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Effects of Replacing Fish Meal with Stickwater Hydrolysate and Meal on the Growth, Serum Biochemical Indexes, and Muscle Quality of Yellow Catfish (*Tachysurus fulvidraco*)

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Abstract: An eight-week feeding experiment was conducted to evaluate the effects of replacing fish meal with stickwater hydrolysate (SWH) or stickwater hydrolysate meal (SWM) on the growth, serum biochemical parameters, intestinal digestive enzyme activity, and muscle quality of yellow catfish (*Tachysurus fulvidraco*). The control diet (CON) contained 30% fish meal and the remaining five diets were substituted for fish meal with 2.5% (SWM2.5), 5% (SWM5) SWM, and 5% (SWH5), 10% (SWH10), and 15% (SWH15) SWH, respectively. The results showed that there were no significant differences in weight gain rate, feed conversion rate, survival rate, hepatosomatic index, and viscerosomatic index among the groups. The substitution of fish meal with SWH significantly augmented the serum triglyceride and total cholesterol levels, whereas urea nitrogen content exhibited a reduction proportional to the replacement ratio. The incorporation of SWH led to a notable rise in glutamate-pyruvate transaminase activity, albeit with a gradual decline as the substitution ratio escalated. Relative to the CON group, the SWH5 group displayed a significant reduction in serum superoxide dismutase activity and a significant elevation in serum catalase activity. The substitution of fish meal with SWM yielded noticeable increments in the activities of complement 3, immunoglobulin M, and alkaline phosphatase. Neither SWH nor SWM exerted a substantial influence on intestinal amylase activity. Regarding muscle characteristics, neither SWH nor SWM showed a marked effect on hardness and springiness; however, adhesiveness, cohesiveness, gumminess, and chewiness properties exhibited enhancement as the proportion of fish meal replacement increased. In conclusion, within this experimental context, substituting fish meal with SWH and SWM did not adversely impact the growth and meat quality of yellow catfish. Specifically, replacing 15% fish meal with stickwater hydrolysate and 5% fish meal with stickwater hydrolysate meal contributed to an enhanced immune capacity in yellow catfish to a certain extent.

Keywords: *Tachysurus fulvidraco*; fish meal substitution; stickwater hydrolysate (meal); growth; serum biochemistry; serum immunity

Key Contribution: Substituting fish meal with SWH and SWM did not adversely impact the growth performance and contributed to an enhanced immune capacity in yellow catfish.

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1. Introduction

Over the last few years, the rapid development and expansion of aquaculture have highlighted a growing concern regarding the shortage of raw materials for aquatic feed, particularly protein feedstuffs [1]. Recently, protein feedstuffs, exemplified by fish meal, are currently experiencing global scarcity, leading to an imbalance between supply and

demand that subsequently drives up fish meal prices and, consequently, escalates aquaculture production costs [2]. These factors seriously restrict the sustainable development of aquaculture, and reducing the content of fish meal in compound feed has become a hot spot in current research. Fish meal has always been an indispensable high-quality protein source in aquatic feed due to its high content of essential amino acids and fatty acids, good palatability, and less anti-nutritional factors [3]. However, the majority of plant protein sources that contain antinutritional factors, such as free gossypol, phytic acid, and soybean antigen proteins, have the potential to stunt growth, reduce digestive enzyme activity, and decrease immunity of aquatic animals [4–6]. Moreover, plant-derived protein sources exhibit markedly lower contents of minor nitrogenous compounds, such as peptides, free amino acids, and taurine in comparison to animal-derived counterparts. Several of these compounds, including antigenic proteins, gossypol, and its derivatives, phytic acid, and tannins, hold the capacity to exert adverse impacts on fish growth and overall health [7]. Therefore, the exploration of innovative animal protein sources as substitutes for fish meal stands as a paramount research focus in contemporary aquaculture [8].

In industrial fish meal production lines, stickwater is extracted from the liquid produced during the fish meal pressing process. Stickwater hydrolysate (SWH) is obtained by adding hydrolytic enzymes, such as pineapple proteinase or a solution of pa-paya protease, to stickwater at temperatures below 50–55 °C for 3–5 h. [9]. Owing to the substantial presence of suspended particulates, encompassing proteins, peptides, amino acids, biogenic amines, and trimethylamine oxides, among others, stickwater also encompasses minor quantities of oil constituents and trace elements, all of which bestow an indispensable impact upon the overall nutritional profile of fish [10]. Research has demonstrated that the addition of stickwater to the feed improves the growth performance and feed utilization of juvenile snakehead (*Ophiocephalus argus*) [11]. Over the years, plenty of studies have demonstrated that hydrolyzed fish proteins obtained by protease-mediated hydrolysis not only induce fish feeding but also promote fish growth [10–12].

