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Original Research Article

Flesh quality of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) fed with hydrolyzed porcine mucosa-supplemented low fishmeal diet

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ABSTRACT

Iso-nitrogenous and iso-lipidic diets containing 0%, 3%, 6%, 9%, and 12% hydrolyzed porcine mucosa (namely, HPM0, HPM3, HPM6, HPM9, and HPM12) were prepared to evaluate their effects on the growth performance, muscle nutrition composition, texture property, and gene expression related to muscle growth of hybrid groupers (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). Groupers were fed to apparent satiation at 08:00 and 16:00 every day for a total of 56 days. It was found that the weight gain percentage in the HPM0, HPM3, and HPM6 groups did not differ ($P > 0.05$). The cooking loss and drip loss of the dorsal muscle in the HPM3 group were lower than those in the HPM6 and HPM9 groups ($P < 0.05$). The hardness and chewiness of the dorsal muscle in the HPM3 group were higher than those in the HPM0, HPM9, and HPM12 groups ($P < 0.05$). The gumminess in the HPM3 group was higher than that in the HPM9 and HPM12 groups ($P < 0.05$). The total essential amino acid content of the dorsal muscle in the HPM12 group was higher than that in the HPM0 group ($P < 0.05$). The contents of total n-3 polyunsaturated fatty acid and total n-3 highly unsaturated fatty acid, as well as the ratio of n-3/n-6 polyunsaturated fatty acid in the dorsal muscle was higher in the HPM0 group than in all other groups ($P < 0.05$). The relative expressions of gene myogenic factor 5, myocyte enhancer factor 2c, myocyte enhancer factor 2a, myosin heavy chain, transforming growth factor-beta 1 (*TGF-β1*), and follistatin (*FST*) were the highest in the dorsal muscle of the HPM3 group. The results indicated that the growth performance of hybrid grouper fed a diet with 6% HPM and 27% fish meal was as good as that of the HPM0 group. When fish ingested a diet containing 3% HPM, the expression of genes *TGF-β1* and *FST* involved in muscle growth were upregulated, and then the muscle quality related to hardness and chewiness were improved. An appropriate amount of HPM could be better used in grouper feed.

1. Introduction

China is one of the largest aquaculture producers globally. According to FAO statistics, the aquaculture production of China reached 47.56 million tons in 2018, accounting for 57.93% of global aquaculture production (FAO, 2020). With rapid developments in the field, the marine fish production of China reached 1.61 million tons in 2019, an increase of 7.41% over 2018 (Fisheries and Fishery Administration Bureau of The Ministry of Agriculture and Rural Affairs et al., 2019). The grouper is one of the important

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mariculture fishes in China, and its production ranks third year-round (Fisheries and Fishery Administration Bureau of The Ministry of Agriculture and Rural Affairs et al., 2018). Among them, the hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) is very popular among farmers and consumers owing to its fast growth, strong disease resistance, and good taste (Kim et al., 2020).

With improvements in people's living standards, consumers have begun to exhibit a greater desire for high-quality fish (Liu et al., 2020). The flesh quality of muscle, which is the main edible part of the fish, is not only affected by the internal factors of the fish, such as strain (Johnston et al., 2006) and size (Shearer, 1994), but also by external factors, such as feed (Sun et al., 2018; Zhao et al., 2019), feeding strategy (Lv et al., 2018; Imsland et al., 2020), temperature (López-Albors et al., 2008), density (Álvarez et al., 2020; Birolo et al., 2020; Silva et al., 2020), salinity (Cheng et al., 2020), dissolved oxygen (Zuanazzi et al., 2019), and other environmental factors. The levels of nutrients, such as mineral elements (Zhang et al., 2016a), fatty acids (Li, 2020a), amino acids (Cai et al., 2018), and proteins (Zhang et al., 2016b) in feed, along with the source of protein (Wang et al., 2017), play an important role in regulating flesh quality.

As more and more feed ingredients are being used to replace fish meals completely or partially, they are affecting the flesh quality of fish. The addition of 3% *Saccharomyces cerevisiae* hydrolysate in the feed significantly increased the crude protein (CP) content and reduced the ether extract (EE) content in grouper (*Epinephelus coioides*) muscle (Yang et al., 2020). Upon substituting a 9% faba bean meal for the soybean meal, the n-3 polyunsaturated fatty acid (n-3 PUFA) and n-6 PUFA contents of grass carp (*Ctenopharyngodon idella*) muscle were significantly reduced, and the monounsaturated fatty acid (MUFA) content, hardness, chewiness, and gumminess were significantly increased (Li et al., 2020b). When partly replacing fish meal with fermented soybean meal or housefly (*Musca domestica*) maggot meal, increased moisture and C18:3n-3 contents in Japanese seabass (*Lateolabrax japonicus*; Liang et al., 2017) and lower levels of thaw loss and improved hardness for Nile tilapia (*Oreochromis niloticus*; Wang et al., 2017) were found. After the fish meal in the feed was completely replaced with rice protein concentrate, the cooking loss of blunt snout bream (*Megalobrama amblycephala*) muscle was significantly increased (Cai et al., 2018). Hydrolyzed porcine mucosa (HPM) is derived from the mucosa of the cleaned small intestine of pigs, and is a by-product of the production of the anticoagulant drug heparin sodium after high-temperature spray drying (Mateos et al., 2014). To increase the yield of heparin sodium, enzymes are added during the extraction process for the reaction (Hai et al., 2013). As a new type of functional nutritive animal protein source, HPM has been widely used in lactating sows (Johnston et al., 2003) and post weaning pigs (Cho et al., 2010; Myers et al., 2014; Figueroa et al., 2016) owing to its high protein content, balanced amino acid composition, richness in small peptides, free amino acids, and high safety profile (Mateos et al., 2014; Wu et al., 2017). There is little research on the use of HPM in aquatic animals, especially its effects on flesh quality. To the best of our knowledge, only one study on carp (*Cyprinus carpio*) has been reported thus far, which showed that replacing fishmeal with 3% HPM equivalents had no significant effect on the growth performance of carp, however there was a significant reduction in intestinal fold depth and villi height. Therefore, the purpose of this study is to evaluate the effects of low fish meal feed containing HPM on the growth performance, flesh quality, and muscle growth-related gene expressions of hybrid groupers.

2. Materials and methods

2.1. Animal ethics

All hybrid groupers and their caretaking procedures conformed to the NIH guidelines (NIH Pub. No. 85 to 23, revised 1996), and were approved by the Institutional Animal Care and Use Committee of Guangdong Ocean University (Zhanjiang, China).

2.2. Experimental design and diets

The by-products obtained from the extraction of sodium heparin from the cleaned small intestines from the mucous of pigs were treated at high temperatures and spray dried to obtain HPM in the form of a brownish powder with good flowability. Five iso-nitrogen and iso-lipidic low fishmeal and soybean meal level diets, with HPM levels of 0% (control group), 3%, 6%, 9%, and 12% were formulated. They are referred to as HPM0, HPM3, HPM6, HPM9, and HPM12, respectively. All powdered ingredients in the experiment were crushed (HNX-350, Beijing Huanya Tianyuan Machinery Technology Co. Ltd., Beijing, China) and passed through a 60-mesh screen. According to the formula shown in Table 1, the powdered ingredients were accurately weighed. The micro constituents were

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

Item	HPM0	HPM3	HPM6	HPM9	HPM12
Ingredients					
Brown fish meal [1]	33.00	30.00	27.00	24.00	21.00
HPM [2]		3.00	6.00	9.00	12.00
Soybean meal [1]	22.50	21.50	20.50	19.50	18.50
Wheat gluten flour [1]	4.28	5.15	6.03	6.90	7.78
Cottonseed protein [1]	6.00	6.00	6.00	6.00	6.00
Spray-dried blood [1]	4.00	4.00	4.00	4.00	4.00
Wheat meal [1]	18.20	18.20	18.20	18.20	18.20
L-Lysine [1]	0.78	0.81	0.84	0.87	0.90
DL-Methionine [1]	0.40	0.41	0.41	0.42	0.43
Anchovy oil [1]	3.35	3.17	3.00	2.82	2.65
Soybean oil [1]	3.00	3.00	3.00	3.00	3.00
Soybean lecithin [1]	1.50	1.50	1.50	1.50	1.50
Vitamin premix [3]	0.20	0.20	0.20	0.20	0.20
Mineral premix [4]	0.50	0.50	0.50	0.50	0.50
Vitamin C [1]	0.05	0.05	0.05	0.05	0.05
$Ca(H_2PO_4)_2$ [1]	1.50	1.50	1.50	1.50	1.50
Choline chloride [1]	0.30	0.30	0.30	0.30	0.30
Ethoxyquin [1]	0.03	0.03	0.03	0.03	0.03
Phytases [1]	0.30	0.30	0.30	0.30	0.30
Microcrystalline cellulose [1]	0.11	0.38	0.64	0.91	1.16
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition [5]					
Moisture	10.39	10.50	11.34	11.40	11.56
Crude protein	50.23	50.37	50.00	50.06	49.85
Ether extract	10.78	10.46	10.57	10.14	10.23
Crude ash	9.28	9.25	9.15	9.22	9.00

HPM = hydrolyzed porcine mucosa.
[1] Purchased from Zhanjiang Ouxun Feed Co., Zhanjiang, China.
[2] Provided by Yichang Huatai Biological Technology Co., Ltd., Yichang, China.
[3] Provided by Qingdao Master Biotech Co., Ltd, Qingdao, China. Each kilogram of premix included the following: vitamin B_1, 17.00 g; vitamin B_2, 16.67 g; vitamin B_6, 33.33 g; vitamin B_{12}, 0.07 g; vitamin K_3, 3.33 g; vitamin E, 66.00 g; retinyl acetate, 6.67 g; vitamin D_3, 33.33 g, nicotinic acid, 67.33 g; D-calcium pantothenate, 40.67 g; biotin, 16.67; folic acid, 4.17 g; inositol, 102.04 g; cellulose, 592.72 g.
[4] Provided by Qingdao Master Biotech Co., Ltd, Qingdao, China. Each kilogram of premix included the following: $CaCO_3$, 350 g; $NaH_2PO_4 \cdot H_2O$, 200 g; KH_2PO_4, 200 g; NaCl, 12 g; $MgSO_4 \cdot 7H_2O$, 10 g; $FeSO_4 \cdot 7H_2O$, 2 g; $MnSO_4 \cdot 7H_2O$, 2 g; $AlCl_3 \cdot 6H_2O$, 1 g; $CuCl_2 \cdot 2H_2O$, 1 g; KF, 1 g; $NaMoO_4 \cdot 2H_2O$, 0.5 g; $NaSeO_3$, 0.4 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; KI, 0.1 g; zeolite powder, 219.9 g.
[5] Proximate compositions were measured values.

mixed homogeneously using the sequential-expansion method. The HPM and other powdery ingredients were mixed evenly (GHJ-V, Jiangyin Weixiang Pharmachemical Machinery Factory, Jiangsu, China). Then, the weighed soybean lecithin, soybean oil, fish oil, and water (30% of the diet weight) were added and stirred evenly (B30, Guangzhou Panyu Lifeng Food Machinery Factory, Guangzhou, China). Subsequently, pellets with a diameter of 3.00 mm were made using a twin-screw extruder (TSE65, Beijing Modern Yanggong Machinery S&T Development Co. Ltd., Beijing, China). The pellets were dried in an air-conditioned room at 25 °C for 48 h and stored at −20 °C. The peptide molecular weight distribution and proximate composition of the HPM are shown in Fig. 1 and Table 2, respectively.

