Effects of Selenium-Enriched Yeast and Bacillus subtilis on Growth Performance, Serum Biochemical Parameters, and Digestive Function in Hu Sheep Lambs (Postprint)

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Abstract

This experiment was conducted to investigate the effects of selenium-enriched yeast and *Bacillus subtilis* on growth performance, serum indices, nutrient apparent digestibility, pancreatic digestive enzyme activity, and rumen fermentation parameters in weaned Hu lambs. Twenty-one weaned Hu lambs in good body condition with a body weight of (9.65$±$0.38) kg were randomly divided into three groups, namely a control group, a selenium-enriched yeast group (supplemented with 100 g/t selenium-enriched yeast in the concentrate), and a *Bacillus subtilis* group (supplemented with 100 g/t *Bacillus subtilis* in the concentrate), with 7 lambs per group. The experimental period lasted 28 days. The results showed that, compared with the control group: 1) The final body weight and average daily gain of lambs in both the selenium-enriched yeast and *Bacillus subtilis* groups were significantly (P<0.05) or extremely significantly (P<0.01) increased, while the feed conversion ratio for concentrate and forage was significantly (P<0.05) or extremely significantly (P<0.01) decreased. 2) The concentrations of immunoglobulin A, immunoglobulin G, immunoglobulin M, and interleukin-6, as well as glutathione peroxidase activity in serum were significantly (P<0.05) or extremely significantly (P<0.01) increased in both the selenium-enriched yeast and *Bacillus subtilis* groups; the serum malondialdehyde concentration in the selenium-enriched yeast group was significantly decreased (P<0.05), while serum interleukin-1 concentration and superoxide dismutase activity were significantly (P<0.05) or extremely significantly (P<0.01) increased; the concentrations of interleukin-2 and interferon-gamma in the *Bacillus subtilis* group were significantly (P<0.05) or extremely significantly (P<0.01) increased. 3) The apparent digestibility of dry matter, crude protein, acid detergent fiber, and neutral detergent fiber in both the selenium-enriched yeast and *Bacillus*

subtilis groups were significantly (P<0.05) or extremely significantly (P<0.01) increased, and the apparent digestibility of crude fat in the *Bacillus subtilis* group was significantly increased (P<0.05). 4) Pancreatic lipase in the *Bacillus subtilis* group and pancreatic trypsin in the selenium-enriched yeast group were significantly increased (P<0.05). 5) The concentrations of ammonia nitrogen, acetate, propionate, isobutyrate, and n-butyrate in rumen fluid of lambs in both the selenium-enriched yeast and *Bacillus subtilis* groups were significantly increased (P<0.05). The results confirmed that dietary supplementation of selenium-enriched yeast and *Bacillus subtilis* in weaned Hu lambs could improve growth performance and antioxidant capacity, and enhance immunity and digestive function.

Full Text

Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Growth Performance, Serum Indices, and Digestive Function of Hu Lambs

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Abstract: This experiment was conducted to investigate the effects of selenium-enriched yeast and *Bacillus subtilis* on growth performance, serum indices, nutrient apparent digestibility, pancreatic digestive enzyme activities, and rumen fermentation parameters in weaned Hu lambs. Twenty-one healthy weaned Hu lambs with an initial body weight of $(9.65\$\pm0.38)kgwererandomlyallocatedintothreegroups:acontrolgroup,aselenium-enrichedyeastgroup(supplementedwith100g/tselenium-enrichedyeastinconcentrate),anda*Bacillussubtilis*group(supplementedwith100g/t*Bacillussubtilis*inconcentrate),withsevenlambspergroup.Theexperimentalperiodlasted28days.Theresultsshowedthatcompa$
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$0.05)andsignificantlyorextremelysignificantlyincreasedseruminterleukin-1(IL-1)contentandsuperoxidedismutase(SOD)activity(P<0.05orP<0.01);the*Bacillussubtilis*groupshowedsignificantlyorextremelysignificantlyincreasedseruminterleukin-$
$2(IL-2)andinterferon-$ \$ (IFN-γ) contents (P<0.05 or P<0.01). (3) Apparent digestibility of dry matter, crude protein, acid detergent fiber, and neutral detergent fiber were significantly or extremely significantly improved (P<0.05

or P<0.01), while the *Bacillus subtilis* group also showed significantly enhanced crude fat apparent digestibility (P<0.05). (4) Pancreatic lipase activity in the *Bacillus subtilis* group and pancreatic trypsin activity in the selenium-enriched yeast group were significantly increased (P<0.05). (5) Rumen fluid ammonia nitrogen, acetic acid, propionic acid, isobutyric acid, and n-butyric acid contents were significantly elevated in both treatment groups (P<0.05). These results demonstrate that dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can improve growth performance, antioxidant capacity, immunity, and digestive function in weaned Hu lambs.

