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Response of growth performance, serum metabolites, intestinal tight junction structure and bacterial microbiomes to the long-term intervention of aflatoxin B_1 in *Lateolabrax maculatus* diets

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ABSTRACT

Dietary inclusion of aflatoxin B_1 (AFB_1) has been reported to induce intestinal damage and dysregulate intestinal microbiota of shrimp, but limited information is available in fish. A 56-day feeding trial was conducted to assess the effects of dietary AFB_1 on growth performance, serum metabolites, intestinal tight junction structure and bacterial microbiomes of *Lateolabrax maculatus*. Four diets were formulated to contain 0 (G0), 0.1 (G0.1), 0.5 (G0.5) and 1.0 (G1.0) mg/kg of AFB_1, and each diet was randomly assigned to three groups of 40 fish per tank. Fish were fed to apparent satiation twice daily. Results indicated that increased dietary AFB_1 concentration from 0 to 1.0 mg/kg decreased ($P < 0.01$) final body weight, weight gain rate, specific growth rate, feed intake, condition factor, viscerosomatic index, hepatosomatic index and intestinesomatic index of fish. The albumin, high density lipoprotein cholesterol and glucose were decreased ($P < 0.05$) but aspartate aminotransferase, diamine oxidase, lipopolysaccharide and D-lactate were increased ($P < 0.01$) as dietary AFB_1 increasing. Intestinal tight junction structure appeared different degrees of ambiguous and damaged as reflected by the clear gap and vacuolated cells in fish fed AFB_1-treated diets. Inclusion of AFB_1 in diets increased ($P < 0.05$) abundance of *Enterobacter* and decreased ($P < 0.05$) abundance of *Plesiomonas*, but did not alter ($P > 0.05$) alpha diversity indexes of ace, chao, shannon and simpson in the intestinal microbiota of fish. In conclusion, inclusion of AFB_1 up to 1.0 mg/kg in diets decreased growth performance, induced liver and intestinal injury, and altered intestinal microbiota composition without impacting the bacterial diversity of *Lateolabrax maculatus*.

1. Introduction

Mycotoxins are deleterious feed contaminants routinely observed in plant ingredients and compound feed during storage especially in hygrothermal environment. The main mycotoxins commonly found in aquaculture feedstuffs are aflatoxin, ochratoxin, fumonisin and zearalenone, among which aflatoxin B_1 (AFB_1) is one of the most powerful carcinogens known to reduce growth and health of aquatic animals (Goncalves et al., 2018). AFB_1 can be genotoxic and carcinogenic, and the toxicity of AFB_1 is acute and can lead to reproduction impairment, immunosuppression, hepatocyte necrosis and impaired function (Deng et al., 2010).

It has been reported that the biological effects of AFB_1 on aquatic animals are directly associated with the dietary toxin concentration and animal species (Anater et al., 2016). Compared with omnivorous fish, carnivorous fish are usually more sensitive and less tolerant to AFB_1 (Anater et al., 2016). Although the effects of AFB_1 on growth and health of aquatic animals have been evaluated in tilapia (*Oreochromis niloticus* × *O. aureus*) (Deng et al., 2010), grass carp (*Ctenopharyngodon idella*) (Zeng et al., 2019), common carp (*Cyprinus carpio*) (Tasa et al., 2020), channel catfish (Jantrarotai and Lovell, 1990), *Clarias batrachus* (Amjad and Durreshahwar, 2016), rainbow trout (*Oncorhynchus mykiss*) (Arana et al., 2002), *Sciaenos ocedatas* (Zeng et al., 2016), *Litopanaeus vannamei* (Wang et al., 2012, 2018; Huang et al., 2021), turbot (*Scophthalmus maximus*) (Yang et al., 2020), gibel carp (Huang et al., 2012) and Indian carp (*Labeo rohita*) (Sahoo et al., 2001), rare information is available in *Lateolabrax maculatus*, one of the popular carnivorous fish cultured in the southern region of China in recent years for its rapid growth and

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great taste (Wang et al., 2017). This study aimed to determine the effects of dietary AFB_1 on growth performance, serum metabolites, intestinal tight junction structure and bacterial microbiomes of *Lateolabrax maculatus*.

2. Materials and methods

2.1. Diet preparation

The ingredients and proximate composition of basal diet in this study were shown in Table 1. Four diets were formulated to contain 0 (G0), 0.1 (G0.1), 0.5 (G0.5) and 1.0 (G1.0) mg/kg of AFB_1 (from *Aspergillus flavus*, Sigma-Aldrich, Canada). All ingredients were smashed to pass through a 320 μm sieve, mixed thoroughly and then extruded into 2 mm pellets, dried at 55 ℃ and stored at − 20 ℃ until use. Dietary AFB_1 concentrations were determined using liquid chromatography tandem-mass spectrometry (LC-MS/MS) method (Li et al., 2011). Actual AFB_1 concentrations in the diets of G0, G0.1, G0.5 and G1.0 were 0, 0.09, 0.47 and 1.02 mg/kg, respectively.

