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Original Research Article

Effects of solid-state fermentation product of yeast supplementation on liver and intestinal health, and resistance of common carp (*Cyprinus carpio*) against spring viraemia carp virus

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ABSTRACT

This study aimed to investigate the effects of solid-state fermentation products of yeast (SFPY) on liver and intestinal health and disease resistance of common carp (*Cyprinus carpio*). A total of 200 common carp with an initial average weight of 2.55 ± 0.004 g were divided into 5 groups (4 replications per group and 10 fish per replication), and were fed with one of five diets, including a control diet and 4 diets supplemented with 2‰ (Y2), 3‰ (Y3), 4‰ (Y4), or 5‰ (Y5) SFPY, respectively, for 8 weeks. Results indicated that, the addition of SFPY to the diet of common carp did not affect the growth performance or survival rate of fish ($P = 0.253$). Interestingly, with the addition of SFPY, the triacylglycerol (TAG) content of the liver presented a linear decreasing tendency ($P = 0.004$), with significantly decreased in Y4 and Y5 groups ($P = 0.035$) compared with control. Serum lipopolysaccharide (LPS) content and diamine oxidase (DAO) activity presented a negative linear relationship with the addition of SFPY ($P = 0.015$, $P = 0.030$), while serum lipopolysaccharide binding protein (LBP) content first decreased and then increased ($P < 0.001$). The total antioxidant capacity (T-AOC) in the intestine of fish increased continuously with increasing SFPY supplementation ($P = 0.026$), reaching the highest level in Y5 group. The villus height in all experimental groups were significantly higher than that in the control group ($P < 0.001$). Furthermore, compared to the control, adding 3‰ SFPY to the control diet of common carp significantly increased the relative abundance of Fusobacteria ($P = 0.018$) and decreased that of Proteobacteria ($P = 0.039$) at phylum level, and increased the relative abundance of *Cetobacterium* ($P = 0.018$) and decreased that of *Shewanella* ($P = 0.013$) at genus level. Compared with the control, the relative mRNA expression level of spring viraemia of carp virus N protein (*SVCV-n*) in the kidney was lower than that of the control group without significance and bottomed out in Y4 group ($P = 0.138$). In conclusion, dietary SFPY enhanced the SVCV resistance capacity of common carp by improving liver and intestinal health and modulating the gut microbiota. Thus, SFPY is a potential feed additive to be used in aquaculture to reduce the huge

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economic loss of common carp due to SVCV disease. Based on liver TAG content and intestinal villus height, the optimal addition level of SFPY was 3.02‰ and 2.72‰, respectively.

1. Introduction

Common carp (*Cyprinus carpio*) is an important economic fish both within China, and across the world (Rahman, 2015), with the cultural history of carp in China dating back over 8,000 years (around 6,000 BC) (Nakajima et al., 2019). In 2020, global carp production reached 4.24 million ton, of which China produced about 68.38% (2.9 million ton) carp, accounting for 8.6% of the total global inland aquaculture (FAO, 2022). Currently, the intensification of the farming system is the major driver of the increase in freshwater aquaculture production (Zhang et al., 2022). Unfortunately, this has led to a series of problems such as disease-driven production drop (Naylor et al., 2021), leading to increased use and misuse of antimicrobial compounds in freshwater farming systems (Cabello et al., 2016; Reverter et al., 2020).

Fish health is vital for sustainability within the fisheries and aquaculture industry. Numerous studies have shown that probiotics could modulate the gut microbiota of the farmed aquatic animals (Pérez et al., 2010) and protect them from pathogens (Hai, 2015; Wang et al., 2019). These provide promising alternatives to antibiotics in aquaculture and are indispensable for the healthy development of fish production. Among them, *Saccharomyces cerevisiae*, one of the most used probiotics in aquaculture, could improve the growth, intestinal morphology and resistance to pathogens of fish and crustaceans (Del Valle et al., 2023). Liang et al. (2023) reported that mannan oligosaccharides derived from yeast increased the survival rate (SR) of zebrafish (*Danio rerio*) through inhibiting spring viraemia of carp virus (SVCV) replication in embryonic fibroblast cell line of zebrafish. Similarly, beta-glucan derived from yeast were reduce SVCV infection in zebrafish (Medina-Gali et al., 2018).

The two major methods used for fermentation are solid-state fermentation (SSF) and submerged fermentation (Subramaniyam and Vimala, 2012). Compared with submerged fermentation, SSF has the advantages of high-volume productivity, low equipment cost, high product yield, less waste generation and short process time (Chen, 2013). Solid-state fermentation process has been widely used for bioethanol (Karimi et al., 2021) and beverage production (Parapouli et al., 2020), but only a few studies have focused on it for aquaculture feed additives (Dawood and Koshio, 2020). Tao et al. (2022) have demonstrated that the supplementation of 2% SSF product fermented by *Bacillus subtilis*, *Lactobacillus plantarum* and *S. cerevisiae* (1.0×10^7 CFU/g) could improve antioxidant capacity via the nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1 signaling pathway and disease resistance while inhibited the inflammation response of juvenile largemouth bass (*Micropterus salmoides*). Meanwhile, supplementation with SSF products significantly improved the growth performance, feed efficiency, the enterocyte height and microvilli length of tilapia (*Oreochromis niloticus*) (Bowyer et al., 2020).

In this study, we prepared SSF products of yeast (SFPY) developed from two *S. cerevisiae* strains (GCC-1 and GCC-2) to determine the impact of supplementation with these products on liver health, intestinal health, and SVCV resistance of common carp.

2. Materials and methods

2.1. Animal ethics statement

During the whole experiment period, all experiments and animal care procedures were conducted in accordance with Institute of Feed Research Institute of Chinese Academy of Agricultural Sciences Animal Care Committee under the auspices of the China Council for Animal Care (No. 2020-AFFRI-CAAS-001). All animal experiments complied with the ARRIVE guidelines.

2.2. Experimental diets

Table 1 provides composition and nutrient levels of diets. According to Zhao et al. (2022), SFPY was prepared as follows: firstly, a single colony of *S. cerevisiae* GCC-1 and GCC-2 each, after activation on a solid medium of yeast extract peptone dextrose (YPD)

Table 1
Composition and nutrient levels of diets for common carp (g/kg, DM basis).[1]

Item	Control	Y2	Y3	Y4	Y5
Ingredients					
Rice bran	100.0	100.0	100.0	100.0	100.0
Wheat 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
Chicken meal	120.0	120.0	120.0	120.0	120.0
DDGS	100.0	100.0	100.0	100.0	100.0
SFPY[2]		2.0	3.0	4.0	5.0
Solid substrate[3]	5.0	3.0	2.0	1.0	0.0
Lysine hydrochloride	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
Predigested premix[4]	10.0	10.0	10.0	10.0	10.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Nutrient levels[5]					
Crude protein	388.60	385.50	390.20	395.30	391.10
Crude fat	105.60	108.60	106.00	109.10	103.60
Crude ash	87.24	86.64	87.30	89.55	87.09
Moisture	55.40	59.60	59.10	49.80	53.60
Gross energy, MJ/kg	16.06	16.12	16.10	16.31	16.03

