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ORIGINAL ARTICLE

Aquaculture

Effects of fish meal replacement by soybean peptide on growth performance, digestive enzyme activities, and immune responses of yellow catfish *Pelteobagrus fulvidraco*

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Abstract The present study was conducted to compare the effects of using soybean peptide as a particle substitution in fish meal on the growth performance, digestive function, and immune responses of yellow catfish. Four isonitrogenous and isoenergetic experimental diets were formulated by replacing 0 % (D-0), 20 % (D-20), 35 % (D-35), and 50 % (D-50) of fish meal with soybean peptide for 8 weeks. The results showed that the final body weight (FBW), weight gain rate (WGR), and specific growth rate (SGR) significantly increased in the dietary D-50 group compared to those of the control group (D-0 group), and the feed conversion ratio (FCR) significantly decreased compared to that of other groups. The D-50 group had higher levels of serum globulin concentration (GLB), alkaline phosphatase activity (ALP), and total nitric oxide synthase activity (tNOS) than the control group, respectively. In addition, the anterior intestine protease activity of the D-20 group was significantly higher than that of the control group and the D-50 group. The serum alanine aminotransferase (ALT) activity of the D-20 dietary group was also significantly higher than that of other groups. Furthermore, the challenge with *Aeromonas hydrophila* caused mortality in all groups but it was lower in the group of fish that received D-50 than that of the control group. Therefore, we concluded that the D-50 diet could be used to improve immune responses and growth performance and to replace the fish meal in the diet of yellow catfish.

Keywords Yellow catfish · Fish meal replacement · Growth performance · Immune response · *Aeromonas hydrophila*

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Introduction

The yellow catfish, *Pelteobagrus fulvidraco*, is an omnivorous freshwater fish, and is one of the most commercially important fish species, highly preferred by Asian and especially Chinese consumers [1, 2, 3]. In recent years, because of excellent meat quality and high market value [4], yellow catfish farming has become an emerging industry in China with an annual production of 256,650 tons in 2012, and a rapid growth trend [5].

In aquaculture, protein source is the largest cost and most important component in the aqua feeds. Traditionally, fish meal (FM) has been deemed as the major protein source due to its abundance of essential nutrients, well-balanced amino acid profile, and unknown growth factors [6]. However, high cost and unpredictable supply of fish meal [7] has made it difficult to meet the growing production demand in the aqua feed industry. Therefore, development of new sustainable protein sources has become a major interest in the aquaculture sector, as it can lessen dependency on fish meal (FM) as the main protein component in aqua feeds [8]. Generally, plant protein sources such as soybean meal [9], cottonseed meal [10], and others have been recognized as alternatives to fish meal due to their widespread availability, reduced cost, and relatively favorable amino acid profiles [11]. Furthermore, their development would mean that fish meal can at least be partially replaced by less expensive plant proteins [12–14].

Soybean meal is considered to be one of the most feasible alternatives for high-quality fish meal in feeds for many aquatic animals [15] thanks to its high protein content, moderately balanced amino acid profile [16, 17], reasonable price, and a steady supply of soybean production. Some studies have reported that it is substantially more effective to partially replace fish meal with soybean meal in diets for certain kinds of fish species, such as juvenile crayfish *Pacifastacus leniusculus, Astacidae* [18], Japanese seabass *Lateolabrax japonicus* [19], juvenile tench *Tinca tinca* L. [20], and gilthead sea bream *Sparus aurata* L. [21]. However, complete fish meal replacement with soybean meal remains challenging because of reduced fish growth performance possibly due to reducing feed intake and protein synthesis in the immune system [22, 23].

Soybean proteins contain numerous peptides, which are derived from soybean protein fraction and obtained by hydrolysis with a protease enzyme during the fermented processing of peptides with a molecular weight below 1000 Da [24]. The soybean peptide has various antioxidant activities based on the amino acid composition of their sequences [25], which include Pro, His, or Tyr [26], and can act as metal-ion chelators, singlet oxygen quenchers, and hydroxyl radicals [27]. In addition, soybean peptide has other distinctive functional characteristics, such as flavor potentiator [28], antitumor [29], water solubility, and higher digestibility [30] properties. Its biological effects on metabolic disorders have also been recognized [31]. However, little is currently known about the utilization of soybean bioactive peptides in fish diets [32]. In particular, no data exists for the replacement of fish meal with soybean peptide in yellow catfish feed. Therefore, this study aimed to evaluate the impact of replacing fish meal with soybean peptide on the growth performance, digestive function, and immune responses for yellow catfish. These findings may provide suggestions for feed formulations in yellow catfish.