Yellow catfish (*Tachysurus fulvidraco*), is one of China's important economic aquaculture fish, and the production of it has been growing steadily, reaching 587,800 tonnes in 2022 [13]. The proportion of fish meal in its compound feed is as high as 28–35%, which is much higher than that of many other freshwater cultured fish [14]. Therefore, this study aimed to evaluate the effects of replacing fish meal with stickwater hydrolysate and its meal on the growth, serum biochemical, immune indexes, intestinal digestive enzyme activity, and muscle quality of yellow catfish. This provides a theoretical basis for improving the application of enzymatic hydrolysis of stickwater hydrolysate and stickwater hydrolysate meal in fish feed and effectively reducing the cost of aquaculture.

2. Materials and Methods

2.1. Preparation of the Experimental Diet Stickwater Hydrolysate (Meal)

The SWH used in this study was supplied by Zhejiang Fengfu Marine Organism Products Co. Ltd. (Zhoushan, China). The chemical composition and peptide content of fish meal (FM), stickwater hydrolysate (SWH), and stickwater hydrolysate (SWM) are shown in Table 1. The free amino acid composition of FM, SWH, and SWM is shown in Table 2. It can be seen from Tables 1 and 2 that the peptide content, acid-soluble protein, and free amino acid content of SWH and SWM are significantly higher than FM.

Table 1. Nutrient levels of FM, SWH, and SWM (DM basis).

Items	FM	SWH	SWM
Moisture%	8.27	42.10	2.69
Crude protein%	72.48	70.64	75.54
Crude fat%	9.72	4.43	1.13
Ash%	12.37	14.72	15.01
ASP% [1]	4.08	65.47	71.09

Table 1. *Cont.*

Items	FM	SWH	SWM
	Peptide molecular weight distribution/u [2]		
<180 Da	1.97 (48.31)	15.76 (24.06)	16.27 (22.89)
500–180 Da	0.81 (19.78)	20.69 (31.61)	29.98 (42.17)
1000–500 Da	0.16 (3.86)	9.4 (14.33)	9.66 (13.59)
2000–1000 Da	0.19 (4.62)	8.32 (12.73)	7.41 (10.43)
3000–2000 Da	0.16 (3.97)	4.04 (6.19)	2.95 (4.15)
5000–3000 Da	0.24 (5.94)	3.81 (5.82)	2.42 (3.40)
10,000–5000 Da	0.25 (6.13)	2.73 (4.18)	1.54 (2.17)
>10,000 Da	0.30 (7.39)	0.72 (1.08)	0.85 (1.20)

[1] The content of acid soluble protein (ASP) was analyzed by the Kjeldahl method (GB/T22729-2008) in the Analysis and Testing Center, Hunan Agricultural University (Changsha, China). [2] The peptide molecular weight distribution was measured by HPLC (GB/T22729-2008) in the Analysis and Testing Center, Hunan Agricultural University (Changsha, China). The values outside brackets were the contents of acid soluble protein, and the values inside brackets were the percentages of peptide content.

Table 2. Composition of free amino acids of FM, SWH, and SWM (g/100 g, dry matter).

Items	FM	SWH	SWM
Valine	0.01	0.13	0.60
Methionine	0.02	0.06	0.18
Isoleucine	0.01	0.72	0.91
Leucine	0.02	1.43	1.21
Phenylalanine	0.01	0.84	0.79
Histidine	0.01	0.41	0.42
Lysine	0.03	0.78	0.75
Arginine	0.01	0.26	0.65
Threonine	0.00	0.42	0.64
Tryptophan	0.01	1.82	0.98
Aspartic acid	0.01	0.08	0.19
Serine	0.01	0.06	0.15
Glutamic acid	0.00	0.02	0.01
Glycine	0.24	0.51	0.47
Alanine	0.02	0.86	0.83
Cystine	ND [1]	ND	0.12
Tyrosine	0.01	0.12	0.32
Proline	0.02	0.27	0.26
Total amino acid	0.44	8.79	9.48

[1] "ND" indicates that it cannot be measured.

This experiment consisted of a total of six treatment groups, designated as the control group, two groups in which SWM replaced 2.5% and 5.0% of fish meal, and three groups in which SWH replaced 5%, 10%, and 15% of fish meal. These groups were labeled as CON, SWM2.5, SWM5, SWH5, SWH10, and SWH15. Before feed preparation, the feed raw materials were crushed, screened through a 60-mesh sieve, and weighed accurately according to the formula. The mixture of raw materials was blended from small to large proportions, and the trace components were evenly mixed using the stepwise expansion method. All dry matter raw materials were mixed first, followed by the oil source and water, which were mixed separately and thoroughly blended. The mixed raw materials were then processed using a single-screw extrusion-type feed puffing machine to produce pellet feed with particle size of 1.5 mm and 2.0 mm, with a ratio of 3:7. The pellet feed was air-dried in a cool place and stored for later use. The composition and nutrient levels of basic diet are shown in Table 3. The free amino acid and total amino acid composition of experimental diet are shown in Tables 4 and 5.

Table 3. Composition and nutrient levels of experimental diet (air-dry basis), percentages.

