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Research Article

Evaluation of Six Selected Commercial Fermented Soybean Meal by Feeding Juvenile Turbot (*Scophthalmus maximus* L.)

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Various kinds of fermented soybean meal are now commercially available, whereas the quality of these products is uneven due to different processing technologies, fermentation strains, and raw soybean meal, which would lead to different effects on bioavailability of nutrients in aquaculture feeds. Thus, a 10-week feeding trial was conducted to evaluate the effects of six different commercially available fermented soybean meal (FSBM) on growth performance of juvenile turbot (*Scophthalmus maximus* L.) and to analyze the correlation between growth parameters and FSBM components. Seven isonitrogenous and isolipidic diets were formulated: fish-meal-based diet (the control group, FM) and FM with 450 g/kg of fish meal substituted by six commercially available FSBM (the replacement groups, FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6). Results showed that specific growth rate (SGR) and weight gain rate (WGR) were significantly higher in groups FMSB3 and FMSB4 than in other replacement groups, but significantly lower in the control group. Besides, no significant differences were observed in feed efficiency ratio (FER) and protein efficiency ratio (PER) among groups FSBM3, FSBM4, and FM. Correlation analysis revealed that SGR, PER, and protein retention (PR) of turbot were all positively correlated with the water-soluble protein and trichloroacetic acid- (TCA-) soluble protein content of FSBM, but negatively correlated with the content of trypsin inhibitor. In summary, the commercial FSBM3 and FSBM4 could yield higher growth performance of juvenile turbot than the rest selected FSBM when replacing 450 g/kg of fish meal in diets, and the content of water-soluble protein, TCA-soluble protein, and trypsin inhibitor could serve as relatively accurate indicators in the quality evaluation of FSBM.

1. Introduction

Over the past few decades, increasing demand and price of fish meal have limited the expansion of aquaculture [1, 2]. To address this concern, considerable studies have been performed to evaluate the effects of replacing fish meal by other protein sources, such as poultry by-product meal [3], meat and bone meal [4], corn gluten meal [5], soybean meal [6], and cottonseed meal [7]. Among these alternative protein sources, soybean meal is a good candidate for its reasonable price, steady supply, and relatively well-balanced amino acid profile [8]. Nevertheless, the antinutritional factors (ANFs) in soybean meal cannot be ignored, since their negative effects on digestion, absorption, and utilization of nutrients by fish would impede growth performance ultimately [9]. Fermentation treatment is regarded as an effective method [10, 11] since it can reduce ANF content and improve nutrient digestibility to a great extent [12, 13]. Li et al. [14] recorded that fermentation treatment could reduce the levels of glycinin, β-conglycinin, and trypsin inhibitors by 24.2%, 22.1%, and 44.9%, respectively. Besides, fermentation treatment not only breaks up high-molecular-weight protein into

polypeptide and small peptide but also produces some unknown bioactive substances that are potentially good for growth and health [15]. Nowadays, numerous kinds of fermented soybean meal (FSBM) products are commercially available on the market. However, the quality of these products is uneven due to different fermentation conditions, fermentation strains, and raw soybean meal [10], which would affect growth performance of aquatic animals. To evaluate the nutritional value of FSBM, a series of trials have been conducted to investigate the optimal levels of fish meal replaced by FSBM in different fish, such as in turbot, *Scophthalmus maximus* L. (450 g/kg) [16, 17], Japanese flounder, *Paralichthys olivaceus* (360 g/kg) [18], black sea bream, *Mylio macrocephalus* (200, 400 g/kg) [11, 19], rainbow trout, *Oncorhynchus mykiss* (600 g/kg) [20], and Japanese seabass, *Lateolabrax japonicus* (400 g/kg) [21].

Turbot (*Scophthalmus maximus* L.) is widely farmed around the world, and the cultivation of which has developed into dominant mariculture industries in northern China [22]. However, turbot has a high dietary protein requirement ranging from 500 to 650 g/kg, and most of it comes from fish meal [23, 24]. Thus, it is of great economic value to choose turbot as experimental subject. Previous experiments on turbot mainly focused on the optimal level of replacing fish meal with soybean meal fermented by a single strain, such as *Lactobacillus plantarum* P8 [17], *Aspergillus awamori* [16], and *Enterococcus faecium* [14], whereas the information on the parallel comparison of different commercial FSBM is very scarce. Hence, this study was performed to evaluate the effects of six major commercial FSBM on the growth performance of juvenile turbot and to analyze the correlation between growth parameters and FSBM components, which would provide reference for FSBM producers to improve product quality and help aquafeed companies to select high-quality FSBM.

2. Materials and Methods

2.1. Experimental Diets. Seven isonitrogenous and isolipidic diets were formulated to contain approximately 520 g/kg crude protein and 110 g/kg crude lipid. These diets were divided into control group FM and six replacement groups FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6 (replacing 450 g/kg of fish meal with six different commercially available FSBM, which were collected from different firms in China through Qingdao Bio-ways Ingredients Biotechnology Co., Ltd.). Each diet containing FSBM was supplemented with coated L-methionine, L-isoleucine, and L-lysine to meet the requirements for juvenile turbot (Tables 1 and 2). Yttrium oxide (Y_2O_3) was also added into each diet at the level of 1 g/kg as the indicator for dry matter and crude protein digestibility analysis. Ingredients were smashed into powder which could pass through 250 μm aperture mesh. All ingredients were thoroughly mixed with fish oil, and water was added in to produce moist dough. The moist dough was then pelleted by an experimental granulator (EL-220, Haiyang City Huatong Machinery Co., Ltd.) with 2 mm die and dried in a ventilated oven (CT-C-1, Jiang

Yin Zhou Yuan Pharmaceutical Equipment Co., Ltd.) at 45°C for 10 h. All the pellets were stored at -20°C for use.

2.2. Experimental Fish and Procedure. Juvenile turbot were purchased from a commercial hatchery in Weihai, China. The feeding trial was conducted in LaiZhou MingBo Aquatic Co., Ltd. (Yantai, China). Turbot were fed with commercial diets for 14 days to acclimate to the experimental conditions. At the start of the feeding trial, all the turbot were fasted for 24 h and some of the fish were randomly weighed to estimate the average weight of the batch. Afterwards, fish of similar weight (6.99 ± 0.02 g) were selected and randomly assigned into 21 experimental fibreglass tanks (600 L capacity). Each diet was randomly assigned to triplicate tanks. Fish were fed twice daily (08:00 and 18:00) to apparent satiation for 10 weeks. After feeding, feces waste was cleaned up and seawater was also renewed. During the feeding trial, the water temperature ranged from 18 to 20°C, salinity ranged from 30 to 32, and dissolved oxygen maintained at approximately 6.0 mg/L. Meanwhile, the photoperiod was maintained on 12 h light : 12 h dark.

2.3. Sample Collection. At the start of formal growth experiment, twenty fish were randomly sampled from the remaining turbot for initial carcass protein analysis. From the seventh week of the growth experiment, feces samples were collected by siphoning after 8 h of feeding. All the collected feces samples were stored at -20°C for analysis. At the termination of the feeding trial, all the fish were fasted for 24 h and anesthetized with eugenol (1 : 12,000) to reduce the stress reaction before sampling. Then, the total number and weight of fish in each tank were first recorded. Six fish were randomly sampled from each tank to measure individual body weight, body length, visceral weight, and liver weight. Intact intestines were sampled from four fish per tank and frozen in liquid nitrogen immediately and then stored at -80°C for digestive enzyme analysis. Moreover, the middle intestine was sampled from three fish each tank for histology analysis. The sampled middle intestine (1 cm in length) were placed in 4% paraformaldehyde solution for fixation and then transferred to 75% ethanol after 24 h.

2.4. Biochemical Analysis. Proximate composition of ingredients, diets, and feces was analyzed according to published standards [25]. Moisture was determined by drying samples to a constant weight at 105°C for 24 h. Ash was measured gravimetrically by placing the samples in muffle furnace at 550°C for 16 h after ignition. Crude lipid was analyzed by petroleum ether extraction using Soxhlet (Buchi 36680, Switzerland). Crude protein was analyzed by the Kjeldahl method (Kjeltec™ 8400, FOSS, Sweden). Amino acids of ingredients and diets were analyzed by an amino acid analyzer (L-8900, Hitachi) after acid hydrolysis (6 N HCl for 24 h at 110°C). The content of water-soluble protein, trichloroacetic acid- (TCA-) soluble protein, and alkali- (KOH-) soluble protein was determined according to the methods described by Sarin [26] and Araba and Dale [27] with some modifications. Among them, the water-soluble protein in

TABLE 1: Formulation and proximate composition of experimental diets (g/kg dry matter)[1].

