Effects of Fermented Okara on Growth Performance, Plasma Biochemical Indices, and Antioxidant Capacity in Jian Carp (Postprint)

Authors: Jiang Yu, Zhao Pengfei, Chen Yongjun, Luo Qiang, Li Hong, Lin Shimei

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Abstract

To investigate the effects of fermented soybean residue on growth performance, plasma biochemical indices, and antioxidant capacity of Jian carp, five isonitrogenous and isolipidic experimental diets were formulated by supplementing 0 (FSR0, as control), 6% (FSR6), 12% (FSR12), 18% (FSR18), and 24% (FSR24) fermented soybean residue to replace soybean meal in the basal diet, and fed to Jian carp with an initial body weight of 8.49 g for 9 weeks. Each diet was assigned three replicates, with 30 fish per replicate. The results showed that the FSR12 group exhibited significantly higher specific growth rate (SGR) and protein efficiency ratio (PER) than other groups ($P<0.05$), along with the lowest feed intake (FI) and feed conversion ratio (FCR). With increasing dietary fermented soybean residue levels, the viscera-somatic index (VSI) of Jian carp decreased significantly ($P<0.05$), whereas the hepatosomatic index (HSI) displayed a trend of initially increasing then decreasing. No significant differences were detected among groups in plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and glutathione peroxidase (GPx) activities, glucose (Glu) and triglyceride (TG) concentrations, or GPx/superoxide dismutase (SOD) ratio ($P>0.05$). Plasma total protein (TP) content in the FSR12 group was significantly higher than that in the control, FSR6, and FSR24 groups ($P<0.05$), but did not differ significantly from the FSR18 group ($P>0.05$). The control group showed significantly higher plasma total cholesterol (TC) and malondialdehyde (MDA) contents than all experimental groups ($P<0.05$), while its plasma SOD and catalase (CAT) activities were significantly lower ($P<0.05$). Based on specific growth rate as the evaluation criterion, regression analysis determined that the optimal supplementation level of fermented soybean residue in Jian carp diets was 10.2%; however, excessive levels would inhibit growth while enhancing systemic antioxidant capacity.

Full Text

Abstract

This study investigated the effects of fermented soybean residue (FSR) on growth performance, plasma biochemical indexes, and antioxidant capacity of Jian carp (*Cyprinus carpio* var. Jian). Five isonitrogenous and isolipidic experimental diets were formulated by supplementing 0% (FSR0, control), 6% (FSR6), 12% (FSR12), 18% (FSR18), and 24% (FSR24) FSR to replace soybean meal in basal diets. These diets were fed to Jian carp with an initial body weight of 8.49 g for 9 weeks. Each diet was assigned to three replicates, with 30 fish per replicate. The results showed that the specific growth rate (SGR) and protein efficiency ratio (PER) of fish in the FSR12 group were significantly higher than those in other groups ($P<0.05$), while feed intake (FI) and feed conversion ratio (FCR) were the lowest in this group. The viscera somatic index (VSI) decreased significantly with increasing dietary FSR levels ($P<0.05$), whereas the hepatosomatic index (HSI) first increased and then decreased. No significant differences were observed among groups in plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and glutathione peroxidase (GPx) activities, glucose (Glu) and triglyceride (TG) contents, or GPx/superoxide dismutase (SOD) ratio ($P>0.05$). Plasma total protein (TP) content in the FSR12 group was significantly higher than that in the control, FSR6, and FSR24 groups ($P<0.05$), but did not differ significantly from the FSR18 group ($P>0.05$). Plasma total cholesterol (TC) and malondialdehyde (MDA) contents in the control group were significantly higher than those in all experimental groups ($P<0.05$), while plasma SOD and catalase (CAT) activities were significantly lower ($P<0.05$). Regression analysis based on SGR indicated that the optimal dietary FSR supplementation level for Jian carp was 10.2%. Excessive supplementation levels inhibited growth but enhanced antioxidant capacity.

Keywords: Jian carp; fermented soybean residue; growth; antioxidant capacity; biochemical indexes

Introduction

Protein sources are critical components of aquafeeds that substantially affect feed costs. Therefore, investigating fish nutritional metabolism mechanisms and identifying alternatives to fishmeal protein have long been research priorities for nutritionists. In recent years, food processing by-products have attracted widespread attention from feed manufacturers [1]. Soybean residue (SR) is a by-product of soymilk or tofu processing, with substantial annual output in Asian countries where soybean consumption is high. Currently, China’ s annual SR production exceeds 2.8 million tons [2], with the Li Rang Town in Liangping District, Chongqing alone producing over 10,000 tons annually. China’ s abundant SR resources offer promising prospects for development and utilization.

