Effects of Sodium Butyrate Supplementation in High Plant Protein Diets on Growth Performance, Apparent Nutrient Digestibility, and Liver Antioxidant Function in Juvenile Turbot [1] Postprint

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Abstract

This experiment aimed to investigate the effects of dietary sodium butyrate supplementation in high plant protein diets on growth performance, apparent nutrient digestibility, and hepatic antioxidant function of juvenile turbot (Scophthalmus maximus), and to explore feasible methods for increasing the proportion of fish meal replaced by plant protein in aquafeeds. Five isonitrogenous and isoenergetic experimental diets were formulated using fish meal, soybean meal, corn gluten meal, wheat gluten meal, peanut meal, and brewer' s yeast as protein sources, fish oil, coconut oil, and soybean lecithin as lipid sources, and wheat flour as carbohydrate source. Among them, a basal diet containing 60% fish meal served as the positive control group (FM group), a substitution diet in which composite plant protein sources replaced 50% of fish meal in the basal diet served as the negative control group (CON group), and diets with 0.15% (D1 group), 0.30% (D2 group), and 0.60% (D3 group) sodium butyrate added to the substitution diet served as treatment groups. The above five experimental diets were used to feed juvenile turbot with initial body weight of (13.00$±$0.01) g for 58 days. The experiment was conducted in an indoor aquaculture system, with each experimental diet fed to three replicates of 30 fish each. The results showed that: 1) With increasing sodium butyrate supplementation levels, weight gain rate, specific growth rate, and feed efficiency of juvenile turbot exhibited a trend of first increasing and then decreasing, among which weight gain rate, feed efficiency, and specific growth rate in FM, D1, and D2 groups were significantly higher than those in CON group ($P<0.05$), while no significant difference in feeding rate was observed among groups ($P>0.05$); except that D2 group showed no significant difference in viscera-somatic index

compared with FM group (P>0.05), all other groups had significantly lower viscera-somatic index than FM group (P<0.05); no significant differences were found in whole-body moisture, crude protein, and crude lipid content among groups (P>0.05). 2) Apparent dry matter digestibility in D1 group was significantly higher than that in CON group (P<0.05), with no significant difference from FM group (P>0.05); apparent protein digestibility in D1 group was significantly higher than those in CON, D2, and D3 groups (P<0.05), with no significant difference from FM group (P>0.05); no significant differences in apparent dry matter and protein digestibility were observed between D2 and D3 groups (P>0.05). 3) Hepatic total antioxidant capacity (T-AOC) and catalase (CAT) activity in D2 group were significantly higher than those in CON group (P<0.05), while hepatic malondialdehyde (MDA) content was significantly lower than that in CON group (P<0.05). It was concluded that under the conditions of this experiment, supplementation of 0.15% sodium butyrate in high plant protein diets could improve growth performance, apparent nutrient digestibility, and hepatic antioxidant function of juvenile turbot, while excessive addition of sodium butyrate would reduce growth performance of juvenile turbot.

Full Text

Effects of Adding Different Levels of Sodium Butyrate in High Plant Protein Diets on Growth Performance, Nutrient Apparent Digestibility Coefficients and Liver Antioxidant Function of Juvenile Turbot (Scophthalmus maximus L.)

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Abstract

This study investigated the effects of dietary sodium butyrate supplementation in high plant protein diets on growth performance, nutrient apparent digestibility coefficients, and liver antioxidant function of juvenile turbot (*Scophthalmus maximus* L.), aiming to explore feasible methods for increasing plant protein substitution levels in aquafeeds. Five isonitrogenous and isocaloric experimental diets were formulated using fish meal, soybean meal, corn gluten meal, vital gluten, peanut meal, and beer yeast as protein sources; fish oil, coconut oil, and soybean lecithin as lipid sources; and wheat meal as carbohydrate source. The basal diet containing 60% fish meal served as the positive control (FM group). A negative control diet (CON group) was prepared by replacing 50% of fish meal with mixed plant protein sources. Three experimental diets were formulated by supplementing the CON diet with 0.15% (D1 group), 0.30% (D2 group), and