2.2. Experimental design and feeding management

The protocols and all procedures performed in the present were approved by the Institutional Animal Care and Use Committee of Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangdong, China). A 56-day feeding trial was conducted by randomly distributing 480 juvenile *Lateolabrax maculatus* (initial body weight 2.9 ± 0.02 g) into 12 tanks (350 L) with 40 fish per tank. Each diet was randomly assigned to three tanks. Fish were fed to apparent satiation twice daily (07:00 and 19:00). Uneaten feed was collected in 1 h after each meal, analyzed for dry matter (DM) and subtracted from feed offered (DM basis) to calculate feed intake (FI). The photoperiod regime was 12 h light and 12 h dark. During the feeding trial, water temperature ranged from 25 to 27 ℃, dissolved oxygen was approximately 6.0 mg/L, pH was between 7.5 and 8.0, and ammonia and nitrite were below 0.01 mg/L.

Table 1
Ingredients and proximate composition (g/kg DM) of the basal diet.

Ingredients	
Fish meal	180
Casein	180
Soy protein concentrate	160
High gluten	280
Monocalcium phosphate	15
Fish oil	40
Soybean oil	20
Soy lecithin	20
Vitamin premix[a]	2
Mineral premix[b]	5
Choline chloride	5
Vitamin C ester	1.5
Lysine	0.3
Methionine	2.2
Betaine	5
Zeolite powder	30
Cellulose	54
Proximate composition	
Dry matter	912.4
Crude protein	406.7
Crude lipid	109.8
Ash	73.0

[a] One kilogram of diet provided VA 3230 IU, VD_3 1600 IU, VE 160 mg, VK_3 4 mg, VB_1 4 mg, VB_2 8 mg, VB_6 4.8 mg, VB_{12} 0.016 mg, nicotinic acid 28 mg, pantothenic acid calcium 16 mg, biotin 0.064 mg, folic acid 1.285 mg and inositol 40 mg.

[b] One kilogram of diet provided Ca 1150 mg, K 180 mg, Mg 45 mg, Fe 50 mg, Zn 40 mg, Mn 9.5 mg, Cu 7.5 mg, Co 1.25 mg, I 0.16 mg and Se 0.25 mg.

2.3. Sampling

At the end of the experiment, survival was determined by counting the individuals remaining in each tank. All fish were fasted for 24 h to empty their guts and anesthetized with 40 mg/L of 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222, Sigma, USA) before sampling. Fish in each tank were counted and weighted to analyze for final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR) and feed coefficient (FC).

Six fish per tank were randomly selected for analysis of condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI) and intestinesomatic index (ISI).

Blood samples were obtained from the caudal veins of six fish per tank using heparinized syringes, and kept at room temperature for 20 min and then centrifuged at 8000 × *g* for 10 min at 4 °C. The resultant serum was immediately stored at − 80 °C for subsequent analysis of serum metabolites.

The distal intestines of three fish in each tank were sampled and fixed in the electron microscope fixative (Wuhan Servieobio Technology Co., Ltd., Wuhan, China) for transmission electron microscope analysis according to Gu et al. (2017).

2.4. Intestinal microbiota DNA extraction and sequencing

The intestinal microbiota DNA extraction and bacterial microbiome characterization were analyzed according to Peng et al. (2021a). Briefly, the intestinal mucosa layer samples from three fish per tank were collected and transferred to a 1 mL sterile tube containing cell lysis buffer. DNA was extracted using the protocol of QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The real-time PCR conditions for 16 S was ran as follows: 3 min at 95 °C, followed by 45 cycles of 30 s at 95 °C, 30 s at 60 °C, and 45 s at 72 °C. Sequencing was conducted on an Illumina MiSeq platform (San Francisco, California, USA) according to the manufacturer's recommendations provided by Shenzhen HaploX Biotechnology Co., LTD (Shenzhen, China). All sequencing data generated in the study have been submitted to the HaploX database under project accession number YYR2021072301.

2.5. Laboratory analyses

The proximate compositions of diets were analyzed using AOAC method (Association of Official Analytical Chemists AOAC, 1999). Dry matter was measured by drying samples to a constant weight at 105 °C. Crude protein was calculated from the determination of the total nitrogen (N × 6.25) using the Kjeldahl method. Crude lipid was determined by gravimetric analysis following ether extraction of the lipids according to the Soxhlet method. Ash was determined by combustion in a muffle furnace at 550 °C for 6 h.

