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Research Article

Dietary Supplementation of Crystalline Amino Acid Improves Growth Performance and Health of Yellow Catfish That Reduced by Plant Proteins Replacement of Fishmeal

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To evaluate the growth and health of yellow catfish (5.50 ± 0.61 g) under the conditions of unbalanced and balanced amino acid composition, an 8-week growth experiment was conducted. Three isoenergetic and isonitrogenous diets were formulated with a graded level of fishmeal: control group was formulated with 560.00 g/kg fishmeal, and 25%PP group and 25%PP + AA group were formulated with 420.00 g/kg fishmeal (replace 25% of fishmeal with plant protein), and then 25%PP + AA group was supplemented with threonine, glycine, alanine, methionine, isoleucine, histidine, and lysine. Compared with control group, weight gain (WG), specific growth rate (SGR), and survival (SUR) in 25%PP group were significantly the lowest ($p < 0.05$), but feed conversion ratio (FCR) was higher than those in control group ($p < 0.05$). After supplementation of crystalline amino acid, the WG, SGR, SUR, and FCR in 25%PP + AA group were recovered to the level of control group ($p > 0.05$). Compared with control group, the activities of trypsin and lipase were significantly increased in 25%PP group and 25%PP + AA group ($p < 0.05$). Liver impairment was exhibited in 25%PP group, mainly manifested as significantly increased contents of aspartate aminotransferase (AST) and alanine transaminase (ALT) ($p < 0.05$). Supplementation of crystalline amino acids based on a 25%PP diet significantly reduced activities of AST and ALT ($p < 0.05$). In addition, total antioxidant capacity (T-AOC), superoxide dismutase (SOD), acid phosphatase (ACP), and alkaline phosphatase (AKP) activities in 25%PP group increased significantly ($p < 0.05$). Data from 25%PP + AA group showed that crystalline amino acid supplementation ameliorated antioxidant (T-AOC and SOD) and immune (ACP and AKP) ($p < 0.05$). One of the characteristics of intestinal damage is shortening of villi height, which was significantly reduced in 25%PP group ($p < 0.05$). In short, unbalanced amino acid intake can impact growth, survival, and physiological status of yellow catfish. Diet supplemented with unbalanced amino acid can cause abnormal accumulation of amino acid content in muscle, resulting in unnecessary consumption of nutrients. Dietary supplementation of crystalline amino acid improves growth performance and health of yellow catfish that reduced by plant proteins replacement of fishmeal.

1. Introduction

In recent years, the primary supply mode of aquatic products in Asia has heavily depended on the development of the aquaculture industry. Aquaculture feed is the material basis of the sustainable development of the aquaculture industry. According to the latest statistics from the Food and Agriculture Organization (FAO) of the United Nations, nearly 1/3 of the yield of catch produced by offshore fishing is processed into fishmeal and fish oil [1]. However, with the rapid decline of fishery resources, reduced fishmeal production, and the rapid development of the aquaculture industry, the demand for fishmeal has risen dramatically, and a sharp rise in prices is inevitable (http://www.globefish.org) [2].

Therefore, the substitution of cheap and efficient plant protein for fishmeal and the production of efficient and environmentally friendly aquatic feed are among the current research projects [3].

Different from plant proteins, fishmeal has a high level of digestible crude protein and balanced essential amino acids (EAAs) that approximate the optimal requirements of fish and crustaceans [4]. A diet with a balanced amino acid profile is one of the most important factors enhancing the protein utilization of a feed for proper growth and metabolic responses of animals including fish [5]. At present, soybean, corn, peanut, and other plant proteins have been widely applied to replace fishmeal in aquatic feed. They all have the advantages of high protein content and low production cost, but there are many antinutritional factors, reduction in digestive enzyme activity, imbalance of amino acid profile, and other problems that are also worrying [6, 7]. Many antinutritional factors in plant proteins may affect the utilization of amino acids, thus reducing the protein utilization efficiency of fish [8]. Ideally, all EAAs in a diet can meet the protein requirements of the target species if they are maintained at a balance and efficient level. However, most of the alternative ingredients commonly used in fish and shrimp diets lack 10 or more amino acids [4]. The most effective approach to solve this problem is to supplement crystalline amino acids (CAAs) to optimize the amino acid profile of aquatic feed, thereby improving the protein utilization and growth performance of fish [4]. Previous studies have shown that adding CAAs to plant-based feed is not only one of the effective approaches to improve the nutritional value of feed, but also can reduce the additive amount of fishmeal, which promotes the sustainable development of the feed industry [9, 10]. Therefore, it is worth considering supplementing an appropriate amount of CAAs into plant protein feed to optimize the protein utilization efficiency of fish [11].

Amino acids are known to play an indispensable role in the growth, performance, and physiological state of fish. For instance, arginine can not only improve the growth and immunity of fish but also directly participate in various physiological metabolic pathways; tryptophan can improve the growth of grass carp (*Ctenopharyngodon idella*), regulate the immune system, and reduce the stress reaction [12]; methionine and lysine can improve the growth performance of rainbow trout (*Oncorhynchus mykiss*) and grass carp [13, 14]. Amino acid imbalance in plant proteins means that the contents of various functional amino acids in plant proteins are considerably lower than those in fishmeal. Due to the imbalance of amino acids in plant protein, containing antinutritional factors and low digestibility of feed, the growth performance of fish decreased significantly when excessive plant protein was added to feed. However, it has been reported that the feed utilization efficiency, protein synthesis efficiency, and growth performance of fish were not decreased when the fishmeal was replaced by plant protein and supplemented with CAAs [15–19]. Previous studies reported that fishmeal can be replaced by up to 80% plant protein without any negative impact on the growth performance of juvenile turbot (*Psetta maxima*) [20]; The Senegal sole (*Solea senegalensis*) can be grown effectively on a fishmeal-free diet if the dietary amino acids component is properly formulated [21]; the CAAs and intact protein are equally effective in meeting the essential EAAs requirements of Nile tilapia (*Oreochromis niloticus* L.) [22]; the CAAs mixture can completely replace the nutrients of fishmeal in the whole plant-based feed of juvenile blunt snout bream (*Megalobrama amblycephala*) [23]. An additional benefit of adding CAAs to the diet is increased protein retention. The results of the study showed that Nile tilapia fed with CAAs had higher protein utilization efficiency [24]. These studies suggest that regardless of the protein source used as raw materials, a balanced intake of amino acids is a critical element in ensuring growth performance and feed efficiency. On the other hand, the latest study has shown that the supplementation of crystalline amino acids to replace part of crude protein components in the diet does not affect the body weight gain, feed efficiency, and protein retention of Nile tilapia. But it can promote protein synthesis and the reduction of fat deposition [25]. In summary, the function of crystalline amino acids is mainly reflected in the following two aspects: (1) as an additive in a high plant protein diet with unbalanced amino acid composition, maintaining balanced amino acid intake to ensure the growth performance of fish is not affected; (2) using crystalline amino acids to regulate muscle amino acid composition and mTOR signaling pathway to promote muscle protein synthesis. Therefore, the purpose of this study was to investigate the effects of unbalanced and balanced amino acid intake on growth performance, physiological state, and muscle amino acid metabolism of yellow catfish.

Yellow catfish (*Pelteobagrus fulvidraco*) is widely distributed in China and has high nutritional value. Therefore, it is favored by consumers and has become an important economic aquaculture animal. The study investigated the effects of mixed plant protein supplemented with CAA substituted fishmeal on growth performance, feed utilization, and physiological indexes of yellow catfish. The results will provide a theoretical and practical basis for the preparation of high efficiency, environmental protection, and cheap feed for yellow catfish.

