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Research Article

Curcumin Supplementation Enhances the Feeding and Growth of Largemouth Bass (*Micropterus salmoides*) Fed the Diet Containing 80 g/kg Fish Meal

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Two feeding trials were conducted to evaluate the influence of dietary curcumin level on fish meal replacement with poultry by-product (PBM) in a largemouth bass (*Micropterus salmoides*) diet. In trial I, the reference diet (R) contained 400 g/kg fish meal, and PBM replaced 60 and 80% of the fish meal (P16 and P8). In trial II, the diet containing 80 g/kg fish meal (P8) served as control (C), and curcumin was added at 5000 and 10000 mg/kg (C5 and C10), respectively. Replacement of fish meal with PBM significantly influenced feed intake, phosphorus retention efficiency (PRE), body lipid content, the ratio of fish meal consumption to fish production (RCP), and wastes of carbon and phosphorus but did not result in significant alternation in weight gain, feed conversion ratio (FCR), retention efficiencies of nitrogen (NRE), carbon (CRE) and energy (ERE), viscerosomatic index (VSI), hepatosomatic index (HSI), and body composition. The RCP, body lipid content, and phosphorus waste were higher in fish fed diet R than in fish fed diets P16 and P8. Curcumin supplementation influenced weight gain, feed intake, FCR, carbon waste, and RCP, but did not result in significant alternation in CRE, NRE, PRE, ERE, condition factor, VSI, HSI, body composition, and nitrogen and phosphorus wastes. The weight gain and feed intake were higher in fish fed diet C10 than in fish fed diet C, while the carbon waste was higher in fish fed diets C5 and C10 than in fish fed diet C. Overall, the minimum dietary fish meal level for largemouth bass could be dropped to 80 g/kg when PBM was used as an alternative ingredient, and 10000 mg/kg curcumin could obviously improve growth of fish fed diet containing 80 g/kg fish meal.

1. Introduction

The contribution of aquaculture to food security and human health has been continuously growing with expansion of the aquaculture industry worldwide [1, 2]. Dietary protein sources and environmental impact are the main challenges threatening sustainability of aquaculture industry since the proportion of fed aquaculture in global aquaculture production increased, and more fed aquaculture production resulted in more dietary protein consumption and waste outputs [3]. Among the protein ingredients used for formulating aquafeed, fish meal is recognized as ideal because of its advantages in digestible protein content, amino acid balance, and growth-promoting factors and is widely used at high levels in carnivorous fish feed. However, fish meal is a limited ocean resource, and replacement of fish meal with economic terrestrial protein ingredients, including animal or plant ingredients, is necessary for sustainable development of aquaculture industry. Based on intensive studies over decades, fish meal content in aquafeed significantly declined, e.g., the average dietary fish meal level for marine finfishes was reduced from 43% in 1995 to 14% in 2017 [4]. Several approaches, such as using previously blended protein ingredients (such as blends of PBM, meat and bone meal,

feather meal, and blood meal) or gamma-ray irradiated protein ingredients (such as irradiated soybean meal and feather meal) as fish meal alternate, supplementation of functional amino acids (such as taurine and glycine) and minerals (selenium) and elevating dietary protein levels, have been demonstrated as efficient in increasing fish meal amount replaced from fish diets [5–12]. De Cruz et al. [13] reported that nucleotide supplementation could enhance growth of hybrid striped bass *Morone chrysops* × *M. saxatilis* fed with low fish meal diets. Therefore, identifying the constituents with the function to improve feeding and growth of fish fed low fish meal diets should be a focus in future studies of fish nutrition and feed technology.

Curcumin (CU) is a polyphenolic and hydrophobic phytochemical component of the turmeric herb *Curcuma longa* and has been recognized as an immunomodulator of human and domestic animals [14]. Previous studies reported that CU supplementation could improve growth of crucian carp *Carassius auratus*, Nile tilapia *Oreochromis niloticus,* and large yellow croaker *Larimichthys crocea* [15–17], and positively affect hematological parameters, immunity, and disease resistance of Nile tilapia, carp *Cyprinus carpio,* and rainbow trout *Oncorhynchus mykiss* [18–20]. However, it is uncertain if CU could be used as a functional constituent in low fish meal diets for carnivorous fishes.

Largemouth bass *Micropterus salmoides* is a carnivorous fish species with commercial importance to freshwater aquaculture. In 2020, largemouth bass aquaculture production achieved 621,300 tons, which ranked 15th in inland finfish aquaculture production and 2nd in inland carnivorous fish aquaculture production in the world [3]. Fish meal content in commercial largemouth bass feed was generally more than 400 g/kg, which could be reduced to 160 g/kg by soybean meal, soy protein concentrate, or cottonseed protein concentrate as an alternative ingredient [21–23] or 80 g/kg by using poultry by-product meal as an alternative ingredient [24]. To our knowledge, the formulated diet containing 160 g/kg fish meal has been successfully used in largemouth bass farming, and 80 g/kg is the minimum fish meal level that could satisfy fast growth of the fish. In the present study, we evaluated if CU could be used as a functional constituent to improve feeding, growth, and feed utilization efficiency of largemouth bass fed a diet containing 80 g/kg fish meal, to explore the potential to further reduce fish meal level in commercial fish feed.

2. Materials and Methods

2.1. Curcumin, Feed Ingredients, and Test Diets. Curcumin (purity >98%) was purchased from Dulai Biotechnology Co., Ltd. (Shanghai, China). Feed ingredients, including animal and plant protein ingredients, were purchased from Hongli Feed Company (Huzhou, China). The proximate composition of the feed ingredients is shown in Table 1.

