Received: 2 December 2019 | Revised: 15 May 2020 | Accepted: 20 May 2020

DOI: 10.1111/anu.13123

ORIGINAL ARTICLE

Aquaculture Nutrition WILEY

Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillago (*Sillago sihama* Forsskál) (1775)

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 31972808; Science and Technology Department of Guangdong Province, Grant/Award Number: 2015A020209170; Earmarked Fund for China Agriculture Research System, Grant/Award Number: CARS-47

Abstract

A 56-day feeding trial was conducted to evaluate the effects on the growth performance, digestive enzyme activity, inflammatory genes expression and intestine histology of silver sillago, *Sillago sihama* (Forsskål 1775), by replacing fish meal (FM) with low-gossypol cottonseed meal (LCSM). Five isonitrogenous and isolipidic diets were formulated, including R0 group (control, containing 550.0 g/kg FM), R16 group (88.5 g/kg LCSM and 461.5 g/kg FM), R32 group (177.0 g/kg LCSM and 373.0 g/kg FM), R48 group (265.5 g/kg LCSM and 284.5 g/kg FM) and R64 group (354.0 g/kg LCSM and 196.0 g/kg FM). Fish fed R0 and R16 groups had a significantly higher weight gain rate (WGR) and specific growth rate (SGR) than R48 and R64 groups ($p < .05$). In contrast to whole-body crude protein, whole-body moisture increased with the FM level of substitution ($p < .05$). With the increased amount of LCSM in the diet, the activity of intestinal amylase (AMS) increased significantly ($p < .05$), and intestinal trypsin (TRP) decreased ($p < .05$). Dietary LCSM substitution upregulated the expression of intestinal tumour necrosis factor-α (TNF-α), the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and interleukin one beta (IL-1β), but downregulated tight junction proteins ZO-1(ZO-1), transforming growth factor beta-3 (TGF-β3) and interleukin 10 (IL-10) expression. Histological analysis revealed progressive morphological damage to the mid-intestine with higher levels of FM replacement. These results showed that 88.5 g/kg (16%) of FM replaced by LCSM with amino acids (methionine and lysine) supplementation did not significantly reduce growth compared with FM-based control.

KEYWORDS

digestive enzyme, inflammatory genes, intestine histology, low-gossypol cottonseed meal, plant protein, Sillago sihama

1 | INTRODUCTION

Fish meal is a vital source of essential amino acids, essential fatty acids and energy in aquaculture diets (Miles & Chapman 2015). However, steadily rising costs in fish meal have become the main limiting factor in aquaculture expansion hence fish meal is being replaced with cheaper plant proteins (Bian et al., 2017). Cottonseed meal (CSM) is much cheaper per unit of protein than fish meal (FM) and other FM alternative protein sources due to global mass production of cotton and cottonseed by products (Anderson et al., 2016; Hu et al., 2007). CSM is a high-quality plant protein source for aquaculture fish diets. However, free gossypol (FG) within CSM has anti-nutritional and anti-fertility effects on warm-blooded animals and fish (Anderson et al., 2016; Romano & Scheffler, 2008). On one hand, FG can bind with the amino group of lysine (Lys), reducing the utilization rate of lysine and emphasizing the characteristics of lysine as the first limiting amino acid of CSM (Wilson, Robinson, & Poe, 2018), while on the other hand, FG affects the growth of aquatic animals (Bu et al., 2017; Cai et al., 2011; Gui, Liu, Shao, & Xu, 2010; Wang et al., 2014; Zhou, Habte-Tsion, et al., 2017). Due to the use of low-temperature primary leaching and two solvent step-by-step extraction processes in the processing process, low-gossypol cottonseed meal (LCSM) has dramatically reduced the denaturation of the protein and removed the gossypol, qualitatively improving nutrient level (Liu, Fang, Zhu, & Wang, 2000). The FG content of LCSM is ≤ 0.06 g/kg (HPLC), the crude protein content is above 500.0 g/kg, and the ratio of total amino acid in the crude protein is 95% (He, 2016). The concentration of CSM added to animal and fish diets is increased by reducing or eliminating gossypol (Alam et al., 2018; He, 2016; Li et al., 2008). LCSM can replace some plant and animal protein sources such as soybean meal and fish meal, is widely used in livestock (Lindsey, Hawkins, & Guthrie, 1980), poultry (He et al., 2015) and aquatic diet (Anderson et al., 2016), is a potential substitute for FM in the fish diet (Yin et al., 2018). The silver sillago *Sillago sihama* Forsskál (1775) (*S. sihama*) is mainly distributed in the shallow seas of India-West Pacific and coastal areas of China (Guo et al., 2014). As an important economic fish in coastal areas of China, the excellent meat quality and extremely high economic nutritional value of *S. sihama* are well accepted by people and play a vital role in fishing activities in coastal waters of China. (Zhou, Huang, Du, & Huang, 2017). In recent years, due to overfishing, the natural resources of *S. sihama* have been decreased and the market prices have been rising (Dian, Xin, & Lu, 2010). To meet market demand, Guangdong Ocean University has successfully cultivated the first batch of artificially breeding *S. sihama*. Current research on *S. sihama* mainly focuses on morphology (Cao, Jain, Huang, & Du, 2010; Wan, 1996), reproductive biology and artificial breeding (Lee, 1981), genetics (Guo et al., 2014), tissue physiology (Cao et al., 2010) and ecology (Lee, Hu, & Hirano, 1981; Zhen, Xiao, & Jian, 2008).

To our knowledge, there are few studies on nutritional requirements in the *S. sihama*. There was currently only one report on the requirement of vitamin A in *S. sihama* (Huang et al., 2018). In addition, the lack of exclusive forage production is due to the paucity of nutritional physiology research, leading to the use of shrimps or marine fish diets to cultivate the species. To expand the cultivation of *S. sihama*, further research on feed production is needed hence in this study, growth performance, digestive enzyme activity, intestine histology and inflammatory genes expression were investigated in *S. sihama*. Fish were fed with isonitrogenous and isolipidic diet, were studied in this experiment, to compare and evaluate the nutritional and intestinal health value of LCSM, to provide a theoretical basis for the scientific application of LCSM in feed production.

2 | MATERIAL AND METHODS

2.1 | Experimental design and Diets preparation

LCSM (free gossypol content is 0.0079 g/kg, tested by SGS, China) in this study was supplied by Hunan Xinrui Biological Technology Co., Ltd. (Changsha, China; 649.0 g/kg crude protein on dry matter basis). The FM was supplied by China National Township Enterprises Corporation (Beijing, China; 680.0 g/kg crude protein and 79.0 g/kg total lipid on a dry matter basis). Five isonitrogenous (approximately 500 g/kg crude protein) diets with isolipidic (90 g/kg total lipid) were formulated to replace 0 g/kg (control), 88.5 g/kg (16%), 177.0 g/kg (32%), 265.5 g/kg (48%) and 354.0 g/kg (64%) of fish meal by a corresponding amount of LCSM to form the experimental diets (R0 (control group), R16, R32, R48 and R64, respectively). Methionine and lysine were added to experimental diets to compensate for the imbalance. All raw materials were crushed and passed 60 mesh screens. Feedstuffs were weighed accurately according to the formula and thoroughly mixed in a Hobart-type mixer. After the addition of oils, choline chloride and water, the compound were made in pellets (1.5 mm) through the F-26 double-screw extruder (South China University of Technology, Guangzhou). The diets were air-dried and sealed in plastic Ziploc bags and stored in −20 ℃ refrigerator moisture content of approximately 100 g/kg.

The proximate composition of the experimental diets contained an average of 496.4 g/kg and 93.0 g/kg of crude protein and lipid, respectively, without any significant difference. The proximate composition of the test diets and essential amino acid (EAA) content was shown in Table 1 and Table 2, respectively.

