Effects of Exogenous Lipase on Growth Performance, Body Composition, Serum Biochemical Indices, Digestive Enzyme Activity, and Apparent Digestibility of Nutrients in Japanese Seabass (Lateolabrax japonicus) Postprint

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Abstract

This study aimed to investigate the effects of adding exogenous lipase to low-protein, high-fat diets on growth performance, body composition, serum biochemical indices, digestive enzyme activities, and apparent nutrient digestibility in Japanese sea bass (Lateolabrax maculatus). Six hundred Japanese sea bass with an initial body weight of (6.26$±$0.02) g were randomly divided into 6 groups, with 4 replicates per group and 25 fish per replicate. The positive control group (G+ group) was fed a normal diet with high protein and low fat (41.76% crude protein and 8.34% crude fat), the control group (G0 group) was fed a low-protein, high-fat diet (37.64% crude protein and 11.15% crude fat), and the experimental groups (G100, G200, G400, and G800 groups) were fed experimental diets supplemented with 100, 200, 400, and 800 mg/kg lipase in the low-protein, high-fat diet, respectively. The experimental period lasted for 8 weeks. The results showed: 1) Compared with the G+ group, the final body weight, weight gain rate, feed intake, and hepatosomatic index of Japanese sea bass in the G0 group increased (P>0.05), the viscerosomatic index and lipid-somatic index increased significantly (P<0.05), serum total antioxidant capacity decreased significantly (P<0.05), the apparent digestibility of dry matter, crude protein, crude fat, and total energy decreased (P>0.05), and the apparent digestibility of fatty acids C16:1-n9 and C18:3-n3 increased significantly (P<0.05). 2) Compared with the G0 group, the final body weight and weight gain rate of the experimental groups decreased; among them, the G800 group showed significantly decreased final body weight and weight gain rate (P<0.05), significantly increased feed conversion ratio (P<0.05), significantly decreased survival rate (P<0.05), decreased viscerosomatic index and lipid-somatic index

(P>0.05), decreased midgut lipase and liver protease activities (P>0.05), and decreased serum cholesterol, high-density lipoprotein cholesterol, and triglyceride contents (P>0.05); the G200 group showed significantly decreased serum alanine aminotransferase activity and malondialdehyde content (P<0.05). It can be concluded that dietary fat can spare some protein, and the low-protein, high-fat diet had no significant effects on growth performance, body composition, and digestive enzyme activities in Japanese sea bass, but significantly increased the viscerosomatic index and lipid-somatic index. Supplementation of lipase in the low-protein, high-fat diet reduced growth performance in Japanese sea bass, had no significant effects on digestive enzyme activities and serum biochemical indices, decreased the apparent digestibility of crude fat and some fatty acids, but reduced the viscerosomatic index and lipid-somatic index to some extent and enhanced the body' s antioxidant capacity.

Full Text

Effects of Exogenous Lipase on Growth Performance, Body Composition, Serum Biochemical Indices, Digestive Enzyme Activity, and Nutrient Apparent Digestibility of Japanese Sea Bass (*Lateolabrax japonicus*)

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Abstract: This experiment was conducted to investigate the effects of dietary exogenous lipase supplementation on growth performance, body composition, serum biochemical indices, digestive enzyme activity, and nutrient apparent digestibility in Japanese sea bass (*Lateolabrax japonicus*) fed low-protein, high-fat diets. A total of 600 Japanese sea bass with initial body weight of (6.26$±$0.02) g were randomly allocated into six groups with four replicates per group and 25 fish per replicate. The positive control group (G+ group) was fed a normal diet with high protein and low fat (41.76% crude protein and 8.34% crude fat). The control group (G0 group) was fed a low-protein, high-fat diet (37.64% crude protein and 11.15% crude fat). The experimental groups (G100, G200, G400, and G800 groups) were fed the low-protein, high-fat diet supplemented with 100, 200, 400, and 800 mg/kg lipase, respectively. The feeding trial lasted for eight weeks.

