Fish Physiol Biochem (2020) 46:2037–2053
https://doi.org/10.1007/s10695-020-00851-3

Comprehensive physiological and transcriptomic analysis revealing the responses of hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) to the replacement of fish meal with soy protein concentrate

Yan Chen · Wenkan Liu · Jun Ma · Yaorong Wang · Hai Huang

Received: 21 October 2019 / Accepted: 28 July 2020 / Published online: 7 August 2020
© Springer Nature B.V. 2020

Abstract Plant proteins are suitable and alternative to fish meals (FMs), with less cost compared with that of all other types of fish feeds. In recent years, soy protein concentrate (SPC) has emerged as a cost-effective alternative to FM; however, little is known regarding the effects of dietary SPC on general fish physiology and well-being. This study aimed to perform comprehensive physiological and transcriptomic analysis for testing the applicability of SPC as fish feeds in hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) [SPC replaced 0% (CK), 30% (SPC30), and 75% (SPC75) of FM protein]. Generally, SPC30 promoted fish survival and had less effects on the phenotype, while SPC75 reduced fish survival, promoted inflammation, and regulated multiple physiological responses. Thousands of differentially expressed genes (DEGs) by SPC were identified in the intestine, liver, and muscle, which were enriched in biological regulation, cellular process, metabolic process, single-organism process, cell, cell part, membrane, binding, and catalytic activity based on RNA-seq. Notably, some DEGs involved in amino acid and lipid metabolism in the digestive system highlighted the modulatory effect of SPC on these metabolic processes, consistent with the physiological responses including enzyme activities. The enriched aspects of these predominant DEGs might be directly related to the different effects of SPC30 and SPC75 on fish growth, digestibility, and underlying enzyme activities and histology. In conclusion, the comprehensive physiological and transcriptomic comparative analysis of CK, SPC30, and SPC75 was also effective in testing the applicability of SPC as fish feeds and in designing a proper diet with the best impact on the growth performance and health of fish in hybrid grouper.

Keywords DEGs · Fish meals · Physiological analysis · Soy protein concentrate · Transcriptomic analysis

Y. Chen · W. Liu · J. Ma · H. Huang
Hainan Key Laboratory for Conservation and Utilization of Tropical Marine Fishery Resources, Sanya 572022 Hainan province, People's Republic of China

Y. Chen · W. Liu · J. Ma (✉) · H. Huang
College of Fisheries and Life Science, Hainan Tropical Ocean University, Sanya 572022 Hainan Province, People's Republic of China
e-mail: majun_hp@hotmail.com

Y. Wang
Fisheries College, Guangdong Ocean University, Zhanjiang 524088 Guangdong Province, People's Republic of China

Introduction

As an essential item, protein is most expensive and needed in sufficient amounts for fish growth (Mambrini et al. 1999; Xiao et al. 2017). Fish meal (FM) is now the main protein source of aquatic feed, particularly for omnivorous and carnivorous fish due to its balanced delivery of essential amino acids, lipid, vitamins, and minerals, and palatability (Bonvini et al. 2018; Ween et al. 2017). However, limited production and increased demand and price of FM have resulted in

an urgent need for alternative proteins from sustainable suppliers at a lower cost. Given that plant foodstuffs are readily available and cost-effective and possess a high amino acid profile, they are a suitable alternative to FM (Antony Jesu Prabhu et al. 2015; Guerreiro et al. 2015).

Among the plant protein sources with high protein, amino acid, and lipid levels and digestibility, soybean has been studied as the best replacement for FM (Bonvini et al. 2018; Hossain and Koshio 2017; Xiao et al. 2017). However, some factors, including low palatability and imbalance in amino acids, seriously affect the replacement of soybean (Imanpoor and Bagheri 2012; Mambrini et al. 1999). Further studies found that soy protein concentrate (SPC) partially and fully replaced FM in fish feeds (Bañuelos-Vargas et al. 2014; López et al. 2015; Mambrini et al. 1999). SPC is made by aqueous alcohol extraction of soy flakes and reduces the levels of both soluble carbohydrates and anti-nutritional factors (Mambrini et al. 1999). Previous studies on SPC use in turbot (*Scophthalmus maximus* L.) (Gonzalez et al. 2008), yellowtail kingfish (*Serioala lalandi*) (Monteith et al. 2013), starry flounder (*Platichthys stellatus*) (Xu et al. 2015), and Florida pompano (*Trachinotus carolinus*) (Lech and Reigh 2012) revealed that fish tolerated ≤ 50% of SPC replacement in their feed. In this study assessing SPC replacement in juvenile hybrid grouper, ≤ 30% SPC could be tolerated.

The relationship between dietary SPC and fish growth remains elusive at the cellular and physiological levels. Recent advances in RNA sequencing approaches have enabled the characterization of these effects at the molecular level (Sun et al. 2016a, b). Hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) is an economic species of large-scale aquaculture in Southern China (Yin et al. 2018; Zhou et al. 2018; Zhu et al. 2018; Zou et al. 2018). The cost of the ingredients in fish feed has increased by as much as 50% in recent years, threatening many fish industries. Therefore, plant proteins are now being marketed, largely reducing the cost.

This study aimed to perform comprehensive physiological and transcriptomic analysis on the important aquaculture species hybrid groupers fed with a low-FM diet in which the FM was partially replaced with SPC and to compare the results with those of fish fed with a high-FM diet. It further aimed to test the applicability of SPC as fish feeds in hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus* ♂) and provide more clues for fish feed and management.

Materials and methods

Feeding trial

Hybrid groupers from a specific-pathogen-free line and free of diseases at about 8 g per fish were fed in the indoor recirculating aquaculture system with flow marine water of 1 L min^{-1}. A commercial feed with 43% crude protein and 10% crude lipid (Nisshin Flour Milling Co., Ltd., Japan) was used to adapt the laboratory breeding environment for 2 weeks. Then, the groupers were fed three experimental diets in this system for 8 weeks, with the diurnal cycle of 12-h light/12-h dark. Every diet had triple replicates (500 L per tank), and each replicate had 50 groupers. Water was controlled at a temperature of 29.2 °C ± 0.4 °C, dissolved oxygen of 7.10 ± 0.2 mg L^{-1}, salinity of 25.8‰ ± 0.5‰, pH 7.2 ± 0.2, and total ammonia nitrogen of 0.3 ± 0.2 mg L^{-1}. No weak and sick or injured groupers were found during the whole feeding trial.

Diet formulation and preparation

In this study, three experimental diets containing different levels of SPC by replacing 0% (CK), 30% (SPC30), and 75% (SPC75) of FM were used as described in previous studies (Ding et al. 2010). FM contained crude protein 60.0%, crude lipid 6.0%, and ash 20.6% (dw), while SPC contained crude protein 65.0%, crude lipid 1.0%, crude fiber 4.00%, ash 4.8%, soluble nitrogen-free extract 2.2%, and insoluble nitrogen-free extract about 15% (dw). In all diets, FM, SPC, and corn gluten meal were used as the main protein sources, fish oil and soy oil were used as lipid sources, wheat flour was used as the carbohydrate source, and three isonitrogenous and isocaloric diets were used for the requirements of protein and energy (Luo et al. 2005; Shiau and Lan 1996).

Sample collection

After 8 weeks of feeding, groupers fed with the three experimental diets (CK, SPC30, and SPC75) were fasted for 24 h and anesthetized with 100 mg L^{-1} tricaine methanesulfonate (MS-222). Then, the tissues (liver, intestine, and muscle) of six fish were collected, rapidly fixed in DNA/RNA protector (Takara, Beijing, Cat. 9750), and stored at − 80 °C. The liver and intestine tissues were homogenized in 50-mM Tris-HCl (pH 7.6), and the supernatant was used for the assay of enzyme

activities. The liver and intestine of five fish from each replicate were cut into 1-cm^2 pieces for histological analysis. Plasma biochemistry was determined using a Modular P800 Automatic Biochemical Analyzer (Roche Diagnostics, Mannheim, Germany) in the clinical laboratory (Guangzhou Kingmed Center for Clinical Laboratory, China).

Assay of enzyme activities

The activities of pepsin, lipase, and amylase were assayed using Pepsin Assay Kit, Lipase Assay Kit, and α-Amylase Assay Kit (Nanjing Jiancheng Bioengineering Institute A080, A054, C016), respectively, following the manufacturer's protocols. Briefly, the protein concentration of each tissue homogenate was quantified by the Bradford (1976) method. The activities of all enzymes were expressed as mg per protein.

Histology

The liver and intestine sample pieces were fixed in aqueous Bouin's fluid (75 mL of the saturated picric acid solution, 25 mL of formaldehyde, and 5 mL of acetic acid) for 12–24 h and then washed in running water for 24–48 h. The histological sections were treated and manufactured according to the method of Drury and Wallington (1980). After the processes of dehydration, embedding (in paraffin), cutting, and staining [hematoxylin and eosin (H&E)] of the tissues, the samples were observed using an optical microscope (CX31; Olympus, Japan). The average thickness was calculated from 30 repeated measurements of 20 slides for each species.

RNA extraction, cDNA library, and sequencing

Total RNA was extracted using TRIzol (Invitrogen, Shanghai, Cat. 15596026), quantified using NanoDrop 2000 (Thermo Scientific, USA) and examined using Agilent 2100 Bioanalyzer (Agilent, USA), following the manufacturers' protocols. After incubation with DNase I (RNase-free; NEB, Shanghai, Cat. M0303S) and purification using a MicroPoly (A) Purist Kit (Ambion, Shanghai, Cat. AM1922), mRNA was used for the synthesis of first-strand and second-strand cDNAs. Thereafter, the final cDNA library was constructed by adding the sequencing adapters to the c6DNA fragments and sequenced using the Illumina sequencing platform (Illumina HiSeq 4000) (Honor Tech Co. Ltd., Beijing, China).

Quality control and de novo assembly

When the content of nitrogen exceeded 10% of the base number of any raw sequencing reads, the paired reads were removed; when the number of the low-quality base ($Q \leq 5$) exceeded 50% of the reads, the paired reads were also removed. Then, all clean reads were assembled into transcripts using Trinity software (Grabherr et al. 2011; Haas et al. 2013).