2.2 | Experimental animals and breeding management

Juvenile *Sillago sihama* was obtained from Guangdong Ocean University (Zhanjiang, China). Before the trial, the juvenile fish were temporarily cultured in the cement pond of 4.5 m (L) × 3.45 m (W) × 1.8 m (H) and fed commercial diets (Zhanjiang Aohua Feed Co., Ltd). The environment and breeding management of acclimation period were similar to those of formal experiment. A total of 450 healthy juvenile *S. sihama* (initial body weight 5.8 ± 0.58 g) were randomly distributed into 15 fibreglass tanks (1m^3). Each diet was randomly

TABLE 1 Ingredient composition and nutrient content of experimental diets (g/kg air-dried basis)

Ingredients (g/kg)	Experimental diets R0	R16	R32	R48	R64
White fish meal	550.00	461.50	373.00	284.50	196.00
Low-gossypol cottonseed meal	0.00	88.50	177.00	265.50	354.00
Soybean protein concentrated	50.00	50.00	50.00	50.00	50.00
Vital wheat gluten	70.00	70.00	70.00	70.00	70.00
High protein flour	189.50	189.50	189.50	189.50	189.50
Fish oil[a]	21.90	27.60	33.30	39.10	44.80
Phospholipid	30.00	30.00	30.00	30.00	30.00
Mineral mixture[b]	5.00	5.00	5.00	5.00	5.00
Vitamin mixture[c]	2.00	2.00	2.00	2.00	2.00
$Ca(H_2PO_4)_2$	15.00	15.00	15.00	15.00	15.00
Antioxidant	0.30	0.30	0.30	0.30	0.30
Choline chloride	5.00	5.00	5.00	5.00	5.00
Microcrystalline cellulose	50.30	41.20	32.10	23.00	13.90
Methionine[d]	0.00	2.30	4.60	6.90	9.20
Lysine[d]	0.00	1.10	2.20	3.30	4.30
Carboxymethyl cellulose sodium	10.00	10.00	10.00	10.00	10.00
Attractant[e]	1.00	1.00	1.00	1.00	1.00
Proximate composition[f]					
Moisture	116.30	119.10	120.20	118.90	122.90
Crude protein	495.60	495.30	492.90	499.40	499.20
Crude lipid	89.70	92.50	94.70	96.10	92.30
Ash	147.90	135.00	120.10	108.30	94.60
Free gossypol content (mg/kg)	0.00	6.99	13.98	20.97	27.96

[a]Semirefined fish oil, Oleaginosa Victoria S.A., Peru.

[b]Mineral mixture (g/kg mixture): $CaCO_3$ 350 g; $NaH_2PO_4.H_2O$ 200 g; KH_2PO_4 200 g; NaCl 12 g; $MgSO_4.7H_2O$ 10 g; $FeSO_4.7H_2O$ 2 g; $MnSO_4.7H_2O$ 2 g; $AlCl_3.6H_2O$ 1 g; $CuCl_2.2H_2O$ 1 g; KF 1 g; $Na_2MoO_4.2H_2O$ 0.5 g; Na_2SeO_3 0.4 g; $CoCl_2.6H_2O$ 0.1 g; KI 0.1 g; zeolite powder 219.9 g (Obtained from Zhanjiang Yuehai Feed Co. Ltd., Guangdong, China).

[c]Vitamin mixture (g/kg mixture): vitamin B_1, 25.50 g; vitamin B_2, 25.00 g; vitamin B_6, 50.00 g; vitamin B_{12}, 0.10 g; vitamin K_3, 5.00 g; vitamin E, 99.00 g; Vitamin A, 10.00 g; vitamin D_3, 50.00 g; nicotinic acid, 101.00 g; D-calcium pantothenate, 61.00 g; biotin, 2.50g; folic acid, 6.25 g; inositol, 102.04 g; cellulose,411.59 g (Obtained from Zhanjiang Yuehai Feed Co. Ltd., Guangdong, China)..

[d]Methionine and lysine were added to balance amino acid with control group.

[e]Attractant composition: taurine: glycine: betaine = 1:3:3; they are obtained from Hangzhou King Techina Technology (Hangzhou, China).

[f]Moisture, crude protein, crude lipid and ash contents were measured value, (g/kg of dry matter).

assigned to three groups of 30 fish. The experiment was carried out in the indoor marine culture system of Marine Biology Research Base of Guangdong Ocean University. The trial lasted 56 days, adjusting the salinity of seawater to 6–8 in the breeding cycle (Zhou, Habte-Tsion, et al., 2017). Before changing the water, the seawater was disinfected with chlorine dioxide and aerated for 24 hr. The daily water temperature was 28.4–31.5°C; the pH was 7.5–8.0. Dissolved oxygen content ≥ 6 mg/L, ammonia nitrogen and nitrite concentration ≤ 0. 5 mg/L. Fish fed twice a day (08:00–09:00, 16:30–17:30) and fed to apparent saturation (At the beginning of feeding, the *S. sihama* was eating diet on the surface of the water, and then the intensity of the eating was reduced. Finally, when the fish sank into the bottom of the water and no longer came to eat, it was regarded as apparent saturation). Procedures involving animals were conducted in conformity with NIH guidelines and were approved by the Animal Care and Use Committee of the Guangdong Ocean University. (NIH Pub. No.85–23, revised 1996) and were approved by Animal Care and Use Committee of the Guangdong Ocean University.

TABLE 2 Amino acid compositions of diets (g/kg dry matter) replacing fish meal by low-gossypol cottonseed meal (LCSM)

	Experimental diets				
Amino acid (g/kg)	R0	R16	R32	R48	R64
NEAA[a,b]					
Ala	25.56	24.02	22.48	20.91	19.40
Asp	40.65	39.92	39.20	38.41	37.75
Tyr	16.90	16.31	15.73	15.12	14.56
Ser	21.26	20.77	20.27	19.75	19.28
Glu	82.09	84.87	87.65	90.32	93.21
Gly	29.29	27.40	25.50	23.57	21.71
Cys	5.60	6.02	6.45	6.86	7.30
Pro	26.16	25.44	24.73	23.98	23.29
EAA[a,b]					0.00
Val	21.88	21.31	20.74	20.15	19.61
Met	12.25	13.44	14.63	15.81	17.02
Ile	19.58	18.74	17.91	17.06	16.25
Leu	33.61	32.28	30.95	29.58	28.30
Thr	19.05	18.12	17.19	16.24	15.33
Phe	19.63	20.26	20.89	21.49	22.15
His	9.90	10.19	10.48	10.76	11.07
Lys	31.30	30.10	28.90	27.66	26.39
Arg	28.89	31.47	34.06	36.58	39.23
Trp	18.50	26.40	10.70	30.80	23.00

[a]EAA: essential amino acid.

[b]NEAA: nonessential amino acid.

2.3 | Sample collection and analysis

At the end of the trial, 24 hr after the last feeding, fish in each tank were counted and weighed to determine survival rate (SR), weight gain rate (WGR), specific growth rate (SGR) and feed conversion ratio (FCR). After the final weighing, the intestine was removed from sixteen randomly sampled fish from each fibreglass tanks and stored at −80°C. Three fish were randomly removed from each replication tank, liver and viscera were dissected and weighed, respectively, to obtain hepatosomatic index (HSI) and viscerasomatic index (VSI). Five fishes were randomly selected and stored in the refrigerator at −20°C for whole-body composition analysis.

Diet and body proximate analysis of dry matter (dried at 105°C), crude protein (by Kjeldahl apparatus, nitrogen × 6.25), crude lipid (extraction with petroleum ether by Soxhlet apparatus), and ash contents (incineration at 550°C) in raw material, experimental diets, and whole body were determined according to the established methods of Association of Official Analytical Chemists (AOAC, 1995).

The raw materials and dietary amino acid profile were measured by an automatic amino acid analyser 433D (SYKAM) after acid hydrolysis in 6 M HCl for 24 hr at 110°C. (Tryptophan in diet was alkaline hydrolysed in 4 M LiOH for 24 hr at 110°C.)

The whole intestinal digestive enzyme (amylase (AMS), lipase (LPS), and trypsin (TRP)) were determined by enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Enzyme-linked Biotechnology Co., Ltd). The whole intestine was first weighed (about 1 g), thawed and homogenized in a 9-volume frozen buffer (10 ml PBS, pH 7.4), then centrifuged at 4°C for 20 min (2000–3000 rpm), and the supernatants were carefully collected where aliquots of samples were used in the testing process. The final tissue concentration (U/g prot and U/mg prot) was obtained by dividing the result by total protein (TP) in the intestine tissue.

2.4 | Calculation formula

$$\text{Weight gain rate (WGR,\%)} = 100 \times (\text{final body weight } (g) - \text{initial body weight } (g)).$$

$$\text{Specific growth rate (SGR,\%)} = 100 \times [\ln \text{final body weight } (g) - \ln \text{initial body weight (g)}] / \text{days}.$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)} / \text{total weight gain (g)}.$$

$$\text{Protein efficiency ratio (PER)} = \text{fish weight gain (g)} / \text{fish protein intake (g)}.$$

$$\text{Survival rate (SR,\%)} = 100 \times \text{final fish number/initial fish number}.$$

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

$$\text{Hepatosomatic indices (HSI,\%)} = 100 \times \text{liver wet weight (g)} / \text{final body weight (g)}.$$

$$\text{Viscerosomatic index (VSI,\%)} = 100 \times \text{viscera wet weight (g)} / \text{final body weight (g)}.$$

$$\text{Daily feed intake (DFI, g/fish/day)} = 100 \times \text{feed offered/average total weight/days}.$$

2.5 | Real-time quantitative RT-PCR analysis of gene expression

Total RNA was extracted from the whole intestine of juvenile *S. sihama* using General RNA Extraction Kit (R1051) (Dongsheng Biotech) according to the manufacturer's instructions. The RNA samples were treated by RQ1 RNase-Free DNase prior to RT-PCR (Takara) analysis. cDNA was generated from 1,000 ng DNase-treated RNA using ExScriptTMRT-PCR kit (Takara). Real-time PCR assays were carried out in a quantitative thermal cycler (Bio-Rad CFX96; Bio-Rad Labs) in a 10 μl reaction volume containing 5 μl SYBR@ Green Real-time PCR Master Mix (Bio- Rad Labs), 1 μl of cDNA, 0.8 μM of each primer and 3.2 μl of sterilized double-distilled water. Each sample in the reaction was performed with triplicate. With data acquisition every 6 s, the thermal programmer included 30 s at 95°C, 40 cycles at 95°C for 5 s, 60°C for 34 s, and a melt curve step from 60°C gradually increasing 0.5°C/s to 95°C. According to the results of our preliminary experiment concerning the evaluation of internal control genes, 60S ribosomal protein L38 was used as a reference gene to normalize cDNA loading. The gene expression results were analysed using the $2^{-\Delta\Delta CT}$ method according to An et al. (2020). Normalized gene expression for the control diet group (R0) was set at 1. All primers were designed using PrimerQuest Tool (Integrated DNA Technologies). The primer sequences are listed in Table 3.