0.60% (D3 group) sodium butyrate, respectively. Juvenile turbot with initial body weight of (13.00$±$0.01) g were fed the experimental diets for 58 days in an indoor aquaculture system, with three replicates per diet and 30 fish per replicate. The results showed: 1) Weight gain rate (WGR), specific growth rate (SGR), and feed efficiency (FE) increased initially and then decreased with increasing sodium butyrate levels. The WGR, SGR, and FE in FM, D1, and D2 groups were significantly higher than those in CON group ($P<0.05$), while no significant differences in feed intake (FI) were observed among groups ($P>0.05$). Except for D2 group, which showed no significant difference in viscerosomatic index (VSI) compared with FM group ($P>0.05$), all other groups had significantly lower VSI than FM group ($P<0.05$). No significant differences were found in whole-body moisture, crude protein, or crude lipid contents among all groups ($P>0.05$). 2) The apparent digestibility coefficient of dry matter in D1 group was significantly higher than that in CON group ($P<0.05$) and showed no significant difference from FM group ($P>0.05$). The apparent digestibility coefficient of protein in D1 group was significantly higher than those in CON, D2, and D3 groups ($P<0.05$) but did not differ significantly from FM group ($P>0.05$). No significant differences in apparent digestibility coefficients of dry matter and protein were observed between D2 and D3 groups ($P>0.05$). 3) Liver total antioxidant capacity (T-AOC) and catalase (CAT) activity in D2 group were significantly higher than those in CON group ($P<0.05$), while liver malondialdehyde (MDA) content was significantly lower than that in CON group ($P<0.05$). In conclusion, under the present experimental conditions, supplementation of 0.15% sodium butyrate in high plant protein diets improved growth performance, nutrient apparent digestibility, and liver antioxidant function of juvenile turbot, whereas excessive supplementation reduced growth performance.

Keywords: sodium butyrate; juvenile turbot; growth performance; apparent digestibility; antioxidant ability

Introduction

Due to the increasing shortage of fish meal resources, replacing fish meal with alternative protein sources such as animal or plant proteins has become an inevitable approach for sustainable aquaculture development [1]. However, the utilization proportion of plant proteins in aquatic animal feeds is often limited, as excessive replacement of fish meal with plant proteins can lead to reduced growth performance, decreased feed intake, lower digestibility [2-4], and oxidative stress responses [5] in cultured fish. These limitations primarily arise from the deficiency of various functional nutrients in plant protein sources compared with fish meal, including amino acid imbalance, presence of indigestible carbohydrates, and anti-nutritional factors [6].

Numerous studies have demonstrated that supplementing functional small-molecule bioactive substances lacking in plant protein sources is crucial for maintaining normal fish growth. For instance, adding 5 g/kg taurine to an all-plant protein diet significantly improved growth performance and promoted

feed intake in rainbow trout [7]. Supplementation of cholesterol [8] and hydroxyproline [9] in high plant protein diets enhanced growth of turbot, while dietary nucleotides improved growth performance of Atlantic salmon [10]. Therefore, strengthening research on functional nutrients may be a viable approach to improve the utilization efficiency of non-fish meal proteins in aquafeeds.

Sodium butyrate, primarily used as an antibiotic alternative additive in animal feeds, has been reported by many scholars to promote growth, enhance digestion, and improve immune function, making it a highly promising functional additive [11]. Studies have shown that sodium butyrate can increase the specific growth rate of grass carp [12], and its active component butyric acid can maintain normal intestinal mucosal epithelial cells [13] and promote small intestinal digestion and absorption [14]. Additionally, sodium butyrate can alleviate oxidative stress and enhance stress resistance in fish. However, no reports have been published on the effects of sodium butyrate supplementation in diets with fish meal replaced by mixed plant proteins on growth performance, digestive capacity, immune function of turbot, or its potential to improve plant protein utilization. Therefore, this study used juvenile turbot as the experimental model to investigate the effects of different dietary sodium butyrate levels on growth performance, physical indices, nutrient apparent digestibility, and antioxidant function, aiming to clarify whether sodium butyrate supplementation can increase the proportion of plant protein replacement for fish meal and provide scientific evidence for the comprehensive utilization of sodium butyrate in aquaculture.

1.1 Experimental Design and Diets

Five isonitrogenous and isocaloric experimental diets were formulated using fish meal, soybean meal, corn gluten meal, vital gluten, peanut meal, and beer yeast as protein sources; fish oil, coconut oil, and soybean lecithin as lipid sources; and wheat meal as carbohydrate source. All feed ingredients were purchased from Qingdao Qihao Biological Technology Co., Ltd. The basal diet containing 60% fish meal served as the positive control (FM group). A negative control diet (CON group) was prepared by replacing 50% of fish meal with mixed plant protein sources. Three experimental diets were formulated by supplementing the CON diet with 0.15% (D1 group), 0.30% (D2 group), and 0.60% (D3 group) sodium butyrate (purchased from Shanghai Hanle Biological Technology Co., Ltd., active ingredient content $ 98.5{2}O{3}$) at 0.1% was added as an indicator for determining nutrient apparent digestibility. Feed ingredients were ground to pass through a 60-mesh sieve, and micro-ingredients were mixed using the progressive enlargement method. Lipid sources including fish oil were then thoroughly mixed, followed by addition of water and choline chloride. The mixture was processed into hard pellet feed using an F-26 twin-screw extruder. The feed was dried in a 45°C oven for 12 hours and stored at -20°C until use. The composition and nutrient levels of experimental diets are shown in Table 1