2.3. Fish and feeding trial

All groupers used in the experiment were purchased from the South East Quay Grouper Hatchery, Zhanjiang, China. Prior to commencing the feeding trial, hybrid groupers were acclimatised to the environment for two weeks, during which they were fed commercial feeds purchased from the Guangdong Yuehai Feeds Group (Zhanjiang, China), with a crude protein level of 50.0% twice (08:00 and 16:00) daily to apparent satiation. At the start of the experiment, all fish fasted for 24 h. The 450 hybrid groupers, with an initial average weight of 7.51 ± 0.03 g, were randomly divided into 15 fibreglass buckets (300 L) for indoor hydrostatic farming, with 30 fish in each bucket. They were fed twice (08:00 and 16:00) to apparent satiation every day for a total of 56 days. Each bucket contained approximately 70% of water, which was replaced daily. During the feeding period, the light conditions followed a natural cycle, the water temperature was 29 to 31 °C, the pH value was 6.9 to 7.2, the salinity was 27 to 30 g/L, the ammonia nitrogen level was less than or equal to 0.05 mg/L, and the dissolved oxygen level was at least 5.0 mg/L.

2.4. Sample collection and analysis

Before beginning the feeding experiment, 30 initial hybrid groupers were randomly collected and stored at −20 °C until the contents of CP and EE were measured, so as to calculate the protein deposition rate (PDR) and lipid deposition rate (LDR) of fish after feeding the experimental feed. At the end of the feeding trial, all hybrid groupers were anaesthetised using MS-222 (100 mg/L) prior to sample collection, and all fish in each tank were counted and weighed to calculate the weight gain percentage (WG), survival rate (SR), and feed efficiency (FE). Three groupers were randomly collected from each tank and stored at −20 °C to calculate the PDR and LDR. Three anaesthetized fish were randomly caught from each tank, skinned, and had their dorsal muscle collected using a scalpel and stored in a sealed bag at −20 °C to detect the moisture, CP, EE, amino acid (AA), and fatty acid (FA) contents of the dorsal muscles.

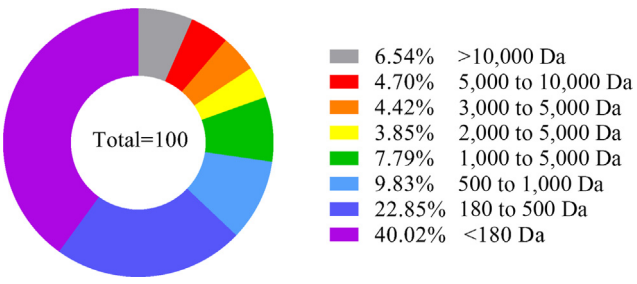

Fig. 1. Peptide molecular weight distribution of hydrolyzed porcine mucosa. Da, Dalton.

The proximate composition of the feed and dorsal muscle, including its moisture, CP, EE, and crude ash contents were analysed using standard methods of the AOAC (Association of Official Analytical Chemists, 1999). Moisture content was determined gravimetrically at a constant weight in an oven at 105 °C. CP content was determined using the Kjeldahl method (Total-N × 6.25). EE content was determined gravimetrically after extraction with ethyl ether. Crude ash content was determined by calcination at 550 °C in a muffle furnace. The amino acid and fatty acid compositions were determined using a modified amino acid analysis method (L-8800, Hitachi, Ltd., Japan) described by Ezaki et al. (2011) and the gas chromatograph method (GC-2010, Shimadzu Corporation, Japan) described by Faudzi et al. (2018), respectively. The results were expressed as a percentage of protein and fatty acids, respectively. The peptide molecular weight distribution of the HPM was determined by the Analysis and Testing Centre of Jiangnan University (Jiangsu, China) following standard national methods (General Administration of Quality Supervision et al., 2008).

Six fish were randomly selected from each bucket to collect dorsal muscle samples for texture property analysis (TPA) including hardness, cohesiveness, springiness, gumminess, and chewiness by using a food property analyser (TMS-PRO, Food Technology Corporation, Rockville, Maryland, USA). Dorsal muscle samples were collected from an area 2.00 cm posterior to the gill cover and 0.50 cm below the dorsal fin, whose size was 3.00 cm (length) × 3.00 cm (width) × 1.00 cm (height). Three fish were randomly selected from each tank, and approximately 3.00 g of dorsal muscle was collected, weighed, and suspended in a refrigerator at 4 °C for 24 h. Subsequently, it was weighed again and the difference was used to calculate the drip loss (Bidner et al., 2004); approximately 3.00 g of dorsal muscle was collected, sealed in cling film, steamed at 100 °C for 5 min, cooled to room temperature, and weighed the difference was used to calculate the cooking loss (Bertram et al., 2003). Three fish were randomly selected from each bucket, and their dorsal muscle collected, quickly placed in the RNA-later (Ambion, Life Technologies, Carlsbad CA, USA) and immersed at 4 °C for 12 h and then stored at −80 °C until the relative gene mRNA expression was analysed.

2.5. Quantitative real-time PCR analysis

The mRNA expressions of myogenic factor 5 (*Myf5*), myogenic differentiation (*MyoD*), myocyte enhancer factor 2c (*MEF-2c*), myogenin (*MyoG*), myocyte enhancer factor 2a (*MEF-2a*), myosin heavy chain (*MyHC*), insulin-like growth factor 1 (*IGF-1*), collagen type I alpha 1 (*Col1α-1*), collagen type I alpha 2 (*Col1α-2*), transforming growth factor-beta 1 (*TGF-β1*), follistatin (*FST*), myostatin (*MSTN*), and insulin-like growth factor 2 (*IGF-2*) in dorsal muscle were determined using quantitative real-time PCR (qRT-PCR). The β-actin gene was used as a housekeeping gene, owing to its highly stable expression (Kortner et al., 2011). The primer sequences of target genes for qRT-PCR are listed in Table 3. After the dorsal muscle samples were thawed, approximately 1.00 g of dorsal muscle was taken, the RNA-later was blotted off their surfaces, and the total RNA was extracted from the dorsal muscle using Trizol Reagent (Beijing Trans Gen Biotech Co., Ltd., China). The integrity was verified using 1% agarose gel electrophoresis. Recombinant DNase I (Takara, Japan) was used to treat the RNA extracts to remove possible DNA contamination, and the quality of RNA extracts was assessed using the spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, USA). A high absorbance ratio, between 1.86 and 2.00 at 260/280 nm, was found for the RNA

Table 2
Proximate composition of fish meal, hydrolyzed porcine mucosa, soybean meal and wheat gluten flour (%, dry matter).

Item	Brown fish meal	HPM	Soybean meal	Wheat gluten flour
Moisture	8.20	10.40	12.30	6.30
Crude protein	70.93	66.39	43.77	74.43
Ether extract	8.06	14.62	1.30	0.30
Essential amino acid				
Lysine	5.08	4.28	2.57	1.22
Methionine	1.91	1.39	0.46	1.01
Arginine	3.80	4.18	2.96	2.57
Isoleucine	2.73	2.82	1.91	2.63
Leucine	5.02	5.70	3.34	5.23
Threonine	2.95	3.17	1.79	2.07
Valine	3.15	3.38	1.96	2.73
Histidine	2.04	1.63	1.13	1.56
Phenylalanine	2.85	3.22	2.30	3.88
Semi-essential amino acid				
Tyrosine	2.33	3.03	1.49	2.66
Cystine	0.70	0.79	0.67	1.65
Non-essential amino acid				
Aspartate	6.15	13.16	4.98	2.58
Glutamine	9.06	9.32	8.21	29.17
Glycine	4.41	3.31	1.94	2.68
Alanine	4.29	3.22	1.84	1.83
Serine	2.75	3.02	2.33	3.83
Proline	2.72	2.73	2.16	9.28

HPM = hydrolyzed porcine mucosa.

Table 3
Sequence of the primers used for quantitative real-time PCR in this study.

