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Effects of Dietary L-glutamic acid on the Growth Performance, Gene Expression Associated with Muscle Growth-Related Gene Expression, and Intestinal Health of Juvenile Largemouth Bass (*Micropterus salmoides*)

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Abstract: The present research examined the impact of L-glutamic acid (Glu) supplementation on the growth performance, muscle composition, gene expression correlated with muscle growth, and intestinal health of largemouth bass. There were 525 fish in total, which were distributed randomly into five groups. Each group had three replicates, and each replicate consisted of 35 fish. Groups with control and experimental diets were assigned glutamic acid amounts of 0.2%, 0.4%, 0.6%, and 0.8%. The findings demonstrated that glutamic acid supplementation enhanced growth performance, feed intake (FI), and condition factor (CF), with the best value being attained at 0.4% Glu. The mean muscle fiber area was increased and the muscle fiber density was decreased in the 0.6% Glu group. The levels of total amino acids and specific amino acids, such as glutamic acid, aspartic acid, leucine, valine, alanine, and glycine, were shown to be higher in the 0.6% Glu group. In the 0.6% Glu group, the mRNA expression levels of *atrogin-1*, *murf-1*, *foxo3a*, and *4e-bp1* were decreased compared to the control group. Conversely, the mRNA expression levels of *myf5*, *myog*, *myod*, *s6k1*, *tor*, *akt*, and *pi3k* were increased in the 0.6% Glu group compared to the control group. The 0.4% Glu group had higher intestinal amylase, lipase, and protease activities and greater villus height, villus width, and muscle thickness. In summary, Glu can support largemouth bass growth, muscular development, intestinal digestion, and absorption.

Keywords: glutamic acid; largemouth bass; muscle; intestinal health

Key Contribution: This study was conducted to investigate the effects of dietary HMB on growth performance, muscle development and intestinal health of largemouth bass and to explore the possible mechanisms.

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1. Introduction

Recently, due to the progress of the economic landscape, there has been a growing demand for aquatic items among individuals [1]. The aquaculture sector has risen rapidly, with worldwide fish production rising from 2.6 million tons in 1970 to 87.5 million tons in 2020 [2]. As people's demand for aquatic products continues to expand, various high-density, high-yield factory farming methods continue to innovate [3]. The increase in the total output of the aquaculture industry has made the competition in the aquaculture market more intense. People are eager to find ways to increase the output of aquatic products, enhance the health of aquatic products, and improve the meat quality of aquatic products [4].

In a previous study, amino acids, as the main component of proteins, undertake a variety of biological functions, including maintaining cell growth [5] and promoting the synthesis of physiological factors [6] and oxidation energy supply [7]. In the study of carnivorous fish, some amino acids can improve the growth performance of the fish, promote muscle development, improve muscle quality [8], improve intestinal digestion [9], and promote intestinal health [10]. L-glutamic acid (Glu) is a functional feed additive [11]. It can provide energy for animal tissues and participate in many physiological regulation processes [12]. Research has demonstrated that adding glutamate to the diet can enhance the growth performance of *Ctenopharyngodon idella*, enhance the antioxidant capacity of the intestinal tract, and facilitate digestion and absorption [13]. And studies have shown that glutamate can affect the growth, muscle development, and muscle quality of *Ctenopharyngodon idellus* by different regulation of protein metabolism, muscle development, and antioxidant-related genes [14]. In summary, glutamic acid as a functional additive has achieved good research results, but it has not been reported in the study of largemouth bass.

As a carnivorous freshwater fish, largemouth bass has high economic benefits [15], which can be well adapted to a variety of breeding modes [16]. It also has the advantages of rapid growth, delicious flavor, and is boneless between the muscles, which makes it popular in the Chinese market [17]. According to the statistics of the Fisheries Administration of the Ministry of Agriculture and Rural Affairs and the Chinese Aquatic Association, the output of largemouth bass reached 802,500 tons in 2022 [18]. Nevertheless, research is scarce about the impact of glutamic acid as a dietary supplement on the growth performance, intestinal health, and muscular growth and development of largemouth bass. Therefore, the present research was performed to examine the impacts of dietary glutamic acid on the developmental performance, intestinal health, and muscular development and growth of largemouth bass.

2. Generally Used Wording ID: Materials and Methods

2.1. Preparation of Experimental Diet

Five experimental diets were established, each containing equal amounts of nitrogen and lipids. The diets included fish meal, chicken meal, cottonseed meal, soybean meal, and soybean protein concentrate as the primary sources of protein. Fish oil and soybean oil were used as the primary sources of fat, while high-gluten flour served as the main source of sugar. Glutamic acid was added at 0.2%, 0.4%, 0.6%, and 0.8%. From McLean Biochemical Technology Co., Ltd Shanghai China., glutamic acid was acquired. After being crushed, the feed components were run through an 80-mesh sieve. The low-dose raw components were gradually pre-mixed before being combined in a V-type mixer for a thorough mixing. The oil-kneading machine was then filled with fish and soybean oils to begin the kneading process. After the oil was mixed, 30% water was added to the mixer to stir and mix, and then the SLX-80 twin-screw extruder (South China University of Technology, Guangzhou, China) was used to make a pellet feed with a particle size of 2.0 mm. The pellet feed was allowed to naturally cool to 55 °C before being wrapped in a bag and refrigerated at −20 °C for subsequent use (Table 1).

