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

Xuanyi Yang, Xinyan Zhi, Ziling Song, Guanghui Wang, Xumin Zhao, Shuyan Chi, Beiping Tan

Laboratory of Aquatic Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China
Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang), Zhanjiang 524025, China
Aquatic Animals Precision Nutrition and High Efficiency Feed Engineering Research Center of Guangdong Province, Zhanjiang 524088, China
Yichang Huatai Biological Technology Co., Ltd., Yichang 443500, China

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% fishmeal 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 tonnes 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 tonnes 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 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 $9 \times$ Epinephelus lanceolatus $\delta$) 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 (Lopez-Albors et al., 2008), density (Alvarez 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 blunit snout bream (Megalobrama amblycephala) muscle was significantly decreased (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 medium, enzymes are added during the extraction process for the drying (Mateos et al., 2014). To increase the yield of heparin so-
mucosa (HPM) is derived from the mucosa of 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 were 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

### 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 hepa-
arin 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 were 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

| Item | HPM0 | HPM3 | HPM6 | HPM9 | HPM12 |
|------|------|------|------|------|-------|
| **Ingredients** | | | | | |
| Brown fish meal | 33.00 | 30.00 | 27.00 | 24.00 | 21.00 |
| HPM | 3.00 | 6.00 | 9.00 | 12.00 |
| Soybean meal | 22.50 | 21.50 | 20.50 | 19.50 | 18.50 |
| Wheat gluten flour | 4.28 | 5.15 | 6.03 | 6.90 | 7.78 |
| Cottonseed protein | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Spray-dried blood | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Wheat meal | 18.20 | 18.20 | 18.20 | 18.20 | 18.20 |
| L-Lysine | 0.78 | 0.81 | 0.84 | 0.87 | 0.90 |
| DL-Methionine | 0.40 | 0.41 | 0.41 | 0.42 | 0.43 |
| Anchovy oil | 3.35 | 3.17 | 3.00 | 2.82 | 2.65 |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Soybean lecithin | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Vitamin premix | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Minemal premix | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin C | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Ca(H2PO4)$_2$.7H2O | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Chlorine chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Ethoxyquin | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Phytases | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Microcrystalline cellulose | 0.11 | 0.38 | 0.64 | 0.91 | 1.16 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **Proximate composition (dry matter)** | | | | | |
| 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 Provided by 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 $B_6$, 17.00; vitamin $B_12$, 16.67; vitamin $B_1$, 33.33; vitamin B$_2$, 0.07; vitamin K$_3$, 3.33; vitamin E, 66.00 g; retinyl acetate, 6.67 g; vitamin D$_3$, 33.33; nicotinic acid, 67.33 g; D-calcium pantothenate, 40.67 g; biotin, 16.67; folic acid, 4.17 g; inositol, 102.04 g; cellulose, 392.72 g.
4 Provided by Qingdao Master Biotech Co., Ltd, Qingdao, China. Each kilogram of premix included the following: CaCO$_3$, 350 g; NaH$_2$PO$_4$.H$_2$O, 200 g; KH$_2$PO$_4$, 200 g; NaCl, 12 g; MgSO$_4$.7H$_2$O, 10 g; FeSO$_4$.7H$_2$O, 2 g; MnSO$_4$.7H$_2$O, 2 g; AlCl$_3$.6H$_2$O, 1 g; CuCl$_2$.2H$_2$O, 1 g; KI, 1 g; NaMoO$_4$.2H$_2$O, 0.5 g; Na$_2$SeO$_3$.0.4 g; CoCl$_2$.6H$_2$O, 0.1 g; KI, 0.1 g; zelite 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 Pharmaceutical 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 &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 fiberglass 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.

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 (Myl5), 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 (Col1a-1), collagen type I alpha 2 (Col1a-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.
extracts, indicating a high degree of purity. Reverse transcription of cDNA was carried out using the Prime Script™ RT reagent kit (Takara, Japan), following the manufacturer’s instructions. The qRT-PCR reactions were performed according to instructions of the SYBR® Premix Ex Taq™ 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-ΔΔCT method, following Mu et al. (2018).

