Effects of Dietary Supplementation with Bacillus subtilis or Enterococcus faecalis on Growth Performance, Nutrient Digestibility, Nitrogen Metabolism, and Fur Quality of Mink during the Winter Fur Period (Postprint)

Authors: Mie Hao, Yang Fuhe, Zhang Tietao, Cui Hu, Si Huazhe, Wang Zhongcheng, Jingjing Xie, Gao Xiuhua

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Abstract

This experiment aimed to investigate the effects of dietary supplementation of Bacillus subtilis or Enterococcus faecalis on growth performance, nutrient digestibility, nitrogen metabolism, and fur quality of minks during the winter fur period. The experiment adopted a single-factor completely randomized design, selecting 70 healthy male minks at 135 days of age with an average body weight of (1,767.94 ± 174.20) g, randomly divided into 7 groups with 10 replicates per group and 1 mink per replicate. Group I was the control group fed the basal diet; Groups II, III, and IV were fed the basal diet supplemented with 1×10 , 1×10 , and 1×10^{1} CFU/kg Bacillus subtilis, respectively; Groups V, VI, and VII were fed the basal diet supplemented with 1×10 , 1×10 , and 1×10^{1} CFU/kg Enterococcus faecalis, respectively. The experimental period was 70 days. The results showed: 1) Compared with the control group, dietary supplementation of 1×10 CFU/kg Enterococcus faecalis significantly increased final body weight ($P < 0.05$); dietary supplementation of 1×10^{1} CFU/kg Bacillus subtilis or 1×10 and 1×10^{1} CFU/kg Enterococcus faecalis significantly increased average daily gain ($P < 0.05$); dietary supplementation of 1×10 and 1×10^{1} CFU/kg Bacillus subtilis or 1×10 , 1×10 , and 1×10^{1} CFU/kg Enterococcus faecalis significantly decreased feed conversion ratio ($P < 0.05$). 2) Compared with the control group, dietary supplementation of 1×10 CFU/kg Enterococcus faecalis significantly increased protein digestibility ($P < 0.05$); dietary supplementation of 1×10 , 1×10 , and 1×10^{1} CFU/kg Bacillus subtilis or 1×10 , 1×10 , and 1×10^{1} CFU/kg Enterococcus faecalis significantly increased fat digestibility ($P < 0.05$). 3) Compared with the control group, dietary supplementation of 1×10^{1} CFU/kg Bacillus subtilis or 1×10 CFU/kg Enterococcus faecalis significantly increased nitrogen retention ($P < 0.05$). 4) The underhair length in the 1×10^{1} CFU/kg Bacillus subtilis group was significantly higher than that in the 1×10 CFU/kg Enterococcus faecalis group, and the guard hair to underhair ratio was significantly lower than that in the 1×10 CFU/kg Enterococcus faecalis group ($P < 0.05$). It can be concluded that dietary supplementation of 1×10^{1} CFU/kg Bacillus subtilis or 1×10 CFU/kg Enterococcus faecalis during the winter fur period resulted in favorable growth performance, nutrient digestibility, nitrogen retention, and fur quality in minks.

Full Text

Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Growth Performance, Nutrient Digestibility, Nitrogen Metabolism and Pelt Quality of Mink during Winter Fur-growing Season

NIE Hao[1], YANG Fuhe[2], ZHANG Tietao[2], CUI Hu[1], SI Huazhe[2], WANG Zhongcheng[1], XIE Jingjing[1], GAO Xiuhua[1]*

[1]National Engineering Research Center of Biological Feed, Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2]Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China

Abstract

This experiment was conducted to investigate the effects of dietary supplementation with *Bacillus subtilis* or *Enterococcus faecalis* on growth performance, nutrient digestibility, nitrogen metabolism, and pelt quality of male mink during the winter fur-growing season. Seventy healthy 135-day-old male mink with an average body weight of (1,767.94±174.20) g were randomly allocated to 7 groups using a single-factor completely randomized design, with 10 replicates per group and one mink per replicate. Group I served as the control and received a basal diet. Groups II, III, and IV received the basal diet supplemented with 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Bacillus subtilis*, respectively. Groups V, VI, and VII received the basal diet supplemented with 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Enterococcus faecalis*, respectively. The experimental period lasted 70 days.