Keywords: Hu sheep; selenium-enriched yeast; *Bacillus subtilis*; growth performance; serum indices; apparent digestibility; rumen fermentation parameters

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At present, antibiotic use in Chinese livestock farms has led to increasing bacterial resistance, reduced disease resistance in animals, and drug residues in animal products, which severely restricts the development of the livestock industry. Consequently, developing green, safe, highly effective, and residue-free antibiotic alternatives has become a research hotspot and an inevitable trend in livestock production.

Microecological preparations are products made from normal microorganisms or substances that promote microbial growth. Due to their non-residual nature in animals and their ability to improve animal health, productivity, and immunity, these preparations are gradually being widely applied [1]. Dietary supplementation with selenium-enriched yeast can improve growth performance, nutrient apparent digestibility, and antioxidant capacity in finishing pigs [2]. *Bacillus subtilis* can improve the intestinal microbial environment, promote adequate nutrient absorption, enhance feed utilization efficiency, and reduce costs [3]. Additionally, *Bacillus subtilis* can improve animal resistance, create better conditions for the growth and reproduction of other beneficial bacteria, and enhance immunity by inhibiting pathogenic bacteria [4].

Weaned Hu lambs often experience significant stress, reduced intake of starter feed and milk replacer, resulting in growth retardation and diarrhea. However, few studies have investigated the effects of selenium-enriched yeast and *Bacillus subtilis* on Hu lambs. Therefore, this experiment used 45-day-old weaned Hu lambs to explore the effects of dietary selenium-enriched yeast and *Bacillus subtilis* supplementation on growth performance, serum indices, nutrient apparent digestibility, pancreatic digestive enzyme activities, and rumen fermentation parameters, providing an experimental basis for the scientific application of microecological preparations in sheep production.

1. Materials and Methods

1.1 Experimental Materials and Animals

The *Bacillus subtilis* preparation used in this experiment was purchased from Zhuzhou Zhihui Biotechnology Co., Ltd., with a viable count of 1$×10^{10}$ CFU/g. Selenium-enriched yeast was purchased from Angel Yeast Co., Ltd., with a selenium content of 2,000 mg/kg. The experimental animals were 45-day-old weaned Hu lambs. Both the basal diet and experimental animals were provided by Jinong Animal Husbandry Co., Ltd. in Ma' anshan City, Anhui Province.

1.2 Experimental Design

Twenty-one 45-day-old weaned Hu lambs with good health status and an average body weight of (9.65$±$0.38) kg were randomly divided into three groups: a control group, a selenium-enriched yeast group, and a *Bacillus subtilis* group, with seven lambs per group. The experimental period was 28 days. The diet was formulated according to the *Feeding Standard of Meat Sheep*. The control group was fed the basal diet, while the experimental groups received the basal diet supplemented with 100 g/t selenium-enriched yeast or 100 g/t *Bacillus subtilis* preparation in the concentrate, respectively. The composition and nutrient levels of the basal diet are shown in Table 1 .

1.3 Feeding Management

The experiment was conducted at the sheep farm of Jinong Animal Husbandry Co., Ltd. in Ma' anshan City, Anhui Province. During the pre-feeding period, all lambs were vaccinated with a triple vaccine and dewormed with ivermectin injection (0.2 mg/kg). Seven lambs were housed per pen with adequate space for movement. Concentrate premix and forage were fed at fixed times daily. The feeding amount was adjusted daily based on the previous day' s residual feed to ensure ad libitum intake, with regular disinfection maintained throughout the trial.

