

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

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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 to three replicates, with 30 fish per replicate. The results showed that the specific growth rate (SGR) and protein efficiency ratio (PER) of Jian carp in the FSR12 group were significantly higher than those in other groups ($P < 0.05$), while its feed intake (FI) and feed conversion ratio (FCR) were the lowest. With the increase of fermented soybean residue supplementation level in the diets, the viscerosomatic index (VSI) of Jian carp decreased significantly ($P < 0.05$), while the hepatosomatic index (HSI) showed a trend of first increasing and then decreasing. 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, and GPx/superoxide dismutase (SOD) ratio ($P > 0.05$). The 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 showed no significant difference from the FSR18 group ($P > 0.05$). The 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 superoxide dismutase (SOD) and catalase (CAT) activities were significantly lower than those in all experimental groups ($P < 0.05$). Using specific growth rate as the evaluation index, regression analysis indicated that the optimal supplementation level of fermented soybean residue in Jian carp

diets was 10.2%; excessive supplementation levels would inhibit the growth of Jian carp, but could improve the antioxidant capacity of the organism.

Full Text

Effects of Fermented Soybean Residue on Growth Performance, Plasma Biochemical Indexes, and Antioxidant Capacity of Jian Carp

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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) fermented soybean residue to replace soybean meal in basal diets. Jian carp with an initial body weight of 8.49 g were fed the experimental diets for nine 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) showed a trend of initial increase followed by decrease. No significant differences were observed among all 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 treatment groups ($P < 0.05$), while plasma SOD and catalase (CAT) activities were significantly lower ($P < 0.05$). Regression analysis based on specific growth rate indicated that the optimal dietary inclusion level of fermented soybean

residue for Jian carp was 10.2%. Excessive inclusion 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. Consequently, investigating fish nutritional metabolism mechanisms and identifying alternatives to fishmeal protein has long been a focus for nutritionists. In recent years, food processing by-products have attracted widespread attention from feed manufacturers. Soybean residue (okara) is a by-product of soy milk and tofu processing, with substantial annual output in Asian countries with high soybean consumption. Currently, China's annual okara production exceeds 2.8 million tons, with the town of Li Rang in Liangping District, Chongqing alone producing over ten thousand tons annually. This abundant resource offers promising prospects for development and utilization.

Okara is rich in protein, fat, fiber, minerals, monosaccharides, oligosaccharides, and dietary fiber, though nutritional composition varies with soybean variety and processing methods. As a high-quality human food, okara exhibits multiple beneficial effects including antioxidant activity, cardiovascular disease prevention, reduced hepatic fat deposition, and health maintenance. Previous studies have demonstrated okara's potential as a protein source in diets for ruminants, pigs, and broilers. However, research on okara application in fish remains limited, possibly due to drying costs and the presence of fiber, phytic acid, and other antinutritional factors.

Research has shown that fermentation of okara using *Lactobacillus*, *Bacillus*, *Aspergillus flavus*, or yeast can enhance its nutritional value. To date, no studies have investigated fermented soybean residue in Jian carp. Therefore, this experiment utilized solid-state fermented okara 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 okara utilization.

1.1 Experimental Diets

Okara was purchased from Liangping District, Chongqing, as a by-product of soybean processing. Solid-state fermentation was conducted 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. and Li et al. The proximate nutrient and amino acid contents of okara and fermented okara are presented in Table 1.

Basal diets were formulated using fish meal, soybean meal, rapeseed meal, and cottonseed protein as primary protein sources, with soybean oil as the lipid source. Five isonitrogenous and isolipidic experimental diets were prepared by

supplementing 0% (control), 6%, 12%, 18%, and 24% fermented soybean residue (designated FSR0, FSR6, FSR12, FSR18, and FSR24, respectively), with soybean meal adjusted to balance crude protein content. Diet composition and nutrient levels are shown in Table 2. All feed 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.

1.2 Feeding Management

A total of 450 healthy Jian carp with uniform size (average body weight 8.49 g) were 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 nine 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 $26.2\pm 0.5^{\circ}\text{C}$, 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 hours and weighed. Three fish per replicate were randomly selected as whole-body samples for body composition analysis. Four fish per replicate were anesthetized with MS-222 to measure body length and height, and to isolate viscera and hepatopancreas for morphological index determination. 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 $1,000\times g$ 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 to constant weight at 105°C . Crude protein content was determined by the Kjeldahl method, crude lipid by Soxhlet extraction, and crude ash by high-temperature incineration at 550°C . Phytic acid content was measured according to Vaintraub et al. Trypsin inhibitor content was determined following Smith et al. Amino acid contents in feed samples were analyzed using a Hitachi 8800 amino acid analyzer with a 4.6 mm \times 60 mm column after hydrolysis with 6 mol/L HCl at 110°C for 22 h.

Plasma metabolic indexes were measured using a Hitachi 7100 automatic biochemical analyzer, including ALT, AST, and 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 measured by the Coomassie brilliant blue method.

