Effects of Replacing Fish Meal with Soybean Protein Concentrate on Growth, Feed Utilization, and Digestive and Antioxidant Enzyme Activities in Yellow Catfish (Pelteobagrus fulvidraco) Postprint

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Abstract

This experiment was conducted to investigate the effects of soybean protein concentrate (SPC) replacing fish meal on growth performance, feed utilization, serum biochemical indices, proximate composition of whole body and muscle, digestive enzymes, and antioxidant activity in yellow catfish. Six isonitrogenous and isolipidic diets were formulated with SPC replacement levels of 0, 10%, 20%, 30%, 40%, and 60%. A total of 540 juvenile yellow catfish with initial body weight of (2.17$±$0.02) g were randomly divided into 6 groups with 3 replicates per group, and 30 juvenile fish were stocked per replicate (culture tank) for an 8-week feeding trial. The results showed that: 1) With increasing SPC replacement level, survival rate, feed efficiency, protein efficiency ratio, condition factor, viscera-somatic index, and intestine-fat ratio showed no significant changes ($P>0.05$); when dietary SPC replacement level did not exceed 20%, weight gain rate and specific growth rate showed no significant changes ($P>0.05$), but when replacement level increased above 30%, both parameters decreased significantly ($P<0.05$). 2) SPC replacement level had no significant effects on whole body dry matter, crude protein, and ash, as well as muscle dry matter, crude protein, and crude lipid contents ($P>0.05$); however, when SPC replacement level increased from 10% to 20% and 30%, whole body crude lipid content decreased significantly ($P<0.05$), and when SPC replacement level increased from 10% to above 20%, muscle ash content decreased significantly ($P<0.05$). 3) SPC replacement level had significant effects on serum glucose, total cholesterol contents, and alanine aminotransferase activity ($P<0.05$); serum glucose content reached the maximum value at 30% SPC replacement level; with increasing SPC replacement level, serum alanine aminotransferase activity

showed an overall upward trend, while total cholesterol content showed an overall downward trend. 4) Serum superoxide dismutase and peroxidase activities reached the maximum values, while serum malondialdehyde content reached the minimum value in the 30% SPC replacement group, which were significantly different from the control group (P<0.05). 5) No significant differences in pepsin activity were observed among all groups (P>0.05); SPC replacement level had significant effects on gastric amylase, foregut amylase, and hepatic amylase activities (P<0.05), with gastric amylase activity reaching the maximum value, foregut amylase activity reaching the minimum value at 10% SPC replacement level, and hepatic amylase activity reaching the minimum value at 40% SPC replacement level. The results of this experiment indicated that replacing 20% fish meal with SPC (SPC inclusion level of 10.72% in diet) had no adverse effects on growth performance, digestive enzymes, antioxidant enzyme activities, etc. in yellow catfish.

Full Text

Effects of Fish Meal Replacement with Soybean Protein Concentrate on Growth Performance, Feed Utilization, and Digestive and Antioxidant Enzyme Activities in Yellow Catfish (Pelteobagrus fulvidraco)

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Abstract

This study investigated the effects of soybean protein concentrate (SPC) replacement of fish meal on growth performance, feed utilization, serum biochemical indices, whole-body and muscle proximate composition, and digestive and antioxidant enzyme activities in yellow catfish. Six iso-nitrogenous and iso-lipid diets were formulated with SPC replacing fish meal at levels of 0, 10%, 20%, 30%, 40%, and 60%. A total of 540 juvenile yellow catfish with an initial body weight of (2.17$±$0.02) g were randomly assigned to six groups with three replicates each, with 30 fish per replicate (tank). The feeding trial lasted for eight weeks. The results showed: 1) Survival rate, feed efficiency, protein efficiency ratio, condition factor, viscerosomatic index, and intraperitoneal fat ratio were not significantly affected by SPC replacement level (P>0.05). Weight gain rate and specific growth rate remained unchanged when fish meal replacement did not exceed 20% (P>0.05), but decreased significantly when replacement exceeded 30% (P<0.05). 2) SPC replacement had no significant effects on whole-body dry matter, crude protein, and ash contents, or muscle dry matter, crude protein,