1.4.1 Growth Performance

Daily feed intake was accurately recorded for each group, and lamb growth was monitored. Body weight was measured on days 1, 7, 14, 21, and 28 of the experimental period to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.2 Serum Biochemical Indices

On days 1, 14, and 28 of the experimental period, 10 mL of blood was collected from the jugular vein of all lambs after overnight fasting. After standing for 30 minutes, serum was collected by centrifugation at 3,000 r/min and stored at

-20°C for subsequent analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities, and total protein (TP), albumin (ALB), globulin (GLB), urea (UREA), total cholesterol (TC), and triglyceride (TG) contents were determined using a Mindray BSL-220 automatic biochemical analyzer. Serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, and malondialdehyde (MDA), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were measured using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits provided by Nanjing Senbeijia Biological Technology Co., Ltd., following the manufacturer' s instructions.

1.4.3 Nutrient Apparent Digestibility

During the last three days of the experimental period, three lambs were randomly selected from each group for a digestion and metabolism trial. Lambs in each group were fed from the same feed trough, and feed intake was accurately calculated based on residual feed. Fecal samples were collected via rectal sampling, accurately weighed, mixed, and 20% of the total weight was taken as a sample. Samples were preserved with 10% hydrochloric acid at 100 mL/kg and stored at -20°C. Samples were then dried at 65°C for 48 hours, ground, and equilibrated at room temperature for 24 hours before being bagged for analysis. Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents in feed and fecal samples were determined according to GB/T 6435-2006, GB/T 6432-1994, GB/T 6433-2006, GB/T 20806-2006, and NY/T 1459-2007, respectively.

Nutrient apparent digestibility (%) = 100 × (feed intake × nutrient content in feed - fecal output × nutrient content in feces) / (feed intake × nutrient content in feed).

1.4.4 Pancreatic Digestive Enzyme Activity

At the end of the experimental period, three lambs from each group were randomly selected for slaughter after fasting and weighing. Pancreas samples were collected according to the methods described in *Livestock Anatomy and Histology* [6], accurately weighed and recorded. Approximately 0.2 g of pancreatic tissue was taken from the head, body, and tail regions, mixed with 1.8 mL of physiological saline, and homogenized to prepare a 10% tissue homogenate using a high-speed homogenizer. The homogenate was centrifuged at 2,500 r/min for 15 minutes at 4°C, and the supernatant was collected for analysis. Pancreatic amylase, lipase, and trypsin activities were determined using kits from Nanjing Senbeijia Biological Technology Co., Ltd., following the manufacturer' s instructions. Amylase activity was defined as: under 37°C conditions, 1 unit (U/g) was defined as the amount of enzyme that hydrolyzed 10 mg of starch in 30 minutes per mg of tissue protein. Lipase activity was defined as: under 37°C conditions, 1 unit (U/g) was defined as the consumption of 1 mol of substrate

per minute per mg of tissue protein. Trypsin activity was defined as: under 37°C conditions, 1 unit (U/g) was defined as the production of 1 g of amino acids per minute per mg of tissue protein.

1.4.5 Rumen Fermentation Parameters

At the end of the experimental period, three lambs from each group were randomly selected for slaughter, and rumen fluid was collected, filtered through three layers of gauze, and centrifuged at 1,500 r/min for 15 minutes. The supernatant was stored at -20°C for analysis.

Rumen fluid ammonia nitrogen (NH_3-N) content was determined using an improved colorimetric method [7]. Volatile fatty acid (VFA) content was detected using gas chromatography-mass spectrometry (GC-MS ISQ LT) [column (TG-WAX, 30 m$×$0.25 mm, 0.25 m); autosampler TriPlus RSH; detector MS ISQ. Standards included: acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric acid, and n-valeric acid].

Chromatographic conditions: carrier gas helium (constant flow mode, total flow 0.8 mL/min); injector temperature 200°C; ion source temperature 200°C; transfer line temperature 250°C; column temperature 120°C (6 min) - 5°C/min - 150°C (2 min); split ratio 75:1; mass spectrometry: EI source; ionization voltage: 70 eV; single ion monitoring mode: quantitative ions 60, 73; temperature program: initial temperature 120°C for 6 min, increased to 150°C at 5°C/min, held for 2 min; injection volume 1 L.