The results showed: (1) Compared with the control group, supplementation with 1×10 CFU/kg of *Enterococcus faecalis* significantly increased final body weight ($P<0.05$). Supplementation with 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 and 1×10^{1} CFU/kg of *Enterococcus faecalis* significantly increased average daily gain ($P<0.05$). Supplementation with 1×10 and 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Enterococcus faecalis* significantly reduced feed-to-gain ratio ($P<0.05$). (2) Supplementation with 1×10 CFU/kg of *Enterococcus faecalis* significantly improved protein digestibility compared with the control group ($P<0.05$). Supplementation with 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Enterococcus faecalis* significantly improved fat digestibility ($P<0.05$). (3) Supplementation with 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 CFU/kg of *Enterococcus faecalis* significantly increased nitrogen retention ($P<0.05$). (4) The 1×10^{1} CFU/kg *Bacillus subtilis* group exhibited significantly higher pile fur length and significantly lower guard hair-to-down hair ratio compared with the 1×10 CFU/kg *Enterococcus faecalis* group ($P<0.05$). These results indicate that dietary supplementation with 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 CFU/kg of *Enterococcus faecalis* during the winter fur-growing season can effectively improve growth performance, nutrient digestibility, nitrogen deposition, and pelt quality in mink.

Keywords: *Bacillus subtilis*; *Enterococcus faecalis*; mink; winter fur-growing season; growth performance; nutrient digestibility; pelt quality

Introduction

Probiotics are defined as live microorganisms that colonize the animal gut and reproductive system, producing definitive health benefits and thereby improving microecological balance and exerting beneficial effects on the host [?]. As feed additives, probiotics promote growth, enhance feed digestibility, strengthen immune function, and prevent or treat diseases [?]. Consequently, probiotics have attracted considerable attention as feed additives due to their advantages of being pollution-free, residue-free, and non-toxic. Hu et al. [?] reported that dietary supplementation with *Bacillus subtilis* KN-42 improved growth performance and gastrointestinal health in piglets. Liu et al. [?] found that adding *Bacillus subtilis* (strain PB6) to weaned piglet diets significantly increased average daily gain (ADG) and protein digestibility while reducing feed-to-gain ratio (F/G), thereby improving intestinal microecological balance and alleviating the adverse effects of weaning stress. Hou [?] demonstrated that supplementing weaned piglet diets with *Enterococcus faecalis* and other probiotics increased average daily feed intake (ADFI) and ADG while reducing diarrhea rates and enhancing immune function and protein utilization. Guo et al. [?] observed that probiotic supplementation (*Bacillus subtilis* and *Enterococcus faecalis*) in Arctic fox diets during the winter fur-growing period improved growth performance and protein and fat digestibility while reducing nitrogen excretion and increasing nitrogen retention. Jing et al. [?] reported that supplementing mink diets with mink-derived *Lactobacillus* and *Enterococcus faecium* significantly improved dry matter and protein digestibility, reduced fecal nitrogen excretion, and enhanced immune function.

While numerous studies have reported on the application of *Bacillus subtilis* and *Enterococcus faecalis* in pigs and other animals [?], no research has been conducted on their use in mink during the winter fur-growing season. Therefore, this study aimed to investigate the effects of dietary supplementation with

Bacillus subtilis and *Enterococcus faecalis* on growth performance, nutrient digestibility, nitrogen metabolism, and pelt quality of mink during this critical period, and to determine the optimal supplementation levels for practical application.

Materials and Methods

1.1 Experimental Materials

The probiotics used in this experiment were provided by Beijing Scitechful Biological Technology Co., Ltd. The *Bacillus subtilis* was isolated from soil, while the *Enterococcus faecalis* originated from pig intestine. Laboratory analysis confirmed that both probiotic preparations contained viable bacteria counts of 1×10^{1} CFU/g.

1.2 Experimental Animals and Diets

Seventy healthy 135-day-old male mink with an average body weight of (1,767.94±174.20) g were randomly selected from the fur animal production base at the Changbai Mountain Wildlife Resources Field Scientific Observation and Research Station of the Ministry of Agriculture. Currently, there is no unified feeding standard for mink in China. Therefore, the basal diet for the winter fur-growing period was formulated according to NRC (1982) [?] nutrient requirements for mink and foxes, combined with relevant domestic research reports [?]. The diet was prepared as a dry powder formulation; its composition and nutrient levels are presented in Table 1 .