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1.2 Experimental Fish and Culture Conditions

Juvenile turbot were purchased from Laizhou Farm in Yantai City and the feeding trial was conducted at Qingdao Yihai Feng Aquatic Products Co., Ltd. Prior to the formal experiment, juvenile turbot were acclimated in the culture system for two weeks and fed commercial feed to adapt to the environment. After acclimation, uniform-sized and healthy juvenile turbot [initial body weight (13.00$±$0.01) g] were randomly distributed into five groups with three replicates per group and 30 fish per replicate. Fish were stocked in 200-L tanks, with each replicate representing one tank. Each experimental diet was randomly assigned to one group of fish. The feeding trial was conducted in an indoor flow-through system, where seawater was pumped to a filtration tank, sand-filtered, and then flowed into culture tanks at a consistent flow rate. During the culture period, fish were fed to apparent satiation twice daily at 07:00 and 19:00. Uneaten feed was collected and quantified after feeding, and water was exchanged to maintain water quality.

1.3 Sample Collection

At the end of the feeding trial, all fish were fasted for 24 hours and anesthetized with eugenol (1:10,000) for weighing and counting. Five fish per tank were randomly selected and stored at -20°C for whole-body composition analysis. Another four fish per tank were randomly selected for individual weighing and length measurement. Viscera and liver were dissected and weighed to calculate hepatosomatic index (HSI) and viscerosomatic index (VSI). Liver samples were placed in centrifuge tubes, frozen in liquid nitrogen, and stored at -80°C after sampling. Samples from two fish were pooled into one tube, with two tubes per tank and six samples per group. Additionally, four fish per tank were randomly selected for feces collection using the extrusion method [15] at 4 hours post-feeding.

1.4 Analytical Methods

1.4.1 Growth Performance Indicators Proximate composition of feed ingredients, experimental diets, and fish were determined according to AOAC (1995) methods. Moisture and dry matter contents were determined by drying samples in a 105°C oven to constant weight. Crude protein content was measured using an automatic Kjeldahl nitrogen analyzer (TM-8400, FOSS, Sweden). Crude lipid content was determined using a Soxhlet extraction apparatus (SOXTEC-8000, FOSS, Sweden). Gross energy was measured using an oxygen bomb calorimeter (Parr1281, Parr, USA).

1.4.2 Nutrient Apparent Digestibility Coefficients Yttrium oxide (Y_2O_3) added at 0.1% in diets served as an indicator. Yttrium content in diets

and feces was determined using a high-frequency inductively coupled plasma optical emission spectrometer (VIATA-MPX-ICP, VARIAN, USA) to calculate apparent digestibility coefficients of dry matter and protein.

1.4.3 Liver Antioxidant Indices Thawed liver tissue was accurately weighed and homogenized with 9 volumes of 0.9% physiological saline (w/v = 1:9) on ice to prepare 10% tissue homogenate. The homogenate was centrifuged at 3,000 r/min for 10 min at 4°C, and the supernatant was collected and diluted with physiological saline to appropriate concentrations for different assays. Liver protein content was determined using the Coomassie brilliant blue method. Liver antioxidant indices measured included total antioxidant capacity (T-AOC), catalase (CAT) activity, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content, all determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Calculation Formulas

Specific growth rate (SGR, %/d) = 100 × (ln final body weight - ln initial body weight) / feeding days;
Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight) / initial body weight;
Feed intake (FI, %/d) = 100 × feed intake / [(initial body weight + final body weight) / 2] / feeding days;
Feed efficiency (FE) = weight gain / feed intake;
Condition factor (CF, %) = 100 × body weight (g) / body length $(cm)^3$;
Hepatosomatic index (HSI, %) = 100 × liver weight / body weight;
Viscerosomatic index (VSI, %) = 100 × viscera weight / body weight;
Apparent digestibility coefficient of dry matter (%) = 100 × [1 - (Y_2O_3 content in diet / Y_2O_3 content in feces)];
Apparent digestibility coefficient of protein (%) = 100 × [1 - (crude protein content in feces / crude protein content in diet) × (Y_2O_3 content in diet / Y_2O_3 content in feces)].

1.6 Data Processing and Statistical Analysis

Data were processed using SPSS 17.0 software and subjected to one-way ANOVA. When significant differences were detected among groups, Tukey' s test was used for multiple comparisons, with $P<0.05$ considered statistically significant. Results are presented as mean ± standard error (SE).

