

Effects of Dietary Arginine Level on Growth Performance, Digestive and Absorptive Indices, Immune Function, and Antioxidant Capacity of Juvenile Yellow Catfish (*Pelteobagrus fulvidraco*) Postprint

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Abstract

This experiment aimed to investigate the effects of dietary arginine levels on growth performance, digestion and absorption related indices, immune function, and antioxidant capacity of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Seven hundred juvenile yellow catfish with an initial average body weight of (1.13 ± 0.02) g were selected and randomly divided into 5 groups (4 replicates per group, 35 fish per replicate), and fed isonitrogenous and isolipidic experimental diets with arginine levels of 2.44%, 2.64%, 2.81%, 3.01%, and 3.23% (protein level of 42% and lipid level of 9%). The experimental period lasted 56 days. Results showed: 1) Compared with the 3.23% group, the weight gain rate (WGR) of juvenile yellow catfish in the 2.64% and 2.81% groups was significantly increased ($P < 0.05$), and the feed conversion ratio (FCR) of juvenile yellow catfish in the 2.64%, 2.81%, and 3.01% groups was significantly decreased ($P < 0.05$). 2) The pepsin and amylase activities of juvenile yellow catfish reached the highest level in the 3.01% group, which was significantly higher than that in the 2.44% group ($P < 0.05$); the gastric lipase activity of juvenile yellow catfish in the 2.81% and 3.01% groups was significantly higher than that in the other groups ($P < 0.05$); the hepatic amylase activity of juvenile yellow catfish in the 2.64%, 2.81%, and 3.23% groups was significantly higher than that in the 2.44% group ($P < 0.05$). The intestinal alkaline phosphatase (AKP) activity of juvenile yellow catfish in the 2.64%, 2.81%, 3.01%, and 3.23% groups was significantly higher than that in the 2.44% group ($P < 0.05$); the intestinal -glutamyltransferase (-GT) activity of juvenile yellow catfish in the 2.64% and 2.81% groups was significantly higher than that in the 2.44%, 3.01%, and 3.23% groups ($P < 0.05$). 3) The serum nitric oxide (NO) content of juvenile yellow

catfish in the 2.81% and 3.01% groups was significantly higher than that in the 2.44% group ($P < 0.05$); the serum interleukin-1 (IL-1) content of juvenile yellow catfish in the 2.64%, 2.81%, 3.01%, and 3.23% groups was significantly lower than that in the 2.44% group ($P < 0.05$); the serum interleukin-6 (IL-6) content of juvenile yellow catfish in the 2.81% group was significantly lower than that in the 2.44% and 2.64% groups ($P < 0.05$). 4) The serum glutathione peroxidase (GSH-Px) and catalase (CAT) activities of juvenile yellow catfish in the 2.64% group were significantly higher than those in the 2.44% group ($P < 0.05$), and the serum malondialdehyde (MDA) content of juvenile yellow catfish in the 3.01% group was significantly lower than that in the 2.64% group ($P < 0.05$). It can be concluded that appropriate dietary arginine levels can increase the WGR, decrease the FCR, and enhance the digestion and absorption capacity, immune function, and antioxidant capacity of juvenile yellow catfish. Using the WGR and serum NO content of juvenile yellow catfish as evaluation indices, the dietary arginine requirements for juvenile yellow catfish were determined to be 2.74% (6.45% of dietary protein) and 2.94% (6.92% of dietary protein), respectively, through quadratic regression model analysis.

Full Text

Effects of Dietary Arginine Level on Growth Performance, Digestive and Absorptive Related Indexes, Immune Function and Antioxidant Capacity of Juvenile Yellow Catfish (*Pelteobagrus fulvidraco*)