Transcriptional expression level analysis and coding potential prediction

The clean reads of different groups were used to map to the reliable reference transcripts. The RSEM (RNA-Seq by Expectation Maximization) method was used to examine the transcripts by the transcripts per million (TPM) (Li and Dewey 2011; Wagner et al. 2012). TPM > 0.5 in at least one sample was considered a screening condition for highly reliable transcripts. These highly reliable transcripts not only contained the mRNA-encoded proteins but also a large number of noncoding RNAs. The CPAT (v.1.22) (Coding Potential Assessment Tool) was used to predict the coding potential of RNA (Wang et al. 2013), and four model organisms were used to evaluate the prediction result. As long as one model supported the coding genes, they were judged to be the encoding genes.

Functional annotation and analysis

The TransDecoder software was used to analyze the encoding region of the transcripts, and the BLASTP (Basic Local Alignment Search Tool program) (v2.2.29) was used to annotate the gene function using nonredundant proteins (Nr), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), evolutionary genealogy of genes and nonsupervised orthologous groups (eggNOG), and Gene Ontology (GO) databases. The GO level 2 classification was used to analyze the GO functional class.

Analysis of differentially expressed genes

The R language (v3.2.1) was used to identify the differentially expressed genes (DEGs) between two groups

(CK vs SPC30, CK vs SPC75, and SPC30 vs SPC75). The transcripts with $|\log_2 FC| > 1$ and q value < 0.05 were defined as DEGs in this study. Moreover, the transcripts with a false discovery rate < 0.05 and $\log_2$ ratio > 1 were regarded as significant abundance. Additionally, a hypergeometric distribution test was used to identify GO and KEGG with significantly enriched DEGs ($P < 0.05$), compared with the total background expressed genes. Enriched GO items were displayed in the topological diagram using the GO TopNET software (v.1.0).

Statistical analysis

The data were presented as means ± standard deviation (SD, $n = 3$). After normality and heterogeneity-of-variance tests, one-way analysis of variance was used for the comparison of the mean values using SPSS23.0 (SPSS Inc., IL, USA). Statistically significant differences were described as $P < 0.05$.

Results

Effects of SPC diet on the phenotype of hybrid grouper

Briefly, the proximate composition of three diets were as follows: crude protein (46.56, 47.11, and 46.30%), crude lipid (9.63, 9.29, and 9.15%), crude ash (15.73, 13.34, and 9.26%), and gross energy (18.91, 18.95, and 19.24 kcal kg^{-1}). The diets had similar levels of digestible protein, lipids, and digestible energy, which varied by 0.33 kcal kg^{-1}, and met the nutritional requirement of hybrid grouper. The crude ash content varied among the diets but remained within the required range. The first- and second-limit amino acids (methionine and lysine) in the three diets based on SPC were as follows: methionine, 0.85, 0.74, and 0.49%; lysine, 2.41, 2.31, and 1.98%, respectively. The growth of hybrid grouper during the 42-day period was higher than 7.9 g, showing that the three diets used in this experiment provided satisfactory growth to the fish. A positive trend was found for final weight, average body weight gain (BWG), weight gain ratio (WGR), specific growth rate (SGR), protein efficiency ratio (PER), daily feed intake (DFI), feed conversion ratio (FCR), and survival (Table 1). However, the survival of hybrid grouper in the SPC75 group was 85.56% compared with 91.11 and 94.44% in the CK and SPC30 groups, respectively, suggesting that replacing up to 75% of FM by SPC reduced fish survival, while 30% SPC promoted fish survival. Moreover, high SPC inclusion (SPC75) clearly reduced the condition factor (CF), hepatosomatic index (HSI), and intraperitoneal ratio (IPR) compared with the other treatments ($P < 0.05$) (Chen et al. 2019).

Effects of SPC diet on the serum biochemistry and digestive enzyme activity of hybrid grouper

Analysis of the fish serum biochemistry suggested that the SPC diet exhibited higher levels of alanine transaminase (ALT), aspartate aminotransferase (AST) albumin (ALB), and serum total protein and urea and higher AST/ALT ratios compared with the CK diet (Table 2). Pepsin in the intestine was responsive to SPC, with values of 253.22 U/(mg protein) observed in the SPC75 group compared with 59.95 and 60.66 U/(mg protein) in the SPC30 and CK groups (Table 3). The amylase activity of the intestine was similarly increased by SPC (Table 3). However, the lipase level was decreased by SPC, with the degree of response significantly higher in the CK [849.76 U/(mg protein)] and SPC30 groups [717.37 U/(mg protein)] compared with the SPC75 group [391.49 U/(mg protein]. Therefore, the levels of liver pepsin, amylase, and lipase significantly decreased in the SPC75 group compared with the SPC30 and CK groups.

Effects of SPC diet on the histology of hybrid grouper

Enhanced plant protein levels in diets can lead to intestinal cell damage. Therefore, histological examination of intestinal and liver tissues was performed in fish fed with the CK, SPC30, and SPC75 diets. Little change was observed in the intestinal tissue of hybrid grouper fed with the CK and SPC30 diets, which displayed no signs of intestinal (Fig. 1) or liver (Fig. 2) inflammation. In contrast, fish fed with the SPC75 diet displayed signs of intestinal and liver damage. This finding suggested that SPC supplementation at 30% did not promote inflammatory processes and tissue damage, while the supplementation at 75% promoted inflammation, consistent with the increased ALT and AST levels and AST/ALT ratios observed in the SPC75 diet.

Table 1 Growth and biometry of juvenile hybrid grouper fed with different diets

Items	Diets		
	CK	SPC30	SPC75
Growth performance			
Wi (g)	7.97 ± 0.00	8.04 ± 0.03	8.01 ± 0.01
Wr (g)	41.29 ± 0.66^{a}	39.34 ± 0.54^{b}	19.53 ± 0.32^{c}
BWG (g)	33.32 ± 0.60^{a}	31.30 ± 0.60^{b}	11.52 ± 0. 32^{c}
WGR (%)	3.76 ± 0.13^{a}	3.62 ± 0.12^{a}	1.04 ± 0.06^{b}
SGR (% d^{-1})	3.42 ± 0.02^{a}	3.24 ± 0.06^{b}	0.78 ± 0.07^{c}
PER	1.69 ± 0.07^{b}	2.21 ± 0.09^{a}	1.11 ± 0.07^{c}
DFI (% d^{-1})	3.98 ± 0.11^{a}	2.95 ± 0.08^{c}	3.09 ± 0.07^{b}
FCR	1.27 ± 0.05^{b}	0.96 ± 0.04^{c}	1.96 ± 0.13^{a}
Survival	91.11 ± 1.92^{a}	94.44 ± 1.93^{a}	85.56 ± 1.93^{b}
Biometric indices			
CF (g cm^{-3})	1.73 ± 0.13^{a}	1.61 ± 0.19^{a}	1.41 ± 0.20^{b}
HSI (%)	2.97 ± 0.38^{a}	2.17 ± 0.56^{b}	2.12 ± 0.61^{b}
IPR (%)	1.41 ± 0.41^{b}	2.03 ± 0.58^{a}	0.93 ± 0.57^{c}

The values (mean ± SD; $n = 3$) within the same row with different letters are significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments

Data referenced from Chen Y, Ma J, Huang H, Zhong H. Effects of the replacement of fishmeal by soy protein concentrate on growth performance, apparent digestibility, and retention of protein and amino acid in juvenile pearl gentian grouper. PLoS One. 2019;14(12):e0222780. Published 2019 Dec 23. doi:https://doi.org/10.1371/journal.pone.0222780

Table 2 Serum biochemistry of juvenile hybrid grouper fed with different diets

Items	Groups		
	CK	SPC30	SPC75
ALT	45.33 ± 2.73^{b}	45.67 ± 2.96^{b}	66.67 ± 1.85^{a}
AST	22.67 ± 0.33^{b}	19.33 ± 0.33^{c}	82.67 ± 0.33^{a}
AST/ALT	0.53 ± 0.33^{b}	0.43 ± 0.33^{b}	1.23 ± 0.33^{a}
TP	37.23 ± 0.15^{b}	36.37 ± 0.03^{c}	55.77 ± 0.24^{a}
GLB	21.53 ± 0.12^{a}	21.73 ± 0.03^{a}	19.93 ± 0.21^{b}
ALB	15.70 ± 0.15^{b}	14.63 ± 0.03^{c}	35.83 ± 0.09^{a}
ALB/GLB	0.70 ± 0.00^{b}	0.70 ± 0.00^{b}	1.80 ± 0.13^{a}
GLU	2.30 ± 0.00^{b}	1.56 ± 0.03^{c}	2.40 ± 0.03^{a}
URE	2.30 ± 0.00^{b}	2.37 ± 0.03^{b}	3.43 ± 0.06^{a}
TG	0.68 ± 0.03^{a}	0.56 ± 0.01^{b}	0.58 ± 0.02^{b}
CHOL	7.20 ± 0.03^{a}	3.83 ± 0.02^{b}	2.45 ± 0.01^{c}
HDL (mmol/L)	2.18 ± 0.03^{a}	2.23 ± 0.07^{a}	1.91 ± 0.01^{b}
LDL (mmol/L)	4.21 ± 0.01^{a}	2.03 ± 0.01^{b}	0.59 ± 0.01^{c}

Values (mean ± SD; $n = 3$) within the same row with different letters were significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments

AKP alkaline phosphatase, *ALB* albumin, *ALT* alanine transaminase, *AST* aspartate aminotransferase, *CHOL* cholesterol, *GLB* globulin, *GLU* glucose, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *LZY* serum lysozyme, *SOD* superoxide dismutase, *TG* triglyceride, *TP* total protein

RNA-seq and data analysis

The summary of the quality of the sequencing results in CK, SPC30, and SPC75 groups is shown in Table 4. A total of 15.232 GB clean data (101.55 million clean reads, average length = 150 bp/read) were obtained by removing low-quality bases from raw sequences. The number of clean reads in the CK (34.37, 33.86, and 33.53 million for liver, intestine, and muscle samples, respectively), SPC30 (33.86, 34.56, and 34.80 million for liver, intestine, and muscle samples, respectively), and SPC75 (33.53, 34.95, and 34.38 million for liver, intestine, and muscle samples, respectively) groups did not differ by more than 10%.

