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Dietary Solid-state-fermentation product of *Bacillus velezensis* T23 alleviate hepatic steatosis, oxidative stress, gut barrier damage, and microbiota dysbiosis in juvenile genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*)

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ABSTRACT

The application of commensal probiotic bacteria is one of the preferable green feed additives for antibiotic substitution in aquaculture, while the application methods and detailed action mechanisms of commensal bacteria isolated from aquatic animals are not consistent with normal mammalian-derived probiotics. The purpose of this study was to evaluate the effects of solid-state fermentation product of autochthonous *Bacillus velezensis* T23 on improving hepatic steatosis, liver antioxidation capacity, gut injury and gut microbiota profile of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). The T23 probiotic was added to the basal diet at a level of 0.00, 0.05, 0.1, 0.2 and 0.3 g/kg to develop five experimental diets, respectively. After 4 weeks of the feeding trial, the results showed that T23 supplementation notably reduced the hepatocytes vacuolization and the content of liver TAGs by downregulated the mRNA expression of the lipogenesis gene (*fas*), while upregulated the mRNA expression of lipid degradation genes (*cpt1* and *pparα*) ($p < 0.05$). Compared with the control, the 0.3T23 group showed significantly upregulated expression levels of anti-inflammatory cytokine genes (*tgf-β* and *il-10*) in the liver of the fish ($p < 0.05$). Besides, the activities of antioxidase SOD and CAT were significantly upregulated in T23 treatment groups, compared with the control ($p < 0.05$). Fish groups fed with 0.2 g/kg and 0.3 g/kg T23 supplemented diet also exhibited significantly lower intestinal injury and intestinal inflammation via upregulating the expression of tight junction protein gene *claudin*, anti-inflammatory factor gene *il-10* ($p < 0.05$). However, markedly downregulation of the mRNA level of pro-inflammatory cytokine *il-1β* ($p < 0.05$) was observed in 0.05 and 0.3T23 groups, compared with the control. 16S rRNA sequencing analysis data on gut microbiota indicated that supplementation of T23 at lower levels (0.05, 0.1 and 0.2) resulted in a remarkable elevation of the abundance of phylum Firmicutes and reduced the abundance of Proteobacteria, and the ratio of (Firmicutes +Bacteroidota +Fusobacteria) /Proteobacteria. On the contrary, feeding with 0.3T23 negatively affects the gut microbiota diversity and structure of tilapia. Therefore, a 0.2 g/kg dose is suggested to supplement the diet of Nile tilapia to improve liver health and modulate the gut microbiota profile of the fish positively. Overall, these findings provide a shred of beneficial evidence of the application of *B. velezensis* T23 as a green feed additive to improve the productivity and profitability of tilapia farming.

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1. Introduction

Aquaculture is one of the important and rapidly growing segments of the global food industry, which provides a high-quality of animal protein for human consumption, huge employment opportunities, and economic development in many countries all over the world (Pradeepkiran, 2019). China is the largest producer and exporter of aquatic products in the world, which accounted for nearly two-thirds of the global aquaculture production in 2021, with a total output of 66.9 million tons (FAO, 2022).

Probiotics are beneficial microorganisms that can contribute to maintaining the homeostasis and growth of hosts both in aquatic species and terrestrial species (El-Saadony et al., 2021; Pérez-Sánchez et al., 2014). As substitutes for antibiotics, probiotics are currently attracting extensive attention in aquaculture nutrition due to their potential function to improve growth, immune response, gut microbiota and as an environmentally friendly and sustainable feed additive (Adama, 2020; Chauhan, Singh, 2018; Dawood et al., 2018; Tran et al., 2022). Numerous studies have demonstrated that probiotics play a crucial role in sustainable aquaculture. For example, Nimrat et al. (2012) reported that *Bacillus* had a significant role in reducing ammonia and nitrite levels in the culture water of white shrimp (*Litopenaeus vannamei*) by converting them into less toxic compounds. Liu et al. (2017) indicated that *B. subtilis* E20 supplementation promoted growth performance by improving nutrient digestion and absorption in parrot fish (*Oplegnathus fasciatus*). Moreover, *Lactobacillus plantarum* supplementation in the diet of Nile tilapia (*Oreochromis niloticus*) enhanced the immunity and resistance to streptococcosis disease (Foysal et al., 2020).

Compared to mammals, there is still limited information on the effect of autochthonous probiotics isolated from aquatic animals on the growth and health of aquatic animals (Zhang et al., 2024). Most probiotics used in aquaculture were derived from mammals. Furthermore, it has been reported that the composition and diversity of the gastrointestinal microbiota of aquatic animals are different from that of terrestrial animals (Xie et al., 2022). The capabilities of probiotics isolated from terrestrial animals to colonize and become functional in the intestinal tract of aquatic animals were limited (Zhang et al., 2019). Furthermore, a study performed by Zhang et al., (2023) demonstrated that some non-host origin probiotics do not influence the fish's physiological functions positively as showed in zebrafish fed with *Lactobacillus rhamnosus GG* triggered intestinal epithelium injury. Therefore, it is crucial to use probiotics for aquatic animals should isolate them from aquatic animals. The *Bacillus velezensis* T23 used in this experiment was isolated from the gastrointestinal tract of the large yellow croaker (*Pseudosciaena crocea)*. A previous study indicated that the autochthonous *B. velezensis* T23 notably improved the non-specific immunity and effectively alleviated liver steatosis and inflammation in zebrafish (*Danio rerio*) (Zhang et al., 2022). Increased disease resistance and improve growth performance of several fish species including, Grass carp (*Ctenopharyngodon idella*) (Wu et al., 2021) and Red Sea stingray (*Hemitrygon akajei*) (Emam and Dunlap, 2020) was also reported.

Tilapia farming is a significant part of China's aquaculture industry. According to data from the Administration of the Ministry of Agriculture and Rural Affairs of China (MARAC), China produced over 1.66 million tons of tilapia in 2021 (Years Book, 2022), which accounted for more than 60 % of the world's total tilapia production (FAO, 2022). Genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*), Mozambique tilapia (*Oreochromis mossambicus*) and Blue tilapia (*Oreochromis aureus*) are the main farmed tilapia species in China (Xiong et al., 2022).

Previous studies indicated that in addition to fermented products of probiotics have a significant role in improving the health of aquatic animals (Li et al., 2024; Wang et al., 2022). This study aimed to evaluate if the solid state fermented product of autochthonous *B. velezensis* T23 mitigate hepatic steatosis and gastrointestinal injury in GIFT. Moreover, its effect on the modulation of the gut microbiota composition and diversity were analyzed using 16S rRNA sequencing.

2. Materials and methods

2.1. Bacteria culture and experimental diets preparation

B. velezensis T23 was isolated from the gut of *Pseudosciaena crocea* and preserved in China General Microbiological Culture Collection Center (GMCCC) with a preservation number of CGMCC No.22029. The colonies of T23 were inoculated to LB Broth medium (Land bridge, Beijing, China) and cultured in the incubator (Jiangnan, Ningbo, China) with 180 rmp in 30℃ for 24 h to attain the primary seed fermentation broth of T23. The primary seed fermentation broth was cultured for 12 h with the same culture conditions as the previous step to obtain the secondary seed fermentation broth of T23. The secondary seed fermentation broth of T23 was then fermented in solid-state fermentation with a 5 % inoculation amount at a culture condition of 30℃ for 96 h to obtain the final fermentation product of T23 with a concentration of 10^8 CFU/g. To obtain 10^8 CFU/g of T23, the active bacterial solution was diluted in a gradient of 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 times, then taken 100 μL of the 10^6, 10^7 and 10^8 diluted solution, and cultured on the solid culture medium, incubated the plate at 30^0 C incubator for bacterial growth. Finally, count the number of colonies on the plate and we use the colony count and the dilution factor to calculate the final concentration. The composition of the solid medium was a mixture of soybean meal (60 %) and rice bran (40 %) with 60 % water, 3.6 % glucose, 2.4 % yeast extract powder, and 0.06 % manganese sulfate. For this experiment, we used 8×10^8 cell/g of product density of T23.