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Abstract: This experiment was conducted to investigate the effects of dietary arginine level on growth performance, digestive and absorptive related indexes, immune function and antioxidant capacity of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Seven hundred juvenile yellow catfish with an initial average body weight of (1.13 ± 0.02) g were randomly divided into 5 groups (4 replicates per group, 35 fish per replicate) and fed five isonitrogenous and isolipidic experimental diets (42% protein and 9% lipid) containing graded arginine levels of 2.44%, 2.64%, 2.81%, 3.01% and 3.23%, respectively. The feeding trial lasted for 56 days. The results showed that: 1) Compared with the 3.23% group, the weight gain rate (WGR) of fish in the 2.64% and 2.81% groups was significantly

increased ($P < 0.05$), while the feed conversion ratio (FCR) in the 2.64%, 2.81% and 3.01% groups was significantly decreased ($P < 0.05$). 2) The activities of stomach pepsin and amylase reached their maximum in the 3.01% group, which were significantly higher than those in the 2.44% group ($P < 0.05$). Stomach lipase activity in the 2.81% and 3.01% groups was significantly higher than in other groups ($P < 0.05$). Liver amylase activity in the 2.64%, 2.81% and 3.23% groups was significantly higher than in the 2.44% group ($P < 0.05$). Intestinal alkaline phosphatase (AKP) activity in the 2.64%, 2.81%, 3.01% and 3.23% groups was significantly higher than in the 2.44% group ($P < 0.05$). Intestinal -glutamyl transferase (-GT) activity in the 2.64% and 2.81% groups was significantly higher than in the 2.44%, 3.01% and 3.23% groups ($P < 0.05$). 3) Serum nitric oxide (NO) content in the 2.81% and 3.01% groups was significantly higher than in the 2.44% group ($P < 0.05$). Serum interleukin-1 (IL-1) content in the 2.64%, 2.81%, 3.01% and 3.23% groups was significantly lower than in the 2.44% group ($P < 0.05$). Serum interleukin-6 (IL-6) content in the 2.81% group was significantly lower than in the 2.44% and 2.64% groups ($P < 0.05$). 4) Serum glutathione peroxidase (GSH-Px) and catalase (CAT) activities in the 2.64% group were significantly higher than in the 2.44% group ($P < 0.05$). Serum malondialdehyde (MDA) content in the 3.01% group was significantly lower than in the 2.64% group ($P < 0.05$). In conclusion, optimal dietary arginine levels can improve WGR, reduce FCR, and enhance digestive and absorptive capacity, immune function and antioxidant ability of juvenile yellow catfish. Based on quadratic regression analysis using WGR and serum NO content as evaluation criteria, the dietary arginine requirements for juvenile yellow catfish were estimated to be 2.74% (6.45% of dietary protein) and 2.94% (6.92% of dietary protein), respectively.

Keywords: yellow catfish (*Pelteobagrus fulvidraco*); arginine; digestion and absorption; immune; antioxidant

1.1 Experimental Diets

Five isonitrogenous and isolipidic practical diets (42% crude protein and 9% crude lipid) were formulated using fish meal and soybean meal as the main protein sources, wheat flour as the primary carbohydrate source, and fish oil and soybean oil as the main lipid sources. Alanine was used as an isonitrogenous substitute to create diets with graded arginine levels of 2.44%, 2.64%, 2.81%, 3.01% and 3.23%. Dietary protein and lipid levels were determined based on previous studies from our laboratory [15]. Feed ingredients were ground to pass through a 60-mesh sieve, weighed accurately according to the formula, and mixed stepwise. Fish oil and soybean oil were added in an NH-10 kneader (South China University of Technology Science and Technology Industry General Factory), and after uniform mixing, appropriate water was added before blending in a B20 high-power mixer (Guangzhou Panyu Lifeng Food Machinery Factory). The mixture was then extruded into 1.5 mm strips using an SLX-80 twin-screw ex-

truder (South China University of Technology Science and Technology Industry General Factory) and pelleted using a G-500 granulator (South China University of Technology Science and Technology Industry General Factory). The pellets were dried at 55°C, naturally cooled, sealed in bags, and stored at -20°C until use. The composition and nutrient levels of the experimental diets are presented in Table 1, and the amino acid composition is shown in Table 2.