Redundancy in the transcriptome sequences of the different groups was removed, and rRNA was filtered out to produce a large number of unigenes (119,124). The 31,038 unigenes were ≥ 1000 bp, and 62,976 transcripts were ≥ 500 bp. According to the similarities of sequences in public databases, the functions of all unigenes were annotated (cutoff E-value of 10^{-5}). A total of 137,080 (approximately, 74.4%) transcripts were annotated using the BLASTP program within five public databases, including Nr, Swiss-Prot, KEGG, egg-NOG, and GO. Among these transcripts, 31,734, 28,590, 31,439, 26,061, and 19,256 were identified, respectively. The remaining 64,782 (25.6%) unmapped unigenes were not identified.

The clean reads were quantitatively mapped to the reliable reference transcripts using the RSEM method. A total of 34,142, 37,009, and 33,809 mutual transcripts were available in different groups of the liver, intestine, and muscle, respectively. In the liver, 25,244, 24,607, and 25,862 transcripts were specifically expressed in the CK, SPC30, and SPC75 groups, respectively. Meanwhile, 19,334, 20,093, and 19,916 were shared by at least two of the groups, namely, CK versus SPC30, CK versus SPC75, and SPC30 versus SPC75, respectively (Fig. 3A). In the intestine, 25,963, 27,601, and 29,784 transcripts were specifically expressed in the CK, SPC30, and SPC75 groups, respectively. Meanwhile, 21,353, 21,412, and 23,346 transcripts were shared by at

Table 3 Enzyme activities of juvenile hybrid grouper fed with different diets

Items		Groups		
		CK	SPC30	SPC75
Intestine	Pepsin (U/mg protein)	60.66 ± 0.74^{b}	56.95 ± 0.47^{c}	253.22 ± 1.67^{a}
	Amylase (U/mg protein)	0.46 ± 0.00^{c}	0.60 ± 0.001^{b}	1.46 ± 0.01^{a}
	Lipase (U/mg protein)	849.76 ± 8.99^{a}	717.37 ± 3.50^{b}	391.49 ± 2.97^{c}
Liver	Pepsin (U/mg protein)	203.44 ± 4.05^{a}	98.92 ± 3.00^{b}	31.69 ± 5.14^{c}
	Amylase (U/mg protein)	0.29 ± 0.07	0.25 ± 0.04	0.16 ± 0.00
	Lipase (U/mg protein)	2695.86 ± 12.38^{a}	527.86 ± 12.87^{b}	102.76 ± 10.09^{c}

Values (mean ± SD; $n = 3$) within the same row with different letters were significantly different ($P < 0.05$). The absence of letters indicates no significant difference between treatments

least two of the groups, namely, CK versus SPC30, CK versus SPC75, and SPC30 versus SPC75, respectively (Fig. 3B). In the muscle, 23,111, 28,651, and 21,258 transcripts were specifically expressed in the CK, SPC30, and SPC75 groups, respectively. Of these, 19,573, 17,426, and 18,760 transcripts were shared by at least two of the groups, namely, CK versus SPC30, CK versus SPC75, and SPC30 versus SPC75, respectively (Fig. 3C).

Function enrichment and classification

All transcripts in this study were used for the analysis of function enrichment and classification through GO and KEGG (Fig. 4A and B). The GO annotation provides a controlled vocabulary to describe gene functions. Through alignment of the GO database, 19,256 (10.45%) unigenes were annotated to 51 terms, including cellular components [cell parts (11,448 unigenes), cell (11,250 unigenes), organelles (8413 unigenes), and membranes (6499 unigenes)], molecular function [binding (11,630 unigenes) and catalytic activity (7508 unigenes)], and biological regulation (8720 unigenes) (Fig. 4A).

The KEGG Orthology (KO) system is a classification system of proteins (enzymes) with highly similar sequences and similar functions in the same pathway. KEGG is categorized into a group of proteins and then labeled with KO. Through the alignment of the KEGG database, 19,256 (10.45%) unigenes were also annotated to 44 terms of the GO classification (Fig. 4B).

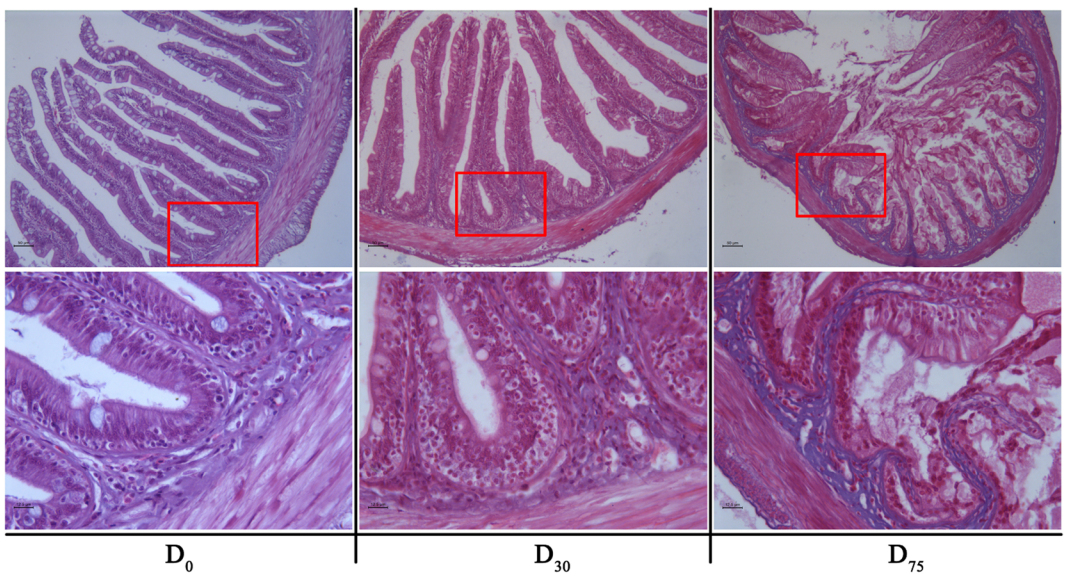

Fig. 1 H&E-stained intestinal tissue sections of juvenile hybrid grouper fed with different diets

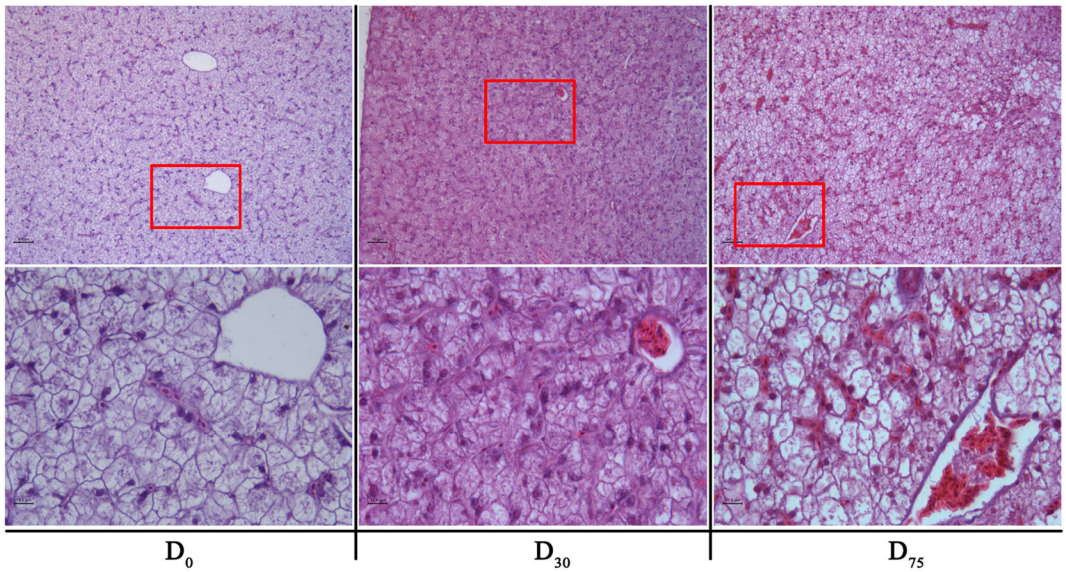

Fig. 2 H&E-stained liver tissue sections of juvenile hybrid grouper fed with different diets

Extensive transcriptional reprogramming and enrichment of functional analysis of DEGs

The summary of the identified DEGs in the intestine, liver, and muscle of juvenile hybrid grouper fed with different diets is presented in Table 5. All transcriptomic responses to the SPC diet were compared with those to the CK diet, with upregulation and downregulation of DEGs in the fish.

In this study, different samples of CK, SPC30, and SPC75 displayed substantially different gene transcription profiles; SPC30 versus SPC75 had 1260, 1731, and 4484 DEGs in the intestine, liver, and muscle, respectively. On a direct comparison of the groups in the intestine, liver, and muscle by GO analysis, the DEGs could be divided into seven major metabolic pathways: (1) transport and catabolism, including secondary metabolites, inorganic ions, lipids, coenzymes, nucleotides, amino acids, and carbohydrates; (2) energy production and conversion; (3) trafficking and protein processing (including posttranslational modifications, protein turnover, chaperones, intracellular trafficking, secretion, and intracellular transport), cytoskeletal rearrangements, and cell membrane biogenesis; (4) signal transduction mechanisms; (5) cell defense mechanisms; (6) cell cycle regulation; and (7) DNA/RNA synthesis, including replication, recombination and repair, transcription, and translational regulation (Fig. 5A). Generally, in the intestine, liver, and muscle, DEGs of all comparisons were mainly distributed in biological regulation, cellular process, metabolic process, single-organism process, cell, cell part, membrane, binding, and catalytic activity (Fig. 5A, B, C). The predominantly enriched DEGs might be directly related to the

Table 4 Basic data of RNA-seq for juvenile hybrid grouper fed with different diets

Sample	Liver			Intestine			Muscle		
	CK	SPC30	SPC75	CK	SPC30	SPC75	CK	SPC30	SPC75
Clean reads ($\times 10^6$)	34.37	33.99	33.19	33.86	34.56	34.80	33.53	34.95	34.38
Average length of reads (bp)	150	150	150	150	150	150	150	150	150
Clean bases (G)	5.156	5.098	4.978	5.078	5.184	5.22	5.03	5.242	5.156
GC (%)	46	47	46	46	47	47	50	49	50
Reads mapping to all unigenes (%)	81.91	80.2	81.53	77.07	79.23	79.49	84.38	85.11	86.64

