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Effects of guar gum supplementation in high-fat diets on fish growth, gut histology, intestinal oxidative stress, inflammation, and apoptosis in juvenile largemouth bass (*Micropterus salmoides*)

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Abstract

The present study aimed to investigate the influence of guar gum supplementation in high-fat diets on the growth performance and intestinal oxidative stress, inflammation, and apoptosis of juvenile largemouth bass. Five isonitrogenous diets were prepared: a control diet (10% crude lipid, C), a high-fat diet (17% crude lipid, HF), and three high-fat diets supplemented with 0.3% guar gum (GG0.3), 1% guar gum (GG1), and 3% guar gum (GG3). Largemouth bass (3.1±0.2 g) were randomly assigned to fifteen tanks (30 fish/tank) and fed for 8 weeks. The results demonstrated that GG0.3 significantly increased specific growth rate (SGR) and increased feed conversion ratio (FCR) compared to HF ($P < 0.05$). For histology, high-fat diets containing guar gum significantly increased intestinal villus length, villus width, and perimeter ratio, compared with HF ($P < 0.05$). Compared with Control, HF significantly decreased reduced glutathione (GSH) contents and increased malondialdehyde (MDA) contents in the intestine ($P < 0.05$). Additionally, HF significantly increased the expression of interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and cysteine-aspartic proteases 9 (Caspase 9) in the intestine ($P < 0.05$). Compared to HF, GG0.3 significantly decreased MDA contents, increased GSH contents, and downregulated the expression of IL-1β, TNF-α, and Caspase 3 than diet HF in the intestine ($P < 0.05$). These results suggest that guar gum can alleviate the adverse effects of high-fat diets on growth and gut health in fish.

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Introduction

With the rapid development of aquaculture, protein shortages in aquafeed have been a severe problem (Boyd et al., 2022). Several strategies have been used to reduce the pressure of protein deficiency in aquafeed, including high-fat diets (Gao et al., 2023). Lipids/fats are essential nutrients for fish, and increasing the lipid/fat contents in the feed within a specific range can promote fish health and save protein to some extent (Wang et al., 2019). However, fish have a limited ability to utilize dietary lipids (Chou et al., 2001). Several studies have shown that dietary lipids, in addition to feeding, can lead to intestinal inflammation and oxidative stress and reduce fish growth in farmed fish (Liu S. et al., 2022; Yu et al., 2020).

Intestinal damage (Dawood, 2021; Matejova et al., 2017) and oxidative stress (Chowdhury and Saikia, 2020; Zhu et al., 2008) are considered the primary cause of growth inhibition in fish. Histological methods are the primary tools for evaluating intestinal health (Rašković et al., 2011; Saraiva et al., 2016). Oxidative stress in fish is regulated by several antioxidant defenses (Martínez-Álvarez et al., 2005) and is closely connected with inflammation and apoptosis (Circu and Aw, 2010; Reuter et al., 2010). Therefore, dietary strategies to improve fish antioxidant defenses and intestinal damage could be of great potential in alleviating the high-fat diet-induced problems in aquaculture.

Guar gum is the main component of the endosperm of the guar bean, a legume, and generally contains 75%~85% polysaccharides (Gao et al., 2023). It can be used as a feed binder to reduce nutrient leaching (Sarathy and Saraswathi, 1983; Gao et al., 2019). Studies on mammals have indicated that guar gum can alleviate oxidative stress and intestinal damage (Hung and Suzuki, 2016; Dar-Chih et al., 2009). At present, research on guar gum in fish mainly focuses on the effect of guar gum as feed binders on fish growth and feed viscosity; little research has been conducted to explore the effect of guar gum on intestinal histology and oxidative stress (Enes et al., 2013; Gao et al., 2019).

Largemouth bass (*Micropterus salmoides*), a carnivorous fish derived from North America, is now widely farmed in China (Coyle et al., 2007; Pei et al., 2021). According to the China Fisheries Statistical Yearbook, the total production of largemouth bass exceeded 702,000 tons in 2021 (FBMA, 2022). Based on the above background, we hypothesized that dietary guar gum addition could improve intestinal antioxidant capacity, inflammation, and apoptosis in juvenile largemouth bass-fed high-fat diets.