DDGS = distiller's dried grains with solubles.
[1] Y2 to Y5: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively.
[2] SFPY = solid-state fermentation products of yeast, reaching 4.76×10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).
[3] Containing 30% rice bran, 30% soybean meal, 40% DDGS.
[4] Contents (g) of trace element per kg of predigested premix (g/kg): thiamine 0.438, riboflavin 0.632, pyridoxine HCl 0.908, D-pantothenic acid 1.724, nicotinic acid 4.583, biotin 0.211, folic acid 0.549, VB_{12} 0.001, inositol 21.053, menadione sodium bisulfite 0.889, retinyl acetate 0.677, cholecalciferol 0.116, DL α-tocopherol-acetate 12.632, $CoCl_2 \cdot 6H_2O$ 0.074, $CuSO_4 \cdot 5H_2O$ 2.5, $FeSO_4 \cdot 7H_2O$ 73.2, NaCl 40.0, $MgSO_4 \cdot 7H_2O$ 284.0, $MnSO_4 \cdot H_2O$ 6.50, KI 0.68, Na_2SeO_3 0.10, $ZnSO_4 \cdot 7H_2O$ 131.93, cellulose 501.09.
[5] Nutrient levels are actually measured.

medium, were cultured in a liquid medium of YPD at 30 °C, 180 r/min for 48 h with constant temperature incubator shaker (MAXQ 6000, China). The seed cultures of GCC-1 and GCC-2 were seeded into SSF medium at the ratio of 5% and shake-cultured at 30 °C for 96 h, reaching 4.76×10^{10} CFU/g to obtain the SFPY. The SSF medium consisted of 30% soybean meal, 30% rice bran, 40% distiller's dried grains with solubles (DDGS), and the water content of the medium was adjusted to 30% by water containing 4% glucose, 0.5% urea, 0.05% magnesium sulfate, 0.05% calcium dihydrogen phosphate and 0.01% biotin, then autoclaved at 121 °C for 20 min. Finally, four experimental diets were prepared by supplementing SFPY obtained in the previous step to the basal diet at 2‰ (Y2), 3‰ (Y3), 4‰ (Y4) and 5‰ (Y5). The amount of SFPY was set as ‰ (thousandth) or (1/1,000) which means 0, 2, 3, 4, and 5 g of SFPY per 1,000 g of basal diet, respectively to form the five diets. They were stored at room temperature (22 to 25 °C) after testing the chemical compositions.

The crude protein, crude fat, crude ash and the moisture of diets were determined according to GB/T 6432-2018, GB/T 6433-2006, GB/T 6438-2007 and GB/T 6435-2014, respectively. The gross energy was determined using an oxygen bomb calorimeter (IKA C 2000, IKA Analysetechnik, Heitersheim, Germany).

2.3. Fish husbandry

Two hundred carps with average initial weights 2.55 ± 0.004 g were taken at the start of the experiment. Juvenile carp were randomly allocated into 5 groups with 4 replicates each and cultured at a density of 10 fish per recirculating aquacultural system tank (90 L). Fish were fed at 3% of their body weight 3 times per day at 08:00, 13:00 and 17:00. During the 8-week feeding trial, water temperature was kept at approximately 26 °C; the dissolved oxygen content was not less than 6.0 mg/L; the total ammonia and the total nitrite contents were lower than 0.5 and 0.01 mg/L, separately. This experiment was carried out at the International Agricultural High-Tech Industrial Park of the Chinese Academy of Agricultural Sciences, Langfang, Hebei, China.

2.4. Growth performance determination

Upon completion of the experimental phase, the fish in each tank were counted, weighed, and sampled after 24 h starvation. The relevant calculation formulas were as follows:

Weight gain (WG, %) = 100 × (final body weight - initial body weight)/initial body weight;

Feed conversion ratio (FCR) = feed intake (g)/WG of fish (g);

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

2.5. Serum biochemical parameter analysis

After fasting for 24 h, blood of 6 fish per group were sampled form caudal vein, stood at 4 °C for 3 h, centrifuged at 825 × *g* for 10 min. Then, the serum was collected into a new Eppendorf tube and stored at −80 °C. According to the manufactural instructions, lipopolysaccharide (LPS), lipopolysaccharide-binding protein (LBP) contents and diamine oxidase (DAO) activity in serum were detected. The contents of serum LPS, LBP and DAO activity were determined using ToxinSensor Chromogenic LAL Endotoxin (Genscript, China), Fish LBP ELISA Kit (Jiangsu Meimian industrial Co., Ltd., China) and Fish DAO ELISA Kit (Jiangsu Meimian industrial Co., Ltd., China), separately. All samples were stored at −80 °C before analysis.

2.6. Intestinal antioxidant enzymes analysis

After fasting for 24 h, hindgut samples of 6 fish per group were collected and stored at −80 °C before analysis. The weighed hindgut samples were homogenized in ice-cold phosphate buffer solution (PBS) to break up the tissue cells, and then centrifuged at approximately 12,000 × *g* for 5 min at 4 °C. The supernatant was taken to evaluate antioxidant capacity using total antioxidant capability (T-AOC) assay kit (Cominbio, Suzhou, China) and the lipid peroxidation malondialdehyde (MDA) assay kit (Beyotime Biotechnology, Shanghai, China), separately.

2.7. Histological analysis

After starvation for 24 h, the posterior intestine and liver were sampled from 3 fish per treatment to obtain 3 replicates. Samples were washed with sterile PBS, fixed with 4% paraformaldehyde, embedded in paraffin, sectioned (4 μm thick sections), and stained with hematoxylin and eosin (HE). The tissue morphology of 5 slides was observed using a light microscope (Leica DMIL-LED, Germany). K-Viewer (1.5.5.10) software were used to observe the histological morphology of HE sections. Villus height was measured using ImageJ (1.51j8) software. The intestinal sections and liver sections were 7.39 × and 20 × magnification, separately.

2.8. Detection of triacylglycerol (TAG) content

After starvation for 24 h, the liver was sampled from 6 fish per group to obtain 6 replicates and pre-weighed separately before homogenization in 1% PBS. Then, TAG was obtained using the previous method (Zhang et al., 2019), and quantified with free glucose reagent (Sigma–Aldrich, Shanghai, China) and triglyceride reagent (Sigma–Aldrich, Shanghai, China).

2.9. Intestinal microbiota diversity analysis

Within 4 to 8 h after the last feeding, the hindgut contents of 3 fish per replicate for a total of 12 fish each group were sampled into Eppendorf tubes and stored at −80 °C. Total microbial genomic DNA was extracted from about 0.5 g hindgut contents using E.Z.N.A. soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). The V3–V4 region of 16S rRNA was amplified and sequenced on Illumina MiSeq platform (Illumina, San Diego, USA). The methods for extracting DNA and conducting real-time quantitative PCR (RT-qPCR) analysis based on 16S rRNA were detailed in the prior research by Wang et al. (2022). The quantity of 16S rRNA gene copies per milligram of intestinal content is indicative of microbiota abundance (copies/mg).