2.6 | Histology of mid-intestine

Histological analyses were performed according to the method described in previous studies (Chen et al., 2012). Mid-intestine samples were fixed for 24 hr in Bouin's solution and then stored in 70% ethanol. After dehydration in graded concentrations of ethanol, the samples were embedded in paraffin wax. Sagittal sections of 5–7 μm thickness were stained with hematoxylin-eosin (H&E) and then prepared for observation under a Nikon ECLIPSE 80i microscope (Nikon Corporation, Kanagawa, Japan). Villus height (VH), villus width (VW), and intestinal epithelial muscle thickness (MT) were measured using the software Image-Pro Plus 6.3 (Media Cybernetics, Inc.). The villi used for measurements were identified by viewing those without ruined edges in their entirety from the tip to the submucosa. The VW was measured at the midpoint of each villus. Intestinal MT was measured from the inner edge of the muscularis mucosae to the outer edge of the serosa. We examined ten random microscope fields for each sample; results from the individual observations were then combined for the overall results (Amoah et al., 2019). As many villi as possible were measured, with up to 10 villi per slide but no fewer than five. If more than 10 could be measured, the villi which were the most evenly spaced within the intestine sample were chosen. Slides with fewer than five suitable villi per slide were excluded.

2.7 | Statistical analysis

One-way ANOVA and correlation statistical analyses were performed using Microsoft Excel and IBM SPSS Statistics 16 (IBM Corp.). Data were checked for homogeneity of variances by the Levene test and, where necessary, transformed via arcsine function. When significant differences between groups were identified, multiple comparisons among means were made using the Tukey HSD test. Differences were considered significant at the level of $p < .05$. All data were presented as means ± *SD* (standard deviation).

3 | RESULT

3.1 | Growth performance

As showed in Table 4, final body weight (FBW), WGR, CF and SGR of fish were significantly affected by the LCSM level in the experimental diet ($p < .05$). The FBW, WGR, SGR, CF, HSI and DFI were the highest in the R16 group. The reverse was observed in the FCR. However, no significant difference of FCR, VSI, HSI, PER and SR were found between R0 group and other groups ($p > .05$).

3.2 | Whole-body proximate composition

The results of the whole-body proximate composition are shown in Table 5. By replacing FM with LCSM, whole-body crude lipid and ash were not significantly affected ($p > .05$), while whole-body crude protein decreased significantly ($p < .05$) and whole-body moisture increased significantly ($p < .05$).

TABLE 3 Primers used in this study

Name	Primer type	Sequence	Genbank no.	Function	Amplicon
60s	Sense	GACAGCCAGGAGGAAGGATG	ACN10033.1	Housekeeping gene	219
	Anti-sense	TGTCTGTGATGACCAGGGTG			
TGF-β3	Sense	TTCAGGTTCTGGCCCTTTAC	XP_010767476.1	Inflammation-related genes`	100
	Anti-sense	TACTCAGTCTCCGTGTTATCCT			
NF-κB	Sense	GGGATGGTTCAGGTTCTACTG	XP_027136364.1		119
	Anti-sense	CTGAGAGAGGACAGCTGATTG			
IL-1β	Sense	GACAGCGACATGGTGAGATT	AQR55700.1		97
	Anti-sense	CCCTTGCTGTGCTGATGTA			
TNF-α	Sense	CTGGTCCACCACATATGGAAA	AAZ20770.1		119
	Anti-sense	ACCAGCTACCCTCATCATCTA			
IL-10	Sense	GCAGATCTTCGACCAGATCAA	AIC33826.1		97
	Anti-sense	GTACGTGGAGTTCAGGGTATTT			
ZO-1	Sense	GCACAGTCACAGAGAACCTATC	XM_022767781.1	Tight junction protein ZO-1	91
	Anti-sense	TCATCTCTCTGTACCACCATCT			

Note: 60S: 60S ribosomal protein L38; C1RA: complement component 1r; C3: complement component 3; C4: complement component 4; C5: complement component 5; CFH: complement factor H; CFB: complement factor B; MASP1: MBL-associated serine protease 1; TGF-β3: transforming growth factor beta-3; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IL-1β: interleukin one beta; TNF-α: tumour necrosis factor-α; IL-10: interleukin 10; TOR: target of rapamycin; IGF-I: insulin-like growth factor I; ZO-1: tight junction protein ZO-1.

TABLE 4 Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance of juvenile *S. sihama*

Items (%)	R0	R16	R32	R48	R64
IBW (g)	5.84 ± 0.01	5.83 ± 0.01	5.82 ± 0.02	5.82 ± 0.02	5.83 ± 0.02
FBW (g)	16.99 ± 0.59^{b}	17.2 ± 0.53^{b}	15.85 ± 0.74^{ab}	15.23 ± 0.18^{a}	15.23 ± 0.84^{a}
WGR (%)	190.59 ± 19.88^{bc}	195.01 ± 9.72^{bc}	171.48 ± 12.15^{abc}	165.62 ± 7.25^{ab}	161.14 ± 14.25^{a}
SGR (%/day)	2.18 ± 0.07^{bc}	2.21 ± 0.07^{c}	2.04 ± 0.09^{ab}	1.99 ± 0.06^{a}	1.96 ± 0.11^{a}
PER	1.88 ± 0.08	1.92 ± 0.05	1.85 ± 0.08	1.77 ± 0.08	1.78 ± 0.12
FCR	1.07 ± 0.05	1.05 ± 0.03	1.09 ± 0.07	1.13 ± 0.05	1.13 ± 0.07
SR (%)	97.78 ± 3.85	100.00 ± 0.00	98.89 ± 1.92	98.89 ± 1.92	100.00 ± 0.00
CF (%)	1.03 ± 0.01^{ab}	1.07 ± 0.04^{b}	1.00 ± 0.01^{ab}	1.02 ± 0.02^{ab}	0.99 ± 0.01^{a}
HSI (%)	0.93 ± 0.22	1.07 ± 0.06	0.89 ± 0.04	0.9 ± 0.13	0.95 ± 0.06
VSI (%)	7.18 ± 0.32	6.70 ± 0.40	6.52 ± 0.34	6.76 ± 0.11	6.37 ± 0.50
DFI	2.24 ± 0.07^{ab}	2.30 ± 0.02^{b}	2.21 ± 0.04^{ab}	2.21 ± 0.04^{ab}	2.15 ± 0.04^{a}

Note: Means ± *SD* ($n = 3$) in the same line not sharing a common superscript letter were significantly different ($p < .05$).

3.3 | Intestinal digestive enzymes activity

As shown in Table 6, the AMS activity was significantly higher in the R48 and R64 groups than the R0 group ($p < .05$). TRP activity of the R32 and R64 groups was significantly lower than that of the control group ($p < .05$). However, there was no significant difference in LPS activity among groups.

3.4 | Histological observation of mid-intestine

Figure 1 shows the photomicrographs of mid-intestinal tract cross-sectioning of *S. sihama* stained with hematoxylin-eosin under light microscopy. In the high substitution group (Figure 1d,e)—as compared with the control group (Figure 1a)—the villi were shortened, the goblet cells reduced, the lamina propria thickened, the sub-epithelial mucosa widened, and the microvilli damaged. Correspondingly, the villus height/villus width (Figure 2) and the thickness of the intestinal tunica muscularis (Figure 3) tended to decrease significantly ($p < .05$) in the high substitution group as compared to the control group.

3.5 | The expression levels of inflammation-related genes in the intestine

As observed in Figure 4, by replacing FM with LCSM, the expression levels of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interleukin one beta (IL-1β) and tumour necrosis factor-α (TNF-α) in the intestine increased significantly ($p < .05$), while the expression levels of tight junction proteins ZO-1 (ZO-1), transforming growth factor beta-3 (TGF-β3) and interleukin 10 (IL-10) showed a significant downward trend ($p < .05$).