Ingredients	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Fish meal	30.00	27.50	25.00	25.00	20.00	15.00
Stickwater hydrolysate meal		2.23	4.46			
Stickwater hydrolysate				5.13	10.26	15.39
Soybean meal	18.00	18.00	18.00	18.00	18.00	18.00
Cottonseed meal	4.00	4.00	4.00	4.00	4.00	4.00
Rapeseed meal	4.00	4.00	4.00	4.00	4.00	4.00
Beer yeast	6.00	6.00	6.00	6.00	6.00	6.00
Corn gluten meal	5.00	5.00	5.00	5.00	5.00	5.00
Fish oil	0.00	0.24	0.49	0.47	0.93	1.40
High-protein flour	24.00	24.00	24.00	24.00	24.00	24.00
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50	1.50
Soybean oil	3.40	3.40	3.40	3.40	3.40	3.40
Premix [1]	1.20	1.20	1.20	1.20	1.20	1.20
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Antioxidants [2]	0.01	0.01	0.01	0.01	0.01	0.01
Mould inhibitor [3]	0.03	0.03	0.03	0.03	0.03	0.03
Wheat middling	2.36	2.39	2.41	1.76	1.16	0.56
Nutrient composition [4]						
Crude protein	38.85	38.74	38.68	38.81	38.64	38.75
Crude fat	8.09	8.15	8.24	8.14	8.08	8.11
Crude ash	11.15	11.23	11.19	11.32	11.27	11.21

[1] Premix provides KCl 200 mg, KI (1%) 60 mg, $CoCl_2 \cdot 6H_2O$ (1%) 50 mg, $CuSO_4 \cdot 5H_2O$ 30 mg, $FeSO_4 \cdot H_2O$ 400 mg, $ZnSO_4 \cdot H_2O$ 400 mg, $MnSO_4 \cdot H_2O$ 150 mg, $Na_2SeO_3 \cdot 5H_2O$ (1%) 65 mg, $MgSO_4 \cdot H_2O$ 2000 mg, Zeolite Powder 3645.85 mg, VB_1 12 mg, Riboflavin 12 mg, VB_6 8 mg, VB_{12} 0.05 mg, VK_3 8 mg, Inositol 100 mg, Pantothenic Acid 40 mg, Niacin Acid 50 mg, Folic Acid 5 mg, Biotin 0.8 mg, VA 25 mg, VD 35 mg, VE 50 mg, VC 100 mg, Ethoxyquin 150 mg, and Wheat Flour 2434.15 mg per kilogram of diets; [2] The main component of Antioxidant is Ethoxyquin; [3] The main component of Mould Inhibitor is Calcium Propionate; [4] Nutrient compositions were calculated values.

Table 4. Free amino acid compositions of experimental diet (air-dry basis, g/kg).

Items	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Essential amino acid						
Valine	0.26	0.46	0.90	0.57	0.91	1.12
Methionine	0.08	0.15	0.27	0.13	0.29	0.36
Isoleucine	0.23	0.37	0.62	0.38	0.56	0.74
Leucine	0.47	0.60	1.07	0.68	1.22	1.39
Phenylalanine	0.21	0.29	0.51	0.37	0.59	0.76
Histidine	1.46	1.51	2.24	1.49	2.46	2.67
Lysine	0.37	0.54	0.92	0.54	0.82	1.08
Arginine	0.97	1.12	1.93	1.67	2.19	2.85
Threonine	0.18	0.22	0.46	0.29	0.41	0.52
Tryptophan	0.19	0.21	0.30	0.22	0.27	0.34
Non-essential amino acid						
Aspartic acid	0.24	0.31	0.50	0.32	0.43	0.67
Serine	0.18	0.24	0.49	0.34	0.52	0.79
Glutamic acid	0.55	0.71	1.12	0.83	1.25	1.69
Glycine	0.25	0.76	1.20	0.62	0.88	1.42
Alanine	1.21	1.46	2.27	1.54	1.87	2.32
Cystine	0.02	0.03	0.10	0.03	0.09	0.14
Tyrosine	0.22	0.33	0.69	0.36	0.63	0.72
Proline	0.39	0.45	0.68	0.44	0.53	0.71
Total amino acid	7.48	9.76	16.27	10.82	15.92	20.29

Table 5. Total amino acid compositions of experimental diet (air-dry basis, g/kg).

Items	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Essential amino acid						
Valine	2.11	2.06	2.01	1.98	2.12	2.06
Methionine	0.83	0.74	0.79	0.68	0.88	0.86
Isoleucine	1.89	1.78	1.75	1.74	1.83	1.81
Leucine	3.50	3.39	3.32	3.40	3.51	3.46
Phenylalanine	1.96	1.91	1.88	1.87	1.99	1.91
Histidine	1.49	1.36	1.42	1.55	1.52	1.58
Lysine	2.56	2.33	2.38	2.37	2.46	2.43
Arginine	2.68	2.73	2.70	2.69	2.62	2.72
Threonine	1.75	1.62	1.64	1.68	1.76	1.69
Tryptophan	ND [1]	ND	ND	ND	ND	ND
Non-essential amino acid						
Aspartic acid	3.98	3.94	4.02	3.85	4.01	3.94
Serine	1.93	1.91	1.88	1.87	1.89	1.81
Glutamic acid	7.43	7.74	7.92	7.44	7.58	7.55
Glycine	2.25	2.57	2.64	2.61	2.40	2.32
Alanine	2.62	2.55	2.76	2.68	2.66	2.61
Cystine	0.12	0.16	0.21	0.19	0.20	0.14
Tyrosine	1.14	1.23	1.19	1.21	1.17	1.30
Proline	1.94	2.02	2.11	2.20	2.13	1.95
Total amino acid	40.18	40.04	40.62	40.01	40.73	40.14

[1] "ND" indicates that it cannot be measured.