1.3 Experimental Design and Management

A single-factor completely randomized design was employed. The 70 mink were randomly divided into 7 groups with 10 replicates per group and one mink per replicate. Group I served as the control and received the basal diet. Groups II, III, and IV received the basal diet supplemented with 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Bacillus subtilis* (equivalent to 0.01, 0.10, and 1.00 g of the probiotic preparation per kg of diet, respectively). Groups V, VI, and VII received the basal diet supplemented with 1×10 , 1×10 , and 1×10^{1} CFU/kg of *Enterococcus faecalis* (equivalent to 0.01, 0.10, and 1.00 g of the probiotic preparation per kg of diet, respectively). Since the mink had already adapted to dry powder diets during the growing period, no pre-feeding period was implemented, and the experiment commenced immediately after grouping. The 70-day trial was conducted from September 14 to November 23, 2016, at the Changbai Mountain Wildlife Resources Field Scientific Observation and Research Station. All mink were housed individually in cages and fed twice daily at 08:00 and 15:00. The probiotics were administered daily by dissolving them in water, mixing with a portion of the diet, and feeding the mixture. Fresh water was available ad libitum.

1.4 Digestion and Metabolism Trial

On day 32 of the experiment, six mink with similar body weights were selected from each group for a digestion and metabolism trial conducted from October 16 to 18, 2016 (3 days). The total feces collection method was employed, with feeding management identical to routine practices. Urine was collected daily, with 2 mL of 10% sulfuric acid and 4 drops of toluene added per 100 mL of urine as preservatives, and stored at -20°C. Fresh feces were weighed daily, and 10% sulfuric acid (5% of fresh weight) and a small amount of toluene were added as preservatives before storage at -20°C. The 3-day collections of urine and feces were thoroughly mixed, and representative samples were taken. Fecal samples were dried to constant weight at 65°C, ground to pass through a 40-mesh sieve, and prepared as air-dried samples for laboratory analysis.

1.5 Measurement Indicators and Methods

Initial body weight was measured after overnight fasting on day 1, and final body weight was measured after fasting at the end of the experiment to calculate average daily gain (ADG). Daily feed allowance and refusals were recorded to determine average daily feed intake (ADFI) and feed-to-gain ratio (F/G) for each group. At the conclusion of the trial, mink were slaughtered and pelt quality was evaluated prior to slaughtering by a panel of four experts from the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences. Quality scores ranged from 1 to 12 points [?], with four grades based on fur density, color, luster, and evenness: 1-3 points for bent guard hairs, hair loss, self-biting injuries, or damage; 4-6 points for dull color, poor evenness, or uneven color distribution; 7-9 points for relatively abundant fur, black color, relatively glossy coat, relatively even and soft hair on the back and abdomen, and no injuries; and 10-12 points for abundant fur, black color, glossy coat, even and soft hair on the back and abdomen, no injuries, no bent guard hairs, and no residual summer hair.

Body length was measured with a flexible ruler as the distance from nose tip to tail base. Guard hair length and down hair length were measured with a straight ruler on hairs selected from the middle of the back, representing the length from hair tip to root.

Dry matter content in diets and excreta was determined by oven drying according to GB/T 6435-2006 [?]. Crude fat content was determined by Soxhlet extraction following GB/T 6433-2006 [?]. Crude protein content was determined by the Kjeldahl method using a FOSS Kjeltec analyzer according to GB/T 6432-1994 [?]. Calcium content was measured by potassium permanganate titration (GB/T 6436-2002) [?], and total phosphorus by vanadium molybdate colorimetry (GB/T 6437-2002) [?]. Amino acid content was determined by hydrochloric acid hydrolysis (GB/T 5009.124-2003) [?]. Nutrient digestibility was calculated using the method described by Zhang [?].

Data are expressed as means ± standard deviation. Statistical analysis was performed using SPSS 22.0 software. Differences among groups were examined by one-way ANOVA, with $P<0.05$ considered statistically significant and $P<0.01$ considered highly significant.