4 | DISCUSSION

Cottonseed meal is a favourite protein feed ingredient used in diets of many fish species in China, including grass carp (*Ctenopharyngodon idellus*) (H. Liu et al., 2016), crucian carp (*Carassius auratus gibelio*) (Gui et al., 2010), black sea bass (*Centropristis striata*)(Anderson et al., 2016), blunt snout bream (*Megalobrama amblycephala*) (Zhou, Habte-Tsion, et al., 2017). Different processing methods, raw material sources of cottonseed protein and fish varieties affect the amount and effect of cottonseed protein in fish diet. Cottonseed protein products were mainly used in aquaculture diet as follows: 1) cottonseed meal (CSM) (Bian et al., 2017; Bu et al., 2017; Cook, Zhou, Rhodes, & Davis, 2016; Hu et al., 2015; Liu et al., 2016; Zhou, Habte-Tsion, et al., 2017); 2) fermented cottonseed meal (FCM) (Sun et al., 2015, 2016); 3) transgenic cottonseed protein with low gossypol (TCM) (Alam et al., 2018); 4) the low-gossypol cottonseed meal which prepared by solvent extraction or under negative pressure and low temperature (LCSM) (A. D. Anderson et al., 2016; Yin et al., 2018); 5) mixed with other plant protein sources to form mixed plant protein sources (MPS) (Jiang, Chen, & Qin, 2018; Song et al., 2014). In this

TABLE 5 Effects of fish meal replacement by low-gossypol cottonseed meal on whole-body proximate composition of juvenile *S. sihama* (g/kg wet matter)

Treatments	Moisture	Crude lipid	Crude protein	Ash
R0	713.2 ± 5.7^{a}	57.7 ± 5.0	168.7 ± 3.4^{b}	39.0 ± 2.1
R16	714.7 ± 9.8^{ab}	58.2 ± 5.6	168.1 ± 1.6^{b}	38.0 ± 0.9
R32	717.9 ± 6.9^{ab}	56.1 ± 3.5	166.9 ± 2.9^{ab}	38.0 ± 1.8
R48	721.7 ± 3.4^{ab}	55.0 ± 3.7	166.2 ± 1.7^{ab}	38.0 ± 1.6
R64	725.8 ± 6.0^{b}	56.7 ± 4.1	160.4 ± 2.3^{a}	37.5 ± 0.6

Note: Means ± *SD* ($n = 3$) in the same column not sharing a common superscript letter were significantly different ($p < .05$).

TABLE 6 Effects of fish meal replacement by low-gossypol cottonseed meal on intestinal digestive enzymes activity of juvenile *S. sihama*

Treatments	AMS (U/g prot)	LPS(U/g prot)	TRP (U/mg prot)
R0	28.39 ± 3.52^{a}	72.79 ± 0.61	238.70 ± 15.33^{c}
R16	30.38 ± 6.50^{ab}	71.75 ± 3.65	219.47 ± 9.52^{bc}
R32	38.46 ± 8.44^{abc}	75.67 ± 9.66	190.24 ± 13.49^{ab}
R48	41.11 ± 9.88^{bc}	75.82 ± 7.28	206.50 ± 14.35^{abc}
R64	43.43 ± 3.24^{c}	70.21 ± 2.21	175.55 ± 10.55^{a}

Note: Means ± *SD* ($n = 3$) in the same column not sharing a common superscript letter were significantly different ($p < .05$).

Abbreviations: AMS, amylase; LPS, lipase; TRP, trypsin.

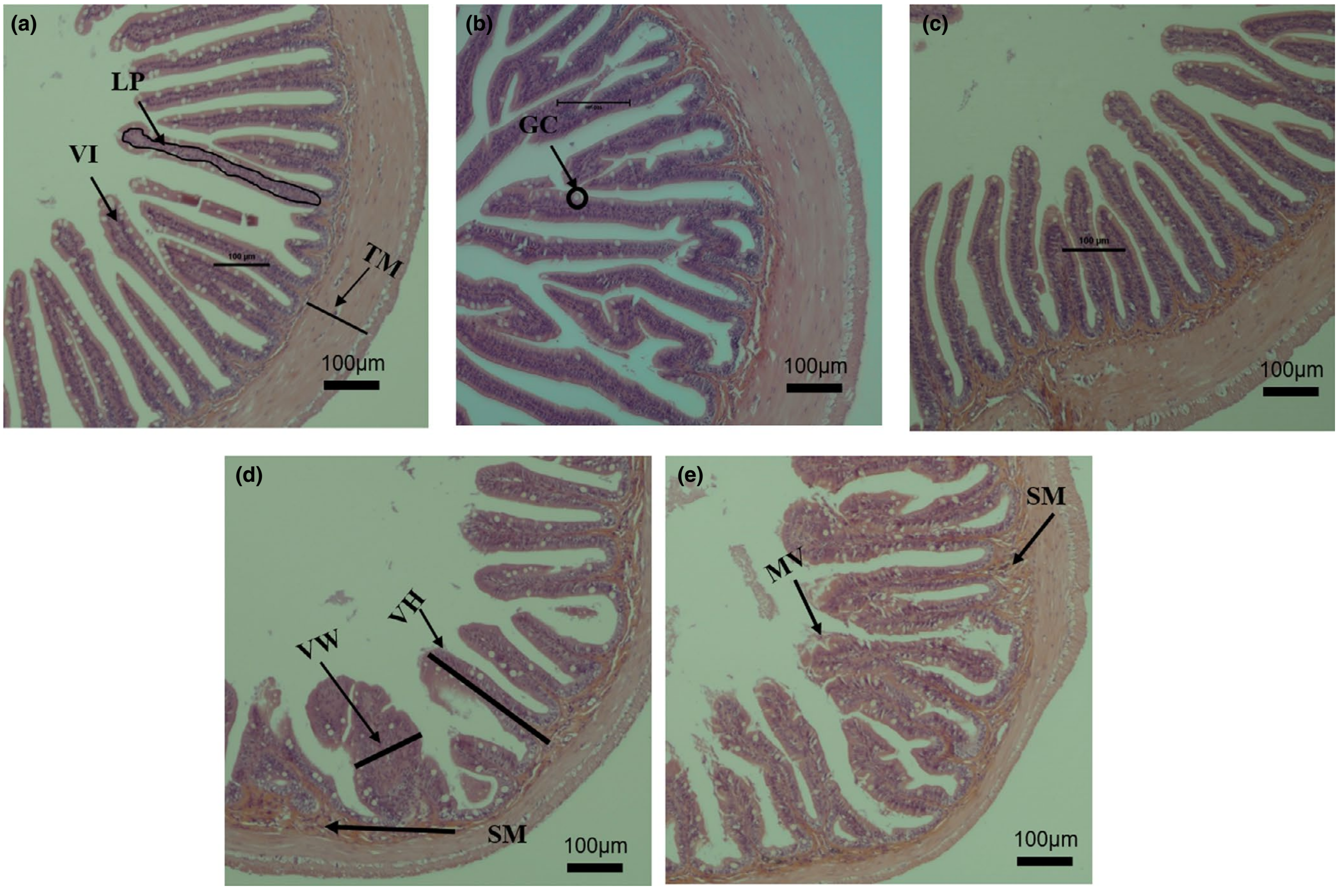

FIGURE 1 Photomicrographs of intestinal tract cross-cutting of *S. sihama* stained with hematoxylin-eosin under light microscopy. Abbreviation: villi (VI), tunica muscularis (TM), villus height (VH), villus width (VW), sub-epithelial mucosa (SM), goblet cell (GC), lamina propria (LP) and microvilli(MV). R0(a) and R16(b): Control group and R16 shows the normal intestinal tract (100×). R32(c): villus and tunica muscularis thickness begin to decrease, and the lamina propria widens (100×). R48(d) and R64(e): A substantial aggravation of the condition was observed which included a shortening of the villus, fewer goblet cells, a thickened lamina propria and widening of the sub-epithelial mucosa as well as impaired microvilli (100×)

experiment, we used LCSM to replace FM. After an 8-week feeding trial, results showed that final body weight, WGR and SGR of juvenile *S. sihama* were similar in the R0 and R16 groups, and this was significantly higher than values in the R48 and R64 groups. However, there were no significant differences in PER and FCR among the groups. Juvenile *S. sihama* could, therefore, use the diet containing 88.5 g/kg LCSM and 461.50 g/kg FM without causing significant adverse effects on growth performance and feed utilization of the fish. In this study, although PER and FCR were not significantly affected by the level of LCSM substitution for fish meal, both PER and SGR showed a rising trend with the level of substitution, while FCR showed the opposite trend with PER. The possible reason was that this experiment was to reduce the adverse effects of LCSM substitution for fishmeal by balancing lysine and methionine in the diet. Similar findings were recently reported in black sea bass (Anderson et al., 2016) and southern flounder (*Paralichthys lethostigma*) (Alam et al., 2018). In addition, some results might require longer experimental periods, and the eight-week experimental period had no significant effect on