and crude lipid contents (P>0.05). However, whole-body crude lipid content decreased significantly when replacement increased from 10% to 20% and 30% (P<0.05), and muscle ash content decreased significantly when replacement exceeded 20% (P<0.05). 3) Serum glucose, total cholesterol, and alanine aminotransferase activity were significantly affected by SPC replacement (P<0.05). Serum glucose peaked at 30% replacement, while alanine aminotransferase activity increased and total cholesterol decreased with higher replacement levels. 4) Serum superoxide dismutase and catalase activities reached maximum values at 30% replacement, while malondialdehyde content reached its minimum, differing significantly from the control (P<0.05). 5) Gastric pepsin activity did not differ among groups (P>0.05), but SPC replacement significantly affected gastric, foregut, and hepatic amylase activities (P<0.05). Gastric amylase peaked at 10% replacement, foregut amylase reached its minimum at 10% replacement, and hepatic amylase was lowest at 40% replacement. These results indicate that replacing 20% of fish meal with SPC (corresponding to 10.72% SPC in the diet) does not adversely affect growth performance, digestive enzyme activities, or antioxidant capacity in yellow catfish.

Keywords: Yellow catfish; Soybean protein concentrate; Growth performance; Feed utilization; Enzyme activity

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Yellow catfish (*Pelteobagrus fulvidraco*) is a small freshwater economic fish species native to inland China, belonging to the order Siluriformes, family Bagridae, and genus *Pelteobagrus*. Commonly known as "gá yá zi," "huáng là dīng," or "gā yú" in China, this warm-water bottom-dwelling species is highly valued for its delicate flesh, delicious taste, high nutritional value, and lack of intermuscular bones, making it popular among consumers and giving it substantial market potential. In recent years, yellow catfish aquaculture has expanded rapidly, establishing it as an important cultured species in inland freshwater regions.

Fish meal is a high-quality protein source widely used in aquafeeds. However, as aquaculture develops, demand for aquafeed and fish meal continues to increase. Resource depletion and overfishing have led to insufficient fish meal supply and high market prices. To effectively reduce aquafeed costs, finding reliable alternative protein sources to partially or completely replace fish meal has become an inevitable trend. Soybean meal has high protein content with relatively balanced amino acids. Rapeseed meal is also widely used as a plant protein source in freshwater fish, often combined with soybean protein to achieve optimal amino acid balance. Studies on Japanese seabass and other species have shown that rapeseed meal can replace a certain proportion of fish meal. Cottonseed meal ranks as the third major plant protein source after soybean and rapeseed meals, with annual production in China exceeding 6 million tons. Research indicates cottonseed meal can replace a certain proportion of fish meal, though its use is limited by high gossypol content, which typically exists as polyphenolic dialdehyde.

Recent studies have found that replacing fish meal with plant protein sources like soybean meal can damage the histological structure of fish digestive and metabolic systems, inhibiting digestive and metabolic capacity. Fish fed soybean meal-containing diets exhibit reduced feed intake and digestibility. Soybean protein concentrate (SPC) is produced from soybeans with stable quality, high amino acid digestibility, and extremely low anti-nutritional factor content. SPC removes beany flavor substances and flatulence factors, with crude protein content generally ranging from 65% to 70%. By removing most soluble carbohydrates, crude fiber, and anti-nutritional factors that affect nutrient utilization, SPC shows excellent application potential in aquafeeds. Although SPC is rich in amino acids, its amino acid balance is far inferior to fish meal, with certain essential amino acids still insufficient, particularly methionine at less than half the content of high-quality fish meal. Studies on various fish species have shown that SPC can replace 25%-100% of fish meal, but results vary considerably with partial or complete replacement. Research on darkbarbel catfish found that SPC replacement did not significantly affect survival but significantly impacted growth and feed intake. Building on previous studies, this trial supplemented diets with lysine and methionine to ensure adequate essential amino acid supply, investigating the effects of SPC replacement levels on growth, feed utilization, digestive enzymes, and antioxidant activity to provide a scientific basis for developing efficient and environmentally friendly yellow catfish feeds.