Materials and Methods

Diet preparation

Shree Ram Gum Chemicals Ltd. (Jodhpur, India) provided guar gum containing 88.5% polysaccharide. The ratio of mannose to galactose in guar gum was 1.64:1. In this study. We prepared five diets with the same protein content: a control diet (10% lipid, C), a high-fat diet (17% lipid, HF), and three high-fat diets supplemented with 0.3%, 1%, and 3% guar gum (GG0.3, GG1, and GG3, respectively) (**Table 1**). All raw materials were crushed by a grinder and sieved through 100 mesh to remove large particles. Then the sieved raw materials were mixed according to the feed formula (**Table 1**). The mixture was extruded into pellets (diameter: 2 mm; length: about 3 mm) using a machine (High power-12B meat grinder). The feed was dried at 20 °C for 12 h and stored at -20 °C until use.

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

Raw materials (%)	Control	HF	0.3%GG	1%GG	3%GG
Fish meal[a]	51.0	51.0	51.0	51.0	51.0
Soybean protein concentrate[b]	10.0	10.0	10.0	10.0	10.0
Soybean meal[c]	9.8	9.8	9.8	9.8	9.8
Flour[d]	10.0	10.0	10.0	10.0	10.0
Fish Oil[e]	3.0	3.0	3.0	3.0	3.0
Soybean oil[f]	2.0	9.0	9.0	9.0	9.0
Vitamin C[g]	0.2	0.2	0.2	0.2	0.2
Premixes[h]	1.0	1.0	1.0	1.0	1.0
Choline[i]	0.5	0.5	0.5	0.5	0.5
Calcium dihydrogen phosphate[j]	1.5	1.5	1.5	1.5	1.5
CMC[k]	1.0	1.0	1.0	1.0	1.0
Guar gum[l]	0.0	0.0	0.3	1.0	3.0
Cellulose[m]	10.0	3.0	2.7	2.0	0.0
Chemical composition (%)					
Crude protein	47.0	47.0	47.1	47.1	47.3
Crude fat	10.0	17.0	17.0	17.0	17.0

[a] Fish meal: White fish meal, American Seafood Company, Seattle, WA, USA.
[b] Soybean protein concentrate: Hamlet Protein Denmark Ltd, Denmark.
[c] Soybean meal: Jinggrain (Tianjin) Grain and Oil Industry Co., Ltd, China.
[d] Flour: Zaozhuang Nongjia Shengyuan Flour Co., Ltd, China.
e Fish Oil: Anchovy oil from Peru.
[f] Soybean oil: Yihai Kerry Food Marketing Co., Ltd, China.
[g] Vitamin C: Shanghai Macklin Biochemical Co., Ltd, China.
[h] Premixes: Vitamin A 1 200 000 IU, Vitamin D 3 25 000 IU, Vitamin E 2 000 IU, Vitamin K 31 000 mg, Vitamin B1 250 mg, Vitamin B2 500 mg, L-methionine 10.000 mg, L-lysine 10.000 mg, Threonine 380 mg, food attractant 7 500 mg, serine 1.200 mg, leucine 660 mg, arginine 400 mg, alanine 600 mg, aspartic acid 800 mg, active yeast polysaccharide peptide 2 000 mg, biotin 50 mg, cystine 120 mg, ethanol 350 mg, growth promoter 2 000 mg, cobalt chloride 6 mg. calcium 230 g, iron 5 g, copper 1 g, manganese 2.5 g, zinc 1.5 g, cobalt 250 mg.
[I] Choline: Cangzhou Yihong Feed Co., Ltd, China.
[j] Calcium dihydrogen phosphate: Tianjin Fenghua Reagent Factory, China.
[k] CMC：Sodium hydroxymethylcellulose，Chongqing Lihong Fine Chemical Co., Ltd, China.
[l] Guar gum: Shree Ram Gum Chemicals Ltd., India.
[m] Cellulose: Huzhou Linghu Xinwang Chemical Co., Ltd, China.