Results

2.1 Effects of Dietary Sodium Butyrate Levels on Growth Performance of Juvenile Turbot

As shown in Table 2 , final body weight, weight gain rate, and specific growth rate in D1 group were significantly higher than those in CON group ($P<0.05$)

but showed no significant differences from FM group (P>0.05). Feed efficiency in D1 and D2 groups was significantly higher than that in CON group (P<0.05) but did not differ significantly from FM group (P>0.05), with no significant difference between D1 and D2 groups (P>0.05). No significant differences in feed intake were observed among all groups (P>0.05).

2.2 Effects of Dietary Sodium Butyrate Levels on Physical Indices of Juvenile Turbot

As shown in Table 3 , dietary supplementation of different sodium butyrate levels had no significant effects on condition factor or hepatosomatic index of juvenile turbot (P>0.05). For viscerosomatic index, except for D2 group which showed no significant difference from FM group (P>0.05), all other groups were significantly lower than FM group (P<0.05).

2.3 Effects of Dietary Sodium Butyrate Levels on Body Composition of Juvenile Turbot

As shown in Table 4 , dietary supplementation of different sodium butyrate levels had no significant effects on whole-body moisture, crude protein, or crude lipid contents of juvenile turbot (P>0.05).

2.4 Effects of Dietary Sodium Butyrate Levels on Nutrient Apparent Digestibility of Juvenile Turbot

As shown in Table 5 , apparent digestibility coefficients of dry matter and protein increased initially and then decreased with increasing sodium butyrate levels. The apparent digestibility coefficients of dry matter and protein in D1 group were 6.93% and 6.64% higher than those in CON group, respectively (P<0.05). No significant differences in dry matter apparent digestibility were observed among experimental groups (D1, D2, D3) or between these groups and FM group (P>0.05). Protein apparent digestibility in D1 group was significantly higher than those in other experimental groups and CON group (P<0.05) but showed no significant difference from FM group (P>0.05).

2.5 Effects of Dietary Sodium Butyrate Levels on Liver Antioxidant Indices of Juvenile Turbot

As shown in Table 6 , liver SOD activity increased initially and then decreased with increasing sodium butyrate levels, reaching the highest value in D2 group. D2 group showed no significant difference from FM group (P>0.05) but was significantly higher than D3 group (P<0.05), while showing an increase compared with CON group without reaching statistical significance (P>0.05). Liver T-AOC showed a gradual increasing trend with sodium butyrate supplementation, with no significant differences among experimental groups (P>0.05). However, all experimental groups had significantly higher T-AOC than CON group (P<0.05) and showed no significant differences from FM group (P>0.05). CON

group had the highest liver MDA content, which was significantly higher than those in FM, D2, and D3 groups ($P<0.05$) but showed no significant difference from D1 group ($P>0.05$). Liver CAT activity increased initially and then decreased with sodium butyrate supplementation, with D1 and D2 groups being significantly higher than CON and D3 groups ($P<0.05$) and showing no significant differences from FM group ($P>0.05$). No significant difference was observed between D1 and D2 groups ($P>0.05$).

Discussion

3.1 Effects on Growth Performance

The growth-promoting effects of sodium butyrate vary among different animal species, and the optimal supplementation level also differs. Studies have shown that diets containing 0.05% sodium butyrate significantly affected growth performance and improved intestinal health in growing rabbits [16]. Dietary supplementation of 0.08% sodium butyrate significantly promoted growth in weaned piglets during the early post-weaning period, with significantly higher weight gain than the control group [17], which is consistent with our findings that 0.15% sodium butyrate supplementation significantly increased weight gain rate in juvenile turbot. Liu et al. [12] reported that dietary supplementation of 1,000 or 2,000 mg/kg sodium butyrate significantly improved specific growth rate of grass carp. However, other studies found that 0.2% dietary sodium butyrate had no significant effects on weight gain rate or specific growth rate of European sea bass but significantly affected immune-related gene expression and enhanced immune function [18]. Our results indicated that replacing 50% of fish meal with mixed plant protein sources negatively affected growth performance of juvenile turbot, resulting in decreased weight gain rate, feed efficiency, and specific growth rate. Sodium butyrate supplementation improved these parameters, with the 0.15% group showing the best growth performance, reaching levels comparable to the FM group. This improvement can be attributed to two main mechanisms: first, sodium butyrate enhanced nutrient digestibility in juvenile turbot; second, it improved their antioxidant capacity.

In this study, excessive sodium butyrate supplementation inhibited growth of juvenile turbot, possibly by suppressing intestinal development and reducing nutrient digestion and absorption. Sodium butyrate did not significantly affect feed intake in juvenile turbot, which is similar to findings by Zhang et al. [19] and indicates that sodium butyrate does not affect feed palatability [20]. Therefore, the growth-promoting effect of sodium butyrate is not achieved by increasing feed consumption but by improving feed utilization efficiency.