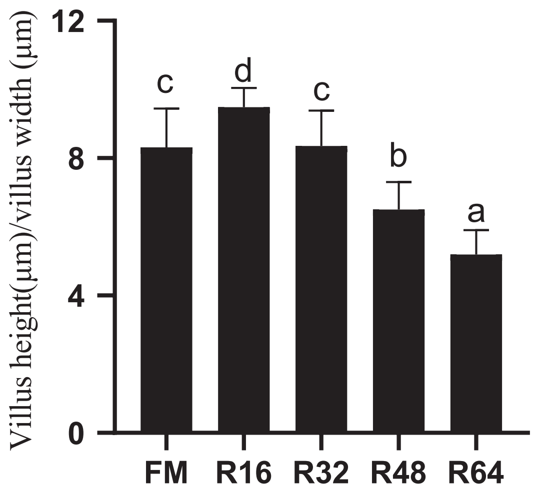

FIGURE 2 Effects of fish meal replacement by low-gossypol cottonseed meal on the villus height/villus width of juvenile *S. sihama*

these results. In the present study, DFI decreased with the increased concentration of free gossypol in diets. Suppression of feed intake is the main reason causing decrease of growth performance, which was similar to the findings of blunt snout bream (Zhou, Habte-Tsion, et al., 2017) and black sea bass (Anderson et al., 2016). SR was found to have no significant difference among groups and maintained at a high level. The results showed that *S. sihama* had certain adaptability to LCSM in diet. Similar results had been reported for southern flounder (Alam et al., 2018), tilapia (*Oreochromis* sp.) (Mbahinzireki, Dabrowski, Lee, El-Saidy, & Wisner, 2001), pacific white shrimp (*Litopenaeus vannamei*) (Siccardi, Richardson, Dowd, Wedegaertner, & Samocha, 2016) and hybrid catfish (*Ictalurus punctatus* × *I. furcatus*) (Li, Robinson, Oberle, Lucas, & Bosworth, 2012) but inconsistent with the results of hybrid grouper (Yin et al., 2018). This inconsistency might be due to fish species, the experimental environment, or other factors, proving *S. sihama* being more tolerant to LSCM supplementation than groupers.

Previous studies showed that different levels of plant protein replacing fish meal in the diet might have caused the differences in body composition on a wet matter basis (Bian et al., 2017; Gui et al., 2010; Liu et al., 2016; Pakravan, Akbarzadeh, Sajjadi, Hajimoradloo, & Noori, 2018). Studies on crucian carp (Gui et al., 2010) found that the level of cottonseed meal protein hydrolysate in the diet had a significant effect on whole-body moisture and crude protein content. In this study, the whole-body crude protein content of fish fed the R0 diet was significantly higher than that of fish fed the R64 diet, and the whole-body moisture also increased with the increase in the level of substitute fish meal. However, whole-body crude lipid content was not affected by the LCSM replacement fish meal levels. Relatively low whole-body crude protein levels in fish fed the high LCSM might reflect more significant catabolism of protein with less storage of protein since this diet was less palatable and consumed at a much lower rate than the other diets. Dietary ash decreased in diets with increasing LCSM, but no trends in whole-body ash were seen (Table 1, Table 5). This result is similar to what was reported in black sea bass fed diets containing up to 100% CSM, which showed no differences in whole-body ash (A. D. Anderson et al., 2016). Fish fed the R64 diet showed the highest moisture (72.58%), while the fish control diet showed the lowest moisture (71.32%) (Table 5). These differences in moisture content were also likely related to reduced palatability and consumption of the high LCSM diet with less storage of protein, resulting in a proportional increase in moisture content as ash levels were unchanged. Experiments using plant protein to replace fish meal have similar results in hybrid snakehead (Lin, Ma, Xu, Chen, & Huang, 2018), cobia (*Rachycentron canadum*) (Luo et al., 2013), red sea bream (*Pagrus major*)(Dossou et al., 2018) and silver crucian carp (*Carassius auratus gibelio*♀×*Cyprinus carpio*♂) (Cai et al., 2011).

Digestive enzyme activities demonstrate the potential impact on feed utilization and growth performance and play a pivotal role in the digestive process (Zhao et al., 2016). Many studies have shown that substituting plant protein for FM reduces the activity of intestinal TRP (Perera & Yúfera, 2017; Shi, Luo, et al., 2017; Taher et al., 2017; Yaghoubi, Mozanzadeh, Marammazi, Safari, & Gisbert, 2016). In starry flounder (Song et al., 2014), tilapia (*Oreochromis niloticus* × *O. aureus*) (Lin & Luo, 2011), yellowtail kingfish (*Seriola lalandi*) (Bowyer et al., 2013), red sea bream (Kader et al., 2012) and crucian carp (Shi, Chen, et al., 2017), the substitution of fish meal with a plant protein source showed that the activity of amylase was not affected by the degree of substitution. In the present study, the TRP activity of R32 and R64 groups was significantly lower than that of the control groups, while AMS activity in the control group was significantly lower than that of the treatment group. This result suggests that high dietary levels of LCSM inhibited intestinal trypsin activity and increased intestinal amylase activity. This might be caused by residual anti-nutritional factors in LCSM that inhibit or antagonize digestive enzymes (Li et al., 2014). Similarly, it has been reported that gossypol in diet can inhibit trypsin activity in Chinese Mitten-handed crab

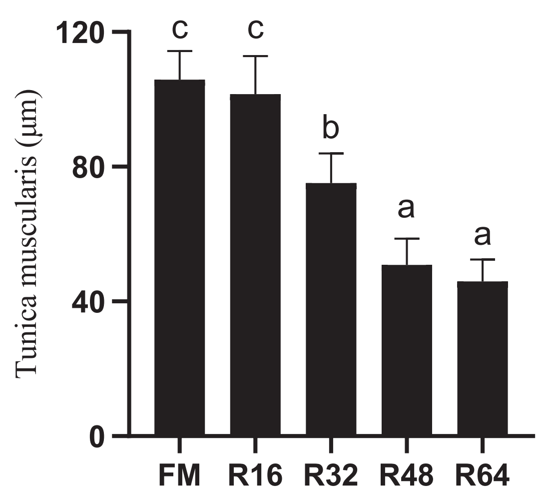

FIGURE 3 Effects of fish meal replacement by low-gossypol cottonseed meal on the thickness of intestinal tunica muscularis of juvenile *S. sihama*

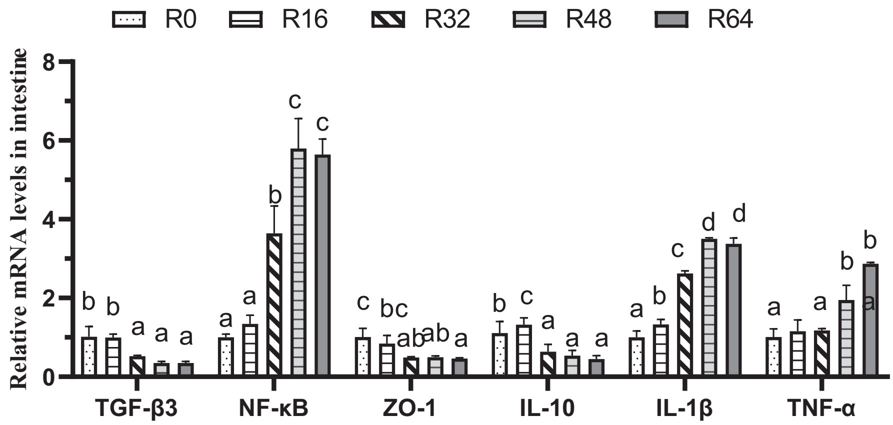

FIGURE 4 Effects of dietary replacement level on relative mRNA level of pro-inflammatory cytokines TNF-α, NF-κB and IL-1β in the intestine as well as anti-inflammatory cytokines TGF-β3 and IL-10. Data represent means of three fish in each group; error bar indicates S. D. Values had different letters are significantly different ($p < .05$). TNF-α: tumour necrosis factor-α; TGF-β3: transforming growth factor beta-3; IL-1β: interleukin one beta; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IL-10: interleukin 10; ZO-1: tight junction proteins ZO-1

(*Eriocheir sinehsis*) (Lu, 2007). In addition, many studies have shown that a diet rich in plant protein reduces trypsin activity in carnivorous fish (Li et al., 2014; Lin et al., 2018; Santigosa et al., 2008; Yaghoubi et al., 2016). *S. sihama* is a small carnivorous fish, and,in this study, excessively high plant protein in the diet inhibited trypsin activity. The increase in intestinal amylase activity seen in this study may also be the result of the shelling process of LCSM during processing, the shallow content of crude fibre, and the relatively high level of sugar and starch in carbohydrates (Guo, Li, Tang, & Liu, 2006).