Experimental fish and experimental conditions

The juvenile largemouth bass was purchased from the farm (Guangzhou, China) and acclimatized under experimental conditions for two weeks. The control diet C was the only food source for largemouth bass during this period. After 24 h of starvation, 450 fish (initial weight 3.1±0.2 g) were randomly allocated to 15 tanks (200 L, 30 fish per tank). Each diet received three duplicate tanks at random. Fish in the experiment received two meals (10:00 and 17:00) daily until they appeared satiated. The experiment lasted for a total of 8 weeks. During the trial, fish were subjected to natural photoperiod (approximately 12 h light/12h darkness); the water temperature was 25.5±0.6 °C, dissolved oxygen concentrations were above 6 mg/L, ammonia, and nitrite concentrations were both below 0.1 mg/L, pH was 7.4±0.3, and the hardness was 166±6 mg/L.

Sample collection

On day 56, after being starved for 24 h, all fish in each tank were counted and weighed to calculate growth-related parameters. Then 9 fish in each tank were randomly collected and anesthetized (benzocaine, 50 mg/L). Intestinal samples of three fish were sampled and fixed in 4% paraformaldehyde for histological analysis. Then intestinal samples of the other six fish were collected immediately, placed in liquid nitrogen for rapid freezing, and stored at a -80 °C refrigerator: three were for the analysis of antioxidant-related parameters; the other three were for the measurement of gene expression.

The following formulae were used to calculate the growth-related parameters:

Feed intake (FI) (g/ind) = dry feed intake/fish number

Specific growth rate (SGR, %/d) =100 × [ln final body weight − ln initial body weight]/days

Feed conversion ratio (FCR) = dry feed intake/wet weight gain

Hepatosomatic index (HSI) (%) = 100 × liver weight/body weight

Survival rate (%) = 100 × initial fish number/final fish number

The study protocol and experimental procedures in the present study were approved by the Animal Protection and Utilization Committee of Henan University of Science and Technology, Luoyang, China.

Analysis of feed chemical composition

The experimental diets were analyzed for composition according to the methods of AOAC (1995). Moisture content was measured by the loss-in-weight method and oven-dried to constant weight at 105 °C. Crude protein was determined by the Kjeldahl method using a 2300 Kjeltec analyzer. Crude fat was extracted with diethyl ether in a Soxtec system (Soxtec System HT6, Tecator, Haganas, Sweden) and measured by weight method. The ash content was determined using a muffle furnace at 550 °C for 12 h.

Determination of the antioxidant capacity of the intestine

Samples were homogenized in saline (6.8 g/L sodium chloride dissolved in double-distilled water at a dilution of 1:10) at 4 °C. The homogenate was centrifuged at 4000 rpm for 10 min, and the supernatant was obtained and stored temporarily at 4 °C until analysis. Antioxidant-related parameters, including malondialdehyde (MDA) (The thiobarbituric acid method; product number: A003-1), total superoxide dismutase (T-SOD) (The hydroxylamine method; product number: A001-1), catalase (CAT) (The ammonium molybdate method; product number: A007-1-1), glutathione peroxidase (GPx) (A spectrophotometric method; product number: A005-1), and reduced glutathione (GSH) (A spectrophotometric method; product number: A006-1-1) were assayed using commercial kits (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China). Protein concentrations in the intestinal homogenates were determined using a detergent-compatible Bradford protein detection kit (BTS Biotechnology Institute, Shanghai, China).

Intestinal histology

Each tank sample (nine per treatment) was dehydrated in ethanol and embedded in paraffin. The paraffin blocks were cut into 4 μm sections and stained with hematoxylin and eosin (HE). The morphological structures of the intestine, including villus height, villus width, and muscular width, were measured by analyzing the micrographs using Image-Pro Plus® 6.0 software (Media Cybernetics, Silver Spring, MD, USA). The calculation of absorptive surface (Perimeter ratio, PR arbitrary units) was performed following Dimitroglou et al. (2010). PR = IP/EP, in which IP and EP denote the intestinal lumen's internal and intestine's external perimeter.