Materials and methods

Experimental diets

Four isonitrogenous and isoenergetic experimental diets (45.5 % crude protein, dry matter) were formulated by replacing 0 % (D-0, control group), 20 % (D-20), 35 % (D-35), and 50 % (D-50) of fish meal with soybean peptide, which was obtained from Wuxi Hanove Animal Health Products Co., Ltd (Wuxi, China). The soybean peptide is a soy protein hydrolysate (approximately 70 % crude protein content), which is produced by converting soybean meal into peptides of lower molecular weight (less than 10 kDa) through bacterial enzymatic hydrolysis. Processing of soy products with modern biological technology helps to improve their protein purity and digestibility and to eliminate the toxic effect of antinutritional factors. Besides, the inclusion levels of flour, soybean meal, corn starch, rapeseed meal, and premixes of minerals and vitamins remained constant in the five diets, whereas fish oil and soy oil levels slightly varied. The formulation and proximate composition of the experimental diets are presented in Table 1.

Table 1 Formulation and proximate composition of the experimental diets (% dry matter)

Ingredients	Diet number			
	D-0	D-20	D-35	D-50
Fish meal[a]	32	25.6	20.8	16
Soybean peptide	0	6.4	11.2	16
Flour	25.6	24.5	22.7	23.2
Soybean meal[b]	15	17	20	17
Corn protein powder	10	8	8	8
Rapeseed meal	6.7	6.8	4.9	6.7
Fish oil	1	1	1	1
Soy oil	5	5.3	5.5	5.8
Zeolite powder	2	2	2	2
Premix[c]	1	1	1	1
Zeaxanthin	0.3	0.3	0.3	0.3
CaH_2PO_4	1.4	2.1	2.6	3
Total	100	100	100	100
Chemical composition				
Dry matter (DM, %)	90.21	90.21	90.23	90.26
Gross energy (kJ/g DM)[d]	22.19	22.50	22.90	23.24
Crude protein (% DM)	45.42	45.06	45.64	45.00
Crude lipid (% DM)	9.66	9.72	9.77	9.91
NFE (% DM)	33.71	33.49	33.01	33.55
Ash (% DM)	9.52	9.85	10.03	10.01
Lys (% DM)	2.56	2.50	2.48	2.34
Met (% DM)	1.41	1.28	1.18	1.08
Total phosphorus (% DM)[e]	1.78	1.80	1.80	1.79
Available phosphorous (% DM)[f]	1.54	1.55	1.55	1.52

[a] Fish meal (CP 68 %), provided by Coprinca Lt (Lima, Peru)

[b] Soybean meal, crude protein 46 %, supplied by Cargill, Shanghai, China

[c] Premix (vitamin and mineral), provided by Wuxi Hanove Animal Health Products Co., Ltd. (Jiangsu, China)

[d] Gross energy was calculated with the following values: protein 23.64 kJ/g, fat 39.54 kJ/g, carbohydrate 17.15 kJ/g; the others are measured in nutrition levels

[e] Total phosphorus: a measure of all the forms including dissolved, inorganic, and organically bound forms

[f] Available phosphorous: Phosphorus that can be absorbed and made available to meet an animal's net nutritional requirements

All ingredients were ground and sieved through a 60-mesh sieve before final mixing with a commercial food mixer, then blended with the oils and water, forced through a pelletizer (Y90L-2, Xinchang Chenshi Machinery Co., Ltd., Zhejiang, China), and dried in a ventilated oven at 30 °C. After drying, all diets were sealed in bags and stored at −4 °C until used.

Experimental fish and feeding trial

Experimental yellow catfish were obtained from the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, China. Prior to beginning the experiment, healthy fish of similar sizes were selected, stocked in round fiberglass tanks (φ820 × 700 mm, $N = 18$ fish/tank), and fed a control diet for 15 days to adapt to the experimental conditions.

