Aquaculture Reports 20 (2021) 100710

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Replacing fishmeal with cottonseed protein concentrate in feed for pearl gentian groupers (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂): Effects on growth and expressions of key genes involved in appetite and hepatic glucose and lipid metabolism

Yuanfa He [a,b,c], Xinwei Guo [a,c], Beiping Tan [a,b,c], Xiaohui Dong [a,b,c], Qihui Yang [a,b,c], Hongyu Liu [a,b,c], Shuang Zhang [a,b,c], Shuyan Chi [a,b,c,*]

[a] Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang), Guangdong Ocean University, Zhanjiang, 524088, China
[b] Aquatic Animals Precision Nutrition and High-Efficiency Feed Engineering Research Center of Guangdong Province, Guangdong Ocean University, Zhanjiang, 524088, China
[c] Key Laboratory of Aquatic, Livestock and Poultry Feed Science and Technology in South China, Ministry of Agriculture, Zhanjiang, Guangdong, 524088, China

ARTICLE INFO

Keywords:
Cottonseed protein concentrate
Glucose metabolism
Growth performance
Lipid metabolism
Pearl gentian groupers

ABSTRACT

This study investigated the effects of replacing the fish meal (FM) in a 55 % FM fish feed with cottonseed protein concentrate (CPC) in pearl gentian groupers (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂). Effects on growth and the expression of key genes involved in appetite and hepatic glucose and lipid metabolism Four isoproteic and isolipidic diets were formulated containing 0 %, 6.06 %, 18.18 %, or 30.30 % CPC by replacing 0 % (FM, control), 10 % (CPC10), 30 % (CPC30), and 50 % (CPC50) of the FM in the feed, respectively. Each diet was fed to fish raised in triplicate groups for 56 days. No significant differences in specific growth rate, feed conversion ratio, or survival rate were found among dietary treatments. The weight gain in CPC groups was no different than in controls ($P < 0.05$). Fish fed the CPC50 diet had higher body lipid contents than others ($P < 0.05$) and the highest activity of hepatic fatty acid synthase, which was significantly higher than in group CPC10 ($P < 0.05$). The livers of CPC50 fish had significantly higher mRNA expression levels of apolipoprotein B-100, carnitine palmitoyltransferase 1, peroxisome proliferator-activated receptor α (PPARα), and PPARγ genes compared to other groups except CPC30 ($P < 0.05$). In conclusion, up to 50 % of the FM in fish feed can be replaced with CPC without adversely affecting the growth and feed utilization of pearl gentian groupers. However, 50 % replacement increased body lipid contents via modulation of hepatic lipid metabolism-related genes and enzyme activity.

1. Introduction

Fish can adapt to different nutritional conditions; for example, carnivorous fish can accommodate a certain level of plant protein in their diet (10–60 %) by shifting their metabolic profile (Messina et al., 2007; Ye et al., 2020; Zhang et al., 2019), including the pathways involved in protein and energy utilization (Kissil et al., 2000; Luo et al., 2012; Messina et al., 2007). There is growing evidence that lipid metabolism in carnivorous fish can be modulated by the level and/or source of plant protein in the diet. Soy protein concentrate (SPC) used as a fish meal (FM) replacement significantly reduced the hepatic lipid contents of totoaba juveniles (*Totoaba macdonaldi*), while malic enzyme and fatty acid synthetase activities were not significantly affected by the SPC replacement level (Bañuelos-Vargas et al., 2014). Increased levels of dietary soybean meal (SBM) elevated cholesterol and/or triglyceride contents in Japanese flounder (*Paralichthys olivaceus*; Ye et al., 2011) and cobia (*Rachycentron canadum*; Zhou et al., 2005). In contrast, plasma CHOL and TG levels decreased in Tiger puffer (*Takifugu rubripes*; Lim et al., 2011) and European sea bass (*Dicentrarchus labrax* L.; Messina et al., 2013) when their diets had elevated levels of dietary SBM and/or wheat gluten meal. The high contents of non-starch polysaccharides and several anti-nutritional factors (ANFs) in plant protein sources may

* Corresponding author at: Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang), Guangdong Ocean University, Zhanjiang, 524088, China.
E-mail address: chishuyan77@163.com (S. Chi).

https://doi.org/10.1016/j.aqrep.2021.100710
Received 15 December 2020; Received in revised form 23 April 2021; Accepted 23 April 2021
Available online 4 May 2021

inhibit fish growth and reduce their disease resistance (Gatlin et al., 2007; He et al., 2020; Jiang et al., 2015).

Cottonseed protein concentrate (CPC) is made from common cottonseed meal with low gossypol, and has been used as a substitute for FM in the feed of southern flounder (*Paralichthys lethostigma*; Alam et al., 2018), black sea bass (*Centropristis striata*; Anderson et al., 2016), and pearl gentian groupers (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂; Yin et al., 2018). Our previous studies found that dietary CPC used as FM replacement significantly affects growth, immune function, and intestinal microflora structure in pearl gentian groupers (Chen et al., 2020; Ye et al., 2020; Yin et al., 2018) and juvenile golden pompano (*Trachinotus ovatus*; Shen et al., 2020). However, little attention has been paid to the effects of replacing FM with CPC on lipid and carbohydrate metabolism in carnivorous fish.

It is worth noting that hepatic fructose-1,6-bisphosphatase (FBPase) activity was significantly reduced by dietary replacement of FM with SPC, while glucokinase (GK) activity increased with increasing dietary SPC levels in totoaba juveniles (Bañuelos-Vargas et al., 2014). In general, carnivorous fish can utilize or tolerate far fewer dietary carbohydrates than omnivorous and herbivorous fish (Polakof et al., 2012). Thus, the imbalanced regulation of glycolysis and gluconeogenesis may be partly responsible for glucose intolerance in carnivorous fish (Kamalam et al., 2017; Li et al., 2019). Carbohydrate does not provide energy to carnivorous fish very well, as they are adapted to use amino acids in preference, and require a high level of dietary protein (30–50 %), which is also required for specific physiological functions (National Research Council (NRC, 2011). In trout hepatocytes, either the size of the amino acid pool or specific amino acids (methionine, leucine, and lysine) have been reported to regulate glycolysis and gluconeogenesis (Lansard et al., 2011). Our previous study made an interesting finding—that dietary methionine affected growth and the expression of key genes involved in hepatic glucose metabolism in cobia (Chi et al., 2020). This prompted us to focus on the responses of glycolysis- and gluconeogenesis-related enzymes to dietary plant proteins at the enzymatic or transcriptional level.

