Effects of Replacing Fish Meal with Fermented Soybean Meal on Growth Performance, Serum Biochemical Indices, and Hepatic Insulin-like Growth Factor-I Gene Expression in Juvenile Yellow Drum (Nibea albiflora) Postprint

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Abstract

This experiment aimed to investigate the effects of fermented soybean meal replacing fish meal on growth performance, serum biochemical indices, and hepatic insulin-like growth factor-I (IGF-I) gene relative expression in juvenile yellow drum (Nibea albiflora), and to determine the appropriate proportion of fish meal replacement by fermented soybean meal in juvenile yellow drum diets. A basal diet containing 45% fish meal was formulated using Peruvian fish meal, soybean meal, and wheat gluten meal as the main protein sources, and fish oil, soybean oil, and soybean lecithin as the main lipid sources. Fermented soybean meal was then used to replace 0 (FSM0 group, as control), 10% (FSM10 group), 20% (FSM20 group), 30% (FSM30 group), 40% (FSM40 group), and 50% (FSM50 group) of fish meal in the basal diet, with appropriate amounts of lysine and methionine added to all groups except the control to maintain consistent lysine and methionine contents across groups, thereby formulating six isonitrogenous (protein level 50%) and isolipidic (lipid level 12%) experimental diets. Three hundred sixty juvenile yellow drum with initial body weight of (31.24±0.02) g were selected and randomly divided into six groups with three replicates of 20 fish each, and the culture experiment lasted for eight weeks. Results showed that weight gain rate, specific growth rate, and feed conversion ratio in FSM10, FSM20, and FSM30 groups were not significantly different from the control group ($P>0.05$), while those in FSM40 and FSM50 groups had significantly lower weight gain and specific growth rates ($P<0.05$) and significantly higher feed conversion ratios ($P<0.05$). No significant differences were found in survival rate among all groups ($P>0.05$). Replacement of fish meal with different proportions of fermented soybean meal had no significant effect on

hepatosomatic index, viserosomatic index, or condition factor of juvenile yellow drum (P>0.05). Serum aspartate aminotransferase (AST) activity in FSM40 and FSM50 groups was significantly higher than in other groups (P<0.05), and serum alanine aminotransferase (ALT) activity in FSM50 group was significantly higher than in control and FSM10 groups (P<0.05). Hepatic IGF-I gene relative expression in FSM50 group was significantly lower than in the control group (P<0.05). Based on comprehensive evaluation of all indicators, replacement of 20%-30% of dietary fish meal with fermented soybean meal was appropriate under the experimental conditions, while higher replacement proportions reduced growth performance and feed utilization in juvenile yellow drum.

Full Text

Abstract

This study investigated the effects of replacing fish meal with fermented soybean meal on growth performance, serum biochemical indices, and hepatic insulin-like growth factor-I (IGF-I) gene expression in juvenile yellow drum (Nibea albiflora) to determine the optimal substitution level. A basal diet containing 45% fish meal was formulated using Peruvian fish meal, soybean meal, and wheat gluten meal as primary protein sources, and fish oil, soybean oil, and soybean lecithin as main lipid sources. Six isonitrogenous (50% crude protein) and isolipidic (12% crude lipid) experimental diets were prepared by replacing 0% (FSM0, control), 10% (FSM10), 20% (FSM20), 30% (FSM30), 40% (FSM40), and 50% (FSM50) of the fish meal with fermented soybean meal. Lysine and methionine were supplemented to all diets except the control to maintain consistent amino acid levels across treatments.