The results showed: (1) Compared with the G+ group, the G0 group exhibited increased final body weight, weight gain rate, feed intake, and hepatosomatic in-

dex (P>0.05), with significant elevations in viscerosomatic index and intraperitoneal fat ratio (P<0.05). Serum total antioxidant capacity decreased significantly (P<0.05), while apparent digestibilities of dry matter, crude protein, crude fat, and gross energy declined (P>0.05). Notably, apparent digestibilities of fatty acids C16:1-n9 and C18:3-n3 increased significantly (P<0.05). (2) Compared with the G0 group, all lipase-supplemented groups showed reduced final body weight and weight gain rate. The G800 group exhibited significantly lower final body weight and weight gain rate (P<0.05), significantly higher feed conversion ratio (P<0.05), and significantly reduced survival rate (P<0.05). Viscerosomatic and intraperitoneal fat indices decreased (P>0.05), while mid-intestinal lipase and hepatic protease activities declined (P>0.05). Serum cholesterol, high-density lipoprotein cholesterol, and triglyceride contents decreased (P>0.05). The G200 group showed significantly reduced serum alanine aminotransferase activity and malondialdehyde content (P<0.05).

These findings indicate that dietary fat can partially spare protein. The low-protein, high-fat diet had no significant effects on growth performance, body composition, or digestive enzyme activity but significantly increased viscerosomatic and intraperitoneal fat ratios. Lipase supplementation in low-protein, high-fat diets reduced growth performance and decreased apparent digestibility of crude fat and some fatty acids, though it modestly reduced viscerosomatic and intraperitoneal fat indices while enhancing antioxidant capacity.

Keywords: Japanese sea bass; low-protein high-fat diet; exogenous lipase; growth performance; serum biochemical indices; digestive enzyme activity; apparent digestibility

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Introduction

The rapid development of aquaculture has exacerbated the shortage of protein feed resources. Fish protein metabolism produces ammonia as the final product, which can deteriorate water quality and trigger fish diseases. Dietary fat has been reported to exert a protein-sparing effect, thereby maintaining water quality and reducing disease incidence. Japanese sea bass (*Lateolabrax japonicus*) requires high dietary protein content. With increasing supply-demand contradictions for fish meal, prices continue to rise, elevating feed costs. Appropriately reducing dietary protein levels while increasing fat content represents one approach to reduce feed costs and alleviate protein resource shortages. However, high-fat diets can induce inflammatory responses and affect intestinal health in rats, possibly because excessive dietary fat cannot be completely digested by the animal intestine.

Lipase exhibits affinity for oil-water interfaces and can catalyze the hydrolysis of water-insoluble lipid substances at high rates, acting at hydrophilic-hydrophobic interface layers. Lipases from different sources vary in amino acid sequences and activity. Supplementing exogenous lipase can improve fat digestion in broiler

chickens and lipid metabolism in yellow-feathered broilers. Adding appropriate amounts of exogenous lipase to high-fat diets can enhance growth performance and improve intestinal health in grass carp. Therefore, exogenous lipase may improve fish growth by enhancing fat digestion, warranting further investigation. In vitro experiments have demonstrated that lipase can catalyze the conversion of polyunsaturated fats into polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Compared with poultry and mammals, fish have extremely low endogenous lipase activity, possibly due to the low metabolic activity of poikilothermic animals. Previous studies found that the apparent digestibility of dietary crude fat in juvenile sea bass (above 80%) was lower than that in juvenile tilapia and shrimp (above 95%), which may be related to limitations in endogenous enzymes, as they are deficient in juvenile or stressed states. Whether supplementing exogenous lipase would further affect growth by hydrolyzing fat remains unknown, and no relevant reports on lipase application in Japanese sea bass feed have been published. Japanese sea bass belongs to Perciformes, Serranidae, and *Lateolabrax* genus, commonly known as sea perch. It is a carnivorous, aggressive fish with tender, delicious meat, fast growth rate, and wide adaptability to salinity and temperature, making it one of the main species in China' s marine aquaculture. This study investigated the effects of lipase supplementation in low-protein, high-fat diets on growth performance, body composition, serum biochemical indices, digestive enzyme activity, and nutrient apparent digestibility in Japanese sea bass to provide reference for lipase application in practical sea bass feed.