Results

2.1 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Growth Performance of Mink during Winter Fur-growing Season

As shown in Table 3 , final body weight and ADG were higher in all treatment groups compared with the control group. Final body weight in group VI was significantly higher than in the control group ($P<0.05$), while ADG in groups IV, VI, and VII was significantly higher than in the control group ($P<0.05$). The feed-to-gain ratio in group II did not differ significantly from the control group ($P>0.05$), but all other treatment groups showed significantly lower values than the control group ($P<0.05$). No significant differences in ADFI were observed among all groups ($P>0.05$).

2.2 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Nutrient Digestibility of Mink during Winter Fur-growing Season

Table 4 shows that no significant differences were detected among groups in dry matter intake, dry matter output, or dry matter digestibility ($P>0.05$). Protein digestibility in group VI was significantly higher than in the control group and groups II and V ($P<0.05$). Fat digestibility exceeded 90% in all groups, with all probiotic-supplemented groups showing significantly higher values than the control group ($P<0.05$).

2.3 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Nitrogen Metabolism of Mink during Winter Fur-growing Season

As presented in Table 5 , no significant differences were observed among groups in nitrogen intake, fecal nitrogen, or urinary nitrogen ($P>0.05$). However, nitrogen retention in groups IV and VI was significantly higher than in the control group ($P<0.05$). Net protein utilization (NPU) and protein biological value (BV) were higher in all treatment groups compared with the control group, though these differences were not statistically significant ($P>0.05$).

2.4 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Pelt Quality of Mink during Winter Fur-growing Season

Table 6 indicates that pelt quality scores and body length were slightly higher in all treatment groups compared with the control group, but these differences were not significant ($P>0.05$). No significant differences in guard hair length were detected among groups ($P>0.05$). Group IV exhibited significantly greater down hair length than group V ($P<0.05$) but a significantly lower guard hair-to-down hair ratio than group V ($P<0.05$).

Discussion

3.1 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Growth Performance of Mink during Winter Fur-growing Season

When used as feed additives, probiotics partially colonize the animal intestine and exert various beneficial effects [?]. Aerobic probiotics such as *Bacillus subtilis* rapidly consume free oxygen in the gut, favoring the growth of dominant anaerobic bacteria while inhibiting pathogenic aerobic bacteria, thereby restoring intestinal health and improving growth performance. Lactic acid bacteria such as *Enterococcus faecalis* produce large amounts of lactic acid in the intestine, reducing intestinal pH, inhibiting pathogen growth, and enhancing acidic protease activity to facilitate digestion and improve growth performance [?]. Cui et al. [?] reported that dietary *Bacillus subtilis* supplementation significantly increased ADG and improved growth performance in pigs. Liu et al. [?] found that probiotic supplementation (containing *Bacillus subtilis* and *Enterococcus faecium*) significantly increased ADG and reduced feed-to-gain ratio in weaned piglets. In the present study, supplementation with 1×10^{1} CFU/kg of *Bacillus subtilis* resulted in the highest ADG and lowest feed-to-gain ratio, indicating that this level was optimal for mink during the winter fur-growing season, consistent with findings from the growing period [?]. However, when *Bacillus subtilis* was supplemented at 1×10^{11} CFU/kg during the growing period, no significant effects on growth performance were observed [?], possibly because excessive and prolonged supplementation disrupted intestinal balance and provided minimal additional benefits. Ross et al. [?] demonstrated that dietary supplementation with *Lactobacillus* and *Enterococcus faecium* (isolated from pig feces) improved ADG in pigs. Shi [?] reported that *Enterococcus faecalis* supplementation increased ADG and ADFI in weaned piglets during days 15-31 of the experiment. In this study, supplementation with 1×10 CFU/kg of *Enterococcus faecalis* significantly increased ADG and final body weight while reducing feed-to-gain ratio compared with the control group, suggesting this level was optimal for mink during the winter fur-growing season.

