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# Can Taurine Supplementation in a Diet with Soybean Meal Instead of Fish Meal Improve the Growth Performance, Feed Utilization, and Antioxidant Capacity of Spotted Knifejaw (*Oplegnathus punctatus*)?

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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Abstract: To determine the impact of replacing fish meal (FM) in the diet with various levels of soybean meal (SBM) on the spotted knifejaw Oplegnathus punctatus, a 56 day feeding trial was done. Seven diets were formulated with SBM to replace 0% (SBM0), 30% (SBM30), 40% (SBM40), 50% (SBM50), 60% (SBM60), and 70% (SBM70) of FM protein, and SBM50 + T was developed on the basis of SBM50 with the addition of 1.2% taurine. There were triplicate groups of 18 fish (initial weight:  $14.62 \pm 0.02$  g). The weight gain (WG), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER) values of the SBM0, SBM30, and SBM50 + T groups were found to be significantly higher than those of the SBM60 and SBM70 groups (p < 0.05). The daily energy gain (DEG), daily nitrogen gain (DNG), daily lipid gain (DLG), energy retention (ER), nitrogen retention (NR), and lipid retention (LR) values decreased significantly with increasing dietary SBM levels (p < 0.05). The highest retention of most amino acids (except lysine) was observed in the SBM30 group (p < 0.05). The lipid content of the whole body and dorsal muscle decreased significantly as dietary SBM levels increased (p < 0.05). Fish fed the SBM70 diet had the lowest serum triglyceride (TG) concentrations (p < 0.05). The effects of different treatments on total cholesterol (T-CHO) were not significant (p > 0.05). Fish fed the SBM0 and SBM30 diets had the highest amylase (AMS) and lipase (LPS) activities (p < 0.05). The lowest liver superoxide dismutase (SOD) and catalase (CAT) activities were observed in the SBM70 group. The malondialdehyde (MDA) concentration of the SBM50 to SBM70 groups were significantly higher than that of other groups (p < 0.05). The levels of interleukin 8 (*il-8*) mRNA were highest in fish fed the SBM0, SBM30, and SBM50 + T diets (p < 0.05), while the level of transforming growth factor  $\beta 1$  (*tgf-\beta 1*) was the opposite (p < 0.05). According to the broken line regression of WG and FE, the highest level of FM substitution by SBM for Oplegnathus punctatus was 24.07-25.31%.

**Keywords:** spotted knifejaw; *Oplegnathus punctatus*; taurine; soybean meal; growth performance; feed efficient; inflammation; antioxidant capacity

# 1. Introduction

Fish meal (FM) has been used as a major protein source in aqua-feeds due to its relatively balanced amino acid pattern, high mineral and vitamin contents, and long-chain omega-3 fatty acids [1]. Over the last 20 years, although the global FM production has remained relatively stable, it has not been able to match the rapid growth of the worldwide aquaculture industry. The limited supply of FM has led to a continuous rise of the price of commercial diets [2–4]. Therefore, it is critical to seek alternative sources of protein to ensure a steady supply of commercial diets. Numerous researchers who use plant protein sources such as soybean meal, canola meal, pea protein, corn gluten meal, and so on to

partially or completely replace FM have reported significant progress in different fish species [5,6].

Compared with other plant proteins, soybean meal (SBM) is regarded as a nutritious feedstuff with a high crude protein content, wide availability, relatively balanced amino acid profile, and a stable supply [7–9]. Some studies have demonstrated considerable success in the partial or complete replacement of FM with SBM in the diets of many fish species [10,11]. However, some species have a limited capacity to use SBM. High dietary levels of soybean meal also significantly reduce the protease, amylase, and lipase activities in the digestive tract of *Oreochromis niloticus* and *Myxocyprinus asiaticus* [11,12]. Even in some fish, such as some salmonids, high levels of SBM can cause severe enteritis [13]. Therefore, it is worth noting that the ability to utilize SBM as a protein source varies among different species.

As a non-proteinogenic essential amino acid or conditionally essential amino acid, taurine has received special attention from nutritionists [14]. Taurine plays a vital role in regulating the physiological functions of fish, including growth promotion, immune response modulation, feeding stimulation, cellular osmoregulation, antioxidant action, and detoxification [15–19]. Although most animals can synthesize taurine from methionine and cysteine, some species have a limited capacity to synthesize this sulfur-containing amino acid due to the lack of L-cysteine sulphinate decarboxylase [14,20,21]. In addition, plant-based ingredients are severely deficient in cysteine, methionine, and serine and do not contain taurine, compared to animal-based ingredients [15]. Thus, diets containing plant protein must provide sufficient taurine to meet the physiological needs of fish for optimal growth, health, and development. It is reported that taurine supplementation can increase growth performance and feed utilization efficiency in various fish fed diets containing high levels of plant proteins, such as *Argyrosomus regius* [22], *Diplodus sargus* [23], *Oreochromis niloticus* [24], *Acanthopagrus schlegelii* [25], and *Oncorhynchus mykiss* [26]. These results suggest the potential for incorporating high levels of SBM into the diets with added taurine.

The spotted knifejaw, *Oplegnathus punctatus*, is a carnivorous marine fish with high economic value due to its beautiful appearance and good taste [27,28]. In recent years, the aquaculture of *Oplegnathus punctatus* has increased due to the continuous expansion of the market demand in China. However, studies on the nutritional requirements of this species are limited. For example, the optimal lipid and protein requirements were estimated to be 10.46–12.83% and 42.92–46.44%, respectively [29,30]. The current culture of *Oplegnathus punctatus* mainly relies on the commercial diets of other species, for example, *Pseudosciaena crocea*. It is incompatible with the development of the emerging aquaculture culture industry. There are currently no studies on alternative protein sources for this species, especially the impact of SBM on the growth, antioxidant capacity, and immunity of juvenile *Oplegnathus punctatus*. Therefore, there is an urgent need to develop an effective diet that provides balanced nutrition for *Oplegnathus punctatus*.

#### 2. Materials and Methods

#### 2.1. Experimental Diets

Seven isonitrogen (43% crude protein) and isoenergy (20.00 kJ g<sup>-1</sup>) diets were prepared. FM protein was replaced with 0%, 30%, 40%, 50%, 60%, and 70% SBM protein, and 1.2% taurine was added on the basis of 50% SBM (designated as SBM0, SBM30, SBM40, SBM50, SBM60, and SBM50 + T, respectively). The composition and formula of the diets used in the experiment are listed in Table 1. Table 2 shows the composition of amino acids in the diets. All dry ingredients were mixed thoroughly for 15 min using a mixer. Subsequently, oil (soybean oil and fish oil) and water were added to the dry mixture sequentially, which was mixed again for 15 min. Afterward, the diets (size, 1.5 mm) were obtained by a twin-screw extruder, air-dried in a 45 °C oven overnight, and then stored in a refrigerator at -20 °C until use.