In view of the above, we designed an 8-week feeding trial to compare the effects of dietary CPC and FM on pearl gentian groupers. Growth and serum biochemical indexes, as well as gene expressions related to feeding intake and carbohydrate and lipid metabolism, were investigated to explore the influences of using CPC as an FM replacement in the feed of pearl gentian groupers.

2. Materials and methods

2.1. Experimental diets

Our previous study found that, for a diet composed of 50 % FM, replacing 60 % of the FM with CPC did not significantly alter the growth performance of pearl gentian groupers (Ye et al., 2020). Based on this background information, four isoproteic (51 % crude protein) and isolipidic (12 % crude lipid) experimental feeds were formulated by replacing the FM in a 55 % FM feed with various amounts of CPC. Feeds were made with 0 %, 6.06 %, 18.18 %, or 30.30 % CPC by replacing 0 % (FM, control), 10 % (CPC10), 30 % (CPC30), or 50 % (CPC50), respectively, of the FM in the feed (Table 1). Methionine was added to the experimental feeds to improve the amino acid balance (Chi et al., 2015). All ingredients were ground into fine powder and weighed accurately according to the dietary formulas. A fish oil lipid source was added and thoroughly mixed, then purified water and soybean lecithin were added to produce homogenous mixtures. Subsequently, pelleted feeds (2.5 mm diameter) were formed using a pelletizer. The feeds were air-dried and stored at −20 °C until feeding.

2.2. Fish husbandry

All work was approved by the Animal Research and Ethics Committee (AREC) of Guangdong Ocean University, China. Pearl gentian grouper juveniles were obtained from a commercial hatchery (Zhanjiang, China). Healthy and similarly-sized fish (mean ± standard error of the mean, SEM, initial weight = 11.97 ± 0.04 g) were randomly divided into four groups in triplicate. Each group comprised 30 fish, which were acclimated for 1 week before the feeding trial. Fish were fed twice daily at 08:00 and 17:00 until apparent satiation for 8 weeks. The water temperature was 29 ± 1 °C with ammonia and nitrate concentrations < 0.03 mg/L and dissolved oxygen > 5 mg/L.

Table 1
Ingredient composition and nutrient level of the diets (Dry matter %).

Ingredients	Experimental diets			
	FM (Control)	CPC10	CPC30	CPC50
Brown fishmeal	55.00	49.50	38.50	27.50
Cottonseed protein concentrate[a]	0.00	6.06	18.18	30.30
Casein	5.00	5.00	5.00	5.00
Wheat gluten meal	9.44	9.44	9.44	9.44
Wheat flour	16.00	16.00	16.00	16.00
Corn starch	5.69	4.54	2.25	0.00
Fish oil	3.62	4.15	5.20	6.20
Soybean lecithin	2.00	2.00	2.00	2.00
Methionine	0.12	0.18	0.30	0.43
Vitamin premix[b]	0.20	0.20	0.20	0.20
Mineral premix[b]	0.50	0.50	0.50	0.50
Yttrium trioxide	0.10	0.10	0.10	0.10
Others[c]	2.33	2.33	2.33	2.33
Total	100.00	100.00	100.00	100.00
Nutrient level (%)				
Crude protein	50.49	51.24	51.07	50.98
Crude lipid	11.96	11.47	11.81	11.95
Nitrogen-free extract	18.23	20.55	22.48	22.86
Energy	19.88	20.28	20.61	20.66
Ash	11.07	10.55	9.80	8.88
Moisture	12.46	13.60	13.17	13.45

[a] Obtained from Hunan Xinrui Biological Technology Co. Ltd (Hunan, China). Crude protein, 60.81 %; Crude lipid, 0.85 %. Essential amino acid contents: methionine, 0.67 %; lysine, 2.51 %; valine, 2.64 %; isoleucine, 1.92 %; phenylalanine, 3.42 %; leucine, 3.36 %; threonine, 1.86 %; histidine, 1.67 %; arginine, 7.64 %.
[b] Obtained from Qingdao Master Biotech (Qingdao, China).
[c] Others: calcium monophosphate, 1.50 %; attractant, 0.50 %; choline chloride, 0.30 %; antioxidants, 0.03 %.

2.3. Digestibility trial

A digestibility trial was conducted during the feeding trial period. Yttrium trioxide (0.1 %; 99.9 % purity, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was used as an external indicator in the four experimental diets. After two weeks of acclimation to the experimental diet, feces from each group were collected by siphoning 6–7 h after feeding according to our previous method (He et al., 2021). Briefly, once feces were observed, they were immediately collected by gentle siphoning, dried for 6 h at 65 °C, and stored at −20 °C until analysis. Fecal collection continued for ten weeks until 6 g dry weight of fecal material had been collected for chemical analysis.

2.4. Sample collection

After eight weeks, all fish were fasted for 24 h before sampling, then all fish populations were counted and their bodyweights calculated. Three fish from each tank were collected and stored at −20 °C for analysis of whole-body composition. Blood samples were collected from three fish from each tank on ice (to keep the samples fresh) by caudal venipuncture with a 1 mL syringe and then centrifuged at 4 °C (Yin et al., 2020). The serum was collected to determine serum biochemical indexes. The livers of two fish were removed, frozen in liquid nitrogen, and stored at −80 °C for analysis of hepatic enzyme activity. The livers and brains of another two fish were collected and stored at −80 °C for

analysis of relative mRNA expressions.

2.5. Chemical composition analysis

The body composition in fish whole-bodies and the proximate nutrient level of the diets were determined according to standard methods (AOAC, Chem, 1992). Moisture was determined by drying to a constant weight at 105 ℃; crude proteins (N × 6.25) were determined by the Kjeldahl method using an Auto Kjeldahl System (Foss-2300, Sweden) after acid digestion; crude lipids were determined by Soxhlet extraction; and crude ash was determined by calcination at 550 °C in a muffle furnace.

2.6. Hepatic enzyme activity analysis

Levels of hepatic carnitine palmitoyltransferase 1, lipoprotein lipase, fatty acid synthase, and hepatic lipase were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-link Biotech Co., Ltd., Shanghai, China) following the manufacturer's instructions. The four enzyme activities were determined by a double-antibody sandwich method using ELLSA kits (Nos. ml036411, ml036370, ml036373, and ml451004). All enzyme activities were recorded as specific activity (U g^{-1} tissue) liver contents.

2.7. Serum biochemical indices analysis

The levels of triglyceride, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, and D-lactic acid in serum were measured using commercial reagent kits (Shanghai Enzyme-link Biotech Co., Ltd., Shanghai, China). Samples were measured in duplicate.