1.1 Experimental Diets

Six iso-nitrogenous and iso-lipid diets were formulated using fish meal, soybean meal, and squid meal as protein sources; fish oil and soybean lecithin as lipid sources; and wheat flour as carbohydrate source. The control diet contained 50% fish meal, while experimental diets replaced fish meal with SPC at 10.0%, 20.0%, 30.0%, 40.0%, and 60.0%. Methionine and lysine were supplemented in replacement groups to match control diet levels. Diet composition and nutrient levels are shown in , and amino acid composition in . Feed ingredients were ground through a 60-mesh sieve, weighed according to formulation, mixed using the progressive enlargement method for micro-additives, and blended with approximately 35% water before being pelleted into 2-3 mm diameter particles. The pellets were air-dried to about 10% moisture, bagged, and stored at -20°C.

1.2 Feeding Management

Juvenile yellow catfish were purchased from Jiaxing, Zhejiang Province, and reared at the freshwater aquaculture facility of the Laboratory of Fish Nutrition at Ningbo University. Prior to the trial, fish were acclimated with commercial feed (crude protein 45%, crude lipid 10%; Tianbang Co., Ltd., Ningbo) for two weeks. After 24-hour fasting, 540 healthy fish of uniform size [initial weight (2.17$±$0.02) g] were randomly stocked into 300-L fiberglass tanks at 30 fish per tank. Each diet was randomly assigned to three tanks (three replicates). Fish were fed twice daily at 07:30 and 17:00 at 4%-6% body weight,

with feeding behavior observed for one hour post-feeding. During early culture, approximately 50% water was exchanged every other day, increasing to 50%-100% daily in mid-to-late culture. Aeration and water temperature were monitored daily, with dead fish recorded. The feeding period lasted eight weeks, with biweekly weighing and counting to adjust feed ration. Dissolved oxygen was maintained above 6.0 mg/L, water temperature at 23.0-29.5°C, and pH at 7.5-8.0.

1.3 Sample Collection and Analysis Methods

At trial termination, fish were fasted for 24 hours. Each tank was weighed and counted to calculate weight gain rate, specific growth rate, survival rate, feed efficiency, and protein efficiency ratio. Three fish per tank were measured for body length and weight, then dissected to weigh viscera and mesenteric fat for calculating viscerosomatic index, intraperitoneal fat ratio, and condition factor. Dorsal muscle (skinless) was collected in sealed bags for proximate analysis. Four fish per tank were collected for whole-body proximate analysis. Three fish per tank were dissected to collect liver, stomach, and foregut in 1.5-mL tubes for antioxidant and digestive enzyme assays. Blood from 5-8 fish per tank was collected with syringes into 1.5-mL tubes, left overnight at 4°C, centrifuged at 5,000 r/min for 10 minutes, and serum stored at -80°C for biochemical analysis.

Proximate composition analysis of feed, muscle, and whole body used standard methods: moisture by 105°C drying, crude protein by protein analyzer (FP-528, LECO, USA), crude lipid by Soxhlet extraction, and ash by 550°C muffle furnace incineration. Amino acids in feed were analyzed by high-speed automatic amino acid analyzer (L-8900, Hitachi, Japan).

Serum biochemical indices (total protein, albumin, globulin, total cholesterol, triglycerides) were measured by automatic biochemical analyzer (7600-110, Hitachi, Japan) at Ningbo University Hospital. Aspartate aminotransferase and alanine aminotransferase activities were determined using assay kits from Nanjing Jiancheng Bioengineering Institute following manufacturer protocols.

