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Growth performance, hepatic enzymes, and gut health status of common carp (*Cyprinus carpio*) in response to dietary *Cetobacterium somerae* fermentation product

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ABSTRACT

Intensive aquaculture practices compromise the health of fish. Probiotics especially those isolated from aquatic animals play important roles in improving fish health. The aim of the study was to evaluate the effects of stabilized fermentation product of *Cetobacterium somerae* (XMX-1) on the growth performance, gut and liver health of common carp. A total of 300 carps (initial weight of 2.32 ± 0.02 g) were divided into the control (fed a basal diet) and XMX-1 groups (fed a basal diet with 2 g/kg, 3 g/kg, 4 g/kg or 5 g/kg XMX-1 diet). After 8-week feeding, growth performance, serum lipopolysaccharide (LPS), diamine oxidase activity (DAO), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated. The intestinal total superoxide dismutase (SOD) activity, the expression of gut health related genes was tested. In addition, the liver triacylglycerol (TAG) and the expression of liver lipid metabolism related genes were conducted. Results showed that XMX-1 addition had no effect on the growth performance of carps at a supplementation level up to 5 g/kg ($P > 0.05$). However, dietary XMX-1 at addition levels ranging from 2 g/kg to 5 g/kg reduced serum LPS and DAO. Furthermore, all XMX-1 additions significantly increased total SOD activity compared with the control group ($P < 0.05$). In contrast, dietary 3 g/kg XMX-1 significantly increased the expression of intestinal hypoxia inducible factor 1 subunit alpha (*HIF1α*), *Occludin*, *Hepcidin* and zonula occludens (*ZO*)-*1* ($P < 0.05$). Dietary 2 g/kg XMX-1 significantly increased the expression of intestinal *Hepcidin* and *ZO-1* ($P < 0.05$). Moreover, dietary XMX-1 decreased the level of serum ALT ($P \leq 0.37$) and liver TAG ($P \leq 0.07$). Furthermore, we found that dietary XMX-1 significantly reduced the expression of genes related to lipid synthesis including sterol regulatory element-binding protein (*SREBP1c*) and peroxisome proliferator-activated receptor gamma (*PPARγ*) ($P < 0.05$). Meanwhile, dietary 3 g/kg XMX-1 significantly increased the expression of carnitine palmitoyltransferase 1 (*CPT1*) and peroxisome proliferator-activated receptor alpha (*PPARα*) compared with the control group ($P < 0.05$). Together, our findings suggest that XMX-1 additions of 3 g/kg had the best effect on fish health.

1. Introduction

Aquaculture has a long history, which can be traced back more than 2000 years (Boyd and Tucker, 2012). In recent years, the intensification of aquaculture and the globalization of seafood trade have led to the significant development of aquaculture (Gephart et al., 2020; Pradeepkiran, 2019). In 2020, global per capita fish consumption has set a new record of 20.5 kg per year and is expected to increase further in the next decade. It is estimated that by 2030, the total fish production will increase to 204 million tons, an increase of 15% over 2018 (FAO. The State of World Fisheries and Aquaculture 2020).

To supply more fish production, higher fat content is included in

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aquafeed formulations to save proteins and increase fish weight gain (Dong et al., 2014). High lipid consumption, on the other hand, has negative consequences on fish. These negative impacts include excess lipid accumulation in fish tissues, the formation of fatty liver syndrome, and the compromised nutritional value of farmed fish (Fuller and Gibson, 1998; Dong et al., 2014; Tocher, 2003). Fatty liver is currently a common concern in aquaculture, impairing growth performance, liver health, immunological response, and nutritional value of fish. (Zhenyu, 2014; Sun et al., 2021). In addition, a high-fat diet also increases intestinal permeability, making the intestinal wall permeable to pro-inflammatory cytokines and lipopolysaccharide (LPS) (Crawford et al., 2019). The integrity of the intestine is vital in maintaining intestinal health since it serves as an infection channel for many harmful microorganisms (Li et al., 2019; Rendueles et al., 2012).

In recent years, probiotics have been widely used in aquatic animal feed because of their non-toxic, harmless, have no residue, low cost and convenient use (Gao et al., 2013). Probiotics are active microorganisms, which can improve the host's immunity by affecting the intestinal microecological balance of aquatic animals or regulating the function of host mucosa and immune system (Kesarcodi-Watson et al., 2008; Wang et al., 2019). A lot of studies in fish have shown that probiotics play a positive effect on obesity, fatty liver and other metabolic syndromes (de La Banda et al., 2010; Feng et al., 2021; Zhao et al., 2020). Furthermore, probiotics have been found to benefit gut health, including sickness resistance, antioxidant status, gut morphology, and gut microbial composition (Dimitroglou et al., 2011; Lauzon et al., 2014; Merrifield et al., 2010).