Materials and Methods

1.1 Experimental Materials Lipase (from *Aspergillus niger*) was provided by a domestic enterprise, with activity measured at 20,000 U/g according to GBT 23535-2009. Experimental Japanese sea bass were purchased from Fujian Zhao' an County Gaoshiqin Aquaculture Cooperative.

1.2 Experimental Design and Diets Six hundred Japanese sea bass with initial body weight of (6.26$±$0.02) g were randomly allocated into six groups with four replicates per group and 25 fish per replicate. The positive control group (G+ group) received a normal diet with high protein and low fat (41.76% crude protein and 8.34% crude fat). The control group (G0 group) received a low-protein, high-fat diet (37.64% crude protein and 11.15% crude fat). The experimental groups (G100, G200, G400, and G800 groups) received the low-protein, high-fat diet supplemented with 100, 200, 400, and 800 mg/kg lipase, respectively. The feeding trial lasted for eight weeks. Dietary composition and nutrient levels are presented in Table 1 .

Feed ingredients were ground through a 40-mesh sieve, weighed according to group requirements, mixed using a stepwise amplification method, further homogenized with a V-shaped mixer, and then processed into pelleted feed (2.00

mm) using a twin-screw extruder (SLX-80). The pellets were air-dried in an air-conditioned room, sealed in plastic bags, and stored at -20°C until use.

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

Items	Groups
Ingredients	
Soybean meal	
White-fish meal	
Peanut meal	
Cottonseed meal	
Rapeseed meal	
Fish oil	
Soybean phospholipid	
Lard oil	
L-Met	
L-Lys · HCl	
High quality flour	
$CaHPO_4$	
Vitamin C phosphate ester	
Choline chloride	
Compound microelement[1])	
Compound vitamin[2])	
Microcrystalline cellulose	
Sodium alginate	
Betaine	
Lipase	
Y_2O_3	
Total	
Nutrient levels[3])	
Crude protein	
Ether extract	
Moisture	
Crude ash	
Gross energy/(MJ/kg)	

[1]) One kilogram of compound microelement contained: $MgSO_4 \cdot H_2O$ 12 g, $Ca(IO_3)_2$ 9 g, KCl 36 g, Met-Cu 1.5 g, $ZnSO_4 \cdot H_2O$ 10 g, $FeSO_4 \cdot H_2O$ 1 g, Met-Co 250 mg, $NaSeO_3$ 0.0036 g.

[2]) One kilogram of compound vitamin contained: VA 3,200,000 IU, VB_1 4 g, VB_2 8 g, VB_6 4.8 g, VB_{12} 16 mg, VD_3 1,600,000 IU, VE 16 g, VK 4 g, calcium pantothenate 16 g, folic acid 1.28 g, nicotinic acid 28 g, inositol 40 g, biotin 64 mg, moisture $ $10%.

[3]) Nutrient levels were measured values.

1.3 Feeding Management The feeding trial was conducted in an indoor recirculating aquaculture system (300 L water volume) at the Aquaculture Research Unit, Institute of Animal Science, Guangdong Academy of Agricultural Sciences. Fish were fed commercial sea bass feed during acclimation. Feeding occurred at 08:30 and 18:00 daily at 6-7% of body weight. Fish health and feeding behavior were monitored daily, with feed intake, mortality, and body weight recorded. The experiment was conducted under natural photoperiod with water temperature maintained at 26.6-31.6°C, dissolved oxygen $ $6 mg/L, pH 7.7-7.9, and ammonia nitrogen $ $0.25 mg/L.

1.4 Sample Collection and Analysis 1.4.1 Growth Performance

Prior to the experiment' s conclusion, fish were fasted for 24 h, then individually weighed and measured for body length. The following parameters were calculated: weight gain ratio (WGR), specific growth rate (SGR), feed conversion rate (FCR), feed intake (FI), survival rate (SR), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and intraperitoneal fat ratio (IPF). Formulas were as follows:

- Weight gain ratio (%) = {[Final body weight (g) - Initial body weight (g)] / Initial body weight (g)} × 100
- Specific growth rate = 100 × [Final body weight (g) - Initial body weight (g)] / Feeding days
- Feed conversion rate = Total feed intake / [Final body weight (g) + Dead fish weight (g) - Initial body weight (g)]
- Feed intake (g/fish) = Dry feed weight consumed (g) / [(Initial fish number + Final fish number) / 2]
- Hepatosomatic index (%) = [Liver weight (g) / Body weight (g)] × 100
- Viscerosomatic index (%) = [Visceral mass weight (g) / Body weight (g)] × 100
- Condition factor (g/cm^3) = [Body weight (g) / Body length (cm)^3] × 100
- Intraperitoneal fat ratio (%) = [Mesenteric fat weight (g) / Body weight (g)] × 100
- Survival rate (%) = (Final fish number / Initial fish number) × 100

1.4.2 Whole-Body Composition and Nutrient Apparent Digestibility

For whole-body composition analysis, two fish per replicate were randomly selected. Crude protein content was determined by the Kjeldahl method (GB/T 6432-1994), crude fat by Soxhlet extraction (GB/T 6433-1994), crude ash by incineration at 550°C (GB/T 6438-1992), and moisture by oven drying at 105°C (GB/T 6435-1986). Gross energy was measured using an oxygen bomb calorimeter (IKA-C2000).

Feces collection: One hour after feeding, uneaten feed was removed by siphoning, and feces were collected with a net. All collected feces were fixed with 6

mol/L HCl for nitrogen preservation, dried at 103°C for 15 min, then at 65°C overnight, pulverized with a micro-grinder, passed through a 40-mesh sieve, homogenized, sealed in bags, and stored at -20°C. Yttrium oxide (Y_2O_3) content in diets and feces was determined by inductively coupled plasma spectrometry at Guangzhou Analysis and Testing Center. Fatty acid content was analyzed by gas chromatography.

Apparent digestibility calculations: - Dry matter apparent digestibility (%) = [1 - (Y_2O_3 in diet / Y_2O_3 in feces)] × 100 - Nutrient apparent digestibility (%) = [1 - (Y_2O_3 in diet × Nutrient in feces) / (Y_2O_3 in feces × Nutrient in diet)] × 100

1.4.3 Digestive Enzyme Activity

Three fish per tank were randomly selected and dissected on ice. Intestines and livers were removed, cleared of contents and fat, rinsed with 4°C pre-cooled physiological saline, and blotted dry. Tissues were homogenized with 0.86% physiological saline at 1:4 (m/V) ratio under ice-water bath conditions to prepare 20% tissue homogenates. After centrifugation, supernatants were collected and stored at -80°C for lipase and amylase activity determination. For protease activity, accurate tissue weights were homogenized at 1:9 (m/V) ratio with homogenization medium, centrifuged at 2,500 r/min for 10 min, and supernatants were collected for analysis. Protease, lipase, and amylase activities in mid-intestine and liver were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to manufacturer instructions.

1.4.4 Serum Biochemical Indices

Ten fish per replicate were randomly selected for blood collection. Blood was drawn from the caudal vein, immediately placed in tubes, allowed to clot at room temperature for 3-4 h, then centrifuged at 3,500 r/min for 15 min using a high-speed refrigerated centrifuge. Serum was aliquoted and stored at -80°C for analysis. Total protein (TP), glucose (GLU), urea nitrogen (UN), cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) contents, and lactate dehydrogenase (LDH), glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) activities were measured by Guangzhou Kingmed Center for Clinical Laboratory. Total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, and acid phosphatase (ACP) and alkaline phosphatase (AKP) activities were determined using kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Data are presented as means ± standard error. Statistical analysis was performed using SPSS 20.0 software. One-way ANOVA was conducted for intergroup comparisons, followed by Duncan' s multiple comparison test if significant differences were detected. $P<0.05$ was considered statistically significant.