2.8. Real-time quantitative PCR

The total RNA, both hepatic and brain, of three samples from each group were prepared using Trizol Reagent. The integrity of total RNA was assessed by agarose gel electrophoresis, while its concentration was determined by a spectrophotometer (NanoDrop® ND-2000). Subsequently, complementary DNA was synthesized using the Prime Script™ RT reagent kit with genomic DNA Eraser (Takara, China). All primer sequences used in this study are listed in Table 2. Quantitative real-time PCR assays were conducted on a CFX96 real-time PCR Detection System (Bio-Rad, Hercules, USA) with 5 μL SYBR Green Master Mix (Takara, China). When PCR amplification was finished, a melting curve analysis was performed to check the specificity of production. The relative mRNA levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.9. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's multiple-range tests using SPSS 24.0 software. A $P < 0.05$ level of significance was used, and data are shown as means ± SEM.

3. Results

3.1. Growth performance

There were no significant differences in specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), and survival rate (SR) among dietary treatments (Table 3). The final body weight (FBW), weight gain (WG), weight gain rate (WGR), and lipid efficiency ratio (LER) were significantly lower in fish fed the CPC50 diet than in other groups except controls ($P < 0.05$). The feed intake (FI) in fish fed CPC-containing feeds showed no significant differences compared with controls, except for the CPC30 group.

Table 2
Primers pair sequences for real-time qPCR.

Genes	Nucleotide sequence (5′-3′)	Genbank accession no.
APOB100	F: CCGAACAGGGTGAGAAGTTGCTC R: GGCCTCTTCAACCTGCATGGTAA	GU982583.1
CPT1	F: GCCGTTTCTACAAGGTGTGG R: CTGCGGTTAGTGCGGCTAGT	HM037343.1
PPARα	F: CTCACGGAGTTCGCAAAGTC R: AAGCCCGTCCTTGTTCATAC	FJ196234.1
PPARγ	F: CCTGTGAGGGCTGTAAGGGTT R: TGTTGCGGGACTTCTTGTGA	KM052849.1
GK	F: ATGTCAAGAGTCCTAAGGGCAAGC R: TCAGGAGACCCCAGGACCAC	JN897396.1
HK	F: TGGGAGGTACAAACTTCAGAGC R: CCCATGTAGTCCAGGAAGTCAC	AF053335.1
6PF1K	F: GATAGGTAGTGCCCGATGTAAGCC R: TCCAGCAGACCGCTCCACTCATT	JX122768.1
PK	F: GGAGCCGACTGCGTAATG R: CAATAATAGGACAGCGAGGG	JN112571.1
PEPCK	F: TGGAAGGTTGAGTGTGTGGG R: AAAAAGCCGTTCTCTGGGTT	FJ196233.1
FBPase	F: GATCAAGTCGTCTTTTACCTCCTG R: CAATGTATTTGCCCCTCGTCTCTG	NM_199942.2
G6Pase	F: GACCAGCAAGAAGAAGTTTGGGAGCAA R: GGGAAGTGGGCGGCGATGAA	FJ196232.1
NPY	F: ACGCTCCCACAGTCAAGATAC R: GCGGCTCATAGAGGTAAAGG	DQ294213.1
Orexin	F: GAGAGAAGATGATCGCTTGC R: GCGTCGTGAAGGTTCTTGAC	HM992945.1
GHRF	F: TTCGGCCAGAGGGATACATC R: TGGCGTCCTGAGCGTAACTC	KR269823.1
β-actin	F: GATCTGGCATCACACCTTCT R: CATCTTCTCCCTGTTGGCTT	AY510710.2

APOB100: apolipoprotein B-100; CPT1: carnitine palmitoyltransferase 1; PPARα: peroxisome proliferator-activated receptor α; PPARγ: peroxisome proliferator-activated receptor γ; GK: glucokinase; HK: hexokinase; 6PF1K: 6-phospho-fructo-1-kinase; PK: pyruvate kinase; PEPCK: phosphoenolpyruvate carboxykinase; FBPase; fructose-1,6-bisphosphatase; G6Pase: glucose-6-phosphatase; NPY: neuropeptide Y; GHRF: growth hormone releasing factor.

3.2. Apparent digestibility and body composition

The ADC of dry matter decreased as the dietary replacement level increased ($P < 0.05$, Table 4). The CPC30 group showed the lowest protein ADC (92.99 %), which was significantly lower than in controls ($P < 0.05$). The lipid ADC in group CPC50 (88.90 %) was significantly lower than those in other groups except CPC30 ($P < 0.05$).

No significant differences in the crude ash and moisture contents of body composition were found among dietary treatments. The crude protein content in fish fed CPC showed no significant difference compared with controls (58.49 %, $P < 0.05$). The crude lipid content was significantly higher in the CPC50 group (27.42 %) than in others ($P < 0.05$).

3.3. Serum biochemical indexes

The triglyceride (TG) contents in CPC-containing groups showed no significant differences compared with controls (0.20 mmol/L; Table 5). No significant differences in cholesterol (CHOL) or D-lactic acid (D-LA) contents were observed among dietary treatments. The high-density lipoprotein cholesterol (HDL-C) contents in CPC-containing groups were significantly lower than in controls (1.19 mmol/L; $P < 0.05$). The low-density lipoprotein cholesterol (LDL-C) content in the CPC50 group (2.00 mmol/L) was significantly lower than in controls (2.64 mmol/L; $P < 0.05$). The glucose (GLU) content was significantly higher in the CPC50 group (10.68 mmol/L) compared with other groups ($P < 0.05$). No significant differences in GLU content were observed among the control, CPC10, and CPC30 groups.

Table 3
Growth performance of juvenile pearl gentian groupers fed the experimental diets.