2.10. RT-qPCR

After fasting for 24 h, the livers and intestines of 6 carps sampled from each tank were treated with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), stand at 4 °C for 2 h, and then broken using a homogenizer before extracting total RNA. One μg RNA was converted into cDNA for each sample. Real-time quantitative PCR reaction procedures followed the descriptions in Zhang et al. (2019). All primers were synthesized by Sangon Biotech (Beijing) Co., Ltd. (Table 2). The reference gene is β-actin. The $2^{-\Delta\Delta Ct}$ method was used to analyze the RT-qPCR data.

Table 2
Primer sequences for real-time quantitative PCR.

Gene	Nucleotide sequence of primers (5′–3′)	Accession number
β-actin	F: GAAGTGTGGTGTGGACATCCGTAA R: AGACTCATCGTACTCCTGCTTGCT	JQ619775.1
TNF-α	F: GCTGTCTGCTTCACGCTCAA R: CCTTGGAAGTGACATTTGCTTTT	JX181982.1
TGF-β	F: ACGCTTTATTCCCAACCAAA R: GAAATCCTTGCTCTGCCTCA	AF136947.1
IL-1β	F: AAGGAGGCCAGTGGCTCTGT R: CCTGAAGAAGAGGAGGCTGTCA	KC008576.1
IL-10	F: GCTGTCACGTCATGAACGAGAT R: CCCGCTTGAGATCCTGAAATAT	KX622693.1

TNF-α = tumor necrosis factor-α; *TGF-β* = transforming growth factor-β; *IL-1β* = interleukin-1β; *IL-10* = interleukin-10.

2.11. Virus challenge

After starvation for 24 h, 18 fish selected randomly from every group were challenged with 50 μL SVCV solution (about 10^7 copies SVCV, non-lethal SVCV dose) (Liang et al., 2023) through intraperitoneal injection. Then, 18 fish were divided randomly into 3 replicates. No feeding was performed during the challenge period. After 7 days of challenge with the virus, the kidneys were taken to extract the RNA, and then the expressions of carp antiviral genes were measured as described as 2.10. All primers used in this experiment were synthesized by Sangon Biotech (Beijing) Co., Ltd., as shown in Table 3.

2.12. Statistical analysis

All statistical analyses were carried out using SPSS 26.0 (IBM SPSS Inc., Chicago, USA). Graphs were developed with GraphPad Prism 8 (GraphPad Software Inc. San Diego, CA, USA). All results are presented as means ± standard error of the mean (SEM). Normality and homoscedasticity assumptions were confirmed prior to any statistical analysis. One-way ANOVA was used for comparisons between multiple groups, followed by Duncan's multiple range test. The statistical significance was set at $P < 0.05$. In addition, to determine if the effect was linear and/or quadratic, a follow-up trend analysis using orthogonal polynomial contrasts was performed (Wei et al., 2019). The optimal level was calculated using regression analysis.

3. Results

3.1. Effects of SFPY on growth performance and SR of common carp

After 8 weeks of the experimental period, the growth performance and SR of common carp were analyzed and presented in Table 4. Unexpectedly, there were no significant differences between all groups in the FI, FBW, WG, SR and FCR ($P > 0.05$).

3.2. Effects of SFPY on the liver health of common carp

The liver HE staining showed that, when compared to the control group, the supplementation with SFPY decreased the infiltration of inflammatory cells and the vacuolization in the liver of common carp, particularly for the Y3 and Y4 groups (Fig. 1A–E). Surprisingly, the content of TAG in liver decreased linearly ($P = 0.004$) with the addition of SFPY especially for the Y4 and Y5 groups ($P = 0.035$, Fig. 1F).

To further reveal the effects of SFPY on the liver inflammation of common carp, the expressions of liver inflammation response-related genes were tested. While there were no significant differences in the relative mRNA expression levels of tumor necrosis factor-α (*TNF-α*, Fig. 2A) and interleukin-1β (*IL-1β*, Fig. 2B) of carp liver ($P > 0.05$), SFPY addition had quadratic effect on the relative mRNA expression level of *IL-1β* bottoming out approximately at Y3 ($P = 0.046$). The relative mRNA expression levels of liver tumor growth factor-β (*TGF-β*, Fig. 2C) and interleukin-10 (*IL-10*, Fig. 2D) (except the group Y3) in Y4 group was markedly higher than those of other groups ($P < 0.05$).

Table 3
Primer sequences for real-time quantitative PCR.

Gene	Forward primer (5′–3′)	Accession number
β-actin	F: GAAGTGTGGTGTGGACATCCGTAA R: AGACTCATCGTACTCCTGCTTGCT	JQ619775.1
SVCV-n	F: TGAGGTGAGTGCTGAGGATG R: CCATCAGCAAAGTCCGGTAT	NC_002823

SVCV-n = spring viraemia of carp virus N protein.

3.3. Effects of SFPY on the intestinal health of common carp

To evaluate the effects of SFPY on the intestinal health of common carp, we examined the hindgut morphology. As shown in Fig. 3, when compared with the control, SFPY treated group showed alleviated lymphoid cell infiltration and loss edema of lamina propria. The intestinal villus height of the SFPY groups was significantly longer than that of the control group, with that of groups Y2 and Y3 being longer that of Y4 and Y5 ($P < 0.001$, Fig. 3F).

As shown in Table 5, the content of LPS in serum showed a linear decrease with addition of SFPY ($P = 0.015$). Correspondingly, serum LBP content decreased first before increasing. To be more specific, the LBP content in the serum of SFPY groups was significantly lower than that of the control ($P < 0.001$), that in the Y3 group was the lowest. Serum DAO activity had a negative correlation with SFPY addition (linear trend, $P = 0.030$), slightly lowered in the Y3 group.

As shown in Table 6, there was a positive relationship between the T-AOC and SFPY addition (linear trend, $P = 0.026$), reaching the highest level in Y5 group. The supplement of SFPY did not significantly change the intestinal MDA content in intestine ($P = 0.082$) but a slight quadratic trend was observed ($P = 0.024$), with Y4 group reaching the lowest level.

Furthermore, we investigated the effects of SFPY on the expression of inflammatory factors related to intestinal health. Solid-state fermentation products of yeast addition did not affect the relative mRNA expression levels of intestine *TNF-α* (Fig. 4A) and *IL-10* (Fig. 4D) ($P > 0.05$), but significantly down-regulated the relative mRNA expression level of *IL-1β* in Y3 to Y5 groups ($P = 0.020$, Fig. 4B). The relative mRNA expression level of *TGF-β* in Y2 and Y5 groups were significantly down-regulated compared with the control and Y4 groups ($P = 0.021$, Fig. 4C).

3.4. Effects of SFPY on the intestinal microbiota of common carp

As shown in Table 7, there were no significances in Simpson, Shannon, Ace and Chao indexes among all groups ($P > 0.05$). At the phylum level, the relative abundance of Fusobacteria in the Y3 group was meaningfully higher, whereas that of Proteobacteria was significantly decreased ($P < 0.05$), compared with the control group (Table 8 and Fig. 5A). At the genus level, the relative abundance of *Cetobacterium* in the Y3 group was noticeably increased ($P = 0.018$), while that of *Shewanella* was significantly reduced ($P = 0.013$, Table 9 and Fig. 5B), compared with the control group. PCoA results showed that the intestinal microbiota composition of the SFPY was different from that of the control group at the operational taxonomic unit (OTU) level, and the Y3 group was completely separated from the control group (Fig. 5C). Furthermore, the ratio of (Fusobacteria + Bacteroidetes + Firmicutes)/Proteobacteria (Li

Table 4
Effects of SFPY on the growth performance and SR of common carp fed different diets.