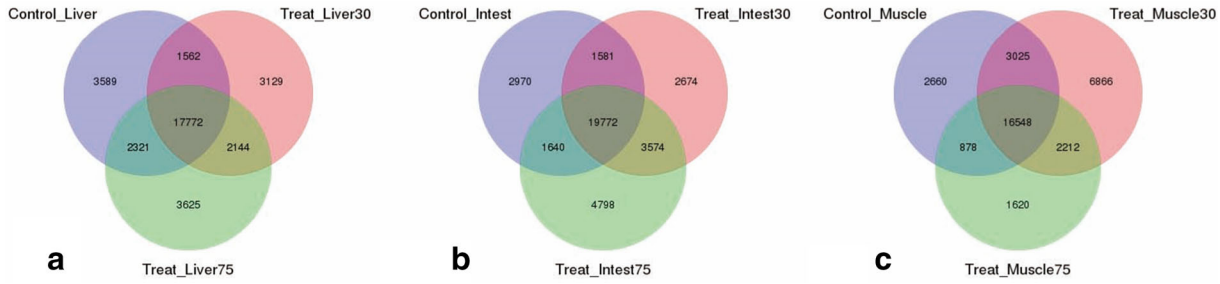

Fig. 3 A Venn diagram showing the number of transcripts in juvenile hybrid grouper fed with different diets by comparing CK versus SPC30, CK versus SPC75, and SPC30 versus SPC75

different effects of SPC30 and SPC75 on fish development and immune response as well as the underlying enzyme activities and histology (Hossain et al. 2018). Notably, the DEGs of CK versus SPC75 groups in the muscle showed no significant differences compared with the other groups (Fig. 5C); the difference of SPC75 might contribute to its effect on physiological response. Then, 30% of SPC might be applicable for fish growth and survival as well as the underlying extensive transcriptional reprogramming in hybrid grouper.

DEGs involved in different pathways

The genes involved in different pathways were chosen for further analysis to investigate the direct link between the DEGs and the effect of dietary SPC. Based on the heat map, multiple genes were significantly affected by SPC30 and SPC75 in the intestine, liver, or muscle (Fig. 6).

Discussion

The RNA-seq is a valuable method to identify novel genes involved in fish dietary responses (Król et al. 2016). In recent years, the need to improve protein supplements that are both cost-effective and can promote fish growth has attracted an increasing interest in the fishing industry (Monteith et al. 2013). SPC is rich in amino acids and fatty acids and provides a cheap nutritional food supplement for fish. However, the response of fish to this dietary protein has been poorly understood. Consistent with previous reports (Dawood et al. 2015; Monteith et al. 2013), SPC dietary manipulation had significant effects on multiple physiological responses. Moreover, the RNA-seq technology was applied in this study for an in-depth analysis of the molecular responses of fish to dietary SPC. Functional annotations were employed that provided valuable information on fish cellular processes, molecular functions, and biological pathways that changed in response to SPC.

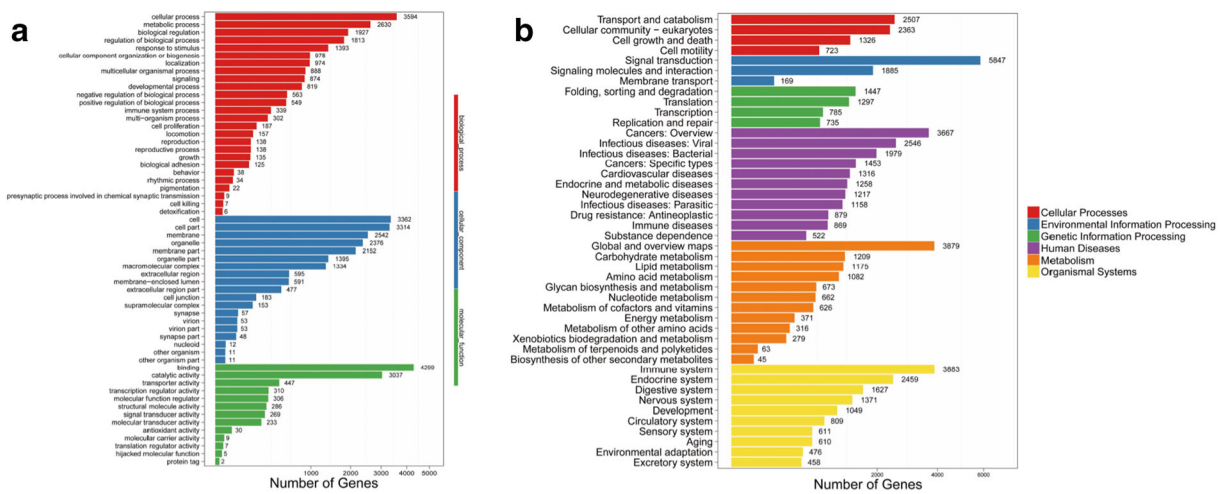

Fig. 4 (A) GO classifications of all unigenes of hybrid grouper. (B) KEGG classifications of all unigenes of hybrid grouper

Table 5 Summary of the identified DEGs for juvenile hybrid grouper fed with different diets

DEG set	All DEGs	Downregulated	Upregulated
CK_vs_SPC30 (intestine)	1793	950	843
CK_vs_SPC75 (intestine)	2102	1274	828
SPC30_vs_SPC75 (intestine)	1260	731	529
CK_vs_SPC30 (liver)	2048	993	1055
CK_vs_SPC75 (liver)	1927	1043	884
SPC30_vs_SPC75 (liver)	1731	964	767
CK_vs_SPC30 (muscle)	3825	917	2908
CK_vs_SPC75 (muscle)	847	471	376
SPC30_vs_SPC75 (muscle)	4484	3331	1153

Using the GO function ontology, the major classifications of the DEGs were metabolic enzymes and cell transport processes. Besides lipid and protein enzyme activity and metabolism, the transport of substances into and out of the cells and the regulation of cell-based secretory and trafficking processes (posttranslational modifications and cell cycle regulation) might also be essential components of the response of fish to SPC. The transcriptional reprogramming and physiological response by SPC might be directly related to its effects on the growth performance, digestibility, and immunity of fish (Hossain et al. 2018; Kokou et al. 2017; López et al. 2015; Metochis et al. 2016; Perera and Yúfera 2016).

DEGs involved in growth performance

In a previous study, the hybrid grouper had a limited ability to use SPC as a protein source, and the FM replacement with SPC was less than 30% (FM 45.5 g 100 g^{-1} and SPC 18 g 100 g^{-1}) (Chen et al. 2019). Moreover, the protein content in the muscle of fish fed with the SPC75 diet was affected based on the concentration of dietary SPC ($P < 0.05$), while other compositions were not influenced compared with those of CK and SPC30.

The sarcomeric structure is the basic contractile unit of skeletal muscle (Squire et al. 1997) and is made of different structural proteins such as troponin T and C (*TnT* and *TnC*) (Vasatova et al. 2013; Gillis et al. 2007), tropomyosin (Perry 2001), and myosin light chain 2 and 3 (Stevens et al. 2013; Kurabayashi et al. 1988). The expression of genes of *myosins*, *TnT* and *TnC*, and *alpha-actinin-2* and *alpha-actinin-3* was downregulated in the muscles of fish fed with the SPC30 diet. Some studies reported that oocyte development might cause a change in the body shape and increase drug resistance and muscle atrophy (Salem et al. 2006a, b). The data on muscle atrophy–related genes in the present study led to the aforementioned conclusion.

Muscle atrophy is a result of various factors, such as diseases, disuse, and nutrition (Bajotto and Shimomura 2006). Muscle atrophy can be influenced by protein-related synthesis and degradation pathways. The mammalian target of rapamycin (*mTOR*) signaling pathway can regulate protein biosynthesis through phosphate downstream regulators such as *4E-binding protein* and *70-kDa ribosomal protein S6 kinases* (Hay and Sonenberg 2004). Under the downstream *mTOR* signaling pathway, *eukaryotic initiation factor 4E* (*eIF4E*) is responsible for the modulation of protein synthesis (Bodine et al. 2001). An increase in the amount of *translation initiation factor 4E-binding protein* (*4EBP1*) bound to *eIF4E* was found in the gastrocnemius muscle of rats suffering from unloading-induced atrophy, indicating the relationship between *eIF4E* and atrophy (Bodine et al. 2001). Protein degradation also could induce atrophy. Three main proteases, including *cytosolic* $Ca2^{+}$*-dependent protease* (*calpains*), *lysosomal protease* (*cathepsins*), and *adenosine triphosphate* (*ATP*)*-dependent protease* (*ubiquitin–proteasome degradation*), were widely studied, and the results suggested that each of them could cause atrophy in different ways (Bajotto and Shimomura 2006). In previous studies, the levels of *calpains* dramatically increased in the atrophying muscle (Tischler et al. 1990; Taillandier et al. 1996). *Cathepsins* are responsible for degrading protein in the cell membranes, such as receptors, transporters, ligands, and channels (Jackman and Kandarian 2004). They may get involved in oxidative stress-induced myocyte apoptotic feedback (Brunk and Svensson 1999) due to altered calcium homeostasis (Hunter et al. 2001; Lawler et al. 2003). The gastrocnemius muscle atrophy *F-box/Atrogin-1* and *muscle ring finger 1* (*MuRF1*) are two muscle-specific ubiquitin ligases found to be related to muscle atrophy in a number of previous studies (Bajotto and Shimomura 2006; Gomes et al. 2001). In the present study, the expression