3.2 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Nutrient Digestibility of Mink during Winter Fur-growing Season

Fat is the primary energy-yielding nutrient in winter fur-growing diets, and mink have high energy requirements during this period. Research indicates that dietary energy level can affect feed intake [?]. In this study, all groups received the same basal diet with identical energy levels, resulting in no significant differences in dry matter intake. Giang et al. [?] reported that probiotic supplementation (*Bacillus* and lactic acid bacteria) significantly improved crude protein and dry matter digestibility in finishing pigs. Wei [?] found that *Enterococcus faecalis* supplementation ($>2\times10$ CFU/kg) significantly improved crude protein and fat digestibility in nursery piglets, with effects comparable to antibiotic treatment. The current results showed no significant differences in dry matter digestibility among groups, though a trend toward improvement was observed with increasing probiotic levels. Protein digestibility was significantly higher in the 1×10 CFU/kg *Enterococcus faecalis* group compared with the control, 1×10 CFU/kg *Bacillus subtilis*, and 1×10 CFU/kg *Enterococcus faecalis* groups. All probiotic-supplemented groups exhibited higher fat digestibility than the control group. These findings align with previous research demonstrating that probiotics improve protein and fat digestibility by secreting proteases, lipases, and lactic acid, reducing intestinal pH, and enhancing intestinal motility. The lower protein digestibility observed in the 1×10 CFU/kg groups may be attributed to insufficient probiotic levels and inadequate mixing during diet preparation. Compared with studies on growing mink, protein digestibility was lower during the winter fur-growing period. Previous research indicates that fur-bearing animals have higher fat digestibility than other animals, often exceeding 90%, with digestibility increasing as dietary fat level rises [?]. Jing et al. [?] reported that *Lactobacillus plantarum* and *Enterococcus faecium* supplementation improved fat digestibility in mink, with values exceeding 90%. The present study yielded similar results, with all groups showing fat digestibility above 90% and probiotic-supplemented groups demonstrating significantly higher values than the control.

3.3 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Nitrogen Metabolism of Mink during Winter Fur-growing Season

Nitrogenous compounds in animal diets are either excreted in feces when not digested and absorbed, or utilized for protein synthesis after absorption, with metabolic products excreted in urine [?]. Nitrogenous excreta from animals cause significant pollution to land and water resources, representing a major environmental concern [?]. The present study found that probiotic supplementation tended to reduce fecal and urinary nitrogen excretion while increasing net protein utilization and protein biological value. Furthermore, nitrogen retention was significantly higher in the 1×10^{1} CFU/kg *Bacillus subtilis* and 1×10 CFU/kg *Enterococcus faecalis* groups compared with the control. These results demonstrate that probiotic supplementation improved protein utilization, reduced nitrogen emissions, and enhanced nitrogen retention.

3.4 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Pelt Quality of Mink during Winter Fur-growing Season

As fur-bearing animals, mink pelt quality directly determines their economic value, which is associated with fur quality, pelt size, evenness, and softness [?]. Piao and Hou [?] established a linear relationship between body surface area and body weight in mink, indicating that greater body weight during the winter fur-growing period yields larger pelts. In this study, probiotic supplementation increased body weight, with the 1×10 CFU/kg *Enterococcus faecalis* group showing significantly higher weight than the control. Additionally, body length tended to increase in all treatment groups compared with the control, suggesting that probiotic supplementation improved pelt size. Gugolek et al. [?] reported that dietary supplementation with *Enterococcus faecalis* and *Lactobacillus acidophilus* significantly improved pelt quality (including body length, color, luster, and pelt size) in Arctic foxes. The present study found that supplementation with 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 CFU/kg of *Enterococcus faecalis* did not significantly affect guard hair length compared with the control, though values were slightly higher than other treatment groups. The 1×10^{1} CFU/kg *Bacillus subtilis* group showed the greatest down hair length, significantly higher than the 1×10 CFU/kg *Enterococcus faecalis* group, and the lowest guard hair-to-down hair ratio, significantly lower than the 1×10 CFU/kg *Enterococcus faecalis* group. A lower guard hair-to-down hair ratio indicates greater pelt evenness. The 1×10^{1} CFU/kg *Bacillus subtilis* group exhibited the longest down hair, relatively long guard hair, and the lowest guard hair-to-down hair ratio, indicating the highest pelt evenness among all groups.

Conclusion

Dietary supplementation with 1×10^{1} CFU/kg of *Bacillus subtilis* or 1×10 CFU/kg of *Enterococcus faecalis* during the winter fur-growing season can effectively improve growth performance, nutrient digestibility, nitrogen retention, and pelt quality in mink.

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