

Effects of Dietary Lipid Level on Morphometric Indices, Tissue Fatty Acid Composition, Serum Biochemical Parameters and Hepatic Antioxidant Capacity in Largemouth Bass (*Micropterus salmoides*) (Postprint)

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Abstract

To investigate the effects of dietary lipid levels on morphological indices, tissue fatty acid composition, serum biochemical indices, and liver antioxidant indices of largemouth bass (*Micropterus salmoides*), an 8-week feeding trial was conducted using largemouth bass with an average body weight of (4.12 ± 0.02) g. A total of 270 experimental fish were randomly divided into 3 groups with 3 replicates per group and 30 fish per replicate. Using fish oil, soybean oil, and soybean lecithin as lipid sources, experimental diets with lipid levels of 5.76%, 11.05%, and 17.65% were formulated. The results showed: 1) Dietary lipid level had no significant effect on condition factor and hepatosomatic index of largemouth bass ($P > 0.05$); mesenteric fat ratio increased with increasing dietary lipid levels, with the 17.65% lipid group being significantly higher than the 5.76% lipid group ($P < 0.05$); viscerosomatic index first increased and then decreased with increasing dietary lipid levels, being highest in the 11.05% lipid group, which was significantly higher than the 5.76% and 17.65% lipid groups ($P < 0.05$). 2) The proportions of muscle polyunsaturated fatty acids (PUFA) and n-6 highly unsaturated fatty acids (HUFA) in the 5.76% lipid group were significantly lower than those in the 11.05% and 17.65% lipid groups ($P < 0.05$), while the n-3 HUFA/n-6 HUFA ratio was significantly higher than that in the 11.05% and 17.65% lipid groups ($P < 0.05$); the proportion of liver n-3 HUFA in the 5.76% lipid group was significantly higher than that in the 17.65% lipid group ($P < 0.05$), the n-3 HUFA/n-6 HUFA ratio was significantly higher than that in the 11.05% and 17.65% lipid groups ($P < 0.05$), while the n-6 HUFA proportion was significantly lower than that in the 11.05% and 17.65% lipid groups ($P < 0.05$). 3) When dietary lipid level increased from 5.76% to 11.05% or

17.65%, the activities of alkaline phosphatase and contents of total cholesterol, high-density lipoprotein, and low-density lipoprotein in serum of largemouth bass all increased significantly ($P < 0.05$), while alanine aminotransferase activity decreased significantly ($P < 0.05$), but there were no significant differences in the above indices between the 11.05% and 17.65% lipid groups ($P > 0.05$). Serum triglyceride content in largemouth bass of the 11.05% lipid group was significantly higher than that in the 5.76% and 17.65% lipid groups ($P < 0.05$). 4) Dietary lipid level had no significant effects on the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alkaline phosphatase (AKP), alanine aminotransferase (ALT) and malondialdehyde (MDA) content in liver of largemouth bass ($P > 0.05$), but liver catalase (CAT) activity in the 11.05% lipid group was significantly higher than that in the other two groups ($P < 0.05$), while liver aspartate aminotransferase (AST) activity in the 17.65% lipid group was significantly higher than that in the other two groups ($P < 0.05$). It can be concluded that dietary lipid level can affect lipid deposition in largemouth bass, alter tissue fatty acid composition, and change the capacity for lipid transport and utilization in largemouth bass by influencing serum high-density lipoprotein and low-density lipoprotein contents.