Five levels of T23 supplemented diets were prepared by mixing 0.00, 0.05, 0.1, 0.2 and 0.3 g/kg of the T23 fermentation product to the basal diet and groups were labelled as Control, 0.05T23, 0.1T23, 0.2T23, and 0.3T23, respectively. Table 1 and Table 2 show the formulation and proximate compositions of the experimental diets.

2.2. Fish rearing conditions and growth parameters measurements

All experimental and animal care procedures were performed following the procedures of the Feed Research Institute of CAAS chaired by the China Council for Animal Care (Assurance No. 2021-AF-FRI-CAAS-001). Three hundred, one month-old tilapia (GIFT) were selected and divided into 5 groups at random, with three replications and with a density of 20 fish per tank. Tilapia were fed with one of the five diets for 4 weeks. The fish were fed three times a day (10:00, 15:00 and 20:00) with 5 % and 10 % of their body weight for first and last 2 weeks, respectively and cultured in a recirculating system with water temperature of 27.5℃, pH 7.0–7.5, dissolved oxygen $>$ 6.5 mg/L, ammonia nitrogen $<$ 0.02 mg/L, nitrite $<$ 0.005 mg/L, and a 12 h/12 h light /dark cycle during the feeding period.

After 4 weeks of the feeding trial, tilapia were weighed and sampled after 24 h of fasting. Meantime, the survival rate (SR), feed conversion ratio (FCR) and weight gain (WG) were calculated according to the following formula:

SR (%) = (the final number of tilapia /the initial number of tilapia) $\times$ 100

FCR = feed intake (g) /total weight gain of tilapia (g)

WG (%) = [(average final weight-average initial weight)/ average initial weight] $\times$ 100

Condition factor (CF), viscerosomatic index (VI), hepatosomatic index (HI) and gut index (GI) were calculated according to the previous work (Meng et al., 2023).

2.3. Hematoxylin and Eosin (H&E) staining

After feeding for 4 weeks, the fish were fasted for 24 h and the intact liver, and hindgut tissues were taken out using ophthalmic forceps and

Table 1
The dietary formulation of feeds for Nile tilapia.

Ingredient (g/kg diet)	Control	0.05 T23	0.1 T23	0.2 T23	0.3 T23
Rice bran	100.00	100.00	100.00	100.00	100.0
Wheat Flour	200.00	200.00	200.00	200.00	200.0
Soybean meal	200.00	200.00	200.00	200.00	200.0
Rapeseed meal	90.00	90.00	90.00	90.00	90.0
Fish meal	80.00	80.00	80.00	80.00	80.0
Poultry meal	120.00	120.00	120.00	120.00	120.0
DDGS	100.00	100.00	100.00	100.00	100.0
T23 fermentation product	0.00	0.05	0.10	0.20	0.30
Bentonite	45.00	44.95	44.90	44.80	44.70
Lys-HCl	2.00	2.00	2.00	2.00	2.00
Methionine	0.50	0.50	0.50	0.50	0.50
Choline chloride	2.00	2.00	2.00	2.00	2.00
Monocalcium phosphate	20.00	20.00	20.00	20.00	20.00
Soybean oil	30.00	30.00	30.00	30.00	30.00
VC phosphate	0.50	0.50	0.50	0.50	0.50
Vitamin premix[a]	5.00	5.00	5.00	5.00	5.00
Mineral premix[b]	5.00	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Crude protein (%, dry matter)	36.60	36.68	36.31	36.66	36.65
Crude fat (%, dry matter)	11.34	11.48	11.65	11.54	11.26
Crude ash (%, dry matter)	19.57	19.48	19.38	19.86	19.61
Moisture (%)	3.49	3.95	3.71	3.54	3.51

[a] Containing the following (g/kg vitamin premix): 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; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; dl-α-tocopherol-acetate, 12.632.

[b] Containing the following (g/kg mineral premix): $CoCl_2{\cdot}6H_2O$, 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.

Table 2
The proximate composition of ingredients of basis diet for Nile tilapia.

Proximate composition	Moisture	CA	CF	CP
Rice bran	12.58	6.63	15.32	12.80
Wheat Flour	13.49	0.96	1.74	15.37
Soybean meal	9.35	6.20	0.76	43.12
Rapeseed meal	11.48	6.43	2.34	33.22
Fish meal	7.49	18.02	10.37	66.42
Poultry meal	4.60	17.01	9.97	65.55
DDGS	9.87	4.74	10.74	26.61
T23 fermentation product	5.75	6.61	1.47	41.05

CP: Crude protein (%, WT); CF: Crude fat (%, WT); CA: Crude ash (%, WT); Moisture (%).

dehydrated in 4 % paraformaldehyde. H&E staining of the hindgut and liver was performed following the previous method (Zhang et al., 2019). The slides were scanned using an automatic digital pathology biopsy scanner (Kfbio, China), and K-Viewer (Kfbio, Ningbo) software was used for image analysis.

2.4. Triacylglycerols assay

The liver tissue from 2 tilapia was weighed and pooled as one sample. A total of 6 replicates were collected per treatment. The liver samples were then homogenized in 1 mL phosphate buffer solution (PBS). Then the homogenate vortexed with 4 mL methanol chloroform solution (chloroform: methanol =2:1) 4 times and centrifuged at 92 g for 10 min to separate the phases. Aspirated the lower organic phase and then dried in the metal bath with nitrogen gas at 70°C to obtain the Triacylglycerols (TAGs), the dried TAGs were emulsified in 1 mL chloroform containing 1 % Triton X-100 at 70°C with nitrogen gas, the dried TAGs were reconstituted in 200 μL distilled water. And then the TAGs levels were quantified using free glucose reagent and triglyceride reagent (Sigma Aldrich, Darmstadt, Germany). The content of TAGs was expressed as mg/g liver tissue.

2.5. Liver antioxidants activity detection

The liver tissues were collected from 6 fish per treatment. The samples were homogenized with 1 mL precooled PBS, then centrifuged with 13201 g at 4℃ for 10 min and then the supernatants were collected. Total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), and total protein were measured using commercial assay kits (Beyotime, Shanghai, China) according to the manufacturer's instructions, respectively. Absorbance was read by microplate reader (Bio Tek, United States). Results were given as the activity unit per liver protein weight (U /mg protein).

2.6. Quantitative real-time PCR analysis (q-PCR)

Hindgut and liver tissues were collected for total RNA isolation using Trizol reagent (Genstar, Beijing, China) and reversed transcribed to cDNA using FastKing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China) as previously described (Hao et al., 2021). The cDNA was used as a template to perform the *q*-PCR on Roche Light Cycler 480 (Roche, Basel, Switzerland) with SYBR Green SuperReal PreMix Plus (Tiangen, Beijing, China) according to the manufacturer's instructions. Lists of primer sequences used for *q*PCR were presented in Table 3. *Beta actin* (*β-actin*) was used as a reference gene and gene expression results were analyzed by $2^{-\Delta\Delta CT}$ method.

2.7. The 16S rRNA gene sequencing

The hindgut contents from 3 tilapia were collected 4 h after the last feeding, and mixed as 1 sample and 6 replicates per treatment were used

Table 3
Lists of primer sequences used for qPCR.

