Evaluation of the Effects of Frozen Fish and Formulated Feed on Muscle Quality and Health Status of Largemouth Bass (Micropterus salmoides) Postprint

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Abstract

This experiment evaluated the effects of chilled mixed fish and formulated feed on muscle quality and health status of largemouth bass through determination of serum biochemical parameters and muscle nutrient composition.

Full Text

Abstract

This study evaluated the effects of frozen fresh fish and artificial compound feed on the muscle quality and health status of largemouth bass (*Micropterus salmoides*) by measuring serum biochemical indices and muscle nutritional components. During the entire culture period, largemouth bass in the frozen fresh group were fed exclusively frozen fresh fish, while those in the feed group received a specialized artificial compound feed formulated for largemouth bass. Prior to market, six fish were randomly sampled from each group for serum biochemical analysis, and another six fish from each group were collected for muscle nutritional component determination. The results showed that serum aspartate aminotransferase (AST) activity in the feed group was significantly higher than in the frozen fresh group ($0.01 \leq P < 0.05$), alkaline phosphatase (ALP) activity was extremely significantly higher ($P < 0.01$), while total protein (TP) and albumin (ALB) contents were extremely significantly lower ($P < 0.01$). Muscle total amino acid (TAA) content in the frozen fresh group was significantly higher than in the feed group ($0.01 \leq P < 0.05$), and the amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) for all amino acids were higher in the frozen fresh group. Muscle linoleic acid (C18:2) content in the feed group was extremely significantly higher than in the frozen

fresh group ($P < 0.01$). These findings indicate that under the experimental conditions, largemouth bass fed frozen fresh fish exhibited superior muscle nutritional quality, particularly protein quality and amino acid composition, along with better health status compared to the feed group. However, the feed group showed extremely significantly elevated linoleic acid levels. Therefore, artificial compound feed formulations for largemouth bass require further optimization to meet consumer nutritional demands.

Keywords: *Micropterus salmoides*; artificial compound feed; frozen fresh fish; serum biochemical indices; muscle nutritional components

Introduction

Micropterus salmoides, commonly known as largemouth bass, belongs to the order Perciformes, family Centrarchidae, and genus *Micropterus*. Native to freshwater rivers and large lakes in America, particularly abundant in the Great Lakes region of the United States, it has been widely introduced and cultured globally. Valued for its tender meat and excellent flavor, it enjoys strong popularity in international markets and is often referred to as "freshwater grouper." According to the *China Fishery Statistical Yearbook 2016*, China' s total production of largemouth bass reached 353,000 tons. Two primary feed types are used in largemouth bass aquaculture: frozen fresh fish and artificial compound feed, both of which can successfully raise fish to market size. However, consumers have long been concerned about differences in fish health status and nutritional value between these two feeding regimes. Current research comparing these feed types has primarily focused on growth performance [1-2], muscle nutritional components [2-3], intestinal protease activity [4], and intestinal microbial community structure [5], without providing a comprehensive evaluation of fish health status and muscle nutritional quality.

Numerous studies have directly analyzed fish muscle nutritional components. International research has concentrated on fatty acids in marine perciform fish [6-9], while domestic studies have covered various edible fish species [11-13]. Alasalvar et al. [6] compared differences in total lipid content, trace element composition, and fatty acid profiles between wild and cultured sea bass muscle. Codier et al. [8] investigated changes in tissue phospholipid fatty acid composition of sea bass throughout the culture cycle. Glover et al. [10] analyzed the effects of feed composition, genetic factors, growth cycle, and environmental conditions on the muscle nutritional components of Atlantic salmon (*Salmo salar* L.). Ji et al. [11] compared muscle nutritional components between pond- and cage-cultured paddlefish (*Polyodon spathula*). Sun et al. [12] studied the effects of dietary protein levels on muscle nutritional components in juvenile paddlefish. Liu et al. [13] compared muscle nutritional components of grass carp (*Ctenopharyngodon idellus*) fed broad beans versus conventional compound feed.

To date, no comparative analysis of health status in largemouth bass fed different feed types has been reported. Previous studies have demonstrated that fish

blood biochemical indices are closely related to nutritional status, metabolic levels, and disease occurrence, and that physiological or pathological changes in fish following external influences are inevitably reflected in blood physiological and biochemical parameters [14]. Therefore, this experiment conducted a comprehensive comparative analysis of serum biochemical indices, muscle amino acid composition, and fatty acid profiles of largemouth bass fed exclusively frozen fresh fish or artificial compound feed, aiming to objectively evaluate the health status and nutritional quality differences between the two feeding regimes.

