

# Bacillus megatherium 1259, Bull Calves

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This study was conducted to investigate the effects of dietary supplementation with *Bacillus megatherium* 1259 (BM1259) on growth performance, nutrient digestibility, rumen fermentation, and blood biochemical parameters in Holstein bull calves. The results demonstrated that the addition of BM1259 to the diets can significantly improve the growth performance and elevate the apparent digestibility of crude protein and neutral detergent fiber. Moreover, supplementation with BM1259 ameliorated rumen fermentation and reduced the emission of both ammoniacal nitrogen and sulfuretted hydrogen in feces and urine. In addition, adding 12 g/head/day of BM1259 had no adverse effect on blood biochemical parameters and the health status of Holstein bull calves. This study demonstrates that BM1259 can be applied as a potential microecologics to improve production performance and nitrogen utilization in Holstein bull calves.

Keywords: *Bacillus megatherium* 1259 ; growth performance ; nutrient digestibility ; rumen fermentation ; blood biochemical parameters ; Holstein bull calves

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## 1. Introduction

In recent years, research and application of microecologics has attracted extensive attention from scholars in domestic fields and abroad. As a new kind of green and environmental protection feed additive, microecologics have been widely used in animal husbandry due to its green, safe, and residue-free function, which can be used to replace antibiotics, to promote the healthy growth of animals, and improve the environment [1][2]. At present, lactic acid bacteria, bacillus, yeast, photosynthetic bacteria, and so on are the most widely studied microecological additives. Among them, bacillus has a history of more than 100 years since its discovery and a large quantity of scientific research data have confirmed the use effect of bacillus [3]. Among bacillus, *Bacillus megatherium* as probiotics has become a research hotspot due to its unique characteristics, such as its strong resistance to stress, high temperature resistance, and easy storage [4][5]. BM1259, as a new-type of microecological additive isolated from chicken manure, is a gram-positive bacterium that can produce spores. BM1259 is usually a rod-shaped bacterium with rounded ends and tends to be arranged in single or chain forms. It is a large bacterium with a diameter of more than 1.0  $\mu\text{m}$  and a volume of more than 60 microcubic meters, which is 100 times the volume of *E. coli*, demonstrating strong resistance to high temperature treatment, ultraviolet radiation, drying, and high concentrations of organic solvents. It plays a very important role in improving animal production performance and product quality, increasing the output of livestock products and reducing feed costs [6][7][8][9][10]. Kritas et al. demonstrated that supplementing ewe feed with bacillus sp. probiotics promoted subsequent milk with yields, fat, and protein contents [11]. Huo et al. showed that feeding 0.04% of BM1259 could improve the digestibility of crude protein and dry matter in the diet of finishing pigs, reduce the production of  $\text{NH}_3$ , and improve the air environment of pig pens to a certain extent [12]. Ding et al. added 100 mg/kg BM1259 to the feed of laying hens and found that the parameters of the total egg weight, egg number, and average egg weight were significantly increased, while the contents of ammonia-nitrogen, uric acid, and urea nitrogen in the excrement were significantly decreased, which could improve economic benefits [13]. Yu et al. supplemented 12 g/head/day of BM1259 to the diet of dairy cows, finding that the concentrations of urea nitrogen and uric acid in feces decreased, the activities of urease and uricase decreased, and the activity of alanine aminotransferase in the serum also decreased [14].

However, few studies concentrated on dietary supplementation with BM1259 in young ruminants and especially the study and application of BM1259 on Holstein bull calves is still unknown. Hence, this work was conducted with the aim to evaluate the effects of BM1259 on growth performance, nutrient digestibility, rumen fermentation, and blood biochemical parameters in Holstein bull calves. Together, these data could represent a useful basis for future applications of BM1259 as ruminant microecologics.