As a genus belonging to Fusobacteria, *Cetobacterium* is present in many fish species, accounting for more than 70% of the gut microbiota in many individuals (Bhute et al., 2020; Hao et al., 2021). *Cetobacterium* has been proved to improve fish health by producing the fermentation product, such as vitamin B12, butyrate and acetate (Bennett and Eley, 1993; Bhute et al., 2020; Sugita et al., 1991). Fermentation product of probiotics has been used as a feed supplement in aquaculture, which showed various beneficial effects. Huang et al. found that supplementing brewer's yeast *Saccharomyces cerevisiae* fermentation product improved the health of common carp (Huang et al., 2015). Similarly, dietary *S. cerevisiae* fermentation product DVAQUA® demonstrated a protective impact on fish by modifying the host gut microbiota, allowing them to fight bacterial pathogens (Zhou et al., 2011). Furthermore, dietary supplementation of the fermentation product DVAQUA® increased nonspecific immunity as well as gut bacterial count and diversity of fish (He et al., 2011). In a previous study, we found that the addition of *C. somerae* can improve the gut and liver health of common carp fed diet with plant proteins (Xie et al., 2021). In zebrafish, dietary supplemented of *C. somerae* stabilized fermentation product can improve gut and liver health as well as antiviral immunity (Xie et al., 2022). In this study, the stabilized fermentation product of *C. somerae* XMX-1 was added to the diet of common carp. To our knowledge, it is the first time that *C. somerae* XMX-1 stabilized fermentation product is directly applied to common carp as probiotics in the context of a regular diet. The effect of XMX-1 on liver health and gut health of common carp was studied, which could provide beneficial reference to the application of *C. somerae* XMX-1 in the aquaculture feed additive.

2. Materials and methods

2.1. Bacteria culture

Cetobacterium somerae XMX-1, with the preservation number CGMCC no.18908 in the China General Microbiological Culture Collection Center, was anaerobically isolated from zebrafish's intestine cultured GAM Broth medium (Haibo, China) in an anaerobic incubator (Electrotek, England) for 24 h. XMX-1 came from the abbreviation of Mingxu Xie, who isolated the strain. Protocols to obtain primary seed fermentation broth and secondary seed fermentation broth were performed according to the previous method (Xie et al., 2022). 3 ml sterilized calcium carbonate (1 M) was added to 100 ml of the secondary seed fermentation broth product of XMX-1 with the concentration of 10^8 CFU/ml. Then, 100 ml of the above fermentation product was mixed with 100 g of rice husk powder. The mixture of XMX-1 and rice husk powder was dried at room temperature and added to the feed. According to the feed formula, the ingredients of feed were mixed step by step, and sterile water was added to the mixture. Finally, the feed was dried at room temperature.

Table 1
Feed formulation and chemical composition of diets for common carp (dry matter, g/kg).

Ingredient	g/kg DM				
	Control	2 g/kg	3 g/kg	4 g/kg	5 g/kg
Rice bran	100.0	100.0	100.0	100.0	100.0
Flour	200.0	200.0	200.0	200.0	200.0
Soybean meal	200.0	200.0	200.0	200.0	200.0
Rapeseed meal	130.0	130.0	130.0	130.0	130.0
Fish meal	80.0	80.0	80.0	80.0	80.0
Poultry by-product meal	80.0	80.0	80.0	80.0	80.0
Pork powder	40.0	40.0	40.0	40.0	40.0
DDGS	100.0	100.0	100.0	100.0	100.0
XMX-1	0.0	2.0	3.0	4.0	5.0
Rice husk powder	5.0	3.0	2.0	1.0	0.0
Lys-HCl	2.0	2.0	2.0	2.0	2.0
Methionine	0.5	0.5	0.5	0.5	0.5
Choline chloride (50%)	2.0	2.0	2.0	2.0	2.0
$Ca(H_2PO_4)_2$	20.0	20.0	20.0	20.0	20.0
Soybean oil	30.0	30.0	30.0	30.0	30.0
VC phosphate	0.5	0.5	0.5	0.5	0.5
Fish premix (1%)[a]	10.0	10.0	10.0	10.0	10.0
Crude protein (%)	38.36	38.28	38.58	37.59	39.12
Crude fat (%)	10.17	10.60	10.98	10.46	10.82
Crude ash (%)	8.45	8.47	8.45	8.52	8.50
Moisture (%)	3.14	3.41	3.58	2.84	2.44
Gross energy (KJ/g DM)	17.83	17.83	17.83	17.83	17.83

[a] Provided by Beijing Sino-Norway Joint Aquaculture Technology Co., Ltd. The product meets NRC standard.

2.2. Animals feeding and sample collection

All fish and experimental protocols in the work were permitted by the Feed Research Institute of the Chinese Academy of Agricultural Sciences chaired by the China Council for Animal Care (Assurance No. 2018-AF-FRI-CAAS-001).

A total of 300 common carp with an average initial weight of 2.32 ± 0.02 g, were randomly divided into 5 treatments: the control, 2 g/kg, 3 g/kg, 4 g/kg and 5 g/kg XMX-1. During 8-week feeding, common carp in the control group were fed a basal diet and in the XMX-1 groups were fed a basal diet supplemented with 2, 3, 4 or 5 g XMX-1/kg diet. The formulation and proximate composition of diets were listed in Table 1. Common carp in each treatment were housed in 6 replicate 100-L cube tanks with 10 common carp in each tank. Fish were fed three times (08:30, 12:00 and 18:00) a day, with 6% of the total body weight. Recirculating system with pH 7.0–7.3, water temperature 26 °C, dissolved oxygen ≥ 6 mg/L, and total ammonia < 0.01 mg/L was performed in the study. The photo-period during husbandry was from 08:00 am to 22:00 pm. After 24 h fasting, fish were weighed and sampled. The survival rate, feed conversion ratio and weight gain of common carp were calculated according to the following formula:

Survival rate = (number of fish at the end of the experiment/number of fish at the start of the experiment) × 100

Feed conversion ratio (FCR) = food intake (g)/ weight gain of fish (g)

Weight gain (WG) = [100 × (final body weight-initial body weight)/ initial body weight]

Table 2
Primer sequences for *q*PCR.