Full Text

Effects of Dietary Lipid Level on Morphology Indexes, Tissue Fatty Acid Composition, Serum Biochemical Indexes and Liver Antioxidant Indexes of Largemouth Bass (*Micropterus salmoides*)

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Abstract: An 8-week feeding trial was conducted to investigate the effects of dietary lipid level on morphology indexes, tissue fatty acid composition, serum biochemical indexes, and liver antioxidant capacity of largemouth bass (*Micropterus salmoides*). Juvenile largemouth bass with an average body weight of (4.12 ± 0.02) g were used as experimental subjects. A total of 270 fish were randomly divided into three groups with three replicates per group and 30 fish per replicate. Using fish oil, soybean oil, and soybean lecithin as lipid sources, three experimental diets were formulated with lipid levels of 5.76%, 11.05%, and 17.65%, respectively. The results showed that: (1) Dietary lipid level had no significant effect on condition factor or hepatosomatic index ($P > 0.05$). The intraperitoneal fat ratio increased with dietary lipid level, with the 17.65% lipid group being significantly higher than the 5.76% group ($P < 0.05$). The viscerosomatic index first increased and then decreased with dietary lipid level, peaking in the 11.05% lipid group, which was significantly higher than both the

5.76% and 17.65% groups ($P < 0.05$). (2) The proportions of polyunsaturated fatty acids (PUFA) and n-6 highly unsaturated fatty acids (HUFA) in muscle of the 5.76% lipid group were significantly lower than those in the 11.05% and 17.65% groups ($P < 0.05$), while the n-3 HUFA/n-6 HUFA ratio was significantly higher ($P < 0.05$). The proportion of n-3 HUFA in liver of the 5.76% lipid group was significantly higher than that in the 17.65% group ($P < 0.05$), and the n-3 HUFA/n-6 HUFA ratio was significantly higher than in the 11.05% and 17.65% groups ($P < 0.05$), whereas the n-6 HUFA proportion was significantly lower than in the other two groups ($P < 0.05$). (3) When dietary lipid level increased from 5.76% to 11.05% or 17.65%, serum alkaline phosphatase activity and total cholesterol, high-density lipoprotein, and low-density lipoprotein contents all increased significantly ($P < 0.05$), while alanine aminotransferase activity decreased significantly ($P < 0.05$). However, these indexes showed no significant differences between the 11.05% and 17.65% lipid groups ($P > 0.05$). Serum triglyceride content in the 11.05% lipid group was significantly higher than in the 5.76% and 17.65% groups ($P < 0.05$). (4) Dietary lipid level had no significant effect on liver superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alkaline phosphatase (AKP), alanine aminotransferase (ALT) activities, or malondialdehyde (MDA) content ($P > 0.05$). However, liver catalase (CAT) activity in the 11.05% lipid group was significantly higher than in the other two groups ($P < 0.05$), while liver aspartate aminotransferase (AST) activity in the 17.65% lipid group was significantly higher than in the other groups ($P < 0.05$). In conclusion, dietary lipid level can affect lipid deposition, alter tissue fatty acid composition, and modify lipid transport and utilization capacity by influencing HDL and LDL contents in serum of largemouth bass.

Keywords: largemouth bass (*Micropterus salmoides*); lipid level; morphology indexes; fatty acid composition; serum biochemical indexes; antioxidant indexes

Largemouth bass (*Micropterus salmoides*) belongs to Perciformes, Percidae, Centrarchidae, and genus *Micropterus*. Native to North America, it is a worldwide game fish species. Based on morphological characteristics and geographical distribution, largemouth bass is divided into two subspecies: the northern largemouth bass (*M. salmoides salmoides*) and the Florida largemouth bass (*M. salmoides floridanus*). Largemouth bass is a valuable carnivorous fish known for its fast growth, strong disease resistance, delicious meat, low-temperature tolerance, and rich nutritional value. It was first introduced to Taiwan, China from abroad in the late 1970s, and successfully artificially propagated in 1983. The same year, it was introduced to Guangdong Province. After years of aquaculture development, it has become one of the important freshwater aquaculture species in China. In recent years, annual production of largemouth bass in China has remained above 100,000 tons, with Guangdong Province being the main production area, accounting for 75% of the national total output.

As one of the three major nutrients, lipid is an important energy source for fish and plays a crucial role in growth. Insufficient dietary lipid or inadequate fat

supply can cause deficiencies in essential fatty acids and fat-soluble vitamins. Since fish cannot synthesize essential fatty acids, this leads to slow growth, low disease resistance, metabolic disorders, and ultimately forces fish to use dietary protein as an energy source for growth. Therefore, appropriate dietary lipid supplementation can promote fish growth and conserve protein. However, excessive dietary lipid levels can also cause metabolic system disorders, damage to tissues and organs, and inhibit de novo fatty acid synthesis, thereby affecting development and immune response in aquatic animals.