At the start of the experiment, fish were fasted for 24 h and weighed. All fish (initial weight 22.28 ± 0.15 g), especially the similar sizes of fish, were randomly chosen and divided into 12 tanks with 20 fish per tank. Each experimental diet was randomly assigned to triplicate tanks in a completely randomized design and hand-fed three times daily (08:00, 12:00, and 16:00) until apparent satiation on the basis of visual observation of fish feeding behavior. The feeding amount increased every other week and the amount was adjusted according to body weight measurement every 2 weeks. During the experimental period, all tanks were maintained under a natural photoperiod, the water flow rate in each tank was 2 l/min, water temperature fluctuated from 26 to 30 °C, and pH ranged from 7.2 to 7.6. Dissolved oxygen concentration was higher than 5 mg/l, and ammonia–nitrogen was lower than 0.01 mg/l. After 56 days, fish from each tank were counted and weighed.

Sample collection

At the end of feeding trial, approximately 24 h after the last feeding, all fish were individually weighed and counted from each tank to calculate the survival, weight gain, and feed efficiency ratio. Nine fish (three fish per tank) of each group were anesthetized by MS-222 (150 mg/l), and then blood samples were collected immediately from the caudal vein with disposable medical syringes. Following centrifugation (3500×g, 10 min, 4 °C), serum samples were separated. Nine fish from each group were killed, and then samples of liver and viscera were collected and weighed. All the samples were stored at −80 °C for further analysis.

Infection experiment

Aeromonas hydrophila (Ah, BSK-10) was acquired from the Key Laboratory of Freshwater Fisheries Research center (Wuxi, China). According to the method described by Liu et al. [33], *A. hydrophila* was incubated in a nutrient broth for 24 h at 28 °C. After, it was centrifuged at 12, 000g for 10 min at 4 °C. The cells were then washed twice in sterile PBS (pH 7.2) and the final concentration was maintained at 1×10^7 CFU/ml.

After the growth experiment, 120 fish from the four groups (three tanks/group, $N = 10$ fish/tank) were moved to 12 respectively labeled new tanks and were infected by intraperitoneal injection with 1×10^7 cfu ml^{-1} *A. hydrophila* (0.5 ml, per 50 body weight). Dead fish were removed and recorded at 24 h.

Sample analysis

Growth performances

The following variables were calculated:

Weight gain rate (WGR) (%) = 100 × (final body weight − initial body weight)/initial body weight;

Feed conversion ratio (FCR) = (dry feed)/(wet weight gain);

Specific growth rate (SGR) = 100 × (ln (final body weight) – ln (initial body weight))/(day);

Survival rate (SR, %) = 100 × (final fish number)/(initial fish number);

Hepatosomatic index (HSI) (%) = 100 × liver wet weight/body wet weight;

Viserosomatic index (VSI) (%) = 100 × viscera wet weight/body wet weight;

Fullness coefficient (FNC) (%) = 100 × final body weight/body wet length^3.

Serum biochemical and immune parameters

Measurements of serum biochemical parameters such as aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin (ALB), globulin (GLB), total protein (TP), and ureophil (UREA) were conducted by an automatic biochemical analyzer Mindary BS-400 (Shenzhen, China) using assay kits purchased from Shenzhen Mindary Bio-medical Electronics Co., Ltd., following a previously described method [5].

Serum malondialdehyde (MDA) assay was conducted using three published high-performance liquid chromatographic methods [34]. Total nitric oxide synthases (tNOS), inducible nitric oxide syntlase (iNOS), and constructive nitric oxide synthase (cNOS) were measured using an immunolocalization method [35] and were estimated by detection kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Enzymes activities

In this assay, most of the procedures of the activities of protease, lipase, and amylase were adopted from previous

Table 2 Effects of fish meal replacement by soybean peptide on the growth performance and feed utilization of yellow catfish *Pelteobagrus fulvidraco* (mean ± S.E.M.)

	Diet number			
	D-0	D-20	D-35	D-50
IBW	22.26 ± 0.13	22.26 ± 0.09	22.32 ± 0.09	22.21 ± 0.08
FBW	60.98 ± 2.77[b]	65.34 ± 1.25[ab]	64.55 ± 2.23[ab]	69.34 ± 1.20[a]
WGR[A]	173.81 ± 10.95[b]	193.51 ± 6.08[b]	189.21 ± 9.14[b]	212.29 ± 6.43[a]
FCR[B]	1.63 ± 0.09[a]	1.56 ± 0.05[a]	1.56 ± 0.02[a]	1.40 ± 0.03[b]
SGR[C]	1.80 ± 0.07[b]	1.92 ± 0.04[b]	1.89 ± 0.06[ab]	2.03 ± 0.04[a]
HSI[D]	1.95 ± 0.08[a]	1.50 ± 0.09[b]	1.47 ± 0.11[b]	1.64 ± 0.08[ab]
VSI[E]	10.13 ± 0.44	9.94 ± 0.34	8.66 ± 0.60	10.13 ± 0.66
FNC[F]	1.74 ± 0.03	1.66 ± 0.07	1.71 ± 0.06	1.79 ± 0.07
SR[G]	100	100	100	100

