Effects of Replacing Fish Oil with Rubber Seed Oil on Growth Performance, Digestive Enzyme Activity, Lipoprotein Content, and Antioxidant Function in Juvenile GIFT Tilapia: A Postprint

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Abstract

This experiment aimed to investigate the effects of replacing fish oil with rubber seed oil on growth performance, digestive enzyme activity, lipoprotein content, and antioxidant function in juvenile GIFT tilapia. Six hundred healthy juvenile GIFT tilapia of uniform size and robust physique were selected and randomly divided into 5 groups with 3 replicates per group and 40 fish per replicate. The five groups of experimental fish were fed five isonitrogenous and isoenergetic diets in which fish oil was replaced by rubber seed oil at 0 (control), 25%, 50%, 75%, and 100%, designated as G0, G25, G50, G75, and G100, respectively. The experimental period lasted 8 weeks. The results showed: 1) Replacement of fish oil with different proportions of rubber seed oil had no significant effects on final body weight, body weight gain, daily weight gain coefficient, feeding rate, feed coefficient, or protein efficiency in juvenile tilapia ($P > 0.05$). 2) The intestinal trypsin and lipase activities in the G25 group were significantly higher than those in the other groups ($P < 0.05$), and the intestinal amylase activity in the G25 group was significantly higher than that in the G75 group ($P < 0.05$). Replacement of fish oil with different proportions of rubber seed oil had no significant effects on hepatic trypsin, amylase, or disaccharidase activities in juvenile tilapia ($P > 0.05$). 3) With increasing replacement proportion of fish oil by rubber seed oil, serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) contents in juvenile tilapia generally showed a decreasing trend, while hepatic free cholesterol (FC) content and FC/TC ratio showed a trend of first decreasing and then increasing, with the lowest values observed in the G25 group. 4) Plasma glutathione reductase (GR) activity in the G0 group was significantly higher than that in the other groups ($P < 0.05$), hepatic GR activity in the G0 group was significantly higher than that in the G50 and

G100 groups ($P < 0.05$), and hepatic total antioxidant capacity in the G100 group was significantly lower than that in the other groups ($P < 0.05$). It can be concluded that replacement of 25%–75% fish oil with rubber seed oil had no obvious negative effects on growth performance or antioxidant function in juvenile tilapia. Replacement of 25% fish oil with rubber seed oil was beneficial for improving intestinal digestive enzyme activity in juvenile tilapia; when 100% fish oil was replaced by rubber seed oil, antioxidant function in juvenile tilapia decreased.

Full Text

Effects of Fish Oil Replacement by Rubber Seed Oil on Growth Performance, Digestive Enzyme Activity, Lipoprotein Content and Antioxidant Function of Juvenile Genetically Improved Farmed Tilapia

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Abstract

This experiment was conducted to investigate the effects of fish oil replacement by rubber seed oil on growth performance, digestive enzyme activity, lipoprotein content, and antioxidant function of juvenile genetically improved farmed tilapia (GIFT). A total of 600 uniformly sized, healthy juvenile GIFT were randomly allocated into five groups with three replicates each (40 fish per replicate). The five groups were fed five isonitrogenous and isoenergetic diets in which fish oil was replaced by rubber seed oil at 0% (control), 25%, 50%, 75%, and 100%, designated as G0, G25, G50, G75, and G100, respectively. The experimental period lasted for 8 weeks. The results showed that: (1) Replacement of fish oil with rubber seed oil at different proportions had no significant effects on final body weight, weight gain, daily growth coefficient, feeding rate, feed conversion ratio, or protein efficiency ratio of juvenile tilapia ($P > 0.05$). (2) The intestinal trypsin and lipase activities in the G25 group were significantly higher than those in other groups ($P < 0.05$), while intestinal amylase activity in G25 was significantly higher than in G75 ($P < 0.05$). No significant differences were observed in hepatic trypsin, amylase, or disaccharidase activities among groups ($P > 0.05$). (3) With increasing replacement levels, serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) contents showed a general downward trend, while hepatic free cholesterol (FC) content and FC/TC ratio first decreased then increased, with the lowest values observed in G25. (4) Plasma glutathione reductase (GR) activity in G0 was significantly higher than in other groups ($P < 0.05$), hepatic GR activity in G0 was signifi-

cantly higher than in G50 and G100 ($P < 0.05$), and hepatic total antioxidant capacity in G100 was significantly lower than in other groups ($P < 0.05$). In conclusion, replacing 25% to 75% of fish oil with rubber seed oil had no obvious negative effects on growth performance or antioxidant function. Replacement at 25% improved intestinal digestive enzyme activity, whereas 100% replacement decreased antioxidant function.