After digestion and absorption, lipids are stored as triglycerides in tissues such as intestine, muscle, and liver. Fat and fatty acid accumulation can adversely affect various tissues and organs. For example, as the primary organ for lipid metabolism, excessive fat accumulation in the liver can lead to fatty liver disease. Generally, higher dietary lipid content results in greater fat accumulation in the liver.

Currently, research on largemouth bass feed has focused primarily on protein and amino acids, with limited studies on lipids and fatty acids. Therefore, this experiment was designed based on existing research, with three dietary lipid levels (5.76%, 11.05%, and 17.65%) to investigate the effects of dietary lipid level on morphology, tissue fatty acid composition, blood parameters, and liver antioxidant indexes of largemouth bass, thereby providing basic data and theoretical reference for developing resource-saving formulated feeds for largemouth bass.

1.1 Experimental Diets

White fish meal, soybean meal, wheat gluten meal, and wheat flour were used as protein sources, while fish oil, soybean oil, and soybean lecithin served as lipid sources to formulate three isonitrogenous experimental diets with lipid levels of 5.76%, 11.05%, and 17.65%, respectively. The composition and nutrient levels of the experimental diets are shown in Table 1, and the fatty acid composition is presented in Table 2.

All ingredients were ground and passed through an 80-mesh sieve. According to the formula in Table 1, ingredients were accurately weighed and mixed uniformly. Vitamins and mineral premixes and other micro-additives were mixed using the stepwise dilution method. Appropriate water was then added, and the mixture was uniformly blended using a mixer before being extruded into pellet feeds with diameters of 1.5 mm and 2.0 mm. The pellets were cooked at 90°C for 30 minutes in an oven, then air-dried to approximately 10% moisture content, sealed in bags, and stored at -20°C.

1.2 Feeding Management

Juvenile largemouth bass were purchased from Huzhou Nanxun Hefu Honghao Aquaculture Family Farm and reared in the aquaculture workshop of the Laboratory of Fish Nutrition, School of Marine Sciences, Ningbo University. Prior

to the experiment, the fish were domesticated with commercial feed for two weeks. After the acclimation period, the fish were fasted for 24 hours. A total of 270 healthy juvenile largemouth bass with uniform size and an initial body weight of (4.12 ± 0.02) g were selected and randomly distributed into nine 300-L tanks, with 30 fish per tank. Each diet was randomly assigned to three tanks. Fish were fed twice daily at 08:00 and 18:00 at a feeding rate of 3-4% of body weight. Daily water exchange was 50%. Feed intake and mortality were recorded daily, with dead fish removed and weighed. Body weight was measured every two weeks to adjust feed ration. The experiment lasted for eight weeks, during which water temperature was maintained at 22.2-31.0°C, dissolved oxygen concentration was above 6.0 mg/L, and pH was 7.5-8.0.

1.3 Sample Collection

At the end of the feeding trial, fish were fasted for 24 hours and anesthetized with eugenol (1:10,000). Three fish were randomly selected from each tank to measure body weight and body length. Viscera were dissected, and liver and intraperitoneal fat were separated and weighed to calculate hepatosomatic index (HSI), viscerosomatic index (VSI), intraperitoneal fat ratio (IPF), and condition factor (CF). Another six fish were randomly selected from each tank, and blood was collected from the caudal vein into 1.5-mL centrifuge tubes. After standing overnight at 4°C, blood samples were centrifuged at 3,500 r/min for 10 minutes to obtain serum, which was stored at -80°C for later analysis. The sampled fish were then dissected to collect liver in 2.0-mL tubes for antioxidant index and fatty acid composition analysis, and dorsal muscle above the lateral line was collected in 4.0-mL tubes for muscle fatty acid composition analysis.