2.2. Experimental Design

Juvenile yellow catfish were purchased from a commercial farm and reared in cages at Chetianjiang Reservoir, Loudi, China. Fish were acclimatized to 2.0 m × 1.5 m × 1.5 m floating cages. Nine-hundred juvenile yellow catfish (initial body weight of 9.99 ± 0.02 g) were divided equally and randomly into six groups. Each group was fed in triplicate for a period of 56 days. The experimental yellow catfish were fed with 1.5 mm grain size feed in the early stage and 2.0 mm grain size feed in the late stage for better palatability. The fish were fed by the experimental diet twice per day according to 3–5% of body weight. The physicochemical parameters of the water were kept at optimal conditions during the culture period: temperature (27.3 ± 3.6 °C), pH (7.1 ± 0.3), dissolved oxygen (6.65 ± 0.25 mg/L), ammonia (0.01 ± 0.005 mg/L), and nitrite (0.01 ± 0.004 mg/L).

2.3. Sample Collection and Determination Method

2.3.1. Growth Parameters

At the end of the breeding trial, the yellow catfish were fasted for 24 h and weighed to determine growth performance. Total mantissa, total body weight, and total feeding volume of experimental fish in each cage were recorded. Five fish were randomly selected from each cage, body length, body weight, visceral mass, and liver mass were measured, and growth indicators were calculated.

The growth performance was evaluated in terms of Survival Rate (SR), Weight Gain Rate (WGR), Feed Conversion Ratio (FCR), Condition Factor (CF), Hepatosomatic Index (HSI), and Viseromatic index (VSI), which were calculated using the following formula.

$$\text{Survival rate (SR, \%)} = \text{Nf}/\text{Ni} \times 100$$

$$\text{Weight gain rate (WGR, \%)} = (\text{Wt} - \text{Wo})/\text{Wo} \times 100$$

$$\text{Feed conversion rate (FCR)} = \text{total amount of the feed consumed (g)}/(\text{Wt} - \text{Wo})$$

$$\text{Condition factor (CF, g/cm}^3\text{)} = \text{Wt} \times 100/(\text{body length})^3$$

$$\text{Hepatosomatic index (HSI, \%)} = \text{liver weight (g)}/\text{Wt} \times 100$$

$$\text{Viserosomatic index (VSI, \%)} = \text{visceral weight (g)}/\text{Wt} \times 100$$

where Ni and Nf are the initial and final numbers of fish, and Wo (g) and Wt (g) are the initial and final weights.

2.3.2. Serum Biochemical, Antioxidant, and Immune Parameters

Five yellow catfish were randomly selected from each cage, and blood was collected from the tail vein with a sterile syringe, placed in a 1.5 mL enzyme-free tube, stood at 4 °C for 24 h, was centrifuged at 3000× *g* for 10 min, and the upper layer of serum was collected and stored at −80 °C until use.

The concentrations of Triglycerides (TG), Total Cholesterol (TCHO), Urea Nitroge (UN), Glutamic Oxalacetic Transaminase (GOT), Glutamate Pyruvic Transaminase (GPT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Complement 3 (C3), Complement 4 (C4), Immunoglobulin M (IgM), Alkaline Phosphatase (AKP), and Catalase (CAT) in serum were determined by the commercial kit from Nanjing Jianxian Biotechnology Co., LTD (Nanjing, China). The determination method refers to the instruction of the kit.

2.3.3. Digestive Enzyme Activity Analysis

At the end of the culture experiment, three yellow catfish were randomly selected from each cage after 24 h of fasting, and the intestinal tissues were rapidly isolated, washed with normal saline, packed in 1.5 mL enzyme-free centrifuge tubes, and stored at −80 °C until use. The activities of trypsin and amylase in the intestine were determined by the commercial kit from Nanjing Jianxian Biotechnology Co., LTD (Nanjing, China).

2.3.4. Muscle Texture Determination

Three yellow catfish were randomly selected from each cage, and the TPA (TMS-PRO, FTC, Sterling, VA, USA) was used to measure the texture parameters of muscle hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness. The test conditions consisted of 2 consecutive compressional sessions at a constant rate of 30 mm/min with a deformation of 60% of the original length and an initial force of 0.1 N.

2.4. Statistical Analysis

The experimental data were analyzed by one-way analysis of variance (ANOVA), and Duncan's multiple-range test was used to compare the difference between the means. All results are expressed as means ± standard deviation of the means, and all statistical analyses were performed using SPSS 19.0. Differences were considered significant at p-value < 0.05.