Hepatic antioxidant indices (superoxide dismutase, catalase, malondialdehyde) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute following manufacturer protocols.

Digestive enzyme activities (pepsin, amylase) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute following manufacturer protocols.

1.4 Calculation Formulas

Feed intake (FI, d^{-1}) = Dry matter intake / [feeding days × (final weight + initial weight)/2]
Weight gain rate (WGR, %) = 100 × (final mean weight - initial mean weight) / initial mean weight

Specific growth rate (SGR, %/d) = 100 × (ln final weight - ln initial weight) / feeding days
Protein efficiency ratio (PER) = (final weight - initial weight) / protein intake
Feed efficiency (FE) = (final total weight + dead total weight - initial total weight) / (total feed × dry matter content)
Condition factor (CF, g/cm^3) = 100 × body weight / body length^3
Intraperitoneal fat ratio (IPF, %) = 100 × intraperitoneal fat / body weight
Viscerosomatic index (VSI, %) = 100 × viscera weight / body weight

1.5 Statistical Analysis

All data were analyzed by one-way ANOVA using SPSS 17.0 software. Duncan' s multiple comparison was performed when significant differences were detected among treatments. Significance was set at $P<0.05$. Results are expressed as mean ± standard deviation (SD).

2.1 Effects of SPC Replacement on Growth Performance, Feed Utilization, and Morphological Indicators

All groups achieved 100% survival. As shown in , feed intake, feed efficiency, and protein efficiency ratio did not differ significantly among groups ($P>0.05$). Weight gain rate and specific growth rate decreased with increasing SPC replacement. When replacement did not exceed 20%, these parameters remained unchanged ($P>0.05$), but decreased significantly when replacement exceeded 30% ($P<0.05$). At 60% replacement, weight gain rate was significantly lower than other levels ($P<0.05$). Condition factor, viscerosomatic index, and intraperitoneal fat ratio showed no significant differences ($P>0.05$) but tended to decrease with higher SPC replacement.

2.2 Effects of SPC Replacement on Whole-Body and Muscle Proximate Composition

As shown in , SPC replacement had no significant effects on whole-body dry matter, crude protein, or ash contents, or muscle dry matter, crude protein, or crude lipid contents ($P>0.05$). However, whole-body crude lipid content was significantly affected ($P<0.05$). Compared with the control, whole-body crude lipid did not differ significantly in treatment groups, but was lower at 20% and 30% replacement than at 10% ($P<0.05$). Muscle ash content was significantly affected by SPC replacement ($P<0.05$). At 10% replacement, muscle ash did not differ from the control, but decreased significantly when replacement exceeded 20% ($P<0.05$).

2.3 Effects of SPC Replacement on Serum Biochemical Indicators

As shown in , serum total protein, albumin, globulin, and triglyceride contents, and aspartate aminotransferase activity did not differ among groups ($P>0.05$).

Serum glucose, total cholesterol, and alanine aminotransferase activity were significantly affected (P<0.05). Serum glucose increased when replacement rose from 10% to 30% (P<0.05), then decreased significantly when replacement exceeded 40% (P<0.05), peaking at 30% replacement. Alanine aminotransferase activity increased, while total cholesterol decreased with higher SPC replacement.

2.4 Effects of SPC Replacement on Hepatic Antioxidant Enzyme Activities

As shown in , hepatic superoxide dismutase and catalase activities and malondialdehyde content changed significantly with SPC replacement (P<0.05). Superoxide dismutase and catalase activities peaked at 30% replacement, while malondialdehyde content reached its minimum. Superoxide dismutase activity was significantly higher than the control when replacement was $ $20% (P<0.05), and malondialdehyde content was significantly lower than the control when replacement exceeded 10% (P<0.05).