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 |            |       |              |                    |
| Asparagine         | 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: TGCTTGACATCAAGGAGAAGC | 61                       | 151                            | AY510710.2        |
|             | R: TCTGGCAACGGAAACCTCT   |                          |                                 |                   |
| Myf5        | F: CGAGACCTGCTGACACTACT | 58                       | 142                            | XM_033614452.1    |
|             | R: ATCCGGCTGGTAGTTGTC    |                          |                                 |                   |
| MyoD        | F: GGCTAAGCAAGTCACAGGA   | 59                       | 113                            | XM_033635008.1    |
|             | R: TCTGATGGTAGTGATGCT    |                          |                                 |                   |
| MEF-2c      | F: CGATTCATCCTGACCACTCTC | 62                       | 113                            | XM_033618691.1    |
|             | R: TGAGCCAGAGTGAAAGCCC   |                          |                                 |                   |
| MyoG        | F: GAGGACCAATGGATGAAACC  | 61                       | 191                            | XM_033625713.1    |
|             | R: TGGGCTACTGAGAAGAGACA  |                          |                                 |                   |
| MEF-2a      | F: TGCTGTATCAATCCCAATG   | 60                       | 206                            | XM_033641861.1    |
|             | R: TGCCATGAAAGCTCTTCGTA  |                          |                                 |                   |
| MyHC        | F: AAGGCTTGCTGAGTACGAAA  | 60                       | 221                            | XM_033644959.1    |
|             | R: TGATGCTCTGCCAGCTCCTA  |                          |                                 |                   |
| IGF-1       | F: GTGCGAAGTGTGACATTCCTG | 61                       | 179                            | XM_033614181.1    |
|             | R: GCCATACGTGATGGTATTAGCT| 59                       |                                 |                   |
| Col1α-1     | F: TGACCGGGAAGGTGATG     | 60                       | 128                            | XM_033645844.1    |
|             | R: CAACCTGAGGCTCGTGA     |                          |                                 |                   |
| Col1α-2     | F: TCTGCGACTCAAAACCCCG  | 60                       | 234                            | XM_033646413.1    |
|             | R: AGTGTAGGTGCTGTTGCTGTA | 59                       |                                 |                   |
| TGF-β1      | F: ACAGGGGACAAACCCGCTAC  | 61                       | 122                            | GQ205390.1        |
|             | R: CGATCTACCTCCTCTCTCTTG | 60                       |                                 |                   |
| FST         | F: CAAAAGCTGCTGGGATGGGCT | 61                       | 174                            | XM_033620608.1    |
|             | R: TGGACTTCCAGCACACCACC  |                          |                                 |                   |
| MSTN        | F: TGATGAATATCGGATGGAAGAG| 60                       | 144                            | XM_033645071.1    |
|             | R: CTTCTCTATTAAACACATTTCTT| 60                      |                                 |                   |
| IGF-2       | F: CGCAGAACGATAGCCACACCA | 62                       | 146                            | XM_033635279.1    |
|             | R: GCCGAGCTTATCCAGAAC    |                          |                                 |                   |

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.
2.6. Calculations and statistical methods

Weight gain percentage (WG, %)

\[ \text{WG} = 100 \times \frac{\text{final body weight (g)} - \text{initial body weight (g)}}{\text{initial body weight (g)}} \]

Survival rate (SR, %)

\[ \text{SR} = 100 \times \frac{\text{number of fish in the final test}}{\text{number of fish in the initial test}} \]

Feed efficiency (FE)

\[ \text{FE} = \frac{\text{weight of muscle after being cooked (g)} - \text{weight of muscle before being cooked (g)}}{\text{weight of fish in the initial test} \times \text{diet weight of group’s fish intake (dry weight, g)}} \]

Protein deposition rate (PDR, %)

\[ \text{PDR} = 100 \times \frac{\text{weight of muscle after being cooked (g)} - \text{weight of muscle before being cooked (g)}}{\text{weight of fish in the initial test} \times \text{diet dry weight of group’s fish intake (g)}} \]

Lipid deposition rate (LDR, %)

\[ \text{LDR} = 100 \times \frac{\text{weight of muscle before being cooked (g)} - \text{weight of muscle after being cooked (g)}}{\text{weight of fish in the initial test} \times \text{diet dry weight of group’s fish intake (g)}} \]

Cooking loss (%)

\[ \text{Cooking loss} = \frac{\text{weight of muscle before being cooked (g)} - \text{weight of muscle after being cooked (g)}}{\text{weight of muscle before being cooked (g)}} \times 100 \]

Drip loss (%)

\[ \text{Drip loss} = \frac{\text{weight of muscle after being suspended (g)} - \text{weight of muscle before being suspended (g)}}{\text{weight of muscle before being suspended (g)}} \times 100 \]

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 the 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).