3. Results

3.1. Growth Performance

There were no significant differences in final weight, WGR, FCR, SR, HSI, and VSI among all groups ($p > 0.05$). However, the WGR and VSI of experimental yellow catfish were reduced by 5% SWM and 5% SWH instead of fish meal (Table 6).

3.2. Serum Biochemical Indexes

The levels of TG, TCHO, and GPT in the SWM2.5 and SWM5 groups were significantly increased ($p < 0.05$), and the levels of UN and GOT were not significantly changed ($p > 0.05$). The TG and TCHO in the SWH5, SWH10, and SWH15 groups were significantly increased ($p < 0.05$), and the UN content showed a downward trend with the increase in replacement ratio ($p < 0.05$). The SWH10 group exhibited a notable rise in GOT activity, while the

introduction of SWH as a replacement for fish meal resulted in a substantial elevation in GPT activity ($p < 0.05$) (Table 7).

Table 6. Growth performance of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Initial weight (g)	9.97 ± 0.01	10 ± 0.01	10 ± 0.02	9.98 ± 0.01	10.01 ± 0.01	9.99 ± 0.001
Final weight (g)	50.37 ± 0.77	50.90 ± 0.51	47.92 ± 4.88	47.7 ± 2.68	50.77 ± 1.35	50.93 ± 0.66
WGR (%)	405.24 ± 8.22	403.52 ± 6.25	379.3 ± 49.48	377.84 ± 27.03	407.38 ± 13.15	409.89 ± 6.67
SGR (%)	2.7 ± 0.03	2.69 ± 0.06	2.63 ± 0.28	2.6 ± 0.09	2.71 ± 0.04	2.71 ± 0.02
SR (%)	100.00	100.00	100.00	100.00	100.00	98.00
FCR	1.48 ± 0.03	1.50 ± 0.07	1.63 ± 0.2	1.6 ± 0.11	1.47 ± 0.05	1.45 ± 0.02
HIS (%)	2.07 ± 0.09	2.14 ± 0.09	1.99 ± 0.21	2.11 ± 0.14	2.16 ± 0.08	1.96 ± 0.09
VSI (%)	18.12 ± 0.4	16.96 ± 0.86	16.02 ± 1.64	15.35 ± 0.7	16.22 ± 0.66	15.44 ± 0.63
CF	1.83 ± 0.03	1.97 ± 0.04	1.89 ± 0.08	1.89 ± 0.11	1.86 ± 0.04	1.98 ± 0.1

Table 7. Serum biochemical indexes of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
TG (mmol/L)	2.92 ± 0.16 ^{a}	4.04 ± 0.51 ^{b}	4.57 ± 0.23 ^{b}	3.47 ± 0.29 ^{a}	6.28 ± 0.6 ^{b}	6.81 ± 1.21 ^{b}
TCHO (mmol/L)	5.58 ± 0.11 ^{a}	6.8 ± 0.2 ^{b}	9.36 ± 0.09 ^{c}	7.92 ± 0.35 ^{b}	6.7 ± 0.92 ^{ab}	7.5 ± 0.22 ^{b}
UN (mmol/L)	5.09 ± 0.16 ^{b}	4.59 ± 0.04 ^{a}	5.27 ± 0.06 ^{b}	4.53 ± 0.08 ^{b}	4.65 ± 0.16 ^{b}	3.36 ± 0.14 ^{c}
GOT (U/L)	23.81 ± 1.59 ^{a}	24.68 ± 0.74 ^{a}	22.7 ± 0.58 ^{a}	23.89 ± 0.55 ^{a}	32.2 ± 2.37 ^{b}	27.81 ± 0.34 ^{ab}
GPT (U/L)	1.19 ± 0.05 ^{a}	2.34 ± 0.15 ^{b}	2.22 ± 0.14 ^{b}	3.24 ± 0.17 ^{c}	2.16 ± 0.35 ^{b}	1.25 ± 0.08 ^{a}

Values with different superscripts in the same row are significantly different ($p < 0.05$).

3.3. Serum Antioxident Indexes

The SOD activity in SWM5 group was significantly lower than that in the CON group ($p < 0.05$). The serum CAT activity in the SWM2.5 and SWM5 groups increased with the increase in replacement ratio, and the activity in the SWM5 group was significantly higher than that in the CON group ($p < 0.05$). MDA content did not change significantly ($p > 0.05$), but its content showed an increasing trend with the increase in substitution ratio (Table 8).

Table 8. Serum antioxidant indexes of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
SOD (U/mL)	66.65 ± 2.8 ^{b}	63.39 ± 2.12 ^{b}	45.01 ± 2.09 ^{a}	64.79 ± 1.42 ^{b}	73.68 ± 2.84 ^{c}	53.16 ± 1.86 ^{a}
CAT (U/mL)	1.84 ± 0.09 ^{a}	2.42 ± 0.37 ^{ab}	3.42 ± 0.11 ^{b}	2.68 ± 0.19 ^{b}	2.8 ± 0.18 ^{b}	2.63 ± 0.3 ^{b}
MDA (mmol/mL)	8.77 ± 0.07	8.77 ± 0.27	8.84 ± 0.22	8.75 ± 0.24	9.06 ± 0.67	9.27 ± 0.44

Values with different superscripts in the same row are significantly different ($p < 0.05$).