Serum metabolites including total cholesterol (TC), triacylglycerol (TG), albumin (ALB), globulin (GLOB), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT), low density lipoprotein cholesterol (LDLC) and high density lipoprotein cholesterol (HDLC) were tested using an automatic biochemical analyzer (Hitachi 7180, Tokyo, Japan). Serum diamine oxidase (DAO), lipopolysaccharide (LPS) and D-lactate (DLA) were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the corresponding manufacturer's instructions.

2.6. Data calculations and statistical analysis

Data was summarized and averaged for each tank. Respective parameters were calculated as follows:

WGR (%) = 100 × (final body weight (g) – initial body weight (g)) / initial body weight (g)

FI (g/fish) = feed intake (g) / ((final number of fish + initial number of fish) / 2)

FC = total feed intake (g) / (final body weight (g) – initial body weight (g))

SR (%) = 100 × (final number of fish) / (initial number of fish)

SGR (%/d) = 100 × (ln final weight (g) – ln initial weight (g)) / days

Sequencing reads were assigned to each sample and analyzed with the QIIME software package. The processed reads were then clustered to OTU (operational taxonomic unit) via the QIIME software package (version 1.9.1) based on 97% sequence similarity (Peng et al., 2018). Representative sequence for each OTU was screened for further annotation using the GreenGene 16 S rRNA gene database (DeSantis et al., 2006). Alpha diversity indexes (ace, chao, shannon and simpson) were calculated using the Phyloseq Package (McMurdie and Holmes, 2013).

All data were statistically analyzed as completely randomized design by ANOVA of mixed procedure of SAS (2012) with treatment as fixed effect and tank as experimental unit. Polynomial contrasts were used to determine linear and/or quadratic responses to the dietary AFB_1 concentrations. Significant differences among treatments were tested using LSMEANS with the PDIFF option and adjusted with a Tukey's test in SAS (2012). Significance was declared at $P < 0.05$.

3. Results

3.1. Growth performance

Increased dietary AFB_1 concentration from 0 to 1.0 mg/kg linearly and quadratically decreased ($P < 0.01$) FBW, WGR, SGR and FI, but did not affect FC, SR and PER ($P > 0.05$) (Table 2). The CF, VSI and ISI were linearly decreased ($P < 0.05$) and HSI was linearly and quadratically decreased ($P < 0.05$) as dietary AFB_1 increasing.

3.2. Serum metabolites

The ALB and HDLC were linearly and quadratically decreased ($P < 0.05$) as dietary AFB_1 increasing from 0 to 1.0 mg/kg and reached significance at the level of 1.0 mg/kg (Table 3). The GLU was linearly decreased ($P < 0.01$), and AST, DAO, LPS and DLA were linearly increased ($P < 0.01$) as dietary AFB_1 increasing. All fish had similar ($P > 0.05$) serum TC, TG, GLOB, ALT and LDLC regardless of treatments.

3.3. Intestinal tight junction structure

The transmission electron microscope observation of intestinal tight junction was shown in Fig. 1. The tight junction structure appears as a dense black electron band (↑) starting from the apex of the epithelium and extending from the villus roots to the basal layer. The tight junction structure as shown in G0 was normal, tightly connected without gap between cells. However, the tight junction structure in G0.1, G0.5 and G1.0 appeared different degrees of ambiguous and damaged as reflected by the clear gap and vacuolated cells.

3.4. Intestinal bacterial microbiomes

A total of 1046,724 clean reads was obtained across all samples, ranging from 60,670 to 157,371 reads in individual sample, resulting in identification of 5155 OTUs with 97% identity from 12 samples in G0, G0.1, G0.5 and G1.0 groups. The OTUs were assigned to 12 phyla, 17 class, 34 orders, 44 families, 60 genera and 20 species (data not shown).

The Venn diagram showed that 458 OTUs were shared by groups G0, G0.1, G0.5 and G1.0, and the number of unique OTUs in G0, G0.1, G0.5 and G1.0 was 199, 151, 157 and 189, respectively (Fig. 2).

At genus level, the *Bradyrhizobium*, *Brevibacillus*, *Lysobacter*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Plesiomonas*, *Aeromonas*, *Bacillus* and *Acinetobacter* composed the top 10 dominant genera of *Lateolabrax maculatus* intestinal microbiota communities among groups (Fig. 3). Inclusion of AFB_1 in diets increased ($P < 0.05$) abundance of *Enterobacter* but decreased ($P < 0.05$) abundance of *Plesiomonas*.(Fig. 4).

Increased dietary AFB_1 concentration from 0 to 1.0 mg/kg did not alter ($P > 0.05$) alpha diversity including ace, chao, shannon and simpson in the intestinal microbiota of fish.