A total of 360 juvenile yellow drum with initial body weight of (31.24±0.02) g were randomly allocated into six groups with three replicates each (20 fish per replicate). The feeding trial lasted for eight weeks. Results showed that weight gain rate, specific growth rate, and feed conversion ratio of fish in FSM10, FSM20, and FSM30 groups did not differ significantly from the control (P>0.05). However, FSM40 and FSM50 groups exhibited significantly lower weight gain and specific growth rates (P<0.05) and significantly higher feed conversion ratios (P<0.05). No significant differences were observed in survival rate among all groups (P>0.05). Hepatosomatic index, viscerosomatic index, and condition factor were not significantly affected by dietary treatments (P>0.05). Serum aspartate aminotransferase (AST) activity in FSM40 and FSM50 groups was significantly higher than other groups (P<0.05), while alanine aminotransferase (ALT) activity in FSM50 group was significantly higher than control and FSM10 groups (P<0.05). Hepatic IGF-I gene relative expression in FSM50 group was significantly lower than the control (P<0.05). Based on these results, replacing 20-30% of fish meal with fermented soybean meal is appropriate under these experimental conditions, while higher replacement levels reduce growth performance and feed utilization in juvenile yellow drum.

Keywords: juvenile yellow drum (Nibea albiflora); fermented soybean meal; fish meal; growth performance; IGF-I gene

Introduction

Fish meal is the most important protein source in aquafeeds. However, with the rapid development of aquaculture, increasing demand for fish meal has driven prices to unprecedented levels, making it the costliest ingredient in aquafeeds [1]. Consequently, identifying cost-effective alternative protein sources to replace fish meal has become a central focus in aquaculture nutrition research. Plant-based protein sources have attracted particular attention due to their relatively abundant supply and stable production. Commonly studied plant proteins include soybean meal, rapeseed meal, and cottonseed meal. Nevertheless, plant protein sources suffer from poor palatability, imbalanced amino acid profiles, and high levels of anti-nutritional factors, which can compromise growth performance in aquatic animals [2].

Fermented soybean meal, produced through microbial fermentation, eliminates many anti-nutritional factors while generating microbial proteins that enrich and balance the nutritional profile of soybean meal, ultimately improving its quality and feed efficiency. As a novel plant protein source, fermented soybean meal promotes animal growth [3] and enhances immune function [4]. Numerous studies have investigated fish meal replacement by fermented soybean meal in various fish species [5-9], though optimal replacement levels vary depending on species, life stage, feed formulation (particularly fish meal content in control diets), and culture duration.

Fish growth is primarily regulated by the growth hormone (GH)/insulin-like growth factors (IGFs) axis. Insulin-like growth factor I (IGF-I), a member of the IGF family, promotes growth and differentiation by inhibiting protein catabolism and suppressing expression of atrophy-related ubiquitin enzymes [10]. In fish, hepatic IGF-I gene expression is influenced by nutritional status [11]. Studies have shown that feeding high levels of plant protein diets significantly reduces hepatic IGF-I gene expression in Japanese seabass (Lateolabrax japonicas) [12] and cobia (Rachycentron canadum) [13]. Additionally, diets deficient in methionine [14] and lysine [15] also decrease hepatic IGF-I expression in aquatic animals.

Yellow drum (Nibea albiflora), belonging to Perciformes, Sciaenidae, and genus Nibea, is widely distributed in coastal waters of China, the Korean Peninsula, and southern Japan, representing a major traditional fishery resource [16]. Valued for its delicious taste, nutritional quality, and high market price, artificial cultivation of yellow drum has gained increasing attention, with commercial farming established in Fujian and Zhejiang provinces. While nutritional studies on yellow drum have been reported [17-21], research on fish meal replacement by fermented soybean meal remains unavailable. Building on our previous research, this study examined the effects of graded levels of fermented soybean meal re-

placement on growth performance, feed utilization, serum biochemical indices, and hepatic IGF-I gene expression in juvenile yellow drum to determine the optimal replacement level and provide fundamental data for developing low-fish meal, high-efficiency formulated feeds.