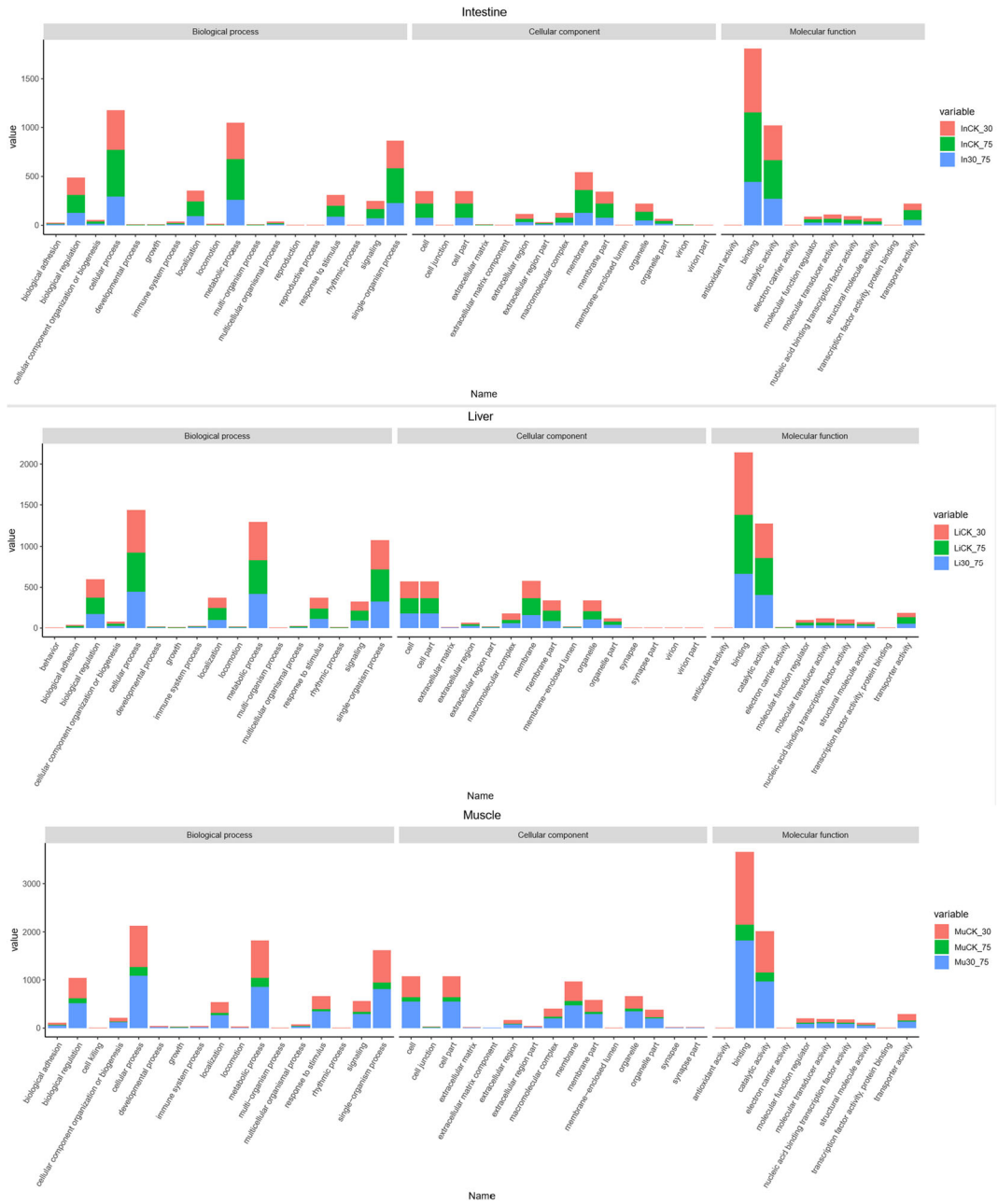

Fig. 5 GO classifications of DEGs in the intestine (A), liver (B), and muscle (C) of juvenile hybrid grouper fed with different diets by comparing CK versus SPC30, CK versus SPC75, and SPC30 versus SPC75

of *eIF4E*, *calpain*, *p38*, *cathepsin*, and *caspase* was found to be upregulated, the expression of *atrogin-1* and *MuRF1* was downregulated in the muscle of fish fed with the SPC30 diet, while the expression of *atrogin-1* in the muscle of fish fed with the SPC75 diet was upregulated. *Myeloperoxidase* (*MPO*) is involved in respiratory burst and released into the phagosome during the degranulation process by neutrophils (Klebanoff 2005). It produces hypochlorous acid through catalytic oxidation of chlorine ions in phagocytic cells to kill microorganisms, destroys a variety of target substances, and plays a role in the production and regulation of inflammatory response in the body. *MPO* in the muscle of fish fed with the SPC30 diet indicated

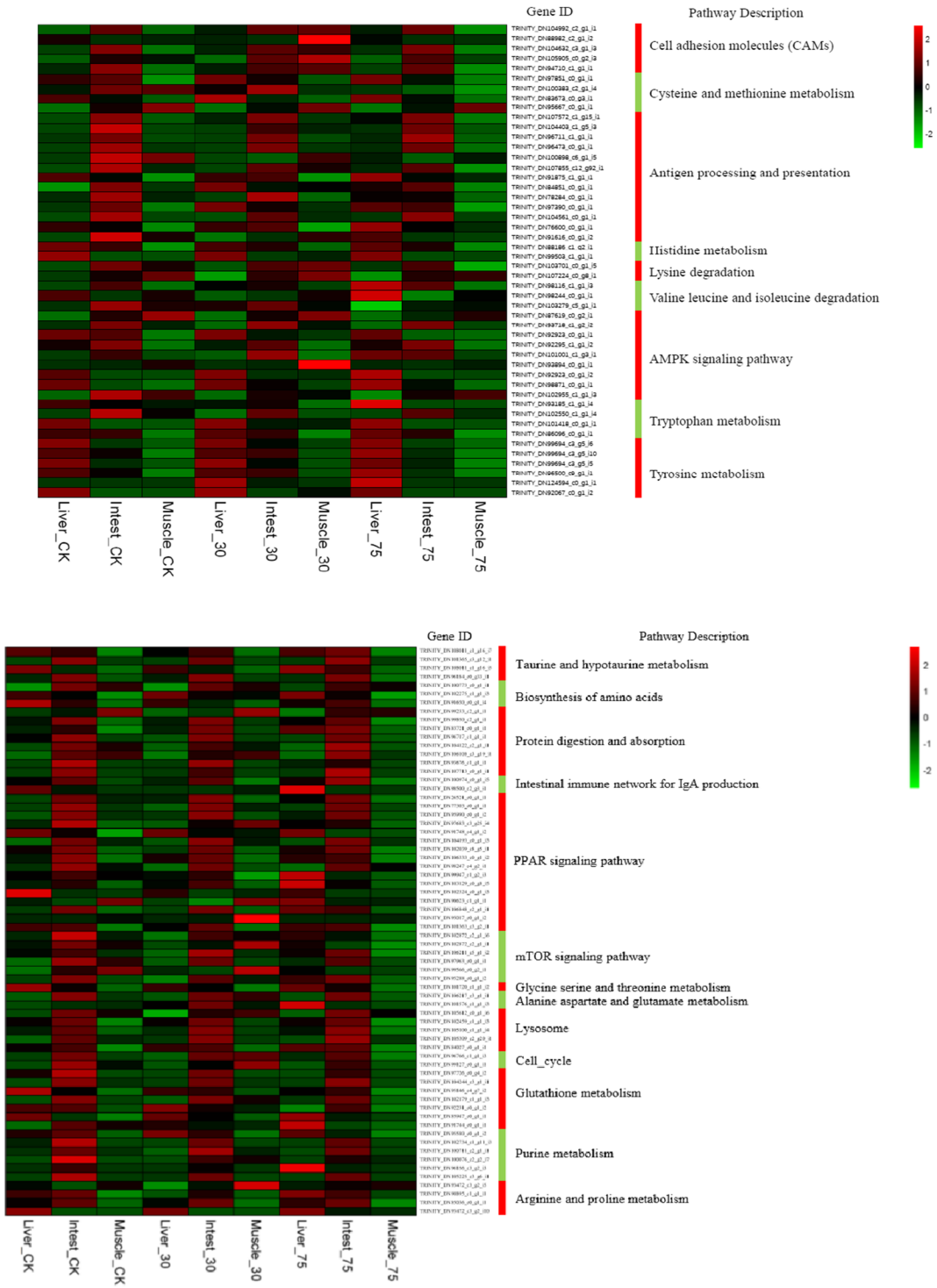

Fig. 6 Heat map of expression levels of DEGs related to different pathways in the hybrid grouper

that the filtration of neutrophils also ameliorated muscle autophagy (Dumont and Frenette 2010). In the SPC75 group, the expression of *atrogin-1* was upregulated, which was greatly responsible for muscle atrophy; the expression of other related genes was downregulated but was not statistically significantly different compared

with that of SPC30. Besides, no inflammation was found in the muscle of fish fed with all the diets; therefore, *atrogin-1* might be highly related to diet-induced muscle autophagy.

DEGs involved in metabolism

In a previous study, with the increasing levels of SPC in diets, the sum of essential amino acids (ΣEAA) decreased and was the lowest in the muscle of fish fed with the SPC75 diet. The muscle of fish fed with the SPC15 and SPC45 diets had the highest sum of amino acids (ΣAA) and the sum of non-essential amino acids (ΣNEAA) ($P < 0.05$), respectively (Chen et al. 2019). Consistently, SPC was found to initiate a regulatory system involving thousands of genes that promote protein and lipid metabolism and cellular rearrangements, especially the DEGs involved in amino acid metabolism and digestive system. For example, *acsbg2*, involved in fatty acid transport, and *carnitine palmitoyltransferase 1* (*CPT1A*) and *acyl-CoA dehydrogenase family member 11(ACAD11)*, involved in fatty acid oxidation, were differentially expressed by SPC, highlighting the modulatory effect of SPC on lipid metabolism. Several genes involved in amino acid biosynthesis, including valine, leucine, isoluecine, arginine, and glutamine synthesis, were also commonly regulated by SPC. Recently, Wu et al. (2018) found that dietary arginine had significant effects on the growth, stress resistance, and immunity of hybrid grouper. Thus, the DEGs involved in arginine biosynthesis might be directly related to the effect of SPC. Moreover, the DEGs involved in the digestive system might also contribute to the underlying enzyme activities.