Gene	Nucleotide sequence of primers (5′ to 3′)	Ref.	GenBank accession No.
β-actin	F: GAAGTGTGGTGTGGACATCCGTAA R: AGACTCATCGTACTCCTGCTTGCT	Xie et al. (2021)	JQ619774.1
HIF1α	F: GTTCTGCGTACCTGGTGCTCATC R: AAAGTGTGGCGGCTGAGAAAGG	Xie et al. (2021)	XM_019125356.1
Occludin	F: GACGCCATGGATGAGTACAA R: GTGGTTGAGTTTGGCTTTCAG	Meng et al. (2018)	NM212832.2
ZO-1	F: CCGAAGCTTTGACAGCAAAC R: GGTTGATCTTCTCCACTGACTC	Meng et al. (2018)	KF193852.1
Hepcidin	F: ACATGCGTCTGCTTCCTCC R: CTGGTTCTCCTGTGGTGCTT	Xie et al. (2021)	JX855261
CPT1	F: CAGATGGAAAGTGTTGCTAATGAC R: TGTGTAGAAGTTGCTGTTGACCA	Meng et al. (2018)	JF728839
PPARα	F: TGAACAAAGCCAAAGCACGC R: TGGAGAGTGTCCATGTCGTG	Hou et al. (2020)	FJ849065.1
SREBP1c	F: CGTCTGCTTCACTTCACTACTC R: GGACCAGTCTTCATCCACAAA	Meng et al. (2018)	KJ162572
PPARγ	F: GTCAAGTCCGAGATGCACC R: GGATGACCTGAGCATTGAAGC	Hou et al. (2020)	FJ849064.1

HIF1α, hypoxia inducible factor 1 subunit alpha; ZO-1, zonula occludens; CPT1, carnitine palmitoyltransferase 1; PPARα, peroxisome proliferator-activated receptor alpha; SREBP1c, sterol regulatory element-binding protein; PPARγ, peroxisome proliferator-activated receptor gamma.

Protocols for collecting liver, intestine, serum and intestinal content samples were conducted as mentioned earlier (Li et al., 2021).

2.3. Determination of triacylglycerol (TAG) content

The samples of liver were collected from 6 fish per treatment to obtain 6 replicates. The liver samples were homogenized in phosphate buffered (PBS), and the TAG was obtained as mentioned earlier (Zhang et al., 2019). Free glucose reagent (Sigma-Aldrich, Shanghai) and triglyceride reagent (Sigma-Aldrich, Shanghai) were used to quantify TAG.

2.4. Analysis of serum parameters

Blood of common carp was collected from caudal vein using a needle. The samples of serum were collected from 6 fish per treatment to obtain 6 replicates. Supernatant serum was transferred to another tube after blood was centrifuged at 1467 g for 10 min. All biochemical parameters of serum were detected using kits according to the manufacturer's instructions. Serum LPS was analyzed using the Serum ToxinSensor™ Chromogenic LAL Endotoxin assay kit (Genscript, China). The serum level of LPS in common carp was expressed as LPS units per milliliter (EU/ml). Serum diamine oxidase activity (DAO) was detected by the kit (Jiangsu Meimian Industrial Co., Ltd. China) according to the manufacturer's instructions, and was expressed as international enzyme activity units per liter (IU/L). Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activity were analyzed using kits (Jiancheng Bioengineering Ins., China) and were expressed as enzyme activity units per liter (U/L).

2.5. Real-time quantitative PCR (RT-qPCR)

TRIzol (Invitrogen) was used to extract total RNA from the liver and intestine. The samples of the liver or intestine were collected from 6 fish per treatment to obtain 6 replicates. Experimental methods include the transformation of cDNA and RT-*q*PCR reaction were performed according to the previous description (Xie et al., 2021). All primers used in the study were listed in Table 2. Tm of all primers was set at 60 ℃. β-actin was used as the reference primer, and all data were statistically analyzed by $2^{-\Delta\Delta CT}$ method.

2.6. Determination of intestinal total superoxide dismutase (SOD) activity content

The samples of intestine were collected from 6 fish per treatment to obtain 6 replicates. Intestinal samples were homogenized at 4 ℃ by adding precooled PBS, followed by centrifugation at 4 ℃. The supernatant was collected. The sample was measured at 560 nm by the assay kit (Beyotime Biotechnology, Shanghai, China) using Synergy H1Multi-Mode Microplate Reader (Bio Tek, USA). The result was presented as the SOD activity unit per tissue weight protein (U / mg protein).

2.7. 16S ribosomal RNA gene sequencing

After the last feeding for 4 h, gut content of common carp was

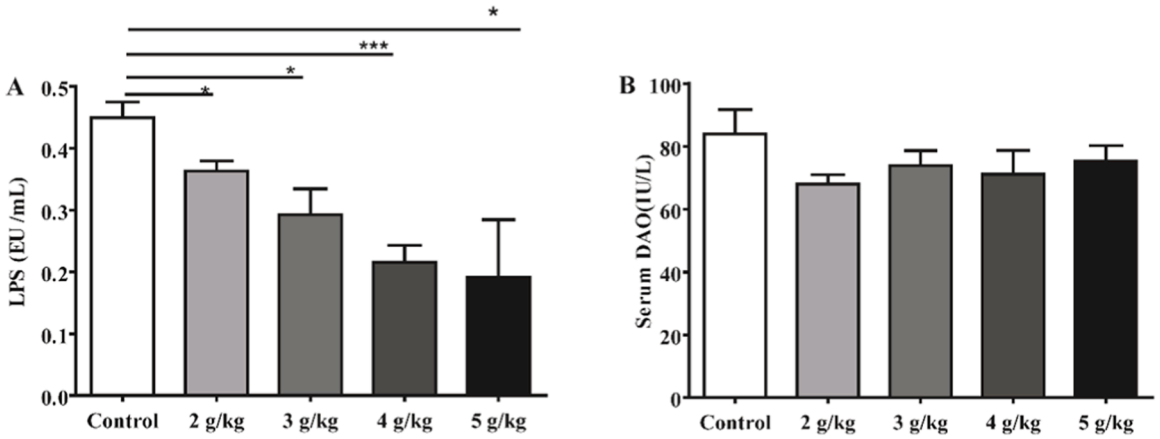

Fig. 1. Effects of dietary control and XMX-1 groups on serum LPS and DAO of common carp (A) Serum LPS. (B) Serum DAO. Data were represented as the means (± SEM) (n = 6). *, $P < 0.05$ and ***, $P < 0.0001$ comparison to the control group. XMX-1 represents *C. somerae*.

