Effects of Selenium-Enriched Yeast and Bacillus subtilis on Small Intestinal Mucosal Morphology and Rectal Microbiota in Hu Sheep Lambs (Post-print)

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Abstract

This experiment aimed to investigate the effects of selenium-enriched yeast and *Bacillus subtilis* on small intestinal mucosal morphology and rectal microbiota in weaned Hu lambs. Twenty-one weaned Hu lambs in good health condition with a body weight of (9.65±0.38) kg were randomly allocated into 3 groups: a control group, a selenium-enriched yeast group, and a *Bacillus subtilis* group, with 7 lambs per group. The control group was fed a basal diet, while the experimental groups received selenium-enriched yeast and *Bacillus subtilis* preparations added to the concentrate at a rate of 100 g/t. The experimental period was 28 days. The results showed that: 1) Compared with the control group, both the selenium-enriched yeast and *Bacillus subtilis* groups exhibited significantly increased villus height in the duodenum, jejunum, and ileum ($P<0.05$), significantly decreased crypt depth in the duodenum and ileum ($P<0.05$), extremely significantly decreased crypt depth in the jejunum ($P<0.01$), extremely significantly increased villus height to crypt depth ratio (V/C) in the duodenum and jejunum ($P<0.01$), and significantly increased V/C in the ileum ($P<0.05$). 2) The supplementation of selenium-enriched yeast and *Bacillus subtilis* influenced the -diversity of the rectal microbiota in Hu lambs, leading to different abundance variations at the class, order, family, genus, and species levels. In conclusion, dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can promote the development of various segments of the small intestine in Hu lambs, increase the abundance of beneficial rectal microbiota, and reduce the proliferation of harmful microbiota.

Full Text

Preamble

Title: Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Small Intestinal Mucosal Morphology and Rectal Microbiota in Hu Sheep Lambs

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Abstract: This study investigated the effects of selenium-enriched yeast and *Bacillus subtilis* on small intestinal mucosal morphology and rectal microbiota in weaned Hu sheep lambs. Twenty-four healthy weaned lambs with good body condition were randomly divided into three groups: control, selenium-enriched yeast, and *B. subtilis* groups, with eight lambs per group. The control group was fed a basal diet, while the treatment groups received selenium-enriched yeast or *B. subtilis* preparations added to the concentrate. The trial period lasted for the duration of the experiment. The results showed: (1) Compared with the control group, both the selenium-enriched yeast and *B. subtilis* groups exhibited significantly increased villus height in the duodenum, jejunum, and ileum, significantly decreased crypt depth in the duodenum and ileum, extremely significantly decreased crypt depth in the jejunum, extremely significantly increased villus height to crypt depth ratio (V/C) in the duodenum and jejunum, and significantly increased V/C ratio in the ileum. (2) Supplementation with selenium-enriched yeast and *B. subtilis* affected the -diversity of rectal microbiota, resulting in different abundance patterns at the class, order, family, genus, and species levels. In conclusion, dietary supplementation with selenium-enriched yeast and *B. subtilis* can promote the development of all small intestinal segments in Hu sheep lambs, increase the abundance of beneficial rectal microbiota, and reduce the proliferation of harmful bacteria.

Keywords: selenium-enriched yeast; *Bacillus subtilis*; Hu sheep lambs; small intestinal mucosal morphology; rectal microbiota

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Species Accumulation Curves and N Curve Analysis

Species accumulation curves describe the increase in species with sampling effort and are widely used to assess sampling adequacy and estimate species richness. Microbial N curves can reveal species diversity in samples.

As shown in [Figure 2: see original paper], the species accumulation curve constructed from the number of randomly sampled sequences versus observed species gradually plateaued, indicating that the effective sequencing depth adequately covered the diversity of intestinal bacterial species in all experimental

lambs. After ranking OTU abundance in each sample, [Figure 3: see original paper] shows that the vertical axis of the N curves for all samples gradually flattened, indicating uniform species distribution suitable for diversity analysis and providing a foundation for subsequent analysis.

Species Annotation Analysis

Through database comparison, OTUs were taxonomically classified and analyzed at phylum, class, order, family, genus, and species levels.

[Figure 4: see original paper] intuitively reflects the relative abundance of different phyla in each sample. The results show that Firmicutes was the most abundant, followed by Actinobacteria, Proteobacteria, and Euryarchaeota, indicating these four phyla are the dominant bacterial groups in the rectum of Hu sheep lambs.

Class Level: As shown in , compared with the control group, the relative abundance of Actinobacteria significantly increased ($P < 0.05$) in both treatment groups, while Clostridia and Gammaproteobacteria significantly decreased ($P < 0.05$). Bacilli, Methanobacteria, and Alphaproteobacteria showed no significant difference but exhibited increasing trends.

Order Level: As shown in , Bifidobacteriales, Clostridiales, Erysipelotrichales, Methanobacteriales, Bacteroidales, and Coriobacteriales were the major orders in each sample. Compared with the control group, the relative abundance of Bifidobacteriales, Methanobacteriales, and Coriobacteriales significantly increased ($P < 0.05$), while Clostridiales significantly decreased ($P < 0.05$).