2. Materials and Methods

2.1. Ethical Statement. All animal use procedures were approved by the Institutional Animal Care and Use Committee of Ningbo University (SYXK (ZHE 2012-011012)). All experimental steps were performed based on the standard operating procedures according to the guidelines for the use of experimental animals at Ningbo University.

2.2. Experimental Diets. Three isoenergetic and isonitrogenous diets were formulated containing approximately 452.60 g/kg protein and 105.60 g/kg lipids. In this experiment, fishmeal was used as the single protein source in the control (Con) group, and 25% fishmeal was replaced by five mixed plant proteins (soybean meal, cottonseed meal, corn gluten meal, sesame meal, and peanut meal) to construct a diet with extremely unbalanced amino acid composition as the 25%PP group. Based on the diet formula of the 25%PP group, supplement deficient CAAs to the control group level as 25%PP + AA group, which refers to the amino acid composition of fishmeal. The 1 : 1 proportion of fish oil to soybean oil was used as a lipid source in the three diets. Wheat meal and microcrystalline cellulose were used as the carbohydrate source. Carboxymethyl was used as the adhesion agent. The composition and primary nutrients of the basal diet are shown in Table 1, and the amino acid composition of the diet is shown in Table 2. The CAA mixture was precoated at 60°C with carboxymethyl to prevent loss and then mixed with other components. All raw materials were pulverized and filtrated through a 60-mesh screen. After thoroughly mixing, the laboratory feedstuff pelleting mechanism was used to produce 2.5 mm pellet feed. Particles of feedstuff are dried at 60°C and stored at -20°C.

2.3. Experimental Fish and Culture Conditions. Juvenile yellow catfish was purchased from a commercial fish farm in Huzhou (Zhejiang, China). Before the formal experiment, all fish were acclimated for 2 weeks to adapt to the unacquainted culture environment and culture water. During the period of domestication, fish were fed twice a day at 09:00 and 18:00. The three experimental feeds were mixed with commercial feeds in the same proportion for domestication and feeding. After domestication, 30 healthy, energetic, and well-developed fish of the same size (initial body weight: 5.50 ± 0.61 g) without obvious scars on the skin surface were randomly assigned to nine cylindrical plastic culture barrels (300 L). Two opaque PVC pipes (10 cm diameter and 15 cm length) were placed in each tank as shelters for yellow catfish to prevent them from being frightened and stabbing each other, and a cylindrical gas stone (5 cm in diameter and 6 cm in height) was hoisted vertically into each tank and aerated continuously for 24 hours by air pump to maintain sufficient dissolved oxygen. During the experiment, 200 L of water was added to each tank. The water was renewed at weekly intervals during the formal experiment. Half of the original amount was exchanged. Water temperature, pH, and dissolved oxygen were measured every two days. The range of water temperature was always maintained at 26.50 ± 1.50°C, the pH value was 7.2-7.5, and dissolved oxygen was greater than 5 mg/L. The concentration of total ammonia nitrogen should be kept below 0.5 mg/L. Yellow catfish were fed twice a day at 09:00 and 18:00, respectively, and the illumination was natural.

2.4. Sampling. All fish stopped feeding for 24 h after 8 weeks of culture experiment before sample collection. Three fish were randomly selected from each tank, and their body weight and body length were measured and recorded. Then, the liver and muscle were anesthetized on ice and quickly preserved with liquid nitrogen at −80°C for further analysis. A little section of the intestine was separated and placed in Bouin's fluid. After 12 hours, it was preserved in 75% ethanol for the comparative analysis of an intestinal histological. In addition, 3 healthy and vibrant yellow catfish were randomly selected from each breeding tank. After anesthesia, they were euthanized and stored in a scientific research refrigerator at -20°C for subsequent body composition analysis. The growth-related parameters are calculated according to the following formula:

$$
\begin{aligned}
\text{Weight gain (WG, \%)} &= \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100,\\
\text{Specific growth rate}\left(\frac{\text{SGR, \%}}{\text{day}}\right) &= \frac{[\text{In (final weight)} - \text{In (initial weight)}] \times 100}{\text{breeding days}},\\
\text{Survival (SUR, \%)} &= \frac{\text{final yellow catfish number}}{\text{initial yellow catfish number}} \times 100,\\
\text{Hepatosomatic index (HIS, \%)} &= \frac{\text{wet hepatopancreatic weight}}{\text{wet body weight}} \times 100,\\
\text{Condition factor (CF, \%)} &= \frac{\text{final weight (g)}}{\text{final length (cm)}^3} \times 100,\\
\text{Feed conversion ratio (FCR)} &= \frac{\text{feed intake}}{\text{final weight} - \text{initial weight}}.
\end{aligned}
\tag{1}
$$

2.5. Analysis of Proximate Composition. According to the standard method provided by the AOAC procedure [26], the approximate crude components of three feedstuffs and the whole body of yellow catfish were determined. The brief steps are summarized as follows: the sample was dried to constant weight at 105°C to determine moisture; the crude protein content was determined by combustion method; crude fat content was determined by the Soxhlet system; ash content was determined in a muffle furnace at 550°C for 6 h.

2.6. Analysis of Amino Acid Composition. Using 6 mol/L hydrochloric acids hydrolyzed the freeze-dried diet and muscle for 24 h (110°C) for amino acid analysis. After filtration and evaporation, all acids were removed. After being treated with 0.05 mol/L HCl, the residue was removed by filtration. After treating with phenyl isothiocyanate, phenylthiocarbamide amino acids were synthesized by derivatization reaction. Finally, the amino acid content in feed and muscle was determined by the amino acid automatic analyzer (Biochrom 20, England) [27].

2.7. Analysis of Digestive Enzyme Activity. The liver of yellow catfish was homogenized with normal saline at a ratio of 1 : 9 and then centrifuged at 2500 r/min for 10 min at 4°C. The supernatant was taken, and the digestive enzyme activities in the liver were determined within 24 h. Trypsin (TRY) and Lipsin (LIP) activities were measured with kits produced by the Nanjing Jiancheng Institute of Biological Engineering.

2.8. Analysis of Antioxidant and Metabolism-Related Enzymes. Total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, superoxide dismutase (SOD), aspartate aminotransferase (AST), alanine transaminase (ALT), acid phosphatase (ACP), and alkaline phosphatase (AKP) activates were selected as a liver antioxidant and immune indexes. All analyses were determined by using commercial kits produced by the Nanjing Jiancheng Institute of Biological Engineering.

2.9. Analysis of Intestinal Histological. Using H&E (hematoxylin and eosin) method, stained sections were obtained

Table 1: The formula and approximate composition of the diets used in the experiment (g/kg dry matter basis).