A previous study (Chen et al. 2019) showed that the levels of alanine, glycine, lysine, methine, and valine decreased with an increase in dietary SPC, compared with the CK group. After the feeding experiment (42 days), free lysine, aspartic acid, glutamic acid, isoleucine, leucine, valine, and alanine levels in the muscle of fish significantly decreased with an increase in dietary SPC ($P < 0.05$). Therefore, how the hybrid grouper compensates for the effect of the imbalanced diet is worth investigating from the genetic aspects. In the SPC30 group, the expression of *ALT*, *glycine hydroxymethyltransferase*, and *methionine synthase* was upregulated, indicating that compared with CK, the SPC30 group had higher levels of free alanine, glycine, and methine levels. Although the expression of other genes, such as *alanine aminotransferase*, *serine hydroxymethyltransferase*, *argininosuccinate synthase*, *tyrosine aminotransferase*, and *phenylalanine-4-hydroxylase*, was upregulated, they still could not compensate for the deficiency of amino acids in the diet. However, in the SPC70 group, most of these genes returned to the normal expression level and the amino acid concentration decreased. Interestingly, although the levels of total amino acids changed, the levels of protein-bound amino acids did not change significantly, implying that free amino acids were consumed when diets contained the imbalanced amino acids.

The DEGs, including *branched-chain amino acid aminotransferase* (*TRINITY_DN99298_c1_g2_i1*) involved in valine, leucine, and isoleucine synthesis, *argininosuccinate lyase* (*TRINITY_DN100244_c0_g2_i4*) involved in arginine synthesis, *acsbg2* involved in fatty acid transport, and *CPT1A* and *acetyl-coenzyme A acyltransferase (THIM)* involved in fatty acid oxidation, might contribute to the modulation of amino acid metabolism and digestive system in SPC30 and SPC75 samples. Glutamic acid and glycine are mainly related to sweetness (Konosu and Yamaguchi 1982). A previous study (Chen et al. 2019) showed that the levels of glutamic acid decreased with the increase in dietary SPC; however, the levels of glycine increased dramatically in the SCP30 group but decreased in the SCP75 group. Glutamic acid and glycine might make the dietary palatability poor in SPC75, but not in SPC30, which was included with the DFI in a previous study (Chen et al. 2019). In conclusion, besides the change in the muscle, the flavor of this sample fish might be impaired and the economical value damaged.

DEGs involved in digestive enzymes

The activities of digestive enzymes in the gastrointestinal tract typically change with feeding habits. Pepsin is the major stomach protease in fish, with optimum activity at pH 2.0–3.3 (Nilsson and Fänge 1969). In the present study, higher pepsin and amylase activities were found in the intestine of fish fed with the SPC75 diet, suggesting that higher levels of digestive enzymes ensured the full use of the SPC in the diets of hybrid grouper, consistent with other fish species (Monteith et al. 2013). Additionally, the induction of lipase activities decreased in the SPC diet, which might be linked to the levels of dietary lipids in the SPC groups.

The intestine is the main organ for nutrition digestion and absorption. In the fish intestine, the expression of *pancreatic alpha-amylase* (*AMYP*) is related to carbohydrate degradation (Janecek et al. 2014). Lipases, such as c*olipase-dependent pancreatic lipase* (*PLRP1*), *bile salt-activated lipase* (*CEL*), and *phospholipase A2* (*PLA2*), are secreted for making the lipids more absorbable (Berton et al. 2009; Xu et al. 2009). The nutrition component in diets could affect the digestive enzyme activities in the intestine of fish by changing the uptaking efficiency via altering the microenvironment of the intestine (Miao et al. 2018; Gatesoupe et al. 2018) or even impairing it to cause inflammation (López et al. 2015). Diets for fish with high levels of proteins led to inflammation or poor growth rate, and the fish responded to it through compensatory behaviors by improving the digestive enzyme secretion, facilitating the transportation, and enhancing the biosynthesis (Król et al. 2016; Kemski et al. 2020). In the present study, the result of KEGG enrichment revealed a dramatic effect of the pathways of pancreatic and bile secretions. In this significant enrichment pathway, an increased activity of pancreatic secretion pathway was found in the SPC30 and SPC75 groups in response to the imbalanced nutrition in the soy protein diet as the soy protein lacked lysine and methionine, which could impair the food uptaking ratio (Rumsey et al. 1995). Besides, in this study, the expression of *amylase* improved in the SPC diet, which was similar to the results of other studies in which plant materials were added to the fish feed (Ali and Kaviraj 2018; Goswami et al. 2020). It might explain that although the anti-nutrition factors in plants significantly influenced the activities of amylase and trypsin (Zevallos et al. 2017), the increased levels of amylase and trypsin compensated the low catalytic quality and led to no significant difference in the final total weight. Similarly, the expression of lipase genes, such as *PLRP*, *CEL*, and *PLA2*, was upregulated, because of the same reason as for amylase and trypsin. Besides, the expression of *hydroxy-3-methyl glutaryl coenzyme A reductase* (*HMG-CoA*), the main cholesterol synthesis gene, in the intestine increased to compensate for the cholesterol deficiency in the SPC diet because the plant-based diet contained plant sterols exclusively and lacked cholesterol (Xu et al. 2019). Although studies showed that both lipid digestion and transportation improved to compensate for the cholesterol deficiency (Gu et al. 2014), the biosynthesis of lipoproteins and the transportation of fatty acids were regulated by the expression of related genes. *Apolipoprotein AI* (*ApoA-1*) works with gene apolipoprotein *B-100-like* (*apoB*), *apolipoprotein A-IV* (*ApoA-4*), *microsomal triglyceride transfer protein* (*mtp*), *acyl-CoA cholesterol acyltransferase* (*acat*), *choline kinase* (*chk*), and *choline-phosphate cytidylyltransferase* (*pcyt1a*) to assemble lipoproteins, and genes like cluster of *differentiation 36* (*cd36*), *fatty acid transport protein* (*fatp*), and fatty acid-binding protein like *fabp2a1* are responsible for fatty acid transportation (Gu et al. 2014). Gu et al. (2014) found that the levels of all these genes increased at different levels when the plant meal was added to the diets for salmon. However, in the present study, an opposing expression pattern of these genes was detected in the intestine. Besides, in the lipoprotein assembly group, the expression of all genes except *mtp* and *acat* was downregulated, and the expression of some genes such as ApoA-1 decreased dramatically in the intestine and ApoA-4 decreased dramatically in the livers of fish fed with the SPC75 diet. This might be because high activities of fatty acid metabolism were responsible for the plant oil in diets.

DEGs involved in inflammatory processes

Previous studies suggested that ≤ 50% SPC content was required to maintain fish growth and survival (Dawood et al. 2015; Hossain et al. 2018; Kokou et al. 2017; López et al. 2015; Metochis et al. 2016; Monteith et al. 2013; Perera and Yúfera 2016). In this study, the comparison between SPC30 and SPC75 groups in terms of physiological and transcriptomic responses might provide new clues to explain why SPC ≥ 50% was not well tolerated. Unlike the SPC30 group, fish in the SPC75 group showed signs of intestinal and liver damage. Although SPC30 had no significant effect on inflammatory processes and tissue damage, SPC75 promoted inflammation, consistent with the increased ALT and AST levels and AST/ALT ratios observed in the SPC75 diet.

Previous studies reported that dietary replacement of FM by SPC increased plasma globulin level and decreased ALB/globulin ratio in totoaba juveniles (*Totoaba macdonaldi*), suggesting that fish fed with the SPC diet might have an intestinal inflammatory process in relation to the detrimental effects of antinutrients or interactions between them (López et al. 2015; Ostaszewska et al. 2005; Escaffre et al. 2007; Urán et al. 2008). In this study, the adipocytes were

positively related to the substitution of the SPC level in diets, consistent with the histology result. Leptin is an adipokine secreted mainly by adipocytes (Asaoka et al. 2013), and the elevation in leptin level is frequently found in nonalcoholic steatohepatitis (Huang et al. 2008). In this study, the expression of leptin was found to be upregulated in the SPC75 group and downregulated in the SPC30 group. Other fatty acid–related genes included *CDP-diacylglycerol-inositol 3-phosphatidyltransferase* (*cdipt*). Studies reported that during a screen for liver defects from a zebrafish insertional mutant library, the mutant cdipt hi559Tg/+ (hi559) was isolated. *Cdipt* is known to play an indispensable role in phosphatidylinositol (PtdIns) synthesis. Besides, *cdipt* is expressed in the developing liver, and its disruption in hi559 mutants abrogates de novo PtdIns synthesis, resulting in hepatomegaly at 5 days postfertilization. The hi559 hepatocytes display features of nonalcoholic fatty liver disease(NAFLD), including macrovesicular steatosis, ballooning, and necroapoptosis(Thakur et al. 2011). *Sec63* is a member of the endoplasmic reticulum translocation machinery, and mutation of *Sec63* might be responsible for endoplasmic reticulum (ER) stress in polycystic liver disease (Thakur et al. 2011). These two genes were detected in the present study. Although the expression of both of them was upregulated in the SPC30 and SPC75 groups (FLog > 1.5), it was not as significantly upregulated as that of the other genes ($P < 0.001$). This was because the liver was abnormal and tried to prevent fatty acid accumulation and adipocytosis as a compensatory effect. Moreover, *sterol regulatory element-binding proteins* (*SREBP1c*) promoted the expression of lipogenic genes fatty acid synthase (*FAS*) and *ACOT4 acyl-coenzyme A thioesterase* from the KEGG result to regulate both saturated and unsaturated fatty acid biosynthesis, which were upregulated in the SPC groups. Under the regulation of *FAS* and *ACOT4 acyl-coenzyme A thioesterase*, fatty acids were synthesized and elongated in the liver of fish fed with the SPC diet.

In the present study, the intestine and liver showed a very high inflammatory response in fish fed with the SPC75 diet, which was consistent with the *MPO* detection. The expression of *MPO* was dramatically upregulated only in the muscle in the SPC30 group, followed by that in the SPC75 group, indicating higher neutrophil infiltration to the liver and intestine compared with the muscles.

Among interleukin (IL) families, IL-8 is responsible for attracting neutrophils to a site of inflammation (Jimenez et al. 2006). Interestingly, two types of IL-8 were detected in this study: one (*TRINITY_DN92154_c0_g1_i1*) was active in the intestine and downregulated with the increase in the SPC diet, while another (*TRINITY_DN106095_c2_g1_i2*) was active in the liver and positively related to the SPC diet. However, none showed activation in muscle samples. Also, two other IL proteins and two IL receptors were detected whose pattern changed besides that of IL-8. The change in IL and its related proteins and receptors indicated that they were responsible for the inflammation in the liver of this fish.

