Effects of Dietary Bacillus subtilis or Enterococcus faecalis Supplementation on Growth Performance, Nutrient Digestibility, and Nitrogen Metabolism in Growing Mink (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with Bacillus subtilis or Enterococcus faecalis on growth performance, nutrient digestibility, and nitrogen metabolism in growing minks. A single-factor completely randomized design was adopted, in which 70 healthy male minks at 60 days of age with an average body weight of
(957.37$±93.96)gwererandomlyallocatedinto7groups, eachconsistingof10replicateswith1minkperreplicate.G
1)ThefinalbodyweightofgroupIIIwassignificantlyhigherthanthatofgroupI(P <
0.05), theaveragedailyfeedintakeofgroupIVwassignificantlylowerthanthatofgroupI(P <
0.05), andtherewerenosignificantdifferencesinaveragedailygainandfeedconversionratioamongallgroups(P
0.05).2)ThedrymatterdigestibilityofgroupsII, III, andVwassignificantlyhigherthanthatofgroupI(P <
0.05), withgroupVbeingsignificantlyhigherthangroupsI, IV, VI, andVII(P <
0.05); theproteindigestibilityofgroupVwassignificantlyhigherthanthatofgroupsIandVII(P <
0.05); therewerenosignificantdifferencesinfatdigestibilityamongallgroups(P >
0.05).3)ThefecalnitrogencontentofgroupsIV, V, andVIwassignificantlylowerthanthatofgroupI(P <
0.05), andtheurinarynitrogencontentofgroupsVandVIwassignificantlylowerthanthatofgroupI(P <
0.05).ThenitrogenretentionofalltreatmentgroupswashigherthanthatofgroupI, butthedifferencewasnotsign
0.05).ThenetproteinutilizationofgroupsIIIandVwassignificantlyhigherthanthatofgroupI(P <
0.05), andtheproteinbiologicalvalueofgroupIIIwassignificantlyhigherthanthatofgroupI(P <
0.05).Inconclusion, supplementationofthedietforgrowingminkswith1×10^10CFU/kgofBacillussubtilisor1×
CFU/kg of Enterococcus faecalis resulted in more desirable growth performance, nutrient digestibility, nitrogen retention, net protein utilization, and protein biological value.

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Full Text

Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Growth Performance, Nutrient Digestibility and Nitrogen Metabolism of Minks during the Growing Period

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Abstract: This experiment investigated the effects of dietary supplementation with *Bacillus subtilis* or *Enterococcus faecalis* on growth performance, nutrient digestibility, and nitrogen metabolism in growing minks. A single-factor completely randomized design was employed using 70 healthy 60-day-old male minks with an average body weight of (957.37$±93.96)$g.Theanimalswererandomlyallocatedinto7groups,eachcomprising10replicateswith1minkper CFU/kg of *Bacillus subtilis*, respectively. Groups V, VI, and VII received the basal diet supplemented with 1$×10^{8}, 1×10^{9}, and 1×10^{10}$ CFU/kg of *Enterococcus faecalis*, respectively. Following a 7-day adaptation period, the experimental period lasted 60 days. The results demonstrated: (1) The final body weight of group III was significantly higher than that of group I (P<0.05), while the average daily feed intake of group IV was significantly lower than that of group I (P<0.05). No significant differences were observed in average daily gain or feed-to-gain ratio among all groups (P>0.05). (2) The dry matter digestibility of groups II, III, and V was significantly higher than that of group I (P<0.05), with group V exhibiting significantly higher dry matter digestibility compared to groups I, IV, VI, and VII (P<0.05). The protein digestibility of group V was significantly higher than that of groups I and VII (P<0.05). No significant differences in fat digestibility were detected among groups (P>0.05). (3) The fecal nitrogen content of groups IV, V, and VI was significantly lower than that of group I (P<0.05), while the urinary nitrogen content of groups V and VI was significantly lower than that of group I (P<0.05). Although nitrogen deposition in all treatment groups was higher than in group I, the differences were not statistically significant (P>0.05). The net protein utilization of groups III and V was significantly higher than that of group I (P<0.05), and the protein biological value of group III was significantly higher than that of group I (P<0.05). In conclusion, supplementation of growing mink diets with 1$×10^{10}$ CFU/kg *Bacillus subtilis* or 1$×10^{8}$ CFU/kg *Enterococcus faecalis* optimally improves growth performance, nutrient digestibility, nitrogen deposition, net protein utilization, and protein biological value.