Keywords: GIFT tilapia; rubber seed oil; fish oil; growth performance; digestive enzyme activity; antioxidant function

Introduction

Lipids not only provide essential fatty acids and energy for animals but also play important roles in regulating immune function. Appropriate dietary lipid sources can conserve protein, reduce environmental pollution, and lower feed costs. Fish oil has long been a premium lipid source in aquafeeds due to its rich content of polyunsaturated fatty acids. However, with the rapid development of aquaculture, demand for fish oil has increased dramatically, driving up prices. Consequently, identifying inexpensive alternatives to fish oil has become a major focus in aquaculture nutrition research.

Rubber seeds, a byproduct of rubber trees, are abundant and inexpensive, yet 95% are discarded in rubber plantations without effective utilization. Rubber seed oil, extracted from these seeds, represents an ideal energy source. It contains up to 74.52% unsaturated fatty acids, with -linolenic acid content reaching 20-24%—three to four times higher than soybean and rapeseed oils, and dozens of times higher than peanut and sunflower oils. Previous studies have demonstrated that long-term consumption of rubber seed oil provides preventive effects against hyperlipidemia, making it a promising alternative to conventional lipid sources such as fish oil and soybean oil in feed formulations.

Genetically improved farmed tilapia (GIFT) is a superior strain developed through selective breeding in recent years, characterized by rapid growth and high fillet yield, and represents an economically important aquaculture species in China. While extensive research has been conducted on its nutritional requirements and health immunity, no studies have reported on the application of rubber seed oil in GIFT diets. Current literature presents conflicting views regarding tilapia' s essential fatty acid (EFA) requirements: (1) tilapia requires only n-6 polyunsaturated fatty acids (PUFA) as EFA; (2) n-6 and n-3 PUFA are equally effective; and (3) in addition to n-6 PUFA, n-3 PUFA provides additive growth benefits. This experiment utilized rubber seed oil to replace fish oil at different proportions, creating varying n-3/n-6 PUFA ratios to investigate effects on growth performance, digestive enzyme activity, lipoprotein content, and antioxidant function, thereby providing a theoretical basis for rubber seed oil application in GIFT diets.

1.1 Experimental Diets

Fish meal, soybean meal, rapeseed meal, and corn gluten meal served as protein sources. Five isonitrogenous and isoenergetic diets (33% crude protein, 19.4 MJ/kg gross energy) were formulated with fish oil replaced by rubber seed oil at 0% (control), 25%, 50%, 75%, and 100%, designated as G0, G25, G50, G75, and G100. Dietary composition and nutrient levels are presented in Table 1 , and fatty acid composition is shown in Table 2 .

Prior to diet preparation, all ingredients were ground using a grinder (SFSP series, Kunming Huaming Grain and Oil Equipment Factory) and passed through a 60-mesh sieve. The ground ingredients were mixed according to formulation, then fish oil, rubber seed oil, and soybean lecithin (pre-dissolved in fish or rubber seed oil) were added. Oil particles were manually dispersed and mixed uniformly before adding appropriate distilled water to form a dough. The mixture was extruded into 1.5 mm diameter pellets using a pellet mill (KS-180, Jiangsu Jinggu Rice Mill Co., Ltd.), dried at 40°C for 12 hours in a constant temperature oven, and stored at -20°C until use.

1.2 Experimental Design and Husbandry

Juvenile GIFT used in the experiment were artificially propagated fry from the same batch, provided by the Xishuangbanna Fisheries Technology Extension Station in Yunnan Province. Prior to the experiment, fish were fasted for 24 hours. Four hundred uniformly sized, healthy fry (initial average body weight 1.92 g) were randomly distributed into five groups with three replicates each (40 fish per replicate). The five groups were fed the five experimental diets corresponding to G0, G25, G50, G75, and G100. Fish were stocked in 15 net cages (0.9 m × 0.9 m × 1.0 m) at 40 fish per cage. The experimental duration was 8 weeks.