2.2. Experimental Design and Feeding Management

The feeding experiment was conducted in an indoor circulating water system at the Guangdong Academy of Agricultural Sciences' Baiyun Experimental Base. Largemouth bass juveniles were purchased from Renzhi (Guangzhou China) Technology Co., Ltd. A total of 525 healthy fish, each weighing 8.00 ± 0.00 g, were selected and categorized into 5 groups. Each group had 3 repetitions, resulting in a total of 15 replicates. For 56 days, the fish were fed satiating meals twice per day (7:30 and 17:30). The ideal environmental conditions are natural light in summer, water temperature 27~31 °C, no heating equipment, ammonia nitrogen concentration below 0.20 mg/L, nitrite concentration below 0.01 mg/L, dissolved oxygen concentration above 5.0 mg/L, and pH levels between 7.8 and 8.2.

Table 1. Formulation and composition of experimental diets (air-dry basis).

Item	CON	0.2% Glu	0.4% Glu	0.6% Glu	0.8% Glu
Fish meal	40	40	40	40	40
Chicken meal	10	10	10	10	10
Cotton meal	7.5	7.5	7.5	7.5	7.5
Soybean meal	10.5	10.5	10.5	10.5	10.5
Soy protein	8	8	8	8	8
Flour	16	16	16	16	16
Fish oil	3.5	3.5	3.5	3.5	3.5
Microcrystalline cellulose	1.3	1.1	0.9	0.7	0.5
Mineral mixture	1	1	1	1	1
Vitamin mix	0.2	0.2	0.2	0.2	0.2
Calciumbiphosphate	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5	0.5
Glutamic acid	0	0.2	0.4	0.6	0.8
Total	100	100	100	100	100
Analyzed chemical composition					
Crude protein	48.26	48.31	49.01	48.22	49.23
Crude lipid	6.14	6.07	6.19	6.03	6.12
Moisture	8.05	8.04	8.03	8.02	8.01
Crude ash	12.57	12.59	12.27	12.42	12.34

Each kilogram of premix contained: vitamin A 4,000,000 IU, vitamin D3 2,000,000 IU, vitamin E 30 g, vitamin K3 10 g, vitamin B_1 5 g, vitamin B_2 15 g, vitamin B6 8 g, calcium pantothenate 25 g, folic acid 2.5 g, biotin 0.08 g, nicotinic acid 40 g. Vitamin B_{12} 0.02 g, inositol 150 g, $MgSO_4 \cdot H_2O$ 12 g, KCl 90 g, Met-Cu 3 g, $FeSO_4 \cdot H_2O$ 1 g, $ZnSO_4 \cdot H_2O$ 10 g, Ca (IO_3) 2 0.06 g, Met-Co 0.16 g, and $NaSeO_3$ 0.003 6 g. The fish oil was provided by China Oil and Foodstuffs Corporation.

2.3. Sample Analysis

2.3.1. Evaluation of the Growth Performance and Physical Composition

Following the feeding experiment, the fish were deprived of food for 24 h. They were then weighed and numbered in each barrel, and the final average weight and survival rate were determined. From each replicate, a total of 10 fish were selected at random, and 3 of them were utilized to determine the overall body composition. Six fish were used for measuring body weight and body length, dissecting visceral mass, separating liver and weighing, and calculating body index. Additionally, 3 fish were picked from each cage. The foregut and liver were then separated and preserved in 4% paraformaldehyde. These samples were then maintained to section the intestinal and hepatic tissues.

2.3.2. Nutrient Composition of Diets and Muscle

The moisture content was evaluated by subjecting the sample to oven drying at a temperature of 105 °C until a consistent weight was achieved. The Kjeldahl method was employed to determine the protein content in both whole fish and muscle [19]. The ether Soxhlet extraction method was used to determine the crude fat content. The ash content was measured by subjecting the sample to combustion in a muffle furnace until a steady weight was achieved at a temperature of 550 °C [20].

2.3.3. Histological Analysis

One fish was randomly selected from each replicate, and the middle part of the dorsal muscle and the foregut were sliced on ice. The muscle and intestine sections were subjected to histological processing using the paraffin procedure and stained with the hematoxylin and eosin (H&E) staining method. The tissue was washed with a 0.01 mol/L PBS solution, treated with 4% paraformaldehyde for 10 min to fix it, slowly dehydrated in 75% ethanol, and finally embedded in paraffin and sliced into sections that were 5 μm thick. Finally, stained with eosin and hematoxylin, the intestinal and muscle tissues were observed under a microscope and photographed. The intestinal villus height, muscle thickness, villus width, muscle fiber density, muscle fiber number, and average muscle fiber area were measured [21].

2.3.4. Analysis of Muscle Amino Acid Content

The analysis revealed the presence of fifteen different amino acids, namely arginine (Arg), lysine (Lys), histidine (His), phenylalanine (Phe), tyrosine (Tyr), leucine (Leu), isoleucine (Ile), methionine (Met), valine (Val), alanine (Ala), glycine (Gly), glutamic acid (Glu), serine (Ser), threonine (Thr), and asparagine (Asp). The mobile phase A was 40 mmol/L sodium dihydrogen phosphate (pH = 7.8); the detection signal was UV 339 nm, fluorescence (EX = 266 nm, EM = 305 nm). Acetonitrile, methanol, and water comprised the mobile phase B; the ratios were 45, 45, and 10, respectively. Every experimental technique was carried out strictly in line with the standard instructions; the measured findings deviate by less than 10% of the arithmetic mean value, and the correlation coefficient of every amino acid's linear regression equation is >0.99.

2.3.5. Determination of Intestinal Enzyme Activity

The foregut was immediately frozen in a refrigerator at −80 °C. After weighing, the samples were homogenized with a high-speed tissue homogenizer and centrifuged with a refrigerated centrifuge (at 3000 r/min for 15 min at 4 °C). Trypsin activity was determined using r-toluenesul-phonyl-l-arginine methyl esther as a substrate in 0.05-mol/LTriseHCl buffer, (pH = 9.0). Amylase was assayed using 1% solublestarch as a substrate in 0.02 mol/L phosphate buffer, (pH = 8.0). Lipase activity was measured at 405 nm by the rate of methyl-resorufin formation.