Results

2.1 Protein-Sparing Effects The effects of dietary protein and fat levels on growth performance, body composition, digestive enzyme activity, serum biochemical indices, and nutrient apparent digestibility are presented in Tables 2 through 6 . Fish fed the low-protein, high-fat diet (G0 group) showed higher final body weight, weight gain rate, and feed intake compared with those fed the high-protein, low-fat diet (G+ group), though differences were not significant ($P>0.05$). The G0 group exhibited increased hepatosomatic, viscerosomatic, and intraperitoneal fat indices compared with the G+ group, with viscerosomatic and intraperitoneal fat indices reaching statistical significance ($P<0.05$). No significant differences were observed in condition factor or survival rate between G0 and G+ groups ($P>0.05$). Hepatic and mid-intestinal protease, amylase, and lipase activities, as well as body composition and serum biochemical indices (except total antioxidant capacity), showed no significant differences between G0 and G+ groups ($P>0.05$). However, serum total antioxidant capacity in the G0 group was significantly lower than in the G+ group ($P<0.05$).

Compared with the G+ group, the G0 group exhibited slight decreases in apparent digestibilities of dry matter, crude protein, crude fat, and gross energy, though differences were not significant ($P>0.05$). Notably, apparent digestibilities of fatty acids C16:1-n9 and C18:3-n3 were significantly elevated in the G0 group ($P<0.05$).

2.2 Effects of Exogenous Lipase on Growth Performance and Body Composition As shown in Table 2 , all lipase-supplemented groups exhibited reduced final body weight and weight gain rate compared with the G0 group. The G100 and G800 groups showed significantly lower final body weight ($P<0.05$), while the G800 group displayed significantly reduced weight gain rate ($P<0.05$) and significantly increased feed conversion ratio ($P<0.05$). All experimental groups had significantly lower survival rates compared with the G0 group ($P<0.05$). No significant differences were observed in feed intake, viscerosomatic index, hepatosomatic index, or condition factor among groups ($P>0.05$). All lipase-supplemented groups showed reduced intraperitoneal fat ratios compared with the G0 group ($P>0.05$).

Table 2. Effects of Exogenous Lipase on Growth Performance and Physical Indices of *Lateolabrax japonicus*

Items	Groups
IBW/g	
FBW/g	
WGR/%	
FI/(g/fish)	
VSI/%	
HSI/%	

Items	Groups
IPF/%	
CF/(g/cm^3)	
Survival rate/%	

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

As shown in Table 3 , no significant differences were observed in whole-body moisture, crude protein, crude fat, or crude ash contents among groups (P>0.05), though crude fat content tended to decrease in lipase-supplemented groups.

Table 3. Effects of Exogenous Lipase on Body Composition of *Lateolabrax japonicus* (Wet Weight Basis) %

Items	Groups
Moisture	
Crude protein	
Ether extract	
Crude ash	

2.3 Effects of Exogenous Lipase on Serum Biochemical Indices
As shown in Table 4 , serum urea nitrogen content increased in all lipase-supplemented groups compared with the G0 group, though differences were not significant (P>0.05). The G100 and G400 groups showed 16.13% and 21.51% increases in serum urea nitrogen, respectively. Serum total protein, cholesterol, HDL-C, and triglyceride contents, as well as alanine aminotransferase activity, decreased in all experimental groups compared with the G0 group. The G200 and G800 groups exhibited significantly lower serum alanine aminotransferase activity (P<0.05). No significant differences were observed in serum lactate dehydrogenase activity among groups (P>0.05). Serum total antioxidant capacity increased in all experimental groups but did not differ significantly from the G0 group (P>0.05). Except for the G100 group, serum malondialdehyde content decreased in all lipase-supplemented groups, with the G200 group showing significantly lower values than the G0 group (P<0.05). No significant differences were observed in serum acid phosphatase or alkaline phosphatase activities among groups (P>0.05).

Table 4. Effects of Exogenous Lipase on Serum Biochemical Indices of *Lateolabrax japonicus*

Items	Groups
TP/(g/L)	
UN/(mmol/L)	
CHO/(mmol/L)	
HDL-C/(mmol/L)	
LDL-C/(mmol/L)	
TG/(mmol/L)	
LDH/(U/L)	
GOT/(U/L)	
GPT/(U/L)	
T-AOC/(U/mL)	
MDA/(nmol/mL)	
ACP/(U/dL)	
AKP (King' s unit)	

2.4 Effects of Exogenous Lipase on Digestive Enzyme Activity As shown in Table 5 , mid-intestinal and hepatic protease activities decreased in all lipase-supplemented groups compared with the G0 group, though differences were not significant (P>0.05). No significant differences were observed in mid-intestinal or hepatic lipase and amylase activities among groups (P>0.05).

Table 5. Effects of Exogenous Lipase on Digestive Enzyme Activity of *Lateolabrax japonicus*

Items	Groups
Mid-intestine	
Protease (U/mg prot)	
Amylase (U/mg prot)	
Lipase (U/mg prot)	
Liver	
Protease (U/mg prot)	
Amylase (U/mg prot)	
Lipase (U/mg prot)	

2.5 Effects of Exogenous Lipase on Nutrient Apparent Digestibility As shown in Table 6 , the G100, G400, and G800 groups exhibited significantly reduced apparent digestibility of crude fat compared with the G0 group (P<0.05). Gross energy apparent digestibility decreased but did not differ significantly (P>0.05). No significant differences were observed in apparent digestibilities of dry matter, crude protein, or phosphorus among groups (P>0.05). The G400 and G800 groups showed significantly lower apparent digestibility of fatty acid C14:0 (P<0.05). The G200 and G800 groups exhibited significantly reduced apparent digestibility of fatty acid C15:0 (P<0.05). The G400 group showed

significantly lower apparent digestibilities of fatty acids C20:0 and C18:3-n3 ($P<0.05$). No significant differences were observed in apparent digestibilities of fatty acids C18:0, C24:0, C16:1-n9, C18:1-n9, C18:2-n6, C20:1-n9, or C22:6-n3 among groups ($P>0.05$), though C20:1-n9 digestibility tended to decrease.

Table 6. Effects of Exogenous Lipase on Nutrient Apparent Digestibilities of *Lateolabrax japonicus* (Dry Matter Basis) %

Items	Groups
Dry matter	
Crude protein	
Ether extract	
Phosphorus	
Gross energy	
Fatty acids	
C14:0	
C15:0	
C18:0	
C20:0	
C24:0	
C16:1-n9	
C18:1-n9	
C18:2-n6	
C18:3-n3	
C20:1-n9	
C22:6-n3	

Discussion

3.1 Protein-Sparing Effects In this study, fish fed the low-protein, high-fat diet showed no significant differences in final body weight, weight gain rate, feed intake, or hepatic and mid-intestinal digestive enzyme activities compared with those fed the high-protein, low-fat diet. These results differ from Liu et al. [8], who reported that grass carp fed low-protein, high-fat diets (26% crude protein and 6% crude fat without lipase) exhibited significantly higher final body weight, weight gain rate, feed intake, and hepatic and intestinal digestive enzyme activities than those fed normal diets (28% crude protein and 5% crude fat). The discrepancy may be attributed to the greater magnitude of protein reduction and fat increase in this study (4% protein reduction and 3% fat increase) compared with Liu et al. [8] (2% protein reduction and 1% fat increase), or to species differences requiring further investigation. In this study, fish fed the low-protein, high-fat diet showed higher hepatosomatic, viscerosomatic, and intraperitoneal fat indices than those fed the normal diet, with viscerosomatic and intraperitoneal fat indices reaching significance. Lu et al. [12] reported that the appropriate dietary fat level for juvenile sea bass is approximately 10%,

suggesting that the fat level in this study was suitable but that high-fat diets more readily caused hepatic burden and visceral fat deposition.

Fish fed the low-protein, high-fat diet exhibited significantly lower serum total antioxidant capacity than those fed the normal diet. While high-fat diets can induce inflammatory responses in rats [5], Liu et al. [8] suggested that high-fat diets could improve intestinal immunity and antioxidant capacity in grass carp. These differences may arise from variations in blood versus intestinal pathways or species differences, warranting further investigation. Compared with the normal diet group, the low-protein, high-fat diet group showed slight decreases in apparent digestibilities of dry matter, crude protein, crude fat, and gross energy, though not significantly. However, apparent digestibilities of fatty acids C16:1-n9 and C18:3-n3 increased significantly, likely because the increased lipid in the low-protein, high-fat diet was primarily lard, which contains high levels of C16:1-n9, and higher substrate content promoted digestion. Overall, these results demonstrate that dietary fat can partially spare protein, but the magnitude of protein reduction and fat increase in feed formulation must be appropriate.

3.2 Effects of Exogenous Lipase on Growth Performance and Body Composition Studies have shown that lipase supplementation in broiler chickens [7,13-16], pigs [17-19], and ducks [20] can improve growth performance, with similar reports in aquatic animals. The effects depend on lipase source. Ye et al. [7] found that lipase from *Aspergillus niger* was more effective than that from *Candida* species, while Gao et al. [15] reported that fungal lipases were superior to bacterial sources, with *Aspergillus niger* lipase showing the best efficacy. Gu et al. [21] demonstrated that 300 mg/kg lipase supplementation in juvenile yellow catfish significantly improved weight gain rate and reduced feed conversion ratio. Yang et al. [22] reported that lipase supplementation in southern catfish feed significantly reduced feed conversion ratio. Liu et al. [8] found that appropriate lipase supplementation (1,193 and 2,560 U/kg) in low-protein, high-fat diets improved growth performance and intestinal immunity in grass carp by increasing anti-inflammatory cytokines, decreasing pro-inflammatory factors, enhancing intestinal antioxidant enzyme activities, and improving intestinal physical barrier function, ultimately promoting growth through improved intestinal health.

The current results differ from these reports, possibly due to excessive lipase supplementation (2,000-16,000 U/kg) reducing endogenous enzyme activity or excessive free fatty acid production from lipolysis [23] affecting fatty acid digestion and absorption, or both. The lipase used in this study was derived from *Aspergillus niger*, a 1,3-position-specific lipase that produces 2-monoglycerides and free fatty acids as final hydrolysis products [24]. High levels of n-3 polyunsaturated fatty acids have been shown to reduce non-specific immunity and immune-related gene expression in large yellow croaker [25]. Excessive saturated fatty acids and n-6 polyunsaturated fatty acids can aggravate inflammatory re-

sponses in humans by activating the inflammatory regulator nuclear factor- B (NF- B) [26]. High fatty acid levels have also been reported to cause oxidative damage in Atlantic salmon [27]. Luo et al. [28] reported that intestinal mucosal damage in Jian carp led to reduced growth performance. In this study, the significantly lower survival rate in lipase-supplemented groups may be attributed to excessive free fatty acids reducing immunity. Liu et al. [8] also found that high-level lipase supplementation (3,730 U/kg) reduced growth performance and intestinal immunity and antioxidant capacity in grass carp. The reduced growth performance in sea bass fed exogenous lipase may be related to decreased digestive enzyme activity, reduced fat and fatty acid apparent digestibility, and oxidative damage.

Ye et al. [7] found that different lipases (from *Aspergillus niger* and *Candida*) affected protein and fat content in breast muscle dry matter of yellow-feathered broilers, indicating that lipase can alter fat deposition in body parts. Gong et al. [14] reported that lipase supplementation reduced abdominal fat ratio in broilers. In this study, lipase supplementation tended to reduce intraperitoneal fat ratio in sea bass, consistent with these results. Yang et al. [22] found that lipase supplementation in southern catfish significantly improved condition factor but had no significant effects on visceral, hepatic, or intestinal fat indices, which aligns with the non-significant reduction in viscerosomatic index observed in this study. These findings are consistent with Wang et al. [29] and Huang et al. [30], who reported that enzyme supplementation in animal feeds had no significant effects on body composition, likely depending on animal species, enzyme type, and activity.

3.3 Effects of Exogenous Lipase on Serum Biochemical Indices Serum biochemical indices are widely used to evaluate fish health status, nutritional condition, and environmental adaptation, serving as important physiological, pathological, and toxicological indicators [31]. Although factors such as sex, growth, exercise, satiation, and health status affect these indices [32], they remain valuable for assessing animal physiological and health conditions. Ye et al. [7] found that dietary lipase reduced serum total cholesterol, HDL, and LDL while increasing the HDL/LDL ratio in late-phase yellow-feathered broilers. In weaned piglets, 2,000 U/kg lipase significantly or extremely significantly reduced serum total cholesterol, triglycerides, HDL-C, and LDL-C [17]. Similar results have been reported in fish: Gu et al. [21] found that lipase significantly reduced serum triglycerides, LDL, and total cholesterol in yellow catfish, possibly related to lipase hydrolysis specificity. However, conflicting results exist: lipase supplementation had no significant effects on serum free fatty acids, HDL, LDL, or HDL/LDL ratio in weaned piglets [18], and no significant effects on serum total cholesterol, triglycerides, HDL, or LDL in southern catfish [22]. This study found that lipase supplementation in sea bass reduced serum total protein, cholesterol, HDL-C, and triglyceride contents without significant differences, consistent with some previous reports [18,22].

This study demonstrated that lipase supplementation significantly reduced serum alanine aminotransferase activity while increasing total antioxidant capacity and significantly decreasing malondialdehyde content. Combined with reduced growth performance and survival rate, these results suggest that lipase supplementation may have induced inflammatory responses due to excessive free fatty acids from fat hydrolysis, subsequently elevating antioxidant indicators. Fat digestion produces fatty acids in mammalian and fish intestines [33], and appropriate fatty acid levels benefit fish growth and health, whereas excessive levels have negative effects. Zuo et al. [25] reported that high dietary n-3 polyunsaturated fatty acids reduced non-specific immunity and immune-related gene expression in large yellow croaker. Wood et al. [26] indicated that excessive saturated and n-6 polyunsaturated fatty acids aggravated inflammatory responses by activating NF- B. High fatty acid levels have also been reported to cause oxidative damage in Atlantic salmon [27].

3.4 Effects of Exogenous Lipase on Digestive Enzyme Activity and Nutrient Apparent Digestibility The intestine is crucial for food digestion [34], and digestive enzyme activity primarily influences digestibility [35]. Gu et al. [21] reported that lipase supplementation significantly improved trypsin and intestinal amylase activities in yellow catfish, with the 100 mg/kg group also significantly enhancing pancreatic lipase and amylase activities. Liu et al. [8] found that lipase supplementation significantly increased hepatic and intestinal protease, lipase, and amylase activities in grass carp. In contrast, this study showed that lipase supplementation in low-protein, high-fat diets reduced mid-intestinal protease and lipase activities and hepatic protease activity while increasing mid-intestinal amylase activity, though not significantly. Lipase supplementation modestly reduced growth performance by decreasing digestive enzyme activity, inconsistent with previous reports, possibly due to differences in lipase source and experimental animals.

Ma et al. [13] found that 1,000 U/kg lipase supplementation in low-energy broiler diets improved dry matter apparent utilization and significantly enhanced crude fat apparent utilization. Gao et al. [15] reported that fungal lipase at 2,000 U/kg significantly improved crude fat and energy apparent metabolic rates in white-feathered broilers, indicating source-dependent effects. In piglets, lipase improved apparent digestibilities of dry matter, crude protein, and gross energy [17], and improved dry matter, crude fat, and gross energy apparent digestibility in weaned piglets [18], differing from chicken studies and suggesting species-specific effects. Yang et al. [22] reported that lipase significantly improved crude fat digestibility in southern catfish. However, this study found that G100, G400, and G800 groups exhibited significantly reduced crude fat apparent digestibility and lower gross energy apparent digestibility, with decreased apparent digestibilities of fatty acids C14:0, C15:0, C20:0, and C18:3-n3. Therefore, lipase supplementation in sea bass feed reduced digestive enzyme activity and fatty acid apparent digestibility, decreasing fat absorption and utilization. These discrepancies may result from excessive lipase supplementation or dif-

ferent microbial sources. Studies in common carp have shown that excessive enzyme supplementation can reduce nutrient absorption [36].

Conclusions

1. Dietary fat can partially spare protein. Low-protein, high-fat diets had no significant effects on growth performance, body composition, or digestive enzyme activity in Japanese sea bass but significantly increased viscerosomatic and intraperitoneal fat ratios.

2. Lipase supplementation in low-protein, high-fat diets had no significant effects on final body weight, weight gain rate, or feed intake in Japanese sea bass, with performance slightly decreasing. Crude fat and some fatty acid apparent digestibilities decreased, though viscerosomatic and intraperitoneal fat ratios were modestly reduced while antioxidant capacity improved.

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