Item	Group[1]					*P*-value		
	Control	Y2	Y3	Y4	Y5	ANOVA	Linear	Quadratic
FI, g/tank	114.50 ± 0.040	114.30 ± 0.030	114.50 ± 0.060	114.10 ± 0.060	114.50 ± 0.040	0.956	0.895	0.662
IBW, g/fish	2.56 ± 0.009	2.55 ± 0.007	2.56 ± 0.012	2.55 ± 0.012	2.56 ± 0.008	0.956	0.895	0.662
FBW, g/fish	12.28 ± 0.203	11.70 ± 0.155	12.33 ± 0.345	12.49 ± 0.183	12.60 ± 0.107	0.829	0.540	0.566
WG, %	369.89 ± 9.17	374.28 ± 4.885	388.74 ± 12.099	385.61 ± 6.820	380.53 ± 13.775	0.667	0.321	0.347
FCR	1.19 ± 0.020	1.20 ± 0.016	1.16 ± 0.038	1.16 ± 0.021	1.18 ± 0.045	0.827	0.558	0.495
SR, %	100.00 ± 0.000	100.00 ± 0.000	100.00 ± 0.000	95.00 ± 5.000	96.67 ± 3.333	0.253	0.077	0.593

SFPY = solid-state fermentation products of yeast; FI = feed intake; IBW = initial body weight; FBW = final body weight; WG = weight gain; FCR = feed conversion ratio; SR = survival rate.
[1] Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

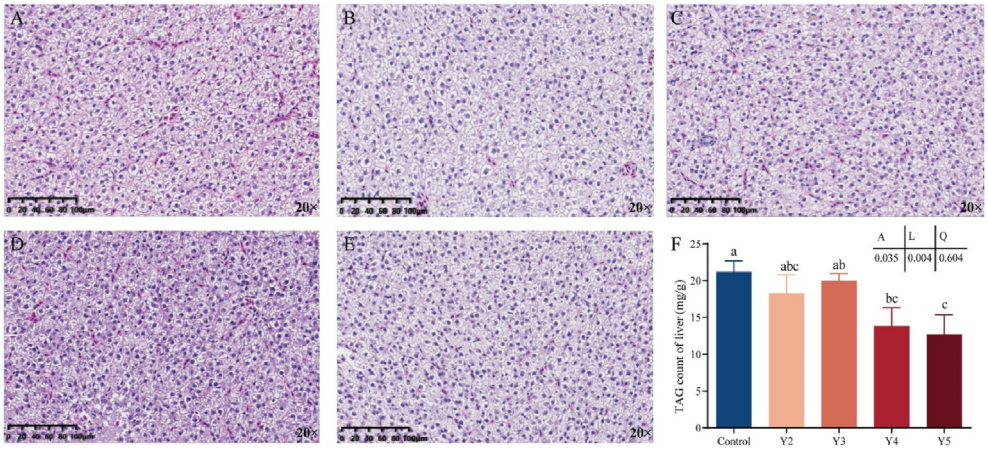

Fig. 1. Liver hematoxylin and eosin (HE)-stained sections and triacylglycerols (TAG) content of common carp after feeding different diets for 8 weeks. (A) Control group. (B) Y2 group. (C) Y3 group. (D) Y4 group. (E) Y5 group. (F) TAG content of liver. The scale bar is 100 μm, slice thickness is 4 μm, at 20 × magnification. Data represent the mean ± SEM (n = 6). Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. [a-c] Bars marked with different letters represent statistically significant results (P < 0.05). SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

et al., 2024) in the Y3 group was significantly higher than that of other groups (P = 0.003, Fig. 5D).

3.5. Effects of SFPY on common carp resistance for SVCV infection

After the challenge, there were no deaths found in all groups. The replication of SVCV in the kidneys of common carp was investigated on the 7th day after the challenging (Fig. 6). The relative mRNA expression level of *SVCV-n* in SFPY groups were lower than that of the control group without significance, reaching the lowest level in Y4 group (P = 0.138).

3.6. The optimal lever of SFPY for the farmed common carp

Because there was no significant difference in growth performance, we selected the intestinal villus height and liver TAG content, representing intestinal health and liver health respectively to do regression analysis. Based on liver TAG content and intestinal villus height, the optimal levels for supplementation with SFPY obtained were 3.02‰ (Fig. 7A) and 2.72‰ (Fig. 7B), respectively.

4. Discussion

At present, the application of yeast in the aquatic farm industry has been widely studied, but due to the different sources and properties of yeast, the effects on aquatic animals have been different for different species. In this study, we studied the effects of two self-isolated yeast complex solid fermentation products on liver health, intestinal health, and SVCV resistance of common carp.

The growth performance of aquatic animals is one of the most important points in the aquaculture industry. Most studies show that supplementation with solid fermentation products fermented with *S. cerevisiae* in the diet could improve the growth performance of aquatic farmed animals such as Nile tilapia (Bowyer et al., 2020) and Rohu (*Labeo rohita*) (Das et al., 2021). However, it was found that the addition 2.0% sea buckthorn solid fermentation product fermented with 3 probiotics, namely *Bacillus subtilis*, *L. plantarum* and *S. cerevisiae*, did not affect the growth performance of large-mouth bass (Tao et al., 2022), similar to the results for Indian prawn shrimp (*Fenneropenaeus indicus*) (Sharawy et al., 2015). This corresponds with the results of the present study, with the addition of SFPY to the diet, except for the Y2 group, WG of common carp presented an upward trend without significant differences, might resulting from the corresponding increasing the relatively high lipid content of the diet (average 10.66%).

A large number of studies have shown that the addition of yeast can effectively reduce the deposition of liver fat. It is reported that TAG content of shrimp fed with diets containing 2% yeast was meaningfully decreased (Ayiku et al., 2020). In addition, we have demonstrated that TAG content in serum was significantly reduced with the addition of 0.5% to 1% SSF product of yeast into zebrafish high-fat diet through the inhibition of expression of lipogenesis related genes (Li et al., 2023). The results of this study demonstrate that with the addition of SFPY to the common lipid diet, the