The experiment was conducted at the Aquaculture Laboratory of the College of Animal Science and Technology, Yunnan Agricultural University. Prior to the experiment, juvenile fish were acclimated for 2 weeks. Fish were hand-fed to satiation twice daily (07:00 and 17:00). Thirty minutes after afternoon feeding, uneaten feed and feces were siphoned out. Aerated tap water was used in a recirculating system with mechanical and biological filtration. Continuous aeration was provided throughout the culture period under natural photoperiod, with water temperature maintained at 26-28°C.

1.3 Sample Collection

At the end of the experiment, fish were fasted for 24 hours. Total weight and number of fish in each cage were recorded to calculate growth performance. Six fish were randomly selected from each cage as whole-body samples for proximate composition analysis. Additionally, six fish per cage were randomly selected, anesthetized, and blood was collected via caudal vein—some into regular centrifuge tubes and some into anticoagulant tubes. Samples were centrifuged at

4,000 r/min for 10 minutes, and serum and plasma were stored at -80°C. From these fish, three were selected to dissect out intact intestines and livers, which were stripped of fat and contents, sealed in bags, and stored at -80°C.

1.4 Analytical Methods

Proximate composition of fish and diets was determined using standard methods: moisture by oven drying at 105°C to constant weight, crude protein by Kjeldahl method (JK9830, Jinan Precision Scientific Instrument Co., Ltd.), ether extract by Soxhlet extraction using petroleum ether, ash by combustion at 550°C for 16 hours (SX-410 muffle furnace, Beijing Yongguangming Medical Instrument Co., Ltd.), and gross energy by oxygen bomb calorimetry (ZDHW-6, Hebi Huatai Instrument Co., Ltd.). Dietary fatty acid composition was analyzed by sulfuric acid-methanol esterification using gas chromatography (GC-2014, Shimadzu, Japan).

Biochemical indices in serum, plasma, liver, and intestine were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute according to manufacturer instructions. Hepatic and intestinal trypsin was measured by UV colorimetry, amylase (AMS) by iodine-starch colorimetry, and lipase (LPS) and disaccharidase by colorimetry. Serum and hepatic total cholesterol (TC) was measured by oxidase method, triglycerides (TG) by glycerol oxidase method, high-density lipoprotein cholesterol (HDL-C) by selective precipitation, free cholesterol (FC) and glutamate dehydrogenase (GDH) by double antibody sandwich method, and cholesterol ester (CE) calculated as TC minus FC. Plasma and hepatic urea nitrogen (UN) was measured by urease method, alkaline phosphatase (ALP) by continuous monitoring, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by Reitman-Frankel method, immunoglobulin M (IgM) and glutathione reductase (GR) by UV colorimetry, nitric oxide (NO), malondialdehyde (MDA), peroxidase (POD), glutathione peroxidase (GSH-Px), -glutamyl transpeptidase (GGT), catalase (CAT), and total antioxidant capacity (T-AOC) by visible light spectrophotometry, and total protein (TP) by Coomassie brilliant blue method.

1.5 Calculations

Weight gain (WG) = (final body weight - initial body weight) / initial body weight
Daily growth coefficient (DGC, %/d) = 100 × (final body weight^(1/3) - initial body weight^(1/3)) / feeding days
Mean metabolic body weight (MBW, kg) = [(initial body weight/1000)^0.75 + (final body weight/1000)^0.75] / 2
Feeding rate (FR, g/kg·d MBW) = total feed intake / (MBW × feeding days)
Feed conversion ratio (FCR) = total feed fed / (final body weight - initial body weight)
Protein efficiency ratio (PER, %) = (final body weight - initial body weight) /

protein intake
Survival rate (SR, %) = 100 × final fish number / initial fish number

1.6 Statistical Analysis

All data were analyzed using SPSS 17.0 software. Percentage data were arcsine-transformed prior to analysis. One-way ANOVA was performed, and Duncan' s multiple range test was applied when significant differences were detected ($P < 0.05$). Results are expressed as means ± SEM.

Results

2.1 Effects on Growth Performance

As shown in Table 3 , survival rates ranged from 98.30% to 100.00% with no significant differences among groups ($P > 0.05$). No significant differences were observed in final body weight, weight gain, daily growth coefficient, feeding rate, feed conversion ratio, or protein efficiency ratio among all groups ($P > 0.05$).

2.2 Effects on Digestive Enzyme Activity in Intestine and Liver

As shown in Table 4 , intestinal trypsin and lipase activities in G25 were significantly higher than in other groups ($P < 0.05$). Intestinal amylase activity in G25 was significantly higher than in G75 ($P < 0.05$) but did not differ significantly from other groups. Intestinal disaccharidase activities in G0 and G25 were significantly higher than in G50, G75, and G100 ($P < 0.05$). No significant differences were found in hepatic trypsin, amylase, or disaccharidase activities among groups ($P > 0.05$).