Ferritin heavy chain is an important inflammation indicator protein upregulated in papillomavirus-associated urothelial tumors of the urinary bladder in cattle (Roperto et al. 2010). In the present study, the expression of ferritin heavy chain (*TRINITY_DN68053_c0_g1_i1*) was found to be upregulated in the intestine of fish fed with the SPC30 and SPC75 diets and in the liver of fish fed with the SPC30 diet but was downregulated in the muscle of fish fed with the SPC30 diet, indicating that the inflammation in the intestine was higher than that in the liver and muscle of this fish.

Although the in vivo roles of these DEGs need to be further investigated, the identification of these DEGs and the underlying GO enrichment might inform about the conventional FM diets that aim to improve costs, fish growth, and survival within the fishing industry. The comprehensive physiological and transcriptomic comparison among the CK, SPC30, and SPC75 diets might also be effective in testing the applicability of SPC as fish feed and designing a proper diet with the best impact on fish development and health.

Acknowledgments This study was supported by the Natural Science Foundation of Hainan Province (No. 20163074, No. 20163071, and No. 2019RC246), the Major Science and Technology Projects in Hainan Province from Science and Technology Department of Hainan Province government, China (No. ZDKJ2016009), the Hainan Key Laboratory for Conservation and Utilization of Tropical Marine Fishery Resources, and Sanya Key Laboratory of Seawater Aquaculture Research (No. L1507).

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical statement All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The use of animal was approved by the

Institutional Animal Care and Use Committee of Hainan Tropical Ocean University.

References

Ali S, Kaviraj A (2018) Aquatic weed *Ipomoea aquatica* as feed ingredient for rearing rohu, *Labeo rohita* (Hamilton). Egypt J Aquat Res 44:321–325. https://doi.org/10.1016/j.ejar.2018.09.004

Antony Jesu Prabhu P, Kaushik SJ, Mariojouls C, Surget A, Fontagné-Dicharry S, Schrama JW, Geurden I (2015) Comparison of endogenous loss and maintenance need for minerals in rainbow trout (Oncorhynchus mykiss) fed fishmeal or plant ingredient-based diets. Fish Physiol Biochem 41(1):243–253

Asaoka Y, Terai S, Sakaida I, Nishina H (2013) The expanding role of fish models in understanding non-alcoholic fatty liver disease. Dis Model Mech 6(4):905–914

Bajotto G, Shimomura Y (2006) Determinants of disuse-induced skeletal muscle atrophy: exercise and nutrition countermeasures to prevent protein loss. J Nutr Sci Vitaminol 52(4):233–247

Bañuelos-Vargas I, López LM, Pérez-Jiménez A, Peres H (2014) Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on hepatic intermediary metabolism and antioxidant status of totoaba juveniles (Totoaba macdonaldi). Comp Biochem Physiol B Biochem Mol Biol 170:18–25

Berton A, Sebban-Kreuzer C, Rouvellac S, Lopez C, Crenon I (2009) Individual and combined action of pancreatic lipase and pancreatic lipase-related proteins 1 and 2 on native versus homogenized milk fat globules. Mol Nutr Food Res 53(12):1592–1602

Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol 3:1014–1019

Bonvini E, Bonaldo A, Mandrioli L, Sirri R, Dondi F, Bianco C, Fontanillas R, Mongile F, Gatta PP, Parma L (2018) Effects of feeding low fishmeal diets with increasing soybean meal levels on growth, gut histology and plasma biochemistry of sea bass. Animal. 12(5):923–930

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brunk UT, Svensson I (1999) Oxidative stress, growth factor starvation and Fas activation may all cause apoptosis through lysosomal leak. Redox Rep 4:3–11

Chen Y, Ma J, Huang H, Zhong H (2019) Effects of the replacement of fishmeal by soy protein concentrate on growth performance, apparent digestibility, and retention of protein and amino acid in juvenile pearl gentian grouper. PLoS One 14(12):e0222780. https://doi.org/10.1371/journal.pone.0222780

Dawood MA, Koshio S, Ishikawa M, Yokoyama S (2015) Effects of partial substitution of fish meal by soybean meal with or without heat-killed Lactobacillus plantarum (LP20) on growth performance, digestibility, and immune response of amberjack, Seriola dumerili juveniles. Biomed Res Int 2015:514196

Ding M, Li W, Zhang Y, Wang X, Zhao A, Zhao X, Wang P, Sheng QH (2010) Amino acid composition of lactating mothers' milk and confinement diet in rural North China. Asia Pac J Clin Nutr 19(3):344–349

Drury RA, Wallington EA (1980) Carleton's histological techniques, 5th edn. Oxford University Press, New York, p 195

Dumont NA, Frenette J (2010) Macrophages protect against muscle atrophy and promote muscle recovery in vivo and in vitro: a mechanism partly dependent on the insulin-like growth factor-1 signaling molecule. Am J Pathol 176(5):2228–2235

Escaffre AM, Kaushik S, Mambrini M (2007) Morphometric evaluation of changes in the digestive tract of rainbow trout (Oncorhynchus mykiss) due to fish meal replacement with soy protein concentrate. Aquaculture 273:127–138

Gatesoupe F-J, Fauconneau B, Deborde C, Madji Hounoum B, Jacob D, Moing A, Corraze G, Médale F (2018) Intestinal microbiota in rainbow trout, Oncorhynchus mykiss, fed diets with different levels of fish-based and plant ingredients: a correlative approach with some plasma metabolites. Aquac Nutr 24(5):1563–1576

Gillis TE, Marshall CR, Tibbits GF (2007) Functional and evolutionary relationships of troponin C. Physiol Genomics 32(1):16–27

Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL (2001) Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc Natl Acad Sci U S A 98:14440–14445

Gonzalez EG, Beerli P, Zardoya R (2008) Genetic structuring and migration patterns of Atlantic bigeye tuna, Thunnus obesus (Lowe, 1839). BMC Evol Biol 8:252

Goswami RK et al (2020) Growth and digestive enzyme activities of rohu *Labeo rohita* fed diets containing macrophytes and almond oil-cake. Anim Feed Sci Technol

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29(7):644–652

Gu M, Kortner TM, Penn M, Hansen AK, Krogdahl Å (2014) Effects of dietary plant meal and soya-saponin supplementation on intestinal and hepatic lipid droplet accumulation and lipoprotein and sterol metabolism in Atlantic salmon (*Salmo salar* L.). Br J Nutr 111:432–444

Guerreiro I, Couto A, Pérez-Jiménez A, Oliva-Teles A, Enes P (2015) Gut morphology and hepatic oxidative status of European sea bass (Dicentrarchus labrax) juveniles fed plant feedstuffs or fishmeal-based diets supplemented with short-chain fructo-oligosaccharides and xylo-oligosaccharides. Br J Nutr 114(12):1975–1984

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, Orvis J, Pochet N, Strozzi F, Weeks N, Westerman R, William T, Dewey CN, Henschel

R, LeDuc RD, Friedman N, Regev A (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc 8(8):1494–1512

Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. Genes Dev 18:1926–1945

Hossain MS, Koshio S (2017) Dietary substitution of fishmeal by alternative protein with guanosine monophosphate supplementation influences growth, digestibility, blood chemistry profile, immunity, and stress resistance of red sea bream, *Pagrus major*. Fish Physiol Biochem 43(6):1629–1644

Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM, Islam MJ (2018) Fishmeal replacement by soya protein concentrate with inosine monophosphate supplementation influences growth, digestibility, immunity, blood health, and stress resistance of red sea bream, Pagrus major. Fish Physiol Biochem. https://doi.org/10.1007/s10695-018-0581-2

Huang XD, Fan Y, Zhang H, Wang P, Yuan JP, Li MJ, Zhan XY (2008) Serum leptin and soluble leptin receptor in non-alcoholic fatty liver disease. World J Gastroenterol 14: 2888–2893

Hunter RB, Mitchell-Felton H, Essig DA, Kandarian SC (2001) Expression of endoplasmic reticulum stress proteins during skeletal muscle disuse atrophy. Am J Physiol Cell Physiol 281:C1285–C1290

Imanpoor MR, Bagheri T (2012) Effects of replacing fish meal by soybean meal along with supplementing phosphorus and magnesium in diet on growth performance of Persian sturgeon, Acipenser persicus. Fish Physiol Biochem 38(2):52152–52158

Jackman RW, Kandarian SC (2004) The molecular basis of skeletal muscle atrophy. Am J Physiol Cell Physiol 287:C834–C843

Janecek S, Svensson B, Macgregor EA et al (2014) α-Amylase: an enzyme specificity found in various families of glycoside hydrolases[J]. Cellular and Molecular Life Sciences, 71(7): 1149–1170

Jimenez N, Coll J, Salguero FJ, Tafalla C (2006) Co-injection of interleukin 8 with the glycoprotein gene from viral haemorrhagic septicemia virus (VHSV) modulates the cytokine response in rainbow trout (Oncorhynchus mykiss). Vaccine, 24(27–28):5615–5626

Kemski MM, Rappleye CA, Dabrowski K et al (2020) Transcriptomic response to soybean meal-based diets as the first formulated feed in juvenile yellow perch (Perca flavescens).[J]. Sci Rep:10(1)

Klebanoff SJ (2005) Myeloperoxidase: friend and foe. J Leukoc Biol 77(5):598–625

Kokou F, Sarropoulou E, Cotou E, Kentouri M, Alexis M, Rigos G (2017) Effects of graded dietary levels of soy protein concentrate supplemented with methionine and phosphate on the immune and antioxidant responses of gilthead sea bream (*Sparus aurata* L.). Fish Shellfish Immunol 64:111–121

Konosu SK, Yamaguchi K (1982) The flavor components in fish and shellfish. In: Martin RE (ed) Chemistry and biochemistry of marine food products. Avi Publishing Co., Westport

Król E, Douglas A, Tocher DR, Crampton VO, Speakman JR, Secombes CJ, Martin SA (2016) Differential responses of the gut transcriptome to plant protein diets in farmed Atlantic salmon. BMC Genomics 17:156