		]	Diets				
Ingredients (g 100 g <sup>-1</sup> )	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T
Fish meal <sup>1</sup>	57.04	39.93	34.22	28.52	22.82	17.11	28.52
Soybean meal <sup>2</sup>	0.00	24.45	32.61	40.76	48.91	57.06	40.76
Corn starch	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Fish oil	4.41	5.79	6.24	6.70	7.16	7.62	6.70
Soybean oil	3.00	2.04	1.72	1.40	1.08	0.76	1.40
Vitamin mix <sup>3</sup>	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix <sup>4</sup>	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin C	0.50	0.50	0.50	0.50	0.50	0.50	0.50
СМС	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Taurine							1.20
Cellulose	18.75	10.99	8.40	5.82	3.23	0.64	4.62
	Proxim	ate compositi	on (g 100 g <sup>-1</sup>	dry matter)			
Moisture	8.04	6.20	6.51	8.64	8.42	9.38	8.46
Ash	7.44	7.28	7.13	7.10	7.02	6.89	6.91
Crude protein	42.29	42.44	43.45	42.61	42.37	42.96	43.40
Crude lipid	13.32	13.62	13.09	13.59	14.11	13.65	12.46
Gross energy (kJ $g^{-1}$ )	20.78	19.63	19.28	18.85	18.32	18.78	19.19
$P/E (mg kJ^{-1})$	20.35	21.62	22.54	22.60	23.13	22.88	22.62
NFE	8.56	8.84	7.28	5.46	4.95	4.24	6.15

Table 1. The formulation and proximate composition of the experimental diets (% dry matter).

Notes: <sup>1</sup> Trident Seafoods Corporation, Seattle, WA, USA. <sup>2</sup> Zhonghai Cereals and Oils Industry Co., Ltd., Zhoushan, China. <sup>3</sup> Vitamin mixture (g/kg mixture): menadione (4.0); riboflavin (5.0); inositol (200.0); biotin (0.6); folic acid (1.5); cyanocobalamin (0.01); D-Ca pantothenate (10.0); nicotinic acid (20.0); pyridoxine hydrochloride (4.0); thiamin hydrochloride (5.0); tocopherol (40.0); axerophthol (5.0); Vitamin D (4.8);  $\alpha$ -cellulose (700.1). <sup>4</sup> Mineral mixture (g/kg mixture): MgSO<sub>4</sub>·7H<sub>2</sub>O (90.43); Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> (122.87); KI (0.02); KH<sub>2</sub>PO<sub>4</sub> (42.03); FeSO<sub>4</sub>·7H<sub>2</sub>O (19.73); CuSO<sub>4</sub>·5H<sub>2</sub>O (0.34); NaCl (32.33); KCl (65.75); CoCl<sub>2</sub>·6H<sub>2</sub>O (0.79); ZnSO<sub>4</sub>·7H<sub>2</sub>O (8.44); ferric citrate (38.26); K<sub>2</sub>SO<sub>4</sub> (163.83); C<sub>6</sub>H<sub>10</sub>CaO<sub>6</sub>·5H<sub>2</sub>O (683.62); MnSO<sub>4</sub>·H<sub>2</sub>O (0.37).

Table 2. Amino acid composition (% total amino acid) of the test diets.

				Diets			
Amino Acids	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T
Arginine	4.38	4.36	4.65	4.24	4.38	4.87	4.84
Histidine	3.08	3.00	3.22	2.83	3.02	3.17	3.13
Isoleucine	6.01	5.56	5.25	5.91	5.73	5.80	6.13
Leucine	7.99	7.36	7.33	7.97	7.66	7.75	6.13
Lysine	6.42	6.98	6.86	6.85	6.53	6.61	6.66
Methionine	4.13	4.00	3.50	3.73	3.63	3.59	4.04
Phenylalanine	4.31	3.76	4.17	4.24	3.98	3.86	4.10
Threonine	3.54	3.87	3.74	3.34	3.14	3.11	2.65
Valine	3.31	3.42	2.91	3.15	3.06	2.96	2.71
Aspartic acid	7.26	7.92	7.68	8.32	7.90	7.80	7.53
Glutamic acid	18.71	19.00	19.94	18.68	19.93	20.39	21.56
Serine	6.78	6.76	6.69	6.25	6.56	6.15	6.79
Proline	5.75	5.47	5.57	5.24	4.92	4.95	5.09
Glycine	4.92	4.50	4.89	4.73	4.88	4.45	4.88
Alanine	6.35	6.36	5.89	6.42	6.41	6.50	5.66
Tyrosine	5.42	6.00	5.97	6.49	6.69	6.48	6.53
Cystine	1.67	1.68	1.75	1.63	1.58	1.58	1.56

### 2.2. Experimental Fish and Feeding Trial

The fish used in the experiments were provided by China Qingdao Mingbo Aquatic Products Co., Ltd., and the feeding experiment was carried out in the Key Laboratory of Mariculture and Improvement, Zhejiang Institute of Marine Fisheries, Zhoushan, China. Before starting the feeding experiments, fish were fed commercial diets for 20 days to adapt to the laboratory environment. After that, 21 circular tanks filled with 300 L of seawater were stocked with 18 fish (average initial body weight,  $14.62 \pm 0.02$  g per). Fish were fed twice (8:30 and 16:30) per day for 8 weeks. During feeding, a root blower was used for uninterrupted aeration to guarantee that dissolved oxygen surpassed 6 mg L<sup>-1</sup>. The water temperature was maintained at  $28.7 \pm 1.4$  °C, and the ammonia nitrogen concentration did not exceed 0.05 mg L<sup>-1</sup>. The salinity and pH value of the water were  $24 \pm 0.8$  g L<sup>-1</sup> and  $7.5 \pm 0.1$  respectively. Experiments were performed under a natural photoperiod, and each tank's lighting was kept consistent.

## 2.3. Sample Collection and Analysis

Before the feeding test, 18 fish were randomly chosen as initial samples and were frozen for the subsequent whole-body composition analysis. At the end of the culture experiment, all fish were starved for 24 h before sampling, and the total number and weight of fish in each tank were subsequently recorded. Three fish were randomly sampled from each tank for whole-body composition and total energy analyses. Blood samples were collected from the caudal vein with a hypodermic syringe and centrifuged at  $4000 \times g$  (4 °C) for 10 min (CT15RE centrifuge, Hitachi, Japan). The viscera, intraperitoneal fat, liver, and intestine were extracted and weighed. All of these samples were processed using liquid nitrogen and then stored at -80 °C.

According to AOAC (1995), the analysis of the whole body and the tissues and the composition of the feed were determined. Moisture was detected by weight removal using a drying oven (diet, 105 °C) or a lyophilizer (LL1500, Thermo Scientific, Waltham, MA, USA) (fish and tissue, -110 °C). The crude protein of the sample was analyzed by an Auto Kjeldahl System (K355/K437, Buchi, Flawil, Switzerland) according to the Kjeldahl method. The ash content was determined in a muffle furnace at 550 °C for 12 h. Crude lipids were determined by ether extraction using a Soxhlet apparatus (E816, Buchi, Flawil, Switzerland). With the use of a calorimeter (HWR-15E, Shangli, Shanghai, China), the total energy of the diet and that of the whole body were assessed. To test the amino acid composition of the samples, the amino acid samples were sent to a professional laboratory for measurement using an automatic analyzer (L-8900, HITACHI, Tokyo, Japan).

