open-circuit calorimetry system, while digestion and metabolism were determined by the total collection of feces and urine method. The results showed that: 1) Compared with the control group, the low- and medium-dose groups significantly reduced methane emission based on dry matter intake and metabolic body weight $[\text{L}/(\text{kg W}^{0.75} \cdot \text{kg DMI})]$ in meat sheep ($P < 0.05$), with reduction rates of 5.18% and 9.33%, respectively. 2) Compared with the control group, all experimental groups significantly improved the apparent digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber (except the low-dose group), and nitrogen, as well as retained nitrogen in meat sheep ($P < 0.05$), and significantly increased dietary digestible energy and metabolizable energy ($P < 0.05$). Under the conditions of this experiment, supplementation with *Bacillus licheniformis* could reduce methane emission from meat sheep to a certain extent, improve nutrient digestibility, and consequently enhance feed energy utilization efficiency.

Full Text

Effects of *Bacillus licheniformis* on Methane Emission, Digestion and Metabolism of Mutton Sheep

XIAO Yi^{1,2}, TAO Dayong¹, ZHAO Mingming², ZHAO Jiangbo², MA Tao², TU Yan², DIAO Qiyu^{2*}

¹College of Animal Science, Tarim University, Alaer 843300, China

²Feed Research Institute, Chinese Academy of Agricultural Sciences, Key Laboratory of Feed Biotechnology of the Ministry of Agriculture, Beijing 100081, China

Abstract: This study investigated the effects of dietary supplementation with different levels of *Bacillus licheniformis* on methane emission, digestion, and metabolism in mutton sheep to provide a basis for using probiotics to reduce methane emissions and improve feed utilization efficiency. Twenty-four healthy adult Dorper () × Small-tailed Han () crossbred wethers (F1 generation) with an average body weight of (45.00±1.96)kg and good body condition were randomly allocated into four groups using a single-factor randomized block design, with six sheep per group and each wether serving as one replicate. The control group received *Bacillus licheniformis* at levels of 2.4×10^8 , 2.4×10^9 , and 2.4×10^{10} CFU/(head · d), designated as low-, medium-, and high-dose groups, respectively. The 20-day experimental period consisted of an 8-day preliminary period followed by a 12-day formal measurement period. Methane emissions were measured using a Sable open-circuit respiratory system, while digestion and metabolism were determined via total fecal and urine collection. The results showed: (1) Compared with the control group, the low- and medium-dose groups significantly reduced methane emission normalized to dry matter intake and metabolic body weight [L/(kg W^{0.75} · kg DMI)] by 5.18% and 9.33%, respectively (P<0.05). (2) All experimental groups significantly improved the apparent digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber (except the low-dose group), and nitrogen, as well as nitrogen retention (P<0.05), and significantly increased dietary digestible and metabolizable energy (P<0.05). Under the conditions of this experiment, supplementation with *Bacillus licheniformis* reduced methane emissions and improved nutrient digestibility, thereby enhancing feed energy utilization efficiency in mutton sheep.

Keywords: probiotic; *Bacillus licheniformis*; methane; digestion and metabolism; mutton sheep

Introduction

Accumulation of greenhouse gases has caused increasing global warming concerns. Methane (CH₄), an important greenhouse gas and the most abundant

organic carbon gas in the atmosphere, accounts for 16% of greenhouse gases and contributes 19% to current global warming, ranking second only to carbon dioxide (CO₂). Ruminants produce substantial CH₄ through microbial fermentation in the rumen, emitting approximately 8×10^7 tons annually and contributing 33% of anthropogenic CH₄ emissions. This not only causes severe environmental pollution but also represents significant energy loss from feed, with 6–10% of total energy typically converted to methane energy in dairy production. However, ruminal CH₄ production is essential for maintaining ruminal hydrogen partial pressure. Therefore, regulating ruminal CH₄ production to reduce emissions while ensuring animal health and safety is critically important. The disadvantages of antibiotics, including dysbiosis, drug resistance, and residues, are well recognized. As a novel green additive, probiotics have been increasingly applied in animal production with significant benefits. *Bacillus licheniformis*, with its stress resistance characteristics, is a promising *Bacillus* species that can regulate gastrointestinal microflora balance, promote beneficial bacteria growth, reduce pathogen populations, enhance immunity, and improve feed utilization. While many reports demonstrate positive effects of *Bacillus licheniformis* on animal growth, reproduction, disease resistance, and feed efficiency, few studies have evaluated its regulation of CH₄ in ruminants, particularly through in vivo trials. The mechanisms by which probiotics regulate CH₄ in ruminants require further investigation. This experiment directly fed *Bacillus licheniformis* to adult mutton sheep, measured CH₄ emissions using a Sable open-circuit respiratory system, and conducted digestion and metabolism trials to explore the effects of *Bacillus licheniformis* on CH₄ emissions and digestive metabolism, providing a theoretical basis for using probiotics to reduce CH₄ emissions and improve feed energy utilization in ruminants.