2.3 Effects on Lipid Metabolism in Serum and Liver

As shown in Table 5 , serum TC and LDL-C contents and LDL-C/HDL-C ratio generally decreased with increasing replacement levels. Specifically, serum TC in G0 was significantly higher than in G75 ($P < 0.05$), while serum LDL-C and LDL-C/HDL-C in G0 and G25 were significantly higher than in G50, G75, and G100 ($P < 0.05$). No significant differences were observed in serum TG or HDL-C among groups ($P > 0.05$).

Hepatic FC content and FC/TC ratio decreased initially then increased with rising replacement levels, with G25 showing the lowest values, significantly lower than G0 and G100 ($P < 0.05$). Conversely, hepatic CE content showed an opposite trend, with G25 exhibiting the highest value, significantly higher than G0 and G100 ($P < 0.05$). No significant differences were detected in hepatic TC or TG contents among groups ($P > 0.05$).

2.4 Effects on Protein Metabolism in Plasma and Liver

As shown in Table 6 , plasma GGT activity in G25 was significantly higher than in G50 and G75 ($P < 0.05$). No significant differences were found in plasma TP, UN, AST, or ALT among groups ($P > 0.05$). Hepatic ALT, AST, and GGT activities did not differ significantly among groups ($P > 0.05$).

2.5 Effects on Antioxidant Indices in Plasma and Liver

As shown in Table 7 , plasma GR activity in G0 was significantly higher than in other groups ($P < 0.05$). Plasma NO content in G50 was significantly lower than in G100 ($P < 0.05$) but did not differ from other groups. No significant differences were observed in plasma CAT, GSH-Px, POD, ALP, GDH, IgM, or MDA among groups ($P > 0.05$).

Hepatic GR activity in G0 was significantly higher than in G50 and G100 ($P < 0.05$). Hepatic T-AOC in G100 was significantly lower than in other groups ($P < 0.05$). No significant differences were found in hepatic CAT, POD, ALP, GDH, or MDA among groups ($P > 0.05$).

2.6 Effects on Body Composition

As shown in Table 8 , no significant differences were observed in moisture, crude protein, ether extract, or ash contents among groups ($P > 0.05$).

Discussion

3.1 Effects on Growth Performance

The present study demonstrated that increasing dietary replacement of fish oil with rubber seed oil did not significantly affect tilapia growth performance. Similar findings have been reported in other studies where vegetable oil replacement of fish oil did not impair fish growth. For instance, Teoh et al. completely replaced fish oil with a blend of vegetable oils (palm, linseed, olive, and sunflower oils) in GIFT diets without negative effects on growth. Complete replacement with linseed oil also did not affect growth performance in catfish (*Silurus asotus*) or rainbow trout (*Oncorhynchus mykiss*). Studies on catfish, Atlantic salmon (*Salmo salar*), and rainbow trout similarly showed that palm oil replacement at various proportions did not affect growth.

However, Turchini et al. reported that fish growth is closely related to dietary n-3 PUFA content, and insufficient n-3 PUFA can reduce growth performance. Yong found that vegetable oils high in linoleic acid were more beneficial for tilapia growth. These conflicting results suggest that tilapia's EFA requirements remain controversial. The current findings indicate that complete replacement with rubber seed oil did not negatively affect GIFT growth, possibly because high replacement levels still met n-3 PUFA requirements or because increased dietary

linoleic acid content was beneficial. This suggests that tilapia may require both n-3 and n-6 PUFA, which may have equivalent effects.

3.2 Effects on Digestive Enzyme Activity

Digestive enzyme activity reflects nutrient digestion and absorption capacity, with higher activity benefiting nutrient utilization. Lipase plays a crucial role in digesting neutral lipids by hydrolyzing ester bonds to produce fatty acids. Rajas et al. demonstrated that increased dietary PUFA enhances trypsin transcription and mRNA expression. The significantly higher intestinal lipase and trypsin activities in G25 indicate that appropriate rubber seed oil replacement improved digestive capacity and nutrient absorption. Zhou et al. reported that fish liver primarily secretes trypsinogen, which is activated by enterokinase in the intestine to promote protein digestion. Ni et al. identified hepatopancreas as the main organ secreting amylase, which is activated in the intestine. The decreasing disaccharidase activity with increasing replacement levels suggests that high rubber seed oil content may impair carbohydrate utilization in tilapia.