3.2 Effects on Body Composition

Previous studies have shown that dietary sodium butyrate supplementation does not affect whole-body moisture, crude protein, crude lipid, or ash contents in grass carp [12]. Zhai et al. [21] reported that tributyrin, a sodium butyrate

analog, did not affect crude protein or crude lipid contents in pufferfish. Our results demonstrated that different levels of sodium butyrate supplementation had no significant effects on whole-body moisture, crude protein, or crude lipid contents in juvenile turbot, consistent with these previous findings. However, some studies found that sodium butyrate significantly increased crude protein content in whole body of tilapia, possibly because sodium butyrate promoted more efficient conversion of ingested food into structural proteins, resulting in greater muscle development [22]. The specific mechanisms underlying this effect require further investigation.

3.3 Effects on Nutrient Apparent Digestibility

Sodium butyrate has been shown to influence nutrient digestibility. Guilloteau et al. [23] demonstrated positive effects of sodium butyrate on nutrient digestibility in calves. Sodium butyrate can stimulate cholecystokinin release to promote pancreatic secretion, and oral administration of sodium butyrate increases pancreatic elastase secretion by 50% [24]. It can also enhance disaccharidase activities in different intestinal segments of piglets, thereby improving intestinal digestive enzyme activities [25]. Furthermore, sodium butyrate promotes activities of total protease, lipase, and ileal amylase in weaned piglets [26], facilitating energy deposition and protein digestion [27]. However, Ribeiro et al. [28] found that sodium butyrate had no significant effects on nutrient apparent digestibility in early-weaned rabbits. Our study revealed that elevated dietary plant protein levels significantly reduced apparent digestibility of dry matter and protein in juvenile turbot, while sodium butyrate supplementation significantly improved these digestibility coefficients, with the 0.15% group showing the best results. However, further increases in sodium butyrate level reduced digestibility. The mechanisms by which sodium butyrate improves nutrient digestibility in juvenile turbot require further investigation.

It should be noted that different feces collection methods can significantly affect apparent digestibility coefficients in fish. The extrusion method used in this study yields significantly lower apparent digestibility coefficients for dry matter and protein than the siphon method [29], because collected feces may be contaminated with undigested feed and body fluids, leading to underestimated digestibility. Conversely, the in vitro feces collection method can cause nutrient leaching into water, which is one reason why the siphon method yields higher apparent digestibility values. Nevertheless, both methods have their validity [30-31]. The relatively low apparent digestibility coefficients obtained in this study are partly attributable to the extrusion method used for feces collection and the cold extrusion processing method employed for feed production.

3.4 Effects on Liver Antioxidant Function

Sodium butyrate enhances the ability to scavenge free radicals and reduces tissue and cellular damage by modulating immune and antioxidant functions [32], thereby alleviating negative impacts of adverse environmental factors. Superox-

ide dismutase (SOD) scavenges superoxide anion radicals to protect cells from damage. Total antioxidant capacity (T-AOC) is closely related to health status and comprises enzymatic and non-enzymatic defense systems that work synergistically to protect the organism from oxidation. Malondialdehyde (MDA) content indirectly reflects the severity of free radical attack on cells, while catalase (CAT) decomposes hydrogen peroxide to reduce peroxidation. Studies have shown that sodium butyrate significantly increased serum SOD activity and T-AOC in dairy cows [33]. Under lipopolysaccharide (LPS) stress conditions, sodium butyrate significantly increased serum and liver SOD and CAT activities while decreasing MDA content in broilers, thereby improving nutrient metabolism, maintaining antioxidant function, and enhancing anti-inflammatory capacity [34]. Dietary supplementation of 0.1% sodium butyrate increased liver T-AOC and CAT activity by 25% and 15%, respectively, while decreasing liver MDA content by 15% in eels [19].

Our results indicated that replacing 50% of fish meal with mixed plant protein sources significantly affected liver antioxidant indices in juvenile turbot. Liver SOD and CAT activities and T-AOC showed initial increases followed by decreases with increasing sodium butyrate levels. The 0.15% group exhibited significantly higher T-AOC and CAT activity than CON group, reaching maximum values at 0.30% supplementation level and achieving levels comparable to FM group. Sodium butyrate supplementation also reduced liver MDA content. These findings suggest that sodium butyrate can alleviate oxidative stress induced by plant protein replacement and enhance antioxidant capacity under stress conditions, which is significant for improving immune function.