Data are means of triplicate observations (n=3). Means in the same column sharing the same lower superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$)

IBW and *FBW* are initial body weight and final body weight

[A] Weight gain rate (WG) = 100 × (FBW − IBW)/IBW

[B] Feed conversion ratio (FCR) = (dry feed)/(wet weight gain)

[C] Specific growth rate (SGR) = 100 × (ln (final body weight) − ln (initial body weight))/(day)

[D] Hepatosomatic index (HSI) (%) = 100 × liver wet weight/body wet weight

[E] Viserosomatic index (VSI) = 100 × (viscera wet weight, g)/(body wet weight, g)

[F] Fullness coefficient (FNC) = 100 × (final body weight, g)/(body wet length^3)

[G] Survival rate (SR, %) = 100 × (final fish number)/(initial fish number)

research [36, 37]. Briefly, the fish gut was divided into two sections: anterior and posterior intestines. The anterior and posterior sections of the intestine of nine individuals from each group (three fish/tank) were carefully weighed and homogenized in 0.01 M Tris buffer, pH 7.4, at a ratio of 1:9 (tissue:buffer) with a Teflon pestle of a motor-driven tissue-cell disruptor under an ice bath. The extract was later centrifuged at 3000×*g* at 4 °C for 10 min, and the supernatant was used as the enzyme source. The protease activity in the intestine was assayed following the Forint phenol-reagent method. The activities of lipase and amylase in the intestine were assayed by the Colorimetric method using commercial kits (Jiancheng Bioengineering Institute, Jiangsu, China).

Statistical analysis

Data were transformed if necessary after evaluating assumptions of normality, equality of variances, and outliers, and were subjected to one way analysis of variance (ANOVA) using the software SPSS 20.0 for Windows. Significant differences in the means between dietary treatments were evaluated by Tukey's multiple range test. Mean differences were considered significant at a P value equal or less than 0.05, and the results were expressed as mean ± standard error.

Results

Growth performance and feed utilization

Growth performance, feed utilization, and morphological index for yellow catfish given graded levels of soybean peptide to replace fish meal for 8 weeks are shown in Table 2. FBW, WGR and SGR in the group of D-50 were significantly ($P < 0.05$) higher than in the control group. Furthermore, FCR in the group of D-50 was lower than that of the D-0, D-20, and D-35 groups.

HSI was significantly ($P < 0.05$) higher in fish fed 0 % dietary soybean peptide than that in fish fed a 20, 35, and 50 % dietary soybean peptide-replacement fish meal diet. On the other hand, no significant ($P > 0.05$) differences were observed in SR, VSI, and FNC of fish fed different dietary replacement levels.

Digestive enzymes

The differences in enzyme activities between the anterior and posterior intestine sections are shown in Table 3. In the anterior intestine, protease activity of the D-20 group was significantly ($P < 0.05$) higher than that of the control group and D-50 group. In addition, amylase activity of the D-20 group was significantly ($P < 0.05$) higher than that

Table 3 Effects of fish meal replacement by soybean peptide on the digestive enzyme activities of yellow catfish *Pelteobagrus fulvidraco* (mean ± S.E.M.)