Materials and Methods

Experimental Design and Diet Preparation

A basal diet containing 45% fish meal was formulated using Peruvian fish meal, soybean meal, and wheat gluten meal as primary protein sources, with fish oil, soybean oil, and soybean lecithin as main lipid sources. Six isonitrogenous (50% crude protein) and isolipidic (12% crude lipid) experimental diets were prepared by replacing 0% (FSM0, control), 10% (FSM10), 20% (FSM20), 30% (FSM30), 40% (FSM40), and 50% (FSM50) of fish meal with fermented soybean meal (purchased from Beijing Xipuzhenghui Biological Feed Co., Ltd.). Based on lysine and methionine contents in the control diet, appropriate amounts of these amino acids were supplemented to all diets except the control to maintain consistent levels across treatments. Nutritional components and amino acid compositions of fish meal and fermented soybean meal are presented in Table 1 . Diet formulations and proximate compositions are shown in Table 2 , and amino acid compositions of experimental diets are provided in Table 3 .

All feed ingredients were ground to pass through a 60-mesh sieve, mixed using a stepwise expansion method, and blended with water in a mixer until thoroughly moistened. The mixture was extruded using a twin-screw extruder (F-26 model, South China University of Technology), pelletized into 2 mm and 4 mm diameter pellets, cooked in a 90°C oven for 30 minutes, air-dried in shade, and stored in sealed bags at -20°C until use.

Experimental Fish and Culture Management

Juvenile yellow drum produced by the Xixuan Fisheries Technology Island of Zhejiang Marine Fisheries Research Institute were initially stocked in indoor 50 m^3 cement tanks for two weeks of acclimation. After acclimation, 360 healthy fish with uniform size and initial body weight of (31.24±0.02) g were randomly distributed into six groups with three replicates each (20 fish per replicate) and cultured in 500 L fiberglass tanks. The feeding trial was conducted in a flow-through system at the China-Norway Joint Laboratory for Marine Fish Nutrition and Feed of Xixuan Fisheries Technology Island. Fish were hand-fed twice daily at 07:00 and 16:00 at approximately 3% of body weight, with feeding rates adjusted every two weeks based on bulk weighing. The trial lasted eight weeks. During the experimental period, water temperature was maintained at (27±2)°C, pH at 7.8-8.0, salinity at 28-29‰, dissolved oxygen >5.5 mg/L, and ammonia nitrogen <0.05 mg/L.

Sample Collection and Analysis

Sample Collection At the end of the feeding trial, fish were fasted for 24 hours, counted, and weighed, then anesthetized with eugenol. Five fish per tank were randomly selected for measurement of body length and weight, followed by dissection and weighing of viscera and liver to calculate hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF). Additionally, three fish per tank were randomly selected for blood collection from the caudal vein using sterile 2 mL syringes. Blood samples were placed in 2 mL centrifuge tubes, centrifuged at 3,000 r/min for 10 minutes at 4°C, and the serum was stored at -80°C for biochemical analysis. After blood collection, livers were excised using sterilized forceps and scissors, placed in sterile RNase-free 2.0 mL centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C for hepatic IGF-I gene expression analysis. All procedures were performed on ice.

Serum Biochemical Indices Serum biochemical parameters were measured using an automatic biochemical analyzer (Beckman DX-800, USA).

Hepatic IGF-I Gene Expression Analysis **Total RNA Extraction:** Total RNA was extracted from liver tissue using a rapid RNA extraction kit (Beijing Solarbio Science & Technology Co., Ltd.) following the manufacturer' s protocol.

cDNA Synthesis: Reverse transcription was performed using the TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) kit (Beijing TransGen Biotech Co., Ltd.) in a 20 L reaction volume. The reaction mixture contained 4 L 5×TransScript All-in-One SuperMix for qPCR, 1 L gDNA Remover, 6 L RNase-free dH2O, and 1 L total RNA, prepared on ice. After brief mixing and centrifugation, reverse transcription was performed under the following conditions: 37°C for 15 minutes, 85°C for 5 seconds, and 4°C hold. The synthesized cDNA was stored at -20°C.