1. Materials and Methods

1.1 Experimental Location and Period The experiment was conducted at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences from December 2014 to January 2015.

1.2 Experimental Materials and Instruments *Bacillus licheniformis* with viable count of 1×10^{10} CFU/g was provided by Beijing Huanong Bioengineering Co., Ltd. The CH₄ measurement system consisted of a Sable open-circuit respiratory system with an LGR gas analyzer purchased from Sable Systems International (USA). The complete apparatus comprised the Sable measurement device, sealed gas metabolism chambers, and supporting computer software.

1.3 Experimental Animals and Diets Twenty-four adult Dorper () × Small-tailed Han () crossbred wethers (F1 generation) with an average body weight of (45.00 ± 1.96) kg, similar age, and good body condition were selected. Before the experiment, ear tags were applied, triple-vaccination was administered, and deworming was performed. Each wether was individually housed in

pens with free access to water and feed. The basal diet was a total mixed pellet (8 mm diameter, 10 mm length) provided by Beijing Zhongnongxing Feed Technology Co., Ltd. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.4 Experimental Design A single-factor randomized block design was employed, with 24 wethers divided into four groups (one control and three experimental groups), six wethers per group, each serving as one replicate. The control group received the basal diet, while experimental groups received the basal diet supplemented with *Bacillus licheniformis* at 2.4×10^8 , 2.4×10^9 , and 2.4×10^{10} CFU/(head · d), designated as low-, medium-, and high-dose groups. The 20-day trial included an 8-day preliminary period followed by a 12-day formal period during which digestion and metabolism trials and gas metabolism measurements were conducted. Methane emissions were measured using the Sable open-circuit respiratory system and LGR gas analyzer, which connected to six respiratory chambers allowing simultaneous measurement of six sheep. During the formal period, on days 1, 4, 7, and 10, wethers were moved into respiratory chambers in four batches (six per batch), adapted for one day, then measured for CH₄ emissions over two days to calculate methane energy. Each wether was weighed when entering and exiting the chamber, with average weight used as the measurement weight. Digestion and metabolism trials employed total fecal and urine collection.

1.5 Sample Collection and Preservation Fecal and urine samples from the previous day were collected daily before morning feeding. Fecal bags were removed and weighed to record fecal output, then mixed thoroughly and subsampled at 10% of total output. For urine collection, buckets containing 100 mL of 10% H₂SO₄ were used to record urine volume, mixed thoroughly, filtered through four layers of gauze, and subsampled at 10% of total volume. All daily fecal and urine samples were stored at -20°C. This process continued for six days, after which all collected samples from each wether were mixed uniformly for analysis. At the end of the digestion trial, fecal samples were dried at 65°C for 48 hours, equilibrated for 48 hours, weighed to determine initial moisture content, then ground and passed through a 40-mesh sieve to prepare analytical samples.

1.6 Animal Management Following NRC (2007) maintenance requirements for sheep, wethers were limit-fed 1,200 g of basal diet daily at 08:00 and 18:00. Different doses of *Bacillus licheniformis* were fed to corresponding groups by sprinkling a small amount of water onto the pellets to adhere the powder, ensuring priority consumption and preventing loss. Each wether received 200 g of Chinese wildrye hay daily at noon with free water access.

1.7 Data Processing and Analysis Data were analyzed using ANOVA in SAS 9.2 software. Duncan' s multiple range test was applied when significant

differences were detected, with $P < 0.05$ as the significance threshold.

2. Results

2.1 Effects of *Bacillus licheniformis* on Methane Emission in Mutton Sheep As shown in Table 2, dietary supplementation with *Bacillus licheniformis* significantly reduced daily total CH_4 emission (L/d), CH_4 emission per dry matter intake (L/kg DMI), CH_4 emission per metabolic body weight (L/kg $W^{0.75}$), and CH_4 emission per both DMI and metabolic body weight [L/(kg $W^{0.75} \cdot \text{kg DMI}$)] in the low- and medium-dose groups compared with the control ($P < 0.05$), with the medium-dose group showing greater reduction. The high-dose group did not differ significantly from the control ($P > 0.05$).