Target gene	Primer sequence (5′-3′)	Annealing Temperature, °C	Length of amplified product, bp	Accession number
β-Actin	F: TGCGTGACATCAAGGAGAAGC	61	151	AY510710.2
	R: TCTGGGCAACGGAACCTCT	60		
Myf5	F: CGAGAGCAGGTGGAAAACTACT	58	142	XM_033614452.1
	R: ATCCGCCGCTGTAGTTTGC	61		
MyoD	F: GGCTCAGCAAGGTCAACGA	59	113	XM_033635008.1
	R: CTCGATGTAGCTGATGGCGT	59		
MEF-2c	F: CGGTTACCCATCTGCCATCTC	62	113	XM_033618691.1
	R: TTGAGCCGAGGTGAAGGGA	61		
MyoG	F: GAGGAGCACCTTGATGAACCC	61	191	XM_033625713.1
	R: TGGGCTCACTTGAAGACGACA	61		
MEF-2a	F: TGCCTGTATCCAATCCCAATG	60	206	XM_033634186.1
	R: TGGCACTGAAAGGTCTGTGGTAT	61		
MyHC	F: AAGGTGTTGGCTGAGTGGAAA	60	221	XM_033644959.1
	R: TGGATGCTCTTGCCAGTCTCA	61		
IGF-1	F: GTGCGATGTGCTGTATCTCCTG	61	179	XM_033614181.1
	R: GCCATAGCCTGTTGGTTTACTG	59		
Col1α-1	F: TGACAGCGGAACGGTGATG	60	128	XM_033645844.1
	R: CAACCTGAGGCTCCGTGAA	59		
Col1α-2	F: TCTGCGACTTCAACACCCG	60	234	XM_033636413.1
	R: AGTGGTAGGTGATGTTCTGGGTAG	59		
TGF-β1	F: ACAGGGACAACAACCCGCTAC	61	122	GQ205390.1
	R: CGCTCATCCTCATTTCCTTTG	60		
FST	F: GAAAAAGTGTCTGTGGGATGCTC	61	174	XM_033620608.1
	R: TTGACTTCCAGCAGCACCC	59		
MSTN	F: TGAGTAAACTGCGGATGAAAGAAG	61	144	XM_033614507.1
	R: CCTCCTCCATAACCACATCCTT	60		
IGF-2	F: CCGCAAAGATACGGACACCA	62	146	XM_033635279.1
	R: GCCGACGCTATTTCCACAAC	61		

Myf5 = myogenic factor 5; *MyoD* = myogenic differentiation; *MEF-2c* = myocyte enhancer factor 2c; *MyoG* = myogenin; *MEF-2a* = myocyte enhancer factor 2a; *MyHC* = myosin heavy chain; *IGF-1* = insulin-like growth factor 1; *Col1α-1* = collagen type I alpha 1; *Col1α-2* = collagen type I alpha 2; *TGF-β1* = transforming growth factor-beta 1; *FST* = follistatin; *MSTN* = myostatin; *IGF-2* = insulin-like growth factor 2.

extracts, indicating a high degree of purity. Reverse transcription of cDNA was carried out using the Prime ScriptTM RT reagent kit (Takara, Japan), following the manufacturer's instructions. The qRT-PCR reactions were performed according to instructions of the SYBR® Premix Ex TaqTM II Kit (Takara, Japan). A 10 μL reaction volume, containing 5 μL of SYBR® Green Real-time PCR Master Mix (Bio-Rad Labs), 0.4 μL of each primer, 1 μL of cDNA, and 3.2 μL of sterilized double distilled water was used for the qRT-PCR assay. Each sample in the reaction was performed in triplicate. The qRT-PCR was performed using a quantitative thermal cycler (Bio-Rad CFX96; Bio-Rad Labs, Hercules, CA, USA), and its parameters were as follows: 30 s at 95 °C, followed by 35 cycles of 5 s at 95 °C, 25 s at 60.0 °C, and 30 s at 72 °C. Gene expression levels were computed using the $2^{-\Delta\Delta CT}$ method, following Mu et al. (2018).

2.6. Calculations and statistical methods

Weight gain percentage (WG, %) = 100 × [final body weight (g) - initial body weight (g)]/initial body weight (g)

Survival rate (SR, %) = 100 × number of fish in the final test/number of fish in the initial test

Feed efficiency (FE) = [final body weight (g) - initial body weight (g)]/diet weight of each group's fish intake (dry weight, g)

Protein deposition rate (PDR, %) = 100 × [final body weight (g) × CP percentage of final test fish body (%) − initial body weight (g) × CP percentage of initial test fish body (%)]/[diet weight of each group's fish intake (dry weight, g) × CP percentage of feed (%)]

Lipid deposition rate (LDR, %) = 100 × [final body weight (g) × EE percentage of final test fish body (%) − initial body weight (g) × EE percentage of initial test fish body (%)]/[diet weight of each group's fish intake (dry weight, g) × EE percentage of feed (%)]

Drip loss (%) = 100 × [weight of muscles before being suspended (g) − weight of muscles after being suspended (g)]/weight of muscles before being suspended (g)

Cooking loss (%) = 100 × [weight of the muscle before being cooked (g) − weight of muscle after being cooked (g)]/weight of muscle before being cooked (g)

All data were statistically validated using one-way analysis of variance (ANOVA) in SPSS 22.0. The homogeneity of variance test was conducted to ensure that the variance was homogeneous. Tukey's test was used to compare the significant differences among mean values of all treatments. All data are shown as the mean value ± standard error of the mean (SEM). *P*-value < 0.05 was considered to be significant.

3. Results

3.1. Growth performance

As the amount of HPM added increased, no significant changes were detected in SR, FE, PDR, and LDR ($P > 0.05$). There was no significant difference in WG among the HPM0, HPM3, and HPM6 groups ($P > 0.05$), whose values were significantly higher than those in other groups ($P < 0.05$, Table 4).

3.2. Muscle composition

There were no significant differences in the muscle compositions in terms of moisture, CP, and ash content ($P > 0.05$). The muscle EE content of the HPM-containing groups was significantly reduced, and the EE content of the HPM9 group was significantly lower than that in other groups ($P < 0.05$, Table 5).

3.3. Water holding capacity of muscle and texture property analysis

There were no significant differences between the muscle cooking loss and drip loss of the HPM0 group and the HPM3 group, but cooking loss and drip loss of the HPM3 group was numerically lower than that in the HPM0 group ($P > 0.05$). The cooking loss and drip loss in the HPM3 group were significantly lower than those in the HPM6 and HPM9 groups ($P < 0.05$). The hardness and chewiness in the HPM3 group were significantly higher than those in the HPM0, HPM9, and HPM12 groups ($P < 0.05$). The gumminess in the HPM3 and HPM6 groups was significantly higher than that in HPM9 and HPM12 groups ($P < 0.05$). The cohesiveness and springiness were not significantly affected by experimental diets ($P > 0.05$, Table 6).

3.4. Amino acid composition of muscle

With increases in HPM content in the feed, the contents of Ile, Lys, Phe, Thr, Val, His, Asp, Glu, and Ala exhibited an upward trend, and their contents in the HPM12 group were significantly higher than those in the HPM0 group ($P < 0.05$). The remaining amino acid contents were not significantly affected by the feed ($P > 0.05$). The total amino acid (TAA) and total essential amino acid (TEAA) contents in the HPM12 group were significantly higher than those in the HPM0 group ($P < 0.05$). The HPM content in the feed had no significant effects on the total delicious amino acid (TDAA) and total aromatic amino acid (TAAA) contents ($P > 0.05$, Table 7).

3.5. Fatty acid composition of muscle

As the content of HPM in the feed increased, the contents of C15:0, C17:0, C18:3n-3, C20:5n-3, C22:6n-3, $\sum$ n-3 PUFA, and $\sum$ n-3 HUFA (n-3 highly unsaturated fatty acid) in muscle gradually decreased ($P < 0.05$). In comparison to the HPM0 group, adding 12% HPM to the feed significantly reduced the C15:0 and C18:3n-3 contents ($P < 0.05$). The C17:0, C20:5n-3, and C22:6n-3 contents in the HPM6, HPM9, and HPM12 groups were significantly lower than those in the HPM0 group ($P < 0.05$). The contents of $\sum$ n-3 PUFA and $\sum$ n-3 HUFA, along with the value of n-3/n-6, in the HPM0 group were the highest ($P < 0.05$). As the proportion of HPM

Table 4
Growth performance of hybrid groupers feed the test diets for 56 days[1].

Index	HPM0	HPM3	HPM6	HPM9	HPM12
IBW, g	7.49 ± 0.01	7.49 ± 0.02	7.49 ± 0.00	7.50 ± 0.02	7.48 ± 0.02
WG, %	847.07 ± 4.61[a]	851.25 ± 13.50[a]	850.63 ± 8.81[a]	805.74 ± 5.32[b]	756.12 ± 9.43[c]
SR, %	97.78 ± 2.22	97.78 ± 2.22	95.56 ± 1.11	97.78 ± 1.11	97.78 ± 1.11
FE	0.75 ± 0.02	0.77 ± 0.01	0.76 ± 0.01	0.79 ± 0.02	0.81 ± 0.02
PDR, %	26.46 ± 0.53	25.39 ± 0.18	26.39 ± 0.60	24.97 ± 0.60	25.52 ± 0.53
LDR, %	54.16 ± 1.70	54.62 ± 1.54	54.11 ± 4.29	51.39 ± 2.27	50.90 ± 0.99

HPM = hydrolyzed porcine mucosa; IBW = initial body weight; WG = weight gain percentage; SR = survival rate; FE = feed efficiency; PDR = protein deposition rate; LDR = lipid deposition rate.
[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] Diets HPM0, HPM3, HPM6, HPM9, and HPM 12 contains 0%, 3%, 6%, 9%, and 12% HPM content, respectively. Mean values ± SEM are presented for each group ($n = 3$).

Table 5
Muscle compositions of hybrid groupers feed the test diets for 56 days (%, wet matter) [1].

Index	HPM0	HPM3	HPM6	HPM9	HPM12
Moisture	65.00 ± 0.12	65.20 ± 0.32	66.09 ± 0.62	66.41 ± 0.58	65.78 ± 0.47
Crude protein	31.39 ± 0.04	31.50 ± 0.29	30.77 ± 0.59	30.77 ± 0.53	31.08 ± 0.43
Ether extract	2.19 ± 0.02^{a}	1.76 ± 0.03^{b}	1.79 ± 0.10^{b}	1.37 ± 0.08^{c}	1.69 ± 0.06^{b}
Crude ash	1.35 ± 0.03	1.42 ± 0.03	1.36 ± 0.03	1.29 ± 0.03	1.37 ± 0.03

HPM = hydrolyzed porcine mucosa.
[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] Diets HPM0, HPM3, HPM6, HPM9, and HPM 12 contains 0%, 3%, 6%, 9%, and 12% HPM content, respectively. Mean values ± SEM are presented for each group ($n = 3$).

Table 6
Water holding capacity and texture property in muscle of hybrid groupers feed the test diets for 56 days[1].