Real-time fluorescence quantitative PCR analysis

The mRNA expressions of apoptotic and inflammatory genes (cysteine-aspartic proteases 3 (Caspase 3), cysteine-aspartic proteases 9 (Caspase 9), tumor necrosis factor α (TNF-α), and interleukin 1β (IL-1β)) in the intestine were measured by quantitative real-time PCR (qPCR). All primers were synthesized by Shanghai General Biotech Co, Ltd (**Table 2**). According to the manufacturer's instructions, total RNA was extracted from gut samples (35 mg) using Trizol (ComWin Biotech, China). The OD260/OD280 proportion was measured utilizing NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). When the A260/A280 values were 1.8~2.0, reverse transcribed using a reverse transcription kit (Vazyme, Nanjing, China). Real-time quantitative PCR was performed in a Mini Opticon Real-Time Detector (Bio-Rad, USA). RT-PCR analyses were performed in 96-well plates, where each well contained 20 μL of reaction mixture consisting of 10 μL ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China), 0.8 μL primers (10 μmol/L), 2 μL cDNA template and 7.2 μL sterilized ddH_2O. The reaction systems were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and annealing for 60 °C for 30 s. After the run, the melting curve of each amplicon was inspected to determine the specificity of the amplification. After the reaction, the fluorescent information was converted into Ct values. Data were quantified by the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001) and subjected to statistical analysis.

Table 2 Primer sequences for RT-PCR in the experiment.

Gene	Primer sequence forward (5′ → 3′)	Primer sequence reverse (5′ → 3′)	Product size (bp)
Caspase 3	GCTTCATTCGTCTGTGTTC	CGAAAAAGTGATGTGAGGTA	98
Caspase 9	CTGGAATGCCTTCAGGAGACGGG	GGGAGGGGCAAGACAACAGGGTG	125
TNF-α	CTTCGTCTACAGCCAGGCATCG	TTTGGCACACCGACCTCACC	161
IL-1β	CGTGACTGACAGCAAAAAGAGG	GATGCCCAGAGCCACAGTTC	166
EF1-α	GGCTGGTATCTCCAAGAACG	GTCTCCAGCATGTTGTCWCC	239

Caspase 3, cysteine-aspartic proteases-3; Caspase 9, cysteine-aspartic proteases-9; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β; EF1α, elongation factor 1α.

Calculations and statistical analysis

All data were shown as means with their standard errors. We used SPSS 19.0 (SPSS) for statistical analyses and checked for normality and homogeneity of variance before analysis. All data were subjected to one-way ANOVA followed by Duncan's multiple-range test. $P < 0.05$ was considered statistically significant.

Results

The growth performance indicators of largemouth bass

Compared to the Control, HF significantly decreased FI and SGR and increased FCR ($P < 0.05$). GG0.3 significantly increased final body weight (FBW) and SGR ($P < 0.05$). GG0.3 and GG1 significantly decreased FCR compared with HF ($P < 0.05$). Fish fed diets containing guar gum did not affect FI, HSI, and survival rates. GG3 did not affect FBW, SGR, and FCR compared to HF (**Table 3**).

Table 3 Growth performance of largemouth bass fed experimental diets (n = 3). Bars assigned with different superscripts are significantly different ($P < 0.05$).

Groups	FBW(g)	FI (g/ind)	SGR (%/d)	FCR	HSI (%)	Survival rate (%)
Control	21.60±1.32^{c}	20.35±0.97^{b}	3.40±0.12^{c}	1.10±0.03^{a}	4.09±0.49	100
HF	14.73±1.25^{a}	14.83±0.97^{a}	2.72±0.15^{a}	1.29±0.06^{b}	4.62±0.56	100
GG0.3	18.28±0.66^{b}	16.17±0.65^{a}	3.13±0.06^{bc}	1.07±0.06^{a}	4.45±0.46	100
GG1	17.84±0.21^{ab}	15.60±0.33^{a}	3.04±0.03^{abc}	1.06±0.03^{a}	4.20±0.36	100
GG3	15.98±1.29^{ab}	15.11±0.94^{a}	2.84±0.14^{ab}	1.19±0.04^{ab}	3.75±0.57	100

FBW, Final body weight; FI, feed intake; SGR, specific growth rate; FCR, feed conversion ratio; HSI, Hepatosomatic index.