Comprehensive analysis revealed that liver antioxidant capacity was strongest at 0.30% sodium butyrate supplementation, but growth performance began to decline at this level, indicating that maximum antioxidant capacity does not necessarily correspond to optimal growth performance. This may be because increased energy expenditure for antioxidant defense reduces energy available for growth, similar to findings that maximum immune capacity did not correspond to optimal growth performance in yellow catfish [35] and turbot [36] fed nucleotide-supplemented diets. Additionally, final body weight and weight gain rate in the 0.30% group were significantly lower than those in the 0.15% group, with decreasing trends in feed efficiency and specific growth rate, possibly related to the significant reduction in protein apparent digestibility. Therefore, based on comprehensive consideration of growth performance and liver antioxidant indices, the recommended sodium butyrate supplementation level is 0.15% under our experimental conditions.

Previous studies have shown that high plant protein diets can cause intestinal damage in juvenile turbot [37], manifested as thinner intestinal muscular layers, shortened and damaged intestinal villi, and increased goblet cells, leading to decreased intestinal weight [38]. This may be the primary reason for the reduced viscerosomatic index. The fact that the 0.30% group showed no significant difference in viscerosomatic index from FM group suggests that sodium butyrate

may have beneficial effects on repairing intestinal damage [39-40].

3.5 Significance of Sodium Butyrate as a Novel Additive in Fish Meal Replacement

Previous studies on fish meal replacement in turbot feeds have shown limited replacement proportions. Xu et al. [41] found that replacing 45% of fish meal protein with single protein source corn gluten meal significantly inhibited growth of juvenile turbot. Soybean meal could replace 30% of fish meal without significantly affecting weight gain rate, protein efficiency, or specific growth rate [42]. Mixed plant proteins could only replace relatively low levels of fish meal in flatfish diets. Wang et al. [43] found that replacing 40% of fish meal with mixed plant proteins affected growth performance of turbot. Imsland et al. [44] successfully replaced 43% of fish meal with mixed plant proteins without affecting growth. Liu et al. [9] reported that replacing 50% of fish meal with mixed plant proteins significantly reduced weight gain rate and specific growth rate of juvenile turbot. Building upon this research, our study supplemented the diet with 0.15% sodium butyrate after replacing 50% of fish meal with mixed plant proteins, successfully maintaining growth performance without significant reduction, thereby increasing the utilization proportion of plant proteins in turbot feeds. The primary reason is that appropriate sodium butyrate levels improved nutrient digestibility without affecting feed palatability. This study not only provides scientific evidence for comprehensive utilization of sodium butyrate in aquaculture but also offers theoretical support for improving utilization efficiency of plant protein sources in feed industry.

Based on our findings: (1) Appropriate sodium butyrate supplementation in high plant protein diets can promote feed digestion, improve growth performance, and enhance antioxidant function without significantly affecting body composition, whereas excessive supplementation reduces growth performance; (2) Under the present experimental conditions, the recommended sodium butyrate supplementation level is 0.15% based on comprehensive evaluation of growth performance and liver antioxidant indices.

References

[1] Xu D D. Preliminary study on nutritional sensing and response mechanisms of different protein sources in juvenile turbot (*Scophthalmus maximus* L.) [D]. PhD thesis. Qingdao: Ocean University of China, 2014: 3-6.

[2] Francis G, Makkar H P S, Becker K, et al. Antinutritional factors present in plant-derived alternate ingredients their effects fish [J]. *Aquaculture*, 2001, 199(3/4): 197-227.

[3] Penn M H, Bendiksen E A, Campbell P, et al. High level of dietary pea protein concentrate induces enteropathy Atlantic salmon (*Salmo salar* L.) [J]. *Aquaculture*, 2011, 310(3/4): 267-273.

[4] Silva F C P, Nicol J R, Zazbonino-Infante J L, et al. Influence of partial substitution of dietary fish meal on the activity of digestive enzymes in the intestinal brush border membrane gilthead bream, *Sparus aurata* goldfish, *Carassius auratus* [J]. *Aquaculture*, 2010, 306(1/2/3/4): 233-237.

[5] Sitjà-Bobadilla A, Peña-Llopis S, Gómez-Requeni P, et al. Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*) [J]. *Aquaculture*, 2005, 249(1/2/3/4): 387-400.

[6] Bonaldo A, Roem A J, Fagioli P, et al. Influence of dietary levels of soybean meal on the performance and gut histology of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.) [J]. *Aquaculture Research*, 2008, 39(9): 970-978.

[7] Gaylord T G, Barrows F T, Teague A M, et al. Supplementation of taurine and methionine all-plant protein diets rainbow trout (*Oncorhynchus mykiss*) [J]. *Aquaculture*, 2007, 269(1/2/3/4): 514-524.

[8] Yun B, Mai K S, Zhang W B, et al. Effects of dietary cholesterol on growth performance, feed intake and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets [J]. *Aquaculture*, 2011, 319(1/2): 105-110.