4. Discussion

4.1. Effect of AFB_1 on growth performance of Lateolabrax maculatus

It has been reported that the biological effects of AFB_1 on fish mainly depend on dietary AFB_1 concentration and fish species (El-Sayed and Khalil, 2009). Dietary AFB_1 concentrations ranged from 0.1 m to 1.0 mg/kg in this study were higher than the limit value (≤ 20 μg/kg in compound feed) according to the Hygienical Standard for Feed (GB/T 13078–2017) issued by the Standardization Administration of China, with a view to evaluating the toxicological effects of AFB_1 on *Lateolabrax maculatus*. The similar survival rate of fish among groups in this study suggested that dietary AFB_1 up to 1.0 mg/kg did not affect survival of *Lateolabrax maculatus*. However, the declined feed intake along with decreased weight gain rate and specific growth rate as dietary AFB_1

Table 2
Effects of AFB_1 on growth performance of *Lateolabrax maculatus*.

Items[3]	Diets[1]				SEM	*P*-value[2]		
	G0	G0.1	G0.5	G1.0		*P*	*L*	*Q*
FBW, g	45.56[a]	45.31[a]	41.34[a]	26.70[b]	2.41	< 0.001	< 0.001	0.002
WGR, %	1464.43[a]	1455.98[a]	1323.00[a]	818.07[b]	82.76	< 0.001	< 0.001	0.001
SGR, %/d	4.10[a]	4.09[a]	3.96[a]	3.31[b]	0.10	< 0.001	< 0.001	< 0.001
FI, g/fish	48.52[a]	46.54[ab]	43.50[b]	27.83[c]	2.50	< 0.001	< 0.001	< 0.001
FC	1.14	1.10	1.13	1.17	0.01	0.301	0.258	0.154
SR, %	86.67	89.52	92.38	90.48	1.38	0.591	0.302	0.435
PER, %	204.26	215.69	207.71	200.81	2.50	0.177	0.377	0.070
CF, g/cm^3	1.65[a]	1.62[ab]	1.56[b]	1.57[b]	0.01	0.036	0.007	0.581
VSI, %	9.27[a]	8.29[b]	7.55[c]	6.77[d]	0.15	< 0.001	< 0.001	0.633
HSI, %	0.84[a]	0.62[b]	0.54[b]	0.56[b]	0.03	0.001	0.001	0.039
ISI, %	0.63[a]	0.64[a]	0.51[b]	0.53[b]	0.01	0.004	0.040	0.148

[1] G0, basal diet; G0.1-G1.0, basal diet supplemented with 0.1–1.0 mg/kg of AFB_1.
[2] *P*, overall effect; *L*, linear effect; *Q*, quadratic effect.
[3] FBW, final body weight; WGR, weight gain rate; SGR, specific growth rate; FI, feed intake; FC, feed coefficient; SR, survival rate; PER, protein efficiency ratio; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index; ISI, intestinesomatic index.
[a, b, c, d] Different letters with a row indicate significant differences ($P < 0.05$).

Table 3
Effects of AFB_1 on serum metabolites of *Lateolabrax maculatus*.

Items[3]	Diets[1]				SEM	*P*-value[2]		
	G0	G0.1	G0.5	G1.0		*P*	*L*	*Q*
TC, mmol/L	3.83	4.28	3.98	3.74	0.10	0.281	0.517	0.111
TG, mmol/L	4.58	4.68	4.00	5.34	0.19	0.066	0.255	0.065
ALB, g/L	15.33^{a}	16.33^{a}	14.33^{a}	11.47^{b}	0.62	0.005	0.002	0.021
GLOB, g/L	28.93	31.83	30.50	28.30	0.73	0.344	0.621	0.107
GLU, mmol/L	9.81^{a}	8.18^{ab}	5.45^{bc}	2.40^{c}	0.96	0.005	0.002	0.054
AST, U/L	111.67^{c}	157.33^{bc}	182.33^{ab}	225.67^{a}	14.82	0.016	0.002	0.952
ALT, U/L	39.67	44.67	38.00	41.00	5.57	0.398	0.363	0.599
LDLC, mmol/L	0.16	0.20	0.25	0.23	0.02	0.284	0.107	0.346
HDLC, mmol/L	0.88^{a}	0.91^{a}	0.92^{a}	0.56^{b}	0.05	0.004	0.004	0.005
DAO, U/L	17.75^{c}	18.16^{b}	19.54^{ab}	21.30^{a}	0.53	0.003	< 0.001	0.185
LPS, EU/L	416.98^{c}	482.64^{b}	508.13^{b}	544.96^{a}	14.88	< 0.001	< 0.001	0.237
DLA, μmol/mL	3.20^{b}	3.77^{b}	4.36^{a}	4.50^{a}	0.26	0.003	< 0.001	0.255

[1] G0, basal diet; G0.1-G1.0, basal diet supplemented with 0.1–1.0 mg/kg of AFB_1.
[2] *P*, overall effect; *L*, linear effect; *Q*, quadratic effect.
[3] ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAO, diamine oxidase; DLA, D-lactate; GLOB, globulin; GLU, glucose; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; LPS, lipopolysaccharide; TC, total cholesterol; TG, triacylglycerol.
[a, b, c] Means with different letters differ ($P < 0.05$).