SR is rich in protein, fat, fiber, minerals, monosaccharides, oligosaccharides, and dietary fiber [2], though nutrient composition varies with soybean variety and processing method [3]. As a high-quality human food, SR exhibits multiple beneficial effects, including antioxidant activity [4], cardiovascular disease prevention [5], reduced hepatic fat deposition, and health maintenance [6-7]. Previous studies have demonstrated SR as a protein source in diets for ruminants [8], pigs [9], and broilers [10]. However, research on SR application in fish remains limited [11], possibly due to drying costs and the presence of fiber, phytic acid, and other antinutritional factors [2].

Research has shown that fermentation of SR with *Lactobacillus*, *Bacillus*, *Aspergillus flavus*, or yeast can enhance its nutritional value [12-13]. To date, no studies have investigated fermented soybean residue in Jian carp (*Cyprinus carpio* var. Jian). Therefore, this experiment utilized solid-state fermented SR to examine its effects on growth performance, body composition, plasma biochemical indexes, and antioxidant capacity of Jian carp, aiming to provide a theoretical basis for SR utilization.

1.1 Experimental Diets

Soybean residue was purchased from Liangping District, Chongqing, as a by-product of soybean processing. The SR was subjected to solid-state fermentation using a mixed culture of *Bacillus subtilis*, lactic acid bacteria, and yeast (fermentation pH 7.0, temperature 28°C, duration 72 h) to produce fermented soybean residue, following methods described by Tang et al. [14] and Li et al. [15]. The proximate nutrient and amino acid contents of SR and FSR are presented in Table 1 .

A basal diet was formulated using fishmeal, soybean meal, rapeseed meal, and cottonseed protein as primary protein sources, with soybean oil as the lipid source. Fermented soybean residue was added at 0% (control), 6%, 12%, 18%, and 24% to replace soybean meal, with adjustments to soybean meal content to balance crude protein levels, resulting in five isonitrogenous and isolipidic experimental diets (designated FSR0, FSR6, FSR12, FSR18, and FSR24). Diet composition and nutrient levels are shown in Table 2 . All dietary ingredients were ground through an 80-mesh sieve, mixed using the stepwise dilution method, and processed into 2.0 mm diameter pellets. The pellets were air-dried and stored at 4°C until use.

The premix provided the following per kg of diet: VA 16.67 mg, VD 3.33 mg, VE 26.67 mg, VC 333.33 mg, VB 8 mg, VB 4 mg, VB 0.03 mg, VK 3.33 mg, riboflavin 3.33 mg, inositol 66.67 mg, pantothenic acid 20 mg, niacin 23.33 mg, folic acid 1.33 mg, biotin 0.04 mg, ethoxyquin 100 mg, wheat middling 9.39 g, KCl 133.33 mg, KI 40 mg, CoCl ·6H O 4.67 mg, CuSO ·5H O 9.33 mg, FeSO ·H O 266.67 mg, ZnSO ·H O 133.33 mg, MnSO ·H O 53.33 mg, Na SeO ·5H O 43.33 mg, MgSO ·7H O 2,000 mg, Ca(H PO) ·H O 13.33 g, and NaCl 90.67 mg.

1.2 Feeding Management

A total of 450 healthy Jian carp with uniform size (average body weight 8.49 g) were selected and randomly divided into five groups, with three replicates per group and 30 fish per replicate. Fish were cultured in indoor freshwater recirculating aquaria (effective volume 250 L) for 9 weeks. The daily feeding rate was 3-5% of body weight, with three feedings daily at 08:00, 12:30, and 17:00. Water source was aerated tap water. During the experimental period, water temperature was maintained at 26.2±0.5°C, pH at 7.3±0.5, dissolved oxygen >6.8 mg/L, ammonia nitrogen <0.48 mg/L, and nitrite nitrogen <0.06 mg/L.

1.3 Sample Preparation and Analysis

At the end of the feeding trial, fish were fasted for 24 h before weighing. Three fish per replicate were randomly selected as whole-body samples for body composition analysis. Four fish per replicate were randomly selected, anesthetized with MS-222, measured for body length and height, and dissected to obtain viscera and hepatopancreas for morphological measurements. Five fish per replicate were randomly selected for blood collection from the caudal vein using disposable sterile syringes. Blood was anticoagulated with heparin and potassium oxalate-sodium fluoride (for plasma glucose determination), immediately centrifuged at 1,000×g for 10 min at 4°C, and plasma was collected and stored at -20°C until analysis.