2.3.6. Real-Time Quantitative PCR Analysis

The protein–nucleic acid approach was utilized to ascertain the total RNA concentration after largemouth bass muscle was treated with Trizol reagent to extract RNA. The process of reverse transcription was employed to convert the total RNA into complementary DNA (cDNA) using the TaKaRa reverse transcription kit (Takara, Tokyo, Japan) [22]. Primer Premier 5.0 software was used for primer design, cDNA sequences from Gen Bank or published papers, and primer sequences from Wang et al. (2021) [23] (Table 2). Using β-actin as an internal reference, the $2^{-\Delta\Delta Ct}$ technique was used to calculate the relative expression of the gene based on the control group.

Table 2. Primer sequences of target genes used in real-time quantitative PCR.

Target Gene	Primer Sequence (5–3′)	Number/Source
pi3k	F-CGCAAGACCAGAGATCAGTATC R-CGTCGTCCACCATAGAGTATTG	XM_038733022.1
akt	F-CACCGTAGAACCGAGCCCGCT R-CGCCATGAAGATCCTAAAGAA	[23]
tor	F-TCAGGACCTCTTCTCATTGGC R-CCTCTCCCACCATGTTTCTCT	[23]
S6k1	F-GCCAATCTCAGCGTTCTCAAC R-CTGCCTAACATCATCCTCCTT	XM_038708508.1
4ebp1	F-ACGAGGTCTGCCCAACATTC R-CAGCGTTGCTGCTATCAGGT	XM_038703879.1
Foxo3a	F-AAGAAGAAAGCCTCGCTACAG R-GTGGGACTTCCTGTCCATTT	XM_038728762.1
myod	F-CCTGCCGCTGATGATTTCTAT R-AGTCGTCCGGCTTCAGTA	EU367961.1
myog	F-GTGACAGGAACAGAGGACAAA R-ACGATCCATGGTAACAGTCTTC	XM_038697403.1
Myf5	F-GGCTGAAGAAGGTCAACCA R-GTCCTGCAGACTCTCAATGTAA	EU555403.1

Table 2. *Cont.*

Target Gene	Primer Sequence (5–3′)	Number/Source
murf-1	F-ACGCCAAAGAGCTGAAGTGT R-TGTCCGAACACCTTGCACAT	XM_038728309.1
atrogin-1	F-CCAAATCAACAGGCCCACAT R-GACAGACGCTGCATGATGTT	XM_038711973.1
β-actin	F-ACTGCTGCTTCCTCTTCATC R-GGATACCGCAAGACTCCATAC	MH018565.1

pi3k, phosphatidylinositol 3-kinase; AKT, protein kinase B; tor, target of rapamycin; S6K1, 70 kDa ribosomal protein S6 kinase 1; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; FoxO3a, forkhead box O3a; MyoD, myoblast determination protein; MyoG, myogenin; Myf5, myogenic factor 5; MuRF-1, muscle-specific RING finger protein 1; β-Actin, muscle-specific F-box protein-regulated; F, forward; R, reverse.

2.3.7. Statistical Analysis

Using SPSS 26.0, a one-way ANOVA was run on each result, and then the Tukey multiple comparisons procedure was applied. The statistical significance threshold was set at $p < 0.05$, and the data were presented as mean ± SD.

3. Results

3.1. Growth Performance

Table 3 displays growth performance and body indexes. Compared to the control group, the FBW, SGR, and WGR of the 0.2% and 0.4% Glu groups were considerably greater ($p < 0.05$). Significant differences were seen between the FCR of the 0.2% and 0.4% Glu groups and that of the control group ($p < 0.05$). Additionally, there was no statistically significant difference in the VSI between the groups, and the HSIs of the 0.4%, 0.6%, and 0.8% Glu groups were all considerably greater than those of the control group ($p > 0.05$).

Table 3. Effects of dietary Glu supplementation on growth performance of largemouth bass.

Item	CON	0.2% Glu	0.4% Glu	0.6% Glu	0.8% Glu
IBW (g/fish)	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
FBW (g/fish)	47.81 ± 1.63 ^{c}	56.13 ± 2.64 ^{ab}	58.94 ± 5.61 ^{a}	49.17 ± 2.48 ^{c}	52.70 ± 1.70 ^{bc}
FCR	1.31 ± 0.05 ^{a}	1.12 ± 0.06 ^{c}	1.20 ± 0.06 ^{abc}	1.27 ± 0.08 ^{ab}	1.18 ± 0.07 ^{bc}
FI (g/fish)	52.18 ± 1.65 ^{a}	53.72 ± 4.86 ^{a}	60.79 ± 4.43 ^{b}	52.33 ± 0.40 ^{a}	52.51 ± 1.35 ^{a}
WGR (%)	497.32 ± 20.37 ^{c}	601.10 ± 33.08 ^{ab}	636.16 ± 69.94 ^{a}	514.22 ± 30.70 ^{c}	558.38 ± 21.37 ^{bc}
SGR (%/day)	3.19 ± 0.06 ^{c}	3.48 ± 0.08 ^{ab}	3.56 ± 0.17 ^{a}	3.24 ± 0.09 ^{c}	3.36 ± 0.06 ^{bc}
CF (g/cm^3)	2.13 ± 0.36 ^{ab}	2.17 ± 0.16 ^{ab}	2.39 ± 0.21 ^{a}	2.11 ± 0.33^{b}	2.13 ± 0.56 ^{ab}
VSI (%)	7.22 ± 0.72	6.98 ± 0.64	6.89 ± 0.6	7.17 ± 0.64	6.7 ± 1.32

IBW, initial body weight; FBW, final body weight; FCR, feed conversion ratio; FI, feed intake; SGR, specific growth rate; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index; Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

3.2. Body Composition

Table 4 displays the body composition of the largemouth bass. The levels of protein, fat, moisture, and ash content were not significantly different across the groups ($p > 0.05$).