	Diet number			
	D-0	D-20	D-35	D-50
Anterior intestine				
Protease (U/mg)	0.24 ± 0.06[b]	0.74 ± 0.25[a]	0.31 ± 0.06[ab]	0.23 ± 0.03[b]
Amylase (U/mg)	0.49 ± 0.07[b]	1.47 ± 0.47[a]	0.54 ± 0.07[b]	0.43 ± 0.09[b]
Lipase (U/g)	93.87 ± 15.24	99.10 ± 85.36	97.59 ± 25.45	89.29 ± 8.47
Posterior intestine				
Protease (U/mg)	0.08 ± 0.01	0.12 ± 0.05	0.14 ± 0.07	0.10 ± 0.04
Amylase (U/mg)	0.26 ± 0.03	0.48 ± 0.13	0.37 ± 0.05	0.39 ± 0.08
Lipase (U/g)	47.56 ± 5.89	49.06 ± 8.85	54.17 ± 14.06	52.71 ± 8.43

Data are means of triplicate observations of each group (n=9). Means in the same column sharing a lower superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$)

Table 4 Effects of fish meal replacement by soybean peptide on the serum biochemical parameters of yellow catfish *Pelteobagrus fulvidraco* (mean ± S.E.M.)

	Diet number			
	D-0	D-20	D-35	D-50
TP (g/l)	32.90 ± 2.53	34.51 ± 0.80	32.82 ± 1.86	37.80 ± 2.09
ALB (g/l)	4.57 ± 1.50	5.29 ± 0.58	4.72 ± 0.74	4.97 ± 0.39
GLB (g/l)	28.33 ± 1.37[b]	29.22 ± 1.13[b]	28.10 ± 2.39[b]	32.83 ± 2.34[a]
A/G	0.16 ± 0.05	0.18 ± 0.03	0.17 ± 0.04	0.16 ± 0.02
ALP (U/l)	30.97 ± 2.83[b]	27.96 ± 0.57[b]	33.76 ± 2.17[ab]	35.64 ± 0.27[a]
AST (U/l)	74.38 ± 8.09	74.61 ± 2.61	89.69 ± 7.86	73.33 ± 12.10
ALT (U/l)	6.91 ± 0.87[b]	10.34 ± 0.70[a]	8.01 ± 0.84[b]	8.09 ± 0.63[b]
UREA (μmol/l)	0.63 ± 0.01	0.65 ± 0.02	0.62 ± 0.03	0.69 ± 0.07

Data are means of triplicate observations of each group (n=9). Means in the same column sharing the same lower superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$)

TP total protein, *ALB* albumin, *GLB* globulin, *A/G* ALB/GLB, *ALP* alkaline phosphatase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *UREA* ureophil

of the other groups. There was no significant difference in the lipase between treatment groups and the control group ($P > 0.05$). As for the posterior intestine, there were no significant differences in protease, amylase, and lipase ($P > 0.05$) among the treatments.

Serum biochemical parameters

The effects of graded levels of fish meal replacement by soybean peptide on the serum biochemical parameters of yellow catfish are described in Table 4. The group of D-50 recorded higher serum GLB and ALP activity among the different groups compared to the D-0 and D-20 groups. In addition, the serum ALT activity in the D-20 group was significantly ($P < 0.05$) higher than that of the other groups. However, the graded soybean peptide levels had no significant impact ($P > 0.05$) on serum TP, AST, UREA, and A/G between the different groups.

Immune parameters

The effects of graded levels of fish meal replacement by soybean peptide on the serum immune parameters of yellow catfish are described in Table 5. The data showed that tNOS activity in the groups that received D-50 and D-20 was higher than that of D-0 group. Furthermore, there were no significant differences ($P > 0.05$) among the groups in terms of iNOS, cNOS, and MDA content, respectively.

Infection test

The effects of fish meal replacement by soybean peptide on the cumulative mortality of yellow catfish challenged with *A. hydrophila* are shown in Fig. 1. At 0 and 6 h, there were no dead fish within the different groups. After 24 h, the cumulative mortality was lower in the group of D-50 than that of the other treatment groups and control group.

Table 5 Effects of fish meal replacement by soybean peptide on the serum immune parameters of yellow catfish *Pelteobagrus fulvidraco* (mean ± S.E.M.)

	Diet number			
	D-0	D-20	D-35	D-50
tNOS (U/l)	44.28 ± 1.53^{b}	51.52 ± 3.40^{a}	48.03 ± 1.83^{ab}	52.92 ± 2.57^{a}
iNOS (U/l)	26.90 ± 0.57	29.00 ± 2.57	25.38 ± 2.69	31.15 ± 2.73
cNOS (U/l)	17.40 ± 1.31	22.53 ± 5.99	22.66 ± 4.47	21.77 ± 5.26
MDA (mmol/l)	5.36 ± 0.60	6.62 ± 0.74	4.70 ± 0.93	6.15 ± 0.42