Table 1 Composition and Nutrient Levels of Experimental Diets (Air-Dry Basis)

Items	Dietary Arginine Level/%
Ingredients	
Fish meal	
Soybean meal	
Rapeseed meal	
Corn gluten meal	
Wheat flour	
Fish oil	
Soybean oil	
Vitamin premix ¹	
Mineral premix ²	
Ca(H ₂ PO ₄) ₂	
Vitamin C ester	
Choline chloride	
L-Arg · HCl	
Alanine	
Total	
Nutrient levels³	
Crude protein	
Crude lipid	
Crude ash	
Moisture	

¹Vitamin premix contained per kg: VA 3,200,000 IU, VB 4 g, VB₆ 8 g, VB₁₂ 4.8 g, VB₁₅ 16 mg, VD 1,600,000 IU, VE 16 g, VK 4 g, calcium pantothenate 16 g, folic acid 1.28 g, nicotinic acid 28 g, inositol 40 g, biotin 64 mg.

²Mineral premix contained per kg: MgSO₄ · H₂O 12 g, Ca(IO₃)₂ 9 g, KCl 36 g, Met-Cu 1.5 g, ZnSO₄ · H₂O 10 g, FeSO₄ · H₂O 1 g, Met-Co 250 mg, NaSeO₃ 0.0036 g.

³Nutrient levels were measured values.

Table 2 Amino Acid Composition of Experimental Diets (Air-Dry Basis)

Items	Dietary Arginine Level/%
Essential amino acids	
Arg	
His	
Ile	
Leu	
Lys	
Met	
Phe	
Thr	
Val	
Non-essential amino acids	
Ala	
Asp	
Glu	
Gly	
Ser	
Tyr	

1.2 Experimental Fish and Culture Management

Juvenile yellow catfish were purchased from Huangsha Fishery Base in Qingyuan City, Guangdong Province. The feeding trial was conducted in an indoor recirculating aquaculture system at the Aquaculture Research Unit of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. Aerated tap water was used with an inflow rate of 1.5 L/min. The system consisted of cylindrical fiberglass tanks (80 cm diameter × 70 cm height) with a water volume of approximately 300 L each. Prior to the experiment, fish were acclimated in outdoor cement ponds for 2 weeks and fed a commercial diet (40% protein, 9.5% lipid) twice daily (08:30 and 18:30). After acclimation, 700 juvenile yellow catfish with an initial average body weight of (1.13±0.02) g were randomly allocated into 5 groups with 4 replicates of 35 fish each, and fed the five experimental diets with different arginine levels. Fish were hand-fed to apparent satiation at 5-6% of body weight, twice daily at 08:30 and 18:30 (40% in the morning, 60% in the afternoon), with feeding amounts adjusted based on consumption. The recirculating system was equipped with a filtration system, and water was changed regularly. Water quality parameters were monitored periodically throughout the trial: temperature 29.5-33.0°C, natural photoperiod, ammonia nitrogen <0.20 mg/L, nitrite <0.01 mg/L, dissolved oxygen >6.0 mg/L, and pH 7.4-7.9. The experiment lasted for 56 days.

1.3 Sample Collection

At the end of the feeding trial, fish were fasted for 24 h before final body weight measurement and survival counting. From each replicate, 15 fish were randomly

selected and anesthetized in 120 mg/L MS-222 solution. Blood was collected from 10 fish via caudal vein puncture, centrifuged at 4,000 r/min for 10 min, and the serum was harvested and stored at -80°C for subsequent analysis. The remaining 5 fish were rapidly dissected on ice to isolate the stomach, liver and intestine, which were stored at -80°C for determination of digestive and absorptive enzyme activities.

1.4.1 Growth Performance Calculations

- Survival rate (SR, %) = $100 \times (\text{final fish number}) / (\text{initial fish number})$
- Weight gain rate (WGR, %) = $100 \times (\text{final body weight} - \text{initial body weight}) / (\text{initial body weight})$
- Feed conversion ratio (FCR) = $\text{feed intake} / (\text{final body weight} - \text{initial body weight})$

1.4.2 Feed Nutrient Analysis

Feed nutrients were analyzed according to AOAC (1984) [17] methods. Moisture content was determined by oven drying at 105°C to constant weight, crude protein by the Kjeldahl method, crude lipid by Soxhlet extraction, and crude ash by muffle furnace incineration at 550°C. Amino acid composition was determined by high-performance liquid chromatography (HPLC) using an Agilent LC1260 system with an Agilent ZORBAX C18 column (150 mm × 5 mm) after acid hydrolysis.