Index	HPM0	HPM3	HPM6	HPM9	HPM12
Cooking loss, %	21.45 ± 0.45^{c}	19.55 ± 0.42^{c}	27.71 ± 0.64^{a}	27.42 ± 0.68^{a}	24.20 ± 0.33^{b}
Drip loss, %	5.80 ± 0.51^{ab}	4.93 ± 0.28^{b}	6.55 ± 0.20^{a}	6.53 ± 0.07^{a}	5.25 ± 0.25^{ab}
Hardness, N	35.23 ± 2.11^{b}	44.17 ± 0.46^{a}	37.23 ± 3.43^{ab}	32.33 ± 0.55^{b}	32.43 ± 0.48^{b}
Cohesiveness ratio	0.46 ± 0.01	0.44 ± 0.01	0.46 ± 0.02	0.45 ± 0.01	0.44 ± 0.01
Springiness, mm	1.93 ± 0.03	1.95 ± 0.05	1.84 ± 0.01	1.83 ± 0.06	1.80 ± 0.08
Gumminess, N	18.05 ± 0.49^{ab}	19.00 ± 0.17^{a}	19.00 ± 0.23^{a}	16.63 ± 0.49^{bc}	15.50 ± 0.52^{c}
Chewiness, mJ	32.74 ± 1.28^{b}	37.72 ± 0.56^{a}	33.58 ± 0.79^{ab}	32.45 ± 0.80^{b}	30.40 ± 0.96^{b}

HPM = hydrolyzed porcine mucosa.
[a, b, c] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] Diets HPM0, HPM3, HPM6, HPM9, and HPM 12 contains 0%, 3%, 6%, 9%, and 12% HPM content, respectively. Mean values ± SEM are presented for each group ($n = 3$).

Table 7
Amino acid composition in muscle of hybrid groupers feed the test diets for 56 days (%, dry matter) [1].

Index	HPM0	HPM3	HPM6	HPM9	HPM12
Ile [2]	3.66 ± 0.07^{b}	3.95 ± 0.13^{ab}	3.98 ± 0.05^{ab}	3.90 ± 0.03^{ab}	4.10 ± 0.08^{a}
Leu [2]	6.55 ± 0.14	6.80 ± 0.16	6.80 ± 0.06	6.71 ± 0.02	7.05 ± 0.16
Lys [2]	7.76 ± 0.10^{b}	8.17 ± 0.21^{ab}	8.20 ± 0.09^{ab}	8.09 ± 0.05^{ab}	8.55 ± 0.21^{a}
Met [2]	2.50 ± 0.05	2.61 ± 0.08	2.61 ± 0.03	2.59 ± 0.01	2.75 ± 0.07
Thr [2]	3.75 ± 0.07^{b}	3.86 ± 0.06^{ab}	3.84 ± 0.04^{ab}	3.82 ± 0.03^{b}	4.07 ± 0.05^{a}
Val [2]	3.82 ± 0.06^{b}	4.13 ± 0.13^{ab}	4.11 ± 0.06^{ab}	4.05 ± 0.03^{ab}	4.25 ± 0.07^{a}
His [2]	1.82 ± 0.04^{b}	1.90 ± 0.03^{ab}	1.91 ± 0.01^{ab}	1.87 ± 0.01^{ab}	1.98 ± 0.04^{a}
Phe [2, 3]	3.47 ± 0.05^{b}	3.64 ± 0.08^{ab}	3.56 ± 0.08^{ab}	3.59 ± 0.02^{ab}	3.78 ± 0.07^{a}
Tyr [3]	2.84 ± 0.04	2.87 ± 0.06	2.85 ± 0.02	2.80 ± 0.01	2.96 ± 0.08
Arg [2, 4]	5.03 ± 0.09	5.26 ± 0.13	5.26 ± 0.08	5.23 ± 0.04	5.45 ± 0.11
Asp [4]	8.68 ± 0.16^{b}	9.02 ± 0.20^{ab}	9.04 ± 0.10^{ab}	8.94 ± 0.04^{ab}	9.43 ± 0.18^{a}
Glu [4]	12.71 ± 0.24^{b}	13.36 ± 0.38^{ab}	13.39 ± 0.13^{ab}	13.28 ± 0.06^{ab}	13.90 ± 0.28^{a}
Gly [4]	4.82 ± 0.14	5.10 ± 0.16	5.29 ± 0.09	5.18 ± 0.13	4.93 ± 0.07
Ala [4]	5.07 ± 0.06^{b}	5.33 ± 0.12^{ab}	5.35 ± 0.08^{ab}	5.28 ± 0.07^{ab}	5.50 ± 0.11^{a}
Ser [4]	3.29 ± 0.08	3.32 ± 0.05	3.25 ± 0.02	3.27 ± 0.01	3.41 ± 0.07
Pro [4]	3.57 ± 0.03	3.51 ± 0.05	3.59 ± 0.07	3.54 ± 0.07	3.74 ± 0.12
TAA	79.33 ± 1.37^{b}	82.83 ± 1.96^{ab}	83.05 ± 0.95^{ab}	82.14 ± 0.50^{ab}	85.88 ± 1.46^{a}
TEAA	38.36 ± 0.67^{b}	40.32 ± 1.01^{ab}	40.28 ± 0.46^{ab}	39.86 ± 0.20^{ab}	41.99 ± 0.85^{a}
TDAA	31.28 ± 0.58	32.81 ± 0.81	33.07 ± 0.39	32.68 ± 0.30	33.77 ± 0.55
TAAA	6.31 ± 0.09	6.51 ± 0.14	6.41 ± 0.10	6.39 ± 0.02	6.75 ± 0.15

HPM = hydrolyzed porcine mucosa; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Thr = threonine; Val = valine; His = histidine; Phe = phenylalanine; Tyr = tyrosine; Arg = arginine; Asp = aspartic acid; Glu = glutamate; Gly = glycine; Ala = alanine; Ser = serine; Pro = proline; TAA = total amino acids; TEAA = total essential amino acids; TDAA = total delicious amino acids; TAAA = total aromatic amino acids.
[a, b] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] Diets HPM0, HPM3, HPM6, HPM9, and HPM 12 contains 0%, 3%, 6%, 9%, and 12% HPM content, respectively. Mean values ± SEM are presented for each group ($n = 3$).
[2] Essential amino acid.
[3] Aromatic amino acid.
[4] Delicious amino acid.

in the feed increased, the contents of C18:1n-9 and $\sum$ MUFA exhibited an upward trend, and reached a maximum in the HPM12 group ($P < 0.05$, Table 8).

3.6. Relative mRNA expression of muscle growth-related genes

With the increases in HPM content in the feed, the expressions of *Myf5*, *MEF-2c*, *MEF-2a*, *MyHC*, and *FST* first exhibited an increasing trend followed by a decreasing trend, reaching a maximum in the HPM3 group ($P < 0.05$). A similar trend was also observed in *MyoD*, *MyoG*, *Col1α-1*, and *TGF-β1*; the maximum expression of *MyoD* was presented in the HPM6 group, and the expressions of the other three reached their maximum values in the HPM3 and HPM6 groups ($P < 0.05$). The expression of *MSTN* in the HPM0 group was significantly higher than that in other groups ($P < 0.05$). The expression of *IGF-2* in the HPM0 group was significantly higher than that in the HPM6, HPM9, and HPM12 groups ($P < 0.05$). With increases in the proportion of HPM in the feed, the expressions of *IGF-1* and *Col1α-2* also exhibited a trend of first increasing and then decreasing, but they were not significantly affected by the feed ($P > 0.05$, Fig. 2A, B, C and D).

Table 8
Fatty acid composition in muscle of hybrid groupers feed the test diets for 56 days (%, dry matter) [1].

Index	HPM0	HPM3	HPM6	HPM9	HPM12
C14:0	4.44 ± 0.10	4.41 ± 0.09	4.22 ± 0.17	4.86 ± 0.10	4.88 ± 0.44
C15:0	0.40 ± 0.01^{a}	0.46 ± 0.01^{ab}	0.46 ± 0.01^{ab}	0.47 ± 0.01^{ab}	0.45 ± 0.01^{b}
C16:0	20.87 ± 0.12^{b}	22.07 ± 0.27^{ab}	22.64 ± 0.48^{a}	21.92 ± 0.24^{ab}	22.26 ± 0.47^{a}
C17:0	1.08 ± 0.01^{a}	1.00 ± 0.01^{ab}	0.97 ± 0.04^{b}	0.94 ± 0.01^{bc}	0.84 ± 0.04^{c}
C18:0	7.67 ± 0.14	7.94 ± 0.44	7.97 ± 0.42	8.27 ± 0.29	8.64 ± 0.21
C20:0	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.01
C22:0	0.26 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.27 ± 0.01	0.25 ± 0.01
C24:0	0.17 ± 0.00	0.17 ± 0.02	0.17 ± 0.02	0.19 ± 0.01	0.17 ± 0.01
∑SFA	45.05 ± 0.09^{b}	46.44 ± 0.46^{ab}	46.89 ± 0.42^{a}	46.14 ± 0.28^{ab}	46.72 ± 0.42^{a}
C16:1n-7	5.27 ± 0.06	5.00 ± 0.11	5.00 ± 0.16	4.64 ± 0.12	4.71 ± 0.25
C17:1n-7	0.75 ± 0.04	0.45 ± 0.10	0.49 ± 0.14	0.58 ± 0.14	0.69 ± 0.24
C18:1n-9	20.20 ± 0.08^{d}	21.14 ± 0.19^{c}	21.71 ± 0.07^{bc}	21.98 ± 0.21^{ab}	22.70 ± 0.20^{a}
C20:1n-9	0.75 ± 0.01	0.84 ± 0.04	0.88 ± 0.09	0.77 ± 0.04	0.78 ± 0.05
C22:1n-9	0.00 ± 0.00	0.08 ± 0.04	0.08 ± 0.04	0.06 ± 0.02	0.07 ± 0.02
C24:1n-9	0.42 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.40 ± 0.01	0.28 ± 0.04
∑MUFA	27.29 ± 0.05^{c}	27.78 ± 0.25^{bc}	28.45 ± 0.12^{b}	28.40 ± 0.21^{b}	29.20 ± 0.21^{a}
C18:2n-6	21.99 ± 0.05	22.16 ± 0.40	21.89 ± 0.10	22.76 ± 0.41	22.10 ± 0.40
C20:2n-6	0.68 ± 0.04	0.65 ± 0.04	0.65 ± 0.02	0.66 ± 0.05	0.61 ± 0.01
C18:4n-6	0.16 ± 0.04	0.12 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.11 ± 0.01
C20:4n-6	0.20 ± 0.01	0.22 ± 0.01	0.22 ± 0.02	0.24 ± 0.04	0.21 ± 0.02
C20:4n-6	0.70 ± 0.02	0.64 ± 0.04	0.67 ± 0.05	0.86 ± 0.08	0.85 ± 0.12
∑n-6 PUHA	24.74 ± 0.02^{ab}	24.78 ± 0.29^{ab}	24.54 ± 0.09^{b}	24.65 ± 0.21^{a}	24.88 ± 0.44^{ab}
C18:4n-4	2.11 ± 0.020^{a}	2.05 ± 0.04^{a}	1.98 ± 0.06^{a}	1.94 ± 0.06^{a}	1.71 ± 0.05^{b}
C20:4n-4	0.09 ± 0.04	0.12 ± 0.02	0.09 ± 0.04	0.06 ± 0.01	0.06 ± 0.02
C20:5n-4 (EPA)	4.00 ± 0.02^{a}	4.62 ± 0.05^{ab}	4.42 ± 0.10^{bc}	4.14 ± 0.06^{c}	2.50 ± 0.14^{d}
C22:6n-4 (DHA)	6.41 ± 0.12^{a}	5.11 ± 0.17^{ab}	4.82 ± 0.21^{b}	4.62 ± 0.21^{b}	4.91 ± 0.51^{b}
∑n-4 PUFA	12.51 ± 0.08^{a}	10.90 ± 0.21^{b}	10.41 ± 0.25^{b}	9.72 ± 0.12^{b}	8.18 ± 0.59^{c}
∑n-4 HUFA	10.41 ± 0.14^{a}	8.74 ± 0.22^{b}	8.25 ± 0.27^{b}	7.74 ± 0.18^{bc}	6.41 ± 0.64^{c}
n-4/n-6 ratio	0.52 ± 0.01^{a}	0.46 ± 0.01^{b}	0.44 ± 0.01^{bc}	0.49 ± 0.01^{cd}	0.44 ± 0.02^{d}
EPA/DHA ratio	0.64 ± 0.01	0.71 ± 0.01	0.71 ± 0.04	0.68 ± 0.04	0.65 ± 0.05