The triglyceride (TG) and total cholesterol (T-CHO) contents in serum were evaluated through the method provided by [31]. The activities of lipase (LPS) and amylase (AMS) in the intestinal tract were determined, as described by [32]. Catalase (CAT) was analyzed using the methods provided by [33]. Superoxide dismutase (SOD) activity was measured according to the method provided by [34]. The concentration of malondialdehyde (MDA) was determined by the method provided by [35]. All parameters above were measured by commercial test kits (Nanjing Jianchen Bioengineering Institute, Nanjing, China) and a microplate reader (Multiskan Go, Thermo Scientific, Waltham, MA, USA).

# 2.4. Gene Expression

Total RNA from liver was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and quantified using 1.0% agarose electrophoresis. Subsequently, according to the manufacturer's scheme, 1 µg of RNA was used for cDNA synthesis using a PrimeScript<sup>TM</sup> RT Reagent kit (perfect Real Time; Takara, Dalian, China). The relative expressions of interleukin-8 (*il-8*) and transforming growth factor  $\beta 1$  (*tgf-\beta 1*) genes were determined by a Real-Time PCR System (QuantStudio<sup>TM</sup> 6 Flex, Life Technologies, Carlsbad, CA, USA) according to [36]. The  $\beta$ -atin gene was chosen as an internal reference. The specific primers used in this study are shown in Table 3. Relative expression levels were calculated based

on the  $2^{-\triangle \triangle CT}$  equation according to [37]. Each treatment was performed in triplicate and was repeated three times per sample.

Table 3. The sequences of primers used in this study.

	Sequence (5' to 3')	Amplicon Length (bp)	References
tgf-β1-F tgf-β1-R	GCCAGACGAGCGTTGATAGTG TTTTTTGCGTGAGGTGAG	161	
<i>il-8</i> -F <i>il-8</i> -R	CACTGCCGCTGCATCC GGTCCAGGCAAACCTCTTGG	142	According to the sequence design of striped knifejaw,
β-actin-F β-actin-R	GCTGTGCTGTCCCTGTA GAGTAGCCACGCTCTGTC	185	Oplegnathus fasciatus

2.5. Calculation and Statistical Analysis

Weight gain (%) =  $100 \times$  (final body weight – initial body weight)/initial body weight. Specific growth rate =  $100 \times (Ln \text{ (final body weight)} - Ln \text{ (initial body weight)})/days.$ Feed efficiency = wet weight gain/dry feed consumed. Protein efficiency ratio = wet weight gain/protein intake. Daily feed intake =  $100 \times$  feed offered/average total weight/days. Viscerosomatic index =  $100 \times$  (viscera weight/whole body weight). Intraperitoneal fat ratio =  $100 \times$  (intraperitoneal fat weight/whole body weight). Hepatosomatic index =  $100 \times$  (hepatosomatic weight/whole body weight). Condition factor =  $100 \times (\text{live weight/length}^3)$ . Average body weight (ABW) = (initial body weight + final body weight)/2. Daily nitrogen intake = feed intake nitrogen/ABW  $\times$  days. Daily nitrogen gain = (final body weight  $\times$  final body nitrogen -initial body weight  $\times$ initial body nitrogen)/ABW  $\times$  days. Nitrogen retention =  $100 \times \text{daily nitrogen gain/daily nitrogen intake}$ . Daily energy intake = feed intake energy/ABW  $\times$  days. Daily energy gain = (final body weight  $\times$  final body energy – initial body weight  $\times$  initial body energy)/ABW  $\times$  days. Energy retention =  $100 \times \text{daily nitrogen gain/daily nitrogen intake}$ . Daily lipid intake = feed intake lipid/ABW  $\times$  days. Daily lipid gain = (final body weight  $\times$  final body lipid -initial body weight  $\times$  initial body lipid)/ABW  $\times$  days. Lipid retention =  $100 \times \text{daily lipid gain/daily lipid intake}$ . Data were expressed as an average (n = 3) and analyzed statistically using SPSS 24.0 (IBM, Chicago, IL, USA). After Levene's test was used to analyze the homogeneity of each treatment, one-way analysis of variance (ANOVA) was used to determine the treatment

effect, and Duncan's multi-range test was used to determine the treatment deviation. Linear and quadratic regression models were constructed to test the correlation between results and dietary SBM substitution FM levels. The statistical significance level was 5% (p < 0.05). Based on WG and FE, a polyline model was applied to determine the maximum substitution level of SBM for FM.

## 3. Results

3.1. Growth Performance, Feed Utilization, and Biometric Parameters

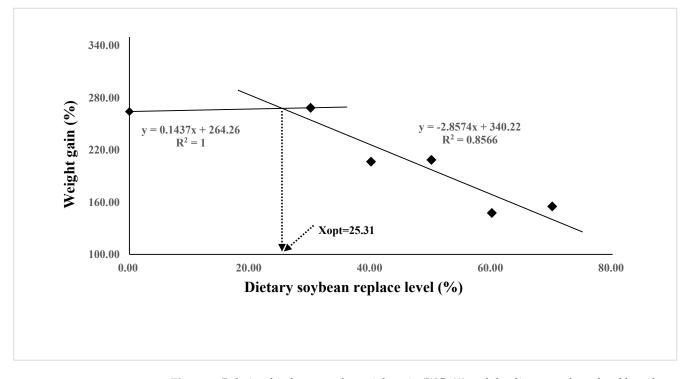
The growth performance and feed utilization of juvenile *Oplegnathus punctatus* were significantly affected by replacing FM in diets with different levels of SBM (Table 4). Although not statistically different, the weight gain (WG), specific growth rate (SGR), and protein efficiency ratio (PER) values of the SBM0 and SBM30 groups were numerically higher than those of the SBM40 and SBM50 groups (p > 0.05) and significantly higher than those of the SBM60 and SBM70 groups (p < 0.05). The regression analysis of WG and FE in the two-slope broken-line model showed that the highest level of FM substitution in

*Oplegnathus punctatus* by SBM was 24.07–25.31% (Figures 1 and 2). In addition, the content of SBM had no significant influence on the viscerosomatic index (VSI), hepatosomatic index (HSI), and condition factor (CF) (p > 0.05).

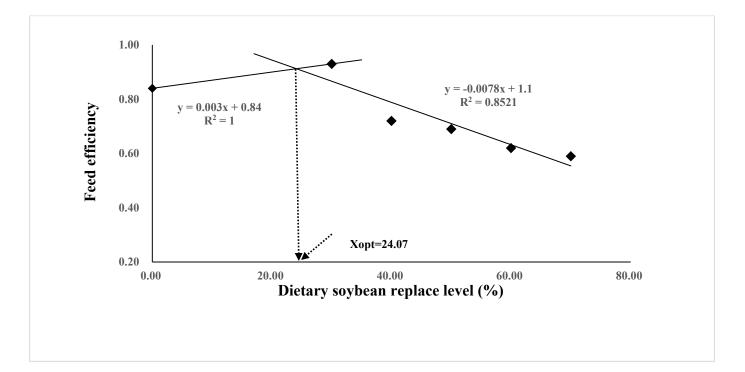
**Table 4.** Growth, feed utilization, and morphometrical parameters of juvenile *Oplegnathus punctatus* fed diets containing different levels of soybean meal.