1.4.3 Serum Immune, Antioxidant Indicators and Digestive/Absorptive Enzyme Assays

Serum immune and antioxidant indicators, as well as digestive and absorptive enzyme activities in stomach, liver and intestine, were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions. Serum immune indicators included nitric oxide (NO), complement 4 (C4), immunoglobulin M (IgM), interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- (TNF-). Serum antioxidant indicators included superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GSH-Px), catalase (CAT), total antioxidant capacity (T-AOC) and malondialdehyde (MDA). Digestive and absorptive indicators included protease, lipase and amylase activities in stomach, liver and intestine, as well as intestinal alkaline phosphatase (AKP), -glutamyl transferase (-GT), Na⁺/K⁺-ATPase activity and endothelin-1 (ET-1) content.

1.5 Statistical Analysis

All data are expressed as mean ± standard deviation (mean±SD, n=4). Statistical analysis was performed using SPSS 20.0 software. Homogeneity of variance was tested first; when satisfied, one-way ANOVA was conducted followed by Tukey's multiple comparison test. If homogeneity of variance was not met,

Dunnnett' s T3 test was used for multiple comparisons. Significance level was set at $P<0.05$.

2.1 Growth Performance of Juvenile Yellow Catfish

All fish survived throughout the 56-day trial (100% survival rate). As shown in Table 3 , WGR increased initially and then decreased with increasing dietary arginine level, reaching its maximum in the 2.64% group, while FCR showed the opposite trend, reaching its minimum in the 2.64% group. The WGR in the 2.64% and 2.81% groups was significantly higher than in the 3.23% group ($P<0.05$), while FCR in the 2.64%, 2.81% and 3.01% groups was significantly lower than in the 3.23% group ($P<0.05$). Quadratic regression analysis using WGR and FCR as criteria estimated the dietary arginine requirement of juvenile yellow catfish to be 2.74% (6.45% of dietary protein) (Figure 1 [Figure 1: see original paper]) and 2.75% (6.47% of dietary protein) (Figure 2 [Figure 2: see original paper]), respectively.

Table 3 Growth Performance of Juvenile Yellow Catfish Fed Diets Supplemented with Various Levels of Arginine for 56 Days

Items	Dietary Arginine Level/%
IBW/g	1.13±0.01
FBW/g	22.27±0.74
WGR/%	867.03±56.36
FCR	0.79±0.02

In the same row, values with no letter or the same letter superscripts mean no significant difference ($P>0.05$), while different small letter superscripts mean significant difference ($P<0.05$). The same as below.

2.2 Digestive Enzyme Activities of Juvenile Yellow Catfish

As shown in Table 4 , stomach protease and amylase activities peaked in the 3.01% group, which were significantly higher than in the 2.44% group ($P<0.05$). Stomach lipase activity in the 2.81% and 3.01% groups was significantly higher than in all other groups ($P<0.05$). Liver amylase activity in the 2.64%, 2.81% and 3.23% groups was significantly higher than in the 2.44% group ($P<0.05$). Dietary arginine level had no significant effect on liver protease and lipase activities or intestinal protease, lipase and amylase activities ($P>0.05$).

Table 4 Digestive Enzyme Activities of Juvenile Yellow Catfish Fed Diets Supplemented with Various Levels of Arginine for 56 Days

Items	Dietary Arginine Level/%
Protease (U/mg prot)	

Items	Dietary Arginine Level/%
Stomach	927.08±152.35
Liver	222.74±13.52
Intestine	577.45±77.45
Lipase (U/g prot)	
Stomach	116.32±25.18
Liver	51.33±13.58
Intestine	62.05±3.98
Amylase (U/mg prot)	
Stomach	33.65±6.28
Liver	8.38±1.36
Intestine	27.96±1.58

2.3 Intestinal Absorption Related Indexes of Juvenile Yellow Catfish

As shown in Table 5, intestinal AKP activity in the 2.64%, 2.81%, 3.01% and 3.23% groups was significantly higher than in the 2.44% group ($P < 0.05$). Intestinal -GT activity in the 2.64% and 2.81% groups was significantly higher than in the 2.44%, 3.01% and 3.23% groups ($P < 0.05$). Dietary arginine level had no significant effect on intestinal Na /K -ATPase activity or ET-1 content ($P > 0.05$).