Two single factor trials (I and II) were designed. In trial I, poultry by-product meal (PBM) replaced 60% (P16) and 80% (P8) of the fish meal in a reference diet (R) that was formulated to contain 400 g/kg fish meal. In trial II, the diet containing 80 g/kg fish meal (P8) served as control (C), and curcumin (CU) was added at 5000 (C5) and 10000 (C10) mg/kg in diet C, respectively. Protein and lipid levels of the test diets were 520 and 120 g/kg, respectively [25]. Formulation and proximate compositions of the test diets are shown in Table 2. Amino acid profile of diets R and P8 are represented as (g/kg) Asp, 43.2 and 39.9; Thr, 20.4 and 19.0; Ser, 22.7 and 21.3; Glu, 76.2 and 72.8; Pro, 22.4 and 25.5; Gly, 26.8 and 29.2; Ala, 28.4 and 27.8; Val, 24.0 and 22.2; Met, 10.7 and 9.1; Ile, 18.4 and 17.0; Leu, 38.2 and 36.0; Tyr, 13.9 and 12.8; Phe, 25.1 and 23.8; His, 21.9 and 21.0; Lys, 37.1 and 33.7; and Arg, 35.9 and 36.4. The amino acid composition of diets C, C5, and C10 was not analysed, but was theoretically the same as that of diet P8.

The feed ingredients were ground with a hammer mill into particles that can pass through a mesh (pore size 500 μm). To prepare the test diets, the feed ingredients of each diet were weighed and mixed as described in Wang et al. [24], and pellets were extruded with an SLP-450 single screw machine (Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China). The extruding temperature was controlled at 80–100°C. After drying under 25°C in an air-conditioned room, the test diets were collected and preserved in plastic bags in a refrigerator (−20°C).

2.2. Fish and Feeding Trials. Trials I and II were conducted in the field station of the Zhejiang Institute of Freshwater Fisheries (Huzhou, China). Largemouth bass fingerlings were purchased from a commercial fish farm located in Deqing (Huzhou, China). The fish were transported to the field station and acclimated in two indoor recirculating aquaculture systems (RAS). Each RAS comprises 24 experimental tanks (water volume, 350 L). Prior to the start of the feeding trials, 720 fish with similar body sizes were selected and reared in a RAS at 30 fish/tank for one week. During the acclimation period, the fish were fed with diet R twice daily.

Trials I and II were initiated and ceased simultaneously. At the start of the trials, the acclimatized fish were pooled in four tanks after 24 h of feed deprivation. Twenty fish were captured from the pooled tanks, weighed in bulk, and randomly released into one of 15 experimental tanks. Each diet treatment had three replicators, and the fish were assigned to diet R, P16, and P8 in trial I and C, C5, and C10 in trial II (diets P8 and C were the same diet but was assigned as different diets in different trial). Initial body weight was 37.8 ± 0.2 g (mean ± SD, $n = 15$). Thereafter, three groups of 15 fish each were collected from the pooled tanks. After the measurement of body weight, body length, liver weight, and viscera weight, the sampled fish were preserved in a refrigerator (−20°C) for analysis of body composition.

The duration of trials I and II was eight weeks. Fish were fed to satiation at 8 : 00 and 16 : 00 every day. To ensure fish were fed adequately and efficiently, several pellets of a test diet were dropped in each tank, and feeding behavior of fish in the tank was observed. Fish in the tanks were fed alternately until no fish swam to the water surface to accept the dropped diet. Dead fish were recorded and weighed. Water

Table 1: Proximate composition (g/kg) of the feed ingredients.

Ingredient	Dry matter	Crude protein[1]	Crude lipid[1]	Ash[1]
Fish meal	894	646	97	149
Poultry by-product meal	931	652	93	184
Protein premix[2]	908	629	7	69
Blood cell meal	913	923	2	39
Soybean meal	903	491	13	61
Cottonseed-protein meal	909	611	6	78
Wheat flour	859	177	21	11

[1]crude protein, crude lipid, and ash are expressed as the situation in natural storage ($n = 2$). [2]protein premix is a commercial product made by the Hongli Feed Company of Deqing county (Huzhou, China). The protein premix comprised blood cell meal, soybean meal, and cottonseed protein meal.

Table 2: Formulation (g/kg), proximate composition (g/kg), and energy content (MJ/kg) of the test diets.

Ingredient	R[1]	P16[1]	P8[1]	C[1]	C5[1]	C10[1]
Fish meal	400	160	80	80	80	80
Poultry by-product meal		238	317	317	317	317
Protein premix[2]	150	150	150	150	150	150
Blood cell meal	23	23	23	23	29	33
Soybean meal	131	131	132	132	121	112
Cottonseed protein meal	50	50	50	50	50	50
Wheat flour	125	125	125	125	125	125
Choline chloride	2	2	2	2	2	2
Ca $(H_2PO_4)_2$	20	20	20	20	20	20
DL-met	2	2	2	2	2	2
L-lysine	5	5	5	5	5	5
Vitamin and mineral premix[3]	15	15	15	15	15	15
Fish oil	77	78	79	79	79	79
Curcumin					5	10
Dry matter	922	943	944	944	938	934
Crude protein[4]	516	519	518	518	518	512
Crude lipid[4]	122	121	121	121	121	123
Ash[4]	108	115	113	113	113	113
Gross energy[4]	21.1	20.2	21.1	21.1	20.0	19.9
Carbon[4]	452	475	447	447	467	484
Phosphorus[4]	21	19	20	20	19	19