Keywords: *Bacillus subtilis*; *Enterococcus faecalis*; minks; growth performance;

nutrient digestibility; nitrogen metabolism

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For decades, dietary antibiotics have substantially promoted livestock development. However, their widespread use has revealed numerous drawbacks, including antimicrobial resistance, compromised animal immunity, disruption of normal intestinal microbiota, food safety concerns, and environmental antibiotic residues [1]. Consequently, identifying alternatives to antibiotics has become critically important. Research indicates that probiotics represent one of the most promising substitutes [1], as they can modulate intestinal microecological balance, promote intestinal villus development, and enhance animal growth performance [2-4]. Lee et al. [2] reported that dietary *Bacillus subtilis* supplementation improved average daily gain and feed conversion ratio while enhancing intestinal health in weaned piglets. Pei et al. [5] found that *Bacillus subtilis* significantly increased average daily gain and reduced feed-to-gain ratio and diarrhea incidence in weaned rex rabbits. Wei [6] demonstrated that different inclusion levels of *Enterococcus faecalis* improved growth performance in nursery piglets, with optimal effects observed at 200 g/t, alongside enhanced immune function. Gong et al. [7] observed that dietary supplementation with *Bacillus subtilis* or *Enterococcus faecalis* improved growth performance and nutrient digestibility while increasing nitrogen deposition in growing blue foxes. While numerous studies have documented the use of *Bacillus subtilis* and *Enterococcus faecalis* in pigs and rabbits [2-10], no reports exist regarding their application in minks. Therefore, this study investigated the effects of these probiotics on growth performance, nutrient digestibility, and nitrogen metabolism in growing minks to determine optimal supplementation levels and provide a theoretical basis for their practical application in mink production.

1.1 Experimental Materials

The probiotics used in this experiment were provided by Beijing Scitechful Bio-Technology Co., Ltd. Laboratory analysis confirmed that *Bacillus subtilis* contained viable bacteria 1×10^{10} CFU/g, and *Enterococcus faecalis* contained viable bacteria 1×10^{10} CFU/g.

1.2 Experimental Animals and Diets

Seventy healthy, 60-day-old male minks with similar body weight [(957.37 ± 93.96) g] were randomly selected from the fur animal production base at the Ministry of Agriculture' s Changbai Mountain Wildlife Resources Field Scientific Observation and Testing Station. As no unified feeding standards for minks currently exist in China, the basal diet for the growing period was formulated based on recent research [11-12]. The composition and nutrient levels are presented in Table 1 , with the diet provided in dry powder form.

1.3 Experimental Design and Management

A single-factor completely randomized design was employed. The 70 minks were randomly divided into 7 groups with 10 replicates per group (1 mink per replicate). Group I served as the control, receiving the basal diet. Groups II, III, and IV received the basal diet supplemented with *Bacillus subtilis* at 1×10^{9}, 1×10^{10}, and 1×10^{11} CFU/kg, equivalent to 0.1, 1.0, and 10.0 g per kg of diet, respectively. Groups V, VI, and VII received the basal diet supplemented with *Enterococcus faecalis* at 1×10^{8}, 1×10^{9}, and 1×10^{10} CFU/kg, equivalent to 0.01, 0.10, and 1.00 g per kg of diet, respectively. The adaptation period lasted 7 days, followed by a 60-day experimental period.

Prior to the experiment, all minks received routine vaccinations. Animals were housed individually in cages and fed twice daily at 08:00 and 15:00. Both probiotics were added daily during the trial period by first dissolving them in water, then mixing with a portion of the diet before feeding. Fresh water was available ad libitum. The experiment was conducted at the Ministry of Agriculture' s Changbai Mountain Wildlife Resources Field Scientific Observation and Testing Station from July 10 to September 14, 2016.

1.4 Digestion and Metabolism Trial

On day 42 of the experiment, six minks with similar body weight were selected from each group for a digestion and metabolism trial conducted from August 27 to August 29, 2016 (3 days). The total fecal collection method was employed, with management practices identical to routine feeding. Urine was collected daily, with 2 mL of 10% sulfuric acid and 4 drops of toluene added per 100 mL as preservative, then stored at -20°C. Fresh feces were weighed daily, and 10% sulfuric acid (5% of fresh weight) was added with a small amount of toluene as preservative, then stored at -20°C. After 3 days, urine and fecal samples were thoroughly mixed. Fecal samples were dried at 65°C to constant weight, 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 recorded after overnight fasting on day 1 of the experimental period, with subsequent weighings every 15 days to calculate average daily gain. Daily feed allowance and refusals were recorded to determine average daily feed intake and feed-to-gain ratio for each group.