At present, there are many reports on the effects of replacing fish meal with plant protein sources on the enzymatic activity of digestive organs in aquatic animals (Perera & Yúfera, 2017; Shi, Luo, et al., 2017; Taher et al., 2017; Yaghoubi et al., 2016), but there are few studies on the mechanism of action. While paying attention to the long-term use of plant protein sources instead of fish meal on the growth performance of fish, impact on digestive organs should also be considered. Damage to a fish's digestive organs may affect the digestion and absorption of nutrients by the fish's body, resulting in a sub-health state, which will eventually compromise healthy growth. Histological change is an essential aspect in the understanding of pathological alteration related to nutritional sources in fish (Shi, Luo, et al., 2017). In the present study, dietary LCSM substitution caused some histopathological changes in the mid-intestine of *S. sihama*. In, for example, the high-substitution group, intestinal tissue structural integrity was damaged: the submucosa was obviously infiltrated by inflammatory cells, including lymphocytes, macrophages and multicellular cells; thickness of the intestinal tunica muscularis was decreased; intestinal villi were widened and shortened; and, intestinal microvilli were detached or damaged. The results of this study showed that excessive substitution of fish meal by LCSM could cause intestinal injury to *S. sihama*. In the study of grass carp, excessive CSM replacement of fish meal was seen to cause damage to intestinal epithelial cells (H. Liu et al., 2016). Nevertheless, a study of crucian carp found that high dietary inclusion of CSM, up to 560 g/kg, did not cause any detrimental effects on gut tissue (Cai et al., 2011). A possible explanation for this finding might be that crucian carp, as an omnivorous fish, has a strong tolerance to plant protein. The literature regarding the effect of LCSM on intestine histology is limited. Contrastingly, many researchers have illuminated damage to the structure of the distal intestine when diets high in plant protein were fed to Asian seabass (*Lates calcarifer*) (Boonyaratpalin, Suraneiranat, & Tunpibal, 1998), rainbow trout (*Oncorhynchus mykiss*) (Merrifield, Dimitroglou, Bradley, Baker, & Davies, 2009) and turbot (*Scophthalmus maximus* L.) (Bian et al., 2017).

A dynamic and well-regulated intestinal barrier are essential to protect the body against dietary antigens and residential intestinal microbiota (Akbari et al., 2017). This barrier is created by an impermeable layer of epithelial cells and sealed by specific TJ proteins, preventing the paracellular diffusion of luminal antigens and microorganisms. Intercellular junctional complexes—mainly claudins, occludin, and ZOs in vertebrates—maintain the physical barrier function of epithelia (Anderson, Van Itallie, & Fanning, 2004). In a normal, healthy state, intestinal epithelial TJs provide an effective barrier against paracellular penetration of noxious substances and antigens present in the gastrointestinal lumen (DeMeo, Mutlu, Keshavarzian, & Tobin, 2002). However, in a diseased state, the intestinal TJ barrier becomes defective allowing increased paracellular permeation of normally excluded luminal antigens. The ‘leaky’ intestinal tight junction barrier allows increased antigenic penetration into the underlying intestinal tissue (Al-Sadi, Boivin, & Ma, 2009; DeMeo et al., 2002). The foreign antigens are then processed by the antigen-presenting cells and helper T-lymphocytes activating an inflammatory response; this leads to an increase in the production and secretion of pro-inflammatory cytokines and pro-inflammatory mediators and recruitment of circulating inflammatory cells. Most pro-inflammatory cytokines, including IFN-γ, TNF-α (a TNF-α induced increase in Caco-2 TJ permeability was mediated in part by activation of nuclear transcription factor NF-κB), IL-12 and IL-1β cause an increase in TJ permeability, while some anti-inflammatory cytokines such as IL-10 and TGF-β protect against the disruption of intestinal TJ barrier and development of intestinal inflammation (Al-Sadi et al., 2009; Wang & Secombes, 2013; Yin et al., 2018). In the present study, feeding fish a high replacement diet significantly upregulated the mRNA levels of pro-inflammatory cytokines TNF-α, NF-κB, and IL-1β and down-regulated the mRNA levels of anti-inflammatory cytokines TGF-β3 and IL-10 indicating that intestinal inflammation was aggravated. In addition, in this experiment, we found that ZO-1 expression level decreased significantly with the increase of LCSM replacement FM level, indicating possible inflammation in the intestine. Downregulation of ZO-1 expression can be observed in chronic intestinal inflammatory diseases (Bertiaux-Vandaële et al., 2011; Drago et al., 2006; Nagy Szakál et al., 2010). Combined with the results of intestinal digestive enzymes, intestinal histology and the expression levels of genes associated with intestinal inflammation, a high-level LCSM diet may have a negative impact on the intestinal health of *S. sihama*.

5 | CONCLUSIONS

Results of the current study demonstrated that 88.5g/kg (16%) of FM replaced by LCSM with amino acids (methionine and lysine) supplementation did not significantly reduce growth or feed utilization compared with FM-based control. An excessively high level of LCSM in the *S. sihama* diet may affect intestinal health. However, an excessively high LCSM in the diet will reduce the growth of *S. sihama* and diet palatability. More studies will be needed to test LCSM at higher replacement levels for fish meal and the subsequent effects on palatability and digestibility in *S. sihama*.

ACKNOWLEDGEMENTS

Science and Technology Department of Guangdong Province (2015A020209170); National Natural Science Foundation of China (31972808); China Agriculture Research System (CARS-47).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical.

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REFERENCES

AOAC (1995). *Official methods of analysis of the association of analytical chemistry* (Vol. *6*), 16th ed. Washington, DC: Association of Official Analytical Chemists. Aoac Official Method.

Akbari, P., Braber, S., Varasteh, S., Alizadeh, A., Garssen, J., & Fink-Gremmels, J. (2017). The intestinal barrier as an emerging target in the toxicological assessment of mycotoxins. *Archives of Toxicology*, *91*(3), 1007–1029. https://doi.org/10.1007/s00204-016-1794-8

Alam, M. S., Watanabe, W. O., Carroll, P. M., Gabel, J. E., Corum, M. A., Seaton, P., … 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. https://doi.org/10.1016/j.aquaculture.2018.02.006

Al-Sadi, R., Boivin, M., & Ma, T. (2009). Mechanism of cytokine modulation of epithelial tight junction barrier. *Frontiers in Bioscience*, *14*(7), 2765–2778. https://doi.org/10.2741/3413

Amoah, K., Huang, Q.-C., Tan, B.-P., Zhang, S., Chi, S.-Y., Yang, Q.-H., … Dong, X.-H. (2019). Dietary supplementation of probiotic Bacillus coagulans ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, *87*, 796–808. https://doi.org/10.1016/j.fsi.2019.02.029

An, W., Dong, X., Tan, B., Yang, Q., Chi, S., Zhang, S., … Yang, Y. (2020). Effects of dietary n-3 highly unsaturated fatty acids on growth, non-specific immunity, expression of some immune-related genes and resistance to *Vibrio* harveyi in hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *Epinephelus lanceolatu*). *Fish and Shellfish Immunology*, *96*, 86–96. https://doi.org/10.1016/j.fsi.2019.11.072

Anderson, A. D., Alam, M. S., Watanabe, W. O., Carroll, P. M., Wedegaertner, T. C., & Dowd, M. K. (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. https://doi.org/10.1016/j.aquaculture.2016.08.006

Anderson, J. M., Van Itallie, C. M., & Fanning, A. S. (2004). Setting up a selective barrier at the apical junction complex. *Current Opinion in Cell Biology*, *16*(2), 140–145. https://doi.org/10.1016/j.ceb.2004.01.005

Bertiaux-Vandaële, N., Youmba, S. B., Belmonte, L., Lecleire, S., Antonietti, M., Gourcerol, G., … Coëffier, M. (2011). The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *American Journal of Gastroenterology*, *106*(12), 2165–2173. https://doi.org/10.1038/ajg.2011.257

Bian, F., Zhou, H., He, G., Wang, C., Peng, H., Pu, 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.). *Aquaculture Nutrition*, *23*(6), 1429–1439. https://doi.org/10.1111/anu.12518

Boonyaratpalin, M., Suraneiranat, P., & Tunpibal, T. (1998). Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. *Aquaculture*, *161*(1–4), 67–78. https://doi.org/10.1016/S0044-8486(97)00257-3

Bowyer, J. N., Qin, J. G., Smullen, R. P., Adams, L. R., Thomson, M. J. S., & Stone, D. A. J. (2013). The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds at different water temperatures: 1. Solvent Extracted Soybean Meal. *Aquaculture*, *384–387*, 35–45. https://doi.org/10.1016/j.aquaculture.2012.12.005

Bu, X., Chen, A., Lian, X., Chen, F., Zhang, Y., Muhammad, I., … Yang, Y. (2017). An evaluation of replacing fish meal with cottonseed meal in the diet of juvenile Ussuri catfish *Pseudobagrus ussuriensis*: Growth, antioxidant capacity, nonspecific immunity and resistance to Aeromonas hydrophila. *Aquaculture*, *479*(2016), 829–837. https://doi.org/10.1016/j.aquaculture.2017.07.032

Cai, C., Li, E., Ye, Y., Krogdahl, A., Jiang, G., Wang, Y., & Chen, L. (2011). Effect of dietary graded levels of cottonseed meal and gossypol on growth performance, body composition and health aspects of allogynogenetic silver crucian carp, *Carassius auratus gibelio♀×Cyprinus carpio♂*. *Aquaculture Nutrition*, *17*(4), 353–360. https://doi.org/10.1111/j.1365-2095.2010.00801.x

Cao, J. X., Jain, J. C., Huang, Y., & Du, T. (2010). Morphology and histology of the digestive system in silver sillago *Sillago sihama*(Forskál). *Fisheries Science*, *29*(6), 339–343.