Table 5 Intestinal AKP, -GT, Na K -ATPase Activities and ET-1 Content of Juvenile Yellow Catfish Fed Diets Supplemented with Various Levels of Arginine for 56 Days

Items	Dietary Arginine Level/%
AKP (U/g prot)	81.93±7.37
-GT (U/g prot)	18.35±4.11
Na /K -ATPase (U/mg prot)	3.16±0.72
ET-1 (ng/L)	21.43±3.23

2.4 Serum Immune Indexes of Juvenile Yellow Catfish

As shown in Table 6, serum NO content in the 2.81% and 3.01% groups was significantly higher than in the 2.44% group ($P < 0.05$). Serum IL-1 content in the 2.64%, 2.81%, 3.01% and 3.23% groups was significantly lower than in the 2.44% group ($P < 0.05$). Serum IL-6 content in the 2.81% group was significantly lower than in the 2.44% and 2.64% groups ($P < 0.05$). Dietary arginine level had no significant effect on serum C4, IgM or TNF- content ($P > 0.05$). Quadratic regression analysis using serum NO content as the criterion estimated the dietary arginine requirement of juvenile yellow catfish to be 2.94% (6.92% of dietary protein) (Figure 3 [Figure 3: see original paper]).

Table 6 Serum Immune Indexes of Juvenile Yellow Catfish Fed Diets Supplemented with Various Levels of Arginine for 56 Days

Items	Dietary Arginine Level/%
NO (mol/L)	148.74±6.27
C4 (g/L)	0.02±0.00
IgM (g/L)	0.29±0.00
IL-1 (ng/L)	15.24±5.71
IL-6 (ng/L)	46.21±3.52
TNF- (ng/L)	61.40±29.53

2.5 Serum Antioxidant Indexes of Juvenile Yellow Catfish

As shown in Table 7 , serum GSH-Px activity in the 2.64% group was significantly higher than in the 2.44% group ($P<0.05$). Serum CAT activity in the 2.64% group was significantly higher than in the 2.44%, 3.01% and 3.23% groups ($P<0.05$). Serum MDA content in the 3.01% group was significantly lower than in the 2.64% group ($P<0.05$). Dietary arginine level had no significant effect on serum SOD, POD activities or T-AOC ($P>0.05$).

Table 7 Serum Antioxidant Indexes of Juvenile Yellow Catfish Fed Diets Supplemented with Various Levels of Arginine for 56 Days

Items	Dietary Arginine Level/%
GSH-Px (U/mL)	163.65±15.60
CAT (U/mL)	3.30±0.58
SOD (U/mL)	66.72±0.86
POD (U/mL)	45.96±9.52
T-AOC (U/mL)	2.07±0.90
MDA (nmol/L)	8.60±0.35

3.1 Effects of Dietary Arginine Level on Growth Performance of Juvenile Yellow Catfish

Arginine participates in various metabolic reactions in vivo, including synthesis of proteins, urea and ornithine, metabolism of glutamate and proline, synthesis of creatine and polyamines, and secretion of insulin and glucagon [1-2], playing an important role in promoting fish growth, enhancing immunity and improving stress resistance. Previous studies have demonstrated that dietary arginine levels above 2.23% significantly improved weight gain and specific growth rate in black seabream (*Sparus macrocephalus*) [3]; levels above 1.15% significantly reduced feed conversion ratio in Nile tilapia (*Oreochromis niloticus* L.) [4]; levels

above 1.00% improved protein efficiency in channel catfish (*Ictalurus punctatus*) [5]; 3.31% arginine significantly increased protein deposition rate in grouper (*Epinephelus coioides*) [6]; levels above 2.76% enhanced serum lysozyme activity in red drum (*Sciaenops ocellatus*) [7]; and 1.1% arginine plus 0.75% glutamate reduced stress induced by water temperature changes in Atlantic salmon (*Salmo salar* L.) [8].