Primer Design and Synthesis: Specific primers for yellow drum IGF-I gene were designed based on partial sequences obtained in our previous study, using -actin as the reference gene. All primers (Table 4) were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

Real-time Quantitative PCR: Real-time PCR was performed using SYBR Green I chemistry with liver cDNA as template. The IGF-I amplicon was 120 bp and -actin amplicon was 161 bp. The reaction was carried out using TransStart Tip Green qPCR Supermix (Beijing TransGen Biotech Co., Ltd.) in a 20 L volume containing 10 L 2×TransStart Tip Green qPCR SuperMix, 0.4 L each of forward and reverse primers, 2 L template, and 7.2 L ddH2O. Cycling conditions were: 95°C for 30 seconds, followed by 40 cycles of 94°C for 5 seconds, 60°C for 15 seconds, and 72°C for 20 seconds. A melting curve was generated by increasing temperature from 60°C to 95°C at 5°C per 5 seconds to verify amplification specificity. Relative gene expression was calculated using the 2-ΔΔCt

method [22].

Calculations

Survival rate (SR, %) = 100 × (final fish number)/(initial fish number)
Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight)/(initial body weight)
Specific growth rate (SGR, %/d) = 100 × (ln final body weight - ln initial body weight)/feeding days
Feed conversion ratio (FCR) = feed intake/total weight gain (wet weight)
Daily feeding rate (DFR, %) = 100 × feed intake/[feeding days × (initial body weight + final body weight)]
Condition factor (CF, g/cm^3) = 100 × body weight/body length^3
Hepatosomatic index (HSI, %) = 100 × liver weight/body weight
Viscerosomatic index (VSI, %) = 100 × viscera weight/body weight

Statistical Analysis

All data were analyzed by one-way ANOVA using SPSS 19.0 software. When significant differences were detected, Turkey' s test was used for multiple comparisons. Significance was set at $P<0.05$. Results are expressed as means ± standard error.

Results

Growth Performance

As shown in Table 5 , weight gain rate, specific growth rate, and feed conversion ratio of fish in FSM10, FSM20, and FSM30 groups did not differ significantly from the control (FSM0) ($P>0.05$). However, FSM40 and FSM50 groups exhibited significantly lower weight gain and specific growth rates ($P<0.05$) and significantly higher feed conversion ratios ($P<0.05$) compared with the control. No significant differences were observed in survival rate among all groups ($P>0.05$).

Morphological Indices

Table 6 shows that replacement of fish meal with fermented soybean meal at different levels did not significantly affect hepatosomatic index, viscerosomatic index, or condition factor of juvenile yellow drum ($P>0.05$).

Serum Biochemical Indices

Table 7 indicates that replacement of fish meal with fermented soybean meal did not significantly affect serum total protein (TP), cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), or low-density lipoprotein cholesterol (LDL-C) levels ($P>0.05$). Serum aspartate aminotransferase (AST) activity in FSM40 and FSM50 groups was significantly higher

than in other groups (P<0.05), while alanine aminotransferase (ALT) activity in FSM50 group was significantly higher than in control and FSM10 groups (P<0.05).

Hepatic IGF-I Gene Expression

Figure 1 [Figure 1: see original paper] shows that hepatic IGF-I gene relative expression in FSM50 group was significantly lower than in the control (P<0.05), with no significant differences among other groups (P>0.05).

Discussion

Effects on Growth Performance

Building on our previous research, this study investigated the effects of fish meal replacement by fermented soybean meal on growth performance of juvenile yellow drum, aiming to develop low-fish meal, high-efficiency formulated feeds. Survival rates exceeded 98% across all groups without significant differences. However, weight gain and specific growth rates declined with increasing replacement levels, being significantly lower in FSM40 and FSM50 groups compared with the control, while feed conversion ratio showed the opposite trend. These results may be attributed to reduced palatability of high plant protein diets, which decreased feed intake [6] and resulted in insufficient nutrient intake, consequently impairing growth performance and feed efficiency. Additionally, compared with fish meal, plant protein sources exhibit essential amino acid imbalances that may inhibit growth as replacement levels increase [23]. Therefore, under our dietary formulation, fermented soybean meal can replace 20-30% of fish meal without affecting growth of juvenile yellow drum (initial weight ~31.2 g, 56-day culture period). Similar findings have been reported in Japanese seabass [8], black sea bream [9], rainbow trout [24], and large yellow croaker [6]. Optimal replacement levels vary depending on species, life stage, feed formulation (particularly fish meal content in control diets), and culture duration. Lee et al. [25] noted that juvenile rockfish (Sebastes schlegeli) showed lower tolerance to fermented soybean meal than adults; while 1.2 g juveniles tolerated only 10% replacement, 148.2 g fish could tolerate up to 40% replacement. This study also found no significant effects on hepatosomatic index, viscerosomatic index, or condition factor, consistent with Feng et al. [6] but contrasting with Liang et al. [8], who reported significantly lower indices in Japanese seabass fed 50% replacement diets. Notably, their study lasted 16 weeks compared to 8 weeks in our trial, suggesting that culture duration may influence the effects of fish meal replacement.