Gene name	Nucleotide sequence of primers (5'–3')	GenBank accession No.
β-actin	F: CAGCAAGCAGGAGTACGATGAGTC R: GTATGAGAAATGTGTGGTGTGTGGTTG	XM_003444484
cat	F: TCCTGAATGAGGAGGAGCGA R: ATCTTAGATGAGGCGGTGATG	XM_019361816
cpt1a	F: GAACTGTCCTCAAGTCCTTGCTGTC R: CAGAGAACACCTTCACTAGAACCATCC	XM_003440354
claudin	F: GTCTGTTTCTGGGCGTGGTGTC R: ACTCCGACTGACTCCTCATCTTCC	XM_019367708
fas	F: GTGTTCGCTCTGTGTTGGAGTCTG R: CGTCCTGGCTCTTCACCTTGTTG	XM_003454056
hif1α	F: AAGCAGACCGCAGATGTGAAGC R: TCCTCCTTCTCCAGTTCAGCCTTC	XM_005477039
il−10	F: GCTTCCCCGTCAGGCTCAA R: CTGTCGGCAGAACCGTGTC	XM_013269189
il−1β	F: ACAAGGATGACGACAAGCCAACC R: GGACAGACATGAGAGTGCTGATGC	XM_019365844
keap1	F: CTTCGCCATCATGAACGAGC R: CACCAACTCCATACCGCACT	XM_003447926
nrf2	F: CTGCCGTAAACGCAAGATGG R: ATCCGTTGACTGCTGAAGGG	XM_003447296
occludin	F: GGAGGAAAGCCGCAGTGTTCAG R: GTCGTAGGCATCGTCATTGTAGGAG	XM_025899615
pparα	F: TGACTCTCTTGAAGTACGGTGTTTACG R: TCGGCGGAGACTCTTGAGGAAC	XM_019346353
sod	F: GGTGCCCTGGAGCCCTA R: ATGCGAAGTCTTCCACTGTC	XM_003449940
tgf-β	F: GCCCATCAGCTCACCTACAAATCC R: ATGACCGAAGAGGAGGAAGAGGAAG	XM_005457932
tnf-α	F: GAGGCCAATAAAATCATCATCCC R: CTTCCCATAGACTCTGAGTAGCG	NM_001279533

for gut microbiota analysis. The 16S rRNA V3–V4 region was amplified using primer pairs of 338 F (5'—ACTCCTACGGGAGGCAGCAG—3') and 806 R (5'—GGACTACHVGGGTWTCTAAT—3'). Purified amplicons were pooled in equimolar amounts and pair-end sequenced on an Illumina MiSeq PE300 platform /NovaSeq PE250 platform (Illumina, San Diego, United States) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Data analysis including alpha diversity, beta diversity and community composition analysis was carried out as described previously (Hao et al., 2021).

2.8. Statistical analysis

GraphPad Prism version 8.0 and SPSS Statistics version 21 were used for drawing the graphs and data analysis, respectively. Normality and homoscedasticity assumptions were confirmed before any statistical analysis was performed. One-way analysis of variance (ANOVA) followed by Turkey's test was used for the comparisons among multiple groups. Differences were considered significant at $p < 0.05$, the results were presented as mean ± standard error means (Mean ± SEMs). Furthermore, a follow-up trend analysis using orthogonal polynomial contrasts was performed to determine if the effect was linear and/or quadratic (Wei et al., 2019; Yossa and Verdegem, 2015).

3. Results

3.1. Effects of B. velezensis T23 on the growth performance of GIFT tilapia

As shown in the results in Table 4, after 4 weeks of the feeding trial, there were no significant effects on the SR, WG and FCR of the fish among the treatment groups. In addition, the condition factor, viscerosomatic, hepatic and gut indexes were similar among the groups.

3.2. B. velezensis T23 alleviated liver lipid metabolism disorder and inflammation in GIFT tilapia

In the control, 0.05T23, and 0.1T23 groups, more inflammatory infiltrate cells and vacuolization were observed, whereas in the 0.2 and 0.3T23 groups, the morphology of the liver was arranged regularly without obvious morphological changes (Fig. 1A, B). T23 supplementation also alleviated hepatic steatosis of tilapia. In addition, the liver TAGs content was significantly decreased in 0.1, 0.2 and 0.3T23 groups, compared with 0.05T23 and the control groups (Table 4). Liver mRNA expression of genes involved in lipid metabolism showed that T23 supplementation reduced the expression of the lipogenesis-related gene, fatty acid synthase (*fas*) in 0.1, 0.2 and 0.3T23 groups, while the expressions of lipolysis-related genes, (carnitine palmitoyltransferase 1 alpha (*cpt1a*) and peroxisome proliferator-activated receptor alpha (*ppar*α) were significantly increased in 0.2 and 0.3T23 groups (Fig. 3A). In addition, supplementation of 0.3T23 resulted in an up-regulation of the relative mRNA expression of anti-inflammatory cytokine genes, transforming growth factor beta (*tgf-*β) and interleukin 10 (*il-10*), compared with the control group (Fig. 3B; $p < 0.05$).

3.3. B. velezensis T23 improved liver antioxidant capacity in GIFT tilapia

Compared to the control, liver T-AOC showed an increased trend in T23 treatment groups, and liver SOD activity was significantly increased in 0.2 and 0.3T23 groups (Table 4; $p < 0.05$). Markedly higher CAT activity was detected in all T23 groups, compared with the control group (Table 5; $P < 0.05$). Moreover, supplementation of 0.2 and 0.3 g/kg T23 also significantly reduced the relative mRNA levels of kelch-like ECH-associated protein 1 (*keap1*) which is the negative regulatory factor of nuclear factor erythroid 2-related factor 2 (*nrf2*) ($p < 0.05$). Furthermore, the mRNA relative level of antioxidant gene catalase (*cat*) was significantly upregulated in 0.3 T23 group, compared to the control (Fig. 3C; $p < 0.05$).

3.4. B. velezensis T23 promoted intestinal structural integrity, barrier function and immune response of GIFT tilapia

In the intestine of fish fed with the control diet, there was vacuolar degeneration of intestinal epithelial cells (IECs) manifested as swelling and redness of the intestinal lining and formation of lesions in the intestine. Furthermore, disorganized microvilli and goblet cells were observed in the control group. Adding T23 at levels of 0.2 and 0.3T23 significantly reduced the number of goblet cells and infiltration of inflammatory cells in the hindgut of tilapia and markedly increased the height and the number of intestinal villi, compared with the 0.05T23 and the control groups (Fig. 4; $p < 0.05$). The relative mRNA level of hypoxia-inducible factor-1 alpha (*hif1*α), and *occludin* genes showed an upward trend in 0.2 and 0.3T23 groups, compared to the control group (Fig. 5A). Significant upregulation of *claudin* gene expression level was observed in 0.3T23 group, compared with the control group (Fig. 5A). The relative expression of the pro-inflammatory gene interleukin 1 beta (*il-1*β) in 0.3 T23 group was noticeably downregulated, while the expression of the anti-inflammatory gene *il-10* was conspicuously upregulated, compared with the control group (Fig. 5B).

3.5. B. velezensis T23 improved the gut microbiota profile of GIFT tilapia

As shown in Table 6 and Fig. 6A, data on gut microbiota structure

Table 4
Growth parameters and survival rate of Nile tilapia fed with different level of T23.