2.2.1 Apparent Digestibility of DM and OM Table 3 shows that under identical intake levels, all experimental groups significantly reduced fecal DM output and increased DM digestion amount and apparent digestibility ($P < 0.05$), with the low-dose group showing the best effect. Experimental groups also significantly reduced fecal OM output and increased OM digestion amount and apparent digestibility ($P < 0.05$), though no significant differences existed among experimental groups ($P > 0.05$).

2.2.2 Apparent Digestibility of NDF and ADF As shown in Table 4, experimental groups significantly reduced fecal NDF output and increased NDF digestion amount and apparent digestibility ($P < 0.05$) compared with the control. Medium- and high-dose groups significantly reduced fecal ADF output and increased ADF digestion amount and apparent digestibility ($P < 0.05$). No significant changes in NDF or ADF apparent digestibility were observed among experimental groups with increasing supplementation levels ($P > 0.05$).

2.2.3 Nitrogen Digestion and Metabolism Table 5 demonstrates that at identical nitrogen intake levels, all experimental groups significantly reduced fecal nitrogen and increased nitrogen apparent digestibility ($P < 0.05$), while medium- and high-dose groups significantly reduced urinary nitrogen ($P < 0.05$). Dietary supplementation with different levels of *Bacillus licheniformis* significantly increased retained nitrogen and retained nitrogen/intake nitrogen ratio compared with the control ($P < 0.05$), though no significant differences existed among experimental groups ($P > 0.05$).

2.2.4 Energy Digestion and Metabolism Table 6 shows that experimental groups significantly reduced fecal energy compared with the control ($P < 0.05$), with the low-dose group lower than the high-dose group ($P < 0.05$) but not differing from the medium-dose group ($P > 0.05$). The high-dose group significantly reduced urinary energy compared with the control ($P < 0.05$), but did not differ from low- or medium-dose groups ($P > 0.05$). Low- and medium-dose groups significantly reduced methane energy and methane energy/gross energy ratio

($P < 0.05$). All experimental groups significantly increased digestible energy, metabolizable energy, gross energy digestibility, and gross energy metabolic rate ($P < 0.05$).

3. Discussion

3.1 Effects of *Bacillus licheniformis* on Methane Emission in Mutton Sheep The rumen is a complex, dynamic microecosystem where ingested feed undergoes anaerobic fermentation. Rumen microorganisms ferment carbohydrates and cellulose into utilizable substances, accompanied by CH_4 production. Rumen-produced CH_4 is poorly utilized by the animal and is expelled through eructation. Studies indicate that CH_4 production in ruminants is primarily influenced by feed type, composition, digesta outflow rate, rumen environment, fermentation pattern, and microbial flora. *Bacillus* species are Gram-positive aerobic bacteria that consume substantial oxygen during growth, reducing intestinal oxygen concentration and creating favorable conditions for obligate anaerobes while inhibiting aerobic pathogens. The rumen resembles an anaerobic fermenter, and this oxygen-scavenging effect of *Bacillus licheniformis* promotes growth of rumen anaerobes, alters microbial balance, improves feed utilization, and indirectly affects CH_4 production. Qiao et al. used short-term artificial rumen methods to study effects of *Bacillus licheniformis* culture on dairy cow rumen fermentation and CH_4 production, reporting a 37.91% reduction in CH_4 emissions. Our in vivo trial directly measured CH_4 production in sheep, showing that supplementation at 2.4×10^8 and 2.4×10^9 CFU/(head · d) reduced CH_4 emissions by 5.18% and 9.33%, respectively. The discrepancy in reduction magnitude may stem from artificial rumen methods failing to simulate the authentic, complex rumen digestion process, where product accumulation affects CH_4 yield, whereas in vivo measurements directly assess live animals.