Table 4. Effects of dietary Glu on body composition of largemouth bass.

Item	CON	0.2% Glu	0.4% Glu	0.6% Glu	0.8% Glu
Crude protein	57.29 ± 0.96	58.96 ± 1.35	57.60 ± 0.55	58.34 ± 2.38	58.55 ± 1.51
Crude lipid	21.29 ± 2.57	21.22 ± 1.90	21.95 ± 0.77	22.83 ± 2.46	21.88 ± 1.06
Moisture	69.91 ± 1.09	69.7 ± 0.15	69.47 ± 0.83	70.09 ± 1.17	68.97 ± 1.18
Crude ash	14.75 ± 0.96	13.63 ± 0.54	13.52 ± 0.17	13.21 ± 0.33	13.75 ± 0.56

3.3. Muscle Amino Acid Composition and Inosine Monophosphate Content

Table 5 displays the inosine monophosphate concentration and amino acid composition of largemouth bass muscle. There was a significant difference between the crude protein levels, total amino acid content, and inosine monophosphate amounts of the 0.2%, 0.4%, 0.6%, and 0.8% Glu groups and the control group ($p < 0.05$). The crude protein level of the 0.6% Glu group was the highest, while the total amino acid and inosine monophosphate contents were the highest at 0.4% Glu. The levels of glutamic acid, aspartic acid, and tyrosine in the 0.4% Glu and 0.6% Glu groups were considerably greater than those in the control group ($p < 0.05$). After adding glutamic acid, the contents of phenylalanine, alanine, and glycine were also increased. The levels of serine, threonine, valine, methionine, isoleucine, leucine, and lysine in Glu solutions with concentrations of 0.2%, 0.4%, 0.6%, and 0.8% were considerably greater than those in the control group ($p < 0.05$).

Table 5. Effects of dietary Glu on muscle amino acid composition of largemouth bass.

Item	CON	0.2% Glu	0.4% Glu	0.6% Glu	0.8% Glu
Crude protein	84.91 ± 0.73 ^{a}	86.89 ± 0.03 ^{b}	87.02 ± 0.04 ^{bc}	87.56 ± 0.03 ^{c}	86.76 ± 0.05 ^{b}
Inosine monophosphate	2.12 ± 0.06 ^{a}	2.80 ± 0.07 ^{bc}	3.10 ± 0.11 ^{bc}	2.69 ± 0.01 ^{bc}	2.58 ± 0.06 ^{abc}
Aspartic acid	9.13 ± 0.11 ^{a}	9.41 ± 0.13 ^{bc}	9.47 ± 0.06 ^{c}	9.40 ± 0.07 ^{bc}	9.38 ± 0.06 ^{bc}
Glutamic acid	14.43 ± 0.26 ^{a}	14.75 ± 0.12 ^{ab}	14.89 ± 0.03 ^{b}	14.92 ± 0.31 ^{b}	14.87 ± 0.09 ^{b}
Phenylalanine	3.83 ± 0.12	3.91 ± 0.07	3.96 ± 0.04	3.91 ± 0.58	3.91 ± 0.02
Alanine	6.04 ± 0.10	6.07 ± 0.18	6.15 ± 0.13	6.21 ± 0.09	6.20 ± 0.02
Glycine	4.15 ± 0.05	4.23 ± 0.15	4.29 ± 0.08	4.24 ± 0.13	4.31 ± 0.02
Tyrosine	4.15 ± 0.05 ^{a}	4.23 ± 0.15 ^{c}	4.29 ± 0.08 ^{c}	4.24 ± 0.13 ^{c}	4.31 ± 0.02 ^{bc}
Serine	3.39 ± 0.12 ^{a}	3.49 ± 0.05 ^{b}	3.51 ± 0.02 ^{b}	3.49 ± 0.04 ^{b}	3.50 ± 0.00 ^{b}
Glycine	4.15 ± 0.05 ^{a}	4.23 ± 0.15 ^{b}	4.29 ± 0.08 ^{b}	4.24 ± 0.13 ^{b}	4.31 ± 0.02 ^{b}
Threonine	3.89 ± 0.02 ^{a}	4.02 ± 0.04 ^{b}	4.05 ± 0.04 ^{b}	4.02 ± 0.05 ^{b}	4.03 ± 0.01 ^{b}
Histidine	2.14 ± 0.08	2.21 ± 0.04	2.18 ± 0.06	2.20 ± 0.06	2.18 ± 0.01
Arginine	5.56 ± 0.45	5.70 ± 0.09	5.73 ± 0.07	5.73 ± 0.02	5.73 ± 0.07
Valine	4.10 ± 0.08 ^{a}	4.23 ± 0.06 ^{b}	4.25 ± 0.05 ^{b}	4.23 ± 0.05 ^{b}	4.17 ± 0.02 ^{ab}
Methionine	2.69 ± 0.03 ^{a}	2.79 ± 0.03 ^{b}	2.81 ± 0.03 ^{b}	2.79 ± 0.01 ^{b}	2.78 ± 0.11 ^{b}
Isoleucine	4.27 ± 0.05 ^{a}	4.43 ± 0.06 ^{b}	4.45 ± 0.04 ^{b}	4.41 ± 0.05 ^{b}	4.37 ± 0.01 ^{b}
Leucine	7.07 ± 0.14 ^{a}	7.35 ± 0.08 ^{b}	7.38 ± 0.12 ^{b}	7.35 ± 0.08 ^{b}	7.35 ± 0.10 ^{b}
Lysine	8.18 ± 0.08 ^{a}	8.48 ± 0.08 ^{b}	8.58 ± 0.11 ^{b}	8.53 ± 0.22 ^{b}	8.66 ± 0.02 ^{b}
Total amino acids	81.80 ± 0.71 ^{a}	84.12 ± 0.64 ^{b}	84.78 ± 0.21 ^{b}	84.45 ± 0.33 ^{b}	84.46 ± 0.02 ^{b}
IMP (mg/kg)	2.12 ± 0.06 ^{a}	2.80 ± 0.07 ^{bc}	3.10 ± 0.11 ^{bc}	2.69 ± 0.01 ^{bc}	2.58 ± 0.06 ^{abc}

Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

3.4. Proximate Composition and Histological Traits of Muscle Fibers

Muscle sections are shown in Figure 1. In comparison to the control group, the muscle fiber size of the 0.4% and 0.6% Glu groups was substantially larger ($p < 0.05$). In comparison to the control group, the muscle fiber density and number of the 0.6% and 0.7% Glu groups were much lower ($p < 0.05$).

3.5. Muscle Growth and Development-Related Gene Expression

pi3k, *akt*, and *tor* mRNA expression levels in the 0.4% Glu, 0.6% Glu, and 0.8% Glu groups were considerably greater than those in the control group (Figures 2–4). In comparison to the control group, the mRNA expression levels of *s6k1*, *myod*, *myog*, and *myf5* in the 0.4% and 0.6% Glu groups were considerably higher ($p < 0.05$). 4e-bp1, foxo3a, murf-1, and atrogin-1 had significantly reduced mRNA expression levels in the 0.6% Glu group compared to the control group ($p < 0.05$).

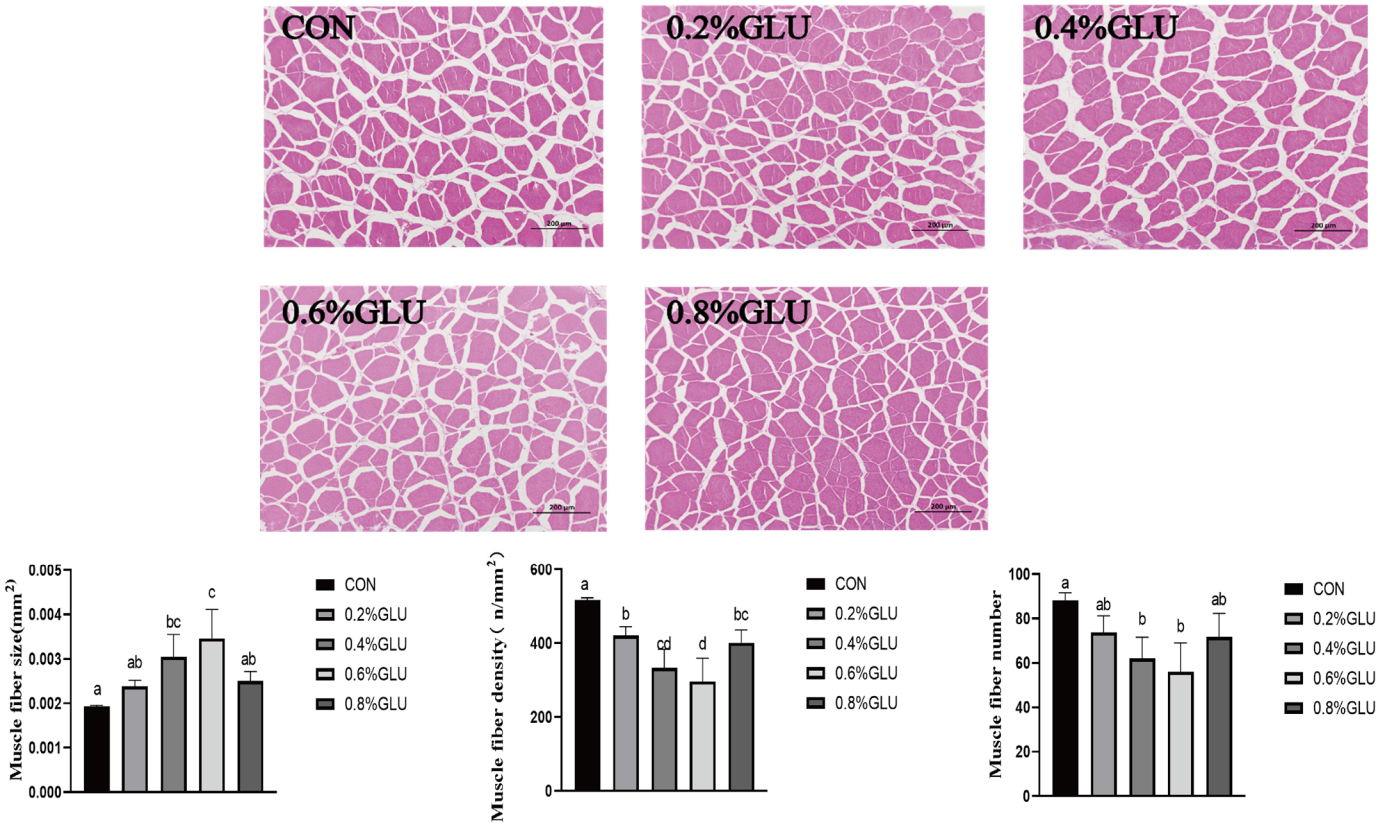

Figure 1. Effects of dietary Glu on muscle histology of largemouth bass. Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