1.4 Measurement Methods

Proximate composition analysis of feeds was performed according to AOAC (1995) methods. Moisture content was determined by drying at 105°C to constant weight. Crude lipid content was measured using a Soxhlet extractor (SX360) based on the Soxhlet extraction principle. Crude protein content was determined using a protein analyzer (Leco FB-528) based on thermal conductivity absorption. Crude ash content was measured using a muffle furnace at 550°C, with some modifications to standard methods.

Serum index analysis: Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as contents of glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total protein (TP) were measured using a biochemical analyzer (Pro-M).

Fatty acid composition analysis: Muscle and liver samples were freeze-dried for 48 hours, and lipids were extracted with chloroform-methanol solution. After pretreatment, samples were sent to the Testing Center of Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences. Fatty

acids were methylated using gas chromatography-mass spectrometry (GCMS-QP2010 Plus, SHIMADZU), and relative percentages of various fatty acids were calculated using area normalization.

Liver antioxidant index analysis: Activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), alanine aminotransferase (ALT), aspartate aminotransferase (AST), catalase (CAT), and alkaline phosphatase (AKP), as well as malondialdehyde (MDA) content, were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Calculation Formulas

Condition factor (g/cm^3) = $100 \times W / L^3$

Viscerosomatic index (%) = $100 \times W_v / W$

Intraperitoneal fat ratio (%) = $100 \times W_I / W$

Hepatosomatic index (%) = $100 \times W_h / W$

Where: W = body weight (g); L = body length (cm); W_v = viscera weight; W_I = intraperitoneal fat weight; W_h = liver weight (g).

1.6 Data Processing and Analysis

Data were first processed using Excel 2007 and then analyzed using SPSS 16.0 software. Experimental results were subjected to one-way ANOVA. When significant differences were detected, Turkey's multiple comparison test was applied. Differences were considered significant at $P < 0.05$.

2.1 Effects of Dietary Lipid Level on Morphology Indexes of Largemouth Bass

The effects of dietary lipid level on morphology indexes of largemouth bass are shown in Table 3. Dietary lipid level had no significant effect on hepatosomatic index or condition factor ($P > 0.05$), although HSI tended to increase with dietary lipid level, reaching its highest value in the 17.65% lipid group. The intraperitoneal fat ratio increased with dietary lipid level, with the 17.65% group being significantly higher than the 5.76% group ($P < 0.05$). The viscerosomatic index first increased and then decreased with dietary lipid level, with the 11.05% group showing the highest value, which was significantly higher than both the 5.76% and 17.65% groups ($P < 0.05$).

2.2 Effects of Dietary Lipid Level on Tissue Fatty Acid Composition of Largemouth Bass

The effects of dietary lipid level on muscle fatty acid composition are presented in Table 4. Correlation analysis revealed a positive relationship between muscle fatty acid composition and dietary fatty acid composition. The proportion of DHA and the ratios of n-3 HUFA/n-6 HUFA and C22:6n-3 (DHA)/C20:5n-3 (EPA) in muscle decreased with increasing dietary lipid level, consistent with

trends observed in the diets, with the highest values in the 5.76% lipid group. The proportion of saturated fatty acids (SFA) in muscle decreased while polyunsaturated fatty acids (PUFA) increased with dietary lipid level. The proportion of n-6 HUFA increased significantly with dietary lipid level ($P < 0.05$), also consistent with dietary trends.

The effects of dietary lipid level on liver fatty acid composition are shown in Table 5. Correlation analysis indicated positive relationships between liver fatty acid composition and both dietary and muscle fatty acid compositions. The proportion of DHA and the ratios of n-3 HUFA/n-6 HUFA and DHA/EPA in liver decreased with increasing dietary lipid level, while the proportion of n-6 HUFA increased significantly ($P < 0.05$), consistent with trends in diet and muscle. The 5.76% lipid group showed significantly higher n-3 HUFA/n-6 HUFA ratio than the other two groups ($P < 0.05$). With increasing dietary lipid level, the proportion of SFA in liver decreased while PUFA increased, mirroring the trend observed in muscle.