3.4. Serum Immune Indexes

Substitution of fish meal by SWM significantly increased serum C3 and IGM activities ($p < 0.05$). The C3 and IGM activities in the SWH5 group were significantly lower than that in the CON group ($p < 0.05$), but its content showed an increasing trend with the increase in substitution ratio. Moreover, the IGM activity was significantly increased in the SWH15 group ($p < 0.05$). The AKP activity was significantly higher in the SWM2.5 and SWH10 groups than in the CON group ($p < 0.05$), but its activity decreased when the substitution ratio increased (Table 9).

3.5. Digestive Enzyme Activities in the Intestine

There was no statistically significant influence ($p > 0.05$) on the Amy activity among the different diets ($p > 0.05$). However, the intestinal Amy activity in the SWH10 group had the highest difference. The substitution of 2.5% and 5% of fish meal with SWM or SWH demonstrated no significant impact on the intestinal Try activity of yellow catfish

($p > 0.05$). However, when 15% of fish meal was replaced by SWH, a notable enhancement in intestinal Try activity was observed ($p < 0.05$). (Table 10).

Table 9. Serum immune indexes of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
C3 (g/L)	0.4 ± 0.04 ^{a}	0.58 ± 0.07 ^{ab}	0.77 ± 0.15 ^{b}	0.24 ± 0.003 ^{b}	0.36 ± 0.03 ^{a}	0.43 ± 0.03 ^{a}
IGM (g/L)	0.7 ± 0.06 ^{a}	0.91 ± 0.07 ^{ab}	1.07 ± 0.14 ^{b}	0.54 ± 0.03 ^{a}	0.76 ± 0.05 ^{ab}	1.04 ± 0.33 ^{b}
AKP (King's unit/100 mL)	4.12 ± 0.23 ^{a}	4.59 ± 0.02 ^{b}	4.26 ± 0.13 ^{ab}	4.35 ± 0.19 ^{ab}	4.56 ± 0.18 ^{b}	4.08 ± 0.28 ^{a}

Values with different superscripts in the same row are significantly different ($p < 0.05$).

Table 10. Digestive enzyme activities in the intestine of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Amy (U/mg)	1.87 ± 0.1	1.7 ± 0.34	1.27 ± 0.12	1.46 ± 0.11	2.02 ± 0.21	1.76 ± 0.27
Try (U/mg)	663.56 ± 35.46 ^{a}	672.95 ± 30.62 ^{a}	589.69 ± 37.69 ^{a}	610.34 ± 34.77 ^{a}	661.36 ± 33.3 ^{a}	463.88 ± 19.73 ^{b}

Values with different superscripts in the same row are significantly different ($p < 0.05$).

3.6. Muscle Textural Indexes

The hardness, adhesiveness, and chewiness of muscle in the SWM2.5 and SWM5 groups did not change significantly ($p > 0.05$). The adhesiveness and cohesiveness of the SWM5 group was significantly higher than the CON group ($p < 0.05$). The SWM2.5 group had the highest gumminess. There was no significant difference in hardness and springiness among the SWH5, SWH10, and SWH15 groups ($p > 0.05$), and SWH5 group had the highest hardness and springiness (Table 11).

Table 11. Muscle textural indexes of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Hardness (N)	5.08 ± 0.33	5.13 ± 0.39	4.75 ± 0.23	5.5 ± 0.28	5.24 ± 0.33	5.35 ± 0.36
Adhesiveness (N.mm)	0.03 ± 0.002 ^{a}	0.02 ± 0.002 ^{a}	0.06 ± 0.002 ^{b}	0.06 ± 0.002 ^{b}	0.07 ± 0.003 ^{b}	0.07 ± 0.001 ^{b}
Cohesiveness (Ratio)	0.49 ± 0.02 ^{a}	0.57 ± 0.02 ^{b}	0.68 ± 0.01 ^{c}	0.65 ± 0.01 ^{c}	0.63 ± 0.01 ^{bc}	0.6 ± 0.01 ^{b}
Springinesse (mm)	1.71 ± 0.07	1.76 ± 0.07	1.82 ± 0.06	1.8 ± 0.04	1.75 ± 0.03	1.7 ± 0.06
Gumminess (N)	3.54 ± 0.26 ^{a}	4.48 ± 0.22 ^{b}	3.63 ± 0.13 ^{a}	3.88 ± 0.19 ^{b}	4.02 ± 0.15 ^{b}	3.28 ± 0.16 ^{a}
Chewiness (mJ)	5.29 ± 0.38 ^{a}	6.1 ± 0.48 ^{a}	5.84 ± 0.27 ^{a}	6.59 ± 0.28 ^{b}	6.82 ± 0.34 ^{b}	5.4 ± 0.32 ^{a}

Values with different superscripts in the same row are significantly different ($p < 0.05$).