Feed ingredients and whole-body samples were dried at 105°C to constant weight. Crude protein content was determined by the Kjeldahl method, crude lipid by Soxhlet extraction, and crude ash by combustion at 550°C. Phytic acid content was determined according to Vaintraub et al. [16], and trypsin inhibitor content according to Smith et al. [17]. Diet samples were hydrolyzed with 6 mol/L HCl at 110°C for 22 h, and amino acid contents were determined using a 4.6 mm×60 mm analytical column on a Hitachi 8800 amino acid analyzer.

Plasma metabolic indexes were measured using a Hitachi 7100 automatic biochemical analyzer, including ALT, AST, ALP activities, and glucose, total cholesterol (TC), and triglyceride (TG) contents. Plasma total protein (TP) and malondialdehyde (MDA) contents, and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were determined using assay kits from Nanjing Jiancheng Bioengineering Institute. Protein content was determined by the Coomassie brilliant blue method.

1.4 Calculation Formulas

Specific growth rate (SGR, %/d) = 100 × [lnW -lnW] / t
Protein efficiency ratio (PER, %) = 100 × (W -W) / (F × F)
Feed conversion ratio (FCR) = F / (W -W)
Feed intake (FI, %/d) = F / [(W + W) / 2] / t
Survival rate (SR, %) = 100 × N / N

Condition factor (CF, g/cm^3) = 100 × W / L^3
Viscera somatic index (VSI, %) = 100 × W / W
Hepatosomatic index (HSI, %) = 100 × W / W

Where: W (g) and W (g) are final and initial body weight; t (d) is the experimental duration; F (g) is average feed intake per fish; F (%) is dietary crude protein content; N and N are final and initial fish number; L (cm) is body length; W (g) is viscera weight; W (g) is hepatopancreas weight.

1.5 Data Processing and Analysis

Data were analyzed using one-way ANOVA in SPSS 17.0. If significant differences were detected, Tukey' s multiple comparison test was performed with significance level set at $P<0.05$. All data are expressed as mean ± standard error (mean ± SE).

2.1 Effects of Fermented Soybean Residue on Growth Performance of Jian Carp

As shown in Table 3 , final body weight, specific growth rate, and protein efficiency ratio of Jian carp increased initially and then decreased with increasing dietary FSR levels, reaching maximum values at 12% FSR supplementation and minimum values at 24% supplementation, with significant differences between these groups ($P<0.05$). Quadratic curve fitting of the relationship between FSR supplementation level (x) and SGR (y) yielded the regression equation $y = -0.0019x^2 + 0.0397x + 2.764$ ($R^2 = 0.9712$) (Figure 1 [Figure 1: see original paper]), from which the optimal FSR level for maximum SGR was calculated as 10.2%. Conversely, feed intake and feed conversion ratio were lowest at 12% FSR supplementation, significantly lower than other groups ($P<0.05$). Survival rate was 100% in all groups.

2.2 Effects of Fermented Soybean Residue on Morphological Measurements and Body Composition of Jian Carp

As shown in Table 4 , viscera somatic index was significantly lower in groups with FSR supplementation levels 12% compared to the control group ($P<0.05$). Hepatosomatic index increased initially and then decreased with increasing FSR levels, reaching its maximum in the FSR12 group, which was significantly higher than all groups except FSR6 ($P<0.05$). No significant differences were observed among groups in condition factor or whole-body moisture, crude protein, crude lipid, and ash contents ($P>0.05$).

2.3 Effects of Fermented Soybean Residue on Plasma Biochemical Indexes of Jian Carp

As shown in Table 5 , no significant differences were detected among groups in plasma ALT, AST, and ALP activities or glucose and triglyceride contents ($P>0.05$). Plasma total protein content in the FSR12 group was significantly higher than that in the control, FSR6, and FSR24 groups ($P<0.05$), but did not differ significantly from the FSR18 group ($P>0.05$). Plasma total cholesterol content in the control group was significantly higher than that in all experimental groups ($P<0.05$).

2.4 Effects of Fermented Soybean Residue on Plasma Antioxidant Indexes of Jian Carp

As shown in Table 6 , plasma superoxide dismutase and catalase activities in all experimental groups were significantly higher than those in the control group ($P<0.05$). Plasma malondialdehyde content in the control group was significantly higher than that in the FSR12, FSR18, and FSR24 groups ($P<0.05$), but did not differ significantly from the FSR6 group ($P>0.05$). The catalase/superoxide dismutase ratio in the FSR18 and FSR24 groups was significantly higher than that in the control group and other experimental groups ($P<0.05$). No significant differences were observed among groups in plasma glutathione peroxidase activity or glutathione peroxidase/superoxide dismutase ratio ($P>0.05$).