HPM = hydrolyzed porcine mucosa; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acid; HUFA = highly unsaturated fatty acid.
[a, b] Values with different superscripts in the same row are significantly different ($P < 0.05$).
[1] Diets HPM0, HPM3, HPM6, HPM9, and HPM 12 contains 0%, 3%, 6%, 9%, and 12% HPM content, respectively. Mean values ± SEM are presented for each group ($n = 4$).

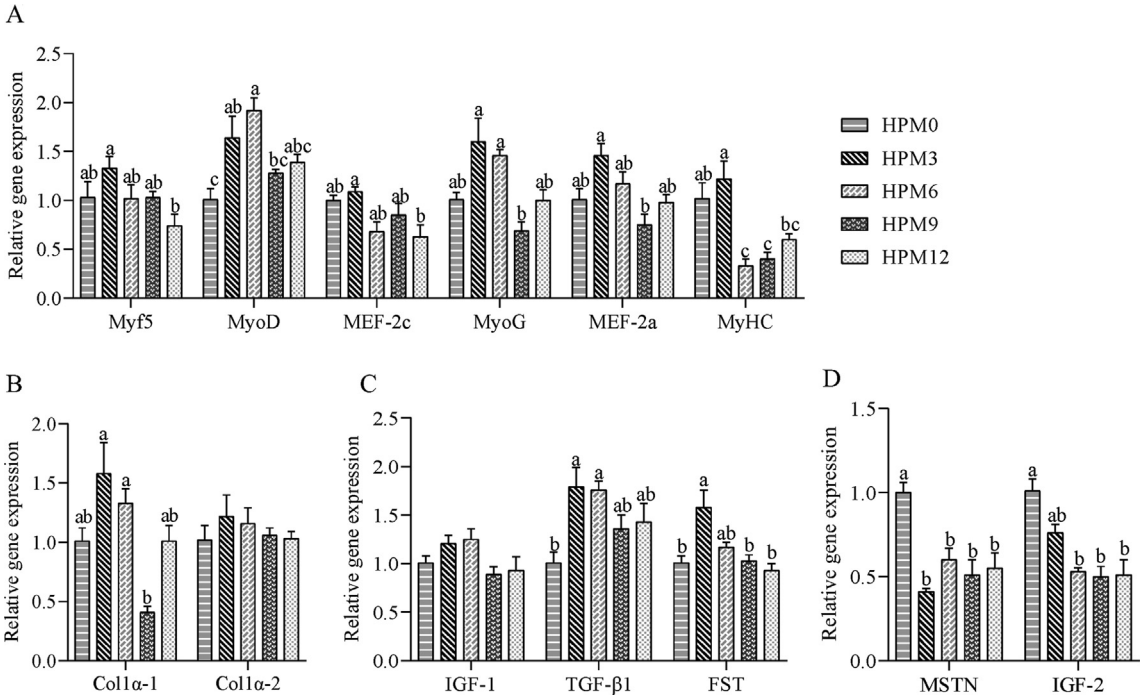

Fig. 2. The genes expressions of myogenic regulatory factors (A), encoding collagen (B), positive regulators of muscle growth (C) and negative regulators of muscle growth (D) in hybrid groupers feed the test diets for 56 days. Hydrolyzed porcine mucosa (HPM) content in feed was 0%,3%, 6%, 9%, and 12% in HPM0, HPM3, HPM6, HPM9, HPM12, respectively. [a, b, c] Different superscripts mean that the difference is significant ($P < 0.05$). *Myf5* = myogenic factor 5; *MyoD* = myogenic differentiation; *MEF* = myocyte enhancer factor; *MyoG* = myogenin; *MyHC* = myosin heavy chain; *Col1* = collagen type I; *IGF* = insulin-like growth factor; *TG* = transforming growth factor; *FST* = follistatin; *MSTN* = myostatin.

4. Discussion

Fish growth performance could be significantly improved by adding appropriate amounts of protein sources containing small peptides, such as cottonseed meal hydrolysate (Xia et al., 2012), hydrolyzed krill meal (Wei et al., 2016), enzymatic hydrolyzed soybean meal (Zhang et al., 2019), and fish protein hydrolysate (Zhou et al., 2019). In this study, WG in the HPM3 and HPM6 groups was not significantly different from the control group. Similar conditions have been found in peptides hydrolyzed from poultry by-products (Jia et al., 2019), anchovy and giant squid hydrolysates (Costa et al., 2020), and size-fractionated fish hydrolysates (Cai et al., 2015). Research in animals has shown that the absorption of small peptides was carried out by a special peptide transport system, which was not easy to saturate, had low energy consumption, fast absorption speed, and was not competitive or inhibitory (Stelzl et al., 2016). In addition, small peptides entered the blood circulation in the form of dipeptides and tripeptides, and were directly absorbed in the skin, kidneys, liver, intestine, and other tissues, which reduced the energy consumption for breaking down protein, allowing more energy to be used for animal growth (Cai et al., 2015; Ki et al., 2015). However, the WG of hybrid grouper was significantly reduced when the dietary HPM content was over 9%. When more hydrolytic or enzymolysis protein sources were used in the feed, fish growth performance was significantly reduced in yellow catfish (*Pelteobagrus fulvidraco*; Zhou et al., 2019), cobia (*Rachycentron canadum*; Han et al., 2010), turbot (*Scophthalmus maximus*; Jiang et al., 2019), and gilthead seabream (*Sparus aurata*; Kolkovski and Tandler, 2000). Owing to the increased number of free amino acids in the enzymatic or hydrolytic process, intestinal amino acid transport system saturation and transport mechanism competition could occur (Plakas and Katayama, 1981), restricting amino acid absorption (Aragão et al., 2004).

The level of delicious amino acids in the fish muscle is an important factor affecting its flavour (Buchtová et al., 2009). Glu and Asp have an umami taste, Gly and Ala have a sweet taste (Cheng et al., 2020). In addition, Ser and Pro are also related to a sweet taste (Park et al., 2002). Studies have shown that the most abundant amino acid in the dorsal muscles of hybrid grouper is Glu, followed by Asp, Lys, and Leu (Yu et al., 2014b; Wang et al., 2015; Fan et al., 2018). These results were confirmed in this experiment. As the content of HPM in the feed increased, the contents of total essential amino acids, aromatic amino acids (Phe), and delicious amino acids (Asp, Glu and Ala) in muscles exhibited an upward trend. This could be related to the composition of HPM amino acids. The HPM contains higher levels of Phe (3.22%), Asp (13.16%), Glu (9.32%), and Ala (3.22%), and the first three amino acids are higher than those in fish meal. Further, more amino acids were absorbed and deposited in the fish muscle (Deng et al., 2016). In addition, Asp, Glu, and Ala are non-essential amino acids that can be synthesized by the fish themselves. It is also possible that the high levels of Asp, Glu, and Ala in the feed promoted their synthesis in the fish. As a result, muscle nutrition and quality were improved.

The water holding capacity, one of the important characteristics to measure muscle quality, reflects the loss of liquid and soluble substances in muscle (Brinkera and Reiteret, 2011). A loss of water will cause losses in water-soluble proteins, soluble flavour substances, and heme in muscle, decreasing muscle quality (Savage et al., 1990; Luciano et al., 2009). In this experiment, in comparison to the control group, there was no significant difference in cooking loss and drip loss of the dorsal muscle when 3% HPM was added to the diet, but a reduced trend in numerical value was found. When low fish meal feed contained 3% HPM, the water holding capacity of the hybrid grouper dorsal muscles was improved.

The hardness, cohesiveness, springiness, gumminess, and chewiness of muscles are their main textural properties (Chen et al., 2019). In this experiment, the hardness, gumminess, and chewiness of the muscles reached their maximum values when 3% HPM was added. In comparison to the control group, the EE content of the muscle was significantly reduced and its chewiness was significantly increased. The lower EE content in muscles caused greater friction between muscle bundles, and increased muscle shear force, which meant that muscles had higher chewiness (Grigorakis and Alexis, 2005).