Indexes	Experimental diets			
	FM (Control)	CPC10	CPC30	CPC50
IBW(g)	11.96 ± 0.04	11.95 ± 0.02	11.98 ± 0.03	11.97 ± 0.02
FBW (g)	70.13 ± 2.90^{ab}	72.46 ± 3.87^{b}	72.46 ± 4.93^{b}	64.38 ± 0.91^{a}
WG[a] (g)	58.17 ± 1.71^{ab}	60.51 ± 2.21^{b}	60.49 ± 4.87^{b}	52.41 ± 0.53^{a}
WGR[b] (%)	486.31 ± 24.26^{ab}	506.32 ± 32.37^{b}	505.32 ± 58.16^{b}	437.70 ± 6.63^{a}
SGR[c] (%/d)	3.16 ± 0.08	3.22 ± 0.09	3.08 ± 0.25	3.00 ± 0.02
FCR[d]	0.90 ± 0.01	0.88 ± 0.04	0.88 ± 0.09	0.91 ± 0.06
FI[e] (% BW/d)	2.27 ± 0.01^{b}	2.21 ± 0.03^{ab}	2.16 ± 0.05^{a}	2.23 ± 0.01^{ab}
PER[f]	2.20 ± 0.02	2.22 ± 0.06	2.24 ± 0.12	2.15 ± 0.01
LER[g]	4.44 ± 0.03^{ab}	4.58 ± 0.11^{b}	4.66 ± 0.26^{b}	4.01 ± 0.02^{a}
PPV [h] (%)	38.26 ± 0.27	38.59 ± 0.88	37.85 ± 1.96	35.84 ± 0.14
SR[i] (%)	98.89 ± 1.92	96.67 ± 3.34	97.78 ± 1.92	100.00 ± 0.00

IBW: initial body weight; FBW: final body weight; WG: weight gain rate; WGR: weight gain rate; SGR: specific growth rate; FCR: feed conversion ratio; FI: feed intake; PER: protein efficiency ratio; LER: lipid efficiency ratio; PPV: protein productive value; SR: survival rate. Values are means ± S.E.M of three replications. Different superscript letters in each row show significant differences among treatments by Tukey's test ($P < 0.05$).

[a] WG (g) =final body weight – initial body weight.

[b] WGR (%) = 100 × (final body weight – initial body weight)/initial body weight.

[c] SGR (%/d) = 100 × (ln (final body weight) – ln (initial body weight))/d.

[d] FCR = total diet intake/total wet weight gain.

[e] FI = 100 × total diet intake/[(initial body weight + final body weight)/2 × days].

[f] PER = (final body weight–initial body weight)/total protein intake.

[g] LER = (final body weight–initial body weight)/total lipid intake.

[h] PPV = 100 × (final body weight × final body protein– initial body weight × initial body protein)/total protein intake.

[i] SR (%) = 100 × the final fish number/the initial fish number.

Table 4
Apparent digestibility and body composition of juvenile pearl gentian groupers fed the experimental diets.

Items	Experimental diets (%)			
	FM (Control)	CPC10	CPC30	CPC50
ADC				
Dry matter	80.15 ± 0.31^{c}	78.36 ± 0.38^{b}	77.06 ± 0.45^{a}	76.40 ± 0.69^{a}
Crude protein	93.68 ± 0.20^{b}	93.14 ± 0.99^{ab}	92.99 ± 0.21^{a}	93.12 ± 0.19^{ab}
Crude lipid	93.39 ± 1.19^{c}	98.41 ± 0.16^{d}	87.12 ± 1.31^{a}	88.90 ± 1.21^{b}
body composition				
Crude protein	58.49 ± 0.65^{ab}	57.47 ± 0.91^{a}	60.18 ± 0.98^{b}	59.39 ± 0.67^{ab}
Crude lipid	24.97 ± 025^{a}	24.86 ± 0.06^{a}	24.51 ± 0.45^{a}	27.42 ± 0.46^{b}
Crude ash	15.37 ± 0.34	15.43 ± 0.07	15.11 ± 0.49	14.89 ± 0.46
Moisture	71.27 ± 0.24	70.85 ± 0.57	71.26 ± 0.10	71.73 ± 0.64

Values are means ± S.E.M of three replications. Different superscript letters in each row show significant differences among treatments by Tukey's test ($P < 0.05$).

ADC of dry matter (%) = 100× [1-(dietary Y_2O_3)/fecal Y_2O_3].

ADC of nutrients (%) = 100× [1-(F/D × D_i/F_i)].

where F is the percent of nutrient in feces, D is the percent of nutrient in the diet, D_i is the percent of yttrium trioxide in diet, and F_i is the percent of yttrium trioxide in feces.

Table 5
Serum biochemical index of juvenile pearl gentian groupers fed the experimental diets.

Items	Experimental diets			
	FM (Control)	CPC10	CPC30	CPC50
TG (mmol/L)	0.20 ± 0.02^{ab}	0.26 ± 0.07^{b}	0.20 ± 0.01^{ab}	0.17 ± 0.01^{a}
CHOL (mmol/L)	6.53 ± 0.92	5.25 ± 0.35	5.45 ± 0.54	5.27 ± 0.24
HDL-C (mmol/L)	1.19 ± 0.04^{c}	0.80 ± 0.05^{a}	0.92 ± 0.02^{b}	0.92 ± 0.04^{b}
LDL-C (mmol/L)	2.64 ± 0.20^{b}	2.43 ± 0.07^{ab}	2.21 ± 0.05^{ab}	2.00 ± 0.28^{a}
GLU (mmol/L)	9.35 ± 0.75^{a}	9.05 ± 0.06^{a}	9.30 ± 0.41^{a}	10.68 ± 0.47^{b}
D-LA (nmol/mL)	9.50 ± 0.59	8.62 ± 0.61	8.98 ± 0.70	9.77 ± 0.90

TG: triglyceride; CHOL; cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; GLU: glucose; D-LA: D-lactic acid. Values are means ± S.E.M of three replications. Different superscript letters in each row show significant differences among dietary treatments by Tukey's test ($P < 0.05$).

3.4. Hepatic key lipid metabolic enzymes

The activities of carnitine palmitoyltransferase I (CPT-1) were significantly higher in the CPC50 group than in controls ($P < 0.05$, Table 6). The fatty acid synthase (FAS) activity in fish fed dietary CPC10 (8.82 U/g tissue) was significantly lower than in other groups except CPC30 ($P < 0.05$). The lipoprotein lipase (LPL) activity in group CPC10 (3.35 U/g tissue) was significantly lower than in others ($P < 0.05$). The hepatic lipase (HL) activity in group CPC50 (335.44 U/g tissue) was significantly higher than in controls ($P < 0.05$).

3.5. Gene expression

3.5.1. Expression of genes related to hepatic fatty acid oxidation and VLDL assembly

No significant differences in APOB100, CPT1, PPARα, or PPARγ expression levels were observed between controls and group CPC10 (Fig. 1). The expression levels of APOB100, CPT1, PPARα, and PPARγ genes in group CPC50 were significantly higher than in others except CPC30 ($P < 0.05$).