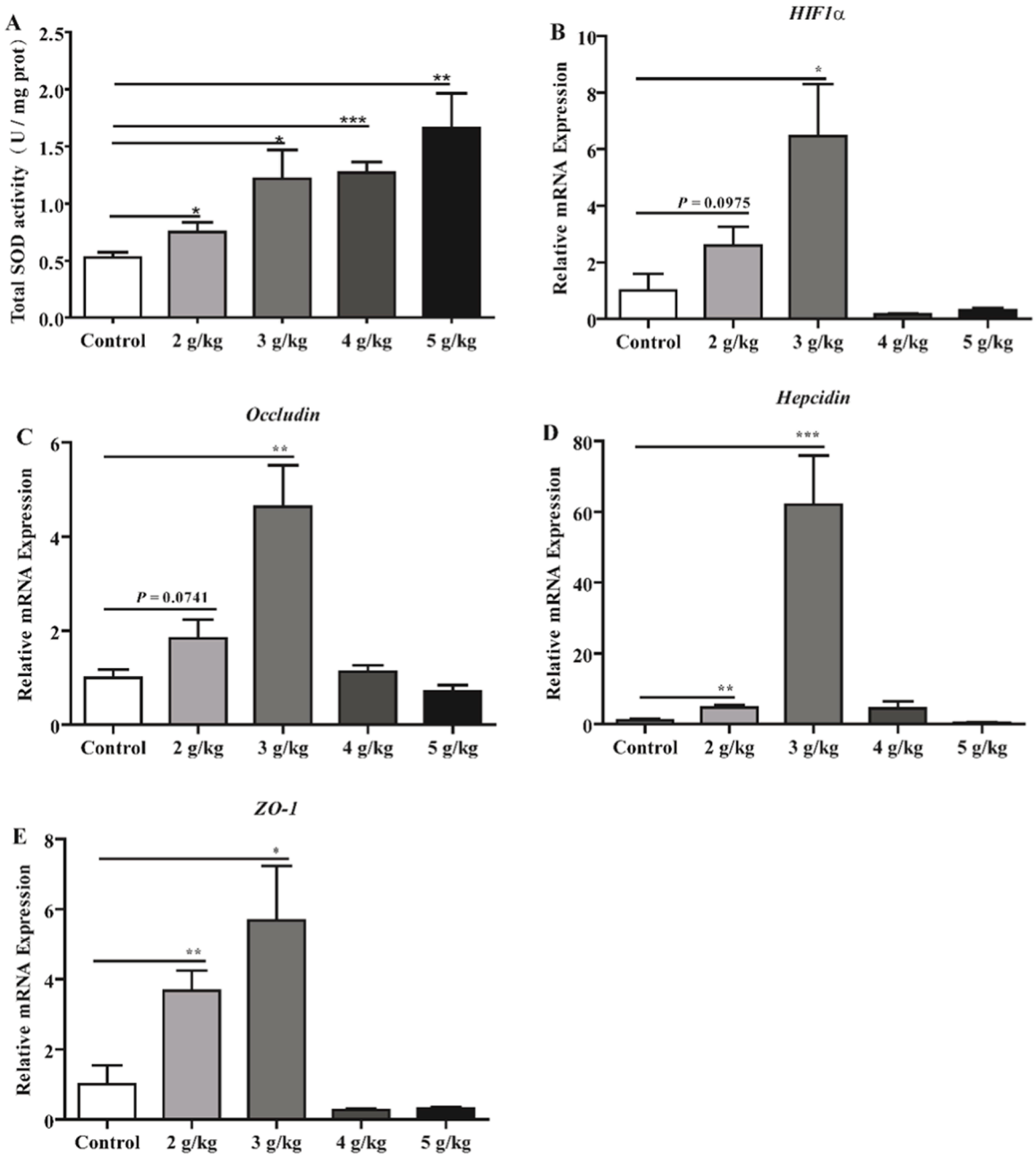

Fig. 2. Effects of XMX-1 diets on the expression of intestinal total SOD activity and intestinal health related genes in common carp (A) Total SOD activity, (B) *HIF1α*, (C) *Occludin*, (D) *Hepcidin*, (E) *ZO-1*. Data were represented as the means (± SEM) (n = 6). *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.0001$ comparison to the control group. XMX-1 represents *C. somerae*.

collected from each treatment group. The samples of gut microbiota were collected from 18 fish per treatment to obtain 6 replicates. DNA was extracted from each pooled sample using a FastDNA® Spin Kit for Soil (MP Biomedicals), according to the manufacturer's instructions. The 16S V3–V4 region was amplified using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Using NEXTFLEX Rapid DNA-Seq Kit to build a library, and then 16S rRNA gene sequencing was performed at the Majorbio (Shanghai, China) using the Illumina Miseq PE300 platform. The raw pair-end readings were then subjected to a quality-control procedure with the use of UPARSE. The qualified reads were clustered to generate operational taxonomic units (OTUs) at the 97% similarity level by using USEARCH. A representative sequence of each OTU was assigned to a taxonomic level in the RDP (Ribosomal Database Project) database by using the RDP classifier. Principal components analysis and heat-map analysis were performed using R software version 3.1.0.