[1]R: reference diet; P16: poultry by-product meal (PBM) replaced 60% of the fish meal in diet R; P8 and C (the same diet): PBM replaced 80% of the fish meal in diet R; C5 and C10: PBM replaced 80% of the fish meal in diet R with 5000 and 10000 mg/kg curcumin supplementation, respectively. [2]protein premix is a commercial product made by the Hongli feed company of Deqing county (Huzhou, China), and comprised of blood meal, soybean meal, and cottonseed protein concentrate. [3]vitamin and mineral premix (per kg diet): vitamin A, 8000 IU; vitamin D_3, 2000 IU; vitamin E, 100 mg; vitamin K_3, 7.5 mg; vitamin B_1, 15 mg; vitamin B_2, 15 mg; vitamin B_6, 12.5 mg; vitamin B_{12}, 0.05 mg; D-biotin, 0.25 mg; D-calcium pantothenate, 40 mg; folic acid, 5 mg; niacinamide, 50 mg; vitamin C, 140 mg; inositol, 120 mg; ethoxyquin, 5 mg; $FeSO_4$, 40 mg; $CuSO_4 \cdot 5H_2O$, 25 mg; $MnSO_4 \cdot 4H_2O$, 10 mg; $ZnSO_4$, 100 mg; $MgSO_4 \cdot 7H_2O$, 200 mg; $CoCO_3$, 0.35 mg; KI, 0.05 mg; and Na_2SeO_3, 0.3 mg. [4]contents of crude protein, crude lipid, ash, gross energy, carbon, and phosphorus reflect the situation of the feed ingredients in natural storage.

in the RAS was continuously aerated, recirculated, and treated with a biofilter, and recirculating rate of water in each tank was 3.0 L/min. Everyday 20% of water in the RAS was renewed with aerated tap water. Water temperature was monitored in the morning and afternoon daily and fluctuated from 25.8 to 30.8°C. Dissolved oxygen was measured weekly and was always higher than 5.0 mg/L.

At the end of trials I and II, fish were deprived of diet for 24 h. Three fish were captured from each tank, anaesthetized with clove oil (100 mg/L), and individually weighed. The blood sample was collected with a 1 ml syringe rinsed with heparin sodium solution (1%, v/v) from the caudal vein of each fish and transferred into a 2 ml Eppendorf tube. After centrifugation (15 min at 3000 rpm), the supernatant (plasma) was isolated and transferred into a 1.5 ml cryogenic vial and preserved in liquid nitrogen. The liver was collected from each fish following plasma sample collection and was preserved in liquid nitrogen. Thereafter, fish were captured from each tank and weighed in bulk. Three fish were sampled from each tank, and the body weight, body length, viscera weight, and liver weight of the fish were measured. The samples of plasma and liver were preserved at −80°C, and the samples of whole fish were preserved at −20°C, until physiological and chemical analyses.

2.3. Physiological and Chemical Assay. Prior to physiological analysis, the samples of plasma and liver were thawed at 4°C. The activity of antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and catalase (CAT)] and malondialdehyde (MDA) content in plasma were assayed with the kits purchased from the Nanjing Jiancheng

Bioengineering Institute (Nanjing, China). The liver was homogenized with 0.86% physiological saline (1 : 4, w/v) in an ice bath. After centrifugation (5 min at 12000 rpm and 4°C), supernatant of the liver sample was isolated, and concentration of the soluble protein was determined with the method described in Bradford [26], using bovine serum albumin as standard. Activities of SOD, GSH-PX, and CAT and MDA content in the liver were assayed with the kits used for plasma assay.

Prior to chemical analysis, the sampled fish were thawed, weighed, autoclaved (20 min at 120°C), homogenized, and dried (6 h at 120°C). Contents of moisture, crude protein, crude lipid, ash and phosphorus of the feed ingredients, test diets, and fish were analyzed with the methods described in AOAC [27]. Contents of crude protein and amino acids were analyzed with an 8400 auto-Kjeldahl-nitrogen analyzer (Foss, Sweden) and 433 amino acid analyzer (Sykam Company with Limited Liability, Germany), respectively. Gross energy content was analyzed with Parr 6200 calorimeter (Parr, USA). Carbon content was analyzed with an EA3000 element analyzer (Euro Vector, Italy).

2.4. Calculation and Statistics. Calculation of feed intake, growth, feed conversion ratio (FCR), nutrient and energy retention efficiencies (carbon: CRE; nitrogen: NRE; phosphorus: PRE; energy: ERE), morphological indexes [condition factor, hepatosomatic index (HSI), and viscerosomatic index (VSI)], waste outputs (carbon, nitrogen, and phosphorus), and fish meal reliance [ratio of dietary consumption to fish production (RCP)] were performed with the equations described by Wang et al. [24]. The retention efficiency of amino acids (ARE) was calculated as follows:

$$ARE(\%) = \frac{100 \times \left[\left(W_t/N_t \times C_{At}\right) - \left(\mathrm{W_0/N_0 \times C_{A0}}\right)\right]}{\left[2 \times I/\left(N_t + N_0\right) \times C_{Af}\right]}, \quad (1)$$

where I (g) is the feed consumption of fish in each tank during the trials; W_0 (g) is the initial body weight of fish in each tank; and W_t (g) is the final body weight; N_t and N_0 are the number of fish alive in each tank at the end and start of the trials; C_{At} (%) is the content of amino acids in the fish body at the end of the trials and C_{A0} (%) at the start; C_{Af} (%) is the content of amino acids in the test diets.