3.3 Effects on Lipoprotein Content

Blood biochemical indices reflect physiological metabolism and nutritional status, responding rapidly to physiological changes. Lipids are transported in blood primarily as LDL-C and HDL-C. TC is transported to the liver as HDL-C for catabolism, reducing vascular deposition, while excess LDL-C can cause atherosclerosis. Therefore, LDL-C/HDL-C reflects cholesterol transport and serves as an atherosclerosis indicator. Cholesterol is essential for synthesizing steroid hormones, bile acids, and vitamin D, and is a major membrane component. Elevated serum TC may cause hypercholesterolemia. Peng et al. reported that soybean oil replacement reduced serum TC in black seabream (*Sparus macrocephalus*). The current study found decreasing serum TC with increasing replacement, possibly due to increased unsaturated fatty acids promoting cholesterol esterification. The significantly higher LDL-C/HDL-C in G0 and G25 suggests high replacement levels may reduce atherosclerosis risk. Torstensen et al. found that complete palm oil replacement did not affect serum TG in Atlantic salmon, consistent with our results. Other studies showed vegetable oil replacement reduced serum LDL-C in Atlantic salmon, aligning with our findings. However, Torstensen also reported no significant effects on serum LDL-C and HDL-C with complete palm oil replacement. The liver is the primary site for cholesterol synthesis. CE is formed from FC and fatty acids by lecithin-cholesterol acyltransferase (LCAT) in the liver and can be hydrolyzed back to FC. The current results showed no significant effects on hepatic TC and TG, while FC and FC/TC decreased then increased, and CE showed the opposite pattern, indicating high replacement levels may affect cholesterol absorption.

3.4 Effects on Protein Metabolism

Protein metabolism primarily occurs in the liver, while transaminases are widely distributed in tissues. ALT and AST are mainly present in hepatocytes with low blood activity; elevated plasma levels indicate nutritional imbalance, environmental stress, or liver damage. No significant differences in plasma or hepatic ALT and AST activities suggest rubber seed oil replacement did not negatively affect the liver. GGT is another indicator of hepatobiliary disease, present in tissues with absorptive and excretory functions, released only under specific pathological conditions. Although plasma GGT in G25 was significantly higher than in G50 and G75, it did not differ from G0, suggesting replacement may affect protein metabolism and amino acid transport without causing liver damage. Urea nitrogen (UN) is a product of amino acid deamination and nitrogen metabolism, excreted primarily as UN. Ammonia concentration changes reflect amino acid oxidation and metabolism, normally correlating negatively with protein deposition. The non-significant upward trend in plasma UN suggests increased protein catabolism, where amino acids were utilized for energy rather than storage, reducing protein deposition. Therefore, high replacement levels may be detrimental to protein deposition.

3.5 Effects on Antioxidant Function

Antioxidant capacity is closely linked to health status, with reduced capacity predisposing animals to disease. T-AOC represents overall antioxidant capability, while GR plays a key role in reactive oxygen species scavenging under oxidative stress. The decreasing hepatic T-AOC and plasma GR with increasing replacement levels align with Pan et al., who reported reduced hepatic T-AOC in common carp (*Cyprinus carpio*) with increasing linseed oil replacement. Complete replacement significantly reduced antioxidant capacity, impairing immune defense. NO scavenges reactive oxygen species but can inhibit antioxidant function when excessive. The lower plasma NO in G50 compared to G100 suggests complete replacement may cause NO overproduction, suppressing antioxidant function. During normal metabolism, fish continuously generate free radicals, which are scavenged by enzymes like CAT and GSH-Px to protect the organism. MDA is a lipid peroxidation product whose level reflects free radical attack severity. The lack of significant effects on plasma and hepatic MDA, CAT, and plasma GSH-Px differs from Peng et al., who found reduced hepatic MDA in black seabream with soybean oil replacement, possibly due to species and lipid source differences. In summary, replacement below 75% did not adversely affect antioxidant capacity, while 100% replacement reduced antioxidant function.

Conclusion

Replacing 25% to 75% of fish oil with rubber seed oil had no significant negative effects on growth performance or antioxidant function in tilapia. Replacement at 25% improved intestinal digestive enzyme activity, whereas 100% replacement decreased antioxidant function.

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