1.5 Statistical Analysis

Experimental data were analyzed using SPSS 17.0 statistical software with one-way ANOVA, followed by LSD multiple comparison tests. Data were expressed as means ± standard deviation. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2. Results

2.1 Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Growth Performance

As shown in Table 2 , initial body weight did not differ significantly among groups ($P>0.05$). Compared with the control group, final body weight was extremely significantly increased in both the selenium-enriched yeast and *Bacillus subtilis* groups ($P<0.01$), while average daily gain was significantly increased ($P<0.05$). Average daily feed intake did not change significantly ($P>0.05$), but feed-to-gain ratios for both concentrate and forage were significantly or extremely significantly decreased ($P<0.05$ or $P<0.01$).

2.2 Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Serum Indices

As shown in Table 3 and Table 4 , on day 28, serum ALB, GLB, TP, TC, TG, and UREA contents, as well as ALT, AST, ALP, and LDH activities, did not differ significantly among groups ($P>0.05$). However, serum IgA, IgG, IgM, and IL-6 contents, and GSH-Px activity were significantly increased in both treatment groups ($P<0.05$). The selenium-enriched yeast group showed significantly reduced serum MDA content ($P<0.05$), significantly increased serum IL-1 content ($P<0.05$), and extremely significantly increased serum SOD activity ($P<0.01$). The *Bacillus subtilis* group exhibited extremely significantly increased serum IL-2 content ($P<0.01$) and significantly increased serum IFN-γ content ($P<0.05$).

2.3 Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Nutrient Apparent Digestibility

As shown in Table 5 , compared with the control group, apparent digestibility of crude protein, acid detergent fiber, and neutral detergent fiber were significantly increased in both treatment groups ($P<0.05$), while dry matter apparent digestibility was extremely significantly increased ($P<0.01$). Additionally, the *Bacillus subtilis* group showed significantly improved crude fat apparent digestibility ($P<0.05$).

2.4 Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Pancreatic Digestive Enzyme Activities

As shown in Table 6 , compared with the control group, pancreatic lipase activity in the *Bacillus subtilis* group and pancreatic trypsin activity in the selenium-enriched yeast group were significantly increased ($P<0.05$). Pancreatic amylase activity did not differ significantly among groups ($P>0.05$), although numerical increases were observed.

2.5 Effects of Selenium-Enriched Yeast and *Bacillus subtilis* on Rumen Fermentation Parameters

As shown in Table 7 , compared with the control group, rumen fluid NH_3-N, acetic acid, propionic acid, isobutyric acid, and n-butyric acid contents were significantly increased in both treatment groups ($P<0.05$). Isovaleric acid and n-valeric acid contents did not differ significantly ($P>0.05$), though numerical improvements were noted.

3. Discussion

3.1 Effects on Growth Performance of Hu Lambs

Microecological preparations can promote the growth and reproduction of dominant anaerobic bacteria in intestinal digestion and absorption, thereby enhanc-

ing nutrient metabolism and absorption and improving growth performance. Shi et al. [8] found that selenium-enriched yeast supplementation significantly improved growth performance in Taibai Black goat kids. Stewart [9] reported that maternal dietary selenium-enriched yeast supplementation significantly increased average daily gain in lambs. Qiu et al. [10] demonstrated that dietary *Bacillus subtilis* reduced feed-to-gain ratio and improved growth performance in lambs. These findings are consistent with our results. However, some studies have reported no significant effects of microecological preparations on growth performance [11]. These discrepancies may be related to strain characteristics, supplementation rates, animal age, physiological status, and nutritional factors. Our results indicate that dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can reduce feed-to-gain ratio and significantly improve lamb growth performance. Further research is needed to determine optimal dosages and application stages for maximum efficacy.

3.2 Effects on Serum Indices of Hu Lambs

No significant differences were observed in serum TP, ALB, and GLB contents or ALT and AST activities among the control, selenium-enriched yeast, and *Bacillus subtilis* groups, indicating that microecological preparations did not significantly affect serum protein levels. Cytokines are important regulatory factors in the immune system that influence the type and level of immune response [12]. Wang [13] found that yeast culture infusion increased serum IgA, IgG, and IL-6 contents and significantly elevated serum IL-2 and IFN-γ contents in sheep. Rajput et al. [14] reported that *Bacillus subtilis* increased serum IL-2 content in Shaoxing ducks. Hall et al. [15] demonstrated that selenium supplementation increased serum IgG content in dairy cows. In our study, serum IgA, IgG, and IgM contents were significantly higher in both treatment groups than in the control group, indicating that microecological preparations can enhance lamb immunity.