The influences of fish meal replacement level (among fish fed diets R, P16, and P8) or CU supplementation (among fish fed diets C, C5, and C10) on survival, growth, feed intake, feed utilization efficiency, morphological indexes, and body composition were examined with one-way ANOVA. The differences in the above variables between the diet treatments in trials I and II were further examined with Duncan's test. The differences in ARE, activity of antioxidant enzymes (SOD, GSH-PX, and CAT) in plasma or liver, and MDA content in plasma or liver between diets R and P8 (trial I) or between diets C and C10 (trial II) were examined with independent t-test. The difference between ARE and NRE for the same diet treatment in trials I and II was examined with paired t-tests. The statistical analysis, including one-way ANOVA, Duncan's test, independent t-test, and paired t-test, were performed with SPSS (version 24.0, SPSS, USA). The significant level was set at $P < 0.05$.

3. Results

3.1. Survival, Feed Intake, Growth, and Feed Utilization Efficiency. Survival was over 98% throughout the feeding trials. In trial I, feed intake and PRE were dependent ($P < 0.05$, Table 3), while final body weight, weight gain, FCR CRE, NRE and ERE were independent ($P < 0.05$), on fish meal replacement level. Fish fed diet R exhibited higher feed intake relative to fish fed diet P8 ($P < 0.05$), and exhibited lower PRE relative to fish fed diet P16 ($P < 0.05$). In trial II, final body weight, weight gain, and feed intake were dependent ($P < 0.05$, Table 3), while FCR, CRE, NRE, PRE, and ERE were independent ($P > 0.05$), on CU supplementation level. The weight gain and feed intake of fish fed diet C10 were higher than that of fish fed diet C ($P < 0.05$) but did not significantly differ from those of fish fed diets C5 ($P > 0.05$).

In trial I, amino acid retention efficiencies except proline were higher in fish fed P8 than in fish fed R ($P < 0.05$, Table 4). In trial II, no significant differences were found in retention efficiencies of Asp, Ser, Pro, Gly, Ala, His, and Lys between fish fed diets C and C10 ($P > 0.05$). In trials I and II, the retention efficiencies of Gly, Met, and Ala were higher, while the retention efficiencies of Arg, Glu, His, Ile, Leu, Lys, Phe, Pro, Ser, Tyr, and Val were lower than NRE in fish fed diets R, P8, C and C10 ($P < 0.05$).

3.2. Morphological Indexes and Body Composition. In trial I, body lipid content was dependent ($P < 0.05$, Table 5), while condition factors, HSI, VSI, and body contents of moisture, crude protein, ash, carbon, phosphorus, and gross energy were independent ($P > 0.05$) on fish meal replacement level. fish fed diets P16 and P8 exhibited lower body lipid content relative to fish fed diet R ($P < 0.05$). In trial II, dietary CU level did not result in significant alternation in condition factors, HSI, VSI, and body composition ($P > 0.05$).

3.3. Fish Meal Reliance and Waste Outputs. In trial I, RCP, carbon waste, and phosphorus waste were dependent ($P < 0.05$, Table 6), while nitrogen waste was independent ($P > 0.05$), on fish meal replacement level. fish fed diet P8 exhibited lower RCP, carbon waste, and phosphorus waste relative to fish fed diet R ($P < 0.05$). In trial II, CU supplementation level influenced RCP and carbon waste ($P < 0.05$) but did not influence wastes of nitrogen and phosphorus ($P > 0.05$). fish fed diet C exhibited lower RCP relative to fish fed diet C5 ($P < 0.05$) and lower carbon waste relative to fish fed diets C5 and C10 ($P < 0.05$).

3.4. Activity of Antioxidant Enzymes and MDA Content. No significant difference was found in SOD activity in plasma either between fish fed diets R and P8 ($P > 0.05$, trial I) or between fish fed diets C and C10 ($P > 0.05$, trial

Table 3: Survival, growth, feed intake, and feed utilization of largemouth bass.

Diet	FBW[1] (g/fish)	Weight gain (g/fish)	Feed intake (%/d)	FCR[1]	CRE[1] (%)	NRE[1] (%)	PRE[1] (%)	ERE[1] (%)	Survival (%)
R[2]	104.7 ± 1.2	66.7 ± 1.4	1.75 ± 0.01^{A}	1.04 ± 0.01	29.92 ± 2.27	31.32 ± 0.27	37.57 ± 2.01^{B}	30.26 ± 0.15	98
P16[2]	103.1 ± 8.3	65.3 ± 8.2	1.67 ± 0.09^{AB}	1.01 ± 0.07	29.96 ± 2.13	32.20 ± 2.28	42.89 ± 0.82^{A}	32.94 ± 2.81	98
P8[2]	101.5 ± 3.4	63.7 ± 3.4	1.59 ± 0.07^{B}	0.98 ± 0.03	33.89 ± 3.3	34.03 ± 0.74	40.00 ± 3.46^{AB}	33.15 ± 0.92	100
C[2]	101.5 ± 3.4^{a}	63.7 ± 3.4^{a}	1.59 ± 0.07^{a}	0.98 ± 0.03	33.89 ± 3.3	34.03 ± 0.74	40.00 ± 3.46	33.15 ± 0.92	100
C5[2]	103.9 ± 2.0^{a}	66.1 ± 2.3^{ab}	1.74 ± 0.16^{ab}	1.03 ± 0.10	30.35 ± 3.55	32.41 ± 3.05	42.40 ± 4.78	33.25 ± 2.74	98
C10[2]	109.0 ± 1.8^{b}	71.1 ± 1.8^{b}	1.74 ± 0.07^{b}	1.00 ± 0.04	31.11 ± 0.31	33.49 ± 0.82	42.83 ± 3.20	34.29 ± 1.65	98

[1]FBW, final body weight; FCR, feed conversion ratio; CRE, NRE, PRE, and ERE are retention efficiencies of carbon, nitrogen, phosphorus, and energy, respectively. [2]R: reference diet; P16: poultry by-product meal (PBM) replaced 60% of the fish meal in diet R; P8 and C (the same treatment): PBM replaced 80% of the fish meal in diet R; C5 and C10: PBM replaced 80% of the fish meal in diet R with 5000 and 10000 mg/kg curcumin supplementation, respectively. Data are presented as mean ± SD ($n = 3$). The superscripts present the results of Duncan's test between R, P16 and P8 (capital letters) or between C, C5, and C10 (small letters). The data in the same column with different superscripts are significantly different ($P < 0.05$).