[9] Liu Y Z, He G, Wang Q C, et al. Hydroxyproline supplementation on the performances of plant protein source based diets turbot (*Scophthalmus maximus* L.) [J]. *Aquaculture*, 2014, 433: 476-480.

[10] Burrells C, Williams P D, Southgate P J, et al. Dietary nucleotides: a novel supplement in fish feeds: 2. Effects on vaccination, salt water transfer, growth rates and physiology of Atlantic salmon (*Salmo salar* L.) [J]. *Aquaculture*, 2001, 199(1/2): 171-184.

[11] Li D D, Feng G Q, Niu H H, et al. Effects of sodium butyrate on growth performance and immune function of weaned piglets [J]. *Chinese Journal of Animal Nutrition*, 2012, 24(2): 307-313.

[12] Liu M M, Guo W, Wu F, et al. Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp (*Ctenopharyngodon idellus*) [J]. *Aquaculture Research*, 2016, doi:10.1111/are.13230.

[13] Claus R, Gunthner D, Letzguß H. Effects of feeding fat-coated butyrate on mucosal morphology and function in the small intestine of the pig [J]. *Journal of Animal Physiology and Animal Nutrition*, 2007, 91(7/8): 312-318.

[14] Piva A, Pizzamiglio V, Morlacchini M, et al. Lipid microencapsulation allows slow release of organic acids and natural identical flavors along the swine intestine [J]. *Journal of Animal Science*, 2007, 85(2): 486-493.

[15] Yang C Z, He G, Zhou H H, et al. Apparent digestibility coefficients of

nutrients in several protein sources and effects of extrusion treatment on them for juvenile turbot (*Scophthalmus maximus* L.) [J]. *Chinese Journal of Animal Nutrition*, 2016, 28(7): 2045-2054.

[16] Hassanin A, Tony M A, Sawiress F A R, et al. Influence of dietary supplementation of coated sodium butyrate and/or synbiotic growth performances, caecal fermentation, intestinal morphometry and metabolic profile of growing rabbits [J]. *Journal of Agricultural Science*, 2015, 7(2): 9752-9760.

[17] Piva A, Morlacchini M, Casadei G, et al. Sodium butyrate improves growth performance of weaned piglets during the first period after weaning [J]. *Italian Journal of Animal Science*, 2002, 1(1): 35-41.

[18] Terova G, Diaz N, Rimoldi S, et al. Effects of sodium butyrate treatment on histone modifications and the expression of genes related to epigenetic regulatory mechanisms and immune response in European sea bass (*Dicentrarchus labrax*) fed a plant-based diet [J]. *PLoS One*, 2016, 11(7): e0160332.

[19] Zhang S L, Chang J B, Ye J D, et al. Effects of sodium butyrate on feed intake, growth performance and antioxidant capacity of American eel (*Anguilla rostrata*) [J]. *Fujian Journal of Agricultural Sciences*, 2011, 26(4): 549-551.

[20] Robles R, Lozano A B, Sevilla L, et al. Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*) [J]. *Fish Physiology and Biochemistry*, 2013, 39(6): 1567-1580.

[21] Zhai Q L, Zhang C X, Sun Y Z, et al. Effects of tributyrin and mannan oligosaccharides on growth performance, body composition and intestinal health indices of pufferfish (*Takifugu flavidus*) [J]. *Chinese Journal of Animal Nutrition*, 2014, 26(8): 2197-2208.

[22] Ahmed H A, Sadek K M. Impact of dietary supplementation of sodium butyrate and/or protexin on the growth performance, some blood parameters, and immune response of *Oreochromis niloticus* [J]. *International Journal of Agriculture Innovations Research*, 2015, 3(4): 985-991.

[23] Guilloteau P, Savary G, Jaguelin-Peyrault Y, et al. Dietary sodium butyrate supplementation increases digestibility and pancreatic secretion in young milk-fed calves [J]. *Journal of Dairy Science*, 2010, 93(12): 5842-5850.

[24] Araujo G, Terré M, Mereu A, et al. Effects of supplementing a milk replacer with sodium butyrate or tributyrin on performance and metabolism of Holstein calves [J]. *Animal Production Science*, 2016, 56(11): 1834-1841.

[25] Gálfi P, Gabel G, Martens H. Influences of extracellular matrix components on the growth and differentiation of ruminal epithelial cells in primary culture [J]. *Research in Veterinary Science*, 1993, 54(1): 102-109.

[26] Chen G S, Xu Z F, Zhao F F, et al. Effects of coated sodium butyrate on growth performance and intestinal digestive enzyme activities of weaned piglets [J]. *Animal Science Abroad: Pigs and Poultry*, 2011, 31(2): 65-68.