3.5.2. Expression of genes related to hepatic glucose metabolism

The expression levels of GK and HK genes in group CPC50 were significantly higher than those in other groups except controls ($P < 0.05$, Fig. 2). The expression levels of HK and 6PF1K genes were significantly higher in group CPC30 than in others except group CPC50 ($P < 0.05$). The PEPCK expression level was significantly lower in group CPC10 than in controls ($P < 0.05$). The FBPase gene expression levels were

Table 6
Hepatic lipid metabolism-related enzymes (U/g tissue) of juvenile pearl gentian groupers fed the experimental diets.

Items	Experimental diets			
	FM (Control)	CPC10	CPC30	CPC50
CPT-1	2.14 ± 0.42^{a}	2.53 ± 0.67^{a}	2.46 ± 0.34^{a}	3.88 ± 0.62^{b}
FAS	14.21 ± 0.21^{b}	8.82 ± 1.37^{a}	11.82 ± 1.21^{ab}	15.28 ± 3.60^{b}
LPL	4.24 ± 0.34^{b}	3.35 ± 0.61^{a}	5.59 ± 0.20^{c}	5.01 ± 0.16^{bc}
HL	264.38 ± 7.52^{a}	305.98 ± 11.22^{ab}	304.59 ± 27.57^{ab}	335.44 ± 43.08^{b}

CPT-1: carnitine palmitoyltransferase I; FAS: fatty acid synthase; LPL: lipoprotein lipase; HL: hepatic lipase. Values are means ± S.E.M of three replications. Different superscript letters in each row show significant differences among treatments by Tukey's test ($P < 0.05$).

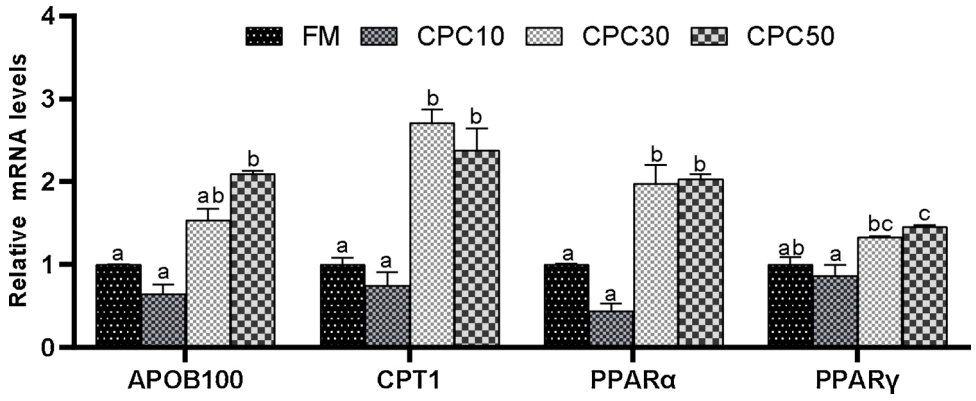

Fig. 1. Relative expression of genes related to hepatic fatty acid oxidation and VLDL assembly of pearl gentian grouper juveniles fed the experimental diets. Values are the means ± S.E.M. ($n = 3$). Bars of the same gene bearing the different letters showed significant difference by Tukey's test ($P < 0.05$).

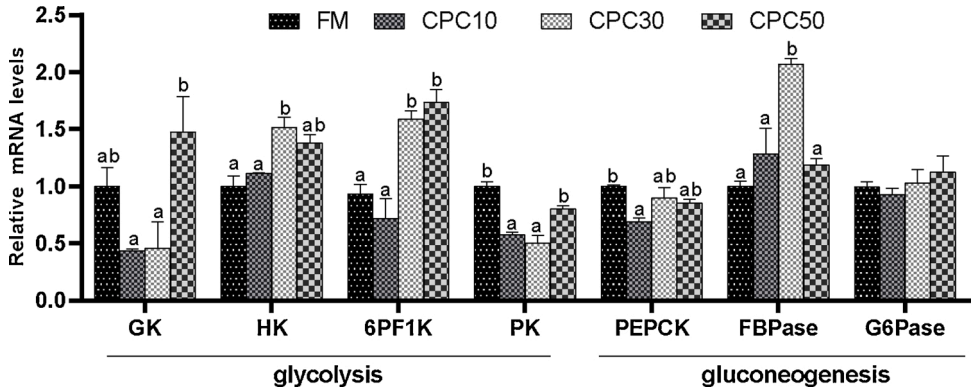

Fig. 2. Relative expression of genes related to glycolysis and gluconeogenesis key enzymes of pearl gentian grouper juveniles fed the experimental diets. Values are the means ± S.E.M. ($n = 3$). Bars of the same gene bearing the different letters showed significant difference by Tukey's test ($P < 0.05$).

significantly upregulated in group CPC30 compared with the other groups ($P < 0.05$). No significant differences in G6Pase expression levels were observed among dietary treatments.

3.5.3. *Expression of genes related to food intake in the brain*

No significant differences in NPY, Orexin, or GHRF expression levels were observed among dietary treatments (Fig. 3).

4. Discussion

4.1. *Effects of replacing dietary FM with CPC on growth, digestibility, and body composition*

In this study, up to 50 % of the FM in the feed could be replaced with CPC without adversely affecting the SGR, FCR, and SR of pearl gentian groupers. Similar findings have been reported before in this species (initial weight 16.91 g; 50 % FM control diet; Ye et al., 2020) and in juvenile golden pompano (initial weight 25 g; 34 % FM control diet; Shen et al., 2020), and rainbow trout (*Oncorhynchus mykiss*; initial weight 17.55 g; 40 % FM control diet; Zhao et al., 2021). However, the WGRs in groups CPC10 and CPC30 were both significantly higher than

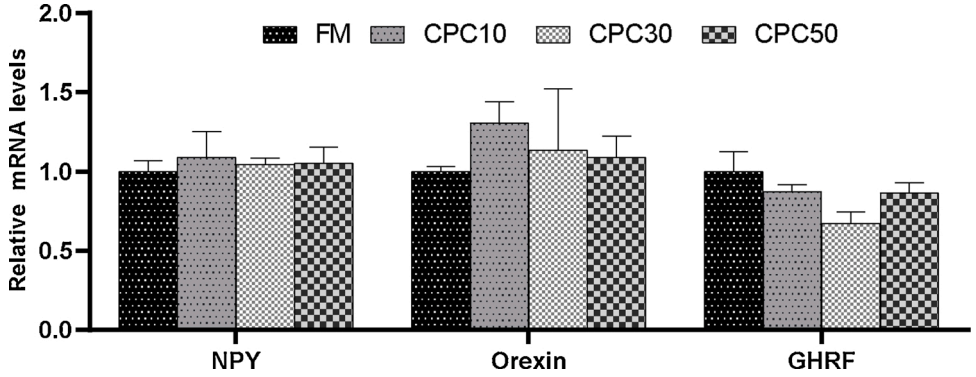

Fig. 3. Relative expression of genes related to feeding intake in brain of pearl gentian grouper juveniles fed the experimental diets. Values are the means ± S.E.M. ($n = 3$). Bars of the same gene bearing the different letters showed significant difference by Tukey's test ($P < 0.05$).