Parameters	Control	0.05 T23	0.1 T23	0.2 T23	0.3 T23	Pr>F[a]		
						A	LT	QT
SR	100.00	100.00	100.00	100.00	100.00	——	——	——
IBW	1.65 ± 0.01	1.65 ± 0.01	1.66 ± 0.01	1.66 ± 0.01	1.65 ± 0.01	0.139	0.040	0.142
FBW	4.71 ± 0.04	4.87 ± 0.16	4.88 ± 0.18	4.83 ± 0.008	4.71 ± 0.145	0.487	0.892	0.087
WG	185.76 ± 3.06	195.37 ± 10.04	194.06 ± 11.43	191.51 ± 0.73	184.68 ± 9.32	0.607	0.747	0.144
FCR	1.13 ± 0.02	1.08 ± 0.05	1.09 ± 0.06	1.1 ± 0.007	1.14 ± 0.06	0.595	0.633	0.145
FI	3.46 ± 0.01	3.46 ± 0.01	3.48 ± 0.01	3.48 ± 0.01	3.47 ± 0.01	0.139	0.108	0.097
CF	3.48 ± 0.22	3.59 ± 0.35	3.29 ± 0.13	3.34 ± 0.12	3.29 ± 0.30	0.187	0.078	0.840
VI	13.62 ± 0.29	14.09 ± 2.43	12.26 ± 4.39	13.83 ± 1.75	12.68 ± 2.62	0.745	0.571	0.963
HI	4.00 ± 1.09	4.70 ± 1.67	3.81 ± 1.12	3.85 ± 1.18	3.44 ± 1.29	0.657	0.340	0.623
GI	5.55 ± 1.62	4.71 ± 1.09	4.16 ± 1.84	4.05 ± 0.77	4.05 ± 0.82	0.290	0.067	0.262

SR: Survival rate, 100 %, IBW: Initial body weight, g; FBW: Final body weight, g; WG: Weight gain, %; FCR: Feed conversion rate; FI: Feed Intake; CF: Condition factor, 100 ×g /cm^3; VI: Viscerosomatic Index, %; HI: Hepatic Index, %; GI: Gut Index, %; Values are presented as mean ± SEMs (SR, IBW, FBW, WG: n = 3 biological replicates; Others: n = 6 biological replicates). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

[a] Significance probability associated with the F-statistic.

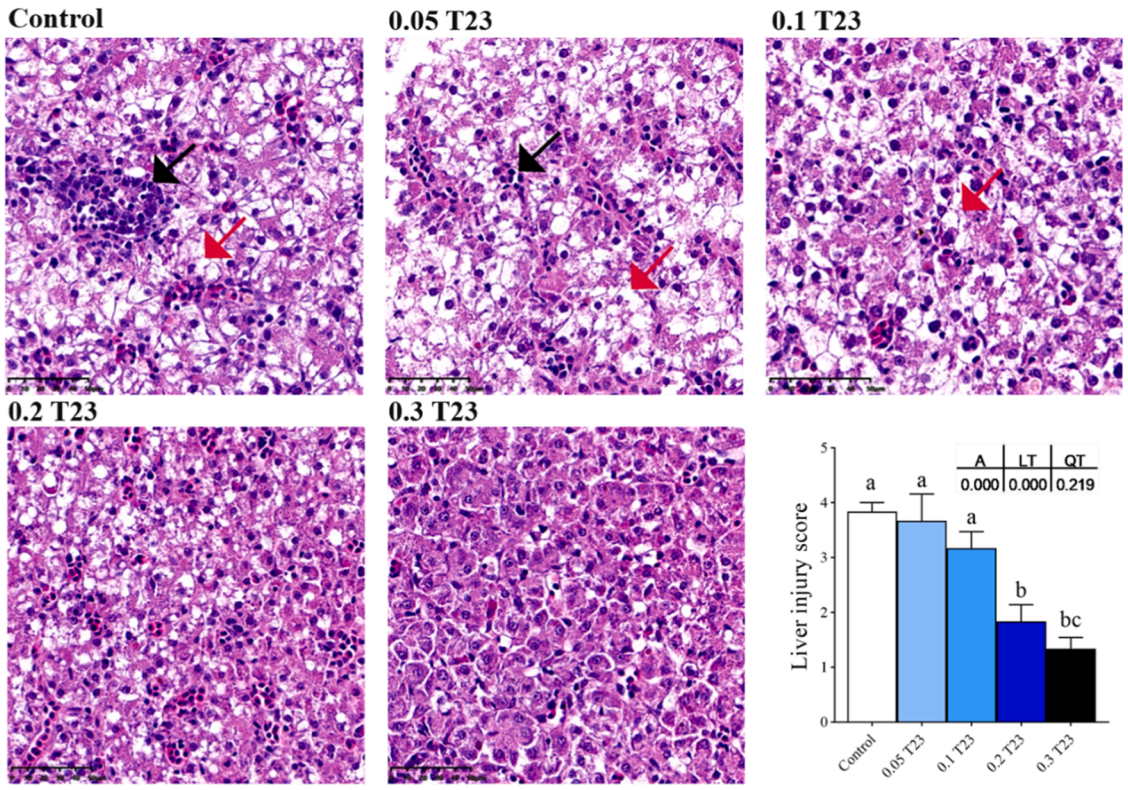

Fig. 1. Effects of T23 on (A) liver morphology (H&E staining) and (B), liver injury score in tilapia. Black arrow: inflammatory infiltrate. Red arrow: lipid vacuoles. The scale bar is 40 μm. Different letter on the different columns indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

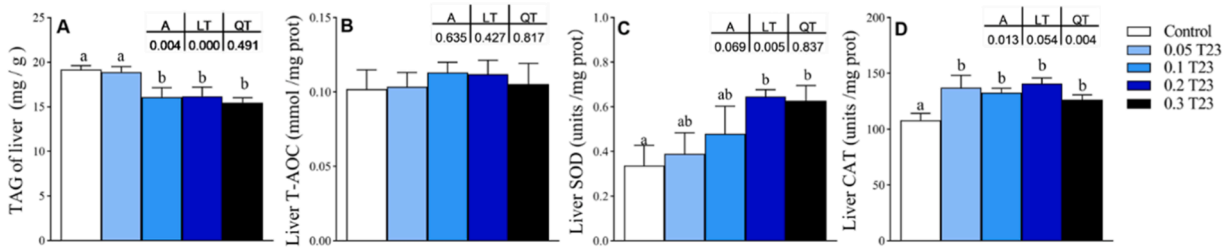

Fig. 2. Effects of T23 on liver TAGs content and liver antioxidant capacity of Nile tilapia, A: TAGs of liver, mg /g; B: Liver Total Antioxidant capacity, mmol /mg prot; C: Liver Total SOD activity, units /mg prot; D: Liver CAT activity, units /mg prot. Values are presented as mean ± SEMs (n = 6 biological replicates). Different letter on the different columns indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

showed that at the phylum level, Firmicutes and Proteobacteria were the most dominant bacteria phyla in all groups. The relative abundance of Firmicutes was remarkably increased in 0.2T23 group compared with the control group ($p < 0.05$). Higher relative abundance of phylum Bacteroidota was detected in the 0.3T23 group, compared with the other groups. At the genus level, the relative abundances of *Lactobacillus, unclassified_Bacilli* and *Pantoea* were significantly increased in 0.1 and 0.2T23 groups, compared with the control. Genera *Staphylococcus* and *Kosakonia* also increased markedly in 0.05, 0.1 and 0.2T23 groups, compared with the control group. The relative abundance of *Rhodobacteraceae* was markedly decreased in T23 groups, compared with the control group (Table 7, Fig. 6B). Furthermore, compared with the control, the ratio of (Firmicutes +Bacteroidota +Fusobacteria) /Proteobacteria showed a significantly increased in 0.2T23 group (Fig. 6C). Alpha diversity results showed that lower ACE and Chao1 indexes were recorded in 0.3T23 group, compared with the other T23 groups. Shannon and Simpson indexes were notably decreased in the 0.3T23 group, compared with the other groups (Table 8). Moreover, PCoA showed a robust separation between the microbiota of the control group and 0.1, 0.2 and 0.3T23 groups (Fig. 6D).