Family Level: As shown in , Bifidobacteriaceae, Lachnospiraceae, Methanobacteriaceae, Ruminococcaceae, and Erysipelotrichaceae were the major families. Compared with the control group, Bifidobacteriaceae and Erysipelotrichaceae significantly increased ($P < 0.05$), while Ruminococcaceae significantly decreased ($P < 0.05$). In the selenium-enriched yeast group, Lachnospiraceae and Methanobacteriaceae significantly decreased ($P < 0.05$).

Genus Level: As shown in , compared with the control group, the relative abundance of *Bifidobacterium*, *Blautia*, and *Lactobacillus* significantly increased ($P < 0.05$), while *Oscillospira*, *Clostridium*, *Coprococcus*, and *Desulfovibrio* significantly decreased ($P < 0.05$). In the selenium-enriched yeast group, *Megasphaera* significantly increased ($P < 0.05$). In the *B. subtilis* group, *Butyrivibrio* and *Methanobrevibacter* significantly increased ($P < 0.05$).

Species Level: As shown in , compared with the control group, the relative abundance of certain species significantly increased while others decreased, consistent with the patterns observed at higher taxonomic levels.

Discussion

Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Small Intestinal Mucosal Morphology

The small intestine is a crucial organ for nutrient digestion and absorption in animals. The developmental status of small intestinal mucosa is not only closely related to nutrient intake but also participates in intestinal mucosal immune responses. Villus height, crypt depth, and the V/C ratio are important indicators for measuring small intestinal absorptive function. Previous studies have demonstrated that dietary yeast supplementation significantly promotes duodenal development in finishing pigs. Research by [citation] showed that selenium-enriched yeast supplementation in ewes' diets significantly promoted jejunal development in their offspring. Qi et al. [citation] reported that *B. subtilis* significantly increased duodenal villus height and V/C ratio in broiler chickens. [citation] found that *B. subtilis* supplementation significantly increased jejunal crypt depth in broilers. Qi et al. [citation] observed that adding *B. subtilis* to the diet of 1-day-old broilers significantly increased duodenal villus height and V/C ratio. Zhou [citation] also reported that *B. subtilis* supplementation significantly increased villus height, V/C ratio, mucosal thickness, and muscular layer thickness in all small intestinal segments of broilers. The present results demonstrate that dietary supplementation with selenium-enriched yeast and *B. subtilis* in Hu sheep lambs significantly increased villus height in the duodenum, jejunum, and ileum, significantly decreased crypt depth in the duodenum and ileum, and extremely significantly increased the V/C ratio in the duodenum and jejunum, indicating a significant promoting effect on the development of all small intestinal segments.

Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Rectal Microbiota

Intestinal microbiota in animals can digest and metabolize nutrients for the host, promote intestinal development, resist pathogens, and participate in immune function development. [citation] investigated the effects of selenium-enriched yeast on gut microecology in piglets under high-temperature conditions and found that *Lactobacillus* content increased extremely significantly. [citation] reported that yeast increased microbial quantity and diversity in rat intestines and improved intestinal immune function. Liu et al. [citation] studied the effects of selenium yeast on broiler intestinal microbiota and found that it enhanced antioxidant capacity and improved microbiota structure. Zheng et al. [citation] reported that *Bacillus* isolated from soil significantly increased intestinal *Peptococcus*, *Bifidobacterium*, *Lactobacillus*, and total anaerobic bacteria in broilers. [citation] confirmed that *B. subtilis* supplementation significantly increased intestinal *Lactobacillus* and decreased *Escherichia coli* in broilers, demonstrating its ability to promote beneficial bacteria proliferation and reduce harmful bacteria. Wu et al. [citation] noted that *B. subtilis* significantly increased *Bacillus* proportion, total anaerobes, *Bifidobacterium*, and *Lactobacillus* in grass carp intestines.

Ren [citation] reported that various *Bacillus* doses significantly increased *Lactobacillus* in cecum or colon. The present results show that both treatment groups exhibited significantly increased relative abundance of Actinobacteria, significantly increased Bifidobacteriales, Methanobacteriales, and Coriobacteriales, and significantly increased Bifidobacteriaceae and Erysipelotrichaceae. Studies have shown that *Bifidobacterium* and *Lactobacillus* can ferment carbohydrates, promote nutrient digestion and absorption, lower intestinal pH, inhibit pathogen growth and invasion, protect the intestinal mucosal barrier, and enhance host immune function. [citation] confirmed that *Clostridium* can produce exotoxins with strong toxic effects on humans and animals. In this study, the relative abundance of Clostridiales in the rectum significantly decreased in both treatment groups. Therefore, these results demonstrate that selenium-enriched yeast and *B. subtilis* can increase beneficial bacteria, inhibit harmful bacteria proliferation, improve rectal microecology, promote digestive function, and enhance immunity in lambs.

Conclusion

Dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can significantly increase small intestinal villus height and V/C ratio, promoting the development of all small intestinal segments in Hu sheep lambs. These supplements can also increase the abundance of beneficial rectal microbiota, inhibit harmful bacteria proliferation, and enhance lamb immunity.

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