			Di	iets				<b>CEN</b> (1	Regress	ion ( <i>p</i> , R <sup>2</sup> )
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
IBW <sup>2</sup>	14.67	14.56	14.53	14.58	14.66	14.71	14.62	0.02		
FBW <sup>3</sup>	53.41 <sup>a</sup>	53.66 <sup>a</sup>	44.58 <sup>ab</sup>	44.95 <sup>ab</sup>	36.29 <sup>b</sup>	37.52 <sup>b</sup>	53.68 <sup>a</sup>	1.83	0.225, 0.077	0.000 0.591
WG <sup>4</sup>	264.26 <sup>a</sup>	268.57 <sup>a</sup>	206.66 <sup>ab</sup>	208.65 <sup>ab</sup>	147.68 <sup>b</sup>	155.19 <sup>b</sup>	267.05 <sup>a</sup>	12.61	0.233, 0.074	0.000 0.580
SGR <sup>5</sup>	2.30 <sup>a</sup>	2.33 <sup>a</sup>	2.00 <sup>ab</sup>	2.00 <sup>ab</sup>	1.61 <sup>b</sup>	1.66 <sup>b</sup>	2.32 <sup>a</sup>	0.07	0.248, 0.070	0.001 0.569
FE <sup>6</sup>	0.84 <sup>a</sup>	0.93 <sup>a</sup>	0.72 <sup>b</sup>	0.69 <sup>b</sup>	0.62 <sup>b</sup>	0.59 <sup>b</sup>	0.84 <sup>a</sup>	0.03	0.354 <i>,</i> 0.045	0.001 0.553
PER <sup>7</sup>	1.93 <sup>a</sup>	2.15 <sup>a</sup>	1.69 <sup>ab</sup>	1.63 <sup>ab</sup>	1.41 <sup>b</sup>	1.40 <sup>b</sup>	1.99 <sup>a</sup>	0.07	0.270, 0.064	0.001 0.542
FI <sup>8</sup>	50.16	45.01	44.90	47.47	38.24	42.57	50.48	1.31	0.215, 0.080	0.031 0.320
VSI <sup>9</sup>	13.29	11.29	11.15	10.82	11.46	11.94	11.14	0.36	0.355, 0.045	0.305, 0.123
HIS <sup>10</sup>	2.80	2.66	2.27	2.43	2.50	2.40	2.79	0.08	0.800, 0.003	0.114, 0.214
IPF <sup>11</sup>	1.80	1.31	1.12	1.25	1.05	1.11	1.43	0.08	0.729, 0.006	0.007, 0.428
CF 12	4.04	4.36	4.14	4.20	4.05	4.05	4.25	0.04	0.922, 0.001	0.993, 0.001

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means; <sup>2</sup> Initial body weight. <sup>3</sup> Finial body weight. <sup>4</sup> Weight gain. <sup>5</sup> Specific growth rate. <sup>6</sup> Feed efficiency. <sup>7</sup> Protein efficiency ratio. <sup>8</sup> Feed intake. <sup>9</sup> Viscerosomatic index. <sup>10</sup> Intraperitoneal fat ratio. <sup>11</sup> Hepatosomatic index. <sup>12</sup> Condition factor.



**Figure 1.** Relationship between the weight gain (WG, %) and the dietary soybean level based on two slope broken-line regression analysis, where Xopt represents the optimal dietary soybean replacement level for the maximum WG.



**Figure 2.** Relationship between the feed efficiency (FE) and the dietary soybean level based on two slope broken-line regression analysis, where Xopt represents the optimal dietary soybean replacement level for the maximum FE.

## 3.2. Whole-Body and Tissue Composition

The proximal components of the body and tissues of the fish fed different treatments are listed in Table 5. The lipid content of the whole body and dorsal muscle decreased significantly as SBM dietary levels increased (p < 0.05). However, the effects of different treatments on the protein content were not significant (p > 0.05). In this study, except for proline, the content of most amino acids in the whole body was not affected by the dietary treatment (p > 0.05; Table 6).

**Table 5.** Whole-body and tissue proximate composition in juvenile *Oplegnathus punctatus* fed diets containing different levels of soybean meal (as a % of the wet weight basis).

			Di	ets				om s 1	Regress	ion (p, R <sup>2</sup> )
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
Whole body										
Moisture	72.68	73.29	76.11	73.88	76.01	76.10	71.69	0.67	0.186, 0.090	0.081, 0.244
Protein	13.62	13.03	13.48	12.59	12.32	14.20	13.43	0.22	0.759, 0.005	0.890, 0.013
Lipid	9.94 <sup>a</sup>	9.01 <sup>a</sup>	7.06 <sup>b</sup>	6.98 <sup>b</sup>	5.94 <sup>bc</sup>	4.94 <sup>c</sup>	6.39 <sup>b</sup>	0.37	0.179, 0.093	0.000, 0.827
Ash	3.48	3.39	3.32	3.39	3.34	3.20	3.34	0.03	0.700 <i>,</i> 0.008	0.128, 0.205
Dorsal muscle										
Moisture	76.46	76.93	77.42	77.42	77.52	77.73	76.45	0.22	0.320, 0.052	0.094, 0.231
Protein	20.47	19.74	19.44	20.15	19.10	19.01	20.45	0.23	0.350, 0.046	0.109, 0.218
Lipid	1.85 <sup>a</sup>	1.97 <sup>a</sup>	1.17 <sup>b</sup>	1.25 <sup>b</sup>	1.08 <sup>b</sup>	0.98 <sup>b</sup>	1.18 <sup>b</sup>	0.09	0.240, 0.072	0.001, 0.554

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means.

			Di	ets		1	SEM <sup>1</sup> Regression ( <i>p</i> ,			
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
Arginine	7.46	8.04	7.86	8.15	7.34	7.70	7.72	0.10	0.933, 0.000	0.956, 0.005
Histidine	2.05	2.13	2.13	2.10	2.18	2.24	2.13	0.02	0.912, 0.001	0.152, 0.189
Isoleucine	4.41	4.51	4.54	4.57	4.55	4.63	4.63	0.03	0.139, 0.111	0.027, 0.331
Leucine	7.43	6.67	6.73	6.76	6.52	6.83	7.27	0.10	0.203,	0.025,
Lysine	7.65	7.56	7.39	7.64	8.06	7.55	6.98	0.12	0.034 0.067,	0.335 0.167,
Methionine	2.86	2.90	2.93	2.81	2.90	2.81	2.94	0.02	0.165 0.243,	0.180 0.390,
Phenylalanine	4.75	4.93	4.77	4.67	4.77	4.79	4.87	0.02	0.071 0.414,	0.099 0.683,
,									0.035 0.605,	0.041 0.065,
Threonine	4.47	4.69	4.60	4.57	4.86	4.68	4.67	0.03	0.014 0.959,	0.261 0.810,
Valine	6.39	7.05	6.96	6.18	6.63	7.02	6.70	0.12	0.000	0.023
Aspartic acid	10.12	10.46	10.84	10.52	10.72	10.59	10.75	0.09	0.057	0.198
Glutamic acid	16.61	15.46	15.30	16.57	15.17	15.78	15.36	0.19	0.350, 0.046	0.344 <i>,</i> 0.112
Serine	3.94	4.09	3.94	4.02	4.11	3.90	4.04	0.02	0.600, 0.015	0.843, 0.019
Proline	3.40 <sup>c</sup>	3.46 <sup>c</sup>	3.78 <sup>a</sup>	3.53 <sup>bc</sup>	3.64 <sup>ab</sup>	3.54 <sup>bc</sup>	3.77 <sup>a</sup>	0.05	0.011, 0.292	0.012, 0.390
Glycine	4.70	4.59	4.73	4.64	4.68	4.69	4.85	0.03	0.018, 0.260	0.063 <i>,</i> 0.265
Alanine	6.55	6.24	6.02	6.25	6.63	6.29	6.26	0.09	0.782, 0.004	0.920, 0.009
Tyrosine	4.10	4.12	4.21	3.94	4.14	3.95	3.91	0.03	0.142, 0.110	0.288, 0.129
Cystine	1.82	1.81	1.89	1.83	1.89	1.69	1.83	0.03	0.972,	0.897,
Tryptophan	1.29	1.30	1.38	1.25	1.23	1.33	1.33	0.02	0.000 0.599, 0.015	0.014 0.865, 0.016