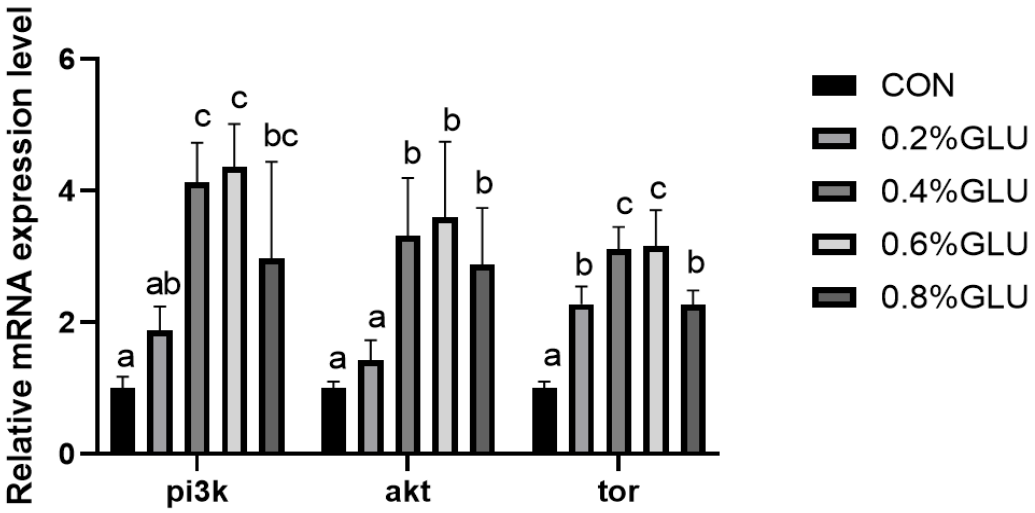

Figure 2. Effects of dietary Glu supplementation on the relative mRNA expression levels of phosphatidyl inositol 3 kinase (*pi3k*), protein kinase B (*akt*), and target of rapamycin (*tor*) in the muscle of largemouth bass. Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

3.6. Intestinal Enzyme Activity

Protease, lipase, and amylase activity in the 0.2% and 0.4% Glu groups were significantly higher than those in the control group ($p < 0.05$). The highest concentrations of lipase, protease, and amylase were found in the 0.6%, 0.4%, and 0.6% Glu groups, respectively (Table 6).

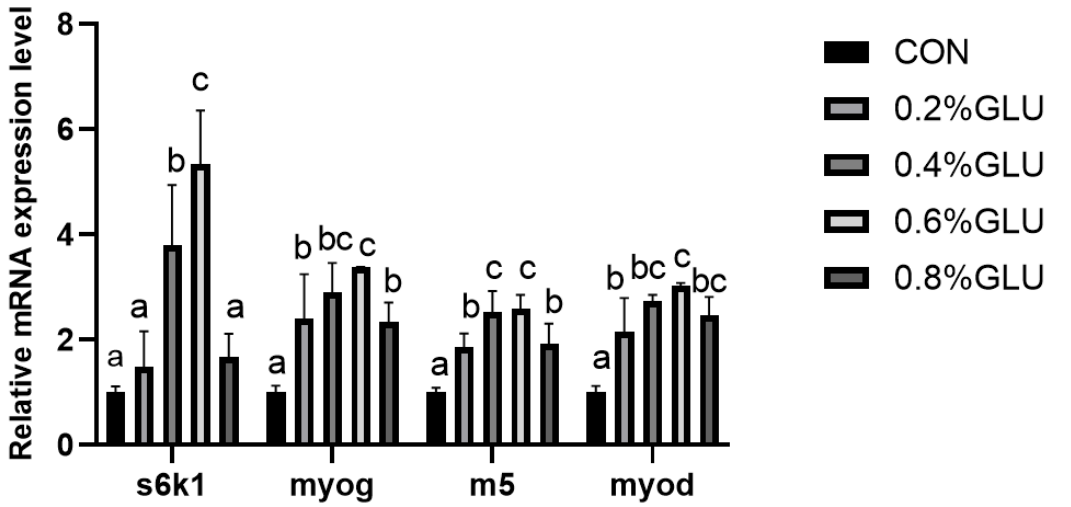

Figure 3. Relative mRNA expression level of 70 kDa ribosomal protein S6 kinase 1 (*S6K1*), myoblast determination protein (*myod*), myogenin (*myog*), myogenic factor 5 (*myf5*) in the muscle of largemouth bass fed Glu diets. Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