Dry matter content in diets and excreta was determined by oven drying according to GB/T 6435-2006 [13]. Crude fat content was measured by Soxhlet extraction using a Soxhlet apparatus according to GB/T 6433-2006 [14]. Crude protein content was determined by the Kjeldahl method using a FOSS Kjeldahl analyzer according to GB/T 6432-1994 [15]. Nutrient digestibility was calculated using the method described by Zhang [16].

Calculation formulas for various indicators were as follows: - Average daily gain (g/d) = (final weight - initial weight) / trial days - Average daily feed intake (g/d) = total feed intake during trial / trial days - Feed-to-gain ratio = average daily feed intake / average daily gain - Dry matter digestibility (%) = [(dry matter intake - dry matter excretion) / dry matter intake] × 100 - Protein digestibility (%) = [(protein intake - fecal protein content) / protein intake] × 100 - Fat digestibility (%) = [(fat intake - fecal fat content) / fat intake] × 100 - Nitrogen deposition (g/d) = nitrogen intake - fecal nitrogen - urinary nitrogen - Net protein utilization (%) = (nitrogen deposition / nitrogen intake) × 100 - Protein biological value (%) = [nitrogen deposition / (nitrogen intake - fecal nitrogen)] × 100

Data were expressed as means ± standard deviation and analyzed using SPSS 22.0 software. One-way ANOVA was performed for significance testing, with $P<0.05$ considered statistically significant.

2.1 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Growth Performance of Growing Minks

As shown in Table 2 , the final body weight of all treatment groups was higher than that of the control group, with group III being significantly higher ($P<0.05$). No significant differences were observed in average daily gain or feed-to-gain ratio among groups ($P>0.05$). However, average daily gain in all treatment groups exceeded that of the control group by 13.19%, 15.65%, 9.37%, 13.98%, 9.61%, and 7.15%, respectively. Feed-to-gain ratios in all treatment groups were lower than the control group by 12.75%, 14.40%, 15.03%, 15.23%, 13.89%, and 5.70%, respectively. Average daily feed intake in all treatment groups was lower than the control group, with group IV being significantly lower ($P<0.05$) but not differing significantly from other treatment groups ($P>0.05$).

2.2 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Nutrient Digestibility of Growing Minks

Table 3 shows that dry matter intake in all treatment groups was slightly lower than the control group, though not significantly different ($P>0.05$). Dry matter excretion in all treatment groups was lower than the control group, with group V being significantly lower ($P<0.05$), while no significant differences existed among treatment groups ($P>0.05$). Dry matter digestibility, protein digestibility, and fat digestibility in all treatment groups were higher than the control group. Specifically, dry matter digestibility in groups II, III, and V was significantly higher than in group I ($P<0.05$), with group V being significantly higher than groups I, IV, VI, and VII ($P<0.05$). Protein digestibility in group V was significantly higher than in groups I and VII ($P<0.05$). No significant differences in fat digestibility were detected among groups ($P>0.05$).

2.3 Effects of Dietary Bacillus subtilis or Enterococcus faecalis on Nitrogen Metabolism of Growing Minks

Table 4 indicates that nitrogen intake, fecal nitrogen, and urinary nitrogen in all treatment groups were lower than the control group, though nitrogen intake differences were not significant (P>0.05). Fecal nitrogen content in groups IV, V, and VI was significantly lower than in group I (P<0.05), while urinary nitrogen content in groups V and VI was significantly lower than in group I (P<0.05). Although nitrogen deposition in all treatment groups exceeded that of the control group, the differences were not statistically significant (P>0.05). Net protein utilization and protein biological value in all treatment groups were higher than the control group, with groups III and V showing significantly higher net protein utilization (P<0.05) and group III demonstrating significantly higher protein biological value (P<0.05).