Table 4: Retention efficiency of amino acids (%) of largemouth bass.

Amino acid	R[1]	P8[1]	C[1]	C10[1]
Met	32.80 ± 0.45^{A}	45.87 ± 1.35^{B}	45.87 ± 1.35^{b}	42.56 ± 1.33^{a}
Gly	40.09 ± 0.55^{A}	42.52 ± 1.25^{B}	42.52 ± 1.25	40.56 ± 1.27
Ala	32.50 ± 0.45^{A}	38.27 ± 1.12^{B}	38.27 ± 1.12	35.84 ± 1.11
Thr	29.34 ± 0.40^{A}	36.55 ± 1.07^{B}	36.55 ± 1.07^{b}	33.46 ± 1.04^{a}
Asp	27.96 ± 0.39^{A}	34.60 ± 1.01^{B}	34.60 ± 1.01	32.39 ± 1.00
Pro	31.31 ± 0.44	31.19 ± 0.91	31.19 ± 0.91	31.79 ± 1.00
Ile	26.89 ± 0.41^{A}	33.61 ± 0.96^{B}	33.61 ± 0.96^{b}	30.92 ± 0.92^{a}
Ser	25.09 ± 0.34^{A}	30.91 ± 0.91^{B}	30.91 ± 0.91	29.07 ± 0.91
Tyr	23.81 ± 0.32^{A}	30.18 ± 0.89^{B}	30.18 ± 0.89^{b}	26.88 ± 0.83^{a}
Lys	24.73 ± 0.34^{A}	32.11 ± 0.94^{B}	32.11 ± 0.94	32.56 ± 1.04
Leu	24.22 ± 0.35^{A}	29.47 ± 0.85^{B}	29.47 ± 0.85^{b}	27.27 ± 0.83^{a}
Glu	23.59 ± 0.34^{A}	28.55 ± 0.83^{B}	28.55 ± 0.83^{b}	26.27 ± 0.80^{a}
Val	23.43 ± 0.36^{A}	29.03 ± 0.83^{B}	29.03 ± 0.83^{b}	27.05 ± 0.81^{a}
Arg	23.53 ± 0.32^{A}	27.04 ± 0.80^{B}	27.04 ± 0.80^{b}	24.73 ± 0.77^{a}
Phe	21.70 ± 0.31^{A}	26.52 ± 0.77^{B}	26.52 ± 0.77^{b}	24.39 ± 0.75^{a}
His	20.52 ± 0.27^{A}	24.51 ± 0.73^{B}	24.51 ± 0.73	24.34 ± 0.78

[1]R: reference diet; P8 and C (the same treatment): PBM replaced 80% of the fish meal in diet R; C10: PBM replaced 80% of the fish meal in diet R with 10000 mg/kg curcumin supplementation. Data are presented as mean ± SD ($n = 3$). The superscripts present the results of independent t-test between R and P8 (capital letters) or between C and C10 (small letters). The data in the same column with different superscripts are significantly different ($P < 0.05$).

Table 5: Morphological indexes and body composition of largemouth bass.

Diet	CF (g/cm^3)	HSI[1] (%)	VSI[1] (%)	Moisture (g/kg)	Crude protein[2] (g/kg)	Crude lipid[2] (g/kg)	Ash[2] (g/kg)	Carbon[2] (g/kg)	Phosphorus[2] (g/kg)	Gross energy[2] (MJ/kg)
Initial	2.10 ± 0.13	1.52 ± 0.38	6.60 ± 0.94	730 ± 8	167 ± 6	60 ± 3	41 ± 2	120 ± 3	7.2 ± 0.7	6.2 ± 0.1
R[3]	2.03 ± 0.08	1.74 ± 0.50	7.08 ± 0.76	713 ± 1	168 ± 1	82 ± 1^{A}	41 ± 0	134 ± 6	7.8 ± 0.2	6.5 ± 0.1
P16[3]	2.10 ± 0.10	1.69 ± 0.33	6.51 ± 0.70	718 ± 2	169 ± 1	72 ± 2^{B}	41 ± 1	135 ± 7	7.9 ± 0.2	6.5 ± 0.1
P8[3]	2.04 ± 0.10	1.84 ± 0.29	6.59 ± 0.72	713 ± 4	170 ± 3	73 ± 3^{B}	42 ± 2	137 ± 6	7.6 ± 0.4	6.6 ± 0.2
C[3]	2.04 ± 0.10	1.84 ± 0.29	6.59 ± 0.72	713 ± 4	170 ± 3	73 ± 3	42 ± 2	137 ± 6	7.6 ± 0.4	6.6 ± 0.2
C5[3]	2.09 ± 0.10	1.52 ± 0.15	6.15 ± 0.35	717 ± 1	171 ± 2	73 ± 4	41 ± 1	137 ± 5	8.0 ± 0.2	6.6 ± 0.1
C10[3]	2.13 ± 0.07	1.57 ± 0.30	6.57 ± 0.78	714 ± 3	170 ± 1	75 ± 5	41 ± 0	140 ± 3	7.9 ± 0.2	6.6 ± 0.3

[1]CF, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index. [2]crude protein, crude lipid, ash, carbon, phosphorus, and gross energy are expressed on a wet weight basis. [3]R: reference diet; P16: poultry by-product meal (PBM) replaced 60% of the fish meal in diet R; P8 and C (the same treatment): PBM replaced 80% of the fish meal in diet R; C5 and C10: PBM replaced 80% of the fish meal in diet R with 5000 and 10000 mg/kg curcumin supplementation, respectively. Data are presented as mean ± SD ($n = 3$). The superscripts present the results of Duncan's test between R, P16, and P8 (capital letters) or between C, C5, and C10 (small letters). The data in the same column with different superscripts are significantly different ($P < 0.05$).