1.4 Calculation Formulas

Specific growth rate (SGR, %/d) = $100 \times [\ln(W_t) - \ln(W_0)] / t$

Protein efficiency ratio (PER, %) = $100 \times (W_t - W_0) / (F \times F_p)$

Feed conversion ratio (FCR) = $F / (W_t - W_0)$

Feed intake (FI, %/d) = $F / [(W_t + W_0) / 2] / t$

Survival rate (SR, %) = $100 \times N_t / N_0$

Condition factor (CF, g/cm³) = $100 \times W_t / L^3$

Viscera somatic index (VSI, %) = $100 \times W_v / W_t$

Hepatosomatic index (HSI, %) = $100 \times W_h / W_t$

Where: W_t (g) and W_0 (g) are final and initial body weight; t (d) is experimental duration; F (g) is average feed intake per fish; F_p (%) is dietary crude protein content; N_t and N_0 are final and initial fish number; L (cm) is body length; W_v (g) is viscera weight; W_h (g) is hepatopancreas weight.

1.5 Data Processing and Analysis

Data were analyzed using one-way ANOVA with 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 \pm standard error.

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% inclusion and minimum values at 24% inclusion, with significant differences between these groups ($P < 0.05$). Quadratic regression analysis of the relationship between FSR inclusion level (x) and SGR (y) yielded the equation $y = -0.0019x^2 + 0.0397x + 2.764$ ($R^2 = 0.9712$) [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 inclusion, 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 inclusion levels of 12% and above compared to the control group ($P < 0.05$). Hepatosomatic index showed an initial increase followed by a decrease with increasing FSR levels, reaching maximum value 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 treatment groups ($P<0.05$).

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

As shown in Table 6 , plasma SOD and CAT activities in all treatment groups were significantly higher than those in the control group ($P<0.05$). Plasma MDA 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 CAT/SOD ratio in the FSR18 and FSR24 groups was significantly higher than that in the control group and other treatment groups ($P<0.05$). No significant differences were observed among groups in plasma GPx activity or GPx/SOD 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 can increase protein and small peptide contents, thereby improving feed nutritional value. As shown in Table 1, fermentation significantly improved okara's nutritional profile by increasing nutrient content and reducing antinutritional factors. Okara has demonstrated satisfactory production effects as a protein feed for livestock. The present results confirm that 10.2% dietary inclusion of fermented okara effectively improved growth performance in Jian carp, indicating its feasibility as a protein source in fish feed. Similar improvements in growth performance have been reported in pigs and broilers fed fermented soybean meal, as well as in Atlantic salmon, rainbow trout, and *Macrobrachium nipponense*. These benefits likely arise from fermentation's ability to increase nutrient content and reduce antinutritional factors, thereby promoting animal growth. Furthermore, studies have shown that dietary inclusion of fermented soybean meal does not alter intestinal morphology in rainbow trout or cause intestinal lesions in Atlantic salmon. Subsequent research confirmed that dietary supplementation with *Bacillus subtilis*-fermented soybean meal significantly improved hepatic and posterior intestinal histomorphology in grouper. These findings suggest that feed fermentation benefits digestive tract health in both terrestrial livestock and aquatic animals.

In this study, 24% fermented okara inclusion significantly reduced growth rate in Jian carp without decreasing feed intake, indicating that palatability was not the limiting factor. While fermentation can enhance digestive enzyme activity and improve digestibility, and the *Bacillus subtilis* and lactic acid bacteria in fermented products are considered probiotics that promote fish growth and health, the high levels of crude fiber and antinutritional factors (trypsin inhibitors) remaining after fermentation may limit high-dose application in Jian carp diets. This warrants further investigation.

The results also showed that fermented okara inclusion significantly affected hepatosomatic index, which peaked at 12% inclusion. Since the experimental fish weighed less than 50 g and were in the liver development stage, this suggests that fermented okara promotes healthy liver development and consequently enhances growth. Studies in mice and hamsters have demonstrated that dietary okara increases expression of hepatic 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. These findings indicate that okara or fermented okara benefits liver health. The observed reduction in viscera somatic index was independent of hepatosomatic index changes, likely 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 okara can regulate plasma glucose in diabetic mice, and fermented mulberry leaves can improve plasma glucose in largemouth bass. However, fermented okara did not significantly affect plasma glucose in Jian carp, possibly due to differences in experimental animals and materials. Nevertheless, fermented okara significantly reduced plasma total cholesterol, consistent with findings in red sea bream. Similar cholesterol-lowering effects have been observed in rats and Syrian hamsters fed okara. Soy protein is known to improve blood lipid profiles, suggesting that fermented okara participates in lipid metabolism regulation. Additionally, okara plays important roles in preventing hypercholesterolemia and hyperlipidemia by enhancing expression of hepatic β -oxidation-related genes. In contrast, Lim et al. found that blood biochemical indexes in tilapia were unaffected by soybean meal fermentation. Therefore, the relationship between fish nutrition and clinical medicine requires further investigation.

Research has demonstrated that fermented plant products can enhance non-specific immune responses and antioxidant capacity in fish. Okara has been identified as a potential source of antioxidant components. The present study found that dietary fermented okara significantly reduced plasma MDA content while increasing SOD and CAT activities. Similar improvements in antioxidant capacity have been reported in black sea bream fed fermented soybean meal and in *Macrobrachium nipponense*. These effects may be attributed to isoflavones

and antioxidants present in fermented okara or fermented soybean meal. Limited research exists on the relationship between fish nutrition and antioxidant capacity. Since okara is rich in antioxidant substances, further investigation into its antioxidant mechanisms is warranted.

4 Conclusions

1. Fermented soybean residue is a feasible protein source in Jian carp diets.
2. The optimal dietary inclusion level of fermented soybean residue for Jian carp is 10.2%, which improves growth performance. Excessive inclusion levels (24%) inhibit growth but enhance antioxidant capacity.

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