**Table 6.** Amino acid composition (% of total amino acids) of the whole body of juvenile *Oplegnathus punctatus* fed diets containing different levels of FM replaced with SBM.

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means.

# 3.3. Retention and Deposition of Energy, Nitrogen, and Lipids

In Table 7, the effects of different treatments on the feed intake (FI), daily nitrogen intake (DNI), and daily energy intake (DEI) values were not significant (p > 0.05). However, the daily energy gain (DEG), daily nitrogen gain (DNG), daily lipid gain (DLG), energy retention (ER), nitrogen retention (NR), and lipid retention (LR) decreased significantly with increasing SBM (p < 0.05). The NR values of the SBM50 + T group were significantly higher than those of the SBM50 group (p < 0.05). Moreover, fish fed the SBM30 diet had significantly higher retention of most amino acids than those fed the SBM50, SBM60, and SBM70 diets, except for lysine (p < 0.05; Table 8).

			Di	ets				ora 1	Regress	ion ( <i>p</i> , R <sup>2</sup> )
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
Nitrogen										
DNI <sup>2</sup>	1.67	1.52	1.72	1.78	1.70	1.79	1.63	0.03	0.532, 0.021	0.201, 0.164
DNG <sup>3</sup>	0.41 <sup>a</sup>	0.39 <sup>a</sup>	0.36 <sup>a</sup>	0.32 <sup>ab</sup>	0.24 <sup>b</sup>	0.33 <sup>ab</sup>	0.41 <sup>a</sup>	0.02	0.284, 0.060	0.006, 0.435
NR <sup>4</sup>	24.64 <sup>a</sup>	25.83 <sup>a</sup>	20.85 ab	18.18 <sup>bc</sup>	14.01 <sup>c</sup>	18.35 <sup>bc</sup>	25.05 <sup>a</sup>	1.08	0.253, 0.068	0.003, 0.486
Lipid DLI <sup>5</sup>	2.95 <sup>abc</sup>	2.82 <sup>bc</sup>	3.10 <sup>abc</sup>	3.24 <sup>a</sup>	3.17 <sup>ab</sup>	3.27 <sup>a</sup>	2.75 <sup>c</sup>	0.05	0.053 <i>,</i> 0.182	0.005, 0.447
DLG <sup>6</sup>	2.56 <sup>a</sup>	2.32 <sup>a</sup>	1.66 <sup>b</sup>	1.53 <sup>b</sup>	1.38 <sup>b</sup>	0.98 <sup>c</sup>	1.58 <sup>b</sup>	0.12	0.334, 0.049	0.000, 0.808
LR <sup>7</sup>	86.78 <sup>a</sup>	82.02 <sup>a</sup>	53.29 <sup>b</sup>	47.50 <sup>b</sup>	43.86 <sup>b</sup>	30.29 <sup>c</sup>	57.20 <sup>b</sup>	4.43	0.611, 0.014	0.000, 0.787
Energy DEI <sup>8</sup>	5.01	4.34	4.89	4.92	4.49	4.96	4.62	0.08	0.503,	0.771,
DEG <sup>9</sup>	3.89 <sup>a</sup>	4.05 <sup>a</sup>	3.67 <sup>a</sup>	4.10 <sup>a</sup>	3.06 <sup>b</sup>	2.42 <sup>c</sup>	4.06 <sup>a</sup>	0.14	0.024 0.361, 0.044	0.028 0.008, 0.413
ER <sup>10</sup>	77.84 <sup>bc</sup>	93.28 <sup>a</sup>	74.97 <sup>bc</sup>	83.50 <sup>bc</sup>	67.98 <sup>c</sup>	49.34 <sup>d</sup>	87.86 <sup>ab</sup>	3.29	0.044 0.278, 0.062	0.025, 0.335

Table 7. Nitrogen, energy, and lipid utilization in juvenile Oplegnathus punctatus fed diets containing different levels of soybean meal.

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means. <sup>2</sup> Daily nitrogen intake. <sup>3</sup> Daily nitrogen gain. <sup>4</sup> Nitrogen retention. <sup>5</sup> Daily lipid intake. <sup>6</sup> Daily lipid gain. <sup>7</sup> Lipid retention. <sup>8</sup> Daily energy intake. <sup>9</sup> Daily energy gain. <sup>10</sup> Energy retention.

			lifference lev	vels of SBM			of juvenile Ople	8		ion ( <i>p</i> , R <sup>2</sup> )
Amino Acids <sup>2</sup>	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
Arginine	75.97 <sup>ab</sup>	83.83 <sup>a</sup>	69.13 <sup>bc</sup>	72.51 <sup>b</sup>	55.46 <sup>d</sup>	60.26 <sup>cd</sup>	71.44 <sup>b</sup>	2.18	0.944, 0.000	0.010, 0.402
Histidine	22.11 ab	23.45 <sup>a</sup>	16.69 <sup>cd</sup>	17.25 <sup>cd</sup>	12.41 <sup>f</sup>	14.07 <sup>ef</sup>	18.88 <sup>bc</sup>	0.92	0.982, 0.000	0.001, 0.552
Isoleucine	32.54 <sup>b</sup>	36.06 <sup>a</sup>	27.35 <sup>c</sup>	26.22 <sup>c</sup>	26.78 <sup>c</sup>	24.88 <sup>c</sup>	28.58 <sup>c</sup>	0.90	0.594, 0.015	0.004, 0.464
Leucine	57.83 <sup>a</sup>	42.75 <sup>b</sup>	33.03 <sup>cd</sup>	31.18 <sup>d</sup>	30.57 <sup>d</sup>	30.71 <sup>d</sup>	36.63 <sup>c</sup>	2.13	0.498, 0.025	0.000, 0.854
Lysine	52.62	58.86	44.89	45.07	41.78	37.00	40.94	1.75	0.130, 0.117	0.002, 0.487
Methionine	24.71 <sup>ab</sup>	27.84 <sup>a</sup>	24.15 <sup>abc</sup>	21.15 <sup>bcd</sup>	19.94 <sup>cd</sup>	19.37 <sup>d</sup>	26.35 <sup>a</sup>	0.81	0.256, 0.067	0.008, 0.416
Phenylalanine	53.15 <sup>b</sup>	60.27 <sup>a</sup>	41.85 <sup>c</sup>	37.53 <sup>c</sup>	36.01 <sup>c</sup>	38.65 <sup>c</sup>	42.40 <sup>c</sup>	1.99	0.444 <i>,</i> 0.031	0.002, 0.492
Threonine	76.48 <sup>a</sup>	70.54 <sup>a</sup>	50.74 <sup>b</sup>	46.38 bc	41.18 <sup>cd</sup>	36.35 <sup>d</sup>	49.02 <sup>b</sup>	3.16	0.310, 0.054	0.000, 0.861
Valine	114.36 <sup>a</sup>	119.04 <sup>a</sup>	86.07 <sup>b</sup>	71.05 <sup>b</sup>	79.30 <sup>b</sup>	69.28 <sup>b</sup>	80.30 <sup>b</sup>	4.78	0.276, 0.062	0.001, 0.546
Aspartic acid	68.32 <sup>a</sup>	69.82 <sup>a</sup>	54.99 <sup>bc</sup>	49.42 <sup>c</sup>	52.38 <sup>c</sup>	47.97 <sup>c</sup>	62.10 <sup>ab</sup>	2.01	0.696, 0.008	0.000, 0.625
Glutamic acid	40.46 <sup>a</sup>	40.59 <sup>a</sup>	31.67 <sup>b</sup>	35.84 <sup>ab</sup>	29.84 <sup>b</sup>	31.16 <sup>b</sup>	35.30 <sup>ab</sup>	1.11	0.796, 0.004	0.006, 0.433
Serine	25 52 <sup>b</sup>	30.10 <sup>a</sup>	19 81 <sup>cd</sup>	21 00 cd	18 26 <sup>de</sup>	16.19 <sup>e</sup>	21.35 °	1.01	0.577,	0.002,