II). In trial I, the activities of GSH-PX and CAT as well as MDA content in plasma were higher in fish fed diet R than in fish fed diet P8 ($P < 0.05$, Figure 1). In trial II, the activities of GSH-PX and CAT as well as MDA content in plasma were higher in fish fed diet C10 than in fish fed diet C ($P < 0.05$).

Table 6: Fish meal reliance and waste outputs of largemouth bass.

Diet	RCP[1]	Waste		
		Carbon	Nitrogen	Phosphorus
	g fish meal/(g fish gain)	g C/(kg fish gain)	g N/(kg fish gain)	g P/(kg fish gain)
R[2]	1.38 ± 0.07^{A}	337.58 ± 18.30^{A}	60.46 ± 2.79	13.94 ± 0.35^{A}
P16[2]	0.52 ± 0.04^{B}	344.75 ± 28.73^{A}	58.50 ± 6.80	11.38 ± 1.10^{B}
P8[2]	0.26 ± 0.00^{C}	288.46 ± 21.68^{B}	53.40 ± 1.62	11.68 ± 0.74^{B}
C[2]	0.26 ± 0.00^{a}	288.46 ± 21.68^{a}	53.40 ± 1.62	11.68 ± 0.74
C5[2]	0.29 ± 0.01^{b}	345.35 ± 35.04^{b}	59.39 ± 5.66	11.78 ± 1.58
C10[2]	0.28 ± 0.00^{ab}	340.61 ± 5.54^{b}	55.66 ± 1.26	11.21 ± 0.57

[1]RCP, ratio of fish meal consumption to fish production. [1]R: reference diet; P16: poultry by-product meal (PBM) replaced 60% of the fish meal in diet R; P8 and C (the same treatment): PBM replaced 80% of the fish meal in diet R; C5 and C10: PBM replaced 80% of the fish meal in diet R with 5000 and 10000 mg/kg curcumin supplementation, respectively. Data are presented as mean ± SD ($n = 3$). The superscripts present the results of Duncan's test between R, P16, and P8 (capital letters) or between C, C5, and C10 (small letters). The data in the same column with different superscripts are significantly different ($P < 0.05$).

4. Discussion

Indeed, dietary fish meal replacement level depends on fish species, alternative ingredients, and basal diet formulation [9, 24]. To our knowledge, the dietary fish meal level for largemouth bass varied from 240 to 80 g/kg [6, 8, 9, 24], and 80 g/kg is the minimum fish meal content for largemouth bass when PBM is used alone as a fish meal alternate [24]. In the present study, no significant difference was found in weight gain between fish fed diets R, P16, and P8. This result is consistent with the conclusion that dietary fish meal content in largemouth bass diet could be reduced to 80 g/kg. The weight gain of fish fed diet C was lower than that of fish fed diet C10 but did not significantly differ from that of fish fed diet C5. These results suggest that the influence of CU supplementation on growth of largemouth bass was positive and dose-dependent, and 10000 mg/kg CU supplementation could improve growth of fish fed the diet containing 80 g/kg fish meal. Therefore, CU could be a functional constituent to improve growth of largemouth bass fed low fish meal diets.

Several studies reported that CU supplementation could promote fish growth, however, the dietary CU level that can accelerate fish growth varied in these studies [16, 20]. For instance, the dietary CU levels that are sufficient to promote growth of Nile tilapia were 50 mg/kg [16] and 200 mg/kg [28]. The dietary CU levels that could improve growth of rainbow trout were 200 mg/kg [29], 400 mg/kg [30], and 10000 mg/kg [20]. Weight gain of tilapia *Oreochromis mossambicus* increased with increasing dietary CU level from 0 to 5000 mg/kg, and then declined with further increase of dietary CU level to 10000 mg/kg [31]. Weight gain of large yellow croaker *Larimichthys crocea* increased with increasing dietary CU level from 0 to 400 mg/kg, and then declined with increasing dietary CU level to 600 mg/kg [17]. Li et al. [32] reported that CU supplementation from 120 to 600 mg/kg could increase feed intake, weight gain, and feed efficiency of grass carp *Ctenopharyngodon idella*, and the suitable dietary CU level was 310 mg/kg. In the present study, the weight gain of largemouth bass increased with increasing dietary CU level from 0 to 10000 mg/kg, suggesting that 10000 mg/kg CU supplementation benefited growth of fish fed diet containing 80 g/kg fish meal. This result is inconsistent with the conclusion that growth *O. mossambicus* declined with increasing dietary CU level from 5000 to 10000 mg/kg [31]. Considering CU is an expensive constituent and the efficacious CU supplementation level (from 50 to 10000 mg/kg) varies greatly among fish species, more studies warrant to determine the minimum dietary CU supplementation level for fishes to identify if CU could be used as an economic dietary functional constituent in the commercial manufacture of fish feed.