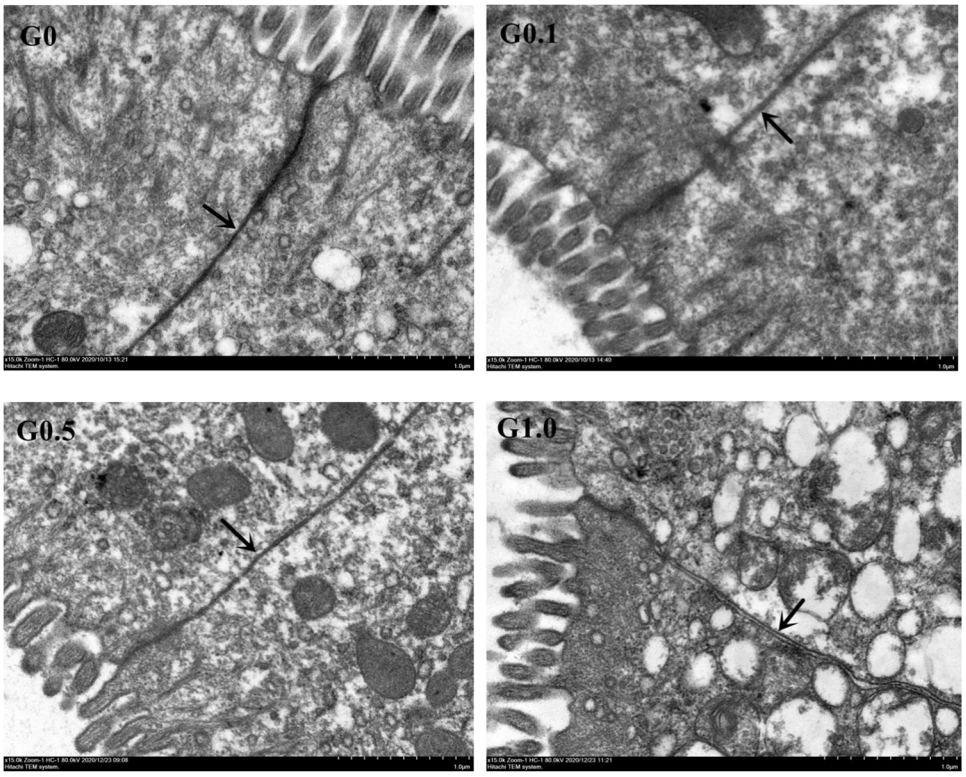

Fig. 1. Effects of AFB_1 on intestinal tight junction (↑) of *Lateolabrax maculatus*. G0, basal diet; G0.1-G1.0, basal diet supplemented with 0.1–1.0 mg/kg of AFB_1. The tight junction structure appears as a dense black electron band starting from the apex of the epithelium and extending from the villus roots to the basal layer. The tight junction structure as shown in G0 was normal, tightly connected without gap between cells, whereas the tight junction structure in G0.1, G0.5 and G1.0 appeared different degrees of ambiguous and damaged as reflected by the clear gap and vacuolated cells.

increasing suggested that inclusion of AFB_1 in *Lateolabrax maculatus* diets adversely impact the palatability of feed and growth performance of fish. Similar results were also observed in tilapia (Deng et al., 2010), grass carp (Zeng et al., 2019), common carp (Tasa et al., 2020), channel catfish (Jantrarotai et al., 1990), *Clarias batrachus* (Amjad and Durreshahwar, 2016), rainbow trout (Arana et al., 2002), *Litopanaeus vannamei* (Wang et al., 2012) and gibel carp (Huang et al., 2012). Inhibition of fish growth is one of the main toxic effects of AFB_1 which is commonly attributed to damaged liver and impaired immune system by AFB_1 (Goncalves et al., 2020). Liver is considered to be the target organ of AFB_1 and the ingested AFB_1 by fish is transformed into carcinogens through metabolism of liver cytochrome P450 family members, which induces liver function impairment and metabolic disorder, thereby inhibiting the growth of fish (Huang et al., 2012). In this study, the reduced hepatosomatic index of *Lateolabrax maculatus* as dietary AFB_1 increasing indicated that AFB_1 inhibited liver development of fish. Our recent study showed that inclusion of 0.1–1.0 mg/kg of AFB_1 in *Lateolabrax maculatus* diets induced degeneration and necrosis of hepatocyte and therefore led to liver injury (Peng et al., 2021b). The decreased growth performance of fish was also regarded as the toxic effects of dietary AFB_1 on liver of fish in other reports (Huang et al., 2012, 2019; Zeng et al., 2019).

4.2. *Effect of AFB_1 on serum metabolites of Lateolabrax maculatus*

The variation of serum metabolites indicated that inclusion of AFB_1 in *Lateolabrax maculatus* diets adversely impacted these metabolite profiles. ALB is a critical protein produced by the liver and its

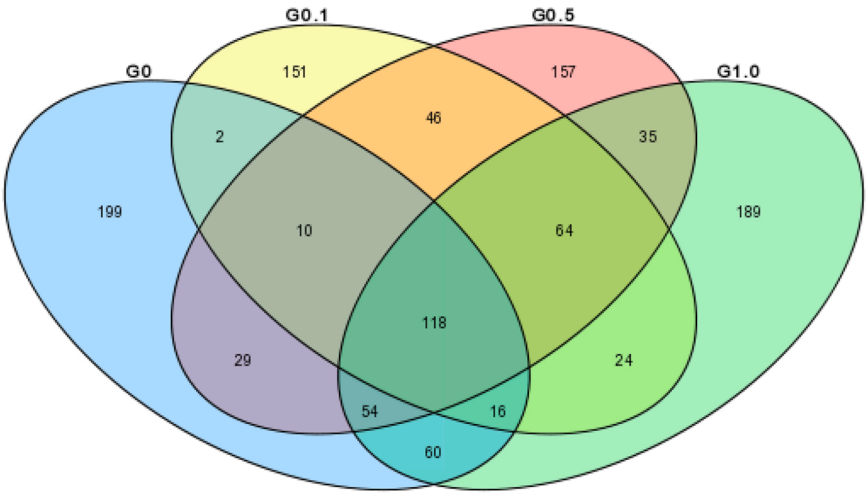

Fig. 2. Venn diagrams showing unique and shared of OTUs (at 97% similarity). Values are the numbers of OTUs calculated using the total data set. G0, basal diet; G0.1-G1.0, basal diet supplemented with 0.1–1.0 mg/kg of AFB_1.

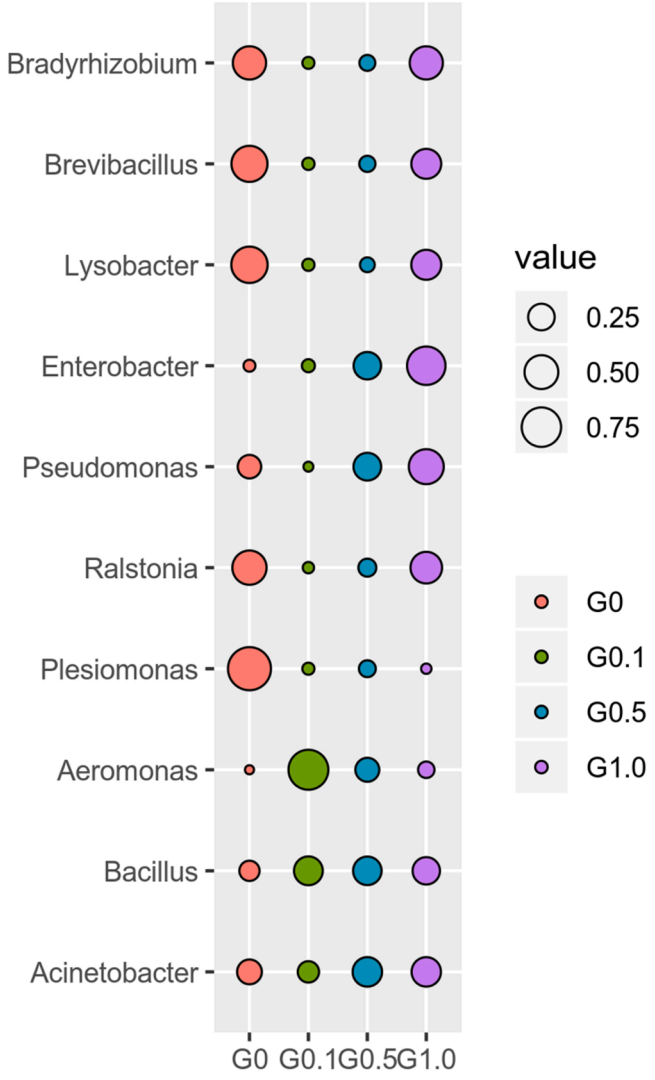

Fig. 3. Abundances of the most 10 most relatively abundant bacterial genera. G0, basal diet; G0.1-G1.0, basal diet supplemented with 0.1–1.0 mg/kg of AFB_1.

concentration in serum indicated the liver health of fish (Wang et al., 2019). Decreased serum ALB in this study suggested that dietary inclusion of AFB_1 led to liver injure. Sahoo and Mukherjee (2001) also reported that AFB_1 exposure declined serum ALB concentration of Indian major carp (*Labeo rohita*). In contrast, activity of serum AST is generally related to liver damage or necrosis when its value increases (Sheikhzadeh et al., 2012; Wang et al., 2014). Increased AST activity in this study suggested that dietary AFB_1 impaired the immune system and liver health of *Lateolabrax maculatus*. It is interesting to note that inclusion of AFB_1 in diets reduced serum GLU concentration of *Lateolabrax maculatus*. Although the mechanism by which AFB_1 decrease serum GLU concentration is not clear, AFB_1 was reported to induce significant decrease in glucose-6-phosphatase activity in the liver of rat (Sakr et al., 2006). The glucose-6-phosphatase is a critical phosphatase found mainly in the liver which plays an important role in releasing liver glucose to the blood of animals (Schaftingen and Gerin, 2002). Therefore, decreased serum GLU concentration as dietary AFB_1 increasing in this study may attribute to the inhibition effect of AFB_1 on the activity of glucose-6-phosphatase in the liver of *Lateolabrax maculatus*, but further study is needed to confirm this. It has been reported that blood concentrations of DAO are positively correlated with the maturity and integrity of intestinal mucosa (Wolvekamp and Bruin, 1994). DAO presents normally small amounts in the blood, whereas the release of DAO to blood is usually increased when the intestinal cells and barrier function are damaged (Li et al., 2015; Song et al., 2009). Similarly, serum concentration of LPS is an important indicator for intestinal inflammation (Peng et al., 2021a). Rhee (2014) reported that even small amounts of LPS in the blood are sufficient to induce potent inflammatory responses. Liu et al. (2018) indicated that serum concentrations of LPS and DAO were both significantly increased in AFB_1-challenged broilers. Serum DLA is commonly regarded as a toxic marker for evaluating intestinal injury (Block et al., 2008; Nielsen et al., 2012). Increased serum levels of DLA were also observed in dairy goats exposed to dietary AFB_1 (Huang, 2016). In this study, the increased DAO and LPS along with raised DLA concentrations in the serum of *Lateolabrax maculatus* suggesting dietary inclusion of AFB_1 had adverse impact on the intestinal integrity and induced enteritis.

4.3. Effect of AFB_1 on intestinal tight junction structure of Lateolabrax maculatus

Intestinal tight junctions are the most apical of the connections between epithelial cells and the most important components of the intracellular junctional complexes regulating cell adhesion and selective

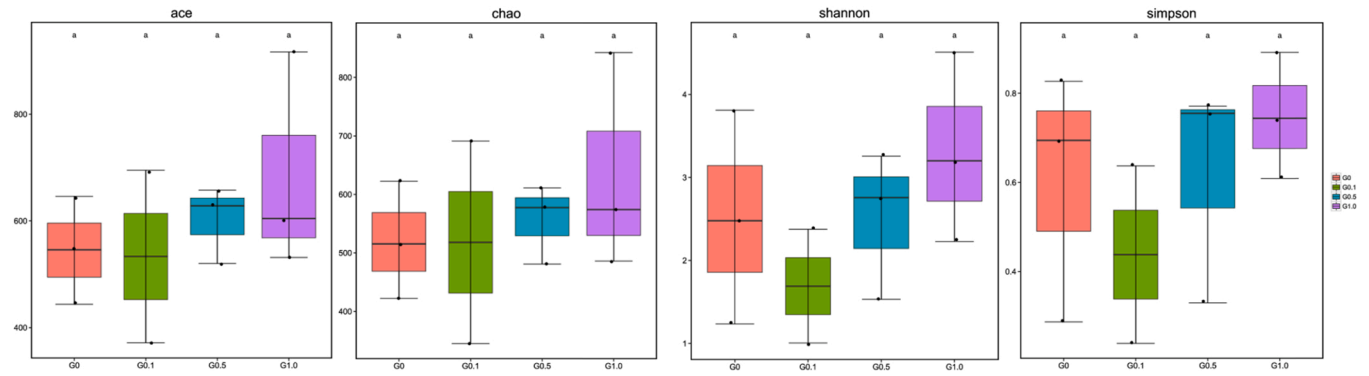

Fig. 4. Alpha diversity of bacterial communities. Each panel represents one alpha diversity measure, as follows: ace and chao, richness estimators to estimate the total number of OTUs present in a community; shannon and simpson, microbial index of diversity. G0, basal diet; G0.1-G1.0, basal diet supplemented with 0.1–1.0 mg/kg of AFB_1.

permeability in the gut (Gonzalez-Mariscal et al., 2003). The intestinal tight junction proteins in vertebrates, including the closed proteins (e.g. claudin family), atresia protein (e.g. occludin) and cytoplasmic proteins (e.g. intracellular zonulae occludens), determine the adhesion and permeability characteristics of the epithelium in terms of specificity and tightness (Anderson et al., 2004). The major function of tight junction proteins was to serve as permeability barriers involved in separating internal and external milieu (Guttman et al., 2006). In this study, the transmission electron microscope observation demonstrated that dietary AFB_1 caused structural disruption of the intestinal tight junctions of *Lateolabrax maculatus*. It has been reported that the physical barrier function of epithelial cells is maintained by tight junction proteins which are susceptible to oxidative damage (Feng et al., 2015; Pan et al., 2018). Romero et al. (2016) documented that AFB_1 reduced the gene expression of the tight junction proteins claudin-3, claudin-4 and occludin in caco-2 cells. Lu et al. (2020) reported that AFB_1 led to impaired epithelial tight junction integrity in porcine intestinal epithelial cell line (IPEC-J2 cells). Dietary inclusion of 40 μg/kg of AFB_1 in broiler diets had negative impact on the intestinal integrity by down-regulating the mRNA expression of claudin-1 and occludin (Liu et al., 2018). Zeng et al. (2019) indicated that dietary AFB_1 damages the structural integrity of immune organs in grass carp via promoting the myosin light chain kinase signalling to down-regulate the mRNA levels of major tight junctions protein. Furthermore, the results of intestinal tight junction structure observed in this study responded to the increased concentrations of serum metabolites DAO, LPS and DLA, suggesting dietary AFB_1 caused intestinal injury by destroying the intestinal tight junction structure of *Lateolabrax maculatus*.

4.4. *Effect of AFB_1 on intestinal bacterial microbiomes of Lateolabrax maculatus*

Although the effect of AFB_1 on intestinal microbiota of shrimp has been evaluated (Wang et al., 2018), rare information is available about the response of intestinal microbiota to dietary AFB_1 in fish. To our best knowledge, this is the first study to evaluate the effects of AFB_1 on the intestinal microbiota composition of *Lateolabrax maculatus*. It has been reported that the genera *Plesiomonas* and *Enterobacter* are the dominant colonizer in the intestine of freshwater fish species (Larsen et al., 2014). The decreased abundance of *Plesiomonas* and increased abundance of *Enterobacter* as dietary AFB_1 increasing from 0 to 1.0 mg/kg in this study suggests AFB_1 may inhibit the growth of *Plesiomonas* and promote the growth of *Enterobacter* in the intestinal bacterial communities of *Lateolabrax maculatus*. Although the *Plesiomonas* is generally regarded as pathogenic bacteria for animals (Santos et al., 2014), it seems to be the most commonly identified genus in the gut of *Lateolabrax maculatus* (Peng et al., 2021a). The *Enterobacter* is part of gram-negative bacteria in the intestinal tract which can cause opportunistic infections and intestinal inflammation (Lawley and Walker, 2012). Moreover, increased abundance of *Enterobacter* responded to the increased serum concentration of LPS as dietary AFB_1 increasing in this study, because LPS are endotoxins found in the cell wall of gram-negative bacteria (Eng et al., 1993). The increased gram-negative bacteria *Enterobacter* in the intestine are able to increase the amount of LPS, resulting in the intestinal inflammation and causing intestinal injury by destroying the intestinal tight junction structure of *Lateolabrax maculatus*.

Alpha diversity refers to diversity within a certain biotope and is usually described in terms of species richness or various alpha diversity indices including ace, chao, shannon, simpson, etc. (Whittaker, 1972). It is interesting to note that dietary AFB_1 increasing from 0 to 1.0 mg/kg did not alter the alpha diversity of bacterial communities in the intestine of *Lateolabrax maculatus*. Similar results were also observed by Liu et al. (2019) that feeding mice with 200 μg/kg of AFB_1 did not impact on the alpha diversity (as reflected by the ace, chao, shannon and simpson indices) of intestinal bacterial communities. However, few studies reported that there is a reduction in the bacterial diversity from *Litopenaeus vannamei* (Wang et al., 2018), *Scophthalmus maximus* (Yang et al., 2020), rats (Wang et al., 2015) and yak (An et al., 2019) exposed chronically to AFB_1. Differences among studies appear to be species specific and may be the result of different AFB_1 concentrations.

5. Conclusion

Inclusion of AFB_1 up to 1.0 mg/kg in *Lateolabrax maculatus* diets decreased growth performance, induced liver and intestinal injury, and altered intestinal microbiota composition without impacting the bacterial diversity.

CRediT authorship contribution statement

Kai Peng conceived and designed the experiments. **Bing Chen, Yuping Sun, Xiaoying Chen, Yuxi Wang and Wen Huang** performed the experiments. **Kai Peng** analyzed the data and wrote the paper. All authors read and approved the final manuscript.

Author contributions

K. Peng conceived and designed the experiments, **B. Chen, Y. Sun, X. Chen, Y. Wang and W. Huang** performed the experiments; **K. Peng** analyzed the data and wrote the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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