Ingredients	Diets[2]						
	FM	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
Brown fish meal[1]	500.0	275.0	275.0	275.0	275.0	275.0	275.0
Fermented soybean meal	0.0	300.4	303.3	293.2	294.8	293.0	297.9
Blood meal[1]	0.0	45.0	45.0	45.0	45.0	45.0	45.0
Wheat gluten meal[1]	134.0	92.0	90.0	90.0	90.0	90.0	92.0
Wheat meal[1]	244.5	146.2	142.4	153.7	150.9	153.9	146.6
Fish oil	45.0	62.0	62.0	63.0	64.0	62.0	64.0
Soy lecithin	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin premix[3]	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin C[3]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mineral premix[4]	15.0	15.0	15.0	15.0	15.0	15.0	15.0
L-Methionine	0.0	1.2	2.4	2.7	2.5	3.4	1.8
L-Isoleucine	0.0	1.1	1.1	0.7	0.9	0.8	0.8
L-Lysine	0.0	0.6	2.3	0.2	0.4	0.4	0.4
Taurine	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mildew preventive[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ethoxyquinoline	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)_2$	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Yttrium oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sodium alginate	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Attractants[6]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Proximate composition							
Crude protein	525.5	529.4	522.2	523.2	525.1	524.5	529.0
Crude lipid	110.4	109.7	109.3	112.1	108.9	109.6	113.0
Ash	113.9	95.2	99.0	96.8	97.5	98.5	96.2

[1]Brown fish meal (g/kg dry matter): crude protein 724.7, crude lipid 98.4; blood meal (g/kg dry matter): crude protein 986.4, crude lipid 12.2; wheat gluten meal (g/kg dry matter): crude protein 791.5, crude lipid 19.3; wheat meal (g/kg dry matter): crude protein 139.3, crude lipid 24.7. These ingredients were provided by Great Seven Bio-tech (Qingdao, China). The commercial fermented soybean meal was collected by Qingdao Bio-ways Ingredients Bio-technology Co., Ltd. (Qingdao, China). [2]Fish meal-based diet was named FM; diets with 450 g/kg of fish meal replaced by six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6. [3]Vitamin premix (mg/kg diet): retinol acetate, 32; cholecalciferol, 5; alpha-tocopherol, 240; thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin B12, 10; pantothenic acid, 60; folic acid, 20; niacin 200; biotin, 60; inositol, 800; microcrystalline cellulose, 13,473. Vitamin C was supplied in the form of vitamin C polyphosphate. [4]Mineral premix (mg/kg diet): $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $MnSO_4 \cdot H_2O$, 45; $CoCl_2 \cdot 6H_2O$, 50; Na_2SeO_3, 20; H_2CaIO_4, 60; zeolite powder, 13,485. [5]Mildew preventive: calcium propionate : boletic acid = 1 : 1. [6]Attractants: glycine betaine : DMPT : glycine : alanine : inosine-5-diphosphate trisodium salt = 4 : 2 : 2 : 1 : 1.

soybean meal was determined after three times of ultrapure water extraction in a shaker. For the determination of TCA-soluble protein, soybean meal was extracted by 15% trichloroacetic acid for 5 min, and supernatant was centrifuged (4000 r/min, 5 min) for protein determination. As to the analysis of alkali-soluble protein, soybean meal was extracted by 0.2% potassium hydroxide for 20 min in a magnetic stirrer; the supernatant used for protein determination was then obtained after centrifugation (2700 r/min, 10 min). The total calcium and phosphorus contents of these six FSBM were measured by an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES, Thermo Fisher 7200).

2.5. Antinutritional Factor Analysis. The trypsin inhibitor, glycinin, and β-conglycinin contents of FSBM were all

analyzed by a microplate reader (SpectraMax i3, Molecular Devices) using competitive enzyme-linked immunosorbent assay (ELISA) kits (Beijing Longkefangzhou Bio-Engineering Technology Co., Ltd., China); all procedures performed in the analysis were in strict accordance with the kits' instructions.

2.6. Apparent Digestibility Coefficients of Dry Matter and Protein. Freeze-dry diets and feces were firstly digested by nitric acid and hydrofluoric acid, and then, the content of yttrium oxide was measured by ICP-OES (Thermo Fisher 7200). The content of yttrium oxide in diets and feces was used in the calculation of apparent digestibility coefficient (ADC) of feeds.

2.7. Digestive Enzyme Activity. Activity of digestive enzymes (lipase, alpha-amylase, and trypsin) was analyzed by a

TABLE 2: Amino acid composition of experimental diets (g/kg dry matter)[1].

Amino acids	Diets[2]						
	FM	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
Essential amino acid							
Threonine	19.1	19.1	17.9	19.3	20.2	18.9	18.5
Valine	24.0	26.0	26.4	26.6	28.9	25.8	26.4
Methionine	12.2	11.7	13.0	13.5	13.7	14.1	11.8
Isoleucine	22.1	21.7	22.0	22.3	24.1	22.0	21.5
Leucine	36.6	39.3	39.4	41.0	43.9	40.1	39.1
Phenylalanine	22.7	25.2	25.9	26.7	28.2	25.2	24.1
Lysine	28.1	27.2	28.1	29.1	30.8	28.0	27.2
Histidine	14.1	14.8	15.0	15.5	16.7	15.0	14.7
Arginine	25.2	25.7	26.1	27.6	29.1	27.0	26.8
Nonessential amino acid							
Proline	26.0	24.1	23.7	24.2	25.6	24.5	24.0
Aspartic acid	36.6	41.8	41.7	44.5	47.6	43.9	43.3
Serine	20.8	21.4	21.0	22.8	23.7	22.6	21.4
Glutamic acid	104.1	96.2	93.5	98.8	106.0	97.5	94.9
Glycine	29.2	27.0	27.3	27.4	29.4	27.5	26.3
Alanine	30.1	30.4	29.3	30.2	32.4	29.9	29.0
Cysteine	5.3	5.2	5.4	5.7	6.3	5.8	5.4
Tyrosine	15.6	15.6	16.8	16.8	17.8	15.9	15.1

[1]Data are means of triplicate. No tryptophan was detected because of acid hydrolysis. [2]Fish meal-based diet was named FM; diets with 450 g/kg of fish meal replaced by six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6.

TABLE 3: Nutritional composition and antinutritional factor content of fermented soybean meal (dry matter basic, means ± S.E.M.)[1].

	Fermented soybean meal[2]					
	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
Crude lipid (g/kg)	25.2 ± 0.1^a	25.5 ± 0.1^a	21.8 ± 0.1^c	18.6 ± 0.1^d	24.5 ± 0.1^b	17.1 ± 0.1^e
Crude protein (g/kg)	542.3 ± 1.2^d	537.1 ± 0.2^e	555.6 ± 0.9^a	552.6 ± 0.3^b	556.0 ± 0.8^a	546.8 ± 1.1^c
Water-soluble protein (g/kg)[3]	57.7 ± 2.2^d	47.9 ± 1.0^e	82.7 ± 0.8^b	91.3 ± 0.1^a	68.1 ± 0.8^c	49.9 ± 1.0^e
TCA-soluble protein (%)[4]	2.9 ± 0.2^d	2.1 ± 0.1^e	5.2 ± 0.1^b	5.8 ± 0.3^a	4.2 ± 0.1^c	2.1 ± 0.1^e
Alkali- (KOH-) soluble protein (%)[5]	85.5 ± 0.2^a	86.5 ± 0.2^a	82.6 ± 0.8^b	75.8 ± 0.3^d	77.8 ± 0.1^c	75.9 ± 0.2^d
Ash (g/kg)	70.3 ± 0.3^c	83.1 ± 0.7^{ab}	84.9 ± 1.5^a	80.3 ± 0.6^b	83.5 ± 1.2^{ab}	82.6 ± 1.0^{ab}
Total calcium (g/kg)	3.6 ± 0.6^b	3.5 ± 0.2^b	4.2 ± 1.0^a	4.0 ± 0.6^a	4.2 ± 0.5^a	4.1 ± 0.9^a
Total phosphorus (g/kg)	7.5 ± 0.5^c	7.7 ± 0.1^c	8.3 ± 0.3^{ab}	8.5 ± 1.2^a	8.2 ± 1.0^b	8.2 ± 0.6^b
Trypsin inhibitor (mg/g)	3.4 ± 0.1^a	3.4 ± 0.2^a	1.5 ± 0.5^c	1.2 ± 0.2^c	2.6 ± 0.1^b	3.3 ± 0.1^a
Glycinin (mg/g)	39.4 ± 0.3^a	40.9 ± 0.1^a	32.3 ± 0.4^c	35.6 ± 0.3^b	15.6 ± 0.2^e	22.6 ± 0.2^d
β-Conglycinin (mg/g)	38.6 ± 0.2^d	47.6 ± 0.1^c	60.7 ± 0.1^a	15.5 ± 0.1^e	59.5 ± 0.2^a	51.9 ± 0.1^b