### Table 4

| 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 | 851.25 ± 13.50 | 850.63 ± 8.81 | 805.74 ± 5.32 | 756.12 ± 9.43 |
| 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.

* Values with different superscripts in the same row are significantly different (P < 0.05).

1 Diets HPM0, HPM3, HPM6, HPM9, and HPM12 contains 0%, 3%, 6%, 9%, and 12% HPM content, respectively. Mean values ± SEM are presented for each group (n = 3).
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, n-3 PUFA, and 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 n-3 PUFA and 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 in the feed increased, the contents of C18:1n-9 and ∑ 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
Table 8
Fatty acid composition in muscle of hybrid groupers fed 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 ± 0.01а| 0.46 ± 0.01й| 0.46 ± 0.01й| 0.47 ± 0.01й| 0.45 ± 0.01й|
| C16:0   | 20.87 ± 0.12б| 22.07 ± 0.27й| 22.64 ± 0.48й| 21.92 ± 0.24й| 22.26 ± 0.47й|
| C17:0   | 1.08 ± 0.01й| 1.00 ± 0.01й| 0.97 ± 0.04й| 0.94 ± 0.01й| 0.84 ± 0.04й|
| 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|

¹ 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 fed the test diets for 56 days. Hydraized porcine mucosa (HPM) content in feed was 0%, 3%, 6%, 9%, and 12% in HPM0, HPM3, HPM6, HPM9, HPM12, respectively.

а, б, в 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; TGF — transforming growth factor; FST — follistatin; MSTN — myostatin.
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).

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; Kt et al., 2015). However, the WG of hybrid grouper was significantly reduced when the dietary HPM content was over 9%. When more hydrolytic or enzymologically 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 (Buchová 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 MUFA and n-6 PUFA in muscle increased, while the n-3 PUFA and n-3 HUFA exhibited a significant decreasing trend. The n-3 PUFA and n-6 PUFA 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 PUFA ratio in food is closely related to human disease, and research has shown that the lower the n-3/n-6 PUFA 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 PUFA ratio of hybrid grouper muscle was 0.34 to 0.52. As the HPM content in the diet increased, the n-3/n-6 PUFA 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 Jaenisch, 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 MyFS, 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 (Col1α1) and one α-2 chain (Col1α2; Gelsema 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 Col1α1 and Col1α2 also exhibited a similar trend, although the change in Col1α2 was not significant. Col1α1 and Col1α2 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 (Rehban 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 firmness and chewiness were improved. An appropriate amount of HPM could be better used in grouper feed.

Acknowledgments

This study was supported by the Fund of The National Key R&D Program of China (2019YFD0900200); Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang) (No. ZJW-2019-06); China Agricultural Research System (CARS-47); Guangdong Modern Agriculture Research System-Innovation Team (2020KJ150).

References

Alhaid 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 hypoxygen and flesh quality of Chilean croaker (Ciltos gilberti) stocked at different densities. Aquaculture 2020;525:735316. https://doi.org/10.1016/j.aquaculture.2020.735316.

Aragão C, Conceição LEC, Martins D, Rennestad I, Gomes E, Dinis MT. A balanced dietary amino acid profile improves amino acid retention in post-larval Sene-galese 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 Official Analytical Chemists International; 1999. 5th revision. Bertram HC, Andersen HJ, Karlsson AH, Horn P, Hedegaard J, Norgaard 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/s0025-774x(03)00022-3.

Bidner BS, Ellis M, Witte DP, Carr SN, McKeeth FK. Influence of dietary lysine level, pre-slaughter fasting, and rendering 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, Tintori D, Delogu G, Sereni L, 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 gut 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.

Buchová H, Svobodová Z, Kocour M, Velisek J. Amino acid composition in fillets of mirror crossesbred common carp (Cyprinus carpio, Linnaeus 1758). Acta Vet 2009;78(2):337–44. https://doi.org/10.2754/avsk89970820337.