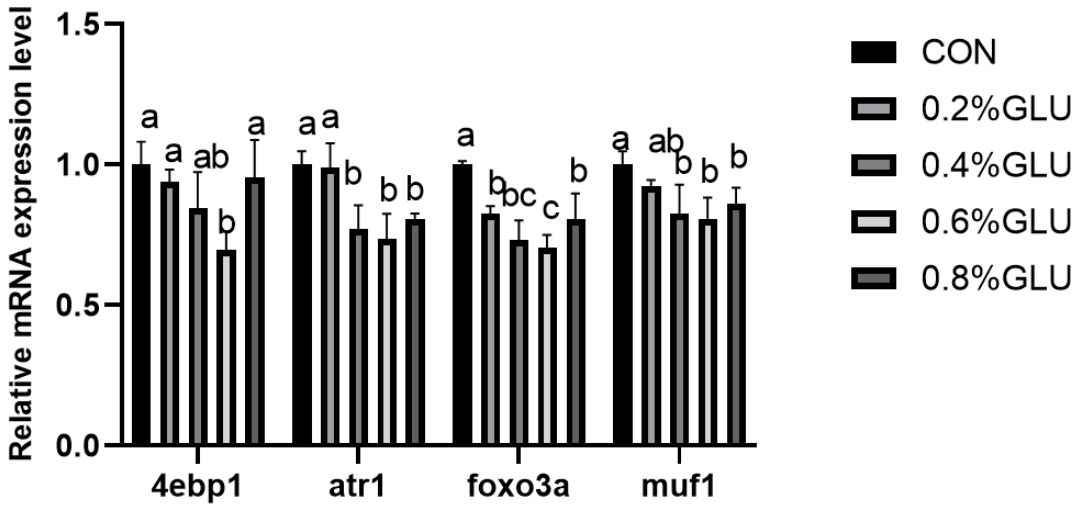

Figure 4. Relative mRNA expression level of eukaryotic translation initiation factor 4E-binding protein 1 (*4e-bp1*), forkhead boxO3a (*foxo3a*), muscle-specific RING finger protein 1 (*murfF-1*), and atrogin-1 in the muscle of largemouth bass fed Glu diets. Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

Table 6. Effects of dietary glutamate on intestinal villus height, villus width, and muscle thickness of largemouth bass.

Item	CON	0.2%Glu	0.4%Glu	0.6%Glu	0.8%Glu
Amylase	369.57 ± 12.46 ^{b}	440.88 ± 6.81 ^{d}	410.48 ± 15.85 ^{cd}	378.68 ± 0.85 ^{bc}	305.37 ± 42.09 ^{a}
Lipase	372.90 ± 37.93 ^{a}	444.75 ± 10.25 ^{b}	495.73 ± 15.04 ^{c}	499.83 ± 26.72 ^{c}	464.88 ± 5.11 ^{bc}
Protease	731.68 ± 25.56 ^{a}	1041.18 ± 57.12 ^{b}	1398.53 ± 12.05 ^{c}	1184.85 ± 156.94 ^{b}	841.77 ± 60.19 ^{a}

Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

3.7. Intestinal Tissue Structure

Compared to the control group, the villus height of the 0.4% Glu group was substantially higher ($p < 0.05$). The width of the 0.6% Glu group was substantially greater than that

of the control group ($p < 0.05$). The thickness of the intestinal walls did not significantly differ across the groups (Table 7 and Figure 5).

Table 7. Effects of dietary glutamate on intestinal villus height, villus width, and muscle layer thickness of largemouth bass after 8 weeks of feeding.

Item	CON	0.2% Glu	0.4% Glu	0.6% Glu	0.8% Glu
Villus height (μm)	225.38 ± 31.76 ^{a}	261.52 ± 32.16 ^{ab}	308.3 ± 29.11 ^{b}	257.29 ± 43.62 ^{ab}	266.28 ± 16.09 ^{ab}
Villus width (μm)	44.64 ± 6.17 ^{ab}	45.27 ± 3.60 ^{ab}	41.26 ± 7.40 ^{a}	51.43 ± 3.61 ^{b}	46.82 ± 1.63 ^{ab}
Intestinal wall thickness (μm)	64.38 ± 4.80	71.93 ± 2.66	76.66 ± 14.19	65.05 ± 10.26	64.14 ± 6.37

Means in the same row with different superscripts are significantly different (mean ± SD; ANOVA, $p < 0.05$; $n = 3$).

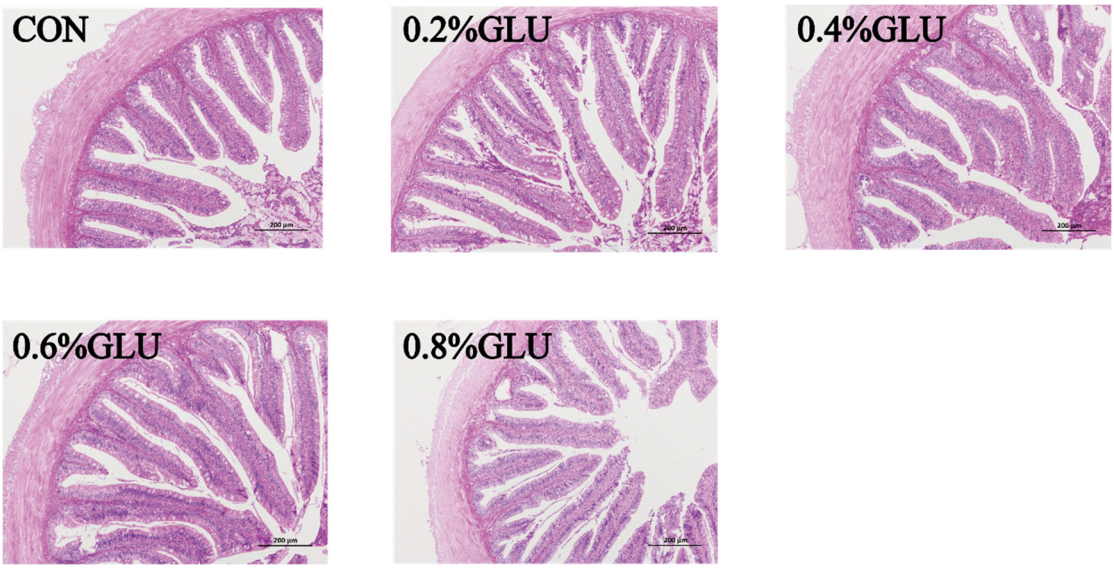

Figure 5. Effects of dietary Glu supplementation on intestinal morphology of largemouth bass.