[1]Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($p > 0.05$). [2]These six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6 to protect corporate privacy. [3]Protein extracted by ultrapure water for three times. [4]Ratio of the protein extracted by 15% trichloroacetic acid to the total crude protein. [5]Ratio of the protein extracted by 0.2% potassium hydroxide to the total crude protein.

spectrophotometer (Amersham Biosciences, Ultrospec 2100 pro) according to the instructions of commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.8. Intestinal Morphology. The middle intestine was sliced into segments in 5 mm and dehydrated in the automatic dehydrator. After dehydration, tissues were embedded in paraffin and sliced in 6 μm by a microtome (RM2235, LEICA). All the slices were stained with haematoxylin and eosin (H & E) using a linear slide stainer (A81500101, Thermo, UK) and enclosed by neutral balsam. Then, these slices were observed and photoed under different magnifications by an imaging microscope (CX31RTSF, Olympus, Japan). All the images were analyzed by the specialized software Imagine-Pro Plus 6.0; the villus height (VH), microvillus height (MH), and thickness of the intestinal wall (TIW) were recorded via the software.

2.9. Calculations and Statistical Analysis. The following parameters were calculated:

$$\text{Survival rate}(\%) = 100 \times \left(\frac{N_t}{N_0}\right),$$

$$\text{Specific growth rate}(\text{SGR, \%/day}) = (\ln W_t - \ln W_0) \times \frac{100}{t},$$

$$\text{Weight gain rate}(\text{WGR, \%}) = 100 \times \frac{(W_t - W_0)}{W_0},$$

$$\text{Feed intake}(\text{FI, \%/day}) = 100 \times \frac{I_d/((W_t + W_0)/2)}{t},$$

$$\text{Feed efficiency ratio}(\text{FER}) = \frac{(W_t - W_0)}{I_d},$$

$$\text{Protein efficiency ratio}(\text{PER}) = \frac{(W_t - W_0)}{(I_d \times P)},$$

$$\text{Protein retention}(\text{PR}) = \frac{(W_t \times P_t - W_0 \times P_0)}{(I_d \times P)},$$

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

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

$$\text{Viscerosomatic index}(\text{VSI, \%}) = 100 \times \frac{\text{viscera weight(g)}}{\text{body weight(g)}},$$

$$\text{Apparent digestibility coefficient}(\text{ADC}) \text{ of dry matter}(\%)$$
$$= \left(100 - \left(\frac{\text{dietary Y}_2\text{O}_3}{\text{fecal Y}_2\text{O}_3}\right) \times 100\right),$$

$$\text{Apparent digestibility coefficient}(\text{ADC}) \text{ of nutrients}(\%)$$
$$= \left(100 - \left(\frac{\text{dietary Y}_2\text{O}_3}{\text{fecal Y}_2\text{O}_3}\right) \times \left(\frac{\text{fecal nutrients}}{\text{dietary nutrients}}\right) \times 100\right),$$

$$(1)$$

where W_0 and W_t are the initial and final body weight, respectively. t is the duration of the feeding trial. I_d is the feed intake as dry matter. P_0, P_t, and P represent the protein content in initial fish body, final fish body, and diet, respectively. N_0 and N_t are the initial and final number of fish, respectively.

Data were analyzed by one-way ANOVA, Pearson's correlation test, and linear regression analysis in software SPSS 17.0. In the analysis, data were initially examined for homogeneity of variances. Differences between means were tested by Tukey's multiple range test. The level of significance was set at $p < 0.05$, and results were presented as means ± S.E.M. (standard error of the mean).

3. Results

3.1. Nutritional Composition and Antinutritional Factor Content of Fermented Soybean Meal. The content of nutrients and ANFs in FSBM, including crude lipid, crude protein, water-soluble protein, TCA-soluble protein, alkali- (KOH-) soluble protein, ash, total calcium, total phospho-

rus, amino acid profile, trypsin inhibitor, glycinin, and β-conglycinin (Tables 3 and 4), was all analyzed. The lipid content of the six tested FSBM ranged from 17.1 to 25.5 g/kg, with values in decreasing order of FSBM2, FSBM1, FSBM5, FSBM3, FSBM4, and FSBM6. Besides, the protein content of FSBM3 and FSBM5 (555.6 and 556.0 g/kg) was significantly higher than that of other FSBM (ranging from 537.1 to 552.6 g/kg) ($p < 0.05$). As to the water-soluble protein and TCA-soluble protein, they were significantly higher in FSBM4 and FSBM3 than in other FSBM ($p < 0.05$). The alkali- (KOH-) soluble protein in FSBM1 and FSBM2 was significantly higher than that in others ($p < 0.05$). In terms of ANFs, significantly higher levels of trypsin inhibitor and glycinin were observed in FSBM1 and FSBM2, while the β-conglycinin content of FSBM3 and FSBM5 was significantly higher than that of other FSBM ($p < 0.05$), whereas FSBM4 showed significantly lower levels of trypsin inhibitor and β-conglycinin ($p < 0.05$).

No significant differences were observed in the content of valine (Val), leucine (Leu), and lysine (Lys) among the six different FSBM ($p > 0.05$), while FSBM1 showed significantly lower levels of isoleucine (Ile), phenylalanine (Phe), histidine (His), and arginine (Arg) than the rest ($p < 0.05$). Meanwhile, the content of Phe in FSBM4 was significantly higher than that of FSBM1 and FSBM2 ($p < 0.05$), and the content of His showed similar pattern as that of Phe.

3.2. Growth Performance and Feed Utilization. The survival rate of fish ranged from 97.78 to 100%, but no significant differences were observed among dietary treatments ($p > 0.05$) (Table 5). FI revealed no significant differences among turbot fed diets with FSBM2, FSBM3, FSBM5, and FM ($p > 0.05$), while fish fed the diet with FSBM1 showed significantly lower FI than the other groups ($p < 0.05$). The SGR was significantly higher in FSBM3 and FSBM4 than in other replacement groups ($p < 0.05$), but significantly lower than in the control group ($p < 0.05$). No significant differences were detected in FER and PER among groups FSBM3, FSBM4, and FM ($p > 0.05$), but their values were significantly lower in groups FSBM1 and FSBM2 than in others ($p < 0.05$). PR showed a similar pattern as FER and PER. HSI and CF were significantly lower in group FSBM1 than in others ($p < 0.05$), while VSI was significantly higher in groups FSBM1 and FSBM2 as compared with the control ($p < 0.05$).

3.3. Apparent Digestibility Coefficients of Dry Matter and Protein. The apparent digestibility coefficient (ADC) of dry matter ranged from 44.65 to 61.46% (Table 6) and its values decreased in the order of FSBM4, FM, FSBM6, FSBM3, FSBM5, FSBM1, and FSBM2, where no significant difference was observed between groups FSBM4 and FM ($p > 0.05$). The ADC of protein ranged from 83.34 to 92.07%, which decreased in the order of FM, FSBM4, FSBM6, FSBM3, FSBM5, FSBM2, and FSBM1.

3.4. Pearson's Correlation Test and Linear Regression Analysis. The correlation analysis showed that SGR and PER were both positively correlated with certain FSBM

TABLE 4: Amino acid profiles of fermented soybean meal (g/kg dry matter, means ± S.E.M.)[1].