With increases in HPM content in the feed, the $\sum$ MUFA and $\sum$ n-6 PUHA in muscle increased, while the $\sum$ n-3 PUFA and $\sum$ n-3 HUFA exhibited a significant decreasing trend. The n-3 PUHA and n-6 PUHA are not only key components in hormone synthesis in vivo, but also play an important role in the regulation of inflammatory responses (Harbige, 2003). Higher levels of n-3 PUFA could volatilize its anti-inflammatory effect (McMurray et al., 2000), and high levels of n-6 PUFA can cause platelet aggregation, vasoconstriction, and inflammatory reactions (Simopoulos and DiNicolantonio, 2016; Huang et al., 2020). The human body cannot synthesize n-3 PUFA and n-6 PUFA by itself, so they must be obtained from food (Xiong et al., 2002). The recommended value for n-3/n-6 PUFA in human food is 0.10 to 0.25 (Zhang et al., 2006). The ratio of n-3/n-6 PUFA in food is one of the most important factors influencing human obesity (Ailhaud et al., 2006). The n-3/n-6 PUHA ratio in food is closely related to human disease, and research has shown that the lower the n-3/n-6 PUHA ratio in food, the higher the risk of humans suffering from hyperlipidemia, obesity, and breast cancer (Jeromson et al., 2015). In this experiment, the n-3/n-6 PUHA ratio of hybrid grouper muscle was 0.34 to 0.52. As the HPM content in the diet increased, the n-3/n-6 PUHA ratio became progressively smaller, approaching the optimal n-3/n-6 ratio in food. The addition of HPM to the feed changed the fatty acid composition of hybrid grouper muscle. Whether these changes will affect human health remains to be further studied.

Myogenic regulatory factors (MRFs) and their related regulatory genes, as the internal factors that affect flesh quality (Cheng et al., 2014), have important regulatory effects on the differentiation of myogenic cells, the development of muscle fibres, and the formation and growth of muscle tissue (Lin et al., 2015; Zheng et al., 2015). The MRFs were expressed in a time sequence (Johnston et al., 2007). During somatic cell formation, the first expression of the MRF family factor is *Myf5*, followed by *MyoD* and *MEF-2c*, then *MyoG* and *MEF-2a*, and finally *MyHC* (Watabe, 1999). Among them, *Myf5* and *MyoD* primarily act as myogenic determinants, and *MyoG* plays an important role in myocyte differentiation (Rudnicki and Jaenish, 1995; Kassar-Duchossoy et al., 2004). In this experiment, the expression levels of *Myf5*, *MyoD*, *MEF-2c*, *MyoG*, *MEF-2a*, and *MyHC* increased when 3% HPM was added to the low fish meal diet. This indicates that 3% HPM can improve the muscle formation and differentiation of hybrid groupers. *IGF-1* is an important growth factor, with many receptors in fish muscle (Castillo et al., 2006), and can promote cell activation, proliferation, and differentiation; moreover, it is a positive regulator of MRFs. In contrast, *IGF-2* is a negative regulator of MRFs (Snijders et al., 2015). In this experiment, although *IGF-1* expression was not significantly affected by feed, it exhibited a trend of first increasing and then decreasing with increases in HPM content. The changing trend of *IGF-1* was consistent with those of *Myf5*, *MyoD*, *MEF-2c*, *MyoG*, *MEF-2a*, and *MyHC*. The collagen content in fish muscle is rich, and the main type of collagen is type I (Yata et al., 2001). Type I collagen is encoded by two α-1 chains (*Col1α-1*) and one α-2 chain (*Col1α-2*; Gelsea et al., 2003). Collagen synthesis and cross-linking are primarily regulated by *TGF-β1*, which mediates the expression of collagen type I (Chen et al., 2005). In this experiment, the expression of *TGF-β1* in the

HPM3 group was significantly increased in comparison to that in other groups. The expressions of *Col1α-1* and *Col1α-2* also exhibited a similar trend, although the change in *Col1α-2* was not significant. *Col1α-1* and *Col1α-2* have high hydrophilicity and strong binding ability with other substances, which can enhance the water holding capacity of meat (Yu et al., 2014a). This is an important reason why the cooking loss and drip loss of the HPM3 group were lower than those of the control group. *MSTN* encodes myostatin protein and is a negative regulator of muscle growth (Zheng et al., 2015). *FST* can inhibit the negative regulation of *MSTN* on muscle growth, restoring muscle growth and development (Rebhan and Funkenstein; 2008). The supplementation of 3% HPM in a low fish meal diet significantly increased *FST* expression, and a significant decrease in *MSTN* expression was observed in all HPM-supplemented groups. These results indicate that 3% HPM supplementation in a low fish meal diet was beneficial to the differentiation, growth, and quality of muscle.

5. Conclusion

The results indicated that the growth performance of hybrid grouper fed a diet with 6% HPM and 27% fish meal was as good as that of the control group. When fish ingested a diet containing 3% HPM, the expression of genes *TGF-β1* and *FST* involved in muscle growth were upregulated, and then the muscle quality related to hardness and chewiness were improved. An appropriate amount of HPM could be better used in grouper feed.

Author contributions

Xuanyi Yang: Conceptualization, Methodology, Investigation, Formal analysis, Writing—Original Draft; **Xinyan Zhi**: Investigation, Formal analysis; **Ziling Song**: Investigation, Formal analysis; **Guanghui Wang**: Methodology, Resources; **Xumin Zhao**: Methodology, Resources; **Shuyan Chi**: Conceptualization, Methodology, Resources, Writing—Review & Editing, Supervision, Project administration, Funding acquisition; **Beiping Tan**: Methodology, Resources, Funding acquisition. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog Lipid Res 2006;45(3):203–36. https://doi.org/10.1016/j.plipres.2006.01.003.

Álvarez CA, Jerez-Cepa I, Cárcamo CB, Toledo P, Flores H, Brokordt K. Growth performance, physiological responses to hypoxia and flesh quality of Chilean croaker (*Cilus gilberti*) stocked at different densities. Aquaculture 2020;525:735316. https://doi.org/10.1016/j.aquaculture.2020.735316.

Aragão C, Conceição LEC, Martins D, Rønnestad I, Gomes E, Dinis MT. A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (*Solea senegalensis*). Aquaculture 2004;233(1–4):293–304. https://doi.org/10.1016/j.aquaculture.2003.08.007.

Association of Official Analytical Chemists (Aoac). In: Cuniff P, editor. Official methods of analysis of AOAC international. 16th ed., vol. 1. Washington: Association of Office Analytical Chemists International; 1999. 5th revision.

Bertram HC, Andersen HJ, Karlsson AH, Horn P, Hedegaard J, Nørgaard L, et al. Prediction of technological quality (cooking loss and *Napole Yield*) of pork based on fresh meat characteristics. Meat Sci 2003;65(2):707–12. https://doi.org/10.1016/S0309-1740(02)00272-3.

Bidner BS, Ellis M, Witte DP, Carr SN, McKeith FK. Influence of dietary lysine level, pre-slaughter fasting, and rendement napole genotype on fresh pork quality. Meat Sci 2004;68(1):53–60. https://doi.org/10.1016/j.meatsci.2003.10.018.

Birolo M, Bordignon F, Trocino A, Fasolato L, Pascual A, Godoy S, et al. Effects of stocking density on the growth and flesh quality of rainbow trout (*Oncorhynchus mykiss*) reared in a low-tech aquaponic system. Aquaculture 2020;529:735653. https://doi.org/10.1016/j.aquaculture.2020.735653.

Brinkera A, Reiter R. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: effects on feed utilization and fish quality. Aquaculture 2011;310(3–4):350–60. https://doi.org/10.1016/j.aquaculture.2010.09.041.

Buchtová H, Svobodová Z, Kocour M, Velisek J. Amino acid composition in fillets of mirror crossbreds common carp (*Cyprinus carpio*, Linnaeus 1758). Acta Vet 2009;78(2):337–44. https://doi.org/10.2754/avb200978020337.

Cai WC, Jiang GZ, Li XF, Sun CX, Mi HF, Liu SQ, et al. Effects of complete fish meal replacement by rice protein concentrate with or without lysine supplement on growth performance, muscle development and flesh quality of blunt snout bream (*Megalobrama amblycephala*). Aquacult Nutr 2018;24(1):481–91. https://doi.org/10.1111/anu.12581.

Cai ZN, Li WJ, Mai KS, Xu W, Zhang YJ, Ai QH. Effects of dietary size-fractionated fish hydrolysates on growth, activities of digestive enzymes and aminotransferases and expression of some protein metabolism related genes in large yellow croaker (*Larimichthys crocea*) larvae. Aquaculture 2015;440:40–7. https://doi.org/10.1016/j.aquaculture.2015.01.026.

Castillo J, Ammendrup-Johnsen I, Codina M, Navarro I, Gutiérrez J. IGF-I and insulin receptor signal transduction in trout muscle cells. Am J Physiol Regul Integr Comp Physiol 2006;290(6):1683–90. https://doi.org/10.1152/ajpregu.00294.2005.

Chen WJ, Wang Y, Han D, Zhu XM, Xie SQ, Han DX, et al. Two filamentous microalgae as feed ingredients improved flesh quality and enhanced antioxidant capacity and immunity of the gibel carp (*Carassius auratus gibelio*). Aquacult Nutr 2019;25(5):1145–55. https://doi.org/10.1111/anu.12930.

Chen YW, Nagaraju K, Bakay M, McIntyre O, Rawat R, Shi R, et al. Early onset of inflammation and later involvement of TGFβ in Duchenne muscular dystrophy. Neurology 2005;65(6):826–34. https://doi.org/10.1212/01.wnl.0000173836.09176.c4.

Cheng JH, Sun DW, Han Z, Zeng XA. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: a review. Compr Rev Food Sci Food Saf 2014;13(1):52–61. https://doi.org/10.1111/1541-4337.12043.

Cheng YM, Zhao JL, Song LY, Wu HY, Zhou HT. Effects of salinity and alkalinity on growth performance and muscle quality of Nile Tilapia *Oreochromis niloticus*. Fish Sci 2020;39(3):341–9. https://doi.org/10.16378/j.cnki.1003-1111.2020.03.006.

Cho JH, Lindemann MD, Monegue HJ, Cromwell GL. Feeding value of dried porcine soluble for weanling pigs. Prof Anim Sci 2010;26(4):425–34. https://doi.org/10.15232/S1080-7446(15)30624-0.

Costa M, Costas B, Machado M, Teixeira C, Fernández-Boo S, Sá T, et al. Anchovy and giant squid hydrolysates can enhance growth and the immune response of European seabass (*Dicentrarchus labrax*) fed plant-protein-based diets. Aquaculture 2020;523:735182. https://doi.org/10.1016/j.aquaculture.2020.735182.