4. Discussion

This research shows that adding glutamic acid to largemouth bass can improve the growth performance of largemouth bass. Additionally, it boosted their feed intake and condition factor. This was similar to the results of Jian carp (*Cyprinus carpio* var. Jian) with dietary glutamate levels of 68.4 and 83.4 g/kg [24]. Administration of 0.25% of glutamic acid to *Ctenopharyngodon idellus* also had the same effect [14]. In recent years, more and more studies have shown that non-essential amino acids such as glutamic acid, glycine [25], and proline [26] can also promote the growth of fish, thereby enhancing the growth performance of fish. The potential cause for the enhancement in growth performance in largemouth bass due to glutamic acid supplementation may be attributed to the observed increase in meal intake. This glutamic acid experiment was carried out in summer and achieved results such as improving fish growth performance.

In this study, the addition of glutamic acid to the feed can increase the crude protein content of largemouth bass. In a prior study, it was found that adding 4% glutamic acid boosted the protein content of *Sparus aurata* [27]. Similarly, adding 3.2% glutamic acid to the diet of triploid crucian carp resulted in the same finding. The protein level in aquatic animals is strongly linked to protein turnover, which is a key element influencing muscle growth and the quality of meat [28]. The important gene-level pathway is the pi3k/akt signaling pathway [29]. mTOR phosphorylation can be enhanced by the control of protein phosphorylation in the pi3k/akt pathway [30]. In addition, s6k1 and 4e-bp1 are downstream targets of tor signal transduction [31]. Two downstream effectors can

be directly phosphorylated by tor, which stimulates the start and translation of protein transcription and controls cell activity to control protein synthesis. Conversely, foxo3a functions as a downstream effector of the pi3k signaling pathway and is essential for the degradation of proteins [32]. Dephosphorylation of foxo3a is induced by inhibition of akt phosphorylation, and dephosphorylated foxo3a enters the nucleus upon activation by stimulating the production of muscle ring-finger gene 1 (murf1) and muscle atrophy Fbox (mafbx/atrogin-1), causing the myofibrillar protein to degrade [33]. The mRNA expression of s6k1, tor, akt, pi3k, myod, myog, and myf5 was elevated in this study by dietary glutamate. Atrogin-1, murf-1, foxo3a, and 4e-bp1 all had downregulated mRNA expression levels. The findings demonstrated that by stimulating the pi3k/akt pathway, Glu might increase the synthesis of muscle protein and decrease its decomposition.

Precursors of flavor compounds include glutamic acid, aspartic acid, leucine, and valine. These compounds are strongly linked to the development of muscle flavor [34]. The sweet amino acids alanine and glycine may help to increase the sweetness of fish during chewing [35]. In this study, the inclusion of glutamic acid in the meal raised the amount of umami amino acids, including aspartic acid, leucine, valine, alanine, and glycine, as well as the overall amino acid content of the largemouth bass muscle. This is consistent with the result of adding 3.2% Glu to crucian carp. Inosine monophosphate is an important flavor substance in fish muscle [36]. The inclusion of glutamic acid in the food resulted in an elevation of inosine monophosphate levels in the muscle tissue of largemouth bass observed in this study.

Muscle growth is related to the number and area of muscle fibers [37]. Diet plays an important role in the balance between muscle fiber hyperplasia and hypertrophy [38]. The study found that incorporating glutamic acid into the diet increased the average size of muscle fibers in largemouth bass and decreased muscle fiber density. This suggests that glutamic acid effectively stimulates muscle growth in largemouth bass. In short, the inclusion of glutamic acid in the meal resulted in an augmentation of the protein composition in the muscle of largemouth bass. This led to the stimulation of muscle growth and development, as well as an increase in the concentration of umami amino acids.

The gastrointestinal system is the primary site for the breakdown and assimilation of nutrients in fish [39]. The morphological structure of the digestive tract is an important basis for maintaining the growth status of largemouth bass [40]. The intestine is the main digestive organ of fish [41]. The integrity of the intestinal structure has a direct impact on the process of digesting and absorbing nutrients [42], as well as on the growth and development of fish [43]. Muscle layer thickness (MT) [44], villus height (VH), and villus width (VW) [45] are usually used to evaluate the intestinal digestion ability of animals [46]. The increase in villus height [47] and muscle layer thickness [48] can increase the surface area of the fish intestine and improve its ability to digest and absorb feed nutrients [49]. The study found that adding glutamic acid to the diet of largemouth bass elevated the thickness of the intestinal muscle, as well as the height and width of the villi. The activity of digestive enzymes is also crucial to the digestive capacity of largemouth bass [50]. After entering the body of largemouth bass, macromolecular nutrients such as protein and fatty acids need to be decomposed into small molecules by digestive enzymes in the digestive tract before they can be absorbed by the digestive tract [51]. As the first step to obtain nutrients, digestive enzyme activity is closely related to nutrient utilization efficiency [52]. The presence of lipase [53], protease [54], and amylase [55] in the fish gut can impact the process of absorbing and utilizing nutrients [56]. As a result, this has an impact on the proliferation and development of the fish [57]. These findings demonstrated that the inclusion of glutamic acid in the meal enhanced the enzymatic activity of amylase, protease, and lipase in the intestine of largemouth bass. The aforementioned findings suggest that including Glu in the meal enhances the digestive capacity and fosters intestinal well-being in largemouth bass. This is comparable to the effects of glutamic acid on Jian carp (Cyprinus carpio var. Jian) [24].

5. Conclusions

In summary, our findings showed that largemouth bass can benefit from Glu in terms of growth performance, muscular development, and intestinal digestion. Taking growth performance as the index, the optimum addition amount of Glu was 0.4%, and taking muscle development as the index, the optimum addition amount of Glu was 0.6%.

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Conflicts of Interest: Author Wenqing Huang was employed by the company Guangzhou Fishteach Biotechnology. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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