 $18.26 \ ^{\rm de}$ 

 $18.32 \ ^{\rm ef}$ 

30.01

16.19 <sup>e</sup>

 $17.35\ ^{\rm f}$ 

31.36

21.35 <sup>c</sup>

23.10 <sup>cd</sup>

37.01

1.01

1.06

1.49

0.017

0.590,

0.016

0.710, 0.007

0.495

0.000,

0.638

0.001, 0.563

19.81 <sup>cd</sup>

24.97 <sup>bc</sup>

33.81

21.00 <sup>cd</sup>

21.02 de

33.36

25.52 <sup>b</sup>

28.22 ab

44.20

Serine

Proline

Glycine

30.10<sup>a</sup>

30.26 <sup>a</sup>

47.92

			Di	ets				<b>GEN (</b> 1	Regress	Regression ( $p$ , $\mathbb{R}^2$ )	
Amino Acids <sup>2</sup>	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	- SEM <sup>1</sup> -	Linear	Quadratic	
Alanine	55.90 <sup>a</sup>	47.16 <sup>b</sup>	34.60 <sup>c</sup>	35.22	38.98 <sup>c</sup>	32.37 <sup>c</sup>	38.77 <sup>c</sup>	1.87	0.416, 0.035	0.000, 0.699	
Tyrosine	27.89 <sup>a</sup>	28.92 <sup>a</sup>	16.45 <sup>c</sup>	19.67 <sup>c</sup>	20.70 <sup>bc</sup>	18.54 <sup>c</sup>	25.60 <sup>ab</sup>	1.14	0.477, 0.027	0.006, 0.438	
Cystine	35.98 <sup>a</sup>	35.67 <sup>a</sup>	24.93 <sup>bc</sup>	22.68 <sup>bc</sup>	15.85 <sup>cd</sup>	12.20 <sup>d</sup>	26.54 <sup>ab</sup>	2.10	0.887, 0.001	0.000, 0.656	

Table 8. Cont.

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means. <sup>2</sup> EAA retention (%) = [(final whole body EAA – initial whole body EAA)/dietary EAA intake] × 100.

## 3.4. Hematological Parameters

Hematological parameter data are listed in Table 9. The effects of different treatments on total cholesterol (T-CHO) were not significant (p > 0.05). Serum triglyceride (TG) concentrations were significantly higher in fish fed the SBM0, SBM30, and SBM50 + T diets compared with the SBM70 diet.

**Table 9.** Biochemical indices in the serum of juvenile *Oplegnathus punctatus* fed experimental diets for eight weeks.

			Di	ets				<b>GEN (</b> 1	Regression ( $p$ , $\mathbb{R}^2$ )	
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
TG (mmol $L^{-1}$ ) <sup>2</sup>	4.24 <sup>ab</sup>	4.16 <sup>ab</sup>	4.84 <sup>a</sup>	3.37 <sup>b</sup>	3.75 <sup>ab</sup>	3.49 <sup>b</sup>	4.87 <sup>a</sup>	0.16	0.094 <i>,</i> 0.141	0.045 <i>,</i> 0.292
T-CHO (mmol $L^{-1}$ ) <sup>3</sup>	8.12	7.61	8.65	7.54	5.57	5.46	8.39	0.43	0.474, 0.027	0.106, 0.220

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means. <sup>2</sup> Triacylglycerol. <sup>3</sup> Total cholesterol.

## 3.5. Activity of the Digestive Enzymes in the Intestine

In the present study, fish fed the SBM0 diet had the highest lipase (LPS) activity (p > 0.05; Table 10). The amylase (AMS) activity of the SBM0 group was significantly higher than that of the other treatment groups (p < 0.05).

**Table 10.** Digestive enzyme activity in the intestine of juvenile *Oplegnathus punctatus* fed experimental diets for eight weeks.

			Di	ets				<b>GEN (</b> 1	Regress	ion ( <i>p</i> , R <sup>2</sup> )
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
AMS (U mgprot <sup><math>-1</math></sup> ) <sup>2</sup>	8.61 <sup>a</sup>	7.02 <sup>ab</sup>	5.42 <sup>b</sup>	6.03 <sup>b</sup>	5.65 <sup>b</sup>	5.55 <sup>b</sup>	5.61 <sup>b</sup>	0.30	0.200, 0.085	0.001, 0.547
LPS (gprot $L^{-1}$ ) <sup>3</sup>	20.89	15.98	17.29	18.78	16.61	13.94	13.34	2.16	0.485 <i>,</i> 0.026	0.654 <i>,</i> 0.046

Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means. <sup>2</sup> Amylase. <sup>3</sup> lipase.

## 3.6. Liver Antioxidant Enzyme and Biochemical Indices

Although there was no significant difference in superoxide dismutase (SOD) under different dietary SBM levels, the lowest SOD value was observed in SBM70 (p > 0.05; Table 11). Catalase (CAT) activity decreased significantly as dietary SBM levels increased (p < 0.05). The concentration of malondialdehyde (MDA) in the SBM0, SBM30, and SBM50 + T groups was significantly lower than that in the SBM50–SBM70 groups (p < 0.05).