Ingredient	Con	25%PP	25%PP + AA
Fish meal	560.00	420.00	420.00
Wheat meal	160.00	160.00	160.00
Soybean meal	0.00	30.00	30.00
Cottonseed meal	0.00	40.00	40.00
Corn gluten meal	0.00	40.00	40.00
Sesame meal	0.00	30.00	30.00
Peanut meal	0.00	30.00	30.00
Casein	10.00	10.00	10.00
Fish oil	10.00	10.00	10.00
Soybean lecithin[a]	10.00	10.00	10.00
Vitamin mix[b]	10.00	10.00	10.00
Mineral mix[c]	5.00	5.00	5.00
Monocalcium phosphate	20.00	20.00	20.00
Choline chloride[d]	5.00	5.00	5.00
Ethoxyquin	0.50	0.50	0.50
Cellulose	189.50	159.50	141.50
Carboxymethyl	20.00	20.00	20.00
Amino acid premix[e]	0.00	0.00	18.00
Total	1000.00	1000.00	1000.00
Proximate composition (g/kg)			
Crude protein	452.80	451.30	453.60
Crude lipid	106.80	105.40	104.70
Ash	108.90	106.90	102.10
Moisture	54.50	56.80	53.60

[a]Shanghai Taiwei, Ltd., Shanghai, China. [b]Vitamin premix (g/kg diet): vitamin A, 0.032; vitamin D, 0.005; vitamin E, 0.24; vitamin K, 0.01; vitamin B1, 0.025; vitamin B2, 0.045; nicotinic acid, 0.2; vitamin B6, 0.02; biotin, 0.06; inositol, 1.8; calcium pantothenate, 0.06; folic acid, 0.02; vitamin B12, 0.01; vitamin C, 2; microcrystalline cellulose, 6.29. [c]Mineral mix (g/kg premix): $CuSO_4 \bullet 5H_2O$, 0.01; Na_2SeO_3, 0.02; $MnSO_4 \bullet H_2O$, 0.045; $CoCl_2 \bullet 6H_2O$, 0.05; $ZnSO_4 \bullet H_2O$, 0.5; $Ca(IO_3)_2$, 0.06; $FeSO_4 \bullet H_2O$, 0.08; $MgSO_4 \bullet 7H_2O$, 1.2; Zeolite powder, 3.485. [d]Sangon Biotech, Ltd., Shanghai, China. [e]Amino acid mix (g/kg premix): Thr, 2; Gly, 3; Ala, 2, Met, 2; Ile, 2; His, 2; Lys, 5. Thr: Threonine; Gly: glycine; Ala: alanine; Met: methionine; Ile: isoleucine; His: histidine; Lys: lysine.

by a 5-μm-thickness rotary microtome. Using light microscopy (Japan, Nikon, Eclipse 200) to observe the slides, using Window Clippings 3 (Version 3.1.131, USA) to photograph.

2.10. Statistical Analysis. Kolmogorov-Smirnov test and Bartlett test were used to test the data for normality and homogeneity of variance. All analyses were performed using SPSS 19.0.0 software (Chicago, USA) for Windows. Duncan's multiple comparisons are used to compare the difference in mean values between groups when the effect of experimental factors is significant; $p < 0.05$ indicates a significant difference. The experimental data were described as mean ± standard deviation. The correlational analyses were conducted by using Pearson correlation; p values of correlational analyses were also adjusted by Duncan's multiple comparisons. Graphs were made by GraphPad Prism 8.0 software.

Table 2: Amino acid composition (g/100 g dry matter) of experimental diets.

Amino acid name	Con	25%PP	25%PP + AA
Asp	3.53	3.79	3.82
Thr	1.70	1.58	1.82
Ser	1.63	1.72	1.77
Glu	6.33	6.82	7.03
Gly	2.30	2.08	2.41
Ala	2.41	2.23	2.42
Val	1.94	2.15	2.10
Met	1.12	0.98	1.17
Ile	1.64	1.51	1.67
Leu	2.96	3.52	3.52
Tyr	1.33	1.55	1.52
Phe	1.75	1.83	2.05
His	1.34	1.22	1.41
Lys	3.01	2.48	3.30
Arg	2.35	2.52	2.70
Pro	1.98	2.14	2.15

Arg: arginine; His: histidine; Ile: isoleucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Thr: threonine; Val: valine; Leu: leucine; Ala: alanine; Asp: aspartic acid; Glu: glutamic acid; Gly: glycine; Pro: proline; Ser: serine; Tyr: tyrosine.

3. Results

3.1. Growth Performance and Body Composition. The data in Table 3 show that compared with the whole fishmeal diet (Con group), replacing 25% fishmeal with mixed plant protein (25%PP group) significantly reduced WG, SGR, and SUR of yellow catfish ($p < 0.05$). Compared with the 25%PP group, the growth performance (WG, SGR, and SUR) of yellow catfish was significantly increased by supplementing the mixed amino acids (25%PP + AA group). Additionally, the FCR was significantly increased in the 25%PP group, while supplementing mixed CAAs could reduce the FCR of the 25%PP + AA group ($p < 0.05$). Replacing 25% fishmeal with mixed plant protein and adding CAAs had no significant effect on the HSI and CF ($p > 0.05$). The crude protein (CP) content in the 25%PP group was significantly lower than Con and 25%PP group ($p < 0.05$), while there were no significant differences in moisture, crude lipid, and ash contents of the whole body of fish among the three groups ($p > 0.05$) (Table 4).

3.2. Amino Acid Composition in Muscle. Compared with the muscle of fish in the control group, the contents of all amino acids in the 25%PP group were significantly increased ($p < 0.05$). In addition, compared with the control group and 25%PP group, the muscle contents of all amino acids in the 25%PP + AA group were significantly higher than in the other two groups ($p < 0.05$) (Table 5).

3.3. Digestive Enzymatic Activity. After substituting 25% fishmeal (25%PP group) with a mixed plant protein source, the TRY and LIP activity of yellow catfish was markedly

Table 3: Growth performances of yellow catfish fed for 8 weeks (mean ± SD).

Parameter	Con	25%PP	25%PP + AA
Weight gain (%)	296.07 ± 3.44^b	256.35 ± 3.26^a	297.56 ± 6.53^b
Specific growth rate (%)	1.94 ± 0.02^b	1.68 ± 0.02^a	1.95 ± 0.04^b
Survival (%)	92.22 ± 1.11^b	81.11 ± 1.11^a	90.00 ± 1.92^b
Hepatosomatic index (%)	1.48 ± 0.20	1.34 ± 0.10	1.38 ± 0.13
Condition factor (%)	0.99 ± 0.06	1.01 ± 0.01	0.94 ± 0.09
Feed conversion ratio	1.97 ± 0.05^a	2.75 ± 0.08^b	2.02 ± 0.04^a

The different and superscript letters ($^{a, b, c}$) represent existing significant differences; lowercase "a" represents the lower value, lowercase "b" represents the higher value, and lowercase "c" represents the highest value ($p < 0.05$). The final data are presented in the table as the mean ± standard deviation of 3 repetitions.

Table 4: Proximate composition of the whole body (% wet weight) (mean ± SD).

Parameter	Con	25%PP	25%PP + AA
Crude protein	669.96 ± 3.12^b	658.50 ± 4.88^a	669.00 ± 2.29^b
Crude lipid	238.32 ± 6.49	235.68 ± 4.16	240.00 ± 5.21
Moisture	748.55 ± 43.29	753.02 ± 2.83	725.40 ± 59.93
Ash	64.51 ± 0.23	64.71 ± 5.92	63.30 ± 5.37

The different and superscript letters ($^{a, b, c}$) represent existing significant differences; the lowercase "a" represents the lower value, and lowercase "b" represents the higher value ($p < 0.05$). The absence of lowercase letters indicates no significant difference. The final data are presented in the table as the mean ± standard deviation of 3 repetitions.

improved compared with Con and 25%PP + AA group ($p < 0.05$). The activity of liver TRY and LIP in the 25%PP + AA group was significantly higher than in the Con group and lower than in the 25%PP group ($p < 0.05$) (Figure 1).