3.1 Effects on Growth Performance

Previous studies have demonstrated that dietary probiotics promote animal growth and improve feed conversion efficiency [5-8]. Zhou et al. [17] reported that various inclusion levels of *Bacillus subtilis* enhanced growth performance and reduced feed-to-gain ratio in weaned piglets. Wei [6] found that different levels of *Enterococcus faecalis* improved growth performance in nursery piglets. Gong et al. [18] observed that dietary *Bacillus subtilis* or *Enterococcus faecalis* increased final body weight and reduced feed-to-gain ratio in growing blue foxes. The current results align with these findings, showing that dietary supplementation with *Bacillus subtilis* or *Enterococcus faecalis* improved growth performance in growing minks. However, Liu et al. [19] reported that probiotic supplementation (0.06 g *Bacillus licheniformis* + 0.06 g *Bacillus subtilis*) did not significantly improve mink growth performance, suggesting no necessity for probiotic inclusion. Such discrepancies may be attributed to variations in probiotic inclusion levels, dietary composition, physiological status, and environmental conditions [20]. The observation that growth rate decreased with increasing *Bacillus subtilis* levels indicates that higher supplementation does not necessarily yield better probiotic effects, consistent with findings from Guo et al. [21] on blue foxes. The optimal growth performance with *Enterococcus faecalis* occurred at 1\$×10^{8}\$ CFU/kg, which was the lowest tested level, suggesting that the optimal inclusion rate requires further investigation.

3.2 Effects on Nutrient Digestibility

Feed intake in minks is influenced by diet palatability and energy level [22]. The current results demonstrate that probiotic supplementation reduced feed-to-gain ratio without significantly affecting dry matter intake. Li et al. [23] reported that microecological preparations (containing *Lactobacillus*, *Bacillus*, and yeast) significantly improved apparent crude protein digestibility and tended to increase apparent crude fat digestibility in growing pigs. Giang et al. [24] showed that dietary probiotics significantly enhanced nutrient digestibility in weaned piglets. Jing et al. [25] observed that *Lactobacillus* supplementation improved dry matter, protein, and fat digestibility in minks. These findings are consistent with the present results, where *Bacillus subtilis* supplementation increased nutrient digestibility across all treatment groups compared to the control, with the 1×10^{10} CFU/kg level showing the best effect–significantly improving dry matter digestibility and increasing protein and fat digestibility by 1.77% and 2.79%, respectively. *Bacillus subtilis* can synthesize amylase, protease, lipase, and cellulase, which work synergistically with the animal' s endogenous digestive enzymes to enhance nutrient absorption and digestibility [18]. Among *Enterococcus faecalis* treatments, the 1×10^{8} CFU/kg level proved most effective, significantly improving dry matter and protein digestibility compared to the control. Higher inclusion levels, however, reduced nutrient digestibility, possibly because excessive supplementation disrupted intestinal microflora balance and decreased beneficial bacteria populations [26-27]. Research indicates that probiotics promote intestinal development in weaned piglets by increasing mucosal thickness, villus length, and crypt depth, thereby expanding absorptive surface area and facilitating growth [3]. Thus, probiotic supplementation likely enhanced intestinal development and absorptive capacity in minks, leading to improved nutrient digestibility.

3.3 Effects on Nitrogen Metabolism

Nitrogen balance serves as a crucial indicator of protein metabolism [22]. Jing et al. [25] demonstrated that *Lactobacillus* supplementation reduced fecal and urinary nitrogen while increasing net protein utilization and decreasing blood urea nitrogen in minks. Guo et al. [21] reported that appropriate probiotic levels improved net protein utilization and protein biological value in blue foxes. The current results show that while nitrogen intake did not differ significantly among groups, fecal and urinary nitrogen in all treatment groups were lower than the control, with groups IV, V, and VI showing significantly reduced fecal nitrogen ($P<0.05$) and groups V and VI exhibiting significantly lower urinary nitrogen ($P<0.05$). Enhanced protein digestibility from probiotic supplementation reduced fecal nitrogen excretion. Studies have shown that probiotics decrease serum urea nitrogen [7,28], and the reduced urinary nitrogen observed here may result from promoted urea cycling and reduced urea excretion. Livestock excreta constitute a major source of environmental nitrogen pollution, which adversely affects animal health and local ecosystems if improperly managed. The reduction in fecal and urinary nitrogen through probiotic supplementation offers a novel approach to mitigating environmental contamination from farming operations. All treatment groups showed higher nitrogen deposition, net protein utilization, and protein biological value than the control, with the 1×10^{10} CFU/kg *Bacillus subtilis* level producing the highest nitrogen deposition and significantly improved net protein utilization and protein biological value. These results confirm that dietary probiotics can enhance nitrogen deposition, net protein utilization, and protein biological value in minks.

In conclusion, dietary supplementation with 1×10^{10} CFU/kg *Bacillus subtilis* or 1×10^{8} CFU/kg *Enterococcus faecalis* optimally improves growth performance, nutrient digestibility, nitrogen deposition, net protein utilization, and protein biological value in growing minks.

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