Amino acids	Fermented soybean meal[2]					
	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
Essential amino acids						
Threonine	24.1 ± 0.4^a	20.9 ± 0.1^b	21.4 ± 0.5^b	22.2 ± 0.1^b	21.9 ± 0.4^b	22.3 ± 0.1^b
Valine	24.5 ± 0.1	24.8 ± 0.2	23.5 ± 1.1	23.4 ± 0.1	23.8 ± 0.6	23.9 ± 0.1
Methionine	4.9 ± 1.4^a	5.1 ± 0.3^a	4.7 ± 0.7^a	3.8 ± 1.1^b	3.7 ± 0.5^b	3.7 ± 1.2^b
Isoleucine	21.9 ± 0.3^b	24.2 ± 0.5^a	22.5 ± 0.9^{ab}	22.7 ± 0.3^{ab}	22.6 ± 0.3^{ab}	22.7 ± 0.3^{ab}
Leucine	39.7 ± 0.8	40.4 ± 0.3	40.2 ± 1.1	41.6 ± 0.3	41.0 ± 0.8	41.5 ± 0.3
Phenylalanine	25.1 ± 0.5^c	26.3 ± 0.1^{bc}	27.0 ± 0.7^{ab}	28.7 ± 0.8^a	27.7 ± 0.5^{ab}	27.6 ± 0.1^{ab}
Lysine	26.7 ± 0.7	27.8 ± 0.1	27.6 ± 0.8	28.0 ± 0.2	27.8 ± 0.6	27.7 ± 0.1
Histidine	12.2 ± 0.4^b	12.6 ± 0.3^{ab}	12.9 ± 0.4^{ab}	13.5 ± 0.1^a	13.1 ± 0.3^{ab}	13.2 ± 0.2^{ab}
Arginine	34.3 ± 0.7^c	36.2 ± 0.1^{bc}	36.7 ± 1.2^b	35.7 ± 0.2^{bc}	36.9 ± 0.7^b	37.7 ± 0.2^a
Nonessential amino acids						
Proline	22.0 ± 1.5^b	23.7 ± 0.5^{ab}	25.6 ± 0.7^a	26.5 ± 0.2^a	25.6 ± 0.5^a	26.4 ± 0.1^a
Aspartic acid	57.5 ± 1.2^b	59.0 ± 0.3^{ab}	59.8 ± 1.4^{ab}	61.6 ± 0.3^a	59.7 ± 1.2^{ab}	61.1 ± 0.2^a
Serine	26.0 ± 0.5^b	26.2 ± 0.3^b	27.4 ± 0.5^{ab}	28.9 ± 0.3^a	29.0 ± 0.7^a	28.8 ± 0.1^a
Glutamic acid	97.2 ± 1.7^b	103.3 ± 0.4^a	104.3 ± 2.5^a	106.1 ± 0.5^a	103.2 ± 2.2^a	105.8 ± 0.2^a
Glycine	24.2 ± 0.7^a	22.4 ± 0.1^b	22.1 ± 0.6^b	23.2 ± 0.1^{ab}	23.2 ± 0.5^{ab}	22.9 ± 0.1^{ab}
Alanine	28.2 ± 0.5^a	22.6 ± 0.1^c	24.1 ± 0.6^b	24.7 ± 0.1^b	24.9 ± 0.5^b	24.5 ± 0.1^b
Cysteine	5.6 ± 0.5^b	6.4 ± 0.1^a	6.4 ± 0.2^a	6.6 ± 0.2^a	6.5 ± 0.1^a	6.5 ± 0.3^a
Tyrosine	16.5 ± 0.7^b	17.5 ± 0.1^b	18.0 ± 0.6^a	19.1 ± 0.5^a	18.0 ± 0.2^a	18.1 ± 0.2^a

[1]Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($p > 0.05$).
[2]These six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6 to protect corporate privacy.

TABLE 5: Growth parameters and feed utilization of juvenile turbot (*Scophthalmus maximus* L.) fed the experimental diets (means ± S.E.M.)[1].

	Diets[2]						
	FM	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
IBW (g)[3]	6.99 ± 0.02	6.99 ± 0.02	6.99 ± 0.02	6.99 ± 0.02	6.99 ± 0.02	6.99 ± 0.02	6.99 ± 0.02
FBW (g)[3]	30.80 ± 1.08^a	15.04 ± 0.88^d	16.06 ± 1.25^d	24.59 ± 0.65^b	24.68 ± 0.31^b	20.20 ± 0.72^c	19.08 ± 0.27^c
WGR (%)[3]	340.06 ± 15.41^a	114.85 ± 12.53^d	129.47 ± 17.81^d	251.24 ± 9.24^b	252.60 ± 4.43^b	188.62 ± 10.35^c	172.53 ± 3.84^c
FI (%/day)[3]	1.50 ± 0.06^a	1.10 ± 0.02^c	1.36 ± 0.08^{ab}	1.39 ± 0.01^{ab}	1.26 ± 0.03^b	1.44 ± 0.04^a	1.28 ± 0.04^b
SGR (%/day)[3]	2.12 ± 0.05^a	1.09 ± 0.08^d	1.18 ± 0.11^d	1.79 ± 0.04^b	1.80 ± 0.02^b	1.51 ± 0.05^c	1.43 ± 0.02^c
Survival rate (%)	100.00 ± 0.00	98.90 ± 0.91	97.80 ± 0.91	100.00 ± 0.00	100.00 ± 0.00	97.78 ± 2.23	100.00 ± 0.00
FER[3]	1.27 ± 0.07^a	0.99 ± 0.06^{cd}	0.87 ± 0.03^d	1.20 ± 0.03^{ab}	1.33 ± 0.02^a	1.00 ± 0.01^{cd}	1.08 ± 0.03^{bc}
PER[3]	2.41 ± 0.14^a	1.86 ± 0.12^{cd}	1.66 ± 0.06^d	2.29 ± 0.06^{ab}	2.54 ± 0.04^a	1.91 ± 0.01^{cd}	2.07 ± 0.06^{bc}
PR[3]	0.34 ± 0.02^a	0.25 ± 0.02^c	0.21 ± 0.01^d	0.30 ± 0.01^b	0.36 ± 0.01^a	0.26 ± 0.01^c	0.27 ± 0.01^{bc}
HSI (%)[3]	0.84 ± 0.02^a	0.53 ± 0.06^b	0.84 ± 0.04^a	0.77 ± 0.02^a	0.79 ± 0.03^a	0.80 ± 0.04^a	0.79 ± 0.04^a
VSI (%)[3]	3.89 ± 0.13^c	4.50 ± 0.08^a	4.35 ± 0.15^{ab}	4.04 ± 0.17^{bc}	3.93 ± 0.07^c	4.22 ± 0.12^{abc}	4.03 ± 0.07^{bc}
CF (g/cm³)[3]	3.52 ± 0.03^a	3.18 ± 0.09^b	3.52 ± 0.09^a	3.67 ± 0.10^a	3.61 ± 0.05^a	3.51 ± 0.11^a	3.56 ± 0.05^a

[1]Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($p > 0.05$).
[2]Fish meal-based diet was named FM; diets with 450 g/kg of fish meal replaced by six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6. [3]IBW: initial body weight; FBW: final body weight; WGR: weight gain rate; FI: feed intake; SGR: specific growth rate; FER: feed efficiency ratio; PER: protein efficiency ratio; PR: protein retention; HSI: hepatosomatic index; VSI: viscerosomatic index; CF: condition factor.

components, such as water-soluble protein, TCA-soluble protein, and total phosphorus (Table 7) ($p < 0.05$), while the PR was only positively correlated with the content of water-soluble protein and TCA-soluble protein ($p < 0.05$).

Besides, the trypsin inhibitor content of FSBM showed negative correlation with SGR, PER, and PR. In addition, SGR, PER, and PR were all positively correlated with the ADC of dry matter and protein (Table 8). Based on Pearson's

TABLE 6: Apparent digestibility coefficients (%, ADC) of dry matter and protein for the experimental diets (means ± S.E.M.)[1].

	Diets[2]						
	FM	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
ADC of dry matter	59.17 ± 0.90[a]	44.66 ± 1.44[d]	44.65 ± 1.09[d]	49.62 ± 0.82[c]	61.46 ± 2.02[a]	48.46 ± 0.86[c]	53.52 ± 0.63[b]
ADC of protein	92.07 ± 0.17[a]	83.34 ± 0.43[e]	84.86 ± 0.30[d]	86.93 ± 0.21[c]	90.86 ± 0.48[b]	86.81 ± 0.22[c]	87.53 ± 0.17[c]

[1]Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($p > 0.05$).
[2]Fish meal-based diet was named FM; diets with 450 g/kg of fish meal replaced by six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6.

TABLE 7: Pearson's correlation test between growth parameters and fermented soybean meal components.