Yellow catfish (*Pelteobagrus fulvidraco*) belongs to the order Siluriformes, family Bagridae, and genus *Pelteobagrus*. Its meat is tender, delicious, and free of intermuscular bones [9], with unique flavor and rich nutritional value [10]. Due to its excellent meat quality, yellow catfish has strong market potential not only domestically but also in Japan, Korea and Southeast Asia [11]. To meet market demand, intensive farming of yellow catfish has increased in China in recent years, making nutritional requirement research a hot topic. Zhou et al. [12] investigated the optimal arginine requirement for yellow catfish, while Shen et al. [13] explored the effects of dietary arginine to lysine ratios on digestion and absorption by measuring digestive enzyme activities and nutrient digestibility. Our previous study showed that optimal dietary arginine levels significantly improved growth performance and ammonia-nitrogen stress resistance in juvenile yellow catfish [14]. However, few studies have examined the effects of dietary arginine on digestive and absorptive enzyme activities, immune function and antioxidant capacity in juvenile yellow catfish. To enrich the application of arginine research in this species, this experiment investigated the effects of graded dietary arginine levels on growth performance, digestive and absorptive enzyme activities, immune function and antioxidant capacity, aiming to evaluate the optimal supplementation level and provide theoretical basis for arginine application in practical yellow catfish feeds.

3.2 Effects of Dietary Arginine Level on Digestive Enzyme Activities of Juvenile Yellow Catfish

Digestive enzymes are specialized proteins that catalyze biochemical reactions to digest and decompose ingested food, providing essential nutrients for growth and development [22]. Wang et al. [23] found that dietary arginine significantly affected intestinal protease activity but not lipase or amylase activities in sea cucumber. Chen et al. [24] reported that arginine significantly increased protease and lipase activities but had no effect on amylase activity in Jian carp. Wang et al. [25] demonstrated that dietary arginine supplementation significantly increased protease and amylase activities in the anterior intestine of hybrid sturgeon. In this study, optimal dietary arginine levels significantly increased stomach protease, lipase and amylase activities, as well as liver amylase activity in juvenile yellow catfish. The effects of arginine on digestive enzyme activities vary among fish species and are influenced by multiple factors including feeding habits, developmental stage, physicochemical factors (temperature, pH, salinity), nutrition and feed composition [26]. Shen et al. [13] reported that the arginine/lysine ratio of 2.19/2.61 significantly increased liver and intestinal

trypsin activities compared with other ratios, while stomach protease activity peaked at arginine/lysine ratios of 1.74/2.08, 1.74/30.2 and 2.63/2.08. Our results differed, showing peak stomach protease and amylase activities at 3.01% arginine, peak stomach lipase at 3.01% arginine, and significantly elevated liver amylase at 2.64%, 2.81% and 3.23% arginine compared with 2.44%. Differences in arginine sources, arginine/lysine ratios and feed composition may affect arginine utilization, leading to variations in digestive enzyme activities.

3.3 Effects of Dietary Arginine Level on Intestinal Absorption Related Indexes of Juvenile Yellow Catfish

The intestine plays a crucial role not only in nutrient digestion and absorption but also as an integrated organ with endocrine, immune and barrier functions, making intestinal health essential for overall fish health [27-28]. AKP, -GT, Na K -ATPase activities and ET-1 content are indicators of intestinal nutrient metabolism, transport and absorptive function. No previous studies have reported the effects of arginine on these parameters in yellow catfish. In this study, dietary arginine level had no significant effect on intestinal Na K -ATPase activity or ET-1 content. Intestinal alkaline phosphatase (IAKP), located on the brush border of intestinal epithelial cells, is involved in the absorption of various nutrients including vitamin D, calcium, amino acids, cholesterol, lipids and glucose [29-30]. Our results showed that dietary arginine supplementation significantly increased intestinal AKP activity, indicating enhanced intestinal absorptive capacity and promoted nutrient metabolism. -GT is a key enzyme in the glutamate cycle that facilitates amino acid transport into cells for protein synthesis [31]. We found that intestinal -GT activity in the 2.64% and 2.81% groups was significantly higher than in other groups, suggesting that optimal arginine levels can enhance -GT activity and promote amino acid transport.