Materials and Methods

1.1 Experimental Design and Management

The experiment was conducted at Foshan Sanshui Huamiao Aquaculture Co., Ltd. Juvenile largemouth bass from the same hatchery were cultured to market size, with the frozen fresh group receiving frozen fresh fish throughout the entire period and the feed group receiving a specialized artificial compound feed for largemouth bass. Frozen fresh fish were purchased from a market in Shunde District, Foshan, while the specialized feed was obtained from a feed manufacturer in Foshan, Guangdong. The basic nutritional components of both feed types are listed in Table 1 . Prior to market, samples were collected from both groups for analysis.

1.2 Serum Biochemical Index Determination

Before market, six fish were randomly collected from each group at the pond site. Blood was drawn from the caudal peduncle, and serum was prepared and stored at 4°C for measurement of 14 serum biochemical indices. Albumin (ALB) content was determined using the bromocresol green method. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and amylase (AMY) activities were measured using rate methods. Glucose (GLU) content was determined by glucose oxidase method. Total cholesterol (TC) content was measured using cholesterol oxidase method. Low-density lipoprotein cholesterol (LDL-CH) and high-density lipoprotein cholesterol (HDL-CH) contents were determined by direct methods. Triglyceride (TG) content was measured by enzymatic method. Total protein (TP) content was determined by biuret method. Lipase (LPS) activity was measured by dry chemistry method. Superoxide dismutase (SOD) and lysozyme (LZM) activities were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.3 Muscle Nutritional Component Determination

Before market, six fish were randomly collected from each group at the pond site. Approximately 200 g of dorsal muscle was sampled from each fish for determination of amino acid and fatty acid composition and other nutritional indices. Seventeen amino acids were measured, including aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala),

valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys), arginine (Arg), proline (Pro), and tryptophan (Trp), using the method specified in GB/T 5009.124-2003. Twenty-one fatty acids were measured, including myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), heneicosanoic acid (C21:0), behenic acid (C22:0), palmitoleic acid (C16:1), heptadecenoic acid (C17:1), oleic acid (C18:1), eicosenoic acid (C20:1), linoleic acid (C18:2), linolenic acid (C18:3), eicosadienoic acid (C20:2), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5, EPA), docosadienoic acid (C22:2), docosatetraenoic acid (C22:4), docosapentaenoic acid (C22:5), and docosahexaenoic acid (C22:6, DHA), using the method specified in GB/T 17377-2008. Crude fat content was determined according to GB/T 5009.6-2003, crude protein content according to GB 5009.5-2010, crude ash content according to GB 5009.4-2010, and moisture content according to GB 5009.3-2010.

1.4 Nutritional Value Evaluation

Muscle nutritional value of largemouth bass fed different feeds was evaluated based on the essential amino acid (EAA) balance pattern established by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) in 1973 [15], and the whole egg protein pattern proposed by the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine in 1991 [16]. Amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) were calculated using the following formulas:

AAS = (mg of amino acid per g N in test protein) / (mg of amino acid per g N in FAO/WHO reference pattern)

CS = (mg of amino acid per g N in test protein) / (mg of amino acid per g N in whole egg protein)

$EAAI = \sqrt[n]{[(t_1/s_1) \times (t_2/s_2) \times \cdots \times (t/s)]}$

where 1, 2, ⋯, n represent different amino acids; t_1, t_2, ⋯, t are the contents of different amino acids in largemouth bass muscle protein (mg/g N); and s_1, s_2, ⋯, s are the corresponding amino acid contents in whole egg protein (mg/g N).

1.5 Data Analysis

Data were processed using Excel 2007 and analyzed using independent samples t-test in SPSS 18.0 software. Results are expressed as mean ± standard deviation (mean ± SD). Differences were considered significant at $0.01 \leq P < 0.05$ and extremely significant at $P < 0.01$.