Serum GSH-Px and SOD activities are important indicators of antioxidant capacity. Liu et al. [16] found that dietary selenium-enriched yeast significantly increased serum GSH-Px and SOD activities while reducing serum MDA content. Faixová et al. [17] reported that dietary selenium-enriched yeast significantly increased serum GSH-Px activity in sheep. Ren et al. [18] demonstrated that dietary *Bacillus subtilis* significantly increased serum GSH-Px activity and reduced serum MDA content. In our experiment, both treatment groups showed significantly increased serum GSH-Px and SOD activities and decreased serum MDA content, indicating that selenium-enriched yeast and *Bacillus subtilis* can improve antioxidant capacity in weaned Hu lambs.

3.3 Effects on Nutrient Apparent Digestibility and Pancreatic Enzymes

Numerous studies have shown that appropriate supplementation with selenium-enriched yeast and *Bacillus subtilis* can significantly improve nutrient appar-

ent digestibility in livestock and poultry. Zhang et al. [19] found that dietary selenium-enriched yeast improved acid detergent fiber apparent digestibility in dairy cows. Molnár et al. [20] reported that dietary *Bacillus subtilis* improved feed conversion efficiency in broilers. Cho et al. [21] demonstrated that dietary *Bacillus subtilis* improved dietary protein digestibility in pigs. Microecological preparations produce digestive enzymes and growth-promoting factors in the intestine that synergistically enhance nutrient digestion and absorption. In our study, nutrient apparent digestibility was significantly improved in both treatment groups. However, some studies have reported no effects on nutrient digestibility [22], possibly due to differences in management practices.

Amylase, protease, and lipase are the three main pancreatic digestive enzymes that, together with electrolytes, enter the proximal small intestine and play crucial roles in digesting proteins, fats, and carbohydrates [23]. Liu et al. [24] found that *Bacillus subtilis* significantly increased pancreatic protease and hindgut amylase activities in juvenile carp. Li et al. [25] reported that *Bacillus subtilis* significantly increased pancreatic lipase and trypsin activities in grass carp. In our experiment, pancreatic trypsin and lipase activities were significantly increased in the treatment groups, indicating a promoting effect on digestive function in Hu lambs.

3.4 Effects on Rumen Fermentation Parameters

Rumen fluid NH_3-N is an important product of dietary protein fermentation in the rumen and provides a nitrogen source for approximately 50% of rumen microbial protein synthesis. Normal NH_3-N concentrations in ruminant rumen fluid generally range from 6.3 to 27.0 mg/dL [26]. Zhou et al. [27] found that dietary yeast culture supplementation increased rumen NH_3-N content in goats, promoting rumen fermentation. Deng [28] reported that dietary *Bacillus natto* supplementation increased rumen NH_3-N content in dairy cows, indicating that *Bacillus subtilis* can improve rumen fermentation parameters. Rumen microorganisms produce large amounts of volatile fatty acids during nutrient digestion and microbial metabolism, which primarily function as energy sources and maintain rumen environment. Zhu et al. [29] found that selenium-enriched yeast increased acetic acid and total volatile fatty acid contents in rumen fluid of Hu ewes, thereby promoting rumen fermentation. Studies have shown that *Bacillus subtilis* can increase rumen NH_3-N and total volatile fatty acid contents in dairy cows, promoting rumen fermentation [30-31]. In our experiment, rumen fermentation parameters were improved in both treatment groups, demonstrating that selenium-enriched yeast and *Bacillus subtilis* can promote rumen fermentation in Hu lambs.

4 Conclusion

1. Dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can increase average daily gain and reduce feed-to-gain ratio in weaned Hu lambs.

2. Dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can increase serum immunoglobulin contents and antioxidant enzyme activities in weaned Hu lambs.
3. Dietary supplementation with selenium-enriched yeast and *Bacillus subtilis* can improve nutrient apparent digestibility, pancreatic digestive enzyme activities, and rumen fermentation parameters, thereby enhancing digestive function in weaned Hu lambs.

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