4. Discussion

It has been shown to cause several hepatic damages such as excessive lipid accumulation, inflammation and oxidative stress in numerous farmed aquatic species with intensive aquaculture development (Guo et al., 2022; Jia et al., 2020a, 2020b; Zhou et al., 2020). Studies indicated that supplementation of fermented *Saccharomyces cerevisiae* in Nile tilapia promoted the growth performance of the fish (Abu-Elala et al., 2020). In the present study, solid-state-fermentation *B. velezensis* T23 have no effect on the growth performance of Nile tilapia. In agreement with this result, supplementation of *L. acidophilus* and *L. plantarum* to the diet of marron (*Cherax cainii*) has no positive effect on the growth performance of the fish (Foysal et al., 2020). Additionally, no

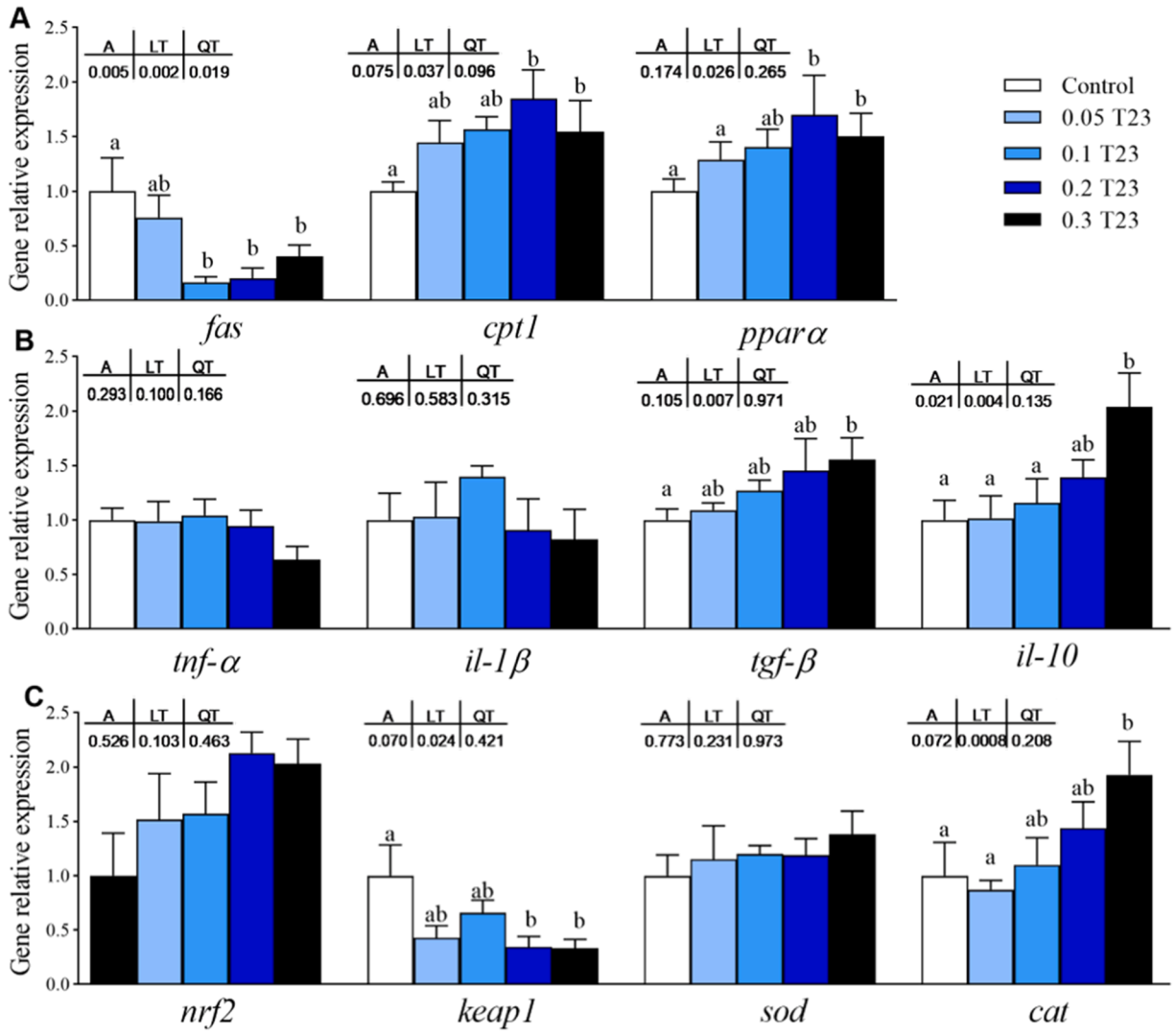

Fig. 3. Effects of T23 on the mRNA relative expressions on tilapia liver. A: Liver lipid metabolism related genes, B: Liver inflammation related genes, C: Liver antioxidant-related genes. Values are presented as mean ± SEMs (n = 6 biological replicates). Different letter on the different columns indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

Table 5
Effects of T23 on liver TAGs content and liver antioxidant capacity of Nile tilapia.

Parameters	Control	0.05 T23	0.1 T23	0.2 T23	0.3 T23	Pr>F[a]		
						A	LT	QT
TAGs	19.18 ± 0.43	18.81 ± 0.60	16.10 ± 1.05	16.19 ± 1.01	14.48 ± 0.55	0.004	0.000	0.491
T-AOC	0.10 ± 0.01	0.09 ± 0.01	0.11 ± 0.02	0.12 ± 0.02	0.10 ± 0.01	0.635	0.427	0.817
SOD	0.34 ± 0.09	0.39 ± 0.10	0.48 ± 0.12	0.65 ± 0.03	0.63 ± 0.07	0.669	0.005	0.837
CAT	108.08 ± 6.22	137.21 ± 11.05	132.70 ± 3.91	140.90 ± 5.07	126.46 ± 4.26	0.013	0.054	0.004

TAGs: Triglyceride, mg/g liver tissue; T-AOC: Total Antioxidant capacity, mmol /mg prot; SOD: Superoxide dismutase, units/mg prot; CAT: Catalase, units/mg prot; A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

[a] Significance probability associated with the F-statistic.

marked changes were observed on the FBW, WG and FCR of farmed tilapia after supplementation of fermented product of *L. rhamnosus* GCC-3 and fermented products of *Cetobacterium somerae* XMX-1 (Zou et al., 2022). Supplementation of solid-state fermentation product of yeast to zebrafish diet reduced the WG of the fish fed with high fat-diet (Li et al., 2024). These different results on the growth performance of aquatic species may be due to the inclusion level of probiotics, type of strain and their different efficacies levels, and host species metabolism efficiencies.

The excess fat in most fish species is deposited as TAGs (Kawano and Cohen, 2013). It has been demonstrated that hepatic TAGs content is caused by up-regulated the relative mRNA expression of lipogenesis-related genes in many aquatic species (Jin et al., 2019; Tang et al., 2019; Zhang et al., 2022). Studies have found that *Bacillus* sp., was