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.12931.

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 amineotransferases 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, Amendumpr-Johnsen I, Codina M, Navarro I, Gutierrez J. IGF-1 and insulin receptor signal transduction in trout muscle cells. Am J Physiol Regul Integr Comp Physiol 2006;290(6):R183–90. https://doi.org/10.1152/ajpregu.00294.2005.

Chen WJ, Wang Y, Han D, Zhu XM, Xie SQ, Han JX, 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(3):1145–55. https://doi.org/10.1111/anu.12930.

Chen YW, Nagaruji 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(8):826–34. https://doi.org/10.1212/01.wnl.0000170383.91786.4a.

Cheng JH, Sun DW, Han Z, Zeng XA. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: a review. Comp Rev Food Sci Food Saf 2014;13(1):52–61. https://doi.org/10.1111/crfs.12104.

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 2019;85(4):965–72. https://doi.org/10.1007/s12265-019-04192-3.

Chen Y, Zeng X, Li X, Wang X, Huang M, Shi T, et al. Nutritional analyses for evaluation of TGFβ in European seabass (Dicentrarchus labrax) fed plant-protein-based diets. Aquaculture 2020;523:735318. https://doi.org/10.1016/j.aquaculture.2020.735318.

Deng YP, Jiang WD, Liu Y, Qu B, Jiang J, Kuang SY, et al. Dietary leucine improves flesh quality and alters mRNA expressions of NR2mediated antioxidant

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; Beijng 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 inappropriate 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.
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, A. and E. and M. and K. and H. and E. and Y. and 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 X, Qin XM, Zhang CH, Chen JP, Zhu QF. Nutritional and volatile flavor component composition of dorsal and ventral muscle from hybrid grouper (Epinephelus fuscoguttatus) × E. lanceolatus). J Guangdong Ocean Univ 2018;38(1):39–46. https://doi.org/10.13697/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 fermented with Polysaccharides from marine fish peptones with enzymatic combined membrane preparation technology. Sci Technol Fish Aquaculture 2020;5(12):1531–41. https://doi.org/10.1007/s42723-020-00052-4.

Figueroa J, Solano-Moreno P, Carballo F, Arboix A, Martínez H, Sunyer X. Yang, X. Zhi, Z. Song et al. Animal Nutrition 8 (2022) 114-120.

Imsland AKD, Roth B, Christensen LB, Siikavuopio SI, Foss A. Effects of short-term dietary porcine mucosa hydrolysate in diets varying in lysine content on growth performance, muscle growth, and muscle chemical composition in Atlantic salmon (Salmo salar L.). Aquaculture 2008;277(1–2):39–51. https://doi.org/10.1016/j.aquaculture.2008.02.015.

Kassar-Duchossoy L, Gayraud-Morel B, Gomis G, Monneret D, Gomis G, Monneret D, et al. Dietary tannins and the effects on muscle fatty acid composition, muscle hypertrophy, muscle performance, stress and the potential of using commercial tannins as a muscle growth promoter in Atlantic salmon (Salmo salar). J World Aquacult Soc 2020b;51(5):71-82. https://doi.org/10.1111/jwas.12871.

Kolkovski S, Tandler A. The use of squid protein hydrolysate as a protein source in Atlantic salmon (Salmo salar) feed. Aquaculture 2006;256(1–4):267–78. https://doi.org/10.1016/j.aquaculture.2006.02.014.

Koruga K, Alexomin M, Bartyk M, Korka J, Janocek R, Koralik J. Yeast protein concentrate as a replacement for soybean meal in the diet of rainbow trout (Oncorhyncus mykiss). J Environ Biol 2016;37(3):355–63. https://doi.org/10.1016/j.jenb.2015.04.038.

Li MM, Zhang M, Ma YC, Ye BK, Wang M, Chen HY, et al. Dietary supplementation with n-3 unsaturated fatty acids decreases serum lipid levels and improves flesh quality in the marine teleost golden pompano Trachinotus ovatus. Aquaculture 2020a;516:734-632. https://doi.org/10.1016/j.aquaculture.2019.734632.