Current mechanisms for ruminal CH_4 regulation include: (1) directly reducing methanogen numbers to decrease CH_4 production; (2) inhibiting CH_4 synthesis by reducing hydrogen substrate; and (3) specifically inhibiting enzymes involved in CH_4 synthesis. *Bacillus* species produce various antimicrobial substances during metabolism that interact with rumen microorganisms, affecting the rumen environment. Yu et al. reported that dietary supplementation with *Bacillus natto* promoted colonization and growth of *Fibrobacter succinogenes*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, and *Anaerovibrio lipolytica* in the digestive tract of post-weaning calves. Fu found that feeding *Bacillus licheniformis* and *Bacillus subtilis* to 0-8-week-old calves increased rumen bacterial species diversity and promoted colonization of fiber-degrading bacteria. Chen et al. observed that *Bacillus licheniformis* supplementation at 50 mg/kg during early growth and 200 mg/kg during late growth significantly optimized intestinal structure and inhibited harmful bacteria in the cecum of broilers. Reports suggest CH_4 production correlates negatively with acetate and positively with propionate. When the acetate/propionate ratio is 0.5, energy loss as CH_4 from substrate fermentation is zero; when carbohydrates ferment exclusively to ac-

etate, energy loss reaches 33%. In the later stage of our experiment, real-time quantitative PCR absolute quantification of rumen methanogens revealed reduced methanogen and protozoal numbers in low- and medium-dose groups, consistent with decreased CH₄ production. Therefore, CH₄ reduction in these groups likely resulted from direct reduction of methanogens and protozoa. The inhibitory effect of *Bacillus licheniformis* on CH₄ first increased then decreased with supplementation level, with low and medium doses significantly reducing CH₄ production (medium dose being most effective), while the high dose showed no significant inhibition. Possible reasons include: (1) *Bacillus licheniformis* interacts with rumen microorganisms, inhibiting methanogens and protozoa at appropriate doses, but excessive supplementation may shift the dominant microbial populations, with complex microbial interactions weakening the inhibitory effect; and (2) energy metabolism results suggest *Bacillus licheniformis* and its metabolites promote nutrient absorption and utilization, but different doses affect this through different pathways, with low and medium doses more effectively reducing methane energy while high doses more effectively reducing urinary energy, leading to differential CH₄ inhibition. The specific mechanism underlying CH₄ reduction by *Bacillus licheniformis* requires further investigation.

3.2 Effects of *Bacillus licheniformis* on Nutrient Digestion and Metabolism in Mutton Sheep Nutrient digestibility indicates gastrointestinal function strength. Dry matter and organic matter digestibility comprehensively reflect dietary digestion characteristics. Ruminants can utilize structural carbohydrates like cellulose and hemicellulose that monogastric animals cannot, thanks to their specialized rumen structure. Neutral detergent fiber and acid detergent fiber digestibility reflect dietary utilization efficiency, with effective cellulose degradation providing energy for both ruminants and rumen microorganisms while promoting saliva secretion, rumination, rumen buffering, and rumen wall health.

Active *Bacillus* cells in the animal digestive tract produce amylase, protease, lipase, xylanase, chitinase, and other metabolites that degrade antinutritional factors, assist digestion, and improve feed conversion efficiency. Zhou reported that feeding *Lactobacillus plantarum* and *Bacillus subtilis* to calves improved nutrient apparent digestibility and liver organ index while reducing feed-to-gain ratio. Dai et al. found that feeding a *Bacillus* preparation containing *Bacillus subtilis* and *Bacillus licheniformis* to yellow-feathered broilers for six weeks increased average daily gain and reduced feed-to-gain ratio. Zhou observed that feeding *Bacillus licheniformis* to 80 Holstein dairy cows at 1×10^9 , 2×10^9 , and 3×10^9 CFU/kg increased milk yield, with 2×10^9 CFU/kg being most effective. Li et al. reported that *Bacillus licheniformis* improved weight gain, specific growth rate, condition factor, and foregut protease concentration in barramundi. These findings align with our results. In this experiment, all three supplementation levels (2.4×10^8 , 2.4×10^9 , and 2.4×10^{10} CFU/(head · d)) improved apparent digestibility of DM, OM, NDF, and ADF. This may relate to the nutritional

function of probiotics, as *Bacillus licheniformis* metabolism supplements the animal with active enzymes including proteases, lipases, amylases, and cellulases that degrade proteins, triglycerides, non-starch polysaccharides, and structural carbohydrates, promoting nutrient digestion and absorption and improving feed utilization. Additionally, retained nitrogen directly reflects nitrogen utilization efficiency. Dietary *Bacillus licheniformis* improved nitrogen apparent digestibility and retention, suggesting it enhances rumen microbial nitrogen utilization and microbial protein synthesis, possibly because colonized *Bacillus* species have nitrogen-fixing capacity that increases nitrogenous substance absorption and reduces nutrient loss in feces and urine.