Data are means of triplicate observations of each group (n=9). Means in the same column sharing the same lower superscript letter are not significantly different as determined by Tukey's test ($P > 0.05$)

tNOS total nitric oxide synthase, *iNOS* inducible nitric oxide synthase, *cNOS* constructive nitric oxide synthase, *MDA* malondialdehyde

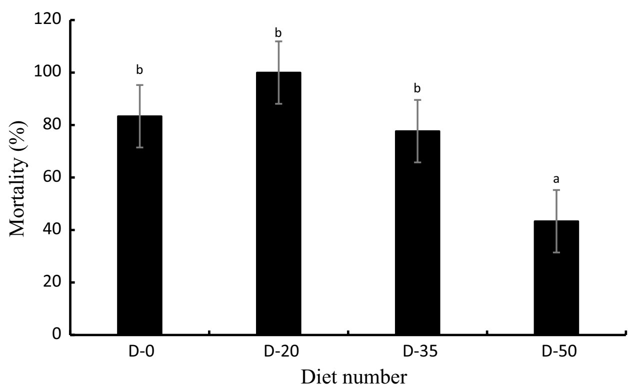

Fig. 1 Effects of fish meal replacement by soybean peptide on cumulative mortality of yellow catfish *P. fulvidraco* challenged with *A. hydrophila* at 24 h. *Note:* Data are expressed as mean ± SEM ($n = 3$). Diverse little letters show significant differences ($P < 0.05$) among the dosage groups according to Tukey's multiple range test

Discussion

No pathological signs and anomalies occurred during the feeding experiment, and the experimental diets were well accepted in all treatments. Test diets had no significant effects on the survival rate of yellow catfish. This was possibly due to a lack of any nutritional deficiencies in the tested diets, which indicated that soybean peptide can be an adequate source of plant protein [38]. The health-promoting properties of soybean peptide have been studied extensively [39], and two previous studies reported that soybean peptides supplemented in diets increased the growth performance of weanling pigs [40] and broilers [41]. In our study, the replacement of fish meal with soybean peptide (20–50 % of dietary soybean peptide) in the diet of yellow catfish promoted FBW, WGR, FCR, and SGR, and replacing up to 50 % of the original content with dietary soybean peptide had significant positively effects on fish growth performance, nutrient utilization, and health parameters compared to the outcomes of a 100 % fish meal-based control diet. This is an indication that partial replacement of fish meal by soybean peptide is also feasible in yellow catfish. These findings were similar to those reported on the partial replacement of fish meal by soybean meal in Atlantic salmon *Salmo salar* [42], Asian sea bass *Lates calcarifer* [43], milkfish *Chanos chanos* [44], and coho salmon [45], and by soy protein concentrate in turbot *Scophthalmus maximus* L. [46], Atlantic halibut *Hippoglossus hippoglossus* [47], and juvenile cobia *Rachycentron canadum* [48].

A reasonable explanation includes several factors, as follows: firstly, soybean peptide has some distinctive functional characteristics, such as various antioxidant activities [25], flavor potentiator [28], antitumor [29], water solubility, and higher digestibility [30] properties. Secondly, soybean peptide (soy protein hydrolysates) might help to eliminate anti-nutritional factors and serve as an immunomodulatory agent, capable of inducing defense genes involved in pathogen attack [49]. According to blood physiological parameters such as ALP and GLB, nitric oxide syntlase, and mortality after *A. hydrophila i*nfection in the group of D-50 group in this study, our findings clearly indicated that 50 % fish meal replacement with soybean peptide may improve the immune response and disease resistance of yellow catfish, and enhance growth performance. This is an indication that partial replacement of fish meal by soybean peptide is also feasible in yellow catfish, which is in accordance with the findings reported in juvenile Japanese Flounder *Paralichthys olivaceus* [50].

In contrast, Nguyen et al. [51] reported a test diet with fish meal replaced by soy protein isolate that resulted in decreased growth performance in yellowtail *Seriola quinqueradiata.* The variation in findings among fish species may be due to differences in aquaculture species, fish age, dietary composition, and feeding strategy. However, complete fish meal replacement with soybean reduced the growth performance of fish possibly by reducing feed intake and protein synthesis in the immune system [22, 23], which indicated that supplemented excess could not be efficiently utilized by fish and that the presence of several anti-nutritional factors limited dietary amino acid utilization [52–54]. Similarly, complete fish meal replacement with soybean peptide in the diet led to poor growth in the juvenile starry flounder [32]. Excessive soybean peptide in the diet might have caused accelerated amino acid oxidation and endogenous excretion [55], resulting in poor cell growth. As for complete fish meal replacement by soybean peptide in the diet of yellow catfish, this strategy needs further study before it can be supported.