3.4. Enzyme Activities. The activities of AST and ALT in the 25%PP group were higher than those in the Con and 25%PP + AA groups ($p < 0.05$); and the activities of AST and ALT in the Con and 25%PP + AA groups exhibited no significant difference ($p > 0.05$) (Figure 2). Substitution of fish meal partially (25%PP group) with the mixture of plant proteins significantly increased the activity of SOD and T-AOC ($p < 0.05$), while the activity of SOD and T-AOC in the 25%PP + AA group showed no difference compared with the Con group ($p > 0.05$). Compared with the 25%PP group, CAA supplementation significantly decreased the MDA content ($p < 0.05$); in addition, the content of MDA in the 25%PP + AA group had no difference compared with the Con group ($p > 0.05$) (Figure 3). Additionally, the activity of ACP had the highest value in the 25%PP group, and the lowest appeared in the Con group ($p < 0.05$). The activity of AKP in the 25%PP group was higher than the Con and 25%PP + AA group ($p < 0.05$), and a significant difference did not appear between the Con and 25%PP + AA group ($p > 0.05$) (Figure 4).

3.5. Intestinal Histology. Intestinal histology is shown in Figure 5. The villus height of the 25%PP group was significantly lower than that of the control group and the 25%PP + AA group ($p < 0.05$), while there was no significant difference between the control group and the 25%PP + AA group ($p > 0.05$).

Table 5: Amino acid composition (g/100 g dry matter) of muscle in yellow catfish (mean ± SD).

Amino acid name	Con	25%PP	25%PP + AA
EAA			
Arg	4.34 ± 0.08^a	4.70 ± 0.01^b	5.11 ± 0.07^c
His	1.76 ± 0.04^a	1.94 ± 0.01^b	2.12 ± 0.01^c
Ile	3.29 ± 0.05^a	3.56 ± 0.01^b	3.91 ± 0.04^c
Lys	6.99 ± 0.11^a	7.59 ± 0.00^b	8.35 ± 0.04^c
Met	1.69 ± 0.27^a	2.19 ± 0.02^b	2.16 ± 0.02^b
Phe	3.32 ± 0.06^a	3.57 ± 0.01^b	3.90 ± 0.02^c
The	3.47 ± 0.06^a	3.73 ± 0.02^b	4.09 ± 0.03^c
Val	3.54 ± 0.05^a	3.82 ± 0.03^b	4.18 ± 0.03^c
Leu	5.99 ± 0.08^a	6.47 ± 0.02^b	7.14 ± 0.03^c
NEAA			
Ala	4.33 ± 0.09^a	4.63 ± 0.04^b	5.03 ± 0.06^c
Asp	7.84 ± 0.16^a	8.47 ± 0.05^b	9.25 ± 0.05^c
Glu	11.80 ± 0.18^a	12.68 ± 0.33^b	14.06 ± 0.11^c
Gly	3.61 ± 0.12^a	3.78 ± 0.04^b	3.91 ± 0.12^c
Pro	2.40 ± 0.06^a	2.52 ± 0.03^b	2.67 ± 0.06^c
Ser	3.19 ± 0.06^a	3.38 ± 0.16^b	3.73 ± 0.05^c
Tyr	2.59 ± 0.05^a	2.84 ± 0.02^b	3.11 ± 0.02^c

The different and superscript letters ($^{a, b, c}$) represent existing significant differences; lowercase "a" represents the lower value, lowercase "b" represents the higher value, and lowercase "c" represents the highest value ($p < 0.05$). The absence of lowercase letters indicates no significant difference. The final data are presented in the table as the mean ± standard deviation of 3 repetitions. Abbreviations: EAA: essential amino acid; NEAA: nonessential amino acid; Arg: arginine; His: histidine; Ile: isoleucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Thr: threonine; Val: valine; Leu: leucine; Ala: alanine; Asp: aspartic acid; Glu: glutamic acid; Gly: glycine; Pro: proline; Ser: serine; Tyr: tyrosine.

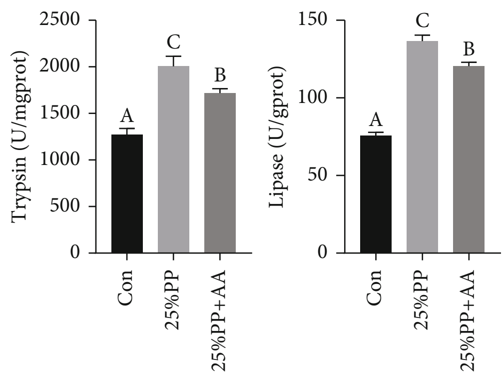


Figure 1: The activities of trypsin and lipase in the liver. The different letters (a, b, and c) represent existing significant differences; lowercase "a" represents the lowest value, and lowercase "c" represents the highest value ($p < 0.05$). The vertical bars represent the mean ± SD ($n = 3$).

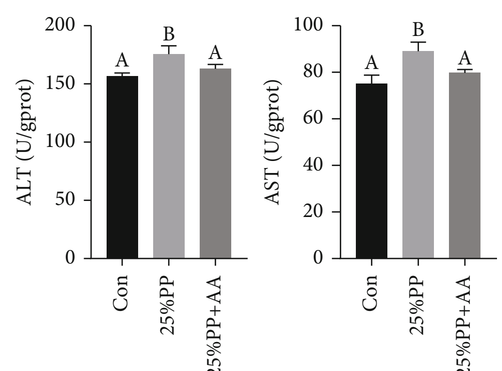


Figure 2: The activities of ALT and AST in the liver of yellow catfish. The different letters (a, b, and c) represent existing significant differences; lowercase "a" represents the lowest value, and lowercase "c" represents the highest value ($p < 0.05$). The vertical bars represent the mean ± SD ($n = 5$). ALT: alanine transaminase; AST: aspartate aminotransferase.

3.6. Correlation Analysis. In the Con vs. 25%PP group, correlation analysis showed that the contents of Tyr, Val, Lys, Ile, Leu, Arg, Phe, His, Asp, Thr, Ala, and Glu in muscle were a positive correlation with FCR, while they were a negative correlation with SGR and WG. In addition, Tyr, Val, Lys, Ile, Leu, Arg, Phe, His, Asp, Thr, and Ala content exhibited a negative correlation with CP and SUR. Met content showed a negative correlation with CP (Figure 6(a)). In the 25%PP vs. 25%PP + AA group, Gly, Tyr, Val, His, Asp, Thr, Leu, Lys, Phe, Glu, Ala, Arg, Ile, Pro, and Ser showed a positive correlation with SUR. His, Asp, Thr, Leu, Lys, Phe, Glu, Ala, Arg, Ile, and Ser showed a positive correlation with CP. Tyr, Val, His, Asp, Thr, Leu, Lys, Phe, Glu, Ala, Arg, and Ile showed a positive correlation with SGR and WG. Tyr, Val, His, Asp, Thr, Leu, Lys, Phe, Glu, Ala, Arg, Ile, and Ser showed a negative correlation with FCR. Met content exhibited a negative correlation with SGR and WG (Figure 6(b)). In the Con vs. 25%PP + AA group, there was no correlation between muscle content and growth performance (Figure 6(c)). In the control group vs. 25%PP group, correlation analysis showed that the contents of His, Thr, Ala, Ile, Gly, Lys, and Met in the diet showed a negative correlation with the contents of Tyr, Val, His, Asp, Thr, Arg, Phe, Lys, Ile, and Leu in muscle (Figure 7(a)). At 25%PP vs. 25%PP + AA group, the content of Thr, Met, Lys Gly, and His in the diet showed a positive correlation with Gly, Thr, Ser, Pro, Leu, Ile, Glu, Asp, Ala, and Arg in muscle (Figure 7(b)). As shown in Figure 8(a), diet Thr, Gly, Lys, Met, His, Ala, and Ile content showed a negative correlation with ALT, ACP, FCR, LIP, T-AOC, AKP, TRY, SOD, and AST while showed a positive correlation with SUR, SGR, WG, CP. As shown in Figure 8(b), diet Thr, Gly, Lys, Met, His, Ala, and Ile content showed a negative correlation with T-AOC, AKP, FCR, SOD, LIP, ACP, MDA, ALT, TRY, and AST while showed a positive correlation with SGR, CP, SUR, and WG.