3.7. Body Composition

There was no significant difference in moisture content, crude fat, and crude ash of experimental yellow catfish in the SWM2.5 and SWM5 groups ($p > 0.05$). Substitution of fish meal by SWH resulted in significantly higher crude fat than that of the CON group, and the substitution group showed an upward trend in crude fat (Table 12).

Table 12. Body composition of experimental yellow catfish.

Index	CON	SWM2.5	SWM5	SWH5	SWH10	SWH15
Moisture content	71.22 ± 0.62	71.59 ± 0.56	71.61 ± 0.52	70.97 ± 1.02	70.34 ± 0.06	70.71 ± 0.42
Crude fat	28.5 ± 0.47	28.2 ± 0.45	29.7 ± 0.52	30.7 ± 0.32	31.1 ± 0.47	31.8 ± 0.52
Crude ash	13.58 ± 0.17	14.05 ± 0.11	14.14 ± 0.3	13.96 ± 0.12	14.11 ± 0.1	14.03 ± 0.5

4. Discussion

SWH and SWM represent new sources of animal protein which are enzymatic hydrolysis products of the industrial production of fish meal by-products. This not only reduces the amount of fish meal but also enhances its utilization rate. A previous study found that

replacing 60% of Peruvian super steam fish meal with stickwater hydrolyzsate combined with plant protein diet did not affect feeding, feed efficiency, or growth performance of pearl gentiana grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀). [15]. In this study, the substitution of 2.5% and 5% of fish meal with SWM, and the replacement of 5%, 10%, and 15% of fish meal with SWH, had no significant effect on the weight gain rate, specific growth rate, survival rate, and feed conversion rate of yellow catfish, which was similar to the results of the study of Atlantic salmon (*Salmo salar* L.) [16]. This may be attributed to SWH and SWM being rich in water-soluble proteins, small peptides, and free amino acids. These ingredients are different from other animal and plant protein sources of unique nutrients, among which taurine [17,18] and small peptides [19] have a certain growth-promoting effect on fish. However, excessive substitution of fish meal with SWM can result in growth inhibition in certain fish species, such as red sea bream (*Pagrus major*) [20] and sea bass larvae (*Dicentrarchus labrax*) [21]. The results of our experiment also revealed that the growth performance of yellow catfish decreased when 5% of fish meal was replaced with SWM, as compared to the CON group. The primary reason for this may be the excessive presence of small peptides and free amino acids in the diet due to high levels of SWH and SWM replacing fish meal, resulting in amino acid saturation and competition in amino acid transport mechanisms [22], leading to an imbalance in amino acid absorption in the bodies of yellow catfish.

Another aspect that can illustrate the growth performance of fish is the activity of intestinal digestive enzymes. Intestinal digestive enzymes degrade macromolecular nutrients in feed into small molecular nutrients, such as amino acids and glucose. Their activity levels directly affect the fish's ability to digest and absorb nutrients [23]. Shi et al. demonstrated that replacing 10% of fish meal with stickwater hydrolysate in the compound diet of rice field eel (*Monopterus albus*) significantly improved the digestive enzyme activities of the fish [24]. In this study, substituting fish meal with SWM did not affect the amylase activity of yellow catfish, but it exhibited a decreasing trend and had no impact on trypsin activity. The substitution of fish meal with SWH did not affect the amylase activity of Pelteobagrus fulvidraco, with the highest activity observed in the SWH10 group. However, when fish meal was replaced by 15% SWH, trypsin activity was significantly lower than that in the CON group. The main reason for this is that SWH contains biogenic amines, which are products of protein degradation. The high concentration of biogenic amine will have certain negative effects on the biological organism [25], and excess free amino acids can reduce the organism's ability to consume and utilize protein [26,27].

Serum physiological indicators can reflect the health and physiological status of fish. Serum cholesterol and triglycerides play a crucial part in human health, and to a certain extent reflect the ability of liver fat metabolism and lipid absorption [28]. The results of this study showed that SWM and SWH significantly increased the serum total cholesterol and triglyceride levels of yellow catfish when replacing fish meal, indicating that SWM and SWH had an inhibitory effect on liver fat metabolism. This finding is consistent with the results reported by Wu et al. in grass carp (*Ctenopharyngodon idella*) [29]. In this study, serum urea nitrogen decreased after fish meal replacement with SWH and SWM. This may be attributed to the increase in small molecular substances in enzymatic hydrolysates, which promoted protein decomposition and accelerated nitrogen excretion, ultimately reducing urea nitrogen production [30,31]. Transaminases related to protein metabolism in aquatic animals mainly exist in the liver cells of the body. These enzymes metabolize and transform proteins through transamination and deamination processes. When the body's liver cells experience inflammation and toxic reactions, damaged liver cells release a significant amount of liver transaminase into the bloodstream, resulting in elevated serum transaminase levels [32,33]. This study demonstrated that the activities of glutamate pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT) in the serum of yellow catfish increased with an increase in SWH replacement rate, suggesting that a high replacement rate may cause some degree of liver damage in yellow catfish. Serum GOT and GPT activities increased with higher levels of dietary SWH and SWM supplementation,

consistent with the findings in turbot [34] and yellow catfish [35]. It is possible that an increase in the substitution ratio leads to an increase in biogenic amines in the diet, causing damage to the fish's liver, subsequently resulting in higher transaminase levels in the bloodstream, which negatively impacts the animals' health [36].