Growth parameters	Fermented soybean meal components	r^2	p^2
SGR[1]	Crude lipid	-0.583	ns[3]
	Crude protein	0.845	<0.05
	Ash	0.562	ns
	Water-soluble protein	0.863	<0.05
	TCA-soluble protein[1]	0.856	<0.05
	Total calcium	0.846	<0.05
	Total phosphorus	0.961	<0.05
	Trypsin inhibitor	-0.928	<0.05
	Glycinin	-0.295	ns
	β-Conglycinin	-0.108	ns
PER[1]	Crude lipid	-0.725	ns
	Crude protein	0.676	ns
	Ash	0.171	ns
	Water-soluble protein	0.864	<0.05
	TCA-soluble protein	0.824	<0.05
	Total calcium	0.607	ns
	Total phosphorus	0.824	<0.05
	Trypsin inhibitor	-0.886	<0.05
	Glycinin	-0.056	ns
	β-Conglycinin	-0.462	ns
PR[1]	Crude lipid	-0.673	ns
	Crude protein	0.674	ns
	Ash	0.086	ns
	Water-soluble protein	0.885	<0.05
	TCA-soluble protein	0.849	<0.05
	Total calcium	0.552	ns
	Total phosphorus	0.805	ns
	Trypsin inhibitor	-0.884	<0.05
	Glycinin	-0.061	ns
	β-Conglycinin	-0.554	ns

[1]SGR: specific growth rate; PER: protein efficiency ratio; PR: protein retention; TCA-soluble protein: ratio of the protein extracted by 15% trichloroacetic acid to the total crude protein. [2]r: correlation index; p: test statistic (significant, $p < 0.05$). [3]ns: not significant.

correlation test, the corresponding linear regression analysis was also performed (Tables 9 and 10).

3.5. Activity of Digestive Enzymes. The trypsin activity of group FSBM6 was significantly lower than that of groups

TABLE 8: Pearson's correlation test between growth parameters and apparent digestibility coefficient (ADC) for feed.

Growth parameters	Apparent digestibility coefficient (ADC)	r^2	p^2
SGR[1]	ADC of dry matter	0.812	<0.05
	ADC of protein	0.915	<0.05
PER[1]	ADC of dry matter	0.901	<0.05
	ADC of protein	0.857	<0.05
PR[1]	ADC of dry matter	0.920	<0.05
	ADC of protein	0.878	<0.05

[1]SGR: specific growth rate; PER: protein efficiency ratio; PR: protein retention. [2]r: correlation index; p: test statistic (significant, $p < 0.05$).

FSBM1 and FM ($p < 0.05$) (Table 11). As to the alpha-amylase, there were no significant differences among fish fed with diets FSBM1, FSBM2, and FM ($p > 0.05$). Lipase showed significantly higher activity in group FSBM2 than in the control group ($p < 0.05$), but no significant differences were observed in lipase activity among groups FSBM1, FSBM3, FSBM5, FSBM6, and FM ($p > 0.05$).

3.6. Intestinal Morphology. The villus height (VH) of group FSBM4 was significantly higher than that of groups FSBM1 and FSBM2 ($p < 0.05$). As to microvillus height (MH) and thickness of the intestinal wall (TIW), they showed decreasing order by groups FM, FSBM4, FSBM6, FSBM3, FSBM5, FSBM2, and FSBM1 (Table 12 and Figure 1), but no significant differences were observed in MH between groups FSBM4 and FM ($p > 0.05$).

4. Discussion

Numerous kinds of fermented soybean meal (FSBM) are now commercially available, whereas the nutritional profiles of these products vary with processing technologies, fermentation strains, and raw soybean meal, which would result in different effects on bioavailability of nutrients in aquaculture feeds [10, 11]. The present study showed that diets containing FSBM3 and FSBM4 yielded higher growth performance of juvenile turbot than diets containing other FSBM, suggesting their higher nutritional quality, whereas growth performance was significantly inhibited in fish fed diets with FSBM1 and FSBM2. According to previous studies on fish meal substitution, the reduced growth performance in replacement groups could generally be attributed to poor palatability, low digestibility, and imbalanced amino acid profile of alternative protein sources [28–30].

TABLE 9: Linear regression analysis between growth parameters and fermented soybean meal components.

Growth parameters	Fermented soybean meal components	Linear regression line[1]	R^2	p[3]
SGR[2]	Crude lipid		0.34	ns[3]
	Crude protein	$y = 0.328x - 16.530$	0.71	<0.05
	Ash		0.32	ns
	Water-soluble protein	$y = 0.145x + 0.508$	0.75	<0.05
	TCA-soluble protein[2]	$y = 0.159x + 0.875$	0.73	<0.05
	Total calcium	$y = 8.794x - 2.004$	0.72	<0.05
	Total phosphorus	$y = 7.510x - 4.602$	0.92	<0.05
	Trypsin inhibitor	$y = -0.274x + 2.167$	0.86	<0.05
	Glycinin		0.09	ns
	β-Conglycinin		0.01	ns
PER[2]	Crude lipid		0.53	ns
	Crude protein		0.46	ns
	Ash		0.03	ns
	Water-soluble protein	$y = 1.55x + 1.029$	0.75	<0.05
	TCA-soluble protein	$y = 0.164x + 1.446$	0.68	<0.05
	Total calcium		0.37	ns
	Total phosphorus	$y = 6.884x - 3.507$	0.68	<0.05
	Trypsin inhibitor	$y = -0.280x + 2.770$	0.79	<0.05
	Glycinin		0.01	ns
	β-Conglycinin		0.21	ns
PR[2]	Crude lipid		0.45	ns
	Crude protein		0.45	ns
	Ash		0.01	ns
	Water-soluble protein	$y = 0.025x + 0.107$	0.78	<0.05
	TCA-soluble protein	$y = 0.027x + 0.175$	0.72	<0.05
	Total calcium		0.31	ns
	Total phosphorus		0.65	ns
	Trypsin inhibitor	$y = -0.045x + 0.389$	0.78	<0.05
	Glycinin		0.01	ns
	β-Conglycinin		0.31	ns

[1]Linear regression lines (where y is the growth parameters and x is the fermented soybean meal components). [2]SGR: specific growth rate; PER: protein efficiency ratio; PR: protein retention; TCA-soluble protein: ratio of the protein extracted by 15% trichloroacetic acid to the total crude protein. [3]p: test statistic (significant, $p < 0.05$); ns: not significant.

TABLE 10: Linear regression analysis between growth parameters and apparent digestibility coefficient (ADC) for feed[1].

Growth performance/protein utilization	Apparent digestibility coefficient (ADC)	Linear regression line[1]	R^2	p
SGR[2]	ADC of dry matter	$y = 0.045x - 0.741$	0.659	<0.05
	ADC of protein	$y = 0.043x - 0.120$	0.812	<0.05
PER[2]	ADC of dry matter	$y = 0.007x - 0.089$	0.846	<0.05
	ADC of protein	$y = 0.109x - 7.950$	0.836	<0.05
PR[2]	ADC of dry matter	$y = 0.089x - 5.653$	0.734	<0.05
	ADC of protein	$y = 0.015x - 1.022$	0.771	<0.05

[1]Linear regression lines (where y is the growth parameters and x is the ADC of dry matter and protein). [2]SGR: specific growth rate; PER: protein efficiency ratio; PR: protein retention.

TABLE 11: Digestive enzyme activity of juvenile turbot (*Scophthalmus maximus* L.) fed the experimental diets (means ± S.E.M.)[1].

			Diets[2]				
	FM	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
Trypsin (U/μg prot)	24.62 ± 2.85[a]	18.59 ± 2.19[b]	14.09 ± 0.21[bc]	14.14 ± 2.18[bc]	14.53 ± 1.28[bc]	13.29 ± 2.17[bc]	10.91 ± 0.94[c]
Alpha-amylase (U/mg prot)	0.34 ± 0.02[a]	0.28 ± 0.02[a]	0.28 ± 0.05[a]	0.17 ± 0.10[b]	0.18 ± 0.05[b]	0.18 ± 0.02[b]	0.15 ± 0.04[b]
Lipase (U/g prot)	1.58 ± 0.34[c]	2.23 ± 0.29[bc]	3.40 ± 0.47[a]	2.04 ± 0.13[c]	3.13 ± 0.94[ab]	2.61 ± 0.28[abc]	2.25 ± 0.15[bc]

[1]Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($p > 0.05$).
[2]Fish meal-based diet was named FM; diets with 450 g/kg of fish meal replaced by six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6.