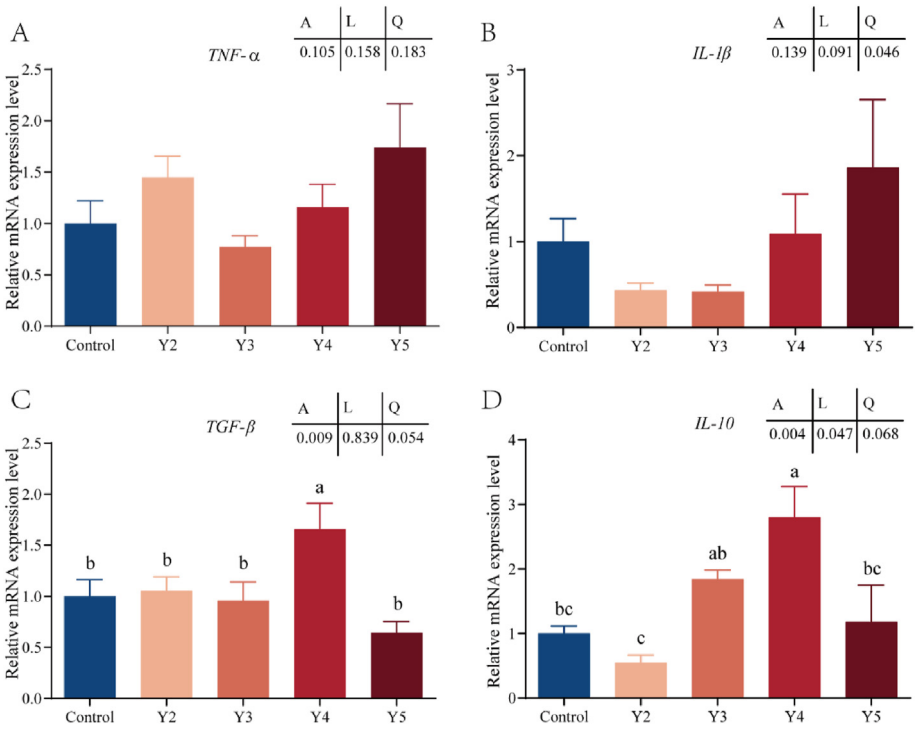

Fig. 2. Relative mRNA expression levels of inflammation-related genes in the liver of common carp fed with different diets for 8 weeks. (A) Tumor necrosis factor-α (*TNF-α*). (B) Interleukin-1β (*IL-1β*). (C) Transforming growth factor-β (*TGF-β*). (D) Interleukin-10 (*IL-10*). Data were represented as the means ± SEM ($n = 6$). Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. [a-c] Bars marked with different letters represent statistically significant results ($P < 0.05$). SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

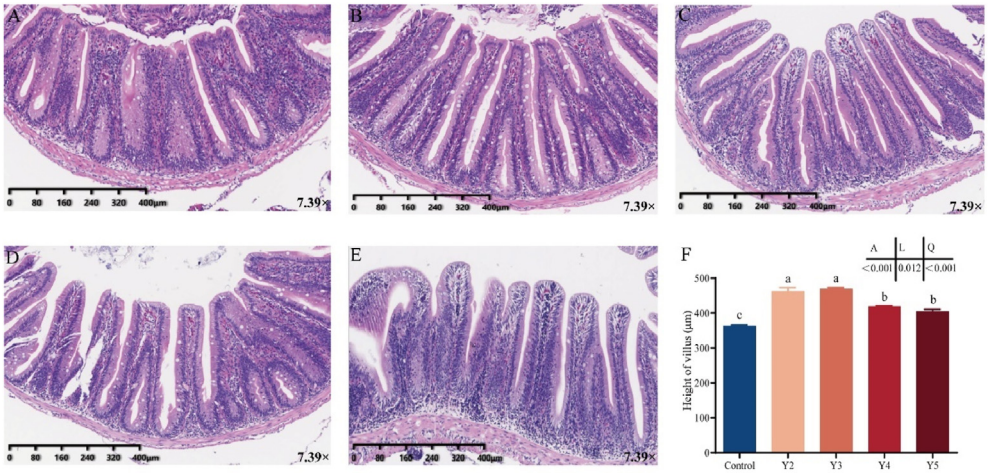

Fig. 3. HE-stained sections of the intestine of common carp fed with different diets for 8 weeks. (A) Control group. (B) Y2 group. (C) Y3 group. (D) Y4 group. (E) Y5 group. (F) Height of villus. The scale bar is 400 μm, slice thickness is 4 μm, at 7.39× magnification. Data were represented as the means ± SEM ($n = 6$). Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. [a-c]Means marked with different letters represent statistically significant results ($P < 0.05$). SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

deposition of liver fat was surprisingly decreased, which was proportional to the dose. Correspondingly, SFPY decreased the infiltration of inflammatory cells and the vacuolization in the liver of common carp (Terzi et al., 2023). Meanwhile, supplementation with SPFY significantly increased the relative abundance of *Cetobacterium somerae* in the hindgut, particularly for the Y3 group. *C. somerae* could decrease liver TAG deposition (Xie et al., 2022a,b, 2021) by suppressing the expression of lipogenesis related genes and promoting fatty acid oxidation with the help of vitamin B_{12} (Sun et al., 2023). Thus, SFPY might decrease the accumulation of TAG in the liver through vitamin B_{12} produced by *C. somerae*. Inflammatory factors are often highly expressed in inflammatory parts of the body to restore body damage (Li et al., 2021) and the occurrence of fatty liver and liver inflammation were often accompanied by the occurrence of vacuolization of hepatocytes (Jiang et al., 2020). The results of HE section of liver showed that there were not obvious differences between all groups, for the expressions of inflammatory factors in liver. The expressions of pro-

Table 5
Effects of SFPY on the LPS, LBP contents and DAO activity in serum of common carp fed different diets.

Item	Groups[1]					*P*-value		
	Control	Y2	Y3	Y4	Y5	ANOVA	Linear	Quadratic
LPS, EU/mL	1.36 ± 0.048	1.24 ± 0.101	1.02 ± 0.170	0.72 ± 0.308	0.79 ± 0.306	0.132	0.015	0.735
LBP, ng/mL	13.05 ± 0.495^{a}	11.51 ± 0.430^{b}	9.38 ± 0.077^{d}	10.00 ± 0.130^{cd}	10.70 ± 0.710^{bc}	<0.001	<0.001	<0.001
DAO, IU/L	70.46 ± 4.413	67.50 ± 4.216	58.06 ± 1.077	60.37 ± 1.972	61.62 ± 2.936	0.074	0.030	0.096

LPS = lipopolysaccharide; LBP = lipopolysaccharide-binding protein; DAO = diamine oxidase.
$^{a-d}$ Different superscripts in the same row indicate significant differences ($P < 0.05$).
[1] Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

Table 6
Effects of SFPY on the T-AOC and MDA content in the intestine of common carp fed different diets.

Item	Groups[1]					*P*-value		
	Control	Y2	Y3	Y4	Y5	ANOVA	Linear	Quadratic
T-AOC, μmol/mg prot	0.05 ± 0.003^{b}	0.06 ± 0.005^{ab}	0.06 ± 0.010^{ab}	0.05 ± 0.015^{b}	0.07 ± 0.005^{a}	0.041	0.026	0.363
MDA, nmol/mg prot	0.98 ± 0.164	0.67 ± 0.223	0.36 ± 0.000	0.26 ± 0.150	0.65 ± 0.034	0.082	0.096	0.024

T-AOC = total antioxidant capacity; MDA = malondialdehyde.
^{a,b} Different superscripts in the same row indicate significant differences ($P < 0.05$).
[1] Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