Chen, Q. L., Luo, Z., Zheng, J. L., Li, X. D., Liu, C. X., Zhao, Y. H., & Gong, Y. (2012). Protective effects of calcium on copper toxicity in *Pelteobagrus fulvidraco*: Copper accumulation, enzymatic activities, histology. *Ecotoxicology and Environmental Safety*, *76*(1), 126–134. https://doi.org/10.1016/j.ecoenv.2011.10.007

Cook, R. L., Zhou, Y., Rhodes, M. A., & Davis, D. A. (2016). Evaluation of various cottonseed products on the growth and digestibility performance in Florida pompano *Trachinotus carolinus*. *Aquaculture*, *453*, 10–18. https://doi.org/10.1016/j.aquaculture.2015.11.029

DeMeo, M. T., Mutlu, E. A., Keshavarzian, A., & Tobin, M. C. (2002). Intestinal permeation and gastrointestinal disease. *Journal of Clinical Gastroenterology*, *34*(4), 385–396. https://doi.org/10.1097/00004836-200204000-00003

Dian, L. J., Xin, Z. L., & Lu, H. S. (2010). Estimation of growth and mortality parameters of the silver sillago (*Sillago sihama*) in beibu gulf. *Journal of Zhejiang Ocean University(Natural Science)*, *1*, 64–69. https://doi.org/10.3969/j.issn.1008-830X.2010.01.014.

Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M. A. O., El Basuini, M. F., … Olivier, A. (2018). Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, *Pagrus major*. *Aquaculture*, *490*, 228–235. https://doi.org/10.1016/j.aquaculture.2018.02.010

Drago, S., El Asmar, R., Di Pierro, M., Grazia Clemente, M., Sapone, A. T. A., Thakar, M., … Fasano, A. (2006). Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scandinavian Journal of Gastroenterology*, *41*(4), 408–419. https://doi.org/10.1080/00365520500235334

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., … Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research*, *38*(6), 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Gui, D., Liu, W., Shao, X., & Xu, W. (2010). Effects of different dietary levels of cottonseed meal protein hydrolysate on growth, digestibility, body composition and serum biochemical indices in crucian carp (*Carassius auratus gibelio*). *Animal Feed Science and Technology*, *156*(3–4), 112–120. https://doi.org/10.1016/j.anifeedsci.2010.01.012

Guo, C., Li, S., Tang, J., & Liu, R. (2006). Application of de-gossypolled cottonseed protein in cow feed. *Chinese Journal of Animal Science*, *42*(6), 63–64. https://doi.org/10.3969/j.issn.0258-7033.2006.06.022

Guo, Y.-S., Wang, Z.-D., Yan, C.-Z., Zhang, Y.-L., Zheng, J.-N., Xu, Y.-M., … Liu, C.-W. (2014). Isolation and characterization of microsatellite DNA loci from Sillago sihama. *Journal of Genetics*, *93*(S1), 32–36. https://doi.org/10.1007/s12041-012-0133-y

He, T. (2016). *Study on the application of low-gossypol cottonseed meal in laying hen diets*. PhD thesis. Beijing, China: Chinese Academy of Agricultural Sciences.

He, T., Zhang, H. J., Wang, J., Wu, S. G., Yue, H. Y., & Qi, G. H. (2015). Application of low-gossypol cottonseed meal in laying hens' diet. *Poultry Science*, *94*(10), 2456–2463. https://doi.org/10.3382/ps/pev247

Hu, Y., Huang, Y., Feng, F., Zhong, L., Ai, Q., Xiao, T., & Wen, H. (2015). Effect of soybean meal replacement by cottonseed meal on growth, feed utilization and some blood physiological/biochemical indices of juvenile black carp, Mylopharyngodon piceus. *Aquaculture Research*, *46*(10), 2490–2500. https://doi.org/10.1111/are.12409

Huang, Q., Kwaku, A., Du, T., Tan, B., Chi, S., Yang, Q., … Yang, Y. (2018). Dietary vitamin A requirement of *Sillago sihama* Forskál. *Aquaculture Research*, *49*(7), 2587–2596. https://doi.org/10.1111/are.13725

Jiang, H.-B., Chen, L.-Q., & Qin, J.-G. (2018). Fishmeal replacement by soybean, rapeseed and cottonseed meals in hybrid sturgeon *Acipenser baerii* ♀ × *Acipenser schrenckii* ♂. *Aquaculture Nutrition*, *24*(4), 1369–1377. https://doi.org/10.1111/anu.12674

Kader, M. A., Bulbul, M., Koshio, S., Ishikawa, M., Yokoyama, S., Nguyen, B. T., & Komilus, C. F. (2012). Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, *Pagrus major*. *Aquaculture*, *350–353*, 109–116. https://doi.org/10.1016/j.aquaculture.2012.04.009

Lee, C. S. (1981). Factors affecting egg characteristics in the fish *Sillago sihama*. *Marine Ecology Progress Series*, *4*(361), 361–363. https://doi.org/10.3354/meps004361

Lee, C. S., Hu, F., & Hirano, R. (1981). Salinity tolerance of fertilized eggs and larval survival in the flsh *Sillago sihama*. *Marine Ecology Progress Series*, *4*, 169–174. https://doi.org/10.3354/meps004169

Li, M. H., Hartnell, G. F., Robinson, E. H., Kronenberg, J. M., Healy, C. E., Oberle, D. F., & Hoberg, J. R. (2008). Evaluation of cottonseed meal derived from genetically modified cotton as feed ingredients for channel catfish, Ictalurus punctatus. *Aquaculture Nutrition*, *14*(6), 490–498. https://doi.org/10.1111/j.1365-2095.2007.00554.x

Li, M. H., Robinson, E. H., Oberle, D. F., Lucas, P. M., & Bosworth, B. G. (2012). Evaluation of corn gluten feed and cottonseed meal as partial replacements for soybean meal and corn in diets for pond-raised hybrid catfish, *Ictalurus punctatus×I. furcatus*. *Journal of the World Aquaculture Society*, *43*(1), 107–113. https://doi.org/10.1111/j.1749-7345.2011.00542.x

Li, Y., Ai, Q., Mai, K., Xu, W., Deng, J., & Cheng, Z. (2014). Comparison of high-protein soybean meal and commercial soybean meal partly replacing fish meal on the activities of digestive enzymes and aminotransferases in juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828). *Aquaculture Research*, *45*(6), 1051–1060. https://doi.org/10.1111/are.12042

Lin, S., & Luo, L. (2011). Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus×O. aureus*. *Animal Feed Science and Technology*, *168*(1–2), 80–87. https://doi.org/10.1016/j.anifeedsci.2011.03.012

Lin, S., Ma, H., Xu, T., Chen, Y., & Huang, X. (2018). Effects of replacing fish meal with compound proteins on the growth, body composition and biochemical indices of hybrid snakehead. *Journal of Fisheries of China*, *42*(5), 744–753. https://doi.org/10.11964/jfc.20170310770.

Lindsey, T. O., Hawkins, G. E., & Guthrie, L. D. (1980). Physiological responses of lactating cows to gossypol from cottonseed meal rations. *Journal of Dairy Science*, *63*(4), 562–573. https://doi.org/10.3168/jds.S0022-0302(80)82972-9

Liu, H., Yan, Q., Han, D., Jin, J., Zhu, X., Yang, Y., & Xie, S. (2016). Effect of dietary cottonseed meal on growth performance, physiological response, and gossypol accumulation in pre-adult grass carp, *Ctenopharyngodon idellus*. *Chinese Journal of Oceanology and Limnology*, *34*(5), 992–1003. https://doi.org/10.1007/s00343-016-4115-4

Liu, J., Fang, S., Zhu, Q., & Wang, Y. (2000). CN00134520.6. CHINA. Retrieved from http://www.wanfangdata.com.cn/details/detail.do?_type=patent&id=CN00134520.6

Lu, Z. (2007). *The study of effect of dietary protein sources and anti-nutritional factors on physiology and chemistry in the Chinese Mitten-handed crab, Eriocheir sinehsis* PhD thesis. Beijing, China: . East China Normal University.