TABLE 12: Middle intestinal histology parameters of juvenile turbot (*Scophthalmus maximus* L.) fed the experimental diets (means ± S.E.M.)[1].

			Diets[2]				
	FM	FSBM1	FSBM2	FSBM3	FSBM4	FSBM5	FSBM6
VH (μm)[3]	847.75 ± 36.11[ab]	607.72 ± 23.48[c]	645.94 ± 24.76[c]	714.26 ± 37.80[ab]	751.38 ± 44.59[ab]	874.48 ± 76.68[a]	818.37 ± 47.61[ab]
TIW (μm)[3]	158.38 ± 3.78[a]	92.43 ± 2.37[d]	101.89 ± 10.63[cd]	122.49 ± 10.10[bc]	128.67 ± 4.19[b]	115.88 ± 7.26[bc]	135.85 ± 4.19[b]
MH (μm)[3]	4.73 ± 0.23[a]	3.36 ± 0.17[c]	3.62 ± 0.05[bc]	3.87 ± 0.06[bc]	4.32 ± 0.46[ab]	3.82 ± 0.29[bc]	3.88 ± 0.05[bc]

[1]Data are means of triplicate. Means in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($p > 0.05$).
[2]Fish meal-based diet was named FM; diets with 450 g/kg of fish meal replaced by six kinds of commercial fermented soybean meal were, respectively, named FSBM1, FSBM2, FSBM3, FSBM4, FSBM5, and FSBM6. [3]VH: villus height; TIW: thickness of the intestinal wall; MH: microvillus height.

The palatability of diets can be reflected by feed intake (FI) [31], and previous studies have revealed that the growth performance in fish was positively correlated with FI when dietary fish meal was substituted by FSBM [32, 33]. In the present study, no significant differences in FI were observed among dietary treatments FM, FSBM2, FSBM3, and FSBM5, which indicated that juvenile turbot had a better adaptability to these FSBM. Coupled with the trend in growth performance, it thus can be inferred that the FI was not responsible for growth reduction.

The digestion and absorption, as important processes in dietary nutrient conversion, have important impacts on growth performance [34]. Apparent digestibility coefficient (ADC) provides an indirect measurement of dietary nutrient digestion [35]. As per Pearson's correlation test, dietary protein utilization reflected by protein efficiency ratio (PER) and protein retention (PR) was positively correlated with the ADC of dry matter and protein. In the present study, the ADC for feed may be affected by digestive enzyme activity, antinutritional factors (ANFs), protein properties of FSBM, and intestinal structure. Results of this study showed that no significant differences in trypsin activity were observed among dietary treatments FSBM1, FSBM2, FSBM3, and FSBM4, while the alpha-amylase and lipase activities were higher in groups FSBM1 and FSBM2 than in FSBM3, which was opposite to the trend of feed digestibility. It thus could be deduced that the reduced digestibility in groups FSBM1 and FSBM2 was not attributed to the digestive enzyme activity, while the increased digestive enzyme activity in groups FSBM1 and FSBM2 might be a compensation for the decreased feed digestibility.

Feed digestibility could also be affected by ANFs in soybean meal [36]. Trypsin inhibitor, as the main ANF in soybean meal, can resist the digestion of trypsin and stimulate the secretion of pancreatic juice, ultimately leading to endog-

enous amino acid depletion [37]. Apart from that, antigenic proteins, such as glycinin and β-conglycinin, are also important components of ANFs in soybean meal, which could lead to intestinal allergies and hinder the development of intestinal villi [38]. In this study, the trypsin inhibitor and glycinin contents of FSBM1 and FSBM2 were higher than those of the rest selected FSBM. Correspondingly, lower ADC of dry matter and protein was shown in diets FSBM1 and FSBM2. In contrast, higher feed digestibility was observed in the diet containing FSBM4 (with the lowest levels of glycinin and β-conglycinin). Within these detected ANFs, the trypsin inhibitor content in FSBM showed negative correlation with the PER, PR, and SGR, which indicated that the ANFs could inhibit growth and feed utilization by affecting feed digestibility. Thus, reducing the content of ANFs plays an important role in improving the quality of FSBM.

According to previous studies, the protein properties of soybean meal can be greatly affected by fermentation treatment, where the high-molecular-weight protein could be degraded into low-molecular-weight protein and polypeptide [15]. Zheng et al. [39] reported that low-molecular-weight protein (<10,000 Dalton) reflected by water-soluble protein and trichloroacetic acid- (TCA-) soluble protein is more readily digested by aquatic animals. In the present study, the water-soluble protein and TCA-soluble protein contents of FSBM, which exhibited consistent trends with ADC of dry matter and protein, were both positively correlated with PER and PR. It could be deduced that the lower-molecular-weight protein in FSBM was responsible for the improved feed digestibility and protein utilization efficiency. Apart from that, the heating degree, reflected by the level of alkali- (KOH-) soluble protein, can also affect the protein properties of soybean meal [40]. Araba and Dale [27] revealed that alkali- (KOH-) soluble protein value in excess of 85% indicated underprocessing of soybean meal. For the
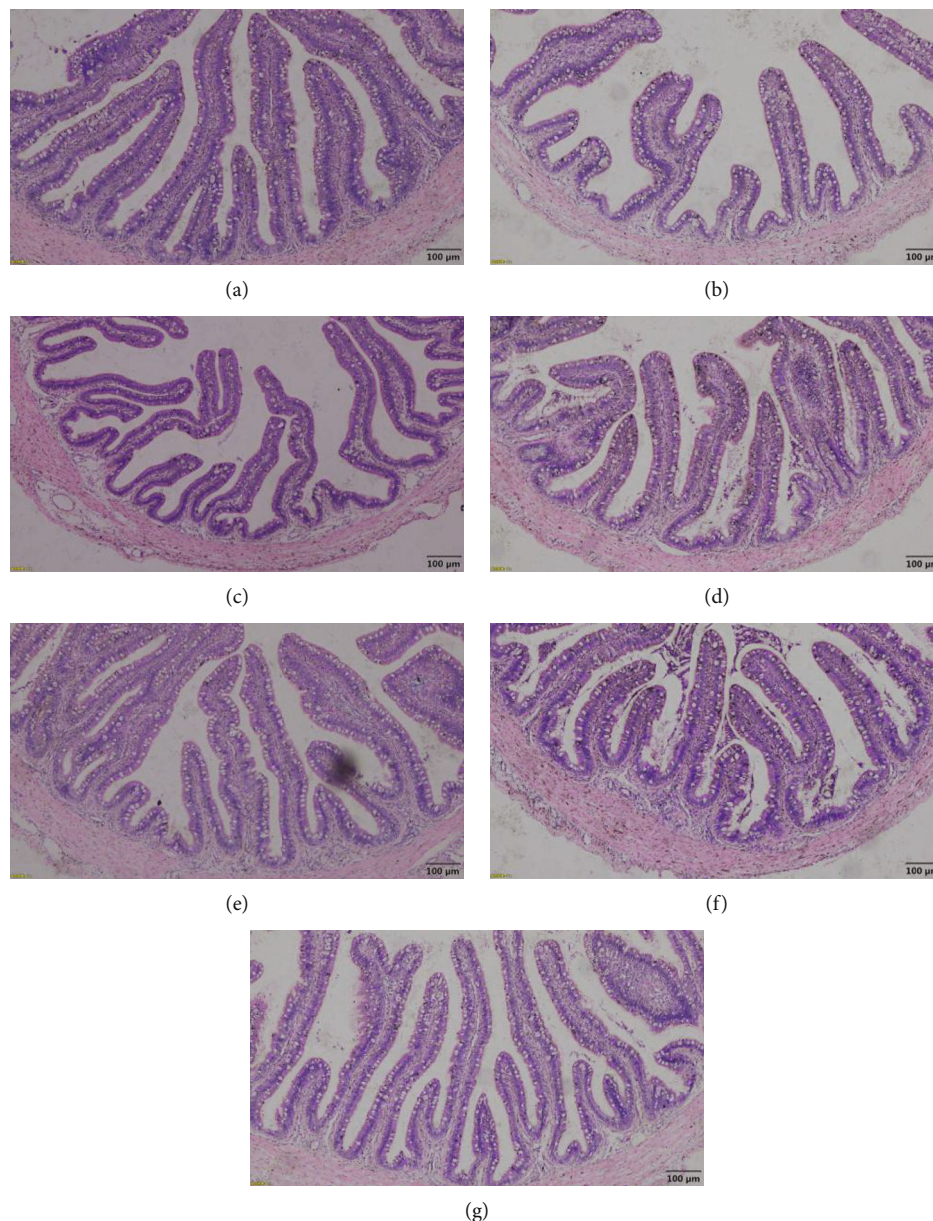
(a)

(b)

(c)

(d)

(e)

(f)

(g)

FIGURE 1: Representative intestine sections of juvenile turbot (*Scophthalmus maximus* L.) fed with (a) fish-meal-based diet FM and diets containing (b) FSBM1, (c) FSBM2, (d) FSBM3, (e) FSBM4, (f) FSBM5, and (g) FSBM6, all the sections were stained by haematoxylin and eosin. Scale bars, 100 μm.

underheated FSBM, it may contain more undenatured protein, which was not conducive to digestion. In the present study, the alkali- (KOH-) soluble protein content of FSBM1 (85.5%) and FSBM2 (86.5%) was significantly higher than that of the rest selected FSBM, indicating lower feed digestibility in groups FSBM1 and FSBM2 may also be attributed to high content of undenatured protein caused by underheated treatment. Therefore, improving the breakdown degree of FSBM protein and the content of low-molecular-weight protein is conducive to improve the utilization efficiency of FSBM in aquafeeds.