Li XX, Chen SJ, Sun JJ, Huang XD, Tang JH, He YH, et al. Partial substitution of soybean meal with faba bean meal in grass carp (Ctenopharyngodon idella) diets, and the effects on fish meat fatty acid composition, flesh quality, and expression of myogenic regulatory factors. J World Aquacult Soc 2020b;51(5):71-82. https://doi.org/10.1111/jwas.12872.

Lin YQ, Zhou JS, 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 (Latesolobus japonicus). Anim Feed Sci Technol 2017;229:1–12. https://doi.org/10.1016/j.anifeedsci.2017.05.007.

Liu JX, Gao F, Tan X, Wang Y, Zhao YY, Hua SH, et al. Effects of different dietary protein hydrolysates (PPh) level of dietary supplements on growth and body composition of larvae of cobia (Rachycentron canadum). Acta Hydrochim Sin 2010;34(1):94–100. https://doi.org/10.7453/1000-0357.2010.0094.

Mateos GG, Mohiti-Asli M, Borda E, Mirzaie S, Frikha M. Effect of inclusion of ruminant digestive enzymes on the growth performance and physiological responses, and the effects on muscle fatty acid composition, meat quality, and aminopeptidase PepA in peptide assimilation by helicobacter pylori. J Microbiol Biotechnol 2015;25(10):1629–38. https://doi.org/10.4162/jmbi.2015.00094.

Mu H, Shen HH, Liu JH, Xie FL, Zhang WB, Mai KS. High level of dietary soybean oil and effect of replacing dietary fish oil with vegetable oils on MRFs expression of on-growing grass carp (Ctenopharyngodon idella) fed non-fish meal diets. Aquaculture 2020;521:735065. https://doi.org/10.1016/j.aquaculture.2020.535065.

López-Albores O, Abdel I, Persia AJ, Ayala MD, Alcaraz AG, Gracia CM, et al. Temperature influence on the muscle growth dynamics of the sea bass Dicentrarchus labrax, L. in Mediterranean conditions. Aquaculture 2008;277(1–2):39–51. https://doi.org/10.1016/j.aquaculture.2008.02.015.

Luciano G, Monahan FJ, Vasta V, Bioni L, Lanza M, Prisolo A. Dietary tannins improve lamb meat colour stability. Meat Sci 2009;81(1):120–5. https://doi.org/10.1016/j.meatsci.2008.07.005.

Lv H, Hu WH, Xiong SB, You J, Fan QX. Depuration and starvation improves flesh quality of grass carp (Ctenopharyngodon idella). Aquaculture 2018;495(9):196–205. https://doi.org/10.1016/j.aquaculture.2018.09.014.

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.aniwatres.2014.01.013.

McMurtry 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. 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 deploys the digestive capacity and induces inflammatory response in large yellow croaker Linotrhys scroce. Fish Shellfish Immunol 2018;77:465–73. https://doi.org/10.1016/j.fsi.2018.04.017.

Nordstrom JJ, Goodland RD, Takahashi D, Dray D, Rouche Y, Neelson J. The effects of porcine intestinal mucosa proteins on nursery pig growth performance. J Anim Sci 2019;92(2):783–92. https://doi.org/10.1093/jas/sky145.

Park JN, Watanabe T, Endoh K, Watanabe K, Abe H. Taste-active components in a Vietnamese fish sauce. Fish Sci 2002;68(4):93–20. https://doi.org/10.1023/A:10134644-2900-2002.x.

Plakas SM, Kamataya T. Apparent digestibilities of amino acids from three regions of the gastrointestinal tract of carp (Cyprinus carpio) after ingestion of a protein and corresponding amino acid diet. Aquaculture 1981;24:304–19. https://doi.org/10.1016/0044-8486(81)90065-x.

Rehban Y, Funkenstein B. Inhibition of myostatin activity by recombinant human myostatin. J Biol Chem 2004;279:39–46. https://doi.org/10.1074/jbc.C303339200.

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.3200700303.

Savage AW, Warriss PD, Jolley PD. The amount and composition of the proteins in fish meal diets. Aquaculture 1990;95(1):203–9. https://doi.org/10.1016/0044-8486(90)90065-X.

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

---

X. Yang, X. Zhi, Z. Song et al.

Animal Nutrition 2018 114–124