3.3 Effects of *Bacillus licheniformis* on Energy Digestion and Metabolism in Mutton Sheep

Ruminants experience greater energy losses during digestion than non-ruminants, directly related to rumen fermentation energy losses, with CH₄ production being the primary pathway. Reports indicate that energy lost as CH₄ accounts for 2-15% of dietary gross energy in ruminants. Our Sable open-circuit respiratory system measured methane energy/gross energy ratios of 7.25% in the control group and 6.96%, 6.76%, and 7.16% in low-, medium-, and high-dose groups, respectively, with the medium dose (2.4×10^9 CFU/(head · d)) showing the most significant reduction. Supplementation reduced methane energy and fecal energy while increasing metabolizable energy and gross energy metabolic rate, demonstrating improved energy utilization efficiency. Under certain conditions, *Bacillus licheniformis* produces stress-resistant endospores and various antibiotics including lipopeptides, peptides, phospholipids, polyenes, and amino acids that effectively inhibit pathogens. Additionally, it produces vitamins, amino acids, organic acids, and growth-promoting factors that enhance metabolism. Possible reasons for improved energy utilization include: (1) reduced methanogen numbers and CH₄ production, increasing gross energy metabolic rate; (2) reduced hydrogen-producing bacteria, decreasing hydrogen substrate for CH₄ synthesis; (3) altered rumen fermentation pattern with decreased acetate/propionate ratio, promoting propionate-type fermentation and energy metabolism; (4) modified rumen microbial flora with increased dominant populations, providing favorable fermentation conditions for nutrient digestion; and (5) enhanced resistance and immune function, reducing environmental stress and improving gross energy utilization.

Under the conditions of this experiment, the following conclusions were drawn: (1) Dietary supplementation with *Bacillus licheniformis* at 2.4×10^8 and 2.4×10^9 CFU/(head · d) reduced CH₄ emissions by 5.18% and 9.33%, respectively. (2) Supplementation improved apparent digestibility of DM, OM, NDF, ADF, and nitrogen, increased retained nitrogen, and enhanced dietary digestible and metabolizable energy, thereby improving energy utilization efficiency.

References

- [1] SCHEEHLE E A, KRUGER D. Global anthropogenic methane and nitrous oxide emissions[J]. *The Energy Journal*, 2006, 27: 33-44.
- [2] LI Shengli, JIN Xin, FAN Xueshan, et al. Ruminant production and carbon emission reduction measures[J]. *Chinese Journal of Animal Nutrition*, 2010, 22(1): 2-9.
- [3] BEAUCHEMIN K A, KREUZER M, O' MARA F, et al. Nutritional management for enteric methane abatement: a review[J]. *Australian Journal of Experimental Agriculture*, 2008, 48(2): 21-27.
- [4] ZHAO Yiguang, DIAO Qiyu, DENG Kaidong, et al. Research progress on measurement and regulation technologies of methane emission from ruminants[J]. *Chinese Journal of Animal Nutrition*, 2011, 23(5): 726-734.
- [5] ZHANG Ju, LI Jinmin, ZHANG Zhiyan, et al. Research progress on *Bacillus licheniformis*[J]. *China Feed*, 2012(17): 9-11.
- [6] HAO Zhengli, LIU Shimin, MENG Xianda. Ruminant Nutrition[M]. Lanzhou: Gansu Nationalities Publishing House, 2000: 5.
- [7] QIAO Guohua, SHAN Anshan. Effects of direct-fed microbial culture on rumen fermentation, methane production, and performance in dairy cows[J]. *China Animal Husbandry & Veterinary Medicine*, 2006, 33(5): 11-14.
- [8] CHEN Dandan, TU Yan, MA Tao, et al. Effects of mulberry leaf flavonoids and resveratrol on gas metabolism and methane emission in mutton sheep[J]. *Chinese Journal of Animal Nutrition*, 2014, 26(5): 1221-1228.
- [9] YU Ping, WANG Jiaqi, BU Dengpan, et al. Effects of dietary *Bacillus natto* on gastrointestinal fiber-degrading bacteria in post-weaning calves[J]. *Journal of China Agricultural University*, 2009, 14(1): 111-116.
- [10] FU Yunqin. Effects of *Bacillus licheniformis* and its composite on growth performance and rumen environment of dairy heifers[D]. Master's Thesis. Beijing: Chinese Academy of Agricultural Sciences, 2012.
- [11] CHEN Jiexiang, ZHANG Renyi, WANG Quanxi, et al. Effects of *Bacillus licheniformis* on intestinal development and cecal microflora balance in broilers[C]//Proceedings of 2009 Annual Conference of Fujian Animal Husbandry and Veterinary Association. Sanming: Fujian Animal Husbandry and Veterinary Association, 2009.
- [12] WOLIN M J, MILLER T L. Microbe-microbe interactions[M]//HOBSON P N. *The Rumen Microbial Ecosystem*. New York: Elsevier Applied Science, 1998.
- [13] XU Guishan, DIAO Qiyu, JI Shoukun, et al. Effects of different feeding levels on energy and protein digestion and metabolism in mutton sheep[J]. *Chinese Journal of Animal Science*, 2012, 48(17): 40-44.