## 2. Discussion

It was found that adding bacillus to the diet of Holstein bull calves could improve the performance of Holstein bull calves. Youssef et al. showed that feeding bacillus subtilis BSN fermentation significantly improved the feed conversion rate and milk yield but had no significant effects on the milk fat percentage and milk protein percentage [15]. Hu et al. demonstrated that feeding  $1.0 \times 10^{10}$  cfu/g bacillus subtilis improved the milk yield of Holstein cows by 11.61% [16]. Deng et al. found that feeding bacillus natto significantly increased the milk yield of 4% milk fat [17]. It was found that the addition of 12 g/head/day of BM1259 could significantly improve the growth performance and rumen fermentation ability, and there was no significant difference in the blood biochemical parameters. In our study, the ADG and DMI were significantly increased but there were no significant differences in FCR, WH, and BL. During the growth and metabolism of bacillus, some digestive enzymes with strong activity can be produced, such as protease, lipase, and starch hydrolase, which can decompose some complex carbohydrates such as gum and xylan, so as to promote the digestion and absorption capacity of nutrients in the rumen [18]. In this study, we found that supplementation of 12 g/head/day of BM1259 could significantly improve the digestion and utilization ratio of CP and glutamate, which was related to 12 coding genes related to nitrogen metabolism in our previous research. In addition, the digestion and utilization rate of NDF were significantly improved after the addition of 12 g/head/day of BM1259, which might be due to the secretion of certain digestive enzymes that promoted the decomposition and digestion of NDF.

The rumen pH value is one of the evaluation parameters of rumen microbial growth, metabolism balance, and fermentation degree, which is mainly affected by the amount of saliva buffer secretion of ruminants [19]. The rumen fibrolytic bacteria had the highest activity at pH 6.7 and the rumen degradability and acetate content decreased with the decrease of pH [20][21][22]. In this study, after adding 12 g/head/day of BM1259, the rumen pH value increased significantly from 6.20 to 6.76 after eight weeks. The results indicated that adding a certain amount of BM1259 could promote the growth of fibrolytic bacteria.  $\text{NH}_3\text{-N}$  is the main nitrogen source (about 20–100%) in the process of rumen microbial flora growth and reproduction of ruminants. The concentration of  $\text{NH}_3\text{-N}$  should be moderate in order to facilitate microbial growth and promote microbial MCP generation. Too high or too low concentration is not suitable for microbial growth [23][24][25]. In this study, it was found that the rumen  $\text{NH}_3\text{-N}$  concentration was significantly decreased after the addition of 12 g/head/day of BM1259, which was in the range of 8.46–12.03 mg/dL, although lower than that of the 10 g/head/day of BM1259 (15.95–25.32 mg/dL) in Yu [26]. However, dietary supplementation of BM1259 has a certain influence on the rumen ammonia–nitrogen  $\text{NH}_3\text{-N}$  concentration. VFA is one of the energy substances used to maintain the normal life activities of ruminants, which can provide more than 70% of the digestive energy and participate in different metabolisms in the body [27]. Diana et al. found that by feeding bacillus subtilis, C2 concentration and C2:C3 were significantly decreased, while TVFA, C3, and  $\text{NH}_3\text{-N}$  contents were significantly increased [28]. Ding et al. found that the C2:C3 could be significantly reduced and both the TVFA and C3 content could be increased with supplementation of bacillus subtilis in the fermentation test [29]. However, our results demonstrated that after the addition of BM1259, the C2 concentration increased significantly and the C2:C3 increased, indicating that the rumen fermentation type changed to acetate fermentation, which was consistent with the significant increase in the content of *Saccharofermentans* in our previous study [2]. The levels of  $\text{NH}_3\text{-N}$  and  $\text{H}_2\text{S}$  in the urine not only affected the health of the livestock and humans, but also reflected the nitrogen utilization capacity of animals [30]. In this study, after the addition of 12 g/head/day of BM1259,  $\text{NH}_3\text{-N}$  in the urine of Holstein bull calves significantly decreased and the content of  $\text{H}_2\text{S}$  in the excreta was significantly reduced.

The level of the blood biochemical parameters can reflect the status of nutrient metabolism and the acid-base balance of the animal body to a certain extent. Meanwhile, the level of serum albumin and total protein is an indicator for measuring the health status of the animal [31][32][33]. In this study, it was found that after adding 12 g/head/day of BM1259, most biochemical parameters in the blood of Holstein bull calves did not significantly change, indicating that BM1259 did not have adverse effects on the body health status.

## 3. Conclusions

In summary, the addition of BM1259 to the diets of Holstein bull calves can significantly improve the growth performance and elevate the apparent digestibility of CP and NDF. Moreover, supplementation with BM1259 increased C2 and C2:C3 in the rumen, and reduced emission of  $\text{NH}_3\text{-N}$  and  $\text{H}_2\text{S}$  in the feces and urine, indicating a possible amelioration in the rumen fermentation and nitrogen utilization. In addition, adding 12 g/head/day of BM1259 had no adverse effect on blood biochemical parameters and health status. However, further research is necessary to uncover the mechanism by which BM1259 improves nitrogen utilization in Holstein bull calves.

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