Effects on Serum Biochemical Indices

Fish blood parameters are closely related to metabolism, nutritional status, and disease [26]. Serum total protein content reflects health status, with reduced levels indicating hepatic dysfunction. Wang et al. [27] reported that 20% fish

meal replacement by soybean meal significantly decreased serum TP in brown-marbled grouper (Epinephelus fuscoguttatus), whereas Feng et al. [6] found no significant effects in large yellow croaker. Our study observed no significant changes in serum TP with increasing replacement levels. Plant proteins containing isoflavones can reduce serum cholesterol when replacing fish meal [28]. Studies on gibel carp (Carassius auratus gibelio) [29] and Japanese flounder (Paralichthys olivaceus) [30] demonstrated significant cholesterol reduction with soybean meal replacement, while Wang et al. [31] reported lower TCHO and HDL-C in gibel carp fed 30% soybean meal. Conversely, no significant effects on cholesterol were observed in large yellow croaker [6] or rice field eel (Monopterus albus) [32] fed fermented soybean meal or extruded soybean. Our study similarly found no significant effects on serum CHOL, HDL-C, or LDL-C levels, possibly due to species differences and culture duration.

AST and ALT are primarily localized in hepatocytes and normally present at low serum levels. Elevated serum activities indicate hepatocellular damage, increased membrane permeability, or necrosis, with the degree of elevation correlating with injury severity [33]. Our study found significantly higher AST activity in FSM40 and FSM50 groups, and significantly elevated ALT activity in FSM50 group compared with control and FSM10 groups, suggesting that high dietary inclusion of fermented soybean meal may cause hepatic damage and consequent growth reduction.

Effects on Hepatic IGF-I Gene Expression

The GH/IGFs axis is a critical endocrine regulator of fish growth. IGF-I directly promotes growth by enhancing amino acid uptake and utilization, stimulating protein and RNA synthesis, and promoting muscle development [34]. Our results showed declining hepatic IGF-I gene expression with increasing replacement levels, with FSM50 group exhibiting significantly lower expression than the control, correlating with observed growth performance. Similar results have been reported in gilthead sea bream [35], Japanese seabass [12], and cobia [13], where high plant protein diets reduced hepatic IGF-I expression. Hepatic IGF-I expression is regulated by nutritional status [11]; fasting decreases expression while refeeding restores it [36]. In our study, daily feeding rate tended to decrease with increasing replacement levels, which may partially explain reduced IGF-I expression. Additionally, amino acid imbalance may contribute to this reduction [12-13]. Although we supplemented limiting amino acids (lysine and methionine) in all diets except the control, differential absorption between crystalline and protein-bound amino acids may have created disparities in amino acid availability [13]. Studies have shown that methionine [14] and lysine [15] can upregulate hepatic IGF-I expression, suggesting that lower absorption of these amino acids in high replacement groups may have contributed to reduced IGF-I expression. Therefore, excessive replacement of fish meal with fermented soybean meal is not recommended, and future research should investigate synchronized absorption of different amino acid forms in high plant protein diets.

Conclusion

Under the conditions of this study, replacing 20-30% of fish meal with fermented soybean meal is appropriate for juvenile yellow drum. Higher replacement levels compromise growth performance and feed utilization efficiency.

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