4. Discussion

After 8 weeks of feeding experiment, the WG, SGR, and SUR of yellow catfish in the 25%PP group were significantly decreased, while the FCR was increased compared with the control group. This suggests that ingestion of unbalanced amino acids may negatively affect growth performance and feed efficiency. A previous study was also consistent with the results of this study that the replacement of fishmeal with mixed plant protein could reduce WG and improve the FCR of blunt snout bream [23]. The other study also showed that partial replacement of fishmeal with mixed plant protein decreased the WG of common carp (*Cyprinus carpio*), and its FCR was also significantly increased [28]. However, in the previous study, the addition of Met to plant protein instead of fishmeal resulted in better growth performance of juvenile gibel carp (*Carassius auratus Gibelio*) [29]; and the addition of Lys, Gly, Thr, and Met to plant feed can also improve the growth performance of rainbow trout [30]. In this study, WG, SGR, FCR, and SUR of yellow catfish were similar to those of the control group after balanced amino acid supplementation in the 25%PP group (25%PP + AA). This suggests that a balanced intake of amino acids is necessary for the growth performance of fish [31, 32]. In this study, the CP content of muscle in the 25%PP group was higher than Con and 25%PP + AA groups; this suggests that unbalanced amino acid intake can reduce muscle crude protein content, which recovered to control levels after CAA supplementation, suggesting that balanced amino acid intake is crucial for muscle protein accumulation. In addition, this study also found that the substitution of mixed plant protein for fishmeal has no effect on the crude lipid, moisture, and ash of yellow catfish, which is consistent with previous studies on tambaqui (*Colossoma macropomum*) and giant tiger prawn (*Penaeus monodon*) [15, 33]. This indicates that the level of partial replacement of fishmeal with mixed plant protein is not enough to affect body composition.

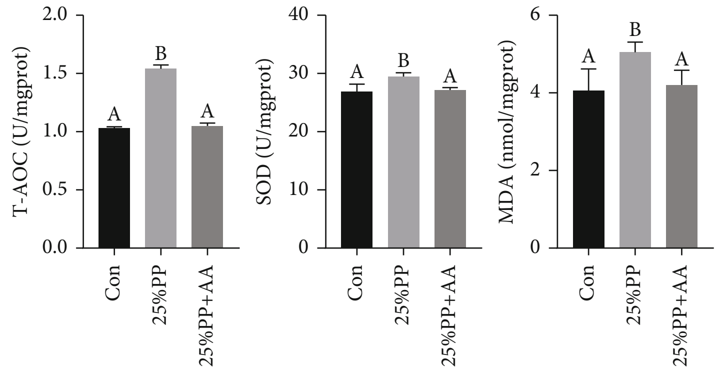


Figure 3: The activities of T-AOC, SOD, and MDA in the liver of yellow catfish. The different letters (a, b, and c) represent existing significant differences; lowercase "a" represents the lower value, and lowercase "b" represents the higher value ($p < 0.05$). The vertical bars represent the mean ± SD ($n = 5$). T-AOC: total antioxidant capacity; MDA: malondialdehyde; SOD: superoxide dismutase.

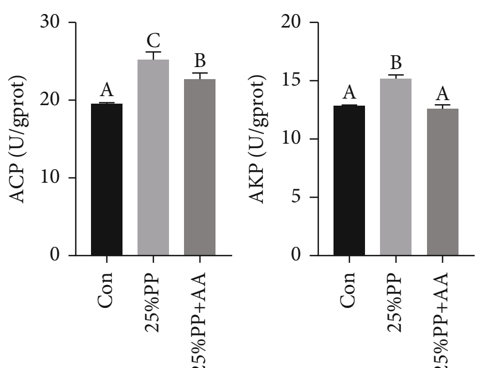


Figure 4: The activities of ACP and AKP in the liver of yellow catfish. The different letters (a, b, and c) represent existing significant differences; lowercase "a" represents the lowest value, and lowercase "c" represents the highest value ($p < 0.05$). The vertical bars represent the mean ± SD ($n = 5$). AKP: alkaline phosphatase; ACP: acid phosphatase.

Digestive enzymes hydrolyze large amounts of ingested nutrients and convert them into nutrient sources that aquatic animals can absorb and utilize [34]. The analysis of animal digestive enzyme activity is helpful to further understand the digestive ability, health status, digestion, and utilization of nutrients of animals [35, 36]. The digestive enzyme activities were decreased with the increase of plant protein content in the diet after replacing fishmeal with plant protein sources [37]. However, in this study, the activities of TRY and LIP were significantly increased in the 25%PP group, and TRY and LIP activity in the 25%PP + AA group was lower than in the 25%PP group. Combined with growth indexes, in the condition of unbalanced amino acid intake (SUR, SGR, and WG decreased in the 25%PP group), even further activation of digestive function (TRY and LIP increased in the 25%PP group) could not improve growth performance. On the contrary, adding CAAs to ensure the balance of amino acid intake could not only significantly improve the digestive capacity of yellow catfish but also restore the growth performance to the level of the Con group (SUR, SGR, WG, and 25%PP + AA group had no significant difference). In conclusion, the introduction of plant protein components can increase the activity of protein digestive enzymes but limit the growth performance, which may be because the unbalanced intake of amino acids limits the absorption and utilization of protein decomposition and digestive products. Therefore, supplementation of deficient amino acids can not only improve the digestive enzyme activities of yellow catfish but also have no negative effect on growth performance.

AST and ALT are crucial indexes for evaluating hepatocyte injury and hepatopancreas function [28]. Unhealthy organisms tend to have higher transaminase activity [34, 38]. The activities of AST and ALT of yellow catfish in the 25%PP group were significantly increased, while they recover to the Con group level in the 25%PP + AA group, suggesting the substitution of mixed plant protein for fishmeal can induce liver injury and adversely affect the health status of yellow catfish; CAA supplements could reverse this tendency. A balanced intake of amino acids is essential for liver health.

In this study, liver T-AOC, SOD activity, and MDA content were significantly increased in the 25%PP group, which may be due to the impact of antinutritional factors on plant proteins. Viana et al. [39] also reported that antioxidant factors (SOD and GPx) in soybean meal can induce the expression of the antioxidant enzyme gene in California yellowtail (*Seriola dorsalis*). Therefore, in this study, the addition of mixed plant protein resulted in higher antioxidant activity and growth restriction of yellow catfish. After CAA supplementation, the T-AOC, SOD activity, and MDA content in the 25%PP + AA group recovered to the level of the Con group. These results suggest that the balanced amino acid intake may have a moderating effect on the oxidative stress response induced by nutritional factors from mixed plant proteins.

Fish as a lower vertebrate and nonspecific immunity is more developed than specific immunity [40, 41]. AKP and ACP can promote the transport and absorption of nutrients and enhance the specific immunity of organisms [42, 43]. Increased activity of ACP and AKP indicates activation and enhancement of aquatic immune function [44, 45].