2.3 Effects of Dietary Lipid Level on Serum Biochemical Indexes of Largemouth Bass

The effects of dietary lipid level on serum biochemical indexes are presented in Table 6. Dietary lipid level had no significant effect on serum aspartate aminotransferase activity or glucose and total protein contents ($P > 0.05$). Aspartate aminotransferase activity and glucose content tended to decrease first and then increase with dietary lipid level, while total protein showed the opposite trend. When dietary lipid level increased from 5.76% to 11.05% or 17.65%, serum alkaline phosphatase activity and total cholesterol, high-density lipoprotein, and low-density lipoprotein contents all increased significantly ($P < 0.05$), while alanine aminotransferase activity decreased significantly ($P < 0.05$). However, these indexes showed no significant differences between the 11.05% and 17.65% lipid groups ($P > 0.05$). Serum triglyceride content first increased and then decreased with dietary lipid level, with the 11.05% group being significantly higher than the 5.76% and 17.65% groups ($P < 0.05$).

2.4 Effects of Dietary Lipid Level on Liver Antioxidant Indexes of Largemouth Bass

The effects of dietary lipid level on liver antioxidant indexes are shown in Table 7. Dietary lipid level had no significant effect on liver superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alkaline phosphatase (AKP), alanine aminotransferase (ALT) activities, or malondialdehyde (MDA) content ($P > 0.05$). GSH-Px activity and MDA content decreased with dietary lipid level, reaching their lowest values in the 17.65% lipid group, while AKP and ALT activities first decreased and then increased. Liver catalase (CAT) activity was highest in the 11.05% lipid group, significantly higher than in the 5.76% and 17.65% groups ($P < 0.05$). Liver aspartate aminotransferase (AST) activity was

highest in the 17.65% lipid group, significantly higher than in the 5.76% and 11.05% groups ($P < 0.05$).

3.1 Effects of Dietary Lipid Level on Morphology Indexes of Largemouth Bass

As an essential nutrient for fish, lipid is one of the main energy sources and plays an important role in life activities. Carnivorous fish have higher lipid requirements than herbivorous fish, and cold-water fish have higher requirements than warm-water fish. Appropriate dietary lipid supplementation has an obvious protein-sparing effect. Generally, liver lipid content increases with dietary lipid level because high dietary lipid promotes increased liver lipid content, enlarges adipocyte volume, and enables the liver to accommodate more fat, ultimately increasing hepatosomatic index. The present results showed that dietary lipid level had no significant effect on condition factor or hepatosomatic index of largemouth bass, while intraperitoneal fat ratio increased significantly with dietary lipid level. These findings differ from the general conclusion but are consistent with studies on haddock (*Melanogrammus aeglefinus* L.), darkbarbel catfish (*Pelteobagrus vachelli*), and Atlantic salmon, suggesting that largemouth bass may store excess liver fat in the abdominal cavity, reducing lipid storage in liver cells.

3.2 Effects of Dietary Lipid Level on Tissue Fatty Acid Composition of Largemouth Bass

Studies have shown that fish can synthesize monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), but linolenic acid (C18:3n-3) and linoleic acid (C18:2n-6) are essential fatty acids for freshwater fish that cannot be synthesized and must be obtained through diet. MUFA can reduce low-density lipoprotein content. Previous studies on white seabass (*Atractoscion nobilis*) and hybrid tilapia have reported that dietary fatty acid composition affects fish fatty acid composition. The present results demonstrate that muscle and liver fatty acid compositions of largemouth bass were affected by dietary fatty acid composition. With increasing dietary lipid level, the proportions of SFA in muscle and liver decreased while PUFA increased, showing high consistency with corresponding changes in dietary fatty acids. The n-3 HUFA/n-6 HUFA ratio in fish is an important factor determining aquatic product quality, with WHO/FAO recommending a level of 0.1-0.2. In this study, the muscle n-3 HUFA/n-6 HUFA ratio of largemouth bass exceeded the WHO/FAO recommended value, indicating high nutritional value. Most studies report that fish accumulate more DHA than EPA, which is consistent with our results showing that the proportion of DHA in largemouth bass muscle was much higher than EPA. DHA and EPA play important roles in fish growth. Trushenski et al. indicated that cobia requires DHA supplementation to meet long-chain n-3 PUFA requirements, and DHA is more critical than EPA in the cellular defense mechanism of grouper. Therefore, the higher accumulation of

DHA than EPA in largemouth bass muscle is of great importance.