Complement is an essential component of antimicrobial defense and is mainly composed of complement 3 (C3) and complement 4 [37]. Alkaline phosphatase (AKP), a phosphohydrolase, is a crucial nonspecific immune marker and an evaluation indicator reflecting the health status of aquatic animals [38]. Various studies have demonstrated that fish have developed specific immune response systems, including cellular and humoral immunity [39]. Immunoglobulins play a significant role in the humoral immunity of fish. Immunoglobulin M (IgM) is one of the most important immunoglobulins in fish and is the first antibody produced when the body is stimulated by antigens [40]. This study revealed that as the proportion of SWH and SWM replacing fish meal increased, the levels of C3, AKP, and IgM in the serum of yellow catfish exhibited an upward trend. In a study involving largemouth bass (*Micropterus salmoides*), it was observed that adding an appropriate amount of small peptides to the diet could enhance its immune capacity [41]. Small peptides generally refer to oligopeptides containing between two and three amino acids, which can be completely absorbed and utilized by the body, playing a pivotal role in amino acid digestion, absorption, and metabolism [42]. These results suggest that substituting a portion of the fish meal with SWM and SWH in the diet can enhance the immunity of yellow catfish, which may be attributed to the presence of small peptides in SWH and SWM.

In addition, the overall antioxidant capacity of the organism's defense system of fish is closely related to the degree of health, including the enzymatic antioxidant system and non-enzymatic antioxidant system [43]. The enzymatic antioxidant system relies on the role of various antioxidant enzymes in the body, while the non-enzymatic antioxidant system relies on the role of metalloproteins, amino acids, and vitamins [44]. Superoxide dismutase (SOD) is a vital part of the enzymatic antioxidant system in animals, and its activity reflects the ability to scavenge reactive oxygen free radicals in animals. SOD can convert harmful superoxide free radicals into H_2O_2 through a reaction [45]. Catalase (CAT) is an antioxidant enzyme present in nearly all organisms. Its primary role is to catalyze the decomposition of hydrogen peroxide into water and oxygen, thereby removing H_2O_2 from the body to prevent cell damage caused by H_2O_2. As one of the key enzymes in the biological defense system, CAT provides an antioxidant defense mechanism for the body [46]. The results indicate that replacing fish meal with 10% SWH significantly improved the antioxidant capacity of yellow catfish. Malondialdehyde (MDA) is a product of lipid peroxidation, and its content indirectly reflects the content of reactive oxygen species and the degree of lipid peroxidation in tissues and cells [47]. The findings from this experiment revealed that the replacement of fish meal with SWH and SWM increased the serum MDA content of yellow catfish as the replacement ratio increased. Xu et al. found that substituting fermented fish soluble pulp for fish meal could significantly reduce MDA content in the serum of juvenile turbot [16]. This may be attributed to the presence of a certain amount of MDA in SWH and SWM. In Cao et al.'s study, it was observed that fish stickwater has the characteristics of high moisture, high fat, and high unsaturation, making its quality more susceptible to destruction due to fat oxidation. MDA is the end-product of lipid oxidation, and the degree of rancidity in fish soluble pulp affects its MDA content [48].

The texture parameters of fish muscle are important indicators for evaluating the taste of fish. These parameters depend on muscle hardness, viscosity, cohesiveness, springiness, gumminess, chewiness, and other factors [49]. Consumers typically prefer meat that is firm and elastic, making muscle texture a key consideration for consumers. In general, the greater the hardness and viscosity of the fish, the better its taste. Hardness is a crucial texture parameter that reflects the internal bonding force within the sample [50]. It has been demonstrated that greater viscosity results in meat that is chewier and crisper, mimicking the mouthfeel of natural muscles [51]. The results indicated that replacing fish meal with SWM had no significant impact on the hardness, viscosity, and chewiness of the meat.

However, with an increase in the replacement ratio, the meat's hardness and chewiness decreased while viscosity increased. The replacement of fish meal with SWH had no significant effect on the hardness and viscosity of yellow catfish meat, with the highest values for hardness and viscosity observed at a replacement ratio of 5%. Substituting 2.5% and 5% of fish meal with SWM, as well as replacing 5%, 10%, and 15% of fish meal with SWH, had no significant effect on the moisture content, crude fat, and crude ash of yellow catfish. This suggests that replacing fish meal with SWH and SWM does not adversely affect the muscle quality of yellow catfish.

5. Conclusions

In conclusion, the substitution of fish meal by 2.5% and 5% SWM, and 5%, 10%, and 15% SWH did not affect the growth performance of yellow catfish. Specifically, the substitution of fish meal by 5% SWM and 15% SWH contributed to an enhanced immune capacity in yellow catfish to a certain extent. However, the substitution of fish meal by 5% SWM and 15% SWH will inhibit the antioxidant capacity of fish, and also cause damage to the liver of fish.

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