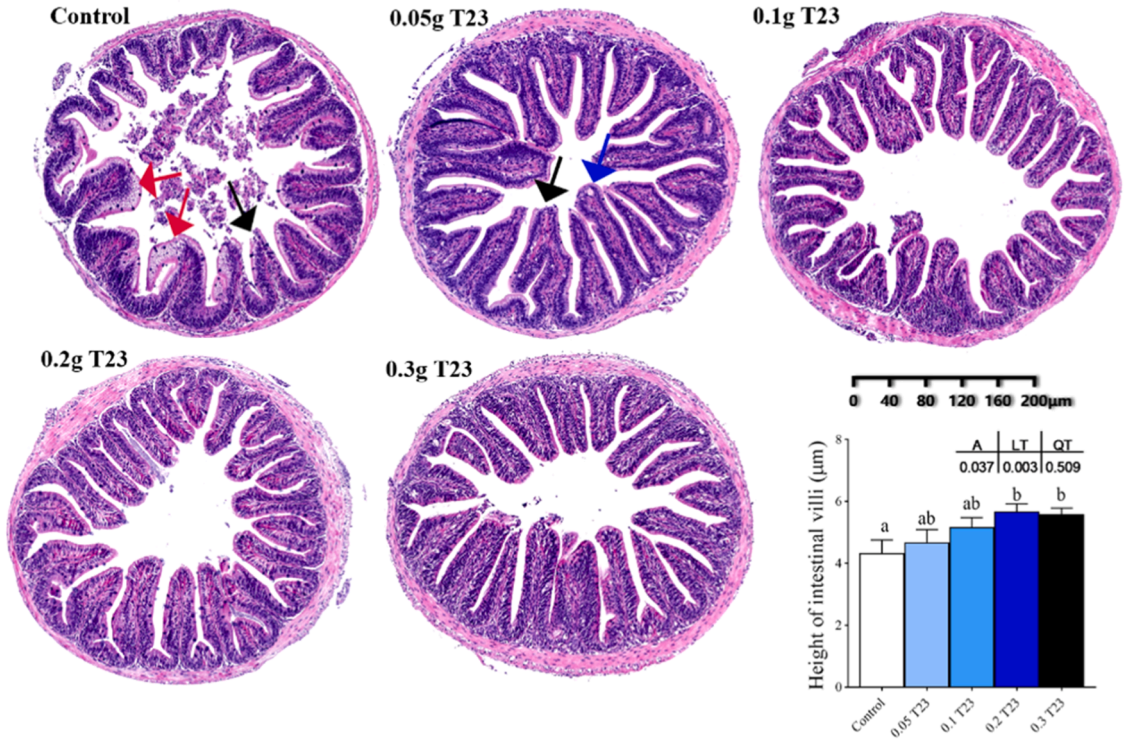

Fig. 4. Effects of T23 on intestinal morphology (H&E staining) and intestinal villi height of tilapia. Black arrow: disorganized microvilli. Red arrow: vacuolar degeneration of IECs.Blue arrow: goblet cell. The scale bar is 40 μm. Values are presented as mean ± SEMs (n = 6 biological replicates). Different letter on the different columns indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

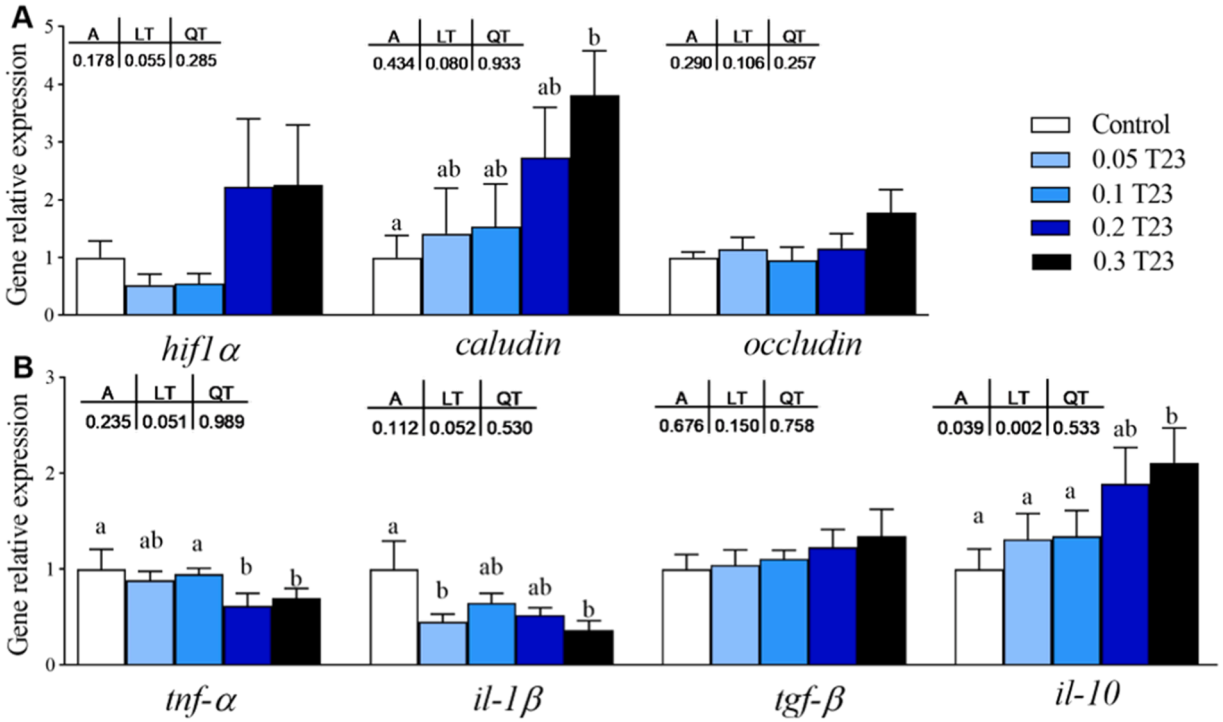

Fig. 5. Effects of T23 on the mRNA relative expression genes related to intestinal integrity, barrier and immune responses of Nile tilapia. A: *hif1α* and tight junction protein genes; B: gut inflammation related genes; Values are presented as mean ± SEMs (n = 6 biological replicates). Different letter on the different columns indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

Table 6
The relative abundance of gut bacteria of Nile tilapia at phylum level (%).

Phylum	Control	0.05 T23	0.1 T23	0.2 T23	0.3 T23	Pr>F[a]		
						A	LT	QT
Firmicutes	34.89 ± 7.99[a]	45.41 ± 13.77[ab]	48.01 ± 13.99[ab]	49.84 ± 11.89[b]	37.01 ± 4.75[ab]	0.099	0.550	0.010
Proteobacteria	37.88 ± 7.23	30.67 ± 7.33	31.56 ± 9.41	29.17 ± 4.57	32.98 ± 3.37	0.241	0.206	0.080
Bacteroidota	8.85 ± 2.84[ab]	8.15 ± 5.05[ab]	6.37 ± 4.27[a]	6.69 ± 4.45[ab]	11.43 ± 0.79[b]	0.181	0.457	0.035
Actinobacteriota	4.89 ± 0.31	5.45 ± 1.11	5.62 ± 1.05	5.87 ± 0.75	5.02 ± 0.22	0.190	0.501	0.030
unclassified	2.74 ± 0.79	3.04 ± 0.52	3.03 ± 0.63	2.96 ± 0.33	3.44 ± 0.70	0.424	0.109	0.737
Cyanobacteria	1.93 ± 0.51	1.64 ± 1.32	1.11 ± 0.83	1.22 ± 0.86	1.45 ± 0.45	0.493	0.226	0.215
Fusobacteriota	1.19 ± 0.45	1.02 ± 0.83	0.88 ± 0.80	0.79 ± 0.68	1.59 ± 0.15	0.246	0.498	0.053
Other	7.43 ± 2.41[a]	4.46 ± 3.32[ab]	3.28 ± 2.87[b]	3.28 ± 2.53[b]	6.83 ± 0.96[a]	0.019	0.472	0.001
Unknown	0.16 ± 0.06	0.15 ± 0.12	0.12 ± 0.13	0.14 ± 0.12	0.22 ± 0.04	0.498	0.421	0.129

Values are presented as mean ± SEMs (n = 6 biological replicates). In the same line, values with different letter superscripts indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

[a] Significance probability associated with the F-statistic.