Intestinal histology

The intestinal histology is shown in **Figure 1**. Villus length, muscular thickness, and perimeter ratio of fish fed the Control diet were higher than those fed diet HF ($P < 0.05$). Fish fed GG1, and GG3 demonstrated higher villus length and muscular thickness than those fed diet HF ($P < 0.05$). Diets containing guar gum significantly increased the perimeter ratio than HF ($P < 0.05$). Experimental diets did not affect the intestinal villus width of largemouth bass (**Figure 1**).

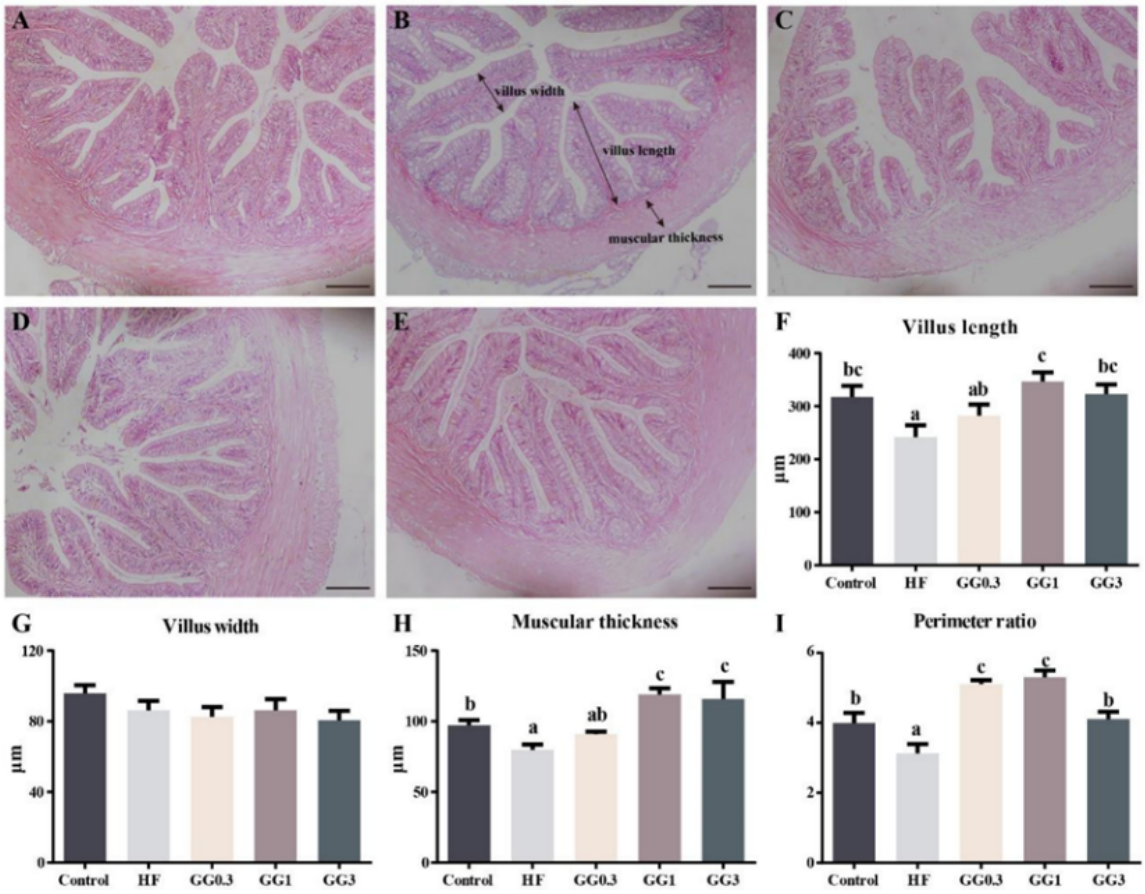

Figure 1 Hindgut morphology (HE, 100×) of largemouth bass fed experimental diets. Scale bar: 100 μm. A, Control. B, HF. C, GG0.3. D, GG1. E, GG3. F, villus length. G, villus width. H, muscular thickness. I, perimeter ratio. For F-I, bars assigned with different superscripts significantly differ ($P < 0.05$).