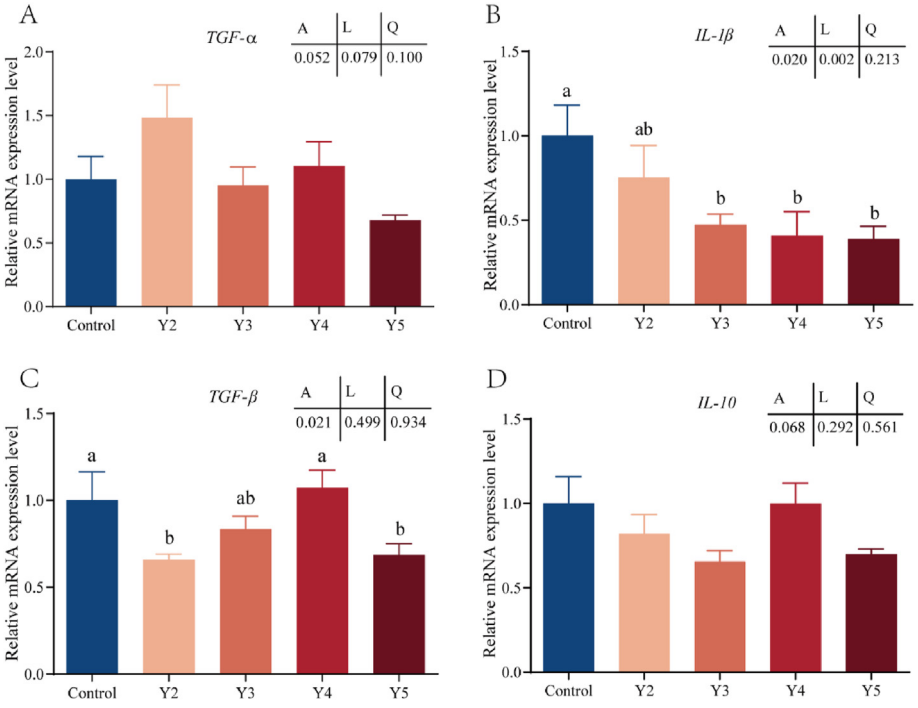

Fig. 4. Relative mRNA expression levels of inflammation-related genes in the intestine of common carp after feeding with different diets for 8 weeks. (A) Tumor necrosis factor-α (*TNF-α*). (B) Interleukin-1β (*IL-1β*). (C) Transforming growth factor-β (*TGF-β*). (D) Interleukin-10 (*IL-10*). Data were represented as the mean ± SEM ($n = 6$). Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. ^{a,b} Means marked with different letters represent statistically significant results ($P < 0.05$). SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

inflammatory factors *TGF-β* and *IL-10* (except Y3) were significantly higher in the Y4 group than that of the other groups, indicating that adding 4‰ SFPY stimulated the immune response of carp. Two anti-inflammatory factors both increased significantly in group Y4 and then decreased noticeably in group Y5, which might be due to excessive addition of SFPY in Y5 group, resulting in some negative immune effects. Therefore, dietary SFPY can effectively reduce liver lipids and regulate liver cell health positively when applied at a rate of less than 4‰.

The intestinal tract is one of the few organs of the body that is in direct contact with the outside world. The intestinal tract is one of the main immune organs which is often referred to as a "firewall" preventing bacterial penetration throughout the body, and it's health status can directly reflect the health status of the body overall (Hooper et al., 2012). Serum LPS, a key component of Gram-negative bacteria (Holen et al., 2021), was increased in serum following intestinal injury (Giamberti et al., 2006). The results of this study indicated a decreasing trend of serum LPS with

Table 7
Alpha diversity analyses of intestinal microbiota of common carp fed different diets.

Item	Groups[1]					*P*-value		
	Control	Y2	Y3	Y4	Y5	ANOVA	Linear	Quadratic
Shannon index	1.76 ± 0.222	1.87 ± 0.141	1.44 ± 0.169	1.70 ± 0.193	1.85 ± 0.484	0.798	0.798	0.798
Simpson index	0.36 ± 0.071	0.31 ± 0.044	0.48 ± 0.026	0.32 ± 0.048	0.36 ± 0.090	0.334	0.334	0.334
Ace index	224.05 ± 39.104	314.04 ± 46.156	296.54 ± 42.825	235.50 ± 16.041	320.74 ± 66.424	0.435	0.435	0.435
Chao index	206.70 ± 33.574	294.34 ± 25.115	250.26 ± 56.956	209.69 ± 11.334	262.19 ± 79.492	0.670	0.670	0.670

[1] Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

Table 8
The relative abundance of main phyla in the intestinal microbiota of common carp fed different diets.

Item	Groups[1]					*P*-value		
	Control	Y2	Y3	Y4	Y5	ANOVA	Linear	Quadratic
Fusobacteria	35.10 ± 10.266^{b}	56.35 ± 6.659^{ab}	78.03 ± 1.919^{a}	53.67 ± 7.339^{ab}	61.78 ± 8.928^{a}	0.018	0.018	0.018
Proteobacteria	52.49 ± 15.951^{a}	32.27 ± 5.013^{ab}	11.48 ± 2.919^{b}	38.5 ± 7.912^{ab}	22.52 ± 3.806^{b}	0.039	0.039	0.039
Firmicutes	4.36 ± 1.944	4.11 ± 0.508	5.81 ± 2.313	3.00 ± 0.803	11.99 ± 5.561	0.236	0.236	0.236
Bacteroidetes	7.69 ± 4.905	6.62 ± 2.685	3.81 ± 2.721	4.63 ± 3.289	0.85 ± 0.284	0.596	0.596	0.596
Other	0.36 ± 0.133	0.65 ± 0.122	0.87 ± 0.656	0.21 ± 0.051	2.86 ± 2.755	0.593	0.593	0.593

[a,b] Different superscripts in the same row indicate significant differences ($P < 0.05$).
[1] Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

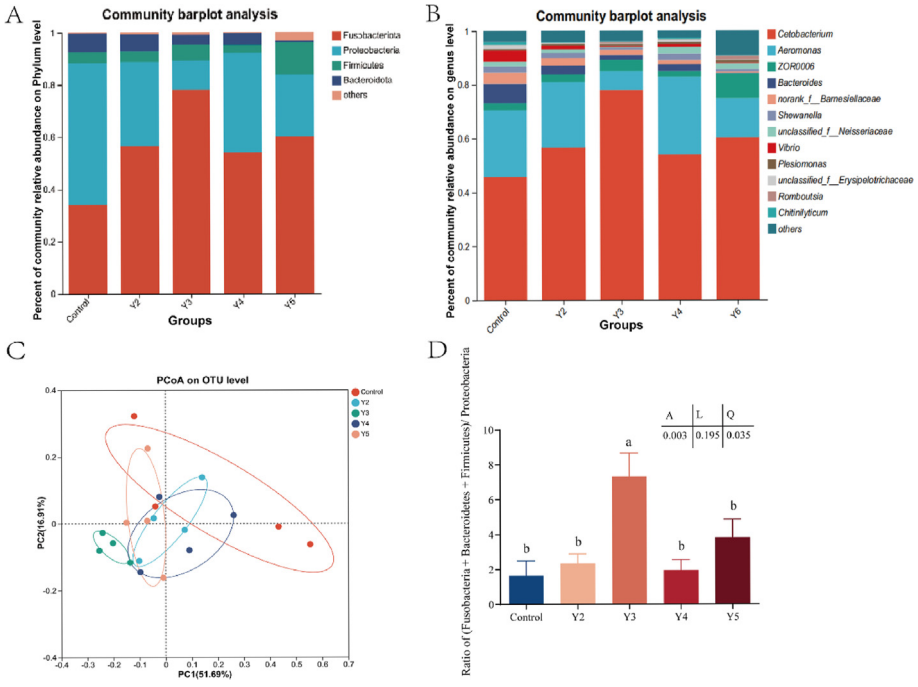

Fig. 5. Effects of SFPY on the intestinal microbiota of common carp. (A) Relative abundance of bacterial phylum of intestinal microbiota. (B) Relative abundance of bacterial genus of intestinal microbiota. (C) Principal coordinates analysis (PCoA) of the gut microbiota OTU level. (D) Ratio of (Fusobacteria + Bacteroidetes + Firmicutes)/Proteobacteria. Data were represented as the mean ± SEM ($n = 6$). Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. [a,b] Means marked with different letters represent statistically significant results ($P < 0.05$). OTU = operational taxonomic unit; SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

increasing SFPY. Consistent with this study, the addition of yeast culture has been shown to down-regulate LPS content in large-mouth bass and reduce the damage on the fish induced by concentrated cottonseed protein (Xv et al., 2021). Serum LBP is mainly responsible for accelerating the binding of monomeric LPS to CD14 (Tang et al., 2015). In this experiment, it was found that the LBP contents in the serum of the groups supplemented with SFPY were significantly decreased, meaning that the expression of the receptor protein was decreased, likely due to the decreased LPS contents in the serum. Serum DAO is mainly distributed in the intestinal mucosa or upper villus, with levels in other organs being much lower than the small intestine. When intestinal mucosal cells are necrotic, the enzyme is released into the blood, resulting in increased DAO activity in the serum. So, the activity of DAO in serum is also a landmark index to judge the intestinal permeability of aquatic animals (Costa et al., 2014; Zhang and Jiang, 2015). The

Table 9
The relative abundance of the main genus in the intestinal microbiota of common carp fed different diets.

Item	Groups[1]					*P*-value		
	Control	Y2	Y3	Y4	Y5	ANOVA	Linear	Quadratic
Cetobacterium	35.09 ± 10.269[b]	56.35 ± 6.658[ab]	78.03 ± 1.919[a]	53.67 ± 7.340[ab]	61.78 ± 8.928[a]	0.018	0.051	0.022
Aeromonas	39.66 ± 16.871	24.45 ± 4.057	7.06 ± 2.479	29.17 ± 6.722	13.74 ± 5.248	0.123	0.108	0.250
ZOR0006	2.37 ± 1.191	2.93 ± 0.376	4.18 ± 2.212	2.07 ± 0.612	9.01 ± 4.342	0.229	0.103	0.286
Bacteroides	4.93 ± 3.577	3.25 ± 1.732	1.81 ± 1.423	2.54 ± 2.168	0.20 ± 0.111	0.618	0.151	0.918
Norank_f__Barnesiellaceae	2.49 ± 1.397	2.83 ± 1.105	1.86 ± 1.220	1.53 ± 1.193	0.39 ± 0.179	0.582	0.137	0.583
Shewanella	2.35 ± 0.377[a]	1.90 ± 0.368[ab]	0.86 ± 0.050[b]	2.47 ± 0.484[a]	0.99 ± 0.314[b]	0.013	0.071	0.657
Unclassified_f__Neisseriaceae	2.16 ± 1.174	1.41 ± 0.375	0.29 ± 0.163	2.58 ± 2.443	2.32 ± 1.321	0.765	0.737	0.404
Vibrio	4.35 ± 2.967	1.30 ± 0.428	0.26 ± 0.163	0.99 ± 0.520	0.32 ± 0.188	0.247	0.072	0.221
Plesiomonas	1.01 ± 0.427	0.73 ± 0.225	0.82 ± 0.306	1.08 ± 0.187	0.93 ± 0.136	0.900	0.826	0.679
Unclassified_f__Erysipelotrichaceae	1.14 ± 0.839	0.48 ± 0.247	0.22 ± 0.165	0.50 ± 0.492	0.15 ± 0.141	0.593	0.200	0.508
Romboutsia	0.23 ± 0.099	0.17 ± 0.050	0.57 ± 0.487	0.18 ± 0.136	1.25 ± 1.054	0.558	0.236	0.460
Others	4.23 ± 0.718	4.21 ± 0.471	4.03 ± 2.266	3.23 ± 0.861	8.92 ± 7.053	0.765	0.441	0.404

[a,b] Different superscripts in the same row indicate significant differences ($P < 0.05$).
[1] Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY = compound yeast solid fermentation product reaching 4.76 × 10^{10} CFU/g developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

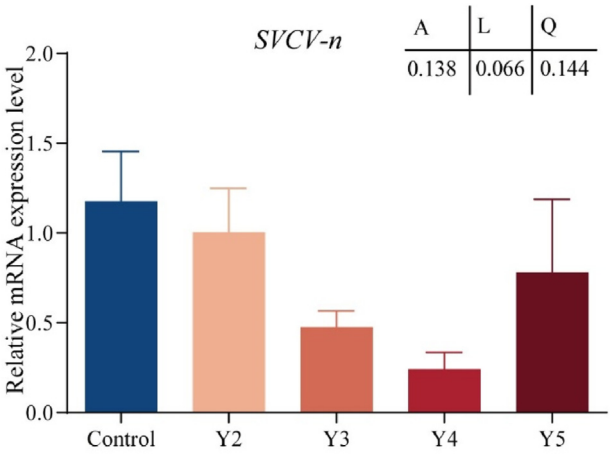

Fig. 6. Relative mRNA expression level of spring viraemia of carp virus N protein (*SVCV-n*) gene in the kidney of the common carp fed with SFPY or control diets, challenged with SVCV after 7 days. Data were represented as mean ± SEM ($n = 6$). Y2 to Y5 groups: adding 2, 3, 4, 5 g/kg SFPY into control diet, respectively. SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

results of this experiment showed that the serum DAO activity of the SFPY supplementation groups showed a decreasing trend, reaching the lowest level in Y3 group. Similar to this study, the addition of yeast cultures to the largemouth bass diet was found to reduce DAO activity in serum (Feng et al., 2022). Considering that decreasing trend of LPS meanwhile both serum LBP contents were decreased first and then increased, the addition of SFPY might decrease intestinal permeability and benefit the intestinal health of carp, with the recommended dose being around 3‰.

Malondialdehyde is one of the most important products of lipid oxidation and an important parameter reflecting the potential antioxidant capacity of the body (Zhu et al., 2022), which reflects the rate and intensity of lipid peroxidation and indirectly reflects the degree of tissue peroxidation damage (Su et al., 2019). In our study, although a significant decrease of MDA content in the intestine was not found with the addition of SFPY, the intestinal MDA content observed to be the lowest in the Y4 group. Lowered contents of MDA indicate an improvement of antioxidant level. In addition, with supplementation with SFPY, T-AOC increased, similar to the results of Chen et al. (2019), indicating SFPY could improve the antioxidant capacity of carp (Meng et al., 2017).