that in group CPC50. Bian et al. (2017) reported that replacement of 45 % of the FM in a 62 % basic FM control diet with cottonseed meals led to the poorest WGR compared with control in juvenile turbot (*Scophthalmus maximus* L., initial weight 5.28 g). Although it is sometimes possible to replace up to 50 % of a diet's FM with CPC without affecting fish growth performance, previous studies indicate that reductions in palatability (Xue et al., 2004), amino acid content, and digestibility (Mbahinzireki et al., 2001) can still reduce growth performance. In this study, dry matter, lipid digestibility, and WGR were lowest in group CPC50. The ADCs of dry matter reflect the overall digestibility of feed ingredients by fish. High or low values are related to the feed's cellulose content and the degrees of absorption of proteins, lipids, and other nutrients (Brunson et al., 1997). High crude cellulose and non-starch polysaccharide (NSP) contents can reduce feed protein digestibility (Mbahinzireki et al., 2001). However, the crude protein content, WG, WGR, and FI in fish fed CPC were not significantly different from those of controls. In addition, PER and PPV were not significantly influenced with up to 50 % FM replacement. This indicates that an FM replacement level of up to 50 % will not negatively affect protein deposition in groupers. The level of D-lactic acid in the serum can be used as a relatively stable marker of the intestinal wall's permeability (Smith et al., 1986). In this study, the D-lactic acid content was not significantly influenced by the FM replacement level, suggesting that replacing 50 % of the FM with CPC does not negatively affect the integrity of the intestinal mucosa.

4.2. Effects of replacing FM with CPC on serum biochemical indexes and hepatic lipid metabolism

To further study the relationship between hepatic lipid metabolism and dietary CPC in fish, we next analyzed the transcriptional levels of lipid metabolism-related genes in the livers of hybrid groupers. Apolipoproteins play a key role in the clearance and metabolism of lipoproteins. Apolipoprotein B-100 (ApoB100) is the key element in the assembly of VLDL particles (Hussain et al., 2008). In this study, ApoB100 gene expression became increasingly up-regulated as the CPC replacement level increased from 10 % to 50 %. It was significantly higher in CPC50 fish, suggesting that there was an increased level of lipid transport and reverse cholesterol transport in the liver. Also, CPC-fed fish had lower serum CHOL, HDL-C, and LDL-C contents than controls. These results indicate that up-regulation of the ApoB100 gene may help to lower serum cholesterol levels, including those of CHOL, HDL-C, and LDL-C. Additionally, high hepatic LPL and HL activities were observed in the 50 % CPC group, implying that these two enzymes in the liver may be fully activated and immediately hydrolyze blood TGs to provide energy. PPARγ is mainly involved in the regulation of lipid storage (Hou et al., 2020). In this study, the gene expression of PPARγ was influenced by dietary CPC level and was highest in group CPC50. Also, the trend in hepatic FAS enzyme activity among the four groups was consistent with that of PPARγ. Group CPC50 had body compositions with significantly higher crude lipid contents than those of other groups. The lipid ADCs were significantly lower in group CPC50 than in others except CPC30. These results suggest that the CPC50 diet might promote lipid synthesis and accumulation.

The PPARα and CPT1 genes regulate fatty acid transport and catabolism to maintain lipid homeostasis (Lu et al., 2014). In the present study, their expression levels were significantly more upregulated in groups CPC30 and CPC50 than in others. Also, CPT1 enzyme activities were affected by diet and were significantly higher in group CPC50. These findings indicate that increases in PPARα and CPT1 enzyme activities at the enzymatic and/or transcriptional levels may be a compensatory mechanism for excessive accumulation of lipid droplets and increased levels of reverse cholesterol transport. Ye et al. (2019) reported that the expression levels of PPARα and CPT1 were upregulated in pearl gentian groupers fed a diet that included rendered animal protein.

4.3. Effects of replacing FM with CPC on serum glucose concentration and hepatic glucose metabolism key gene expression

One major objective in the field of fish nutrition is to improve carbohydrate utilization (Kirchner et al., 2003). The effects of dietary protein on carbohydrate metabolism have been studied in fish. For example, hepatic FBPase activity was significantly reduced in totoaba juveniles when dietary FM was replaced with SPC, while HK activity was unaffected (Bañuelos-Vargas et al., 2014). This contrasts with the results of the present study, in which FBPase gene expression levels were significantly more upregulated in the CPC30 group than in other groups. This inconsistency may be due to differences in the fish species and feed formulations used. In the present study, CPC50 fish showed higher expression levels of the glycolytic GK, HK, 6PF1K, and PK genes compared with CPC10 fish. However, the CPC50 fish had significantly enhanced serum glucose contents, which was also accompanied by the lowest WGRs. This may be attributed to the presence of NSPs. Previous studies have reported that plant proteins contain high levels of NSPs and crude cellulose, which reduce feed protein digestibility (Mbahinzireki et al., 2001; Sinha et al., 2011). The inclusion of NSPs in the basal diet of monogastric animals, including fish, has been reported to delay the intestinal absorption of glucose (Sinha et al., 2011). Carbohydrate is known to provide little energy to carnivorous fish and, in the current study, groupers fed a high-CPC diet tended to exhibit high blood glucose. A potential hypothesis is that, in response to persistent high blood glucose, elevated blood glucose concentrations lead to increases in the levels of mRNA related to the hepatic glycolysis enzyme. The related enzymes are responsible for the uptake of glucose by hepatocytes but not for the uptake of G6Pase, an enzyme involved in the last step of glucose hepatic release.

4.4. Effects of replacing FM with CPC on appetite regulation key gene expression

Appetite regulation occurs in the central nervous system (CNS); mainly in the hypothalamus, which is crucial in the control of food intake and energy homeostasis in mammals and fish (Yao et al., 2018). Some of the significant neuropeptides involved in appetite regulation have been identified, including agouti-related protein (AgRP), NPY, and orexin/hypocretin (Arora, 2006; Volkoff et al., 2005; Yan et al., 2011). In this study, no significant changes in orexin, NPY, or GHRF expression levels were observed among dietary treatments. Our previous study also showed that diets with up to 60 % CPC had no significant effect on the feed intake of pearl gentian groupers (Ye et al., 2020). This is slightly inconsistent with our current results—that the FIs of fish fed CPC-containing diets were not significantly different to that of controls, except for group CPC30. This difference may be related to the experimental trial time, as groupers were fed for 8 weeks in the present study and 10 weeks in Ye et al. (2020). These results suggest that up to 50 % of the FM in feed can be replaced with CPC without adversely affecting its palatability or the appetite of pearl gentian groupers.