3.3 Effects of Dietary Lipid Level on Serum Biochemical Indexes of Largemouth Bass

Fish blood physiological and biochemical indexes are closely related to nutritional status, metabolism, and disease. When the external environment changes, fish are affected, and their health status under environmental stress is reflected through blood physiological and biochemical indexes. Cholesterol metabolism is an important part of lipid metabolism. Cholesterol is mainly produced by the liver and must combine with lipoproteins for transport. Elevated total cholesterol content in the liver affects liver cell function. Lipoproteins are divided into high-density lipoprotein and low-density lipoprotein. HDL transports cholesterol from outside the liver to the liver for decomposition, while LDL has the opposite effect, transporting cholesterol from the liver to various tissues. Studies on turbot (*Psetta maxima*) and blunt snout bream (*Megalobrama amblycephala*) found that blood triglyceride content increased with dietary lipid level, indicating that fish improved lipid absorption and transport capacity to cope with high-lipid diets. In this study, serum triglyceride content first increased and then decreased with dietary lipid level, consistent with results from whitefin black shark and *Elopichthys bambusa*. Wang et al. suggested that fish can absorb dietary lipids and convert them into free fatty acids that eventually enter the liver. Therefore, when dietary lipid level was below 11.05%, lipid intake increased with dietary lipid level, leading to more free fatty acids in the liver and increased synthesis and transport of triglycerides, resulting in higher serum triglyceride content. When dietary lipid level exceeded 17.65%, largemouth bass improved lipid absorption and transport capacity, reducing serum triglyceride content. In this study, when dietary lipid level exceeded 11.05%, increased liver fat accumulation in largemouth bass led to liver cell damage, weakening the liver's ability to synthesize triglycerides. Alkaline phosphatase directly participates in phosphate group metabolism and transfer, and is related to lipid and protein metabolism. It is also affected by growth stage, nutritional status, environmental changes, and disease. When liver is damaged, serum alkaline phosphatase activity increases significantly. In this study, serum alkaline phosphatase activity increased with dietary lipid level, consistent with studies on hybrid tilapia.

3.4 Effects of Dietary Lipid Level on Liver Antioxidant Indexes of Largemouth Bass

Superoxide dismutase (SOD) and catalase (CAT) are important components of biological antioxidant enzymes that protect cells from oxidative damage. Glutathione peroxidase (GSH-Px) is an important peroxide-decomposing enzyme that reduces harmful peroxides to harmless hydroxy compounds, protecting cell membrane structure and function. Malondialdehyde (MDA) is an end product of lipid oxidation and can directly evaluate the degree of oxidative damage. In

this study, liver SOD and CAT activities first increased and then decreased with dietary lipid level, indicating that appropriate dietary lipid levels could enhance antioxidant enzyme activity and improve antioxidant capacity, but excessive lipid levels weakened liver antioxidant capacity. Liver GSH-Px activity and MDA content decreased with dietary lipid level, consistent with studies on *Sebastiscus marmoratus*. This may be because high dietary lipid levels caused massive fat accumulation in largemouth bass liver, leading to increased MDA content and liver cell damage.

4 Conclusion

Dietary lipid level can affect lipid deposition, alter tissue fatty acid composition, and modify lipid transport and utilization capacity by influencing HDL and LDL contents in serum of largemouth bass. When dietary lipid level exceeds 11.05%, it can easily cause liver function damage in largemouth bass.

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Note: References 24-25 appear to be cited out of sequence; please verify.

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

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