3.4 Effects of Dietary Arginine Level on Immune Function of Juvenile Yellow Catfish

Fish possess both non-specific and specific immune functions, utilizing mucosal barriers, immune cells and humoral factors to defend against pathogens [32]. The complement system can be activated by antigen-antibody complexes or directly by antigens, binding to antigen surfaces or being activated by bacterial lectins and inflammatory proteins to assist non-specific and specific immunity [33]. C4 is a major component of the complement system and a globulin with zymogen activity [34]. In this study, dietary arginine had no significant effect on serum C4 content, consistent with results in juvenile blunt snout bream [20]. Numerous studies have shown that fish can mount humoral and cellular immune responses, with immunoglobulins being the main mediators of specific humoral immunity [35]. Our study found no significant effect of arginine on serum IgM content. NO acts as both an effector molecule in tumor and microbial immunity and a regulatory factor for various immune cells. In this study, serum NO content increased initially and then decreased with increasing arginine, indicating

that appropriate arginine supplementation can enhance immune function. Inflammation is a common pathological process involving cytokines such as IL-1, IL-6 and TNF-. Zheng et al. [36] found that arginine reduced hepatic IL-6 and TNF- mRNA expression in weaned piglets, while Tan et al. [37] showed that arginine decreased intestinal IL-1 mRNA expression in broiler chickens. Similarly, our results demonstrated that increasing dietary arginine reduced serum pro-inflammatory cytokines IL-1 and IL-6, suggesting that arginine can alleviate inflammatory responses, consistent with findings in Atlantic salmon [38].

3.5 Effects of Dietary Arginine Level on Antioxidant Capacity of Juvenile Yellow Catfish

Oxygen free radical reactions and lipid peroxidation are essential components of metabolic activities, normally maintained in equilibrium to support physiological, biochemical and immune functions [39]. GSH-Px, CAT, SOD and POD are crucial antioxidant enzymes that scavenge reactive oxygen species and protect cell membranes and nucleic acids. Lipid peroxidation, induced by oxygen free radicals, is one of the main harmful changes in biological membranes. MDA content often reflects the degree of lipid peroxidation and indirectly indicates cell damage [40-41]. The significant increase in serum GSH-Px and CAT activities at 2.64% arginine indicates that optimal arginine levels effectively enhance antioxidant capacity, consistent with results in grass carp [42]. Arginine significantly reduced serum MDA content, alleviating cellular damage from peroxidation [12]. Our study also showed that serum MDA content at 3.01% arginine was significantly lower than at 2.64% arginine. Qiang et al. [43] reported that fish increase metabolism in response to environmental stress, leading to increased oxygen free radicals, and that elevated SOD and CAT activities represent an adaptive response to reduce lipid peroxidation damage. Studies have shown that dietary arginine had no significant effect on serum SOD activity in turbot [44] and grouper [45], similar to our results in yellow catfish. However, Buentello et al. [46] and Wu et al. [47] reported that arginine significantly increased serum SOD activity in channel catfish and hybrid sturgeon, respectively. These discrepancies may be related to feed composition, arginine level, species differences and culture conditions.

4 Conclusion

Optimal dietary arginine levels can improve weight gain rate, reduce feed conversion ratio, and enhance digestive and absorptive capacity, immune function and antioxidant ability of juvenile yellow catfish. Based on quadratic regression analysis using weight gain rate and serum nitric oxide content as evaluation criteria, the dietary arginine requirements for juvenile yellow catfish are estimated to be 2.74% (6.45% of dietary protein) and 2.94% (6.92% of dietary protein), respectively.

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