In conclusion, the results of this study indicate that up to 50 % of the FM in fish feed can be replaced with CPC without adversely affecting the growth performance and feed utilization of pearl gentian groupers. However, 50 % FM replacement increased body lipid contents via modulation of lipid metabolism-related genes and enzyme activity, which provides new evidence for CPC-induced lipid metabolism in carnivorous fish.

Author statement

Conceptualization, Shuyan Chi and Beiping Tan; methodology, Yuanfa He; software, feeding experiment, Xinwei Guo; formal analysis, Xiaohui Dong, Qihui Yang, Hongyu Liu and Shuang Zhang; writing—original draft preparation, Yuanfa He; writing—review and editing, Yuanfa He and Shuyan Chi; funding acquisition, Shuyan Chi and

Beiping Tan. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgement

This study was financially supported by the National Key R&D Program of China (2019YFD0900200), the China Agriculture Research System (CARS-47), the National Natural Science Foundation of China (NSFC 31772864), and the Fund of Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang) (ZJW-2019-06).

References

Alam, M., Watanabe, W., Carroll, P., Gabel, J., Corum, M., Seaton, P., Wedegaertner, T. C., Rathore, K.S., Dowd, M.K., 2018. Evaluation of genetically-improved (glandless) and genetically-modified low-gossypol cottonseed meal as alternative protein sources in the diet of juvenile southern flounder *Paralichthys lethostigma* reared in a recirculating aquaculture system. Aquaculture 489, 36–45.

Anderson, A., Alam, M., Watanabe, W., Carroll, P., Wedegaertner, T., Dowd, M., 2016. Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *Centropristis striata*. Aquaculture 464, 618–628.

Arora, S., 2006. Role of neuropeptides in appetite regulation and obesity–a review. Neuropeptides 40, 375–401.

Bañuelos-Vargas, I., López, L.M., 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 170, 18–25.

Bian, F., Zhou, H., He, G., Wang, C., Peng, H., Pu, X., Jiang, H., Wang, X., Mai, K., 2017. Effects of replacing fishmeal with different cottonseed meals on growth, feed utilization, haematological indexes, intestinal and liver morphology of juvenile turbot (*Scophthalmus maximus* L.). Aquacult. Nutr. 23, 1429–1439.

Brunson, J.F., Romaire, R.P., Reigh, R.C., 1997. Apparent digestibility of selected ingredients in diets for white shrimp *Penaeus setiferus* L. Aquacult. Nutr. 3, 9–16.

Chem, A., 1992. Analytical Chemistry, vol. 90. AOAC International.

Chen, G., Yin, B., Liu, H., Tan, B., Dong, X., Yang, Q., Chi, S., Zhang, S., 2020. Effects of fishmeal replacement with cottonseed protein concentrate on growth, digestive proteinase, intestinal morphology and microflora in pearl gentian grouper (♀ *Epinephelus fuscoguttatus*×♂ *Epinephelus lanceolatu*). Aquac. Res. 1–15. https://doi.org/10.1111/are.14626.

Chi, S.Y., Wang, X.W., Tan, B.P., Yang, Q.H., Dong, X.H., Liu, H.Y., Zhang, S., 2015. Effects of dietary methionine on the growth performance, anti-oxidation and acivities of gluconeogenesis-related enzyme in juvenile groupers, *Epinephelus coioides*. Acta Hydrobiol. Sin. 4, 645–652 (In Chinese with English abstract).

Chi, S., He, Y., Zhu, Y., Tan, B., Dong, X., Yang, Q., Liu, H., Zhang, S., 2020. Dietary methionine affects growth and the expression of key genes involved in hepatic lipogenesis and glucose metabolism in cobia (*Rachycentron canadum*). Aquacult. Nutr. 26, 123–133.

Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551–579.

He, Y., Ye, G., Chi, S., Tan, B., Dong, X., Yang, Q., Liu, H., Zhang, S., 2020. Integrative transcriptomic and small RNA sequencing reveals immune-related miRNA-mRNA regulation network for soybean meal-induced enteritis in hybrid grouper, *Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂. Front. Immunol. 11, 1502.

He, Y., Guo, X., Tan, B., Dong, X., Yang, Q., Liu, H., Zhang, S., Chi, S., 2021. Partial fishmeal protein replacement with peptides from swine blood modulates the nutritional status, immune response, and intestinal microbiota of hybrid groupers (female *Epinephelus fuscoguttatus*× male *E. lanceolatus*). Aquaculture 533, 736154.

Hou, Q., Thidza, I.Z., Miao, L., Dong, Z., Pan, W., Zhu, W., Wang, L., Fu, J., Zhao, W., Li, L., 2020. Lipid metabolism responses of common carp (*Cyprinus carpio* L.) to mulberry leaf meal in diet. Aquac. Res. 51, 719–727.

Hussain, M.M., Rava, P., Pan, X., Dai, K., Dougan, S.K., Iqbal, J., Farrah, L., Irani, K., 2008. Microsomal triglyceride transfer protein in plasma and cellular lipid metabolism. Curr. Opin. Lipidol. 19, 277–284.

Jiang, W., Hu, K., Zhang, J., Liu, Y., Jiang, J., Wu, P., Zhao, J., Kuang, S., Tang, L., Tang, W., Zhang, Y., Zhou, X., Feng, L., 2015. Soyabean glycinin depresses intestinal growth and function in juvenile Jian carp (*Cyprinus carpio* var Jian): protective effects of glutamine. Brit. J. Nutr. 114, 1569–1583.

Kamalam, B.S., Medale, F., Panserat, S., 2017. Utilisation of dietary carbohydrates in farmed fishes: new insights on influencing factors, biological limitations and future strategies. Aquaculture 467, 3–27.

Kirchner, S., Kaushik, S., Panserat, S., 2003. Effect of partial substitution of dietary protein by a single gluconeogenic dispensable amino acid on hepatic glucose metabolism in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. A 134, 337–347.

Kissil, G.W., Lupatsch, I., Higgs, D., Hardy, R., 2000. Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata* L. Aquac. Res. 31, 595–601.