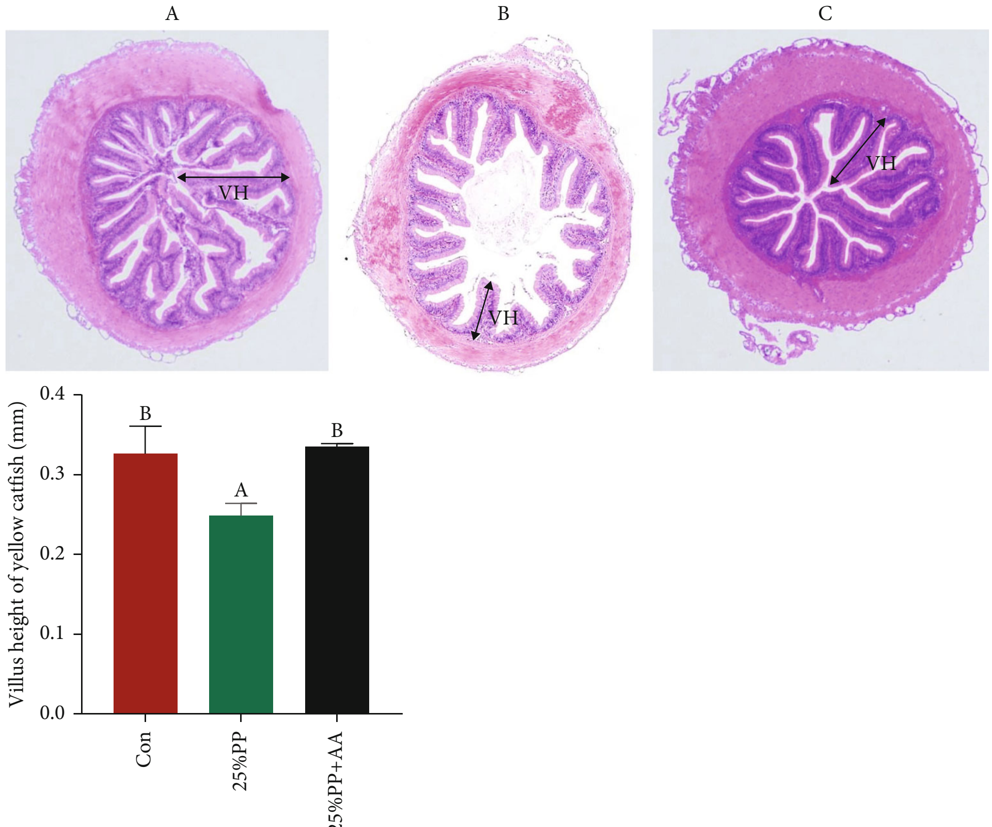


Figure 5: Intestinal histology of yellow catfish. Histological comparison of intestinal differences of yellow catfish in the control, 25%PP, and 25%PP + AA group (a–c). The different letters (a, b, and c) represent existing significant differences, with lowercase "a" representing the lower value and lowercase "b" representing the higher value ($p < 0.05$). The magnification was 200×. VH: villus height.

The activities of AKP and ACP in the liver were increased in the 25%PP group compared with the Con group, which may be due to the antinutritional factors in plant proteins that activate the immune system of yellow catfish. Nevertheless, ACP and AKP activities in the 25%PP + AA group were lower than in the 25%PP group, suggesting that balanced amino acid intake also has certain positive effects on the immune response.

In this study, intestinal morphology was investigated, and the results showed that there was no intestinal inflammation or degeneration caused by mixed plant proteins in the intestinal biopsy of yellow catfish. The only significant difference was found in the 25%PP group, where VH was significantly decreased compared to the control group and the 25%PP + AA group. Higher VH meant greater surface area for absorption of nutrients and metabolites [46]. Interestingly, even in the presence of plant proteins, the intestinal structure is not damaged by ensuring a balanced intake of amino acids. Therefore, we speculate that unbalanced amino acid intake may decrease the height of VH, which may disrupt the absorption and utilization of nutrients and induce a decrease in growth performance [6].

The contents of all amino acids in muscle in the 25%PP group were significantly and slightly higher than those in the Con group. Correlation analysis showed that there was a significant negative correlation between some amino acids in muscle (Tyr, Val, Lys, Ile, Leu, Arg, Phe, His, Asp, Thr, Ala, and Glu) and growth performance (CP, SUR, SGR, and WG). These results indicate that the accumulation of amino acids in muscle showed a significant correlation to the inhibition of growth and development of yellow catfish. This may be due to the antagonistic action between amino acids. The previous study has shown that the growth performance, antioxidant capacity, and antistress ability of Japanese flounder (*Paralichthys olivaceus*) were significantly affected by the antagonistic effect of Arg and His on juvenile Japanese flounders fed with different ratios of Arg and His for 70 days [47]; Yamamoto et al. [48] showed that excessive Leu from corn gluten meal had a significant antagonistic effect on other branch chain amino acids, which reduced the growth performance of rainbow trout. In conclusion, the increase of amino acid content in muscle was not the result of nutrient accumulation but probably resulted from the abnormal accumulation of amino acids due to the decrease of amino acid utilization capacity of yellow catfish under the antagonistic action of various amino acids. On the other hand, in 25%PP and 25%PP + AA groups, the increase of amino acids (Tyr, Val, Lys, Ile, Leu, Arg, Phe, His, Asp, Thr, Ala, Glu, Pro, Gly, and Ser) content in muscle exhibited a significant positive correlation tendency with growth indexes (CP, SUR, SGR, and WG). This suggests that there is no antagonism of amino acids in muscle, so the