The intestine is a major digestive organ for nutrients; thus, its structure has important effects on digestion [41, 42]. In the present study, the villus height (VH) was significantly lower in groups FSBM1 and FSBM2, which agree well with relatively lower feed digestibility and growth performance in both groups. Also, the microvillus height (MH) and thickness of the intestinal wall (TIW) showed the same pattern as ADC of protein, which was in agreement with some previous studies on Atlantic salmon, *Salmo salar* [43], Asian seabass, *Lates calcarifer* [44], and turbot [45]. It is thus deduced that different FSBM could result in varied effects on intestinal structure, thereby affecting the digestion capacity of turbot.

Amino acid is the basic nutrient for protein metabolism, and an adequate supply of essential amino acid can facilitate protein anabolism and growth [34]. In the present study, each diet containing FSBM was supplied with crystalline L-

lysine, L-methionine, and L-isoleucine to meet the essential amino acid requirements reported for turbot [46], which seemed that the dietary amino acids would not limit growth performance of turbot. Nevertheless, previous studies showed that 22%-80% of dietary crystalline amino acids could be leached in a few minutes after water immersion [47, 48]. Besides, the absorption of crystalline amino acids and protein-bound amino acids in the intestine is not synchronized [34, 47], which would compromise the efficiency of crystalline amino acids utilized by turbot. Thus, the original amino acid composition of FSBM may have crucial implications for growth performance. The isoleucine (Ile), phenylalanine (Phe), histidine (His), and arginine (Arg) levels of FSBM1 were all significantly lower than those of the rest selected FSBM, which was consistent with growth performance of fish fed diet with FSBM1. As to FSBM2, the content of its essential amino acids was not significantly different from that of FSBM3 and FSBM4, but the growth performance of fish fed diet with FSBM2 was significantly reduced, which may be attributed to the low digestibility of diet containing FSBM2. On the whole, the growth performance was significantly reduced in all the replacement groups as compared with the control, suggesting that the addition of crystal amino acids cannot make up for the lack of essential amino acids in FSBM, and further treatment (e.g., addition of coated amino acids and amino acids in peptide form) may be required to effectively improve the utilization efficiency of FSBM.

In conclusion, this study demonstrated that commercially available FSBM3 and FSBM4 could yield higher growth performance of juvenile turbot than the rest selected FSBM when replacing 450 g/kg of fish meal in diets, while FSBM1 and FSBM2 significantly reduced the growth performance due to low digestibility and imbalanced amino acid supply. In aquafeed production, the content of water-soluble protein, TCA-soluble protein, and trypsin inhibitor could serve as relatively accurate indicators in the quality evaluation of FSBM.

Data Availability

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

Ethical Approval

The present study was conducted strictly according to the recommendations in the Guide for the Use of Experimental Animals of the Ocean University of China.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All authors have read and approved the final manuscript.

References

[1] X. Bu, X. Lian, Y. Zhang et al., "Effects of replacing fish meal with corn gluten meal on growth, feed utilization, nitrogen and phosphorus excretion and IGF-I gene expression of juvenile Pseudobagrus ussuriensis," *Aquaculture Research*, vol. 49, no. 2, pp. 977–987, 2018.

[2] F. Natale, J. Hofherr, G. Fiore, and J. Virtanen, "Interactions between aquaculture and fisheries," *Marine Policy*, vol. 38, pp. 205–213, 2013.

[3] X. Li, S. Zheng, X. Ma, K. Cheng, and G. Wu, "Use of alternative protein sources for fishmeal replacement in the diet of largemouth bass (Micropterus salmoides). Part I: effects of poultry by-product meal and soybean meal on growth, feed utilization, and health," *Amino Acids*, vol. 53, no. 1, pp. 33–47, 2021.

[4] Y. Wang, S. Tao, Y. Liao et al., "Partial fishmeal replacement by mussel meal or meat and bone meal in low-fishmeal diets for juvenile Ussuri catfish (Pseudobagrus ussuriensis): growth, digestibility, antioxidant capacity and IGF-I gene expression," *Aquaculture Nutrition*, vol. 26, no. 3, pp. 727–736, 2020.

[5] T. Ismail, E. Hegazi, M. A. Dawood et al., "Using of betaine to replace fish meal with soybean or/and corn gluten meal in nile tilapia (Oreochromis niloticus) diets: histomorphology, growth, fatty acid, and glucose-related gene expression traits," *Aquaculture Reports*, vol. 17, article 100376, 2020.

[6] K. S. Peng, N. Wu, Z. W. Cui et al., "Effect of the complete replacement of dietary fish meal by soybean meal on histopathology and immune response of the hindgut in grass carp (Ctenopharyngodon idellus)," *Veterinary Immunology and Immunopathology*, vol. 221, article 110009, 2020.

[7] X. B. Mo, C. L. Huang, S. Jiang et al., "Effects of concentrated dephenolic cottonseed protein instead of fish meal on stress resistance of Penaeus monodon," *The Israeli Journal of Aquaculture-Bamidgeh*, vol. 73, 2021.

[8] Q. C. Zhou, K. S. Mai, B. P. Tan, and Y. J. Liu, "Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (Rachycentron canadum)," *Aquaculture Nutrition*, vol. 11, no. 3, pp. 175–182, 2005.

[9] R. W. Hardy, "Alternate protein sources for salmon and trout diets," *Animal Feed Science and Technology*, vol. 59, no. 1-3, pp. 71–80, 1996.

[10] C. C. Chen, Y. C. Shih, P. W. S. Chiou, and B. Yu, "Evaluating nutritional quality of single stage- and two stage-fermented soybean meal," *Asian-Australasian Journal of Animal Sciences*, vol. 23, no. 5, pp. 598–606, 2010.

[11] F. Zhou, W. Song, Q. Shao et al., "Partial replacement of fish meal by fermented soybean meal in diets for black sea bream, Acanthopagrus schlegelii, juveniles," *Journal of the World Aquaculture Society*, vol. 42, no. 2, pp. 184–197, 2011.

[12] M. Egounlety and O. C. Aworh, "Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms)," *Journal of Food Engineering*, vol. 56, no. 2-3, pp. 249–254, 2003.

[13] B. N. Kim, "Physiological functions of chongkukjang," *Food Industry and Nutrition*, vol. 4, pp. 40–46, 1999.

[14] C. Li, B. Zhang, C. Liu et al., "Effects of dietary raw or *Enterococcus faecium* fermented soybean meal on growth, antioxidant status, intestinal microbiota, morphology, and inflammatory responses in turbot (*Scophthalmus maximus* L.)," *Fish & Shellfish Immunology*, vol. 100, pp. 261–271, 2020.

[15] G. Francis, H. P. Makkar, and K. Becker, "Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish," *Aquaculture*, vol. 199, no. 3-4, pp. 197–227, 2001.

[16] C. Li, B. Zhang, H. Zhou et al., "Beneficial influences of dietary *Aspergillus awamori* fermented soybean meal on oxidative homoeostasis and inflammatory response in turbot (*Scophthalmus maximus* L.)," *Fish & Shellfish Immunology*, vol. 93, pp. 8–16, 2019.