Results and Analysis

2.1 Serum Biochemical Indices of Largemouth Bass in the Two Groups

Independent samples t-test analysis of 14 serum biochemical indices is presented in Table 2 . Serum AST activity, SOD activity, and GLU content in the feed group were significantly higher than in the frozen fresh group ($0.01 \leq P < 0.05$). ALP activity was extremely significantly higher ($P < 0.01$), while ALB and AMY activities and TP content were extremely significantly lower ($P < 0.01$). The remaining seven serum biochemical indices showed no significant differences between groups ($P > 0.05$).

2.2 Proximate Nutritional Components in Muscle of Largemouth Bass in the Two Groups

Independent samples t-test analysis of muscle proximate nutritional components is shown in Table 3 . Muscle moisture content in the frozen fresh group was extremely significantly lower than in the feed group ($P < 0.01$), while crude protein content was significantly higher ($0.01 \leq P < 0.05$). No significant differences were observed in crude ash or crude fat content between groups ($P > 0.05$).

2.3 Amino Acid Contents in Muscle of Largemouth Bass in the Two Groups

As shown in Table 4 , both groups contained a complete profile of 17 amino acids. Total amino acid (TAA) contents were $(19.57 \pm 0.11)\%$ and $(18.80 \pm 0.26)\%$ in the frozen fresh and feed groups, respectively. Independent samples t-test revealed that Ser and His contents in the frozen fresh group were extremely significantly higher than in the feed group ($P < 0.01$). TAA, Thr, Leu, Tyr, Lys, Arg, and Pro contents were significantly higher ($0.01 \leq P < 0.05$). No significant differences were found for the remaining nine amino acids, essential amino acids (EAA), or delicious amino acids (DAA) between groups ($P > 0.05$).

2.4 Nutritional Value Evaluation of Muscle of Largemouth Bass in the Two Groups

As shown in Table 5 , based on AAS, Met + Cys received the lowest score in both groups, indicating that Met + Cys was the first limiting amino acid. Similarly, based on CS, Met + Cys also had the lowest score. Under both AAS and CS evaluation modes, all amino acid scores in the frozen fresh group were higher than in the feed group, and the EAAI was also higher, demonstrating that largemouth bass fed frozen fresh fish had more reasonable amino acid composition and superior muscle nutritional quality.

2.5 Fatty Acid Contents in Muscle of Largemouth Bass in the Two Groups

Independent samples t-test analysis of 21 fatty acids is presented in Table 6 . The frozen fresh group showed extremely significantly higher contents ($P < 0.01$) for eight fatty acids: C14:0, C15:0, C16:1, C17:0, C17:1, C21:0, EPA, and DPA. Five fatty acids were extremely significantly lower ($P < 0.01$): C18:2, C18:3, C20:1, C20:2, and C22:0. Two fatty acids were significantly higher ($0.01 \leq P < 0.05$): C22:2 and C22:4. No significant differences were observed for the remaining six fatty acids ($P > 0.05$). Additionally, no significant differences were found in total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) between groups ($P > 0.05$).

Discussion

3.1 Differences in Serum Biochemical Indices Between the Two Groups

Fish blood biochemical indices are closely related to nutritional status, metabolic levels, and disease occurrence, and physiological or pathological changes in fish following external influences are inevitably reflected in these parameters [14]. Serum transaminases are important indicators of liver function, and elevated activity signifies hepatocellular damage [17]. In this study, serum AST activity in the feed group was significantly higher than in the frozen fresh group, and ALP activity was extremely significantly higher, suggesting that artificial compound feeding may cause abnormal liver metabolism in largemouth bass. Zhang et al. [18] found that dietary vitamin A supplementation significantly increased serum ALP activity in Japanese seabass (*Lateolabrax japonicus*). Lan et al. [19] reported that largemouth bass fed high vitamin A diets showed significantly elevated serum AST activity, with excessive vitamin A causing hepatic toxicity. Serum proteins primarily maintain plasma colloidal osmotic pressure and pH stability while serving transport and nutritional functions [20-21]. Reduced serum ALB content greatly affects blood osmotic pressure and can reflect hepatic issues [22]. Wu et al. [23] found that biotin deficiency in Nile tilapia (*Oreochromis niloticus*) significantly decreased serum ALB and TP contents, causing anorexia and growth retardation. In this study, the extremely significantly lower serum ALB and TP contents in the feed group further indicate that current artificial compound feeds are nutritionally imbalanced and can cause abnormal liver metabolism in largemouth bass, suggesting that feed formulations require further optimization.