Intestinal oxidative stress

The effect of guar gum addition on the antioxidant-related parameters in the intestine is shown in **Table 4**. Diet HF significantly decreased GSH contents and increased MDA contents compared with Control ($P < 0.05$). Diet GG0.3 significantly decreased MDA contents compared to diet HF ($P < 0.05$). Moreover, diets GG0.3 and GG1 significantly increased GSH contents compared to diet HF ($P < 0.05$). Dietary guar gum supplementation in the high-fat diet did not affect T-SOD and CAT activity levels (**Table 4**).

Table 4 Intestinal antioxidant-related parameters of largemouth bass fed experimental diets.

Groups	T-SOD (U/mg prot)	CAT （U/mg prot）	GSH （mg/g prot）	MDA (nmol/mg prot)
Control	2.81±1.44	3.43±0.84^{b}	22.58±3.18^{b}	2.66±0.48^{a}
HF	2.00±0.58	1.49±0.47^{ab}	12.39±1.74^{a}	6.33±0.33^{c}
GG0.3	2.90±1.05	3.31±0.92^{b}	23.89±4.14^{b}	3.84±1.00^{ab}
GG1	2.26±0.83	3.35±0.57^{b}	22.84±3.57^{b}	4.88±0.76^{bc}
GG3	2.15±0.44	1.18±0.36^{a}	13.54±1.76^{a}	5.55±0.93^{bc}

Data are means ± SE (n = 9). Data in the same column with different superscript letters differ significantly ($P < 0.05$). T-SOD, total superoxide dismutase; CAT, catalase; GSH, reduced glutathione; MDA, malondialdehyde.

Intestinal inflammation and apoptosis

The expression of inflammatory and apoptotic genes in the intestine is presented in **Figure 2**. Compared to Control, HF significantly increased the expression of IL-1β, TNF-α, and Caspase 9 ($P < 0.05$). Diet GG0.3 significantly downregulated the expression of IL-1β, TNF-α, and Caspase 3 than diet HF ($P < 0.05$). In addition, diet GG1 also significantly decreased the expression of TNF-α and IL-1β compared with HF ($P < 0.05$). Diet GG3 did not affect the expression of these genes compared to diet HF (**Figure 2**).

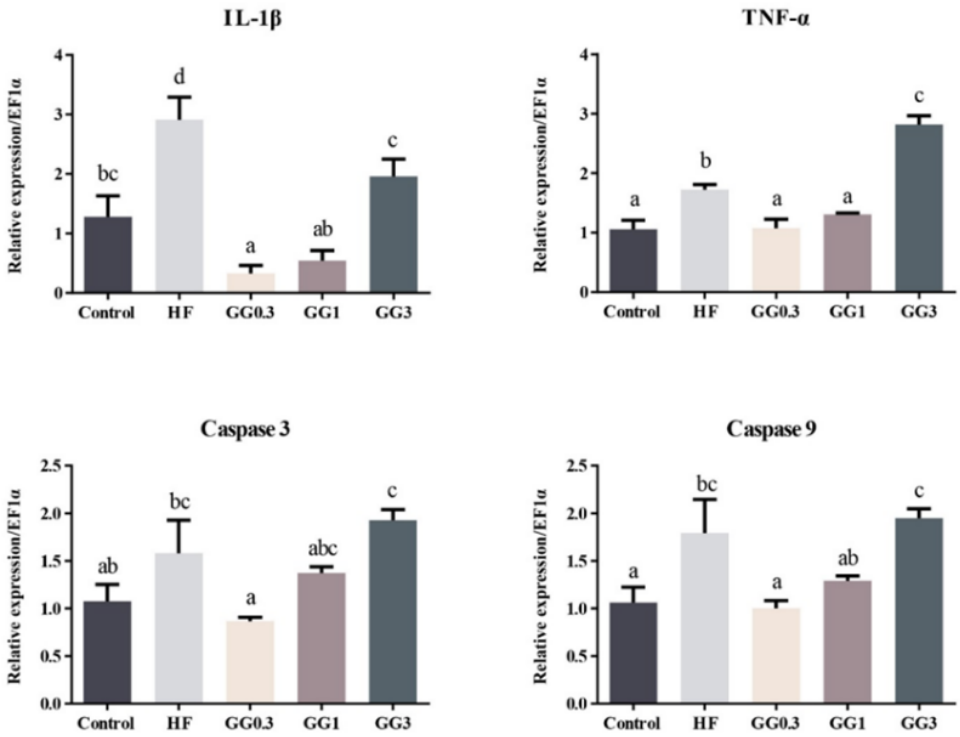

Figure 2 Relative expression of inflammatory and apoptotic genes in the intestine of largemouth bass fed experimental diets (n = 9). Bars assigned with different superscripts are significantly different ($P < 0.05$). IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; Caspase 3, cysteine-aspartic proteases 3.