2.8. Statistical analysis

GraphPad Prism 5 software (GraphPad Software Inc. CA, USA) was used to analyze all data, which was shown as mean±SEM. Comparisons between groups were analyzed using one-way ANOVA followed by a Duncan's post hoc test. Differences were considered to be significant at $P < 0.05$ (*), $P < 0.01$(**) and $P < 0.0001$ (***).

3. Results

3.1. Growth performance and feed utilization of common carp

After 8-week feeding, results of survival rate, feed conversion ratio

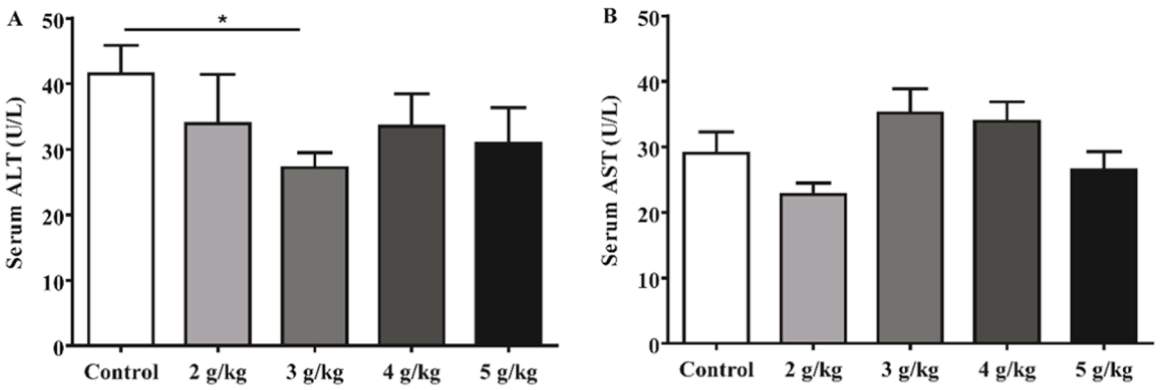

Fig. 3. Effects of control and XMX-1 diets on serum ALT and AST of common carp. (A) Serum ALT, (B) Serum AST. Data were represented as the means (± SEM) (n = 6). *, $P < 0.05$ comparison to the control group. XMX-1 represents *C. somerae*.

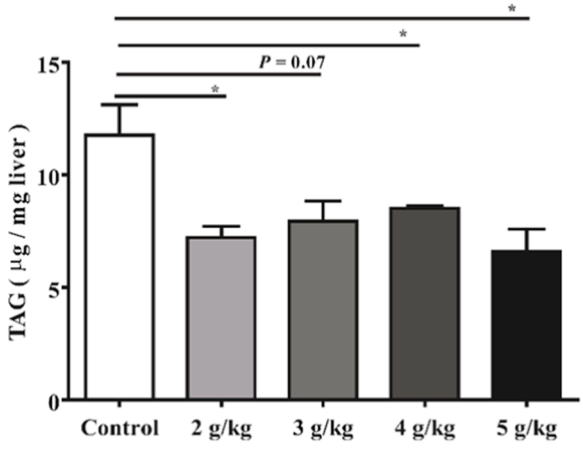

Fig. 4. Effects of control and XMX-1 diets on the liver TAG of common carp. Data were represented as the means (± SEM) (n = 6). *, $P < 0.05$ comparison to the control group. XMX-1 represents *C. somerae*.

and weight gain of common carp were presented in Table 4. Compared to the control group, survival rate, feed conversion ratio and weight gain showed no differences in the XMX-1 groups ($P > 0.05$, Table 4).

3.2. Effects of XMX-1 diets on the gut health of common carp

The effect XMX-1 supplementation on the gut health of common carp was investigated. As can be seen from Fig. 1, dietary 2 g/kg, 3 g/kg, 4 g/kg and 5 g/kg XMX-1 significantly reduced serum LPS of common carp ($P < 0.05$, Fig. 1A). Additionally, serum DAO has a downward trend in common carp fed diet supplemented with XMX-1 than in fish fed the control diet ($P > 0.05$, Fig. 1B). Furthermore, we also found that the intestinal total SOD activity was significantly higher in common carp fed diet supplemented with XMX-1 than in fish fed the control diet ($P < 0.05$, Fig. 2A). Then, we detected the effects of dietary XMX-1 on the expression of intestinal health related genes of common carp. Results showed that dietary 3 g/kg XMX-1 significantly up-regulated the expression of intestinal hypoxia inducible factor 1 subunit alpha (*HIF1α*), *Occludin*, *Hepcidin* and zonula occludens (*ZO*)-*1* compared with the control group ($P < 0.05$, Fig. 2B–E). Dietary 2 g/kg XMX-1 significantly increased the expression of intestinal *Hepcidin* and *ZO-1* ($P < 0.05$, Fig. 2D, E). However, compared to the control group, the expression of intestinal health related genes showed no differences in the 4 g/kg XMX-1 group and 5 g/kg XMX-1 ($P > 0.05$, Fig. 2A–D).

3.3. Effects of XMX-1 diets on the liver health of common carp

Then we detected the effects of XMX-1 diet on the liver health of common carp. It could be seen from Fig. 3, XMX-1 supplementation decreased the level of serum ALT of common carp. In addition, 3 g/kg XMX-1 significantly reduced the level of serum ALT in common carp ($P < 0.05$, Fig. 3A). However, compared to the control group, the serum AST showed no significant difference in XMX-1 groups ($P > 0.05$, Fig. 3B).