The mechanisms by which CU promotes growth of fishes remains unclear. The bioavailability of CU is limited by its low solubility in water, low absorption, chemical instability, and rapid metabolism [33]; therefore, the biological activity of CU might be dependent on its content and retention time in blood and tissues. Mahmoud et al. [14] indicated that CU might increase feed intake by improving diet palatability due to its attractive flavor. Ahmed et al. [34] reported that CU supplementation could enhance antibacterial capacity of gilthead seabream. In the present study, fish fed diet C10 exhibited higher feed intake, but nonsignificantly altered FCR, CRE, NRE, PRE, and ERE, relative to fish fed diet C. These results illustrate that increasing feed intake, rather than improving feed utilization efficiency, should be the mechanism that CU supplementation improved growth of largemouth bass. No significant differences were found in condition factors, HSI, VSI, and body composition between fish fed diets C, C5, and C10, suggesting that CU supplementation could not obviously alter morphological indexes and body composition of largemouth bass. On the other hand, it is noted that retention efficiency of Met and Gly was high relative to that of the other dispensable and indispensable amino acids in fish fed diets R, P8, C, and C10. The retention efficiency, of amino acids (ARE) varied from 20 to 46%, while NRE varied from 31% to 34%. The average ARE (30%) was close to average of NRE (33%). These results are consistent with the conclusion that Gly retention efficiency was high in AREs and average ARE was close to NRE, in Japanese seabass fed the diets with fish meal replaced by PBM [35]. According to the present and previous studies, the retention efficiency of single amino acid could reflect the suitability of the dietary amino acid profile, and Met and Gly might be the important amino acids that play a crucial role in

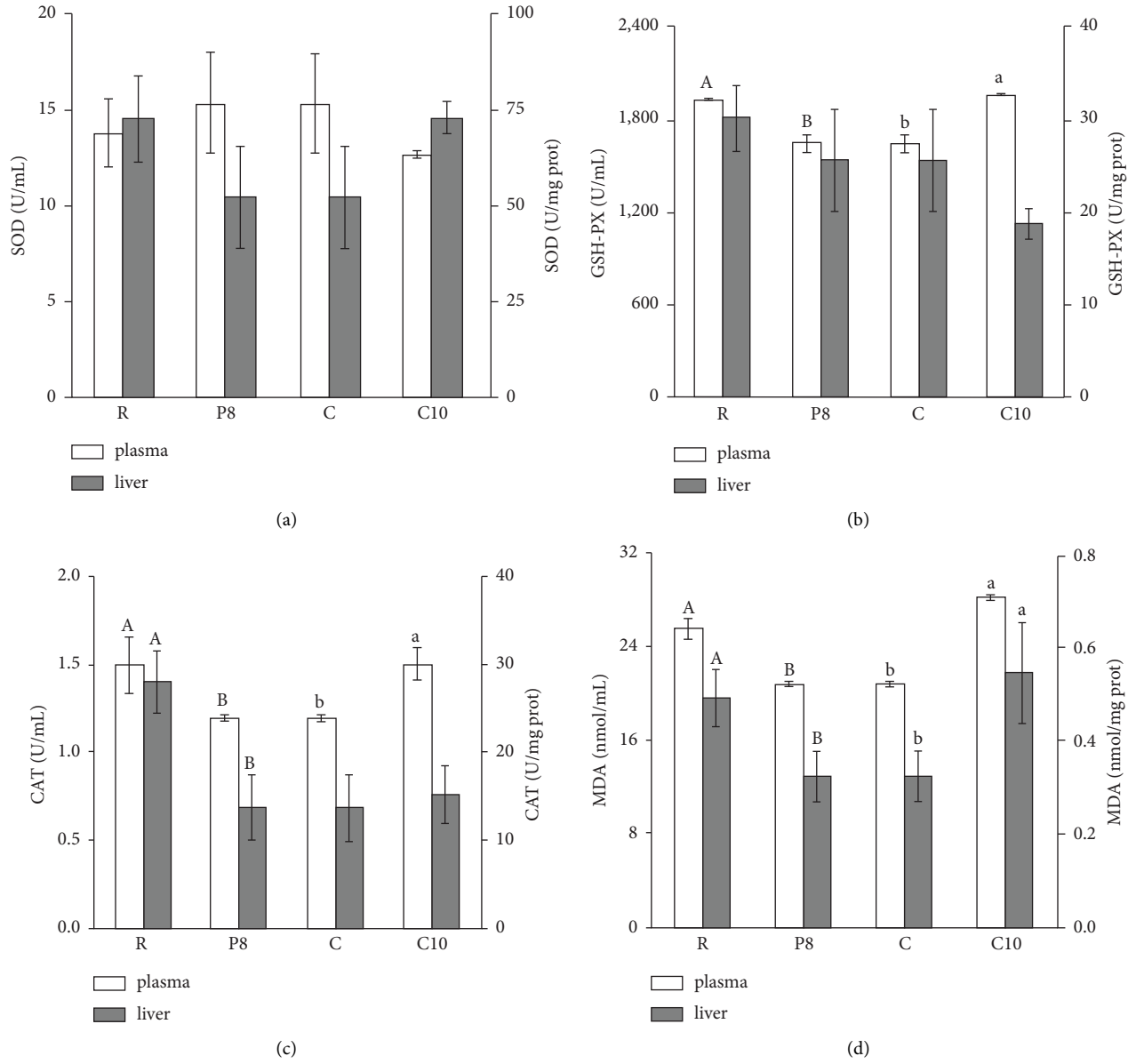

Figure 1: Antioxidant enzyme activities and malondialdehyde content in plasma and liver of largemouth bass. (a) Superoxide dismutase (SOD); (b) glutathione peroxidase (GSH-PX); (c) catalase (CAT); (d) malondialdehyde (MDA). R: reference diet; P8 and C (the same treatment): poultry by-product meal (PBM) replaced 80% of the fish meal in diet R; C10: PBM replaced 80% of the fish meal in diet R with 10000 mg/kg curcumin supplementation. Data are presented as mean ± SD ($n = 3$). The superscripts present the results of independent t-test between R and P8 (capital letters) or between C and C10 (small letters). The data with different superscripts are significantly different ($P < 0.05$).

modulating growth of largemouth bass. Rossi et al. [12] reported that glycine supplementation could improve growth of largemouth bass fed a diet in which fish meal is almost completely replaced by soybean meal.