Deng YP, Jiang WD, Liu Y, Qu B, Jiang J, Kuang SY, et al. Dietary leucine improves flesh quality and alters mRNA expressions of Nrf2-mediated antioxidant enzymes in the muscle of grass carp (*Ctenopharyngodon idella*). Aquaculture 2016;452:380–7. https://doi.org/10.1016/j.aquaculture.2015.11.007.

Ezaki J, Matsumoto N, Takeda-Ezaki M, Komatsu M, Takahashi K, Hiraoka Y, et al. Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. Autophagy 2011;7:727–36. https://doi.org/10.4161/auto.7.7.15371.

Fan XP, Qin XM, Zhang CH, Chen JP, Zhu QF. Nutritional and volatile flavor components of dorsal and ventral muscle from hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). J Guangdong Ocean Univ 2018;38(1):39–46. https://doi.org/10.3969/j.issn.1673-9159.2018.01.006.

FAO (Food and Agriculture Organization of the United Nations). The state of world fisheries and aquaculture 2020. Rome: Sustainability in action; 2020. https://doi.org/10.4060/ca9229en.

Faudzi NM, Yong ASK, Shapawi R, Senoo S, Biswas A, Takii K. Soy protein concentrate as an alternative in replacement of fish meal in the feeds of hybrid grouper, brown-marbled grouper (*Epinephelus fuscoguttatus*) × giant grouper (*E. lanceolatus*) juvenile. Aquacult Res 2018;49(4):1–11. https://doi.org/10.1111/are.13474.

Figueroa J, Solà-Oriol D, Guzmán-Pino SA, Chetrit C, Borda E, Pérez JF. The use of porcine digestible peptides and their continuity effect in nursery pigs. J Anim Sci 2016;94(4):1531–40. https://doi.org/10.2527/jas.2015-0019.

Fisheries and Fishery Administration Bureau of The Ministry of Agriculture and Rural Affairs, National Fishery Technology Promotion Station, China Society of

Fisheries. China Fishery statistics yearbook 2019. Beijing: China Agriculture Press; 2019.
Fisheries and Fishery Administration Bureau of The Ministry of Agriculture and Rural Affairs, National Fishery Technology Promotion Station, China Society of Fisheries. China Fishery statistics yearbook 2018. Beijing: China Agriculture Press; 2018.
Gelsea K, Pöschlb E, Aignera T. Collagens-structure, function, and biosynthesis. Adv Drug Deliv Rev 2003;55(12):1531–46. https://doi.org/10.1016/j.addr.2003.08.002.
General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of China. Oligopeptides powder of marine fish, GB/T 22729-2008. Beijing, China: National Standards of the people's Republic of China; 2008.
Grigorakis K, Alexis MN. Effects of fasting on the meat quality and fat deposition of commercial-size farmed gilthead sea bream (*Sparus aurata*, L.) fed different dietary regimes. Aquacult Nutr 2005;11(5):341–4. https://doi.org/10.1111/j.1365-2095.2005.00351.x.
Hai WY, Wan DJ, Wu ZQ, Yang T, Xiao SY. Research on high efficiency price heparin sodium with enzymatic combined membrane preparation technology. Sci Technol Eng 2013;13(11):3069–72. https://doi.org/10.3969/j.issn.1671-1815.2013.11.030.
Han T, Wang JT, Wang Y, Tian LX, Liu YJ. Effect of different fish protein hydrolysate (FPH) level of dietary supplements on growth and body composition of larvae of cobia (*Rachycentron canadum*). Acta Hydrobiol Sin 2010;34(1):94–100. https://doi.org/10.3724/SP.J.1035.2010.00094.
Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. Lipids 2003;38(4):323–41. https://doi.org/10.1007/s11745-003-1067-z.
Huang WQ, Ruan SJ, Zhou FF, Ni JC, Shi L, Hu YB, et al. Effect of *Pseudostellaria heterohpylla* extracts on nutrient quality in muscle of large yellow croaker. Feed Res 2020;43(9):50–5. https://doi.org/10.13557/j.cnki.issn1002-2813.2020.09.014.
Imsland AKD, Roth B, Christensen LB, Siikavuopio SI, Foss A. Effects of short-term starvation periods on flesh quality in Arctic charr (*Salvelinus alpinus*) in different seasons. Aquacult Res 2020;51(10):4022–9. https://doi.org/10.1111/are.14745.
Jeromson S, Gallagher IJ, Galloway SDR, Hamilton DL. Omega-3 fatty acids and skeletal muscle health. Mar Drugs 2015;13(11):6977–7004. https://doi.org/10.3390/md13116977.
Jia GW, Guo R, Zhang Y, Li XH, Xia H, Gong CG, et al. Effects of partial replacement of fish meal by peptides hydrolyzed from poultry by-products on growth performance, digestive indices and specific immune indices of turbot. Chinese Journal of Animal Nutrition 2019;31(5):2201–11. https://doi.org/10.3969/j.issn.1006-267x.2019.05.027.
Jiang LS, Chen W, Li BS, Sun YZ, Wang JY, Huang BS, et al. Effects of replacing fishmeal by protein hydrolysates on the growth performance and physiological metabolism of *Scophthalmus maximus*. Marine Fisheries 2019;41(5):596–605. https://doi.org/10.13233/j.cnki.mar.fish.2019.05.007.
Johnston IA, Bickerdike R, Li XJ, Dingwall A, Nickell D, Alderson R, et al. Fast growth was not associated with an increased incidence of soft flesh and gaping in two strains of Atlantic salmon (*Salmo salar*) grown under different environmental conditions. Aquaculture 2007;264(1–4):148–55. https://doi.org/10.1016/j.aquaculture.2007.01.045.
Johnston IA, Li XJ, Vieira VLA, Nickell D, Dingwall A, Alderson R, et al. Muscle and flesh quality traits in wild and farmed Atlantic salmon. Aquaculture 2006;256(1–4):323–36. https://doi.org/10.1016/j.aquaculture.2006.02.048.
Johnston LJ, Pettigrew JE, Baidoo SK, Shurson GC, Walker RD. Efficacy of sucrose and milk chocolate product or dried porcine solubles to increase feed intake and improve performance of lactating sows. J Anim Sci 2003;81(10):2475–81. https://doi.org/10.2527/2003.81102475x.
Kassar-Duchossoy L, Gayraud-Morel B, Gomès D, Rocancourt D, Buckingham M, Shinin V, et al. Mrf4 determines skeletal muscle identity in Myf5: myod double-mutant mice. Nature 2004;431(7007):466–71. https://doi.org/10.1038/nature02876.
Ki MR, Lee JH, Yun SK, Choi KM, Hwang SY. Roles of the peptide transport systems and aminopeptidase PepA in peptide assimilation by helicobacter pylori. J Microbiol Biotechnol 2015;25(10):1629–33. https://doi.org/10.4014/jmb.1505.05099.
Kim KM, Lee HJ, Yun HB, Cho JH, Kim SR, Lee KM, et al. Changes of hematological parameters and plasma components in the hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) by acute ammonia exposure. Environmental Biology Research 2020;38(1):40–6. https://doi.org/10.11626/KJEB.2020.38.1.040.
Kolkovski S, Tandler A. The use of squid protein hydrolysate as a protein source in microdiets for gilthead seabream *Sparus aurata* larvae. Aquacult Nutr 2000;6(1):11–5. https://doi.org/10.1046/j.1365-2095.2000.00125.x.
Kortner Valen EC, Kortner H, Marjara IS, Krogdahl Å, Bakke AM. Candidate reference genes for quantitative real-time PCR (qPCR) assays during development of a diet-related enteropathy in Atlantic salmon (*Salmo salar L.*) and the potential pitfalls of uncritical use of normalization software tools. Aquaculture 2011;318:355–63. https://doi.org/10.1016/j.aquaculture.2011.05.038.
Li MM, Zhang M, Ma YC, Ye RK, Wang M, Chen HY, et al. Dietary supplementation with n-3 high unsaturated fatty acids decreases serum lipid levels and improves flesh quality in the marine teleost golden pompano *Trachinotus ovatus*. Aquaculture 2020a;516:734632. https://doi.org/10.1016/j.aquaculture.2019.734632.
Li XX, Chen SJ, Sun JJ, Huang XD, Tang HJ, He YH, et al. Partial substitution of soybean meal with faba bean meal in grass carp (*Ctenopharyngodon idella*) diets, and the effects on muscle fatty acid composition, flesh quality, and expression of myogenic regulatory factors. J World Aquacult Soc 2020b;51(5):1145–60. https://doi.org/10.1111/jwas.12671.
Liang XF, Hu L, Dong YC, Wu XF, Qin YC, Zheng YH, et al. Substitution of fish meal by fermented soybean meal affects the growth performance and flesh quality of Japanese seabass (*Lateolabrax japonicus*). Anim Feed Sci Technol 2017;229:1–12. https://doi.org/10.1016/j.anifeedsci.2017.03.006.
Lin YQ, Zhou JS, Li RW, Zhao YY, Zheng YC. Cloning and expression patterns of MRFs and effect of replacing dietary fish oil with vegetable oils on MRFs expression in grass carp (*Ctenopharyngodon idellus*). Turk J Fish Aquat Sci 2015;15(2):257–66. https://doi.org/10.4194/1303-2712-v15_2_07.
Liu XW, Feng L, Jiang WD, Wu P, Jiang J, Yang DM, et al. (2-Carboxyethyl) dimethylsulfonium Bromide (Br-DMPT) improves muscle flesh quality and antioxidant status of on-growing grass carp (*Ctenopharyngodon idella*) fed non-fish meal diets. Aquaculture 2020;521:735065. https://doi.org/10.1016/j.aquaculture.2020.735065.
López-Albors O, Abdel I, Periago MJ, Ayala MD, Alcázar AG, Graciá CM, et al. Temperature influence on the white muscle growth dynamics of the sea bass *Dicentrarchus labrax*, L. Flesh quality implications at commercial size. Aquaculture 2008;277(1–2):39–51. https://doi.org/10.1016/j.aquaculture.2008.02.015.
Luciano G, Monahan FJ, Vasta V, Biondi L, Lanza M, Priolo A. Dietary tannins improve lamb meat colour stability. Meat Sci 2009;81(1):120–5. https://doi.org/10.1016/j.meatsci.2008.07.006.
Lv H, Hu WH, Xiong SB, You J, Fan QX. Depuration and starvation improves flesh quality of grass carp (*Ctenopharyngodon idella*). Aquacult Res 2018;49(9):3196–206. https://doi.org/10.1111/are.13784.
Mateos GG, Mohiti-Asli M, Borda E, Mirzaie S, Frikha M. Effect of inclusion of porcine mucosa hydrolysate in diets varying in lysine content on growth performance and ileal histomorphology of broilers. Anim Feed Sci Technol 2014;187:53–60. https://doi.org/10.1016/j.anifeedsci.2013.09.013.
McMurray DN, Jolly CA, Chapkin RS. Effects of dietary n-3 fatty acids on T cell activation and T cell receptor-mediated signaling in a murine model. JID (J Infect Dis) 2000;182:103–7. https://doi.org/10.1086/315909.
Mu H, Shen HH, Liu JH, Xie FL, Zhang WB, Mai KS. High level of dietary soybean oil depresses the growth and anti-oxidative capacity and induces inflammatory response in large yellow croaker *Larimichthys crocea*. Fish Shellfish Immunol 2018;77:465–73. https://doi.org/10.1016/j.fsi.2018.04.017.
Myers AJ, Goodband RD, Tokach MD, Dritz SS, Rouchey JMD, Nelssen JL. The effects of porcine intestinal mucosa protein sources on nursery pig growth performance. J Anim Sci 2014;92(2):783–92. https://doi.org/10.2527/jas.2013-6551.
Park JN, Watanabe T, Endoh K, Watanabe K, Abe H. Taste-active components in a Vietnamese fish sauce. Fish Sci 2002;68(4):913–20. https://doi.org/10.1046/j.1444-2906.2002.00510.x.
Plakas SM, Katayama T. Apparent digestibilities of amino acids from three regions of the gastrointestinal tract of carp (*Cyprinus carpio*) after ingestion of a protein and a corresponding free amino acid diet. Aquaculture 1981;24:309–14. https://doi.org/10.1016/0044-8486(81)90065-X.
Rebhan Y, Funkenstein B. Inhibition of fish myostatin activity by recombinant fish follistatin and myostatin prodomain: potential implications for enhancing muscle growth in farmed fish. Aquaculture 2008;284(1–4):231–8. https://doi.org/10.1016/j.aquaculture.2008.07.007.
Rudnicki MA, Jaenisch R. The MyoD family of transcription factors and skeletal myogenesis. Bioessays 1995;17(3):203–9. https://doi.org/10.1002/bies.950170306.
Savage AW, Warriss PD, Jolley PD. The amount and composition of the proteins in drip from stored pig meat. Meat Sci 1990;27(4):289–303. https://doi.org/10.1016/0309-1740(90)90067-G.
Shearer KD. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 1994;119(1):63–88. https://doi.org/10.1016/0044-8486(94)90444-8.
Silva VA, Trushenski J, Schwarz MH, Cavalli RO. Effects of rearing density on growth, physiological responses, and flesh quality in juvenile cobia (*Rachycentron canadum*). J World Aquacult Soc 2020;51(6):1301–12. https://doi.org/10.1111/jwas.12721.
Simopoulos AP, DiNicolantonio JJ. The importance of a balanced ω-6 to ω-3 ratio in the prevention and management of obesity. Open Heart 2016;3(2):e000385. https://doi.org/10.1136/openhrt-2015-000385.
Snijders T, Nederveen JP, McKay BR, Joanisse S, Verdijk LB, Loon LJC, et al. Satellite cells in human skeletal muscle plasticity. Front Physiol 2015;6:283–304. https://doi.org/10.3389/fphys.2015.00283.
Stelzl T, Baranov T, Geillinger KE, Kottra G, Daniel H. Effect of N-glycosylation on the transport activity of the peptide transporter PEPT1. Am J Physiol Gastrointest Liver Physiol 2016;310(2):128–41. https://doi.org/10.1152/ajpgi.00350.2015.
Sun WT, Xu XY, Li XQ, Pan WQ, Leng XJ. Effects of dietary geniposidic acid on growth performance, flesh quality and collagen gene expression of grass carp, *Ctenopharyngodon idella*. Aquacult Nutr 2018;24(3):1112–21. https://doi.org/10.1111/anu.12650.
Wang JY, Zhang DR, Ma JJ, Li BS, Zhang LM. Nutritional components analysis and nutritive value evaluation of ♀ *Epinephelus Fuscoguttatus* × ♂ *E. Lanceolatus* muscles. Trans Oceanol Limnol 2015;4:61–9. https://doi.org/10.13984/j.cnki.cn37-1141.2015.04.010.