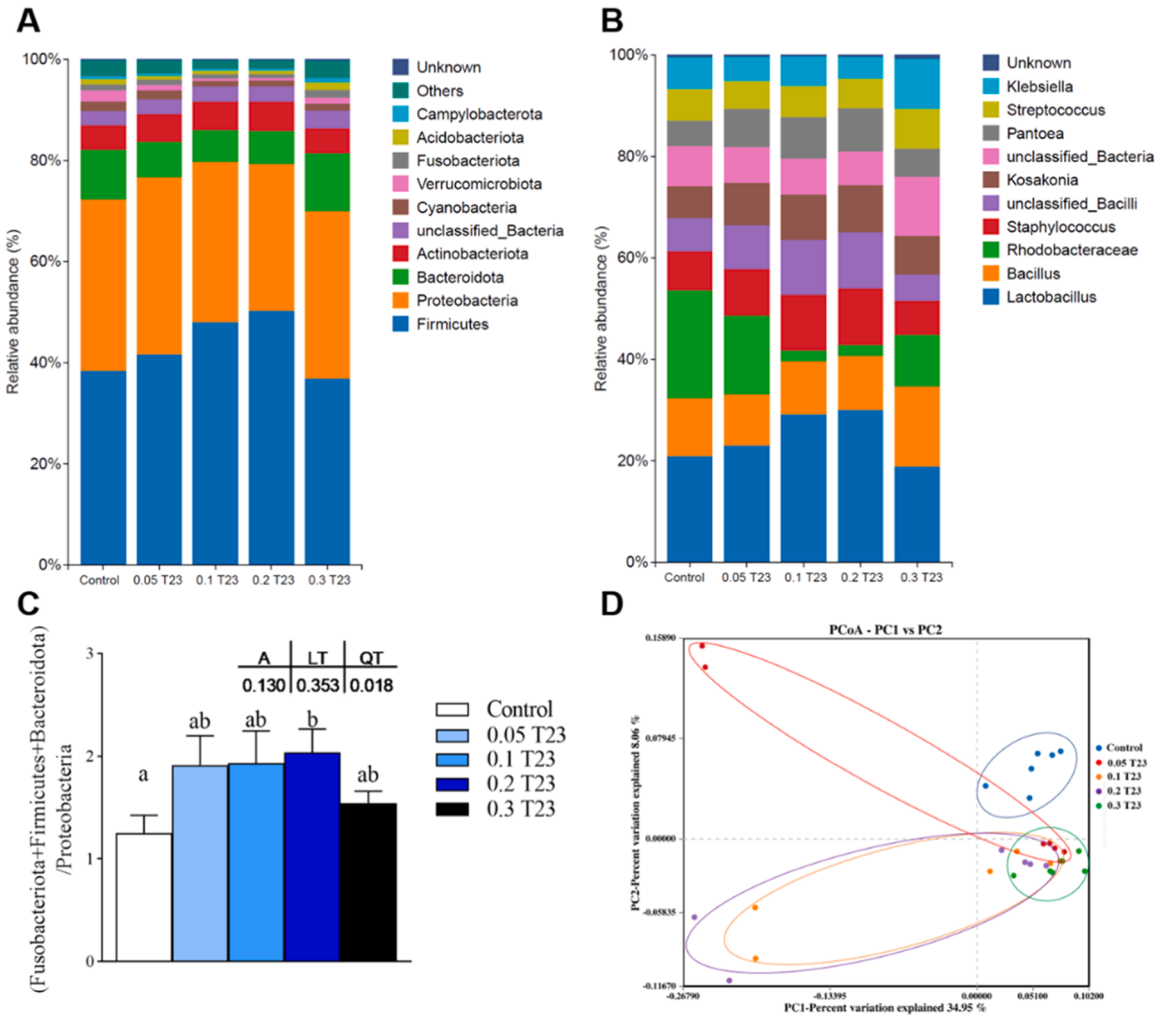

Fig. 6. Effects of T23 supplementation on gut microbial community of Nile tilapia. A: Relative abundance at the phylum level of gut bacterial community; B: Relative abundance at the genus level of gut Bacterial community; C: Ratio of "(Firmicutes+Bacteroidetes+Fusoacteria) /Protobacteria"; D: PCoA analysis of gut bacteria of Nile tilapia. Values are means ± SEMs (n = 6 biological replicates); Different letter on the different columns indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

improved liver lipid metabolism in grass carp (*Ctenopharyngodon idellus*) and zebrafish fed with high-fat diets (Wang et al., 2022; Zhao et al., 2019). In the present study, supplementation of solid state fermented product of T23 remarkably reduced liver TAGs content without affecting the growth of Nile tilapia and significantly reduced the hepatocyte vacuolization. Furthermore, supplementation of the fermented product of T23 decreased the expression of lipogenesis gene (*fas*) and increased lipolysis genes (*cpt* and *ppar*α), which indicated that the fermentation

Table 7
The relative abundance of the gut bacteria of Nile tilapia at genus level (%).

Genus	Control	0.05 T23	0.1 T23	0.2 T23	0.3 T23	Pr>F[a]		
						A	LT	QT
Lactobacillus	17.48 ± 6.12[a]	23.77 ± 7.90[ab]	27.08 ± 7.41[b]	28.34 ± 5.91[b]	18.70 ± 5.00[a]	0.027	0.416	0.002
Bacillus	11.52 ± 3.28[a]	11.49 ± 3.52[a]	11.55 ± 3.88[a]	11.86 ± 4.11[a]	15.77 ± 2.14[b]	0.171	0.058	0.137
Staphylococcus	6.37 ± 2.02[a]	9.32 ± 3.21[b]	10.26 ± 2.62[b]	10.45 ± 2.22[b]	6.59 ± 2.06[a]	0.014	0.624	0.001
unclassified_Bacilli	5.27 ± 2.31[a]	8.25 ± 4.42[ab]	9.61 ± 3.83[b]	10.01 ± 3.45[b]	5.09 ± 1.81[a]	0.038	0.747	0.003
Kosakonia	5.86 ± 1.04[a]	8.25 ± 2.31[b]	8.49 ± 1.90[b]	8.97 ± 1.52[b]	7.57 ± 1.01[ab]	0.027	0.062	0.007
Rhodobacteraceae	27.37 ± 17.60[a]	11.50 ± 14.09[b]	3.13 ± 4.55[b]	2.89 ± 2.99[b]	10.45 ± 9.79[b]	0.006	0.007	0.004
Pantoea	4.52 ± 1.24[a]	7.06 ± 3.22[ab]	7.41 ± 2.67[b]	7.78 ± 2.77[b]	5.49 ± 0.98[ab]	0.113	0.391	0.013
Others	21.08 ± 8.49	19.84 ± 6.32	22.04 ± 9.81	19.23 ± 7.78	29.54 ± 4.16	0.157	0.108	0.130
Unknown	0.50 ± 0.24	0.48 ± 0.40	0.42 ± 0.48	0.44 ± 0.42	0.77 ± 0.17	0.470	0.293	0.171

Values are presented as mean ± SEMs (n = 6 biological replicates). In the same line, values with different letter superscripts indicate significant differences ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

[a] Significance probability associated with the F-statistic.

Table 8
Alpha diversity indexes of gut bacteria of Nile tilapia.

Parameters	Control	0.05 T23	0.1 T23	0.2 T23	0.3 T23	Pr>F[a]		
						A	LT	QT
ACE	1168.37 ± 27.94[ab]	1192.69 ± 44.94[b]	1201.13 ± 30.64[b]	1200.87 ± 40.01[b]	1129.04 ± 74.55[a]	0.067	0.151	0.017
Chao 1	1202.94 ± 23.34[ab]	1236.88 ± 45.70[b]	1238.04 ± 26.32[b]	1221.86 ± 41.30[b]	1152.43 ± 92.12[a]	0.022	0.013	0.020
Shannon	7.84 ± 0.57[a]	8.19 ± 0.06[a]	7.97 ± 0.59[a]	7.89 ± 0.56[a]	6.76 ± 1.28[b]	0.022	0.016	0.015
Simpson	0.98 ± 0.01[a]	0.99 ± 0.001[a]	0.98 ± 0.01[a]	0.98 ± 0.01[a]	0.97 ± 0.02[b]	0.049	0.096	0.009

Values are presented as mean ± SEMs (n = 6 biological replicates). In the same line, values with different letter superscripts indicate significant differences between groups ($p < 0.05$). A: the variance analyzed by one-way ANOVA; LT: Linear trend analyzed by orthogonal polynomial contrasts; QT: Quadratic trend analyzed by orthogonal polynomial contrasts.