3 Discussion

3.1 Effects of Fermented Soybean Residue on Growth Performance of Jian Carp

Numerous studies have demonstrated that biological fermentation effectively enhances the nutritional value of plant proteins. Fermentation reportedly increases protein and small peptide contents, thereby improving feed nutritional value [3,18]. As shown in Table 1, fermentation significantly improved SR nutritional quality by increasing nutrient content and reducing antinutritional factors. SR has achieved favorable production results as a protein feed for livestock [10,19]. The present study confirms that 10.2% dietary FSR supplementation effectively improved growth performance in Jian carp, indicating that FSR is a feasible protein source for fish feeds. Similar improvements in growth performance have been reported in pigs [20] and broilers [21] fed fermented soybean meal, as well as in Atlantic salmon [22], rainbow trout [23], and *Macrobrachium nipponense* [24]. This may be attributed to fermentation enhancing nutrient content [18] and reducing antinutritional factors [25-26], thereby promoting animal growth. Furthermore, studies have shown that dietary fermented soybean meal neither altered intestinal morphology in rainbow trout [23] nor caused intestinal lesions in Atlantic salmon [22]. Subsequent research confirmed that dietary supplementation with *Bacillus subtilis*-fermented soybean meal significantly improved hepatic and posterior intestinal histomorphology in grouper [27]. These findings suggest that feed fermentation benefits digestive tract health in both terrestrial livestock and aquatic animals.

In this experiment, 24% FSR supplementation significantly reduced growth rate in Jian carp without decreasing feed intake, indicating that FSR palatability was not the limiting factor. While fermentation can enhance digestive enzyme activity and digestibility, and the *Bacillus subtilis* and lactic acid bacteria in fermented products are considered probiotics that promote fish growth and health [28], the high crude fiber and antinutritional factor (trypsin inhibitor) contents remaining after fermentation may limit high-dose application in Jian carp feeds, warranting further investigation.

The results also showed that FSR supplementation level significantly affected hepatosomatic index, which peaked at 12% supplementation. Given that experimental fish weighed less than 50 g and were in the liver development stage, this suggests that FSR promoted healthy liver development and consequently enhanced growth. Studies in mice [6] and hamsters [7] found that dietary SR increased hepatic expression of sterol 14 -demethylase (CYP51) and peroxisome proliferator-activated receptor (PPAR) genes, thereby reducing hepatic fat deposition. Soy protein is known to be rich in isoflavones that decrease hepatic lipogenesis [29]. These results indicate that SR or FSR benefits liver health. The observed reduction in viscera somatic index was independent of hepatosomatic index changes, possibly reflecting reduced mesenteric fat deposition in fish, which merits further attention and investigation.

3.2 Effects of Fermented Soybean Residue on Plasma Biochemical Indexes and Antioxidant Capacity of Jian Carp

Plasma biochemical indexes reflect fish physiological and nutritional status. Studies have shown that SR can regulate plasma glucose in diabetic mice [30], and fermented mulberry leaves can improve plasma glucose in largemouth bass [31]. However, FSR supplementation did not significantly affect plasma glucose in Jian carp, possibly due to differences in experimental animals and materials. Nevertheless, FSR significantly reduced plasma total cholesterol, consistent with findings in red sea bream [32] and studies showing cholesterol reduction in rats [33] and Syrian hamsters [7]. Soy protein has been reported to improve blood lipid profiles [30], indicating that FSR participates in lipid metabolism regulation. Additionally, SR plays important roles in preventing hypercholesterolemia [34] and hyperlipidemia [7], associated with enhanced hepatic -oxidation-related gene expression [6]. Conversely, Lim et al. [35] found that blood biochemical indexes in tilapia were unaffected by soybean meal fermentation, suggesting that further research is needed on the relationship between fish nutrition and clinical medicine.

Studies have demonstrated that fermented plant products can enhance nonspecific immune responses and antioxidant capacity in fish [21,36]. Soybean residue has been identified as a potential source of antioxidant components [2,37-38]. The present study found that dietary FSR supplementation significantly reduced plasma malondialdehyde content while increasing superoxide dismutase and catalase activities. Similar improvements in antioxidant capacity have been reported in black sea bream fed fermented soybean meal [38] and in *Macrobrachium nipponense* [25], possibly due to the presence of isoflavones and antioxidants in fermented products. Limited research exists on fish nutrition and antioxidant capacity [39], and since SR is rich in antioxidant substances, further investigation into its antioxidant mechanisms is warranted.

4 Conclusions

1. Fermented soybean residue is a feasible protein source for Jian carp diets.

2. The optimal dietary supplementation level of fermented soybean residue for Jian carp is 10.2%, which improves growth performance. Excessive supplementation (24%) inhibits growth but enhances antioxidant capacity.

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