Digestive enzyme activities demonstrate the potential impact on feed utilization and growth performance, especially protease, amylase, and lipase, which play a pivotal role in the digestive process. In the present study, enzyme activity of the anterior and posterior intestinal sections showed different responses. The protease and amylase activities in the anterior intestine were higher in the group that received D-20 than those of the control group. This indicated that the partial replacement of fish meal by soybean peptide might affect the digestive enzyme activities in the anterior intestine of yellow catfish. With each increase of fish meal replacement by soybean peptide, we observed no significant differences in levels of protease, amylase, and lipase activities in the anterior intestine between the D-50 group and the control group, which may be due to the higher digestibility of the soybean peptide that consequently did not stimulate the digestive enzyme activities. This aspect needs further study.

As for the posterior intestine, no significant difference in protease, amylase, and lipase activities of the posterior intestine was observe among the different soybean peptide diets. Some reports involving freshwater crayfish *Cherax quadricarinatus* [56] and common carp *Cyprinus carpio* L. [57] have shown similar results. This finding was also proven by Sire [58], who proposed that amino acid and peptide absorption was limited in the posterior intestine.

Soybean peptide is effective in stimulating macrophage phagocytosis and immunomodulating activity against lymphocyte proliferation [59], which benefits animal health through modulating cellular immune systems. Those peptide fractions have high antioxidant activity, hydroxyl radical scavenging capacity, and trolox-equivalent antioxidant capacity [60]. In this experiment, GLB and ALP levels were significantly increased in the D-50 group compared with the control group, which suggested that the immune system might be affected by dietary soybean peptide in the diet. The findings consist with sardinella *Sardinella aurita* [61]. In addition, serum ALT is treated as an important metabolizing enzyme in the protein metabolism of liver and kidney. In this experiment, the serum ALT level of the D-20 group was significantly higher than that of the control group, and we observed significant increases in the protease and amylase activities in the anterior intestine, which indicated that the serum level of ALT might improve the protein absorption in a suitable range. This result was supported by previous research in which a high-protein diet significantly increased the serum level of ALT in rainbow trout [62]. However, along with the increased level of soybean peptide, the ALT values tended to decrease. This might be due to the adaptive responses toward an excess of soybean peptide.

Nitric oxide syntlase (NOS) is one of the smallest known molecular mediators, which play important roles in a variety of immune processes ranging from innate immunity to acquired immunity [63], and include tNOS, iNOS, and cNOS. In this study, tNOS enzyme activity was significantly increased in the groups fed with 20 and 50 % soybean peptide diet compared to the D-0 and D-35 groups, which suggested that replacing fish meal with soybean peptide in the diet might serve as an immunomodulatory agent and impact the immune response of yellow catfish [52].

In this study, the immunity benefits of soybean peptide replacement were further confirmed by the use of pathogenic infection. According to previous studies, bacteria-caused diseases have resulted in high levels of mortality in freshwater fish culture, such as those responsible for hemorrhagic septicemia and ulcerative diseases [64], thus resulting in significant economic loss [65]. This study demonstrated that appropriate and economical feedstuff material could positively affect the health status of fish and improve fish resistance against bacterial infection. In this study, based on our infection challenge, the preferable immunity benefit of yellow catfish receiving a 50 % soybean peptide diet was supported by the relatively low cumulative mortality seen after *A. hydrophila* infection. Our results clearly indicated that a 50 % fish meal replacement with soybean peptide may improve the immune response and enhance disease resistance of yellow catfish. This result may be further supported by Gregory et al. [49], who reported on the peptide from soybean as being capable of inducing defense genes involved in pathogen attack.

In conclusion, the optimum requirements of dietary soybean peptide replacement for fish meal in feed materials for yellow catfish was estimated to be 50 % soybean peptide on the basis of SGR, FCR, and cumulative mortality rate, respectively. This study provides evidence that a temporally optimized dietary replacement pattern with soybean peptide improves upon the growth performance, digestive function, and immune responses of yellow catfish.

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