2.5 Effects of SPC Replacement on Digestive Enzyme Activities

As shown in , gastric pepsin activity did not differ among groups (P>0.05), remaining stable up to 30% replacement but tending to decrease at $ $40% replacement. SPC replacement significantly affected gastric, foregut, and hepatic amylase activities (P<0.05). Gastric amylase was significantly higher at 10% and 30% replacement than other levels (P<0.05). Foregut amylase was lowest at 10% replacement. Hepatic amylase was significantly lower at 40% replacement than the control and 10%, 20%, and 30% replacement levels (P<0.05).

3.1 Effects of SPC Replacement on Growth Performance, Feed Utilization, and Morphological Indicators

In this trial, SPC replacement did not significantly affect survival, feed intake, feed efficiency, or protein efficiency ratio. However, weight gain rate and specific growth rate were significantly affected, decreasing with higher SPC replacement. When replacement did not exceed 20%, weight gain and specific growth rates did not differ from the control, but decreased significantly when replacement exceeded 30%, indicating that SPC can replace 20% of fish meal without affecting growth, while higher levels inhibit growth. These results align with studies on Senegalese sole, cobia, and darkbarbel catfish, where SPC partially replaced fish meal without affecting growth. Differences from studies showing complete replacement without growth effects may be due to variations in fish age, species, feeding methods, and diet composition. In contrast to studies on salmonids and marine fish showing reduced feed efficiency with SPC replacement, our results may reflect that freshwater fish face fewer challenges with fish meal replacement, and our supplementation of methionine and lysine ensured essential amino acid balance. Additionally, diet composition affects growth; our

formulation included squid meal, which has strong feeding attractant properties and can improve spawning quality in broodstock diets. Studies on turbot found that SPC affected feed intake as the main limiting factor for fish meal replacement. In our trial, feed intake did not change with increasing SPC replacement, suggesting that squid meal in the formulation mitigated palatability issues caused by SPC. Including feeding attractants when using plant protein sources to replace fish meal is crucial.

Studies have shown that feeding soybean meal-based diets caused enteritis and health issues in Atlantic salmon and rainbow trout. Non-starch polysaccharides in soybean protein sources can cause enteritis and reduce fat absorption. In our trial, whole-body crude lipid content decreased with increasing SPC replacement (10%-60%), with similar trends in condition factor, viscerosomatic index, and intraperitoneal fat ratio, likely due to plant protein sources affecting lipid metabolism. These results are consistent with studies on turbot and black seabream.

3.2 Effects of SPC Replacement on Whole-Body and Muscle Proximate Composition

Our results showed that whole-body crude lipid content in yellow catfish decreased with increasing SPC replacement (10%-60%). This aligns with studies on soybean meal protein in fish diets and different protein levels in yellow catfish. The likely reason is that SPC contains high levels of non-starch polysaccharides that cause enteritis, reducing fat absorption and indirectly affecting body crude lipid content. Muscle ash content decreased with increasing replacement (10%-60%), while other body and muscle proximate components did not change significantly, consistent with findings by López et al.

3.3 Effects of SPC Replacement on Serum Biochemical Indicators

Serum total protein and cholesterol contents are affected by protein, lipid, and carbohydrate metabolism. As an important component of lipid metabolism, total cholesterol is closely related to normal liver cell function and lipid metabolism, with changes reflecting liver cell status. In our trial, total cholesterol decreased with increasing SPC replacement, consistent with studies on Chinese sturgeon and European seabass showing decreased triglycerides and cholesterol with SPC replacement. Animal protein ingredients contain high cholesterol, while plant protein ingredients contain very little, and dietary cholesterol affects blood cholesterol in cultured animals. Using plant protein sources in sea bass diets reduced serum total cholesterol, possibly due to increased bile salt excretion, limited cholesterol absorption, or insufficient dietary cholesterol from plant sources. Most scholars believe that anti-nutritional factors in plant protein sources reduce blood cholesterol. Whether these factors interfere with cholesterol metabolism in fish remains inconclusive.