- [14] NAZMI A R, REINISCH T, HINZ H J. Calorimetric studies on renaturation by CaCl_2 addition of metal-free α -amylase from *Bacillus licheniformis* (BLA)[J]. Journal of Thermal Analysis and Calorimetry, 2008, 91(1): 141-149.
- [15] ROZS M, MANCZINGER L, VÁGVÖLGYI C, et al. Fermentation characteristics and secretion of proteases of a new keratinolytic strain of *Bacillus licheniformis*[J]. Biotechnology Letters, 2001, 23(23): 1925-1929.
- [16] BRADDOO S, SAXENA R K, GUPTA R. Two acidothermotolerant lipases from new variants of *Bacillus* spp.[J]. World Journal of Microbiology and Biotechnology, 1999, 15(1): 87-91.
- [17] DAMIANO V B, BOCCHINI D A, GOMES E, et al. Application of crude xylanase from *Bacillus licheniformis* 77-2 to the bleaching of eucalyptus Kraft pulp[J]. World Journal of Microbiology and Biotechnology, 2003, 19(2): 139-144.
- [18] TOHARISMAN A, SUHARTONO M T, SPINDLER-BARTH M, et al. Purification and characterization of a thermostable chitinase from *Bacillus licheniformis* Mb-2[J]. World Journal of Microbiology and Biotechnology, 2005, 21(5): 733-738.
- [19] RISIEY C. Pelleting direct fed microbial[J]. Feed International, 1992(5): 45-49.
- [20] ZHOU Meng. Application of *Lactobacillus plantarum* and *Bacillus subtilis* and their combination in diets for weaned piglets and calves[D]. Master's Thesis. Urumqi: Xinjiang Agricultural University, 2013.
- [21] DAI Qiuzhong, WANG Xiangrong, JIANG Guitao, et al. Effects of dietary *Bacillus* preparation on growth performance and nutrient utilization in yellow-feathered broilers[J]. Journal of Domestic Animal Ecology, 2011, 32(4): 18-22.
- [22] ZHOU Zhenfeng. Effects of *Bacillus licheniformis* preparation on performance and health status of lactating dairy cows[D]. Master's Thesis. Xianyang: Northwest A&F University, 2006.
- [23] LI Zhuojia, YUAN Fenghua, LIN Heizhao, et al. Effects of *Bacillus licheniformis* on growth and digestive enzyme activity of barramundi[J]. Journal of Oceanography in Taiwan Strait, 2011, 30(1): 43-48.
- [24] LIU Xiaolin, CHEN Lechao, YU Xinjing, et al. Effects of *Bacillus licheniformis* on performance of weaned piglets[J]. Guangdong Feed, 2008, 17(1): 27-28.
- [25] LOU Can, JIANG Chenggang, MA Tao, et al. Effects of feeding level on digestion and metabolism in pregnant mutton sheep[J]. Chinese Journal of Animal Nutrition, 2014, 26(1): 134-143.
- [26] HOLTER J B, YOUNG A J. Methane prediction in dry and lactating Holstein cows[J]. Journal of Dairy Science, 1992, 75(8): 2165-2175.

[27] KIM Y, CHO J Y, KUK J H, et al. Identification and antimicrobial activity of phenylacetic acid produced by *Bacillus licheniformis* isolated from fermented soybean, Chungkook-Jang[J]. Current Microbiology, 2004, 48(4): 312-317.

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

Source: ChinaXiv –Machine translation. Verify with original.