Discussion

Guar gum improved the growth performance of largemouth bass-fed high-fat diets

The present study dramatically reduced the SGR of largemouth bass fed the high-fat diet. Previous studies have also demonstrated the adverse effects of a high-fat diet on fish growth, including those on grass carp (*Ctenopharyngodon idella*) (Du et al., 2006), Nile tilapia (*Oreochromis niloticus*) (Zhang et al., 2020), largemouth bass (Zhou et al., 2020), and blunt snout bream (*Megalobrama amblycephala*) (Dai et al., 2019). Guar gum positively impacted the growth performance of fish fed the high-fat diet, as demonstrated by the rise in SGR. Supplementing the high-fat diet with 1% and 3% guar gum did not affect fish growth, which agreed with our previous study on gibel carp (Gao et al., 2019). Storebakken (1985) investigated the effects of dietary guar gum supplementation on the growth performance of rainbow trout (*Oncorhynchus mykiss*) and found that adding 2.5% guar gum did not affect fish growth and FCR.

Long-term feeding of high-fat diets can result in damage to the intestinal structure, which affect the ability of fish to develop (Chen et al., 2023; Li et al., 2013; Sargent et al., 2003). Guar gum may improve fish growth by alleviating the damage of high-fat diets to the intestinal structure. We next evaluated the intestinal histology of fish fed experimental diets.

Guar gum alleviated the intestinal damage of largemouth bass fed high-fat diets

The intestine is an essential organ for fish in digesting and absorbing food nutrients (Bakke et al., 2010). For this reason, monitoring the morphology and health of the intestine is the key to explaining the difference in fish growth performance. Histological methods are usually employed to assess the impact of experimental feed on fish intestines (Rašković et al., 2011). In the present study, morphometric and semiquantitative methods were adopted to evaluate

the histology structure of the intestine. The increased villus height and muscle thickness of the intestine reflect strong utilization of nutrients and contraction force, respectively (Xu et al., 2020; Yang et al., 2022). The perimeter ratio is also a standard indicator for aquaculture's absorptive surface of the intestine (Dimitroglou et al., 2010). High-fat diets significantly reduced villus height, muscular thickness, and perimeter ratio. In agreement with our results, reduction in villus height and muscular thickness have also been found in rice field eel (*Monopterus albus*) (Shi et al., 2022) and largemouth bass (Yin et al., 2021) fed high-fat diets. However, supplementing the high-fat diet with guar gum significantly increased the villus height, muscular thickness, and perimeter ratio, indicating that guar gum can alleviate the high-fat diet-induced damage to the intestinal structure and health.

Guar gum can reduce the intestinal oxidative stress of largemouth bass fed high-fat diets

In aquaculture, several studies have shown that excessive adding lipids to feed can lead to intestinal oxidative stress in farmed fish (Liu S. et al., 2022; Yu et al., 2020). MDA is a lipid peroxidation marker that can produce biofilm damage and crosslinking with protein and nucleic acid molecules, impairing the organism's normal metabolic metabolism (Torun et al., 2009). The antioxidant enzyme system of organisms, which consists of T-SOD, CAT, and GSH, may eliminate reactive oxygen species (ROS), hydroxyl radicals (HO·), superoxide anions ($O2^{\cdot-}$), and hydrogen peroxide (H_2O_2) and protect cells from the toxicity of lipid peroxidation and the damage caused by oxidative stress (Matés and Sánchez-Jiménez, 1999).

In this study, high-fat diets raised the levels of MDA. They decreased T-SOD and CAT activity levels in the intestine, suggesting that high-fat diets caused oxidative stress and reduced the antioxidant defense of largemouth bass. Other research on the common carp (*Cyprinus carpio*) (Abasubong et al., 2022) and the blunt snout bream (Lu et al., 2017) have shown similar findings. Our research demonstrates that GG0.3 significantly boosted the activities of GSH contents while decreasing the levels of MDA in the intestine. These findings suggested that guar gum might enhance ’fish antioxidant capacity and lessen oxidative stress caused by high-fat diets. Guar gum has been proven to eliminate $O2^{\cdot-}$ and H_2O_2 produced by high-fat diets in hamsters (Dar-Chih et al., 2009). Relevant studies have found that guar gum can increase mice's mitochondrial AMP/ATP ratio, thus activating AMP-activated protein kinase (AMPK) (Den Besten et al., 2015). According to the research by wang et al. (2022), activating the AMPK/nad-dependent deacetylase sirtuin 1 (SIRT1)/forkhead transcription factor 1 (FOXO1) pathway can reduce oxidative stress (D-galactose-induced) in mice. A study on blunt snout bream indicated that AMPK may reduce hepatic oxidative stress by activating antioxidant enzymes CAT and SOD (Chen et al., 2021). One study on largemouth bass showed that guar gum could increase nuclear factor E2-related factor 2 (Nrf2) expression levels (Liu Y. et al., 2022). Zebrafish research has revealed that Nrf2 is essential for controlling the genes associated with antioxidants (Nakajima et al., 2011). Therefore, guar gum might increase antioxidant capacity and decrease oxidative stress in fish fed high-fat diets by activating AMPK and Nrf2. Further studies were needed to elucidate the underlying mechanism.

Guar gum relieved intestinal inflammation, and apoptosis of largemouth bass fed high-fat diets

IL-1β and TNF-α are common proinflammatory cytokines and good markers of inflammation (Chen et al., 2023; Covello et al., 2009). In the present study, the high-fat diet significantly increased the expression of IL-1β and TNF-α in the intestine, indicating intestinal inflammation in fish fed the high-fat diet. However, adding 0.3%~1% guar gum in the high-fat diet significantly decreased the expression of IL-1β and TNF-α in the intestine, implicating the anti-inflammatory effects of guar gum. Few studies have reported guar gum's anti-inflammatory effect in aquaculture. However, numerous studies on mice have proven the anti-inflammatory function of guar gum (Jhundoo et al., 2021; Naito et al., 2006). In a murine

model of sodium sulfate-induced colitis, Hung and Suzuki (2016) investigated the effects of guar gum on intestinal inflammation. They found that dietary 10% guar gum supplementation significantly decreased the expression of TNF-α and reduced intestinal inflammation in colitic mice.

Apoptosis is a common intestinal injury in fish fed high-fat diets (Guicciardi and Gores, 2005; Jia et al., 2020). In the process of apoptosis, Caspase 9 is responsible for initiating caspase activation cascades; Caspase 3 is a critical molecule in the execution of apoptosis (Logue and Martin, 2008). In this study, fish fed the high-fat diet demonstrated higher expression of Caspase 3 and 9 in the intestine than controls, indicating that the high-fat diets induced apoptosis. Similar results were found in blunt snout bream (Lu et al., 2017) and tilapia (Jia et al., 2020). Nevertheless, the administration of 0.3% guar gum in the high-fat diet significantly downregulated the expression of Caspase 3 and Caspase 9 in the intestine, suggesting an essential role of guar gum in counteracting apoptosis. Despite few studies to report the anti-apoptotic effect of guar gum in fish, a study on mice has reported that the dietary supplementation with partially hydrolyzed guar gum (2,000 mg/kg/day) can significantly reduce cleavage of Caspase 3 and suppress intestinal cell apoptosis induced by alcohol administration (Wu et al., 2019). Further investigation is needed to elucidate the mechanism of action of guar gum in attenuating inflammation and apoptosis in aquaculture.

Conclusion

In conclusion, this study shows that dietary 0.3% guar gum can mitigate the adverse effects of a high-fat diet on fish growth and gut histology in juvenile largemouth bass. Supplementation of 0.3% guar gum in the high-fat diet reduced intestinal oxidative stress, inflammatory responses, and apoptosis in juvenile largemouth bass.

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GRediT

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