Kurabayashi M, Komuro I, Tsuchimochi H, Takaku F, Yazaki Y (1988) Molecular cloning and characterization of human atrial and ventricular myosin alkali light chain cDNA clones. J Biol Chem 263(27):13930–13936

Lawler JM, Song W, Demaree SR (2003) Hindlimb unloading increases oxidative stress and disrupts antioxidant capacity in skeletal muscle. Free Radic Biol Med 35:9–16

Lech GP, Reigh RC (2012) Plant products affect growth and digestive efficiency of cultured Florida pompano (Trachinotus carolinus) fed compounded diets. PLoS One 7(4):e34981

Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. BMC Bioinformatics 12(1):323

López LM, Flores-Ibarra M, Bañuelos-Vargas I, Galaviz MA, True CD (2015) Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (Totoaba macdonaldi). Fish Physiol Biochem 41(4):921–936

Luo Z, Liu YJ, Mai KS, Tian LX, Liu DH, Tian XY, Lin HZ (2005) Effect of dietary lipid level on growth performance, feed utilization and body composition of grouper Epinephelus coioides juveniles fed isonitrogenous diets in floating netcages. Aquac Int 13(3):257–269

Mambrini M, Roem AJ, Carvèdi JP, Lallès JP, Kaushik SJ (1999) Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, Oncorhynchus mykiss. J Anim Sci 77(11):2990–2999

Metochis CP, Spanos I, Auchinachie N, Crampton VO, Bell JG, Adams A, Thompson KD (2016) The effects of increasing dietary levels of soy protein concentrate (SPC) on the immune responses and disease resistance (furunculosis) of vaccinated and non-vaccinated Atlantic salmon (*Salmo salar* L.) parr. Fish Shellfish Immunol 59:83–94

Miao S et al (2018) Dietary soybean meal affects intestinal homoeostasis by altering the microbiota, morphology and inflammatory cytokine gene expression in northern snakehead. Sci Rep 8(1):1–10

Monteith KL, Stephenson TR, Bleich VC, Conner MM, Pierce BM, Bowyer RT (2013) Risk-sensitive allocation in seasonal dynamics of fat and protein reserves in a long-lived mammal. J Anim Ecol 82(2):377–388

Nilsson S, Fänge R (1969) Adrenergic and cholinergic vagal effects on the stomach of a teleost (Gadus morhua). Comp Biochem Physiol 30(4):691–694

Ostaszewska T, Dabrowski K, Palacios ME, Olejniczak M, Wieczorek M (2005) Growth and morphological changes in the digestive tract of rainbow trout (Oncorhynchus mykiss) and pacu (Piaractus mesopotamicus) due to casein replacement with soybean proteins. Aquaculture 245:273–286

Perera E, Yúfera M (2016) Soybean meal and soy protein concentrate in early diet elicit different nutritional programming effects on juvenile zebrafish. Zebrafish 13(1):61–69

Perry SV (2001) Vertebrate tropomyosin: distribution, properties and function. J Muscle Res Cell Motil 22(1):5–49

Roperto S, Borzacchiello G, Brun R, Costanzo F, Faniello MC, Raso C, Rosati A, Russo V, Leonardi L, Saracino D, Turco MC, Urraro C, Roperto F (2010) Ferritin heavy chain (FHC) is up-regulated in papillomavirus-associated urothelial tumours of the urinary bladder in cattle. J Comp Pathol 142(1):9–18

Rumsey GL et al (1995) Soy protein in diets of rainbow trout: effects on growth, protein absorption, gastrointestinal

histology, and nonspecific serologic and immune response. Nutr Utiliz Technol Aquac:166–188

Salem M, Kenney PB, Rexroad CE, Yao J (2006a) Micro-array gene expression analysis in atrophying rainbow trout muscle: a unique nonmammalian muscle degradation model. Physiol Genomics 28:33–45

Salem M, Kenney PB, Rexroad CE, Yao J (2006b) Molecular characterization of muscle atrophy and proteolysis associated with spawning in rainbow trout. Comp Biochem Physiol D 1: 227–237

Shiau SY, Lan CW (1996) Optimum dietary protein level and protein to energy ratio for growth of grouper (*Epinephelus malabaricus*). Aquaculture 145:259–266

Squire, John M (1997) Architecture and function in the muscle sarcomere. Current opinion in structural biology 7(2):247–257

Stevens, Laurence, Bastide Bruno, Hedou Julie, Cieniewski-Bernard Caroline, Montel Valérie, Cochon Laetitia, Dupont Erwan, Mounier Yvonne "Potential regulation of human muscle plasticity by MLC2 post-translational modifications during bed rest and countermeasures." Arch Biochem Biophys 540.1–2 (2013): 125–132, 132

Sun Y, Guo CY, Wang DD, Li XF, Xiao L, Zhang X, You X, Shi Q, Hu GJ, Fang C, Lin HR, Zhang Y (2016a) Transcriptome analysis reveals the molecular mechanisms underlying growth superiority in a novel grouper hybrid (Epinephelus fuscogutatus♀ × E. lanceolatus♂). BMC Genet 17:24

Sun Y, Huang Y, Hu G, Zhang X, Ruan Z, Zhao X, Guo C, Tang Z, Li X, You X, Lin H, Zhang Y, Shi Q (2016b) Comparative transcriptomic study of muscle provides new insights into the growth superiority of a novel grouper hybrid. PLoS One 11(12):e0168802

Taillandier D, Aurousseau E, Meynial-Denis D, Bechet D, Ferrara M, Cottin P, Ducastaing A, Bigard X, Guezennec CY, Schmid HP, Attaix D (1996) Coordinate activation of lysosomal, Ca2-activated and ATP-ubiquitin-dependent proteinases in the unweighted rat soleus muscle. Biochem J 316:65–72

Thakur PC, Stuckenholz C, Rivera MR, Davison JM, Yao JK, Amsterdam A, Sadler KC, Bahary N (2011) Lack of de novo phosphatidylinositol synthesis leads to endoplasmic reticulum stress and hepatic steatosis in cdipt-deficient zebrafish. Hepatology 54:452–462

Tischler ME, Rosenberg S, Satarug S, Henriksen EJ, Kirby CR, Tome M, Chase P (1990) Different mechanisms of increased proteolysis in atrophy induced by denervation or unweighting of rat soleus muscle. Metabolism 39:756–763

Urán PA, Goncalves AA, Taverne-Thiele JJ et al (2008) Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.) [J]. Fish Shellfish Immunol 25(6):0–760

Vasatova M et al (2013) Current applications of cardiac troponin T for the diagnosis of myocardial damage. Adv Clin Chem 61. Elsevier:33–65

Wagner GP, Kin K, Lynch VJ (2012) Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. Theory Biosci 131(4):281–285

Wang L, Park HJ, Dasari S, Wang S, Kocher JP, Li W (2013) CPAT: coding-potential assessment tool using an alignment-free logistic regression model. Nucleic Acids Res 41(6):e74

Ween O, Stangeland JK, Fylling TS, Aas GH (2017) Nutritional and functional properties of fishmeal produced from fresh by-products of cod (*Gadus morhua L.*) and saithe (*Pollachius virens*). Heliyon 3(7):e00343

Wu M, Wu X, Lu S, Gao Y, Yao W, Li X, Dong Y, Jin Z (2018) Dietary arginine affects growth, gut morphology, oxidation resistance and immunity of hybrid grouper (*Epinephelus fuscoguttatus*♀×*Epinephelus lanceolatus*♂) juveniles. Br J Nutr 120(3):269–282

Xiao W, Jiang W, Feng L, Liu Y, Wu P, Jiang J, Zhang Y, Zhou X (2017) Supplementation of enzyme-treated soy protein saves dietary protein and promotes digestive and absorptive ability referring to TOR signaling in juvenile fish. Fish Physiol Biochem 43(6):1657–1675

Xu W, Yi L, Feng Y, Chen L, Liu J (2009) Structural insight into the activation mechanism of human pancreatic prophospholipase A2. J Biol Chem 284(24):16659–16666

Xu Y, Zang K, Liu X, Shi B, Li C, Shi X (2015) Insulin-like growth factors I and II in tarry flounder (*Platichthys stellatus*): molecular cloning and differential expression during embryonic development. Fish Physiol Biochem 41(1): 139–152

Xu W, Jin J, Han D, Liu H, Zhu X, Yang Y, Xie S (2019) Physiological and transcriptomic responses to fishmeal-based diet and rapeseed meal-based diet in two strains of Gibel carp (Carassius gibelio). Fish Physiol Biochem 45(1): 267–286

Yin B, Liu H, Tan B, Dong X, Chi S, Yang Q, Zhang S, Chen L (2018) Cottonseed protein concentrate (CPC) suppresses immune function in different intestinal segments of hybrid grouper ♀Epinephelus fuscoguttatus×♂Epinephelus lanceolatus via TLR-2/MyD88 signaling pathways. Fish Shellfish Immunol 81:318–328

Zevallos VF, Raker V, Tenzer S, Jimenez-Calvente C, Ashfaq-Khan M, Rüssel N, Pickert G, Schild H, Steinbrink K, Schuppan D (2017) Nutritional wheat amylase-trypsin inhibitors promote intestinal inflammation via activation of myeloid cells. Gastroenterology 152(5):1100–1113

Zhou S, Song D, Zhou X, Mao X, Zhou X, Wang S, Wei J, Huang Y, Wang W, Xiao SM, Qin Q (2018) Characterization of Bacillus subtilis from gastrointestinal tract of hybrid Hulong grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) and its effects as probiotic additives. Fish Shellfish Immunol 84: 1115–1124

Zhu Z, Dong C, Weng S, He J (2018) Identification of outer membrane protein TolC as the major adhesin and potential vaccine candidate for Vibrio harveyi in hybrid grouper, Epinephelus fuscoguttatus (♀) × E. lanceolatus (♂). Fish Shellfish Immunol 86:143–151

Zou C, Tan X, Ye H, et al (2018) The hepatoprotective effects of Radix Bupleuri extracts against D-galactosamine/lipopolysaccharide induced liver injury in hybrid grouper (Epinephelus lanceolatus♂ × Epinephelus fuscoguttatus♀). Fish Shellfish Immunol 83:8–17. https://doi.org/10.1016/j.fsi.2018.08.047

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.