[17] L. Wang, H. Zhou, R. He, W. Xu, K. Mai, and G. He, "Effects of soybean meal fermentation by *Lactobacillus plantarum* P8 on growth, immune responses, and intestinal morphology in juvenile turbot (*Scophthalmus maximus* L.)," *Aquaculture*, vol. 464, pp. 87–94, 2016.

[18] M. Abdul Kader, S. Koshio, M. Ishikawa et al., "Can fermented soybean meal and squid by-product blend be used as fish-meal replacements for Japanese flounder (Paralichthys olivaceus)?," *Aquaculture Research*, vol. 43, no. 10, pp. 1427–1438, 2012.

[19] H. M. Azarm and S. M. Lee, "Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream Acanthopagrus schlegeli," *Aquaculture Research*, vol. 45, no. 6, pp. 994–1003, 2014.

[20] M. E. Barnes, M. L. Brown, K. A. Rosentrater, and J. R. Sewell, "An initial investigation replacing fish meal with a commercial fermented soybean meal product in the diets of juvenile rainbow trout," *Open Journal of Animal Sciences*, vol. 2, no. 4, pp. 234–243, 2012.

[21] S. Rahimnejad, K. Lu, L. Wang et al., "Replacement of fish meal with *Bacillus pumillus* SE5 and *Pseudozyma aphidis* ZR1 fermented soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*)," *Fish & Shellfish Immunology*, vol. 84, pp. 987–997, 2019.

[22] F. Bian, H. Zhou, G. He et al., "Effects of replacing fishmeal with different cottonseed meals on growth, feed utilization, haematological indexes, intestinal and liver morphology of juvenile turbot (Scophthalmus maximus L.)," *Aquaculture Nutrition*, vol. 23, no. 6, pp. 1429–1439, 2017.

[23] S. H. Cho, S. M. Lee, S. M. Lee, and J. H. Lee, "Effect of dietary protein and lipid levels on growth and body composition of juvenile turbot (Scophthalmus maximus L) reared under optimum salinity and temperature conditions," *Aquaculture Nutrition*, vol. 11, no. 4, pp. 235–240, 2005.

[24] J. K. Lee, S. H. Cho, S. U. Park, K. D. Kim, and S. M. Lee, "Dietary protein requirement for young turbot (Scophthalmus maximusL.)," *Aquaculture Nutrition*, vol. 9, no. 4, pp. 283–286, 2003.

[25] Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis of Official Analytical Chemists International*, Association of Official Analytical Chemists, Arlington, VA, 16th edition, 1995.

[26] V. K. Sarin, S. B. Kent, J. P. Tam, and R. B. Merrifield, "Quantitative monitoring of solid-phase peptide synthesis by the ninhydrin reaction," *Analytical Biochemistry*, vol. 117, no. 1, pp. 147–157, 1981.

[27] M. Araba and N. M. Dale, "Evaluation of protein solubility as an indicator of underprocessing of soybean meal," *Poultry Science*, vol. 69, no. 10, pp. 1749–1752, 1990.

[28] G. M. Berge, G. Baeverfjord, A. Skrede, and T. Storebakken, "Bacterial protein grown on natural gas as protein source in diets for Atlantic salmon, *Salmo salar*, in saltwater," *Aquaculture*, vol. 244, no. 1-4, pp. 233–240, 2005.

[29] B. S. Hauptman, F. T. Barrows, S. S. Block et al., "Evaluation of grain distillers dried yeast as a fish meal substitute in practical-type diets of juvenile rainbow trout, *Oncorhynchus mykiss*," *Aquaculture*, vol. 432, pp. 7–14, 2014.

[30] H. Mundheim, A. Aksnes, and B. Hope, "Growth, feed efficiency and digestibility in salmon (*Salmo salar* L.) fed different dietary proportions of vegetable protein sources in combination with two fish meal qualities," *Aquaculture*, vol. 237, no. 1-4, pp. 315–331, 2004.

[31] M. R. Yeomans, J. E. Blundell, and M. Leshem, "Palatability: response to nutritional need or need-free stimulation of appetite?," *British Journal of Nutrition*, vol. 92, no. S1, pp. S3–S14, 2004.

[32] X. F. Liang, L. Hu, Y. C. Dong et al., "Substitution of fish meal by fermented soybean meal affects the growth performance and flesh quality of Japanese seabass (Lateolabrax japonicus)," *Animal Feed Science and Technology*, vol. 229, pp. 1–12, 2017.

[33] Q. L. Zhou, H. M. Habte-Tsion, X. Ge et al., "Graded replacing fishmeal with canola meal in diets affects growth and target of rapamycin pathway gene expression of juvenile blunt snout bream, Megalobrama amblycephala," *Megalobrama amblycephala. Aquaculture Nutrition*, vol. 24, no. 1, pp. 300–309, 2018.

[34] D. Xu, G. He, K. Mai et al., "Effect of fish meal replacement by plant protein blend on amino acid concentration, transportation and metabolism in juvenile turbot (Scophthalmus maximusL.)," *Aquaculture Nutrition*, vol. 23, no. 5, pp. 1169–1178, 2017.

[35] S. J. Irvin and S. J. Tabrett, "A novel method of collecting fecal samples from spiny lobsters," *Aquaculture*, vol. 243, no. 1-4, pp. 269–272, 2005.

[36] H. F. Gemede and N. Ratta, "Antinutritional factors in plant foods: potential health benefits and adverse effects," *International Journal of Nutrition and Food Sciences*, vol. 3, no. 4, pp. 284–289, 2014.

[37] W. J. Pacheco, C. R. Stark, P. R. Ferket, and J. Brake, "Effects of trypsin inhibitor and particle size of expeller-extracted soybean meal on broiler live performance and weight of gizzard and pancreasEffects of trypsin inhibitor and particle size of expeller-extracted soybean meal on broiler live performance and weight of gizzard and pancreas1," *Poultry Science*, vol. 93, no. 9, pp. 2245–2252, 2014.

[38] S. Wilson, K. Blaschek, and E. G. De Mejia, "Allergenic proteins in soybean: processing and reduction of P34 allergenicity," *Nutrition Reviews*, vol. 63, no. 2, pp. 47–58, 2005.

[39] K. Zheng, T. Xu, C. Qian, M. Liang, and X. Wang, "Effect of low molecular weight fish protein hydrolysate on growth

performance and IGF-I expression in Japanese flounder (Paralichthys olivaceus) fed high plant protein diets," *Aquaculture Nutrition*, vol. 20, no. 4, pp. 372–380, 2014.

[40] S. W. Han, K. M. Chee, and S. J. Cho, "Nutritional quality of rice bran protein in comparison to animal and vegetable protein," *Food Chemistry*, vol. 172, pp. 766–769, 2015.

[41] E. H. Harrison and M. M. Hussain, "Mechanisms involved in the intestinal digestion and absorption of dietary vitamin A," *The Journal of Nutrition*, vol. 131, no. 5, pp. 1405–1408, 2001.

[42] J. X. Zhang, L. Y. Guo, L. Feng et al., "Soybean β-conglycinin induces inflammation and oxidation and causes dysfunction of intestinal digestion and absorption in fish," *PLoS One*, vol. 8, no. 3, article e58115, 2013.

[43] G. Baeverfjord and Å. Krogdahl, "Development and regression of soybean meal induced enteritis in Atlantic salmon, Salmo salar L., distal intestine: a comparison with the intestines of fasted fish," *Journal of Fish Diseases*, vol. 19, no. 5, pp. 375–387, 1996.

[44] M. Boonyaratpalin, P. Suraneiranat, and T. Tunpibal, "Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*," *Lates calcarifer. Aquaculture*, vol. 161, no. 1-4, pp. 67–78, 1998.

[45] M. Peng, W. Xu, Q. Ai, K. Mai, Z. Liufu, and K. Zhang, "Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.)," *Aquaculture*, vol. 392-395, pp. 51–58, 2013.

[46] S. J. Kaushik, "Whole body amino acid composition of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles," *Aquatic Living Resources*, vol. 11, no. 5, pp. 355–358, 1998.

[47] A. Pérez-Jiménez, H. Peres, and A. Oliva-Teles, "Effective replacement of protein-bound amino acids by crystalline amino acids in Senegalese sole (Solea senegalensis) juveniles," *Aquaculture Nutrition*, vol. 20, no. 1, pp. 60–68, 2014.

[48] M. Yúfera, S. Kolkovski, C. Fernández-Dıaz, and K. Dabrowski, "Free amino acid leaching from a protein-walled microencapsulated diet for fish larvae," *Aquaculture*, vol. 214, no. 1-4, pp. 273–287, 2002.