Lansard, M., Panserat, S., Plagnes-Juan, E., Dias, K., Seiliez, I., Skiba-Cassy, S., 2011. L-leucine, L-methionine, and L-lysine are involved in the regulation of intermediary metabolism-related gene expression in rainbow trout hepatocytes. J. Nutr. 141, 75–80.

Li, S., Li, Z., Zhang, J., Sang, C., Chen, N., 2019. The impacts of dietary carbohydrate levels on growth performance, feed utilization, glycogen accumulation and hepatic glucose metabolism in hybrid grouper (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂). Aquaculture 512, 734351.

Lim, S.J., Kim, S.S., Ko, G.Y., Song, J.W., Oh, D.H., Kim, J.D., Kim, J.U., Lee, K.J., 2011. Fish meal replacement by soybean meal in diets for Tiger puffer, *Takifugu rubripes*. Aquaculture 313, 165–170.

Lu, K.L., Xu, W.N., Wang, L.N., Zhang, D.D., Zhang, C.N., Liu, W.B., 2014. Hepatic β-oxidation and regulation of carnitine palmitoyltransferase (CPT) I in blunt snout bream Megalobrama amblycephala fed a high fat diet. PLoS One 9, e93135.

Luo, Y., Ai, Q., Mai, K., Zhang, W., Xu, W., Zhang, Y., 2012. Effects of dietary rapeseed meal on growth performance, digestion and protein metabolism in relation to gene expression of juvenile cobia (*Rachycentron canadum*). Aquaculture 368, 109–116.

Mbahinzireki, G.B., Dabrowski, K., Lee, K.J., El-Saidy, D., Wisner, E.R., 2001. Growth, feed utilization and body composition of tilapia (*Oreochromis sp.*) fed cottonseed meal-based diets in a recirculating system. Aquacult. Nutr. 7, 189–200.

Messina, M., Tulli, F., Messina, C., Tibaldi, E., 2007. Varying plant protein sources in the diet of sea bass *Dicentrarchus labrax* differently affects lipid metabolism and deposition. Ital. J. Anim. Sci. 6, 806–808.

Messina, M., Piccolo, G., Tulli, F., Messina, C., Cardinaletti, G., Tibaldi, E., 2013. Lipid composition and metabolism of European sea bass (*Dicentrarchus labrax* L.) fed diets containing wheat gluten and legume meals as substitutes for fish meal. Aquaculture 376, 6–14.

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, D.C., USA.

Polakof, S., Panserat, S., Soengas, J.L., Moon, T.W., 2012. Glucose metabolism in fish: a review. J. Comp. Physiol. B 182, 1015–1045.

Shen, J., Liu, H., Tan, B., Dong, X., Yang, Q., Chi, S., Zhang, S., 2020. Effects of replacement of fishmeal with cottonseed protein concentrate on the growth, intestinal microflora, haematological and antioxidant indices of juvenile golden pompano (*Trachinotus ovatus*). Aquacult. Nutr. 26, 1119–1130.

Sinha, A.K., Kumar, V., Makkar, H.P., De Boeck, G., ecker, K., 2011. Non-starch polysaccharides and their role in fish nutrition–a review. Food Chem. 127, 1409–1426.

Smith, S.M., Eng, R.H., Buccini, F., 1986. Use of D-lactic acid measurements in the diagnosis of bacterial infections. J. Infect. Dis. 154, 658–664.

Volkoff, H., Canosa, L., Unniappan, S., Cerda-Reverter, J., Bernier, N., Kelly, S., Peter, R., 2005. Neuropeptides and the control of food intake in fish. Gen. Comp. Endocr. 142, 3–19.

Xue, M., Xie, S., Cui, Y., 2004. Effect of a feeding stimulant on feeding adaptation of gibel carp *Carassius auratus gibelio* (Bloch), fed diets with replacement of fish meal by meat and bone meal. Aquac. Res. 35, 473–482.

Yan, A., Zhang, L., Tang, Z., Zhang, Y., Qin, C., Li, B., Li, W., Lin, H., 2011. Orange-spotted grouper (*Epinephelus coioides*) orexin: molecular cloning, tissue expression, ontogeny, daily rhythm and regulation of NPY gene expression. Peptides 32, 1363–1370.

Yao, W., Wu, X., Gao, Y., Wu, M., Lu, S., Li, X., Dong, Y., Jin, Z., Zhou, Z., 2018. Effects of replacing fishmeal protein by hemoglobin powder protein on growth performance, food intake, feeding-related gene expression and gut histology of hybrid grouper (*Epinephelus fuscoguttatus*× *Epinephelus lanceolatus*) juveniles. Aquaculture 488, 235–243.

Ye, J., Liu, X., Wang, Z., Wang, K., 2011. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. Aquac. Int. 19, 143–153.

Ye, H., Zhou, Y., Su, N., Wang, A., Tan, X., Sun, Z., Zou, C., Liu, Q., Ye, C., 2019. Effects of replacing fish meal with rendered animal protein blend on growth performance, hepatic steatosis and immune status in hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂). Aquaculture 511, 734203.

Ye, G., Dong, X., Yang, Q., Chi, S., Liu, H., Zhang, H., Tan, B., Zhang, S., 2020. Low-gossypol cottonseed protein concentrate used as a replacement of fish meal for juvenile hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂): effects on growth performance, immune responses and intestinal microbiota. Aquaculture 524, 735309.

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 lanceolatu* via TLR-2/MyD88 signaling pathways. Fish Shellfish Immunol. 81, 318–328.

Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., Zhang, S., 2020. MHC II-PI3K/Akt/mTOR signaling pathway regulates intestinal immune response induced by soy glycinin in hybrid grouper (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂): protective effects of sodium butyrate. Front. Immunol. 11, 3458.

Zhang, W., Tan, B., Ye, G., Wang, J., Dong, X., Yang, Q., Chi, S., Liu, H., Zhang, S., Zhang, H., 2019. Identification of potential biomarkers for soybean meal-induced

enteritis in juvenile pearl gentian grouper, *Epinephelus lanceolatus*♂× *Epinephelus fuscoguttatus*♀. Aquaculture 512, 734337.
Zhao, W., Liu, Z.L., Niu, J., 2021. Growth performance, intestinal histomorphology, body composition, hematological and antioxidant parameters of *Oncorhynchus mykiss* were not detrimentally affected by replacement of fish meal with concentrated dephenolization cottonseed protein. Aquacult. Rep 19, 100557.
Zhou, Q.C., Mai, K.S., Tan, B.P., Liu, Y.J., 2005. Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). Aquacult. Nutr. 11, 175–182.