[27] Mallo J J, Puyalto M, Rama Rao S V. Evaluation of the effect of sodium butyrate addition to broiler diet on energy and protein digestibility, productive parameters and size of intestinal villi of animals [J]. *Feed Livestock*, 2012, 8: 26-30.

[28] Ribeiro J, Gaspar S, Pinho M, et al. Sodium butyrate in growing and fattening diets for early-weaned rabbits [J]. *World Rabbit Science*, 2012, 20(4): 199-207.

[29] Shi D J, Sun X J, Liang Y J, et al. Effects of different feces collection methods on apparent digestibility determination in koi carp (*Cyprinus carpio*) [J]. *Fisheries Science & Technology Information*, 2013, 40(5): 266-271.

[30] Shipton J A, Britz P J. An assessment of the use of chromic oxide as a marker in protein digestibility studies with *Haliotis midae* L. [J]. *Aquaculture*, 2001, 203(1/2): 69-83.

[31] Ramsay J M, Castell J D, Anderson D M, et al. Effects of fecal collection methods on estimation of digestibility of protein feedstuffs by winter flounder [J]. *Northern American Journal of Aquaculture*, 2000, 62(3): 168-173.

[32] Yue M, Xu L, Fang C L, et al. Effects of sodium butyrate on reproductive performance, antioxidant function of sows and growth of their offspring [J]. *Chinese Journal of Animal Science*, 2014, 50(23): 44-47.

[33] Aminiguli Yasheng, Yu Xiong, Li Shengli, et al. Effects of sodium butyrate on immune and antioxidant functions of perinatal dairy cows [J]. *Feed Research*, 2015, 5(5): 30-34.

[34] Ju T T, Guo X Y, Sui J J, et al. Effects of different forms of sodium butyrate on serum biochemical indices, antioxidant and anti-inflammatory functions of broilers challenged with lipopolysaccharide [J]. *Chinese Journal of Animal Nutrition*, 2015, 27(10): 3146-3154.

[35] Zhao H X, Cao J M, Huang Y H, et al. Effects of dietary nucleotides on growth, physiological parameters and antioxidant responses of juvenile yellow catfish *Pelteobagrus fulvidraco* [J]. *Aquaculture Research*, 2017, 48(1): 214-222.

[36] Meng Y, Ma R, Ma J, et al. Dietary nucleotides improve the growth performance, antioxidative capacity and intestinal morphology of turbot (*Scophthalmus maximus*) [J]. *Aquaculture Nutrition*, 2016, doi:10.1111/anu.12425.

[37] Romarheim O H, Zhang C, Penn M, et al. Growth and intestinal morphology in cobia (*Rachycentron canadum*) fed extruded diets with two types of soybean meal partly replacing fish meal [J]. *Aquaculture Nutrition*, 2008, 14(2): 174-180.

[38] Cai C F, Wang W J, Ye Y T, et al. Effect of soybean meal, raffinose and stachyose on the growth, body composition, intestinal morphology and intestinal microflora of juvenile allogynogenetic silver crucian (*Carassius auratus gibelio* × *Cyprinus carpio*) [J]. *Aquaculture Research*, 2012, 43(1): 128-138.

[39] Liu W S, Yang Y O, Zhang L L, et al. Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil [J]. *British Journal of Nutrition*, 2014, 112(1): 15–29.

[40] Iraporda, Errea A, Romanin D E, et al. Lactate and short chain fatty acids produced by microbial fermentation downregulate proinflammatory responses in intestinal epithelial cells and myeloid cells [J]. *Immunobiology*, 2015, 220(10): 1161–1169.

[41] Xu D D, He G, Zhou H H, et al. Effects of partial replacement of fish meal by corn gluten meal on growth performance and free amino acid metabolism of juvenile turbot (*Scophthalmus maximus* L.) [J]. *Periodical of Ocean University of China: Natural Science Edition*, 2016, 46(2): 26–34.

[42] Wang L, Zhou H H, He R J, et al. Effects of soybean meal fermentation by *Lactobacillus plantarum* P8 on growth, immune responses, and intestinal morphology in juvenile turbot (*Scophthalmus maximus* L.) [J]. *Aquaculture*, 2016, 464: 87–94.

[43] Wang Q, He G, Mai K, et al. Fishmeal replacement by mixed plant proteins and maggot meal on growth performance, target of rapamycin signalling and metabolism in juvenile turbot (*Scophthalmus maximus* L.) [J]. *Aquaculture Nutrition*, 2016, 22(4): 752–758.

[44] Imsland A K D, Helmvig T, Kristjansson G Ö, et al. Effect of fish protein replacement in diets for juvenile turbot (*Scophthalmus maximus* L.) [J]. *Turkish Journal of Fisheries and Aquatic Sciences*, 2016, 16(2): 267–273.

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