[a] Significance probability associated with the F-statistic.

product of T23 could alleviate liver fat accumulation by promoting β-oxidation of fatty acids and inhibiting of lipid synthesis. In agreement with this study, addition of *L. rhamnosus* GCC-3 fermentation product in the diet of farmed tilapia resulted in a marked increase in the expression of lipolysis genes (*cpt* and *pparα*) (Zou et al., 2022). A study revealed that excess fat accumulation has been established as an inducer of liver inflammation in teleosts (Li et al., 2021). As signaling molecules, inflammatory cytokines interact with immune cells and play a leading role in response to disease and infection (Ong et al., 2010). Among them, TNF-α, IL-6, and IL-1β are the key pro-inflammatory factors, while IL-10 and TGF-β are the most conventional anti-inflammatory factors, which regulate the inflammatory response (Yamamoto et al., 2013). Mitigating inflammation is also one of the main functions of probiotics and their products. In relation to this, supplementation of solid-state fermented product of yeast zebrafish fed with high-fat diet resulted in down-regulation of the inflammatory response (Li et al., 2024). Moreover, Wang et al. (2022) investigated the role of solid state fermented product of *B. subtilis* HGcc-1 in the immune response of zebrafish which showed a significant reduction in the expression of pro-inflammatory genes in zebrafish liver. In line with these results, the present study showed that dietary solid state fermented probiotics of T23 significantly up-regulated the expressions of *il-10* and *tgf-β* genes, which indicate that fermented product of T23 supplementation could mitigate the inflammatory response of Nile tilapia by increasing the expression of anti-inflammatory cytokines in the liver.

Oxidative stress is caused by the accumulation of excessive reactive oxygen species (ROS) (Ding et al., 2021). The antioxidant system is an indispensable mechanism for scavenging ROS and maintaining redox homeostasis (Birnie-Gauvin et al., 2017). Antioxidase enzymes such as CAT and SOD played a major role in eliminating ROS from the body (Meng et al., 2020). The liver is undoubtedly the most important organ to control the antioxidative processes (Zhu et al., 2012). Fermented product of probiotics has been shown to relieve oxidative stress in teleost induced by excessive lipid accumulation (Xie et al., 2022). Similarly, the results of the present study showed that the dietary fermented product of T23 improved the antioxidant capacity of the liver by increasing the activities of SOD and CAT enzymes. Besides, compared with the control group, the liver mRNA expression of antioxidant-related gene *nrf2* was increased, while *keap1* was notably decreased at high doses of T23. Nrf2 is an important protective transcription factor regulating antioxidant responses, and Keap1 is one of the negative regulators of Nrf2 (Ken Itoh and Yamamoto, 2010). The above results indicated that the dietary fermented product of T23 could effectively alleviate oxidative stress induced by excessive lipid accumulation in the liver of Nile tilapia by enhancing the antioxidant capacity, and the potential action mechanism for this physiological function could be the activation of Nrf2 signaling pathway.

Moreover, previous research results revealed that oxidative stress could impair gut health by damaging intestinal integrity, inducing inflammation and dysbiosis of gut microbiota in aquatic species (Gatesoupe et al., 2018; Wang et al., 2021; Yu et al., 2020). Intestinal tight junction proteins, which include, *Claudin* and *Occludin*, are important proteins for the regulation of the permeability and barrier functionality of the gut (Wang et al., 2022). In the current study, the fermented product of T23 significantly increased the height of villi. In addition, the mRNA expression of *occludin* gene was notably increased in the 0.3T23 group. In consistence with the present results, Wang et al. (2022) reported that supplementation of *B. subtilis* HGcc-1 in the diet of zebrafish significantly up-regulated the expression of *claudin* and *occludin* genes. *B. licheniformis* increased the expression of *zonula occludens-1* (*zo-1*) and *occludin* in grass carp (Qin et al., 2020). Hypoxia-inducible factors 1α (HIF1α) play a key role in intestinal barrier protection (Fachi et al., 2019). Consistent with previous results (Robrahn et al., 2020), in the present study the mRNA relative expression of *hif1α* was increased in 0.2 and 0.3 T23 groups. A plethora of studies regarding teleost immunity demonstrated that intestinal integrity is beneficial for intestinal immune responses (Thoo et al., 2019; Turner, 2009). In this study, the mRNA expression of gut

pro-inflammatory cytokine *il-1β* was decreased while the expression of anti-inflammatory cytokine *il-10* was noteworthy increased in the intestine of tilapia fed with diet amended with the fermented product of 0.3 T23. In line with our results, dietary *B. subtilis* DSM 32315 has been reported the same beneficial effects on largemouth bass (Du et al., 2021). All our results indicated that the addition of fermented product of T23 to the diet of Nile tilapia improved the intestinal barrier function and immune responses which play a great role in fish physiological functions.

Gut microbiota has been reported to affect the growth, metabolism, as well as immune responses of the host (Butt and Volkoff, 2019; Morais et al., 2021). There are a plethora of evidence that demonstrated that gut microbiota imbalance affects gut homeostasis in several aquatic species (Peng et al., 2019; Zou et al., 2018). Adding exogenous probiotics to feed is an effective strategy for maintaining the balance of gut microbiota (Hemarajata and Versalovic, 2013). Tachibana et al., (2020) reveale that dietary *B. subtilis* and *B. licheniformis* increased the proportion of Firmicutes in the gut microbiota of Nile tilapia. Besides, fermented products of probiotics were also played a remarkable role in modulating the gut microbiota of aquatic animals positively (Li et al., 2024; Wang et al., 2022). Firmicutes and Proteobacteria were the dominant phyla in the gut microbiota of several fish species (Bereded et al., 2020; Foysal., 2020). In line with these results, our gut microbiota data also demonstrated that supplementation of the fermented product of T23 significantly altered the gut microbiota composition with an increase in the relative abundance of Firmicutes at the phylum level. Firmicutes and Bacteroidota are typically dominant phyla in the gut of humans and other mammals (Arumugam et al., 2011; Mahowald et al., 2009; Wu et al., 2022). By contrast, the fish gut microbiota is dominated by phylum Proteobacteria, which is known as containing several pathogenic or opportunistic pathogenic genera/species and are normally considered as negative components of the symbiotic microbiome (Egerton et al., 2018; Llewellyn et al., 2014; Li et al., 2019). Thereby the ratio of Functional Group 2/Functional Group 1 ((Firmicutes +Bacteroidetes +Fusoacteria) /Proteobacteria) was constructed to evaluate the structural and functional characteristics of the microbiota with a higher ratio were positively correlated with better health (Li et al., 2024). Of note, in the current study, the ratio of Functional Group 2 /Functional Group 1 showed a remarkable increase in 0.2T23 group, compared with the control which indicated that supplementation of 0.2T23 positively improved the gut microbiota structure of Nile tilapia. The relative abundance of *Lactobacillus* genus was markedly increased in 0.1, and 0.2T23 groups, compared with the control group. Similar to this result, Atlantic salmon fed with a diet supplemented with Lactic acid bacteria revealed a higher relative abundance of genus *Lactobacillus* (Gupta et al., 2019). It has been reported that *Lactobacillus* played a significant role in enhancing the immune response of several fish species, inducing grouper (*Epinephelus coioides*), cobia (*Rachycentron canadum*), roho labeo (*Labeo rohita*), Nile tilapia and zebrafish (Son et al., 2009; Geng et al., 2012; Giri et al., 2014; Nwanna and Bamidele, 2014; He et al., 2017). Nile tilapia fed with a diet amended with probiotic bacteria *L. plantarum* showed a relatively high abundance of *Lactobacillus* in the gut of the fish (Foysal et al., