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Assessing effects of guar gum viscosity on the growth, intestinal flora, and intestinal health of *Micropterus salmoides*

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ABSTRACT

A 56-day feeding trial was conducted to assess the effects of different viscous guar gum on the growth, intestinal flora, and intestinal health of *Micropterus salmoides*. Four practical diets with 42.5 % crude protein and 13.7 % crude lipid were formulated to contain 8 % cellulose and three different viscosities (2500, 5200, and 6000 mPa·s) of guar gum. Dietary guar gum inhibits fish growth and feed utilization, decreases the α-diversity of the intestinal flora, and negatively alters the intestinal flora structure and metabolite composition. High viscous guar gum down-regulated the intestinal tight junction, anti-inflammatory, and anti-apoptotic related gene's expression, decreased digesta butyrate/histamine ratio; and increased the abundance of *Plesiomonas shigelloides*. These results suggest that dietary guar gum adversely affects intestinal health by disrupting intestinal flora structure and metabolite composition, and that viscosity should be considered when using guar gum as a binder in aquafeeds.

1. Introduction

Largemouth bass (*Micropterus salmoides*) is widely farmed in China because of its rapid growth, strong adaptability, and high nutritional value [1,2]. According to reports, 619,519 tons of largemouth bass were produced in China in 2020, with is an increase of 29.66 % as compared to that in 2019 (477,808 tons) [3]. As a typical carnivorous fish, largemouth bass has high dietary protein (48–51 %) [4] and fishmeal requirements (40–50 %) [5] and poorly utilizes dietary carbohydrates [6]. However, limited fishmeal sources have increased the use of plant-based materials in aquafeeds over the last decades, and more binders or fillers are being used to produce aquafeeds to improve the physical properties or reduce the production cost [7]. These strategies ultimately cause aquafeeds to carry high levels of carbohydrates, including starch, oligosaccharides, and non-starch polysaccharides (NSPs) [8], forcing largemouth bass to face high dietary carbohydrate challenges. To date, carbohydrate-related studies on largemouth bass have focused on starch [9–11], whereas limited attention has been paid to NSPs. NSPs, mainly cellulose, hemicellulose, and pectin, which are formed by linking multiple glycosidic bonds [e.g., β-(1, 4), β-(1, 3), α-(1, 5), and α-(1,6)] [12]. Because of the absence of NSPs-degrading enzymes (e.g., β-glucanase, xylanases, and cellulase), NSPs cannot be directly utilized by fish and are considered as non-nutritive components in their diets [13]. Moreover, studies have shown that dietary NSPs remain in the gastrointestinal tract and have various detrimental effects on fish, such as inhibited nutrient utilization, decreased growth rate, induced metabolic disorders, and tissue damage [13–16]. Generally, these detrimental effects are primarily observed in the intestine and are believed to originate from soluble NSPs (SNSPs, e.g., hemicellulose and pectin), as they are naturally viscous and tend to increase digesta viscosity [12–14,17].

The intestine is an important digestive and immune organ in fish, and a healthy intestine is beneficial to fish health and growth [18]. In addition, the intestinal morphology and health are inevitably affected by dietary ingredients [19]. Previous studies have shown that dietary NSPs can be used as carbon sources by the intestinal flora and act as regulators of the flora community [12,20]. Dietary NSPs can also be used as substrates to produce short-chain fatty acid-based metabolites through fermentation [21], which in turn plays an essential role in improving intestinal function and health [22]. Moreover, changes in the intestinal microbial structure can alter metabolite composition, such as neurotransmitters, which are closely associated with gut function [23]. Consequently, dietary NSPs may significantly influence the intestinal health of fish by regulating the intestinal flora or their metabolites; however, studies in fish relevant to this issue are limited.

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Guar gum, an SNSPs (hemicellulose) mainly consisting of galactomannan, is extracted from *Cyanopsis tetragonolobus* [24]. It is commonly used as a stabilizer and thickener in food, cosmetics, and medicinal processing, owing to its excellent binding properties [25]. Recently, guar gum has been used as a binder in aquafeeds [26,27] and has shown the ability to increase feed hardness and reduce water solubility and nitrogen loss. This has contributed to the mitigation of water pollution in intensive aquaculture [26]. Although increasing dietary guar gum levels can improve the physical quality of aquafeeds [26], studies have found that it is detrimental to intestinal morphology, nutrient utilization, lipid metabolism, and growth performance in fish [26,28,29]. However, no systemic study has been conducted for investigating the effects on fish intestinal health. Moreover, previous studies have only considered the influence of guar gum supplementation levels and have not considered the potential influence of differences in the physicochemical properties of guar gum, such as molecular weight and viscosity. Therefore, this study aimed to assess the influences of different viscous guar gums on the growth, intestinal flora, and intestinal health of the largemouth bass. The findings can bridge the gap in the study of dietary NSPs on largemouth bass and serve as a reference for the application of guar gum as an aquafeed binder.

2. Materials and methods

2.1. Characterization of guar gum

The three types of guar gum, low-viscosity guar gum (Lvs-GG), middle-viscosity guar gum (Mvs-GG), and high-viscosity guar gum (Hvs-GG) used in this trial were purchased from Guangrao Liuhe Chemical Co., Ltd. (Dongying, China) with viscosities of 2500, 5200, and 6000 mPa·s, respectively. Additional product information is provided in Table S1. The molecular weight and composition of the three types of guar gum were determined using a liquid chromatography system (ICS5000, Thermo, USA) with a Dionex™ CarboPac™ PA20 (150 × 3.0 mm, 10 μm), and the results are shown in Fig. 1.

2.2. Diets preparation

Four isonitrogenous (42.5 % crude protein) and isolipidic diets (13.7 %) containing 8 % cellulose, and 8 % Lvs-GG, Mvs-GG, and Hvs-GG were formulated separately. The diets were prepared and stored according to the laboratory method described by Liu et al. [17]. The diet formulation and approximate composition are presented in Table S2.

2.3. Feeding trial

The largemouth bass used in this trial was purchased from the freshwater farming base of Guangdong Ocean University and was approved by the Animal Research and Ethics Committee of Guangdong Ocean University. All experimental procedures were performed in strict accordance with relevant guidelines. A total of 640 healthy largemouth bass of uniform size (6 g) were randomly assigned to four groups, with 4 replicates and 40 fish per replicate. Fish were farmed in 16 net cages of uniform size (1.2 m × 0.8 m × 1.0 m) and placed in the same cement pond. The feeding trial lasted 56 days, and the fish were fed twice daily until satiation. The water temperature, dissolved oxygen, and ammonia nitrogen were all maintained at 28–31 °C, above 6.0 mg/L, and below 0.02 mg/L, respectively. The feed consumption and fish mortality of each net cage were accurately recorded.

2.4. Sample collection

Fish were fasted for 24 h at the end of the trial, weighed, and counted for calculating growth performance. Subsequently, sampling was initiated by anesthetizing the fish with a 0.1 g/L solution of MS-222 (Sigma, USA). Sampling strategies, sample preparation, and preservation were

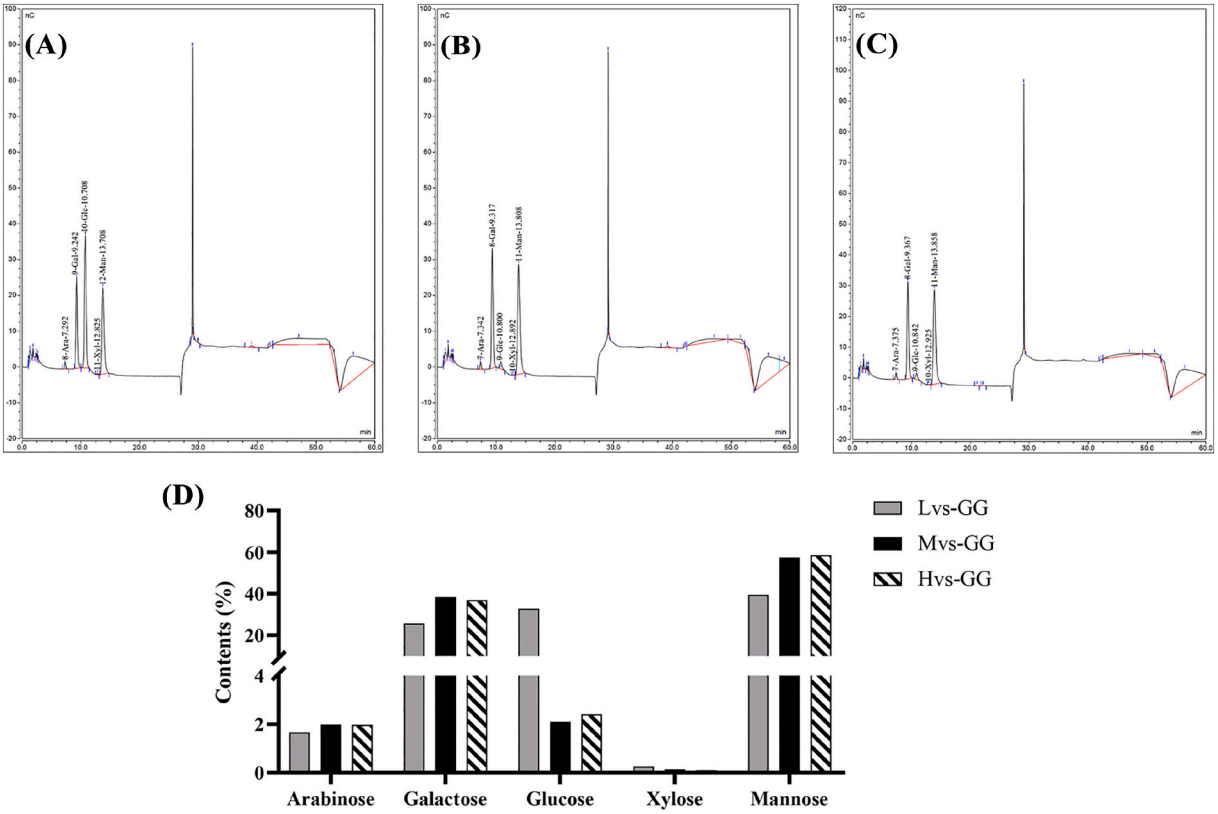

Fig. 1. Liquid chromatographic analysis of different viscous guar gum. Lvs-GG, Mvs-GG, and Hvs-GG are present in (A), (B), and (C), respectively.

performed according to the methods described in a previous study [17].

2.5. Biochemical indices analysis

Plasma diamine oxidase (DAO), and intestinal catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities and plasma D-lactic acid (D-LA), lipopolysaccharide (LPS), endothelin-1 (ET-1), and intestinal mucoprotein 2 (MUC2), immunoglobulin T (IgT), immunoglobulin receptor (IgR), and immunoglobulin M (IgM) concentrations were determined using commercially available kits (ELISA, Shanghai Enzyme Link Biotechnology Co., Ltd., Shanghai, China) according to the methods described in a previous study [17].

2.6. Gene expression analysis

Total RNA was extracted using the TRIzol™ Reagent kit (TransGen Biotech, Beijing, China) following the manufacturer's instructions, and the integrity and concentration of total RNA were detected using a 1.2 % denatured agarose gel and spectrophotometer, respectively. Qualified samples were used for cDNA synthesis. Quantitative real-time PCR analysis was conducted using a high-throughput fluorescent quantitative PCR instrument with a 10 μL SYBR® Green Premix *Pro Taq HS qPCR Kit II* reaction system (Accurate Biology, China), following the method described previously [30]. Specific primer sequences used in this study are listed in Table S3. The expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method [18] and normalized to the expression level of *ef1α* in the control group.

2.7. Microbiota structure analysis and functional prediction

The bacterial DNA was extracted using HiPure Soil DNA kits, following a quality test performed using an ultraviolet (UV)-spectrophotometer (Thermo Fisher Scientific, USA). Subsequently, the qualified samples were amplified using the universal primers 27F (5′–AGRGTTTGATYNTGGCTCAG–3′) and 1492R (5′–TASGGHTAC CTTGTTASGACTT–3′). The products were used to construct a gene library and perform long fragment sequencing using the PacBio platform. Sequencing and data analysis were conducted by Guangzhou Genedenovo Biotechnology Co., Ltd.

The high-quality bacterial and archaeal genomes in the digesta samples were compared and sorted using the PICRUSt2 software combined with the Integrated Microbial Genomes (IMG) database, and then a phylogenetic tree was constructed based on the sequences for functional prediction.

2.8. Digesta short-chain fatty acids and neurotransmitters analyses

Digesta short-chain fatty acid (SCFA) concentrations were determined using gas chromatography–mass spectrometry (GC–MS, Thermo Fisher Scientific, USA) following the method described by Zhang et al. [31]. Briefly, 50 mg of digesta sample was homogenized with 50 μL phosphate buffer (15 %), 400 μL ether, and 100 μL isocaproic acid (internal standard, 125 μg/mL) for 1 min, and then the supernatant was collected by centrifugation at 4 °C, 14,000 ×*g* for 10 min, followed by GC–MS analysis. Standard curve samples were prepared using a gradient mixture of caproate, valerate, propionate, butyrate, and acetate (Sigma, USA). All samples were injected and separated using an Agilent HP-INNOWAX capillary column (30 m × 0.25 mm I.D. × 0.25 μm; Agilent Technologies Inc., USA) and subsequently analyzed by mass spectrometry. Finally, the digesta SCFA content was calculated using a standard curve. This analysis work was conducted at Suzhou PANOMIX Biomedical Tech Co., Ltd.

Additionally, digesta neurotransmitter concentrations were determined using liquid chromatography-mass spectrometry (LC-MS, Thermo Fisher Scientific, USA) accoeding to the method described by Huang et al. [32]. Briefly, the 10 mg of digesta sample was homogenously mixed with 10 μL ascorbic acid stock (10 mg/mL) and 500 μL methanol evenly, and then centrifuged at 4 °C, 16,400 ×*g* for 10 min. Subsequently, the supernatant was blown dry with nitrogen and dissolved by adding 1000 μL methanol (20 %), and then centrifuged at 4 °C, 16,400 ×*g* for 10 min. Finally, 100 μL of supernatant was collected for analysis. Standard curve samples were prepared using gradient mixtures of 27 neurotransmitters (Table S4). All samples were injected and separated using an Agilent RP column (100 × 3 mm ID × 2.5 μm; Agilent Technologies Inc., USA) and subsequently analyzed by mass spectrometry. Sample testing and data analysis were performed by Anachro Technologies (Wuhan, China).

2.9. Correlation analysis

The correlation between intestinal flora (at the species level) and metabolites (SCFA and neurotransmitters) was assessed using the Spearman correlation analysis (using the psych software package) [33].

2.10. Calculation and statistical analysis

The calculations used in this study are presented in Section S1. Data are presented as means ± standard error of measurement (means ± SEM) and analyzed using one-way variance analysis (ANOVA) with SPSS software (version 20.0; Chicago, USA), followed by a Tukey's HSD test. $P < 0.05$ indicates a significant difference between the data.

3. Results

3.1. Growth performance

The weight gain rate, specific growth rate, protein efficiency ratio, and protein productive value in the guar gum groups were significantly lower than those in the control group, and these parameters in the Mvs-GG and Hvs-GG groups were significantly lower than those in the Lvs-GG group; however, the feed intake and feed conversion ratio showed the opposite result ($P < 0.05$; Fig. 2).

3.2. Intestinal mucosal barrier status

Plasma D-LA and DAO concentrations were not significantly affected by the experimental diets ($P > 0.05$; Table S5). The plasma LPS and ET-1 concentrations in the Hvs-GG group were significantly higher than those in the control group ($P < 0.05$). The expression levels of Occludin and Caludin-4 in the Mvs-GG group and the expression levels of Caludin-1 in the Lvs-GG and Hvs-GG groups were significantly lower than those in the control group ($P < 0.05$; Fig. 3A). The expression level of *ZO-1* in the Mvs-GG and Hvs-GG groups was significantly lower than that in the control group ($P < 0.05$).

3.3. Intestinal antioxidant capacity

The intestinal MDA and GSH concentrations and POD activity were not significantly affected by the experimental diets ($P > 0.05$; Table S6). The intestinal CAT activity in the guar gum groups was significantly lower than that in the control group, and the intestinal SOD and GPX activities in the Lvs-GG and Mvs-GG groups were significantly higher than those in the control groups ($P < 0.05$). The expression levels of *Keap1* and *Nrf2* in the Lvs-GG and Hvs-GG groups were significantly higher than those in the control group, and were highest in the Hvs-GG group ($P < 0.05$; Fig. 3B).

3.4. Intestinal immune status

Intestinal IgR, IgT, MUC2, and NO concentrations were not significantly affected by the experimental diets ($P > 0.05$; Table S7), whereas

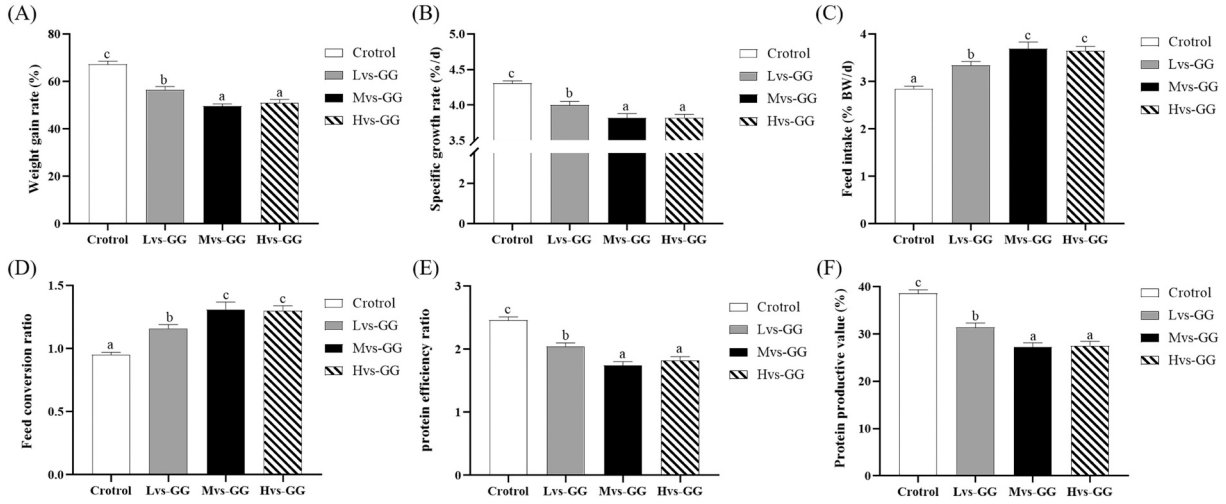

Fig. 2. Effects on the growth performance of largemouth bass fed with different viscous guar gum diets. Values of each column with different superscripts indicates significant difference ($P < 0.05$; $n = 4$).

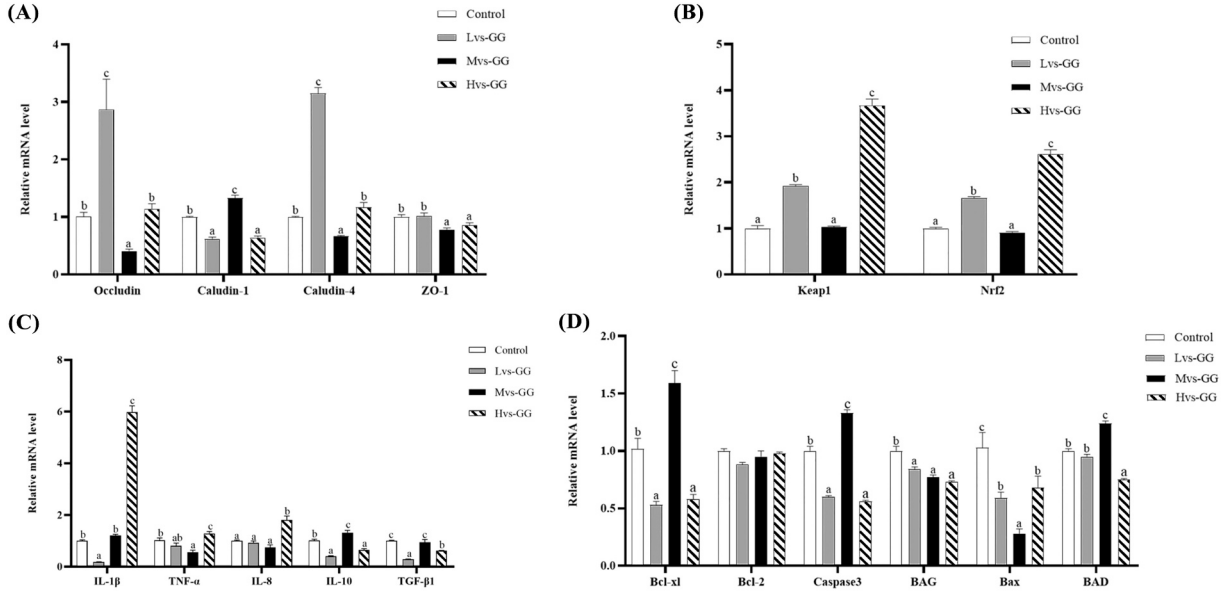

Fig. 3. Intestinal gene expression level of largemouth bass fed with different viscous guar gum diets. (A) tight junction-related gene expression; (B) antioxidant-related gene expression; (C) inflammation-related gene expression, and (D) apoptosis-related gene expression. Values of each column with different superscripts indicates significant difference ($P < 0.05$; $n = 4$). Keap1, Kelch-1ike ECH- associated protein l; Nrf2, nuclear factor erythroid 2-related factor 2, IL-8, interleukin-8; IL-1β, interleukin-1 beta; TNF-α, tumour necrosis factor alpha; IL-10, interleukin-10; TGF-β1, transforming growth factor β1; ZO-1, zona occludns protein 1; Bcl-2, b-cell lymphoma 2; Bcl-xl, b-cell lymphoma-xl; BAG, bcl-2-associated athanogene; Caspase3, cysteine-aspartic protease-3; Bax, bcl-2 associated X; BAD, bcl-2-associated death protein.

intestinal IgM concentration in the Lvs-GG group was significantly higher than that in the control group ($P < 0.05$). The expression levels of *IL-8*, *IL-1β*, and *TNF-α* in the Hvs-GG group were significantly higher than those in the control group, and the expression levels of *IL-1β* in the Lvs-GG group and the expression level of TNF-α in the Mvs-GG group were significantly lower than those in the control group ($P < 0.05$; Fig. 3C). The expression levels of *IL-10* and *TGF-β1* in the Lvs-GG and Hvs-GG groups were significantly lower than those in the control group, whereas the expression level of *IL-10* in the Mvs-GG group was significantly higher than that in the control group ($P < 0.05$).

3.5. Intestinal apoptosis-related gene expression

The expression level of Bcl-2 was not significantly affected by the experimental diets ($P > 0.05$; Fig. 3D). The expression levels of *Bcl-xl* and *Caspase-3* in the Lvs-GG and Hvs-GG groups were significantly lower than those in the control group, whereas the expression levels of these genes were significantly up-regulated in the Mvs-GG group ($P < 0.05$). Additionally, the expression levels of *BAG* and *Bax* in the guar gum groups were significantly lower than those in the control group ($P < 0.05$). The expression level of *BAD* in the Hvs-GG group were significantly lower than that in the control group ($P < 0.05$).

3.6. Intestinal flora structure and functional prediction

A total of 232,034 effective tags were derived from 16 samples, with an average of 14,502.13 tags per sample (Table 1). The number of operational taxonomic units (OTUs) in the Mvs-GG and Hvs-GG groups

Table 1
Effects on α-diversity in intestinal flora and OTUs of largemouth bass fed with different viscous guar gum diets.

Item	Group			
	Control	Lvs-GG	Mvs-GG	Hvs-GG
Reads	14,572.75 ± 755.31	13,574.25 ± 1854.87	15,356.00 ± 461.09	14,842.00 ± 334.10
Effective tags	14,504.75 ± 768.91	13,520.75 ± 1854.45	15,284.50 ± 449.48	14,698.50 ± 332.28
OTU	43.00 ± 3.03^{b}	45.75 ± 6.41^{b}	24.25 ± 1.89^{a}	31.00 ± 3.74^{a}
Sobs	38.00 ± 3.03^{b}	40.50 ± 4.92^{b}	23.25 ± 1.60^{a}	27.50 ± 2.99^{a}
Shannon	1.99 ± 0.21^{c}	1.08 ± 0.19^{b}	0.53 ± 0.10^{a}	0.74 ± 0.08^{ab}
Simpson	0.62 ± 0.06^{b}	0.28 ± 0.04^{a}	0.15 ± 0.03^{a}	0.24 ± 0.03^{a}
Chao	55.87 ± 4.28^{b}	50.61 ± 6.82^{b}	48.54 ± 12.29^{ba}	39.16 ± 7.76^{a}
Ace	48.36 ± 3.05^{b}	52.56 ± 6.52^{b}	36.18 ± 4.99^{a}	40.88 ± 8.25^{ba}
Goods coverage	1.00	1.00	1.00	1.00

Data are shown as mean ± SEM ($n = 4$), and data in the same row with different superscripts indicate that there is a significant difference between each other ($P < 0.05$).

was significantly lower than that in the control and Lvs-GG groups ($P < 0.05$), whereas no significant difference was observed between the Lvs-GG and control groups ($P > 0.05$). Alpha-diversity analysis showed that the Sobs index in the Mvs-GG and Hvs-GG groups was significantly lower than that in the control and Lvs-GG groups, and the Shannon and Simpson indices in the guar gum groups were significantly lower than those in the control group ($P < 0.05$). Moreover, the Chao index in the Hvs-GG group and Ace index in the Mvs-GG group were significantly lower than those in the control group ($P < 0.05$). Principal component analysis (PCA) showed that dietary guar gum caused significant changes in intestinal flora structure (Fig. 4A). Subsequent compositional analysis showed that the four dominant phyla in the control group were Fusobacteria (55.06 %), Firmicutes (37.11 %), Proteobacteria (7.15 %), and Tenericutes (0.66 %) (Fig. 4B). The four domain phyla in the Lvs-GG

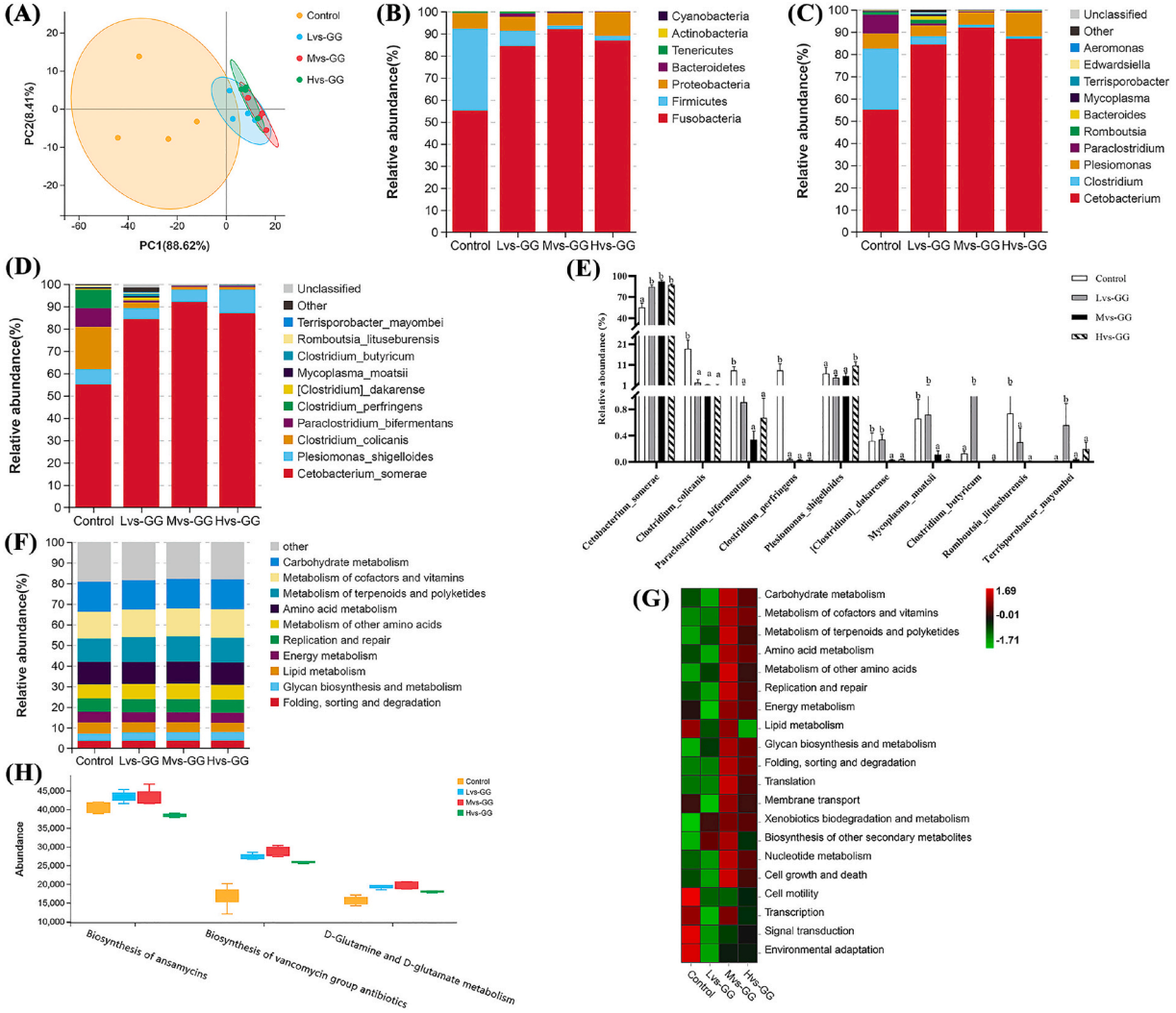

Fig. 4. Intestinal flora composition analysis of largemouth bass fed with different viscous guar gum diets. (A) PCA analysis results; (B) phylum level; (C) genus level; (D) species level; (E) top 10 dominant species; (F) functional stacking diagram of intestinal flora; (G) functional clustering heat map of intestinal flora, and (H) significantly different functions of intestinal flora.

group were Fusobacteria (84.34 %), Firmicutes (6.98 %), Proteobacteria (6.25 %), and Bacteroidetes (1.61 %). The four domain phyla in the Mvs-GG group were Fusobacteria (92.01 %), Proteobacteria (5.59 %), Firmicutes (1.68 %), and Bacteroidetes (0.61 %). The four domain phyla in the Hvs-GG group were Fusobacteria (86.89 %), Proteobacteria (10.77 %), Firmicutes (2.09 %), and Bacteroidetes (0.21 %).

At the genus level, *Cetobacterium* (55.06 %), *Clostridium* (27.44 %), *Paraclostridium* (8.37 %), and *Plesiomonas* (6.86 %) were the four dominant genera in the control group (Fig. 4C). The four dominant genera in the Lvs-GG group were *Cetobacterium* (84.34 %), *Plesiomonas* (4.86 %), *Clostridium* (3.70 %), and *Romboutsia* (1.70 %). The four dominant genera in the Mvs-GG group were *Cetobacterium* (92.01 %), *Plesiomonas* (5.55 %), *Clostridium* (1.24 %), and *Bacteroides* (0.61). The four dominant genera in the Hvs-GG group were *Cetobacterium* (86.89 %), *Plesiomonas* (10.61 %), *Clostridium* (1.15 %), and *Paraclostridium* (0.67 %).

At the species level, the four domainant species in the control group were *Cetobacterium somerae* (55.06 %), *Clostridium colicanis* (18.87 %), *Clostridium perfringens* (8.38 %), and *Paraclostridium bifermentans* (8.37 %) (Fig. 4D). The four domain species in the Lvs-GG group were *C. somerae* (84.34 %), *Plesiomonas shigelloides* (4.86 %), *C. colicanis* (2.47 %), and *[Clostridium] dakarense* (1.59 %). The four domain species in the Mvs-GG group were *C. somerae* (92.01 %), *P. shigelloides* (5.55 %), *C. colicanis* (1.19 %), and *P. bifermentans* (0.34 %). The four domain species in the Hvs-GG group were *C. somerae* (86.89 %), *P. shigelloides* (10.66 %), *C. colicanis* (1.06 %), and *P. bifermentans* (0.67 %). The top 10 dominant species in each group are shown in Fig. 4E. Specifically, the abundance of *C. somerae* in the guar gum groups was significantly higher than that in the control group, in contrast to the abundances of *C. colicanis*, *P. bifermentans*, and *C. perfringens* ($P < 0.05$). The abundances of *R. lituseburensis*, *T.mayombei*, and *P. shigelloides* were highest in the control, Mvs-GG, and Hvs-GG groups, respectively, and were significantly higher than those in the other groups ($P < 0.05$). The abundances of [*Clostridium*] *dakarense* and *Mycoplasma moatsii* in the Mvs-GG and Hvs-GG groups were significantly lower than those in the Lvs-GG and control groups ($P < 0.05$). The results of the functional prediction analysis of the intestinal flora are shown in Fig. 4F. The clustering map (Fig. 4G) more intuitively presented changes in the function of intestinal flora, and functions with significant differences are presented in Fig. 4H, mainly in the biosynthesis of ansamycins, biosynthesis of vancomycin group antibiotics, and D-glutamine and D-glutamate metabolism.

3.7. Digesta SCFA and neurotransmitters composition

The composition of SCFA in the digesta is shown in Fig. 5A. Specifically, the concentrations of valerate and caproate were not significantly affected by the experimental diets ($P < 0.05$). The total SCFA, acetate, and propionate concentrations in the guar gum groups were significantly higher than those in the control groups ($P < 0.05$). The butyrate concentration in the Mvs-GG and Hvs-GG groups was significantly lower than that in the control and Lvs-GG groups ($P < 0.05$).

A total of 19 neurotransmitters were detected in the digesta samples divided into two categories: amines (10 types) and amino acids (9 types), as presented in Fig. 5B and C, respectively. The 5-hydroxyindole-3-acetic acid, L-glutamine, and arginine concentrations in the Lvs-GG and Hvs-GG groups were significantly lower than those in the control group; however, the histamine concentration in the Lvs-GG and Hvs-GG groups was significantly higher than that in the control group ($P < 0.05$).

3.8. Correlation analysis results

The correlations between intestinal flora and SCFA concentration and intestinal flora and neurotransmitters concentration, are shown in Fig. 6A and B, respectively. The results showed a clear correlation between intestinal flora and metabolites, as shown in Section S2.

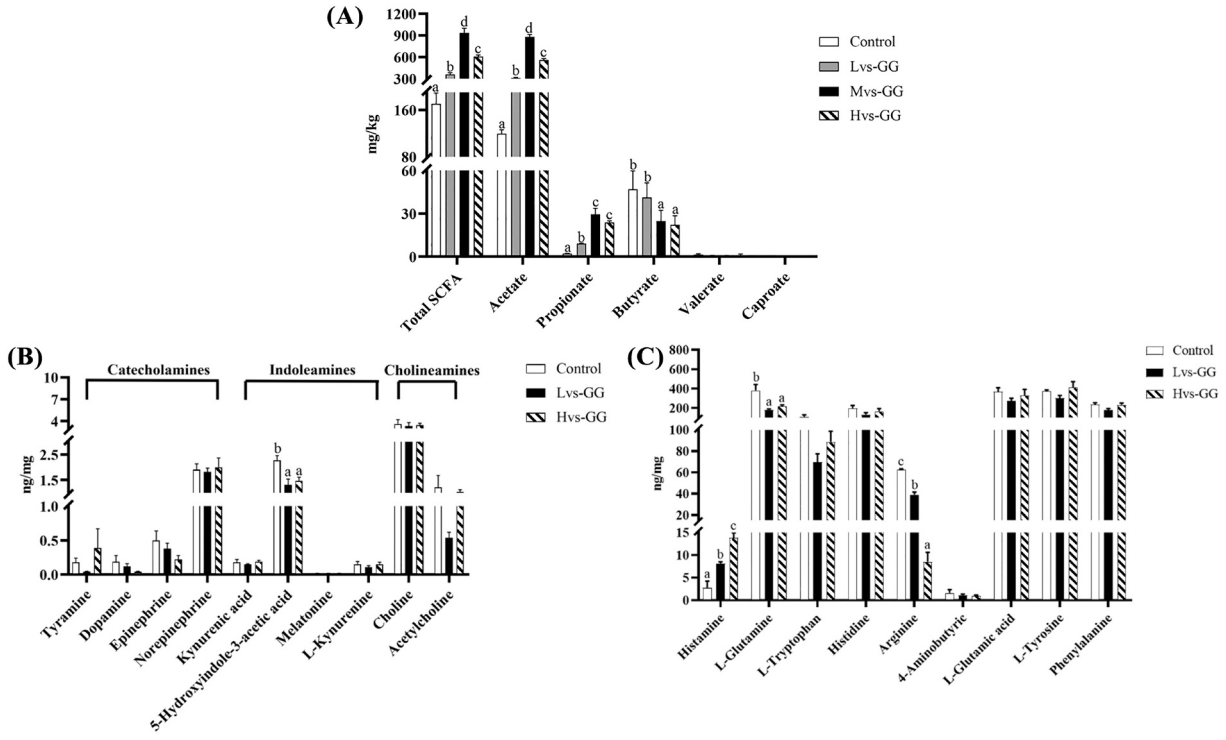

Fig. 5. Digesta SCFA and neurotransmitters concentrations in largemouth bass fed with different viscous guar gum diets. (A) SCFA concentrations; (B) amine neurotransmitters concentrations, and (C) amino acid neurotransmitters concentrations. Values of each column with different superscripts mean a significant difference ($P < 0.05$; $n = 4$).

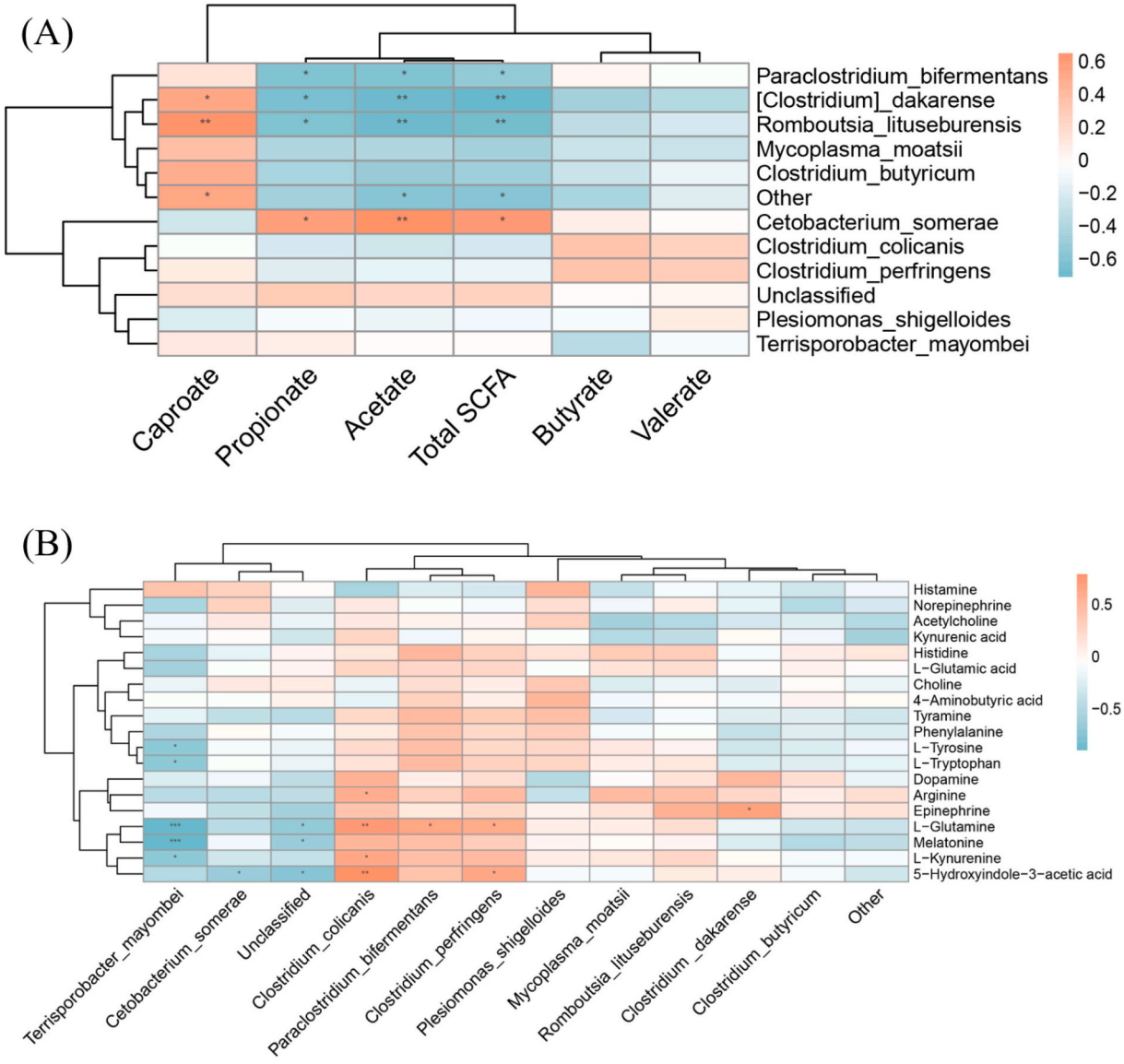

Fig. 6. The association of SCFA and neurotransmitters with the top 10 dominant species, and results are presented in (A) and (B), respectively (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

4. Discussion

Growth performance and feed utilization have been widely used to assess the potential effects of dietary components on aquatic animals [35]. In this study, guar gum diets reduced the growth rate of the test fish, and high-viscosity guar gum exhibited a stronger inhibitory effect than low-viscosity guar gum, suggesting that high viscosity diets are detrimental to fish growth. Moreover, our data demonstrated that viscosity is the main anti-nutritional characteristic of guar gum. This may explain the observation in previous studies that high doses of guar gum inhibited fish growth as increasing doses also increased dietary viscosity [26,36,37]. Sinha et al. [12] concluded that high-viscosity diets increase digesta viscosity, thereby interfering with the digestion and absorption process of nutrients. In this study, dietary supplementation with guar gum significantly decreased the growth performance of largemouth bass juveniles and was accompanied by worse feed utilization (increased FI and FCR), suggesting that dietary guar gum may reduce fish growth by inhibiting feed utilization; however, the exact mechanism needs further investigation.

It is well known that a healthy gut plays an essential role in fish growth and health and that gut health is affected by dietary components [38]. The intestinal mucosal barrier consists of chemical, mechanical, biological, and immune barriers [39], which are extensively involved in maintaining intestinal health [40]. Therefore, the integrity of the intestinal mucosal barrier is often used to assess the intestinal health. Plasma LPS, ET-1, and D-LA concentrations as well as DAO activity are indicators that can efficiently reflect the permeability of the intestinal mucosal barrier; generally, an elevated concentration or activity of these indicators represents intestinal mucosal damage [13,41]. In this study, dietary inclusion of high-viscosity guar gum caused a significant increase in plasma LPS and ET-1 concentrations, indicating that dietary guar gum damaged the intestinal mucosal barrier, and high-viscosity guar gum exhibited stronger adverse effects. Moreover, the tight junction structure is closely associated with intestinal mucosal barrier function [42]. *Occludin*, *ZO-1*, *Claudin-1* and *Claudin-4* are tight junction structure-related genes, and changes in their expression levels represents the integrity of the intestinal mucosal barrier [17]. In this study, *Occludin*, *Claudin-4*, and *ZO-1* expression levels were downregulated by the addition of Mvs-GG or Hvs-GG, suggesting that high-viscosity guar gum may disrupt the intestinal mucosal barrier integrity by

downregulating tight junction structures. Conversely, dietary Lvs-GG supplementation upregulated the expression of *Occludin* and *Claudin-4*, suggesting that low-viscosity guar gum may facilitate the enhancement of intestinal mucosal barrier function; however, the exact mechanism requires further investigation.

Oxygen radicals are continuously generated during aerobic metabolism causing oxidative damage in organisms [43]. The antioxidant system consists of several antioxidant enzymes (SOD and CAT) that can effectively scavenge oxygen radicals and avoid oxidative stress [44,45]. Therefore, a normal antioxidant system is essential for maintaining the health of an organism. *Keap1* and *Nrf2* are key genes involved in regulating antioxidant capacity; *Nrf2* gene expression can promote the transcription and translation of antioxidant enzyme genes [46], whereas the *Keap1* gene can effectively regulate the nuclear translocation process of the *Nrf2* [47]. Hence, changes in their expression levels can reveal the antioxidant status of the body [11,17]. In this study, dietary inclusion of high-viscosity guar gum up-regulated intestinal Keap1 and Nrf2 gene's expression levels and induced a reduction in intestinal CAT activity, indicating that high-viscosity guar gum decreased intestinal antioxidant capacity. Conversely, increased intestinal SOD and GPX activities suggest that low- and middle-viscosity guar gum exhibit antioxidant capacity enhancing effects.

As previously mentioned, the intestinal mucosa also has an immune defense function [48]. For example, intestinal epithelial cells can modulate the activity of immune cells by secreting active factors (cytokines and chemokines), thereby resisting infection and invasion by exogenous pathogenic microorganisms [49]. The intestinal inflammatory response is closely related to intestinal health and is commonly assessed by the expression levels of pro- and anti-inflammatory factors [18]. Additionally, the interaction between pro- and anti-inflammatory factors is essential for maintaining intestinal immune homeostasis [50]. In this study, our data showed that dietary inclusion of high-viscosity guar gum caused upregulation of pro-inflammatory factors and downregulation of anti-inflammatory factors, indicating that high-viscosity guar gum induced intestinal inflammation in largemouth bass. Previous studies have reported that an increase in tight junction proteins suppresses intestinal inflammation [51], and histamine tends to damage intestinal mucosal barrier [52]. Therefore, combined with the downregulation of tight junction protein structure-related genes and increased digesta histamine contents in fish fed high-viscosity guar gum diets, it can be speculated that high-viscosity guar gum increases the digesta histamine content and intestinal mucosal barrier permeability, thereby inducing intestinal inflammation. However, low-viscosity guar gum may inhibit intestinal pro-inflammatory factors (*IL-1β* and *TNF-α*) expression by upregulating the intestinal tight junction-related genes expression (Occludin and Caludin-4) [51], thereby alleviating intestinal inflammation. It is well known that apoptosis is important for maintaining normal physiological functions of tissues [53,54]. The activation of apoptotic signaling is often accompanied by the upregulation of pro-apoptotic factors and the downregulation of anti-apoptotic factors [55,56]. In this study, dietary inclusion of high-viscosity guar gum significantly downregulated the expression levels of anti-apoptotic factors, suggesting that high-viscosity guar gum may induce apoptosis in intestinal cells. Moreover, apoptosis is usually accompanied by the activation of lysosomal function and degradation of organelles [57] that may explain the poor epithelial cell morphology in the Hvs-GG group (Fig. 7).

Innate immunity is the first line of defense against exogenous pathogens and is extensively involved in immune signaling and immune responses in teleost fish, which are essential for maintaining health [58]. Immunoglobulins are important components of the innate immune system and consist of three isoforms: IgM, IgT/Z, and IgD [59]. Among these, IgM is the most important type of immunoglobulin in teleost fish [60]. In this study, dietary inclusion of guar gum resulted in a significant

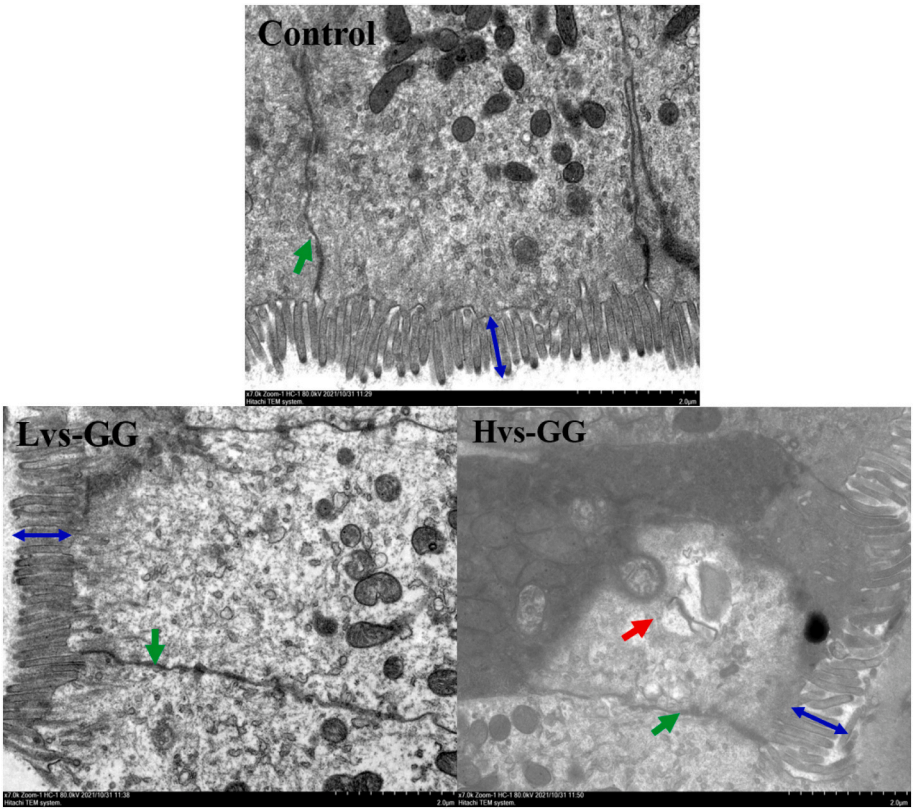

Fig. 7. Intestine transmission electron microscope observation of largemouth bass fed with different viscous guar gum (magnification × 7000). Blue double-side arrow: microvillus height; green arrow: epithelial cell interval; red arrow: intestinal epithelial cell death. Fig. 7 has been published in our previous study [34] together with the preparation and observation methods.

increase in the intestinal IgM concentration, suggesting that dietary guar gum may beneficial for improving intestinal innate immunity in largemouth bass. However, considering that dietary guar gum induces intestinal mucosal barrier damage and intestinal inflammation, we believe that the increase in IgM concentration is an adaptive physiological change that maintains intestinal health. IgM is also considered the main antibody against pathogenic microbial infections in fish [61]. In this study, we noticed that the Hvs-GG diet increased the abundance of pathogenic microbes (*P. shigelloides*) in the intestine, as well as intestinal IgM activity, whereas intestinal IgR, IgT, and MUC2 activities showed limited changes, suggesting that IgM is the major immunoglobulin type in largemouth bass against intestinal pathogenic microbial infection.

The intestinal flora is extensively involved in maintaining intestinal health and can be regulated by diets [62]. Sobs, Simpson, Shannon, Chao, and Ace indices are generally used to assess the α-diversity of the intestinal flora [63]. In this study, dietary inclusion of guar gum induced a significant reduction in the Shannon and Simpson indices, and these parameters showed a decreasing trend with increasing guar gum viscosity, suggesting that dietary guar gum decreases the microbial α-diversity, and that high-viscosity guar gum exhibits a stronger negative effect. Notably, microbial α-diversity is closely associated with their homeostasis [64], and a decrease in α-diversity usually implies a decrease in community stability. Thus, our data suggest that high-viscosity guar gum disrupts intestinal flora homeostasis. Fusobacteria, Proteobacteria, and Firmicutes were the dominant phyla (accounting for >95 % of the gut flora) in all groups, which is highly consistent with previous studies on largemouth bass [11,17,18,65], implying that these three phyla comprise the core flora of largemouth bass. The core flora plays an essential role in maintaining intestinal function and health [66]. In this study, although dietary inclusion of different viscous guar gums did not change the dominant phyla, it dramatically altered the relative abundance of each dominant phylum, mainly including an increase in Fusobacteria and a reduction in Firmicutes. This suggests that different viscous guar gums induced a functional migration of the intestinal flora. Furthermore, this was confirmed by subsequent functional prediction of the intestinal flora.

As a fermentable anaerobic species, *C. somerae* has been reported to produce acetate, propionate and vitamin B_{12} by fermenting peptones [67,68], which are extensively involved in improving gut and liver health, and lipid and glucose metabolism [69–71]. In this study, *C. somerae* was identified as the dominant species of the Fusobacteria phyla, and its abundance in the guar gum groups was dramatically higher than that in the control group. This suggests that guar gum diets facilitate an increase in the abundance of fermentable bacteria. Sinha et al. [12] suggested that increasing dietary viscosity tends to decrease the oxygen tension in the gut. This evidence suggests that guar gum diets may promote the proliferation of *C. somerae* by reducing the intestinal oxygen tension. Moreover, the increasing abundance of *C. somerae* may explain the increased acetate and propionate concentrations of digesta in the guar gum groups.

Clostridium sp. have been reported to produce butyric acid through the fermentation process [72] and can improve intestinal health [73,74]. In this study, *Clostridium* sp. (mainly *C. colicanis* and *C. perfringens*) was identified as the dominant genus of Firmicutes phyla, and the abundance of these bacteria in the guar gum groups was significantly lower than that in the control group, as well as the digesta butyrate concentration in the guar gum groups. Previous studies have shown that butyrate has many benefits to intestinal health, such as providing energy for epithelial cell differentiation, proliferation, and damage repair, regulation of intestinal immunity, and alleviation of intestinal inflammation [75,76]. Combined with the observation that guar gum diets upregulated the expression of intestinal pro-inflammatory and pro-apoptotic factors of largemouth bass in this study, it can be hypothesized that dietary guar gum decreased the abundance of butyrate-producing bacteria and butyrate concentration, thereby negatively affecting intestinal health.

Proteobacteria are generally regarded as biomarkers of the structural instability of the intestinal flora, relying on exogenous proteins as energy sources and can lead to metabolic disturbances, and intestinal inflammation in the host [77,78]. In this study, *Plesiomonas* sp., mainly *P. shigelloides*, was identified as the dominant Proteobacteria species. This species has been reported to cause tissue infections and lesions in fish, which are thought to be a pathogenic microorganisms [79,80], and to produce histamine that damages the intestinal mucosal barrier [52]. In this study, the abundance of *P. shigelloides* in the Hvs-GG group was significantly higher than that in the other three groups, suggesting that high-viscosity guar gum is more detrimental to intestinal health. Moreover, the increased abundance of *P. shigelloides* reasonably explains the increased digesta histamine concentration in the Lvs-GG and Hvs-GG groups and the decreased α-diversity of intestinal flora in the guar gum groups [52].

As mentioned above, metabolites are the primary components by which the intestinal flora regulates the host's gut health. In this study, we observed that the concentration of digesta SCFAs was closely associated with the abundance of Fusobacteria and Firmicutes, which was highly consistent with a previous study, showing that these two phyla are the dominant producers of SCFAs [81]. Specifically, the subsequent correlation analysis showed that *C. somerae* was positively associated with acetate and propionate, whereas *P. bifermentans*, *[Clostridium] dakarense and R. lituseburensis* showed a negative correlation. This suggests that digesta acetate and propionate concentrations were not only associated with producing bacteria but also affected by non-producing bacteria. The exact mechanism needs to be further explored. Intestinal microbial metabolites act as neuro-active substances that can communicate with the host and play a crucial regulatory role in the gut–brain axis [82]. Generally, these metabolites are also considered to be neurotransmitters. Importantly, neurotransmitter homeostasis has positive implications for normal fish behavior and physiological functions [23,83,84]. In this study, we observed that dietary inclusion of guar gum significantly decreased the concentrations of 5-hydroxyindole-3-acetic acid, L-glutamine, and arginine in the digesta but increased histamine concentration, suggesting that dietary guar gum may alter intestinal physiological functions by regulating neurotransmitter homeostasis. For instance, increased histamine concentrations may disrupt intestinal health and normal physiological functions [52]. Arginine plays an important role in gut functionally, including cell migration, growth, and proliferation [85], and increasing dietary arginine levels upregulates the expression and phosphorylation levels of the intestinal target of rapamycin and tight junction-related genes and reduces the expression of pro-inflammatory related genes [86,87]. Therefore, the decreased digesta arginine concentration in the Hvs-GG group also indicates that high-viscosity guar gum is detrimental to intestinal health. Our data provided evidence that dietary guar gum may affect the intestinal health of juvenile largemouth bass by altering the intestinal flora and its metabolites.

5. Conclusion

In summary, dietary guar gum adversely affects growth performance and alters the intestinal flora structure and intestinal health in largemouth bass juveniles. Moreover, different viscous guar gums altered the proportions of acetate, butyrate-producing bacteria and pathogenic microorganisms, and the composition of neurotransmitters in the gut of largemouth bass to varying extents. High-viscosity guar gum showed the strongest adverse effects, downregulating the expression levels of intestinal tight junction, anti-inflammatory, and anti-apoptotic related genes, decreasing digesta butyric acid concentration, and increasing the abundance of *P. shigelloides* and digesta histamine concentration. These results demonstrate that the adverse effects of guar gum on fish are closely related to its viscosity, which should be fully considered when developing guar gum as an aquafeed binder.

CRediT authorship contribution statement

Yu Liu: Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Hang Zhou:** Investigation, Formal analysis, Data curation. **Jiongting Fan:** Formal analysis, Data curation. **Huajing Huang:** Formal analysis, Data curation. **Junming Deng:** Funding acquisition, Supervision, Writing – review & editing. **Beiping Tan:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors of this study declare that there is no conflict of interest or competing interests in the manuscript.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2022.09.220.

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