As polyphenols, CU is recognized as an antioxidant and may be involved in reactive oxygen species (ROS) generation and scavenging. Some ROS generated in the process of cellular metabolisms, such as hydrogen peroxide (H_2O_2), are signal molecules responsible for growth regulation [36]. Excessive ROS can induce oxidative stress that results in growth retard and diseases, and the situation of oxidative stress is generally evaluated with MDA accumulation [37]. Several enzymatic antioxidants, including SOD, GSH-PX and CAT, and non-enzymatic antioxidants, including vitamin E, vitamin C, and polyphenols, are responsible for ROS scavenging [38]. Previous studies reported that CU supplementation could protect a freshwater teleost *Anabas testudineus* from oxidative damage [39], inhibit lipid peroxidation, and reduce MDA accumulation in liver of crucian carp and Nile tilapia [15, 16, 40], suppress inflammatory response of crucian carp [41], and enhance activity of antioxidant enzymes (GSH-PX and CAT in plasma and SOD in liver) of large yellow croaker [17]. In the present study, activities of GSH-PX and CAT in plasma as well as MDA content in plasma and liver were higher in fish fed diet diet C10

than in fish fed diet C. These results suggest that 10000 mg/kg CU supplementation might increase metabolism intensity of largemouth bass and generate more H_2O_2, and the H_2O_2 in return stimulated feeding and growth as a signal molecule. In fish fed diets C10 and C, the higher activities of GSH-PX and CAT in plasma and MDA content in plasma and liver are consistent with the higher feed intake and weight gain. Therefore, it is assumed that modulating ROS generation and scavenging might be a mechanism by which dietary CU influenced feeding and growth of largemouth bass. This hypothesis remains to be tested in the future studies.

Deficiency in dietary protein source (such as fish meal) and environmental pollution are two obstacles limiting the sustainability of fish aquaculture [8, 35, 42]. In the present study, the RCP of fish fed diets diet P8 was 0.26, which was obviously lower than that (1.38) of fish fed diet R but did not significantly differ from that of fish fed diets C (0.26), C5 (0.29), and C10 (0.28). These results indicate that CU supplementation could not further improve the situation of fish meal reliance in largemouth bass farming despite reducing dietary fish meal level from 400 to 80 g/kg considerably saving fish meal in feed manufacture. There was no significant difference in nitrogen waste either between fish fed diets R, P16, and P8 or between fish fed diets C, C5, and C10 and wastes of carbon and phosphorus were lower in fish fed diet P8 than in fish fed diet R. These results are consistent with the report that replacing fish meal with PBM did not increase waste outputs of carbon, nitrogen, and phosphorus in largemouth bass farming [24]. Carbon waste of fish fed diet C was lower than that of fish fed diets C5 and C10, suggesting that CU supplementation at 5000 and 10000 mg/kg could not benefit to reduce waste outputs. In comparison with previous studies, the RCP (0.28), nitrogen waste [56 g·N/(kg fish gain)], and phosphorus waste [11 g·P/(kg fish gain)] of fish fed diet C10 was similar to those [RCP was 0.29, nitrogen waste was 56 g N/(kg fish gain), and phosphorus waste was 17 g P/(kg fish gain)] of Japanese seabass fed a diet with fish meal replaced by PBM [35] and those (RCP was 0.25, nitrogen waste was 49 g·N/(kg fish gain), and phosphorus waste was 10 g·P/(kg fish gain)) of largemouth bass fed a diet with fish meal replaced by PBM [24], but was obivously lower than those (RCP was 0.72, nitrogen waste was 102 g N/(kg fish gain), and phosphorus waste was 28 g·P/(kg fish gain)) of golden pompano fed a diet with fish meal replaced by PBM [9]. Therefore, fish meal reliance and waste outputs of largemouth bass farming are similar are to those of Japanese seabass farming, but lower than those of golden pompano farming.

In summary, the minimum fish meal content could be declined to 80 g/kg in largemouth bass diet, and CU supplementation at 10000 mg/kg could enhance feed intake and growth of fish fed the diet containing 80 g/kg fish meal. Dietary CU might increase feed intake of largemouth bass by modulating the generation and scavenging of ROS.

Data Availability

The data that support the findings of this study appear in the submitted article.

Ethical Approval

All the fish were treated following the guideline of Administration of Laboratory Animals published by the State Science and Technology Commission of China.

Conflicts of Interest

The authors declare that they have no conflicts of interest with respect to research, authorship, and/or publication of this article.

Authors' Contributions

Li Wang conducted the feeding trials, chemical analysis, and data statistics and wrote the preliminary draft. Anlan Yu, Cong Yu, and Jianming Chen were assistants in conducting the feeding trials and sample collection. Umar Bashir Ibrahim participated in preparing preliminary draft. Yan Wang conceived this research, designed the feeding trials, and modified the paper.

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