Solid-state fermentation substrates used in this study contained 30% soybean meal, 30% rice bran, 40% corn meal. It is reported that rice-bran fermented by *S. cerevisiae* is rich in phenolic compounds, including protocatechuic and gallic acids (Christ-Ribeiro et al., 2020). Additionally a report found that SSF of soybean okara using *S. cerevisiae* produced plenty of total phenolics and also promoted the bioconversion of isoflavone β-D-glucosides to aglycone form through the action of β-glucosidases (Queiroz Santos et al., 2018). Therefore, the addition of SFPY may lead to the enhancement of antioxidant capacity in the carp gut through production of phenolic antioxidants by SSF (De Villa et al., 2023), thus improving

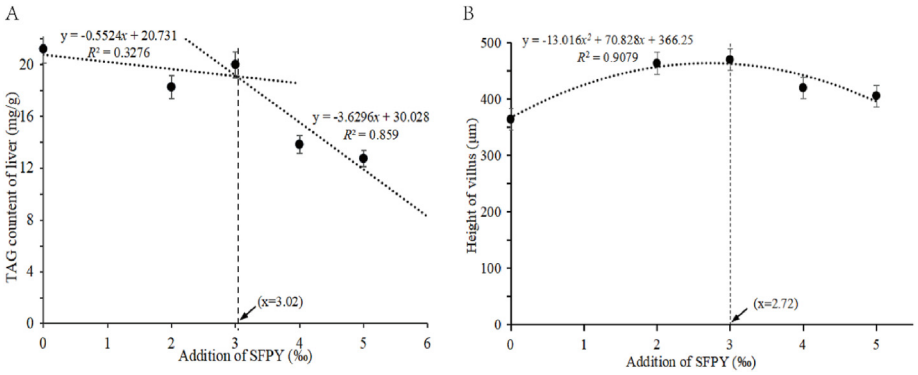

Fig. 7. The relationship between dietary SFPY levels and (A) liver triacylglycerols (TAG) content, (B) height of villus of common carp. The two models for estimating the optimal addition lever of SFPY were compared by broken line model and quadratic regression. The estimated equations and R^2 for broken line model and quadratic regression are provided. SFPY = solid-state fermentation products of yeast, reaching 4.76 × 10^{10} CFU/g yeast developed from two *Saccharomyces cerevisiae* strains (GCC-1 and GCC-2).

carp gut health. Corresponding with these results, we found that the expression of inflammatory factors in the intestine was decreased when compared to the control group, suggesting that the inflammation in the intestine was resolved with the addition of SFPY. Therefore, the results for the expression of inflammatory factors also indicated that the addition of SFPY may lead to the reduction of intestinal inflammation in carp, which is beneficial to intestinal health.

It has been reported that gut microbiota plays a crucial role in many physiological processes such as nutrient metabolism, immune response and disease resistance of the host (Liu et al., 2021). The results of this study showed that dietary SFPY could lead to a significant increase in the abundance of Fusobacteria and a significant reduction of Proteobacteria in the intestinal tract of carp grouped under Y3, which was similar to the results reported by Zhao et al. (2022). Our previous findings suggested that the functional significance of Proteobacteria is generally negative because they contain fewer genes for carbohydrate degradation and short chain fatty acid production while encoding more virulence factors and antibiotic resistance genes (Zhang et al., 2019). In addition, Li et al. (2023) demonstrated that the ratio of (Fusobacteria + Bacteroidetes + Firmicutes)/Proteobacteria can reflect the health of fish. Our results showed that dietary SFPY supplementation could effectively improve the ratio of carp intestinal flora, especially in the Y3 group, which fully indicated the positive effect of dietary SFPY supplementation on carp intestinal flora. Furthermore, it has been shown that *Cetobacterium* has multiple positive effects on teleost fish, concluding improving intestinal and liver health (Xie et al., 2021), reducing hepatic lipid deposition (Xie et al., 2022a), promoting glucose homeostasis (Xv et al., 2021; Wang et al., 2021) and enhancing antiviral immunity (Xie et al., 2022b; Zhou et al., 2022). Additionally, *Shewanella* is known to be an opportunistic pathogen in marine and freshwater fish (Jiang et al., 2022; Khashe and Janda, 1998). A significant increase in *Cetobacterium* relative abundance and a decrease in *Shewanella* relative abundance was observed, indicating a positive effect of the changes in gut microbiota due to the addition of SFPY. In addition, the results of the β-diversity analysis showed that the control group and the SFPY group showed a relatively obvious separation trend. Taken together, these results suggest that dietary SFPY supplementation can significantly alter the gut microbiota profile of common carp positively. Spring viraemia of carp virus is a highly pathogenic virus that frequently causes excessive losses in carp pond fisheries (Liu et al., 2021). We challenged carp with non-lethal SVCV through intraperitoneal injection and found that the viral loads in the immune organs, kidney, of Y4 group was the lowest, which directly indicated that the addition of dietary SFPY could enhance the antiviral ability of carp, blocking virus entry and decreasing viral load. Studies have demonstrated that yeast β-glucan (Medina-Gali et al., 2018) and mannan (Liang et al., 2023) could inhibit viral replication by enhancing expression of interferon (*IFN*) and inflammatory factors, decreasing viral adsorption. Zhou et al. (2018) reported that *C. somerae* was the dominant genus in the experimental groups fed a diet adding yeast hydrolysate, similar to the results of this study. Furthermore, adding 10 g *C. somerae* into 1 kg of feed could enhance SVCV resistance of zebrafish (Xie et al., 2022b). Qi et al. (2023) demonstrated that Vitamin B_{12} which was produced by *C. somerae* improved zebrafish resistance against *Aeromonas hydrophila* infection through increasing the diversity and complexity of gut microbial (Qi et al., 2023). In conclusion, dietary supplementation with 3‰ to 4‰ SFPY could effectively improve the resistance of carp to SVCV infection, which might be achieved through increasing the abundance of *C. somerae*.

5. Conclusions

The addition of SFPY in the common carp diet can improve the health of the intestine through alleviating the lymphoid cell infiltration and increase the villi length. It also enhanced the liver health through reducing the infiltration of inflammatory cells, vacuolization, and TAG content. Furthermore, gut microbiota was modulated positively by increasing the relative abundance of *Cetobacterium* and decreasing that of *Shewanella*, and additionally SFPY supplementation was found to inhibit the replication of SVCV in common carp. Therefore, SFPY can be used as a green feed additive for the farmed common carp, with a suggested level of being 3‰. Moreover, according to regression analysis, the optimal levels of SFPY based on liver TAG content and intestinal villus height were 3.02‰ and 2.72‰, respectively.

Author contributions

Zhigang Zhou: Supervision. **Zhen Zhang:** Conceptualization, Methodology. **Mengxin Wang:** Data curation, Writing-Original draft preparation, Visualization. **Dongmei Xia:** Data curation, Writing-Original draft preparation. **Qiang Hao:** Data curation. **Mingxu Xie:** Data curation, **Qingshuang Zhang:** Validation. **Delong Meng:** Validation. **Lijuan Yu:** Data curation. **Yalin Yang:** Supervision. **Chao Ran:** Supervision. **Tsegay Teame:** Software, Writing-Original draft preparation.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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