We detected the content of liver TAG in common carp. It could be seen from Fig. 5, dietary 2 g/kg, 4 g/kg and 5 g/kg XMX-1 significantly reduced the level of liver TAG in common carp ($P < 0.05$). Additionally, TAG has a downward trend in common carp fed diet supplemented with 3 g/kg XMX-1 than in fish fed the control diet ($P = 0.07$, Fig. 4). Furthermore, we found that dietary XMX-1 significantly down-regulated the expression of genes related to lipid synthesis including sterol regulatory element-binding protein (*SREBP1c*) and peroxisome proliferator-activated receptor gamma (*PPARγ*) ($P < 0.05$, Fig. 5A, B). Meanwhile, 3 g/kg XMX-1 supplementation significantly up-regulated the expression of fatty acid oxidation-related genes fatty acid carnitine palmitoyltransferase 1 (*CPT1*) and peroxisome proliferator-activated receptor alpha (*PPARα*) compared with the control group ($P < 0.05$, Fig. 5C, D). However, dietary 4 g/kg and 5 g/kg XMX-1 down-regulated the expression of *CPT1* and *PPARα* (Fig. 5C, D).

3.4. Effects of XMX-1 diets on the gut microbiota of common carp

Results of gut microbiota in all groups were presented in Table 3 and Fig. 6. We can see from Table 3 and Fig. 6A, Fusobacteria was the most abundant phylum with the relative abundances being 87.24%, 91.86%, 84.05%, 92.22% and 82.21%, in the control, 2 g/kg, 3 g/kg, 4 g/kg and 5 g/kg XMX-1 groups, respectively. Compared to the control group, the relative abundance of Chloroflexi was significantly higher in 3 g/kg ($P < 0.05$, Table 3).

At the phylum level, results of PCoA showed that there was no significant difference in gut microbiota between the control group and the 2 g/kg, 3 g/kg, 4 g/kg and 5 g/kg XMX-1 groups (Fig. 6B).

4. Discussion

With the rapid development of aquaculture and the increasing demand for seafood, intensive mode of aquaculture has led to a series of problems and compromised fish health in recent years (Sahu et al., 2008).

Growth promoting effect of probiotics has attracted increasing attention in recent years. Xu et al. (2014) showed that the addition of *B. coagulans* could improve the growth performance of common carp. Supplementation of potential *Enterococcus casseliflavus* improved growth

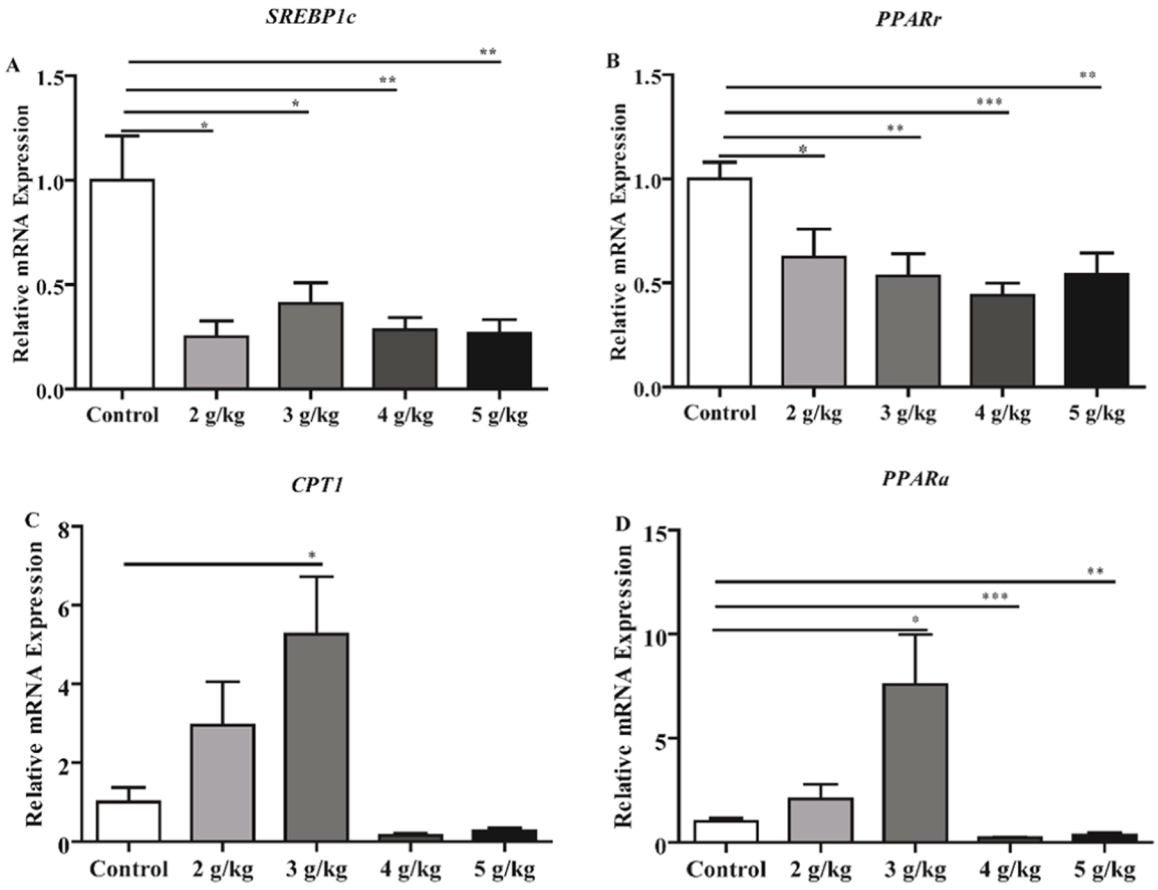

Fig. 5. Effects of control and XMX-1 diets on the expression of hepatic lipid metabolism related genes of common carp. The expression of lipid synthesis genes in liver (A) *SREBP1c*, (B) *PPARγ*. The expression of lipid oxidation genes in liver (C) *CPT1*, (D) *PPARα*. Data were represented as the means (± SEM) (n = 6). *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.0001$ comparison to the control group. XMX-1 represents *C. somerae*.