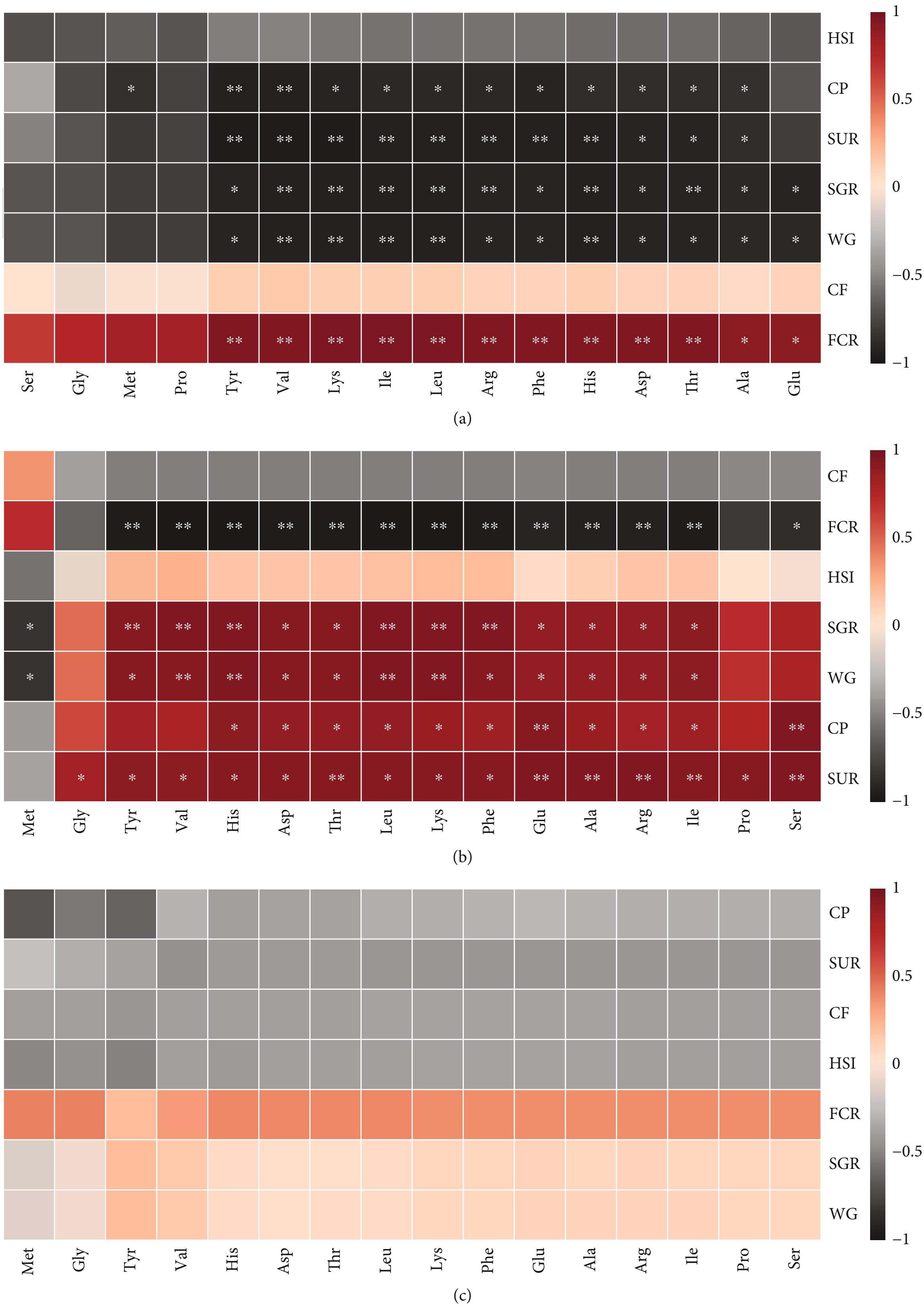
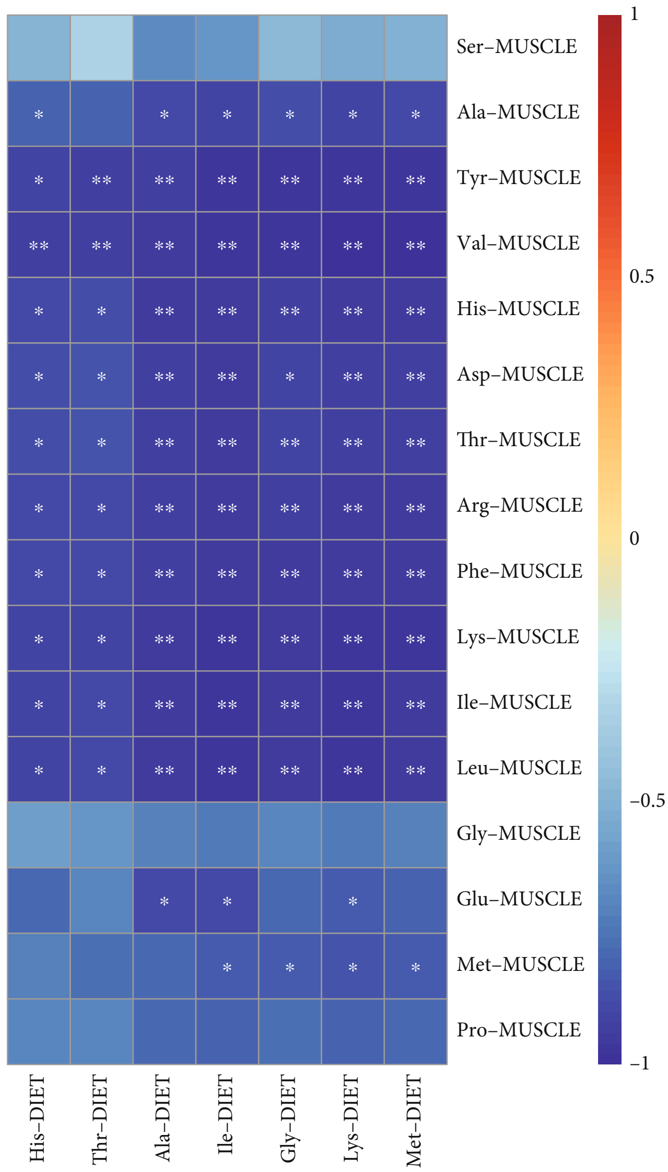
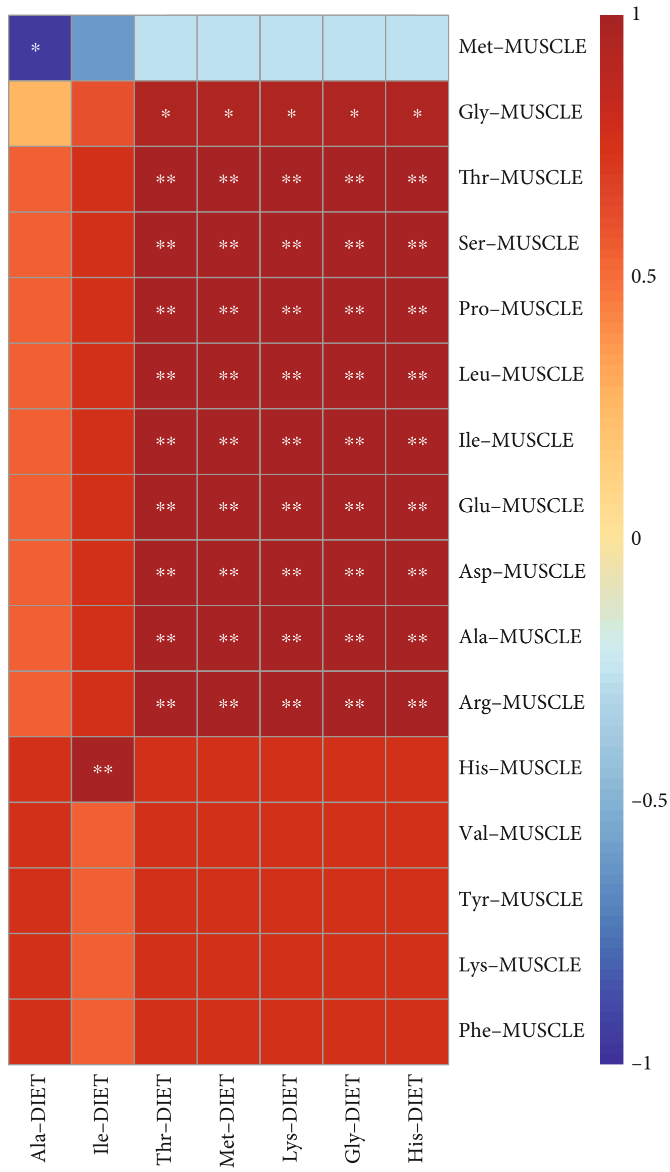


Figure 6: Significant correlations between muscle amino acid content and growth performance in Con vs. 25%PP group (a), 25%PP group vs. 25%PP + AA group (b), and Con vs. 25%PP + AA group (c). A significant difference was marked as $*$ at $p < 0.05$ and $**$ at $p < 0.001$. Arg: arginine; His: histidine; Ile: isoleucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Thr: threonine; Val: valine; Leu: leucine; NEAA: nonessential amino acid; Ala: alanine; Asp: aspartic acid; Glu: glutamic acid; Gly: glycine; Pro: proline; Ser: serine; Tyr: tyrosine.



(a)

Figure 7: Continued.