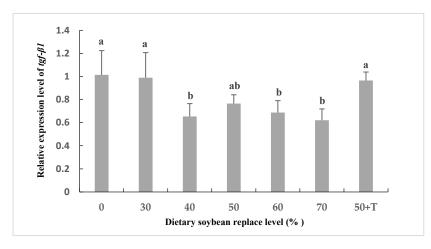
			Di	ets				ora (1	Regress	ion (p, R <sup>2</sup> )
	SBM0	SBM30	SBM40	SBM50	SBM60	SBM70	SBM50 + T	SEM <sup>1</sup>	Linear	Quadratic
SOD (U mgprot <sup><math>-1</math></sup> ) <sup>2</sup>	11.46	10.56	11.90	10.71	10.48	10.09	12.04	0.31	0.256, 0.067	0.224, 0.153
CAT (U mgprot <sup><math>-1</math></sup> ) <sup>3</sup>	10.73 <sup>a</sup>	9.10 <sup>ab</sup>	7.38 <sup>bcd</sup>	7.63 <sup>bcd</sup>	6.31 <sup>cd</sup>	5.59 <sup>d</sup>	8.60 <sup>abc</sup>	0.43	0.856, 0.002	0.000, 0.683
MDA (nmol ml $^{-1}$ ) $^4$	2.69 <sup>c</sup>	3.09 <sup>bc</sup>	3.37 <sup>bc</sup>	6.96 <sup>ab</sup>	9.10 <sup>a</sup>	6.90 ab	2.57 <sup>c</sup>	0.68	0.293, 0.058	0.005, 0.451

**Table 11.** Antioxidant parameters in the liver of juvenile *Oplegnathus punctatus* fed experimental diets for eight weeks.

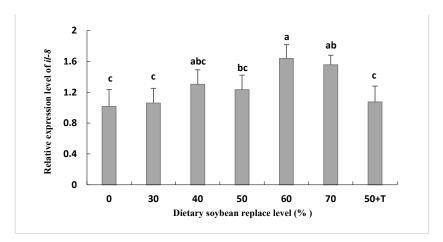
Notes: Means in the same row with different superscripts are significantly different (p < 0.05). <sup>1</sup> SEM: pooled standard error of means. <sup>2</sup> Superoxide dismutase. <sup>3</sup> Catalase. <sup>4</sup> Malondialdehyde.

## 3.7. The mRNA Expression Levels of il-8 and $tgf-\beta 1$

The mRNA levels of *il-8* were higher in the livers of the SBM40 to SBM70 groups compared to those in the SBM0 and SBM30 groups. However, the mRNA expression of tgf- $\beta$ 1 was reversed (Figure 3). In addition, the mRNA level of *il-8* in the SBM50 + T group was lower than that in the SBM40–SBM70 group, which was equivalent to that in the SBM0 and SBM30 groups (Figure 4).



**Figure 3.** Relative expression of the mRNA for transforming growth factor  $\beta 1$  (*tgf-\beta 1*) in the liver of *Oplegnathus punctatus* fed the seven diets. Means in the same row with different superscripts are significantly different (p < 0.05).



**Figure 4.** Relative expression of the mRNA for cytokine interleukin-8 (*il-8*) in the liver of *Oplegnathus punctatus* fed the seven diets. Means in the same row with different superscripts are significantly different (p < 0.05).

## 4. Discussion

After an eight week feeding trial, the growth performance and feed utilization of Oplegnathus punctatus were significantly affected by different dietary treatments. The highest WG values were observed in the SBM0 (264.26  $\pm$  49.45%) and SBM30 (268.57  $\pm$  24.37%) groups. Although not statistically different, the WG values of the SBM0 and SBM30 groups were numerically higher than those of the SBM40 and SBM50 groups and were significantly higher than those of the SBM60 and SBM70 groups. Similar results were also found in other fish species such as *Takifugu rubripes* [38], *Hemibagrus wyckioides* [39], and *Channa striata* [40], with a maximum replacement level of 30%. In addition, the highest FE value occurred in the SBM30 group, which was significantly higher than that in the SBM40 and SBM50 groups. According to the broken line regression analysis of WG and FE, the optimal SBM replacement FM level for spotted knifejaw ranged from 24.07% to 25.31%. These values are similar to those reported for other fish species, such as 24% for juvenile Paralichthys olivaceus [41], 25% for Oreochromis niloticus [42], and 25% for Rhabdosargus sarba [43]. Notably, the SGR and WG values of the SBM50 + T group (with 1.2% taurine added) were significantly higher than those of the corresponding SBM50 group but were not statistically different from those in the SBM30 group. This indicated that FM replacement levels could reach 50% using SBM with 1.2% taurine added to the diet without negatively affecting the growth performance of Oplegnathus punctatus. The beneficial influences of dietary taurine on enhancing the efficacy of plant proteins have also been reported for other carnivorous fish such as *Dicentrarchus labrax* [44] and *Channa striata* [45].

When using high levels of SBM instead of FM in the diet, the decrease in feed intake (FI) caused by poor palatability was probably one of the key factors for the decline in the growth performance in some fish species. In this investigation, the FI values of all groups were not significantly different. Consistent with this, there were no significant differences in DNI values (from 1.52 to 1.79) among fish groups treated with increasing dietary SBM. In aquaculture practice, it was observed that the species actively feed on algae attached to the cage. Therefore, it can be concluded that palatability should not be a major factor in the decline of the growth performance of *Oplegnathus punctatus* or can be ignored. Similar results were also found in studies on *N. miichthioides* [46] and *Lutjanus campechanus* [47].

SBM ingredients commonly contain various anti-nutritional factors (ANFS), such as protease inhibitors, phytate, saponins, lectins, and oligosaccharides. In the industrial production of SBM, the activity of some ANFs in soybean can be reduced by heat treatment, such as trypsin inhibitors and other heat-sensitive ANFs [48]. However, this treatment is basically ineffective in destroying NSPs, lectins, saponins, and phytic acid [49,50]. In fact, the main antigenic components existing in SBM, such as glycinin and  $\beta$ -conglycinin, may be mainly responsible for inducing abnormal intestinal structural changes and activating the immune system, leading to harmful inflammatory reactions [51–53]. In Atlantic salmon, numerous studies reported that plant feedstuffs can result in changes in intestinal histomorphology and gene expression related to immune responses within four weeks [54–57]. In the present study, after eight-week culture experiments, no symptoms of intestinal inflammation were observed histologically in all groups (data not provided). However, it was found that the mRNA levels of the pro-inflammatory cytokine interleukin-8 (il-8) were higher in the livers of fish fed high replacement levels of SBM (SBM40 to SBM70) compared with the SBM0 and SBM30 groups, and the expression of the anti-inflammatory cytokine transforming growth factor  $\beta 1(tgf-\beta 1)$  was suppressed. *il-8* is an important proinflammatory cytokine that recruits and activates macrophages and neutrophils to clear cellular debris and invading microorganisms and promotes the regeneration of damaged tissues [58]. Meanwhile, tgf- $\beta$  acts as an anti-inflammatory cytokine, counteracting the production of pro-inflammatory cytokines and limiting the inflammatory response [59]. Significantly, the mRNA level of the pro-inflammatory cytokine il-8 in the SBM50 + T group was lower than that in the SBM40 to SBM70 groups, which was equivalent to that in the SBM0 and SBM30 groups, whereas the expression of the anti-inflammatory cytokine  $tgf-\beta 1$ was the opposite. Researchers [60] have demonstrated that the primary role of taurine is to

protect and maintain the homeostasis of cells involved in acute and chronic inflammation. Therefore, in this study, the supplementation of taurine in the SBM50 + T group may improve the health status of *Oplegnathus punctatus*. However, [61] found that changes in gene expression associated with immune responses were detected on the third day of a plant-based diet, which was much earlier than the signs of inflammation in histological assessment. Since different species have different levels of susceptibility to ANFs, the effect of supplementing taurine in SBM instead of FM on the digestive tract structure of *Oplegnathus punctatus* needs long-term evaluation.