Luo, Y., Ai, Q., Mai, K., Zhang, W., Xu, W., Zhang, Y., & Liufu, Z. (2013). Effects of dietary corn gluten meal on growth performance and protein metabolism in relation to IGF-I and TOR gene expression of juvenile cobia (*Rachycentron canadum*). *Journal of Ocean University of China*, *12*(3), 418–426. https://doi.org/10.1007/s11802-013-2021-3

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 with cottonseed meal-based diets in a recirculating system. *Aquaculture Nutrition*, *7*(3), 189–200. https://doi.org/10.1046/j.1365-2095.2001.00172.x

Merrifield, D. L., Dimitroglou, A., Bradley, G., Baker, R. T. M., & Davies, S. J. (2009). Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, *32*(9), 755–766. https://doi.org/10.1111/j.1365-2761.2009.01052.x

Miles, R. D., & Chapman, F. (2015). The Benefits of Fish Meal in Aquaculture Diets. University of Florida IFAS Extension, (May 2006), 1–6. Retrieved from http://edis.ifas.ufl.edu/pdffiles/FA/FA12200.pdf .

Nagy Szakál, D., Győrffy, H., Arató, A., Cseh, Á., Molnár, K., Papp, M., … Veres, G. (2010). Mucosal expression of claudins 2, 3 and 4 in proximal and distal part of duodenum in children with coeliac disease. *Virchows Archiv*, *456*(3), 245–250. https://doi.org/10.1007/s00428-009-0879-7

Pakravan, S., Akbarzadeh, A., Sajjadi, M. M., Hajimoradloo, A., & Noori, F. (2018). Chlorella vulgaris meal improved growth performance, digestive enzyme activities, fatty acid composition and tolerance of hypoxia and ammonia stress in juvenile Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, *24*(1), 594–604. https://doi.org/10.1111/anu.12594

Perera, E., & Yúfera, M. (2017). Effects of soybean meal on digestive enzymes activity, expression of inflammation-related genes, and chromatin modifications in marine fish (*Sparus aurata* L.) larvae. *Fish Physiology and Biochemistry*, *43*(2), 563–578. https://doi.org/10.1007/s10695-016-0310-7

Romano, G. B., & Scheffler, J. A. (2008). Lowering seed gossypol content in glanded cotton (*Gossypium hirsutum* L.) lines. *Plant Breeding*, *127*(6), 619–624. https://doi.org/10.1111/j.1439-0523.2008.01545.x

Santigosa, E., Sánchez, J., Médale, F., Kaushik, S., Pérez-Sánchez, J., & Gallardo, M. A. (2008). Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (Sparus aurata) in response to dietary fish meal replacement by plant protein sources. *Aquaculture*, *282*(1–4), 68–74. https://doi.org/10.1016/j.aquaculture.2008.06.007

Shi, X., Chen, F., Chen, G., Pan, Y., Zhu, X., Liu, X., & Luo, Z. (2017). Fishmeal can be totally replaced by a mixture of rapeseed meal and Chlorella meal in diets for crucian carp (*Carassius auratus gibelio*). *Aquaculture Research*, *48*(11), 5481–5489. https://doi.org/10.1111/are.13364

Shi, X., Luo, Z., Chen, F., Wei, C. C., Wu, K., Zhu, X. M., & Liu, X. (2017). Effect of fish meal replacement by Chlorella meal with dietary cellulase addition on growth performance, digestive enzymatic activities, histology and myogenic genes' expression for crucian carp *Carassius auratus*. *Aquaculture Research*, *48*(6), 3244–3256. https://doi.org/10.1111/are.13154

Siccardi, A. J., Richardson, C. M., Dowd, M. K., Wedegaertner, T. C., & Samocha, T. M. (2016). Digestibility of glandless cottonseed protein in diets for pacific white shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, *47*(1), 97–106. https://doi.org/10.1111/jwas.12252

Song, Z., Li, H., Wang, J., Li, P., Sun, Y., & Zhang, L. (2014). Effects of fishmeal replacement with soy protein hydrolysates on growth performance, blood biochemistry, gastrointestinal digestion and muscle composition of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture*, *426–427*, 96–104. https://doi.org/10.1016/j.aquaculture.2014.01.002

Sun, H., Tang, J., Yao, X., Wu, Y., Wang, X., & Liu, Y. (2016). Effects of replacement of fish meal with fermented cottonseed meal on growth performance, body composition and haemolymph indexes of Pacific white shrimp, Litopenaeus vannamei Boone, 1931. *Aquaculture Research*, *47*, 2623–2632. https://doi.org/10.1111/are.12711

Sun, H., Tang, J., Yao, X., Wu, Y., Wang, X., Liu, Y., & Lou, B. (2015). Partial substitution of fish meal with fermented cottonseed meal in juvenile black sea bream (*Acanthopagrus schlegelii*) diets. *Aquaculture*, *446*, 30–36. https://doi.org/10.1016/j.aquaculture.2015.04.020

Taher, S., Romano, N., Arshad, A., Ebrahimi, M., Teh, J. C., Ng, W.-K., & Kumar, V. (2017). Assessing the feasibility of dietary soybean meal replacement for fishmeal to the swimming crab, *Portunus pelagicus*, juveniles. *Aquaculture*, *469*, 88–94. https://doi.org/10.1016/j.aquaculture.2016.11.036

Wan, R. J. (1996). Morphology of early development of *Sillago sihama* Forsskál. *Marine Fisheries Research*, *17*, 35–41.

Wang, T., & Secombes, C. J. (2013). The cytokine networks of adaptive immunity in fish. *Fish and Shellfish Immunology*, *35*(6), 1703–1718. https://doi.org/10.1016/j.fsi.2013.08.030

Wang, X.-F., Li, X.-Q., Leng, X.-J., Shan, L.-L., Zhao, J.-X., & Wang, Y.-T. (2014). Effects of dietary cottonseed meal level on the growth, hematological indices, liver and gonad histology of juvenile common carp (*Cyprinus carpio*). *Aquaculture*, *428–429*, 79–87. https://doi.org/10.1016/j.aquaculture.2014.02.040

Wilson, R. P., Robinson, E. H., & Poe, W. E. (2018). Apparent and true availability of amino acids from common feed ingredients for channel catfish. *The Journal of Nutrition*, *111*(5), 923–929. https://doi.org/10.1093/jn/111.5.923

Yaghoubi, M., Mozanzadeh, M. T., Marammazi, J. G., Safari, O., & Gisbert, E. (2016). Dietary replacement of fish meal by soy products (soybean meal and isolated soy protein) in silvery-black porgy juveniles (*Sparidentex hasta*). *Aquaculture*, *464*, 50–59. https://doi.org/10.1016/j.aquaculture.2016.06.002

Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., … 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 Immunology*, *81*, 318–328. https://doi.org/10.1016/j.fsi.2018.07.038

Zhao, Z.-X., Song, C.-Y., Xie, J., Ge, X.-P., Liu, B. O., Xia, S.-L., … Zhu, S.-H. (2016). Effects of fish meal replacement by soybean peptide on growth performance, digestive enzyme activities, and immune responses of yellow catfish *Pelteobagrus fulvidraco*. *Fisheries Science*, *82*(4), 665–673. https://doi.org/10.1007/s12562-016-0996-6

Zhen, L. U., Xiao, E. N., & Jian, D. U. (2008). The population dynamics and parameter of growth and mortality of *Sillago sihama* in the Minnan-Taiwan fishing grounds. *Marine Fisheries Research*, *29*(5), 47–53.

Zhou, Q., Habte-Tsion, H.-M., Ge, X., Liu, B., Xie, J., Ren, M., … Pan, L. (2017). Growth performance and TOR pathway gene expression of juvenile blunt snout bream, *Megalobrama amblycephala*, fed with diets replacing fish meal with cottonseed meal. *Aquaculture Research*, *48*(7), 3693–3704. https://doi.org/10.1111/are.13195

Zhou, Y., Huang, H., Du, T., & Huang, Y. (2017). Study on adaptability of juvenile of *Sillago sihama* to salinity. *Journal of Fisheries Research*, *39*(1), 22–26. https://doi.org/10.14012/j.cnki.fjsc.2017.01.004.

How to cite this article: Liu H, Dong X, Tan B, et al. Effects of fish meal replacement by low-gossypol cottonseed meal on growth performance, digestive enzyme activity, intestine histology and inflammatory gene expression of silver sillago (*Sillago sihama* Forsskál) (1775). *Aquacult Nutr.* 2020;00:1–12. https://doi.org/10.1111/anu.13123