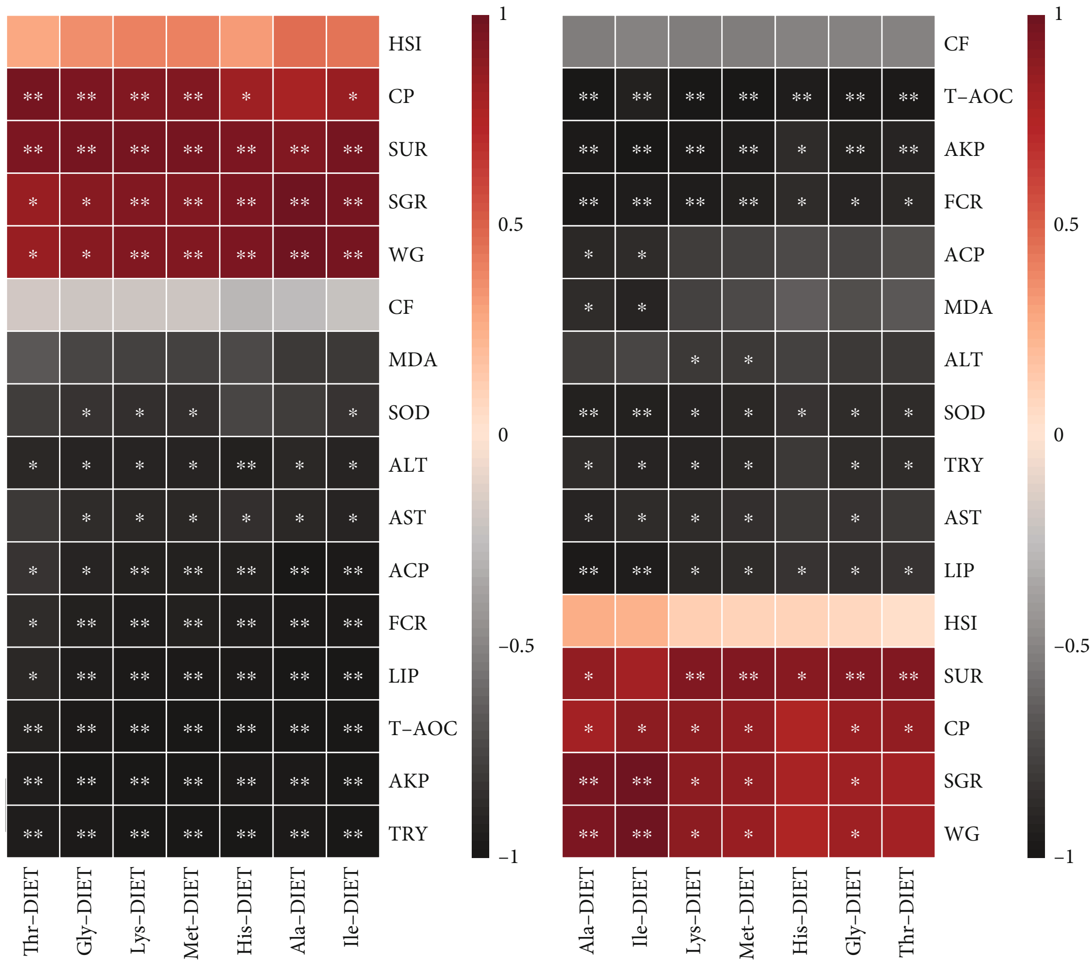


(b)

Figure 7: Significant correlations between diet amino acid content and muscle amino acid content (a) control group vs. 25%PP group and (b) 25%PP group vs. 25%PP + AA group; a significant difference was marked as ∗ at $p < 0.05$ and ∗∗ at $p < 0.01$. Arg: arginine; His: histidine; Ile: isoleucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Thr: threonine; Val: valine; Leu: leucine; NEAA: nonessential amino acid; Ala: alanine; Asp: aspartic acid; Glu: glutamic acid; Gly: glycine; Pro: proline; Ser: serine; Tyr: tyrosine; WG: weight gain (%); SGR: specific growth rate (%); SUR: survival (%); FCR: feed conversion ratio.

increase in amino acids in muscle may be a normal accumulation of nutrients. Previous studies on kuruma shrimp (*Marsupenaeus japonicus*) showed that dietary supplementation of CAA can overcome the negative effects caused by high plant protein diets, and the supplementation of CAAs significantly improved the growth performance and protein deposition of kuruma shrimp [5, 9]. In the Con group and 25%PP + AA group, there was no correlation between the increase of muscle amino acid content and growth performance, and there was no significant difference in growth indexes between the two groups, suggesting that the two diets have similar effects on yellow catfish. However, the amino acid content in muscle of the 25%PP + AA group was slightly higher than that of the con group, suggesting that the dietary pattern in which fish meal is replaced by partial plant protein and supplementation of CAAs may promote the accumulation of amino acids in muscle.

Correlation analysis indicated that the decrease of dietary amino acid content may be associated with the abnormal increase of amino acid content in muscle, the decrease of crude protein content, the decrease in survival rate, the activation of the antioxidant and immune system, the decrease in growth performance, and the increase of digestive enzyme activity. This suggests that amino acids and physiological parameters of muscle are regulated by dietary amino acid composition. After supplementing with CAAs



(a) (b)

Figure 8: Significant correlations between diet amino acid content and growth parameters, physiological index, antioxidant status, and immunologic parameters in the liver: (a) control group vs. 25%PP group and (b) 25%PP group vs. 25%PP + AA group; a significant difference was marked as ∗ at $p < 0.05$ and ∗∗ at $p < 0.001$. His: histidine; Ile: isoleucine; Lys: lysine; Met: methionine; Thr: threonine; Ala: alanine; Gly: glycine; WG: weight gain (%); SGR: specific growth rate (%); SUR: survival (%); HSI: hepatosomatic index (%); CF: condition factor (%); FCR: feed conversion ratio; TRY: trypsin; LIP: lipase; SOD: superoxide dismutase; T-AOC: Total antioxidant capacity; MDA: malondialdehyde; ALT: alanine transaminase; AST: aspartate aminotransferase; AKP: alkaline phosphatase; ACP: acid phosphatase; CP: rude protein.

in the 25%PP group, the increase of dietary amino acid content was related to the accumulation of various amino acids in muscle, the increase of crude protein content, and the increase of growth parameters of yellow catfish. This suggests that plant protein diets with balanced amino acid composition can promote muscle nutrient accumulation to achieve similar growth performance as whole fish meal diets.

5. Conclusion

Based on the findings of this study, compared with other treatment groups, an amino acid imbalance diet has adverse effects on the growth, performance, and survival of yellow catfish. The content of amino acids in muscle was significantly increased compared to the control group indicating the imbalance of amino acid intake can not only harm growth performance, activate the antioxidant and immune systems, cause liver and intestinal damage, but also cause abnormal accumulation of amino acid content in muscle due to the antagonistic effect of amino acid. After being supplemented with CAAs in the 25%PP group, balanced amino acid intake resulted in better growth performance and nutrient accumulation in muscle than in the control group. Although the addition of plant protein has a limiting factor in the physiological status of yellow catfish, the substitution of fish meal with part of plant protein and the supplement of CAAs is also a reasonable dietary pattern.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no competing or financial interests.

Authors' Contributions

Shidong Wang and Ming Li designed the experiments; Shidong Wang, Xue Li, and Muzi Zhang carried out the experimental work; Shidong Wang wrote the manuscript under the direction of Ming Li and Haibo Jiang.

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