In this study, the content of most amino acids in whole body remained unchanged except proline, which is consistent with the fact that the composition of essential amino acids in the whole body is relatively stable and is hardly affected by fish size or dietary composition, as the biosynthesis of body protein is genetically determined [62,63]. It is well known that the essence of amino acid balance is that the ratio of each amino acid constituting a protein should be appropriate. If one amino acid is deficient, it will adversely affect the synthesis of intact protein molecules and will affect the availability of other amino acids [64]. The retention of protein and essential amino acids is considered to be the most sensitive indicator of insufficient amino acid supply [65]. In the current research, the methionine content (1.77% to 1.41%) of the experimental diets gradually decreased with increasing levels of dietary SBM, which significantly affected the retention of essential amino acids. The retention of most amino acids was significantly higher in the SBM30 group than in the SBM50, SBM60, and SBM70 groups, except lysine. This was also confirmed by higher DNG and NR values in the SBM30 group compared to the SBM40 to SBM70 groups. These results suggest that methionine deficiency affected the utilization of other amino acids in the high SBM groups (SBM50 to SBM70). In these groups, due to the lack of methionine, there was a relative surplus of other amino acids, which were used for oxidative breakdown rather than synthesis, ultimately inhibiting growth. Therefore, the imbalance of amino acids in the diet is probably the key factor for the decreased growth performance of Oplegnathus punctatus in the high SBM groups. A similar situation was observed in Pseudobagrus ussuriensis [66], Epinephelus fuscoguttatus [67], and Gadus morhua L. [68] fed soybean meal-based diets. Interestingly, although not statistically different from the SBM50 group, the retention values of most essential amino acids were higher in the SBM50 + T group than in the SBM50 group. Moreover, the NR and SGR values of the SBM50 + T group were significantly higher than those of the SBM50 group. When taurine is insufficient in the diet, part of methionine may be converted into taurine to meet the physiological needs of fish, thus aggravating the lack of methionine and adversely affecting growth. After taurine addition, methionine is saved to a certain extent. More importantly, the saved methionine will be used for growth [15,69–72].

The inclusion of SBM in the diet may have a negative impact on the digestibility of fish, for example, adding SBM to the diet decreased lipid deposition, digestible protein, and digestible energy in Salmo salar [73]. In this study, although DNI and DEI were not significantly different in all treatment groups, DEG, DNG, DLG, ER, NR, and LR decreased notably with increasing levels of SBM. A similar situation was observed in Liza H. [74]. In addition, the amylase (AMS) activity of the SBM0 and SBM30 groups was significantly higher than that of the SBM40 to SBM70 groups, which may partly be responsible for the decreased dietary digestibility after SBM replaced FM. Triglycerides (TGs) and total cholesterol (T-CHO) are mainly synthesized in the liver, and their changes reflect the lipid metabolism in body to a certain extent. Increased levels of TG and T-CHO indicate that the endogenous fat transport is active [75,76]. Several studies have found that nonstarch polysaccharides (NSP) can reduce plasma and liver T-CHO levels in fish, possibly because they interfere with fat digestion and absorption [49,77]. In this study, the levels of TG and T-CHO in the high SBM groups were lower than those in the low SBM and control groups. Therefore, the significant reduction in the whole body and muscle lipid content in the high replacement groups may be related to NSP. A similar result was seen for redlip mullet Liza haematocheila [74]. Interestingly, the T-CHO and TG values of the SBM50 + T

group were higher than those of the SBM50 group. Researchers [78] showed that taurine has a beneficial effect on fat metabolism. However, there were no significant differences in the whole fish and muscle lipid content between the SBM50 + T and SBM50 groups. The specific reason is not clear, so the effect of taurine on lipid metabolism in *Oplegnathus punctatus* needs further evaluation.

Antioxidant enzymes (such as SOD and CAT) in the antioxidant defense system can effectively remove excess reactive oxygen species and protect cells from oxidative damage [79]. The plant protein in the diet may have a harmful influence on the antioxidant status of fish. For example, when feeding a diet containing high levels of SBM, the antioxidant capacity of Monopterus albus decreased significantly [80]. SOD activity was not significantly different among the groups in the study, but the SBM70 treatment group had the lowest SOD activity. As the SBM content in the diet increased, CAT activity decreased significantly. The results were similar to those of *Ctenopharyngodon I.* [81]. In addition, malondialdehyde (MDA) produced by endogenous oxidative damage in vivo is one of the final metabolites of lipid peroxidation, which can reflect the extent of lipid peroxidation and the extent of cell injury [82]. Our study found that the supply of high levels of SBM (SBM50 to SBM70) significantly increased the concentration of MDA in the liver of Oplegnathus punctatus, indicating that the health status of Oplegnathus punctatus could be affected by using SBM instead of FM. Many studies showed that taurine can have beneficial effects in diets containing plant proteins. For example, supplementation of taurine in plant protein diets significantly improved the growth performance as well as the feed conversion ratio of Diplodus sargus [23]. In Totoaba macdonaldi, supplementation of 1.2% taurine in a diet that replaced 60% FM with soybean protein concentrate restored the level of lipid peroxidation and increased the activities of catalase and the key enzymes of intermediate metabolism to normal levels [83]. According to current results, taurine supplementation significantly improved the growth and feed efficiency of Oplegnathus punctatus. Compared with the SBM50 group, the SOD and CAT enzyme activities were higher in the SBM50 + T group, while the MDA level was lower. Importantly, taurine has a wide range of biological effects, including membrane stability, neurotransmitter regulation, and antioxidant effects, especially in osmotic pressure regulation and hormone release [15]. The effects of taurine added to a high-level SBM diet on the growth rate, feed efficiency, and antioxidant stress of Oplegnathus punctatus should be studied further. These findings can significantly change the quantities and types of substitute proteins that could be effectively added to the diets of juvenile Oplegnathus punctatus and could reduce the dependency of the industry on FM supplies.

## 5. Conclusions

The results of this study showed that the use of soybean meal instead of FM in the diet could reach a level of up to 30% without obvious adverse impacts on *Oplegnathus punctatus* growth and feed utilization. However, a higher SBM content significantly decreased the growth performance, feed utilization, digestive enzyme activity, as well as the antioxidant capacity of fish, which may primarily be related to the existence of ANFS and the lack of essential amino acid in diets including SBM. Through the broken line regression analysis of WG and FE, it is suggested that replacing 24.07–25.31% FM with SBM is appropriate for *Oplegnathus punctatus*. Moreover, the addition of 1.2% taurine increased the replacement level to 50% without negatively affecting the performance in *Oplegnathus punctatus*, but longer-term experiments are needed for further validation. Finally, we suggest that future research should explore the feasibility of replacing fishmeal with different plant proteins, which can help save the production cost of feed.

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