Wang L, Li J, Jin JN, Zhu F, Roffeis M, Zhang XZ. A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): growth performance, flesh quality, innate immunity and water environment. Aquacult Nutr 2017;23(5):983–93. https://doi.org/10.1111/anu.12466.

Watabe S. Myogenic regulatory factors and muscle differentiation during ontogeny in fish. J Fish Biol 1999;55:1–18. https://doi.org/10.1111/j.1095-8649.1999.tb01042.x.

Wei JL, Wang JY, Song ZD, Huang Y, Sun YZ, Zhang LM. Effects of the partial substitute for fish meal by hydrolyzed krill meal on growth performance, the body composition and the serum biochemical parameters of juvenile pearl gentian grouper. Progress in Fishery Sciences 2016;37(1):100–10. https://doi.org/10.11758/yykxjz.20141211002.

Wu Y, Pan L, Tian QY, Piao XS. Comparative digestibility of energy and ileal amino acids in yeast extract and spray-dried porcine plasma fed to pigs. Arch Anim Nutr 2017;72(1):1–9. https://doi.org/10.1080/1745039X.2017.1413827.

Xia W, Liu WB, Qiao QS, Li GF, Zhang YJ. Efects of cottonseed meal hydrolysate on growth performance and biochemical indices of *Cyprinus carpio* var. Jian. Freshwater Fisheries 2012;42(1):46–51. https://doi.org/10.3969/j.issn.1000-6907.2012.01.009.

Xiong ZY, Zhang CX, Zheng Y. PUFA and exercise. Journal of Qinghai Normal University (Natural Science Edition) 2002;3:67–70. https://doi.org/10.16229/j.cnki.issn1001-7542.2002.03.020.

Yang XY, He YF, Chi SY, Tan BP, Lin S, Dong XH, et al. Supplementation with *Saccharomyces cerevisiae* hydrolysate in a complex plant protein, low-fishmeal diet improves intestinal morphology, immune function and *Vibrio harveyi* disease resistance in *Epinephelus coioides*. Aquaculture 2020;529:735655. https://doi.org/10.1016/j.aquaculture.2020.735655.

Yata M, Yoshida C, Fujisawa S, Mizuta S, Yoshinaka R. Identification and characterization of molecular species of collagen in fish skin. J Food Sci 2001;66(2):247–51. https://doi.org/10.1111/j.1365-2621.2001.tb11325.x.

Yu EM, Liu BH, Wang GJ, Yu DG, Xie J, Xia Y, et al. Molecular cloning of type I collagen cDNA and nutritional regulation of type I collagen mRNA expression in grass carp. J Anim Physiol Anim Nutr 2014a;98(4):755–65. https://doi.org/10.1111/jpn.12132.

Yu H, Wan GT, Cheng MJ, Xing KZ, Chen CX, Wang QK, et al. Analysis of the nutritive components of muscle from pearl gentian grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). Journal of Guangdong Ocean University 2014b;34(6):83–7. https://doi.org/10.3969/j.issn.1673-9159.2014.06.014.

Zhang GG, Li X, Cai XB, Zhang SX, Hua XM, Huang ZY, et al. Effects of enzymatic hydrolyzed soybean meal on growth performance, liver function and metabolism of largemouth bass (*Micropterus salmoides*). Acta Hydrobiol Sin 2019;43(5):1001–12. https://doi.org/10.7541/2019.119.

Zhang HT, Shan L, Bi YP. Functional relationship between n-6 and n-3 polyunsaturated fatty acids in the bodies of human and animal. Shandong Agricultural Sciences 2006;2:115–20. https://doi.org/10.3969/j.issn.1001-4942.2006.02.044.

Zhang L, Feng L, Jiang WD, Liu Y, Jiang J, Li SH, et al. The impaired flesh quality by iron deficiency and excess is associated with increasing oxidative damage and decreasing antioxidant capacity in the muscle of young grass carp (*Ctenopharyngodon idellus*). Aquacult Nutr 2016a;22(1):191–201. https://doi.org/10.1111/anu.12237.

Zhang YL, Song L, Liu RP, Zhao ZB, He H, Fan QX, et al. Effects of dietary protein and lipid levels on growth, body composition and flesh quality of juvenile topmouth culter, *Culter alburnus* Basilewsky. Aquacult Res 2016b;47(8):2633–41. https://doi.org/10.1111/are.12712.

Zhao Y, Li JY, Yin L, Feng L, Liu Y, Jiang WD, et al. Effects of dietary glutamate supplementation on flesh quality, antioxidant defense and gene expression related to lipid metabolism and myogenic regulation in Jian carp (*Cyprinus carpio var.* Jian). Aquaculture 2019;502:212–22. https://doi.org/10.1016/j.aquaculture.2018.12.050.

Zheng GD, Sun CF, Pu JW, Chen J, Jiang XY, Zou SM. Two myostatin genes exhibit divergent and conserved functions in grass carp (*Ctenopharyngodon idellus*). Gen Comp Endocrinol 2015;241:68–76. https://doi.org/10.1016/j.ygcen.2015.03.008.

Zhou LY, Wu DW, Gao MM, He J, Sun F, Yu N, et al. The effects of fish meal replacement on growth performance of yellow catfish (*Pelteobagrus fulvidraco*). Acta Hydrobiol Sin 2019;43(3):504–16. https://doi.org/10.7541/2019.062.

Zuanazzi JSG, Lara JAF, Goes ESR, Almeida FLA, Oliveira CAL, Ribeiro RP. Anoxia stress and effect on flesh quality and gene expression of tilapia. Food Sci Technol 2019;39(1):195–202. https://doi.org/10.1590/fst.00518.