Effects of Dietary Microencapsulated Sodium Butyrate on Growth Performance, Nonspecific Immunity, and Liver Function of Blunt Snout Bream (Megalobrama amblycephala) under High-Density Aquaculture Conditions: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary microencapsulated sodium butyrate on growth performance, non-specific immunity, and liver function of blunt snout bream (Megalobrama amblycephala) under high-density culture conditions. A total of 1,500 healthy blunt snout bream with similar size [initial body weight of (200.78$±$0.46) g] were selected and randomly divided into 5 groups with 6 replicates per group and 50 fish per replicate. The control group was fed the basal diet, while the experimental groups were fed test diets supplemented with 200, 400, 600, and 1,000 mg/kg microencapsulated sodium butyrate based on the basal diet, respectively, for a trial period of 60 days. The results showed: 1) Compared with the control group, dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased the weight gain rate ($P<0.05$); dietary supplementation with 1,000 mg/kg microencapsulated sodium butyrate significantly decreased the feed conversion ratio ($P<0.05$). 2) Compared with the control group, dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased the activities of total superoxide dismutase (T-SOD) in serum, mucus, and liver, as well as lysozyme (LSZ) activity in serum ($P<0.05$); dietary supplementation with 400 mg/kg microencapsulated sodium butyrate significantly increased T-SOD activity in liver and LSZ activity in serum ($P<0.05$); dietary supplementation with 1,000 mg/kg microencapsulated sodium butyrate significantly increased LSZ activity in mucus ($P<0.05$). 3) Compared with the control group, dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased the activities of glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) in liver ($P<0.05$). It can be concluded that dietary supplementation

with appropriate levels of microencapsulated sodium butyrate is beneficial for improving feed utilization, non-specific immunity, and liver function of blunt snout bream under high-density culture conditions.

Full Text

Effects of Microencapsulated Sodium Butyrate on Growth Performance, Nonspecific Immunity and Hepatic Function of Blunt Snout Bream under High-Density Culture Conditions

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Abstract

This experiment was conducted to investigate the effects of dietary microencapsulated sodium butyrate on growth performance, nonspecific immunity, and hepatic function of blunt snout bream (*Megalobrama amblycephala*) under high-density culture conditions. A total of 1,500 healthy blunt snout bream with similar specifications [initial body weight of (200.78$±$0.46) g] were randomly divided into 5 groups with 6 replicates per group and 50 fish per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 200, 400, 600, and 1,000 mg/kg microencapsulated sodium butyrate for 60 days. The results showed: 1) Compared with the control group, dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased the weight gain rate ($P<0.05$), and 1,000 mg/kg supplementation significantly decreased the feed conversion ratio ($P<0.05$). 2) Supplementation with 600 and 1,000 mg/kg significantly increased total superoxide dismutase (T-SOD) activity in serum, mucus, and liver, as well as lysozyme (LSZ) activity in serum ($P<0.05$). Supplementation with 400 mg/kg significantly increased hepatic T-SOD activity and serum LSZ activity ($P<0.05$), while 1,000 mg/kg supplementation significantly increased mucus LSZ activity ($P<0.05$). 3) Supplementation with 600 and 1,000 mg/kg significantly increased hepatic alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) activities ($P<0.05$). These findings indicate that appropriate dietary supplementation of microencapsulated sodium butyrate can improve feed utilization, nonspecific immunity, and hepatic function in blunt snout bream under high-density culture conditions.

Keywords: microencapsulated sodium butyrate; blunt snout bream; growth performance; nonspecific immunity; hepatic function

Sodium butyrate, also known as sodium n-butyrate, is currently applied in feed primarily as an antibiotic alternative additive. Its active component, butyric acid, is a short-chain volatile fatty acid mainly derived from the fermentation of carbohydrates and proteins by anaerobic bacteria in the large intestine and serves as the primary energy source for colonocytes. Numerous studies have confirmed that sodium butyrate can stimulate intestinal mucosal growth and cell proliferation in monogastric animals, inhibit pathogenic microorganisms and parasites in the digestive tract, alleviate or prevent diarrhea caused by various factors, and promote the secretion of leptin and glucagon-like peptide 2 (GLP-2). It has demonstrated significant effects on promoting growth, digestion, and antioxidant capacity in piglets, broiler chickens, and juvenile fish. Under premixing and pelleting conditions, microencapsulated sodium butyrate exhibits better stability and lower loss rates compared to powdered sodium butyrate, with reduced solubility in the stomach, enabling gradual release in the intestine to exert its physiological effects. Animal trials in pigs and chickens have verified that microencapsulated sodium butyrate produces better feeding effects than the powdered form. Blunt snout bream is an excellent freshwater aquaculture species in China, prized for its delicious meat and strong disease resistance. However, with the development of intensive aquaculture, increased stocking density has led to elevated organic matter, ammonia nitrogen, and microbial counts in water, along with decreased dissolved oxygen concentrations, creating significant stress for blunt snout bream. Currently, no studies have reported the effects of microencapsulated sodium butyrate on growth performance, nonspecific immunity, and hepatic function of adult blunt snout bream. Therefore, this study investigated the effects of dietary supplementation with different levels of microencapsulated sodium butyrate on growth, nonspecific immunity, and hepatic function in adult blunt snout bream to provide a reference for the comprehensive utilization of sodium butyrate in high-density aquaculture.

1.1 Experimental Materials

Microencapsulated sodium butyrate was provided by Hangzhou Kondron Feed Co., Ltd., containing 30% sodium butyrate.

1.2 Experimental Design and Management

The experiment was conducted in concrete tanks (2.0 m × 1.5 m × 1.5 m) at a test station in Yueyang. After a 2-week acclimation period, 1,500 healthy blunt snout bream with similar specifications [initial body weight of (200.78$±$0.46) g] were randomly divided into 5 groups with 6 replicates per group and 50 fish per replicate. The control group (Group I) was fed the basal diet (composition and nutrient levels shown in Table 1), while experimental groups (Groups II–V) were fed the basal diet supplemented with 200, 400, 600, and 1,000 mg/kg microencapsulated sodium butyrate, respectively, for 60 days. All feed ingredients were ground to pass through a 40-mesh sieve and processed into pellet feed (diameter 1.5 mm, length 2.0 mm) using a domestic ring-die pellet mill.

The culture water was underground well water that was filtered and settled before flowing into a reservoir, then aerated and temperature-controlled before being pumped to the fish tanks. Feed was administered at 08:00, 12:00, and 18:00 daily at a rate of 3.0%–3.5% of body weight. Water exchange and conditioning were performed regularly according to conventional aquaculture procedures. During the experimental period, water temperature was 24–31°C, dissolved oxygen concentration >5.0 mg/L, pH 7.0–7.5, ammonia nitrogen concentration <0.5 mg/L, and nitrite concentration <0.05 mg/L.

1.3 Sampling and Detection Indicators

During the experiment, daily feed intake and mortality were recorded. At the end of the trial, fish were fasted for 24 hours before counting and weighing to calculate survival rate (SR), weight gain rate (WGR), feed conversion ratio (FCR), and condition factor (CF). Five fish were randomly selected from each group, and blood was collected from the caudal vein. Serum was obtained by centrifugation at 3,500 r/min for 15 minutes and stored at 4°C for determination of GPT, GOT, T-SOD, and LSZ activities. Mucus was collected from different body surface areas using a scalpel after drying with filter paper, weighed, and mixed with 5 volumes of 0.02 mol/mL phosphate buffer (pH 7.4), homogenized, centrifuged at 10,000 r/min for 20 minutes at 4°C, and the supernatant was snap-frozen in liquid nitrogen and stored at -40°C for T-SOD and LSZ activity analysis. Fresh hepatopancreas was weighed, mixed with 10 volumes of 0.2 mol/mL phosphate buffer (pH 7.4), homogenized, centrifuged at 10,000 r/min for 20 minutes at 4°C, and the supernatant was snap-frozen in liquid nitrogen and stored at -40°C for GPT, GOT, and T-SOD activity analysis.

1.4 Measurement Methods

Growth performance indices were calculated as follows:

- Survival rate (%) = 100 × (initial fish number -final fish number) / initial fish number
- Weight gain rate (%) = 100 × (final body weight -initial body weight) / initial body weight
- Feed conversion ratio = feed intake / (final body weight -initial body weight)
- Condition factor (g/cm^3) = body weight / body length^3

T-SOD activity in serum, hepatopancreas, and mucus, and GPT and GOT activities in hepatopancreas were determined using kits from Nanjing Jiancheng Bioengineering Institute with a microplate reader (Multiskan GO), centrifuge, constant temperature water bath, and rapid mixer. Serum GPT and GOT activities were measured using an automatic biochemical analyzer (Mindray BS-200) with corresponding reagents. Serum and mucus LSZ activities were determined according to the method of Hultmark et al. using a UV-1600 UV-Vis spectrophotometer (Beijing Rayleigh). Briefly, *Micrococcus lysodeikticus* was

suspended in 0.1 mol/L potassium phosphate buffer [initial absorbance (A_0) 0.3], incubated in test tubes for 30 minutes, then heated for 10 minutes to terminate the reaction, and final absorbance (A) was measured. LSZ activity = (A_0 -A) / A.

1.5 Statistical Analysis

Experimental data were analyzed by one-way ANOVA using the ANOVA procedure in SAS 9.2 software. Differences among groups were compared using Duncan' s multiple range test, with $P<0.05$ considered statistically significant. Data are presented as means ± standard deviation.

2.1 Effects of Dietary Microencapsulated Sodium Butyrate on Growth Performance

As shown in Table 2 , dietary supplementation with 200, 400, 600, and 1,000 mg/kg microencapsulated sodium butyrate had no significant effects on condition factor or survival rate of blunt snout bream ($P>0.05$), although survival rate increased with increasing supplementation levels. Supplementation with 1,000 mg/kg significantly increased final body weight ($P<0.05$). Supplementation with 600 and 1,000 mg/kg significantly increased weight gain rate ($P<0.05$). Supplementation with 1,000 mg/kg significantly decreased feed conversion ratio ($P<0.05$), which showed a trend of initial increase followed by decrease with increasing supplementation levels.

2.2 Effects of Dietary Microencapsulated Sodium Butyrate on Non-specific Immune Indices

As shown in Table 3 , compared with the control group, dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased T-SOD activity in serum, mucus, and hepatopancreas, as well as LSZ activity in serum ($P<0.05$). Supplementation with 400 mg/kg significantly increased hepatic T-SOD activity and serum LSZ activity ($P<0.05$). Supplementation with 1,000 mg/kg significantly increased mucus LSZ activity ($P<0.05$).

2.3 Effects of Dietary Microencapsulated Sodium Butyrate on Hepatic Function

As shown in Table 4 , compared with the control group, dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased GPT and GOT activities in hepatopancreas ($P<0.05$).

3.1 Effects on Growth Performance

The growth-promoting effects of sodium butyrate vary among different animal species, and the optimal supplementation level differs substantially. Studies

have reported that sodium butyrate can promote fish growth, with optimal levels of 0.1%-0.2% in juvenile grass carp, 0.25% in juvenile crucian carp [initial weight $(6.02\$\pm0.16)g], 0.15\pm0.01)g], 0.1\pm\$3.13)$ g], and 1.0% in juvenile American eel. Other studies found that 0.2% sodium butyrate supplementation in sea bass had no significant effect on weight gain rate or specific growth rate but significantly affected immune-related gene expression and enhanced immune function. He Jiao reported that dietary supplementation with 500 mg/kg microencapsulated sodium butyrate reduced mortality and improved welfare in broiler chickens. The present study found that in adult blunt snout bream, supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased weight gain rate, and 1,000 mg/kg supplementation significantly decreased feed conversion ratio, similar to results reported by Liu et al. in juvenile grass carp. Moreover, survival rate increased with increasing sodium butyrate levels, consistent with He Jiao' s findings in broiler chickens, indicating that appropriate sodium butyrate supplementation positively affects digestive capacity and health status in blunt snout bream.

3.2 Effects on Nonspecific Immunity

Previous studies have demonstrated that sodium butyrate promotes immune organ development and enhances mucosal immune function in piglets and chickens, improves intestinal mucosal morphology and nonspecific immunity in carp, increases serum nonspecific immune enzyme activities (phenoloxidase, acid phosphatase, and alkaline phosphatase) in *Litopenaeus vannamei*, and enhances immunity and antioxidant capacity in perinatal dairy cows. As lower vertebrates with relatively weak specific immune responses, fish rely heavily on nonspecific immunity for defense, making LSZ and SOD key indicators of immune capacity. LSZ, also known as muramidase or N-acetylmuramide glycanohydrolase, is an alkaline enzyme that hydrolyzes mucopolysaccharides in pathogenic bacteria. SOD is a scavenger of superoxide anion radicals ($O_2^- \cdot$) and a critical enzyme in the anti-peroxidation defense system. The present study found that supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate enhanced T-SOD activity in serum, mucus, and hepatopancreas and LSZ activity in serum, while 400 mg/kg supplementation increased hepatic T-SOD and serum LSZ activities, and 1,000 mg/kg supplementation increased mucus LSZ activity. These results align with previous reports, suggesting that appropriate dietary sodium butyrate supplementation enhances nonspecific immunity in adult blunt snout bream, possibly by regulating intestinal mucosal homeostasis to strengthen autoimmune responses.

3.3 Effects on Hepatic Function

GPT and GOT are important indicators of normal hepatic function and key enzymes in amino acid metabolism, primarily existing in hepatocytes and playing crucial roles in protein metabolism. Under normal conditions, serum transaminase activities are low and relatively stable. When hepatocytes are damaged by

foreign substances, increased cell membrane permeability leads to substantial GPT and GOT release into the bloodstream, significantly elevating serum activities above normal levels. Yang Lianghui et al. found that dietary sodium butyrate had no adverse effects on Nile tilapia. Wei Chaoqing et al. reported that 0.15% sodium butyrate supplementation in high-plant-protein diets improved hepatic antioxidant function in juvenile turbot. The present results showed that supplementation with 200, 400, 600, and 1,000 mg/kg microencapsulated sodium butyrate had no significant effects on serum GPT and GOT activities, while 600 and 1,000 mg/kg supplementation significantly increased hepatic GPT and GOT activities. This suggests that 600 and 1,000 mg/kg sodium butyrate supplementation enhances hepatic detoxification function and accelerates urea production, thereby reducing the toxicity of amino acid metabolites, though the specific molecular mechanisms require further investigation.

Conclusions

1. Dietary supplementation with 600 and 1,000 mg/kg microencapsulated sodium butyrate significantly increased weight gain rate, and 1,000 mg/kg supplementation significantly improved feed utilization in blunt snout bream.

Supplementation with 600 and 1,000 mg/kg significantly increased T-SOD activity in serum, mucus, and hepatopancreas and LSZ activity in serum. Supplementation with 400 mg/kg significantly increased hepatic T-SOD activity and serum LSZ activity, while 1,000 mg/kg supplementation significantly increased mucus LSZ activity.

Supplementation with 600 and 1,000 mg/kg significantly increased hepatic GPT and GOT activities.

In summary, appropriate dietary supplementation of microencapsulated sodium butyrate can improve feed utilization, nonspecific immunity, and hepatic function in blunt snout bream under high-density culture conditions.

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