3.2 Differences in Muscle Nutritional Components Between the Two Groups

The frozen fresh group exhibited significantly higher muscle crude protein content than the feed group. Wang et al. [3] reported that crude protein content in adult largemouth bass fed frozen fish was 5.94% higher than in those fed artifi-

cial feed. Gao et al. [24] found that Japanese flounder (*Paralichthys olivaceus*) fed trash fish had significantly higher crude protein content than those fed artificial feed. These consistent results indicate that the carnivorous largemouth bass experiences reduced muscle quality when switched to artificial feed, necessitating further optimization of feed formulations to meet consumer nutritional demands.

3.3 Differences in Muscle Amino Acid Composition Between the Two Groups

Both groups contained a complete profile of 17 amino acids. The frozen fresh group showed extremely significantly higher Ser and His contents, significantly higher TAA, Thr, Leu, Tyr, Lys, Arg, and Pro contents, and significantly higher total TAA content, indicating superior muscle nutritional quality, particularly protein quality, compared to the feed group. Shi et al. [25] found that 10 of 18 amino acids were significantly higher in paddlefish fed compound feed compared to live feed, with no differences in the remaining eight. Zhuang et al. [26] observed significant changes in nine amino acids when Chinese sturgeon (*Acipenser sinensis* Gray) juveniles were switched from artificial feed to tubifex worms, suggesting that muscle nutritional changes relate not only to feed composition but also to palatability. Zhang et al. [2] proposed that live feed may contain trace active substances that induce enzyme secretion and provide exogenous enzymes (such as trypsin) that directly or indirectly participate in food digestion [27], thereby enhancing protein digestibility and absorption, particularly of essential amino acids, and ultimately improving muscle protein quality.

Protein nutritional value depends on amino acid composition [28]. Evaluation using FAO/WHO and whole egg protein standards revealed Met + Cys as the first limiting amino acid in both groups, consistent with findings for Yellow River carp [29]. The higher amino acid scores and EAAI in the frozen fresh group confirm its more reasonable amino acid composition and superior nutritional quality.

3.4 Differences in Muscle Fatty Acid Composition Between the Two Groups

The frozen fresh group showed extremely significantly higher contents of eight fatty acids (C14:0, C15:0, C16:1, C17:0, C17:1, C21:0, EPA, DPA) and extremely significantly lower contents of five fatty acids (C18:2, C18:3, C20:1, C20:2, C22:0), particularly C18:2 (1.77% vs. 16.53%). This likely reflects fish inability to synthesize C18:2 and varying capacities among species to synthesize long-chain polyunsaturated fatty acids from C18 precursors, making C18:2 an essential dietary component [30]. As an n-6 series fatty acid and primary fatty acid in freshwater fish, the difference in C18:2 content likely stems from different dietary lipid sources. Tidwell et al. [31] demonstrated that dietary fatty acid composition affects largemouth bass fatty acid profiles. Xue et al. [32] found that soybean oil diets significantly reduced fatty acid content in Japanese

seabass juveniles. Chen et al. [33] reported positive correlations between muscle and dietary fatty acid compositions in gibel carp (*Carassius auratus gibelio*). Gao et al. [34] showed that fish oil increased n-3 fatty acid content in loach (*Misgurnus anguillicaudatus*) larvae. The principle that fish fatty acid composition reflects dietary fatty acid composition [35] suggests that the artificial feed used in this study contained high C18:2 levels. However, freshwater fish generally possess elongation and desaturation capabilities to convert C18:2 to DHA, as confirmed in pikeperch (*Sander lucioperca*) [36], Japanese seabass [32], and gibel carp [33]. The extremely high C18:2 content in the feed group warrants further investigation into the conversion capacity of largemouth bass.

Conclusion

Under the experimental conditions, largemouth bass fed frozen fresh fish demonstrated superior muscle nutritional quality, particularly protein quality and amino acid composition, compared to those fed artificial compound feed, as evidenced by higher muscle crude protein content, individual amino acid contents, and amino acid scores. However, compared to frozen fresh fish feeding, artificial compound feeding may cause abnormal liver metabolism and suboptimal health status in largemouth bass, although it significantly increases muscle C18:2 content. These results underscore the need for further optimization of artificial compound feed formulations for largemouth bass to better satisfy consumer nutritional requirements.

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