Glucose is a nutritional indicator reflecting carbohydrate metabolism, tissue

cell function, and endocrine status. Within a certain threshold, higher blood glucose indicates active feeding and good health, but exceeding this threshold causes nutritional physiological stress and health damage. In our trial, serum glucose was significantly higher than the control at 20% and 30% replacement, while weight gain and specific growth rates were significantly lower when replacement exceeded 30%, suggesting that >30% SPC replacement affects fish growth through altered glucose and lipid metabolism.

Aspartate aminotransferase and alanine aminotransferase are important enzymes in amino acid, protein, lipid, and carbohydrate metabolism. Alanine aminotransferase normally resides in hepatocytes, while aspartate aminotransferase is mainly in hepatocyte mitochondria, with low serum levels that increase only when liver function is impaired. In our trial, serum alanine aminotransferase and aspartate aminotransferase activities did not differ from the control when replacement was $ $40%, but alanine aminotransferase was significantly higher at 60% replacement. This indicates that $ $40% SPC replacement did not cause liver damage, likely because SPC contains minimal anti-nutritional factors. Excessive soybean meal can cause liver-pancreas damage and functional disorders in cobia. Anti-nutritional factors in plant protein sources may be the main cause of immune index changes. At 60% replacement, enhanced amino acid metabolism may produce more metabolic waste, burdening the liver and increasing serum alanine aminotransferase activity. Further investigation is needed.

3.4 Effects of SPC Replacement on Antioxidant Enzyme Activities

Superoxide dismutase and catalase are key enzymes in biological defense systems that scavenge superoxide anions and hydrogen peroxide, reducing free radical damage and reflecting stress resistance. Malondialdehyde is the end product of lipid peroxidation by free radicals and is cytotoxic, with its content reflecting lipid peroxidation and cell damage. In our trial, SPC replacement significantly affected serum superoxide dismutase and catalase activities and malondialdehyde content. Superoxide dismutase activity was significantly higher than the control when replacement was $ $20%. Catalase activity was significantly higher at 20% and 30% replacement, and malondialdehyde content was significantly lower when replacement exceeded 10%. These results indicate that 20% and 30% SPC replacement enhanced antioxidant capacity, possibly because SPC contains isoflavones with antioxidant activity and strong superoxide anion radical scavenging capacity.

3.5 Effects of SPC Replacement on Digestive Enzyme Activities

Digestive enzymes are key indicators of fish digestive capacity and feed utilization, with activities varying by location and feeding habits. Amylase catalyzes starch hydrolysis, degrading carbohydrates to sugars and improving carbohydrate utilization. In our trial, intestinal amylase activity was markedly higher than hepatic-pancreatic amylase, consistent with studies on other fish species.

Yellow catfish are carnivorous, with amylase production primarily in the intestine rather than hepatopancreas. Hepatic amylase was lower than the control when replacement was 30%-60%. Histological observation showed that increasing SPC replacement caused enteritis and progressive liver tissue damage in darkbarbel catfish. We speculate that in our trial, SPC damaged hindgut structural stability, inhibiting enzyme activity. Gastric pepsin activity remained stable up to 30% replacement but decreased at 30%-60%, indicating that the stomach is sensitive to SPC. When replacement was <30%, gastric digestive capacity was minimally affected. Future evaluation of plant protein sources should consider their effects on liver and gastrointestinal mechanisms.

4 Conclusion

SPC is a high-quality plant protein source. Replacing fish meal with SPC at levels not exceeding 20% (corresponding to 10.72% SPC in the diet) does not significantly affect growth performance, feed utilization, morphological indices, body composition, digestive enzyme activities, or total cholesterol content in yellow catfish, while maintaining good antioxidant capacity and health status. This replacement level is recommended for practical production.

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