Table 3
The intestinal microbiota differences at phylum level of common carp fed on different diets.

Phylum	Control	2 g/kg	3 g/kg	4 g/kg	5 g/kg
Fusobacteria	87.24 ± 12.03	91.86 ± 9.09	84.05 ± 10.76	92.22 ± 2.74	82.21 ± 8.76
Firmicutes	6.08 ± 3.60	5.62 ± 6.38	4.77 ± 3.59	5.11 ± 2.63	7.10 ± 3.56
Proteobacteria	4.34 ± 5.96	2.06 ± 1.95	5.35 ± 4.56	1.68 ± 1.13	4.52 ± 4.33
Chloroflexi	0.83 ± 1.31^{b}	0.13 ± 0.25^{b}	2.98 ± 2.08^{a}	0.23 ± 0.22^{b}	3.1 ± 2.57^{b}
Actinobacteria	0.90 ± 1.56	0.12 ± 0.21	1.79 ± 1.49	0.23 ± 0.23b	1.33 ± 1.25

Values represent the means (± SEM) of 6 replicates. Different letters mean significant differences ($P < 0.05$).

Table 4
Effects of XMX-1 diet on the survival rate, weight gain (%) and feed conversion ratio of common carp.

	Control	2 g/kg	3 g/kg	4 g/kg	5 g/kg
IBW, g	2.32 ± 0.02	2.33 ± 0.01	2.34 ± 0.00	2.33 ± 0.01	2.32 ± 0.02
FBW, g	10.69 ± 0.31	10.32 ± 0.47	10.86 ± 0.23	10.49 ± 0.40	10.58 ± 0.43
WG, %	360.08 ± 8.97	343.90 ± 18.56	363.27 ± 10.34	350.3 ± 15.48	355.4 ± 16.69
FCR	1.28 ± 0.03	1.34 ± 0.07	1.27 ± 0.04	1.32 ± 0.06	1.30 ± 0.06
FI, g	10.70 ± 1.09	10.71 ± 0.51	10.80 ± 0.12	10.73 ± 0.62	10.70 ± 0.92
SR, %	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

IBW, initial body weight; FBW, final body weight; WG, weight gain; FCR, feed conversion ratio; FI, feed intake; SR, survival rate; XMX-1 represents C. somerae.

performance and had the best effect with a level of 10^9 CFU g^{-1} in common carp (Akbari et al., 2021). However, the present work showed that the growth performance in common carp had no significant difference between the groups of *C. somerae* XMX-1 and control group. Deng et al. (2022) found that dietary *B. subtilis* couldn't significantly improve growth and apparent feed conversion in Nile tilapia (*Oreochromis niloticus*) larvae. Similar results were also found that dietary *B. cereus* var. *toyoi* and *B. subtilis* C-3102 didn't affect the growth performance of Nile tilapia juvenile (Garcia-Marengoni and Menezes-Albuquerque, 2015). These results suggest that the effects of probiotics on fish growth may involve fish species, probiotics and the interaction between them.

A large number of studies have shown that the addition of probiotics can promote gut health of fish (Wang et al., 2021; Gatesoupe, 2016;

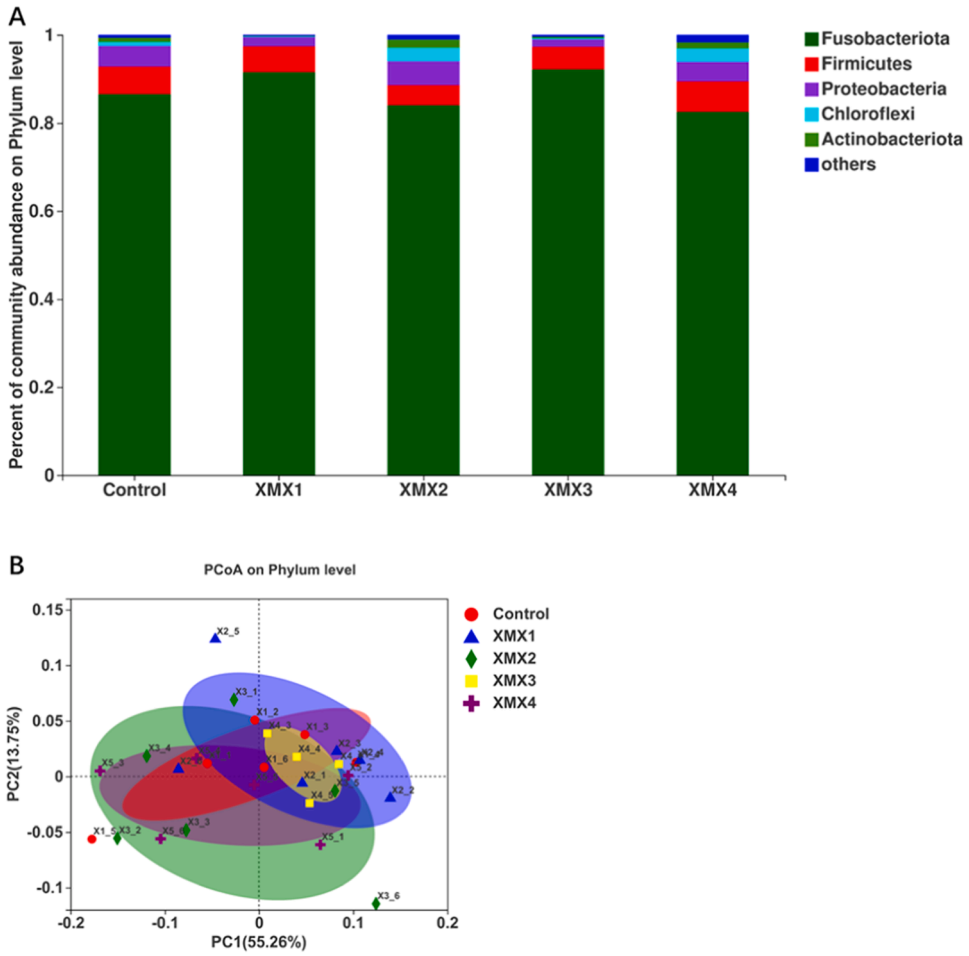

Fig. 6. Effects of control and XMX-1 diets on the gut microbiota of common carp. (A) Relative abundance, (B) Principal coordinates analysis (PCoA) at phylum level of the gut microbiota (n = 6). XMX1, 2 g/kg group; XMX2, 3 g/kg group; XMX3, 4 g/kg group; XMX4, 5 g/kg group. XMX-1 represents *C. somerae*.

Poolsawat et al., 2020). Serum LPS level reflects integrity of intestinal epithelial barrier (Zhang et al., 2019). Serum DAO is also one of the indications of intestinal mucosal integrity, and higher serum DAO levels indicate compromised intestinal mucosal barrier (Zhang et al., 2018). In the present work, dietary XMX-1 stabilized fermentation product reduced serum LPS and DAO of common carp compared to the control group, suggesting improved intestinal barrier. Tight junction proteins including Occludin and ZO-1 are important for the intestinal epithelial barrier (Ulluwishewa et al., 2011). Commane et al. found that dietary fermentation product of probiotics could enhance the strength of the tight junction, which are related to the physical tightness of the epithelial layer (Commane et al., 2005; Otte and Podolsky, 2004). Similarly, our current work showed that the supplementation of 3 g/kg XMX-1 stabilized fermentation product significantly increased the expression of *Occludin* and *ZO-1* in the gut of common carp. It was reported that hepcidin has various action related inhibiting the growth of bacteria, yeast and the activity of immunomodulatory (Álvarez et al., 2016). In the current work, the expression of *Hepcidin* had the highest level in the group of the addition of 3 g/kg XMX-1 stabilized fermentation product. Hypoxia inducible factor (HIF) can regulate intestinal homeostasis (Shao et al., 2018). Supplementation of 3 g/kg XMX-1 stabilized fermentation product up-regulated the expression of *HIF1α*in gut of common carp. SOD activity is intracellular antioxidant defense mechanism associated enzymes and is used to evaluate the level of antioxidant defense (Weksler-Zangen et al., 2003). In the current work, the level of SOD activity was increased in the gut of common carp by XMX-1 fermentation product supplementation. Consistent with this finding, Ali et al. (2020) found that dietary *B. subtilis* could increase SOD activity of Nile tilapia. The study in grouper *Epinephelus coioides* also showed that the addition of *B. pumilus* or *B. clausii* increased the concentration of SOD (Sun et al., 2010).

Probiotics attracted more and more attention in improving liver health in recent years (Looijer-van Langen and Madsen, 2010; Yoo et al., 2013). Many studies in mammals have proved that probiotics can attenuate liver diseases and some research are related to their effect on fatty liver (Kelishadi et al., 2013). For example, the addition of probiotic VSL#3 decreased the content of fatty acid in the liver of mice (Ma et al., 2008). Furthermore, Nido et al. found that supplementation of *Lactobacillus acidophilus* and *S. cerevisiae* could increase the expression of lipolysis related genes, such as *CPT1*, *CPT2*, and *PPARα*, as well as decrease the expression of lipid synthesis related genes, such as *PPARγ* and *SREBP1* (Nido et al., 2016). Yoo et al. (2013) found that dietary *L. curvatus* HY7601, or in combination with *L. plantarum* KY1032 can reduce the content of TAG compared to the control group. In the present study, all treatments of XMX-1 stabilized fermentation product supplementation significantly decreased the content of TAG of the liver in common carp. Consistently, all doses of XMX-1 down-regulated the expression of *PPARγ* and *SREBP1* and the addition of 3 g/kg XMX-1 stabilized fermentation product significantly up-regulated the expression of *CPT1* and *PPARα*. Furthermore, XMX-1 supplementation decreased the level of serum ALT of common carp. The increase of serum ALT is usually used to indicate liver damage in many research (Zhang et al., 2019). These results indicated that adding XMX-1 stabilized fermentation product has a positive effect in improving lipid metabolism and health of the liver in common carp.

5. Conclusions

In conclusion, our results showed that dietary XMX-1 stabilized fermentation product exhibited no effects on the growth performance of common carp. However, the supplementation of XMX-1 stabilized fermentation product improved the gut and liver health of common carp. And XMX-1 addition of 3 g/kg had the best effect on fish health. These results show that *C. somerae* XMX-1 fermentation product benefits the health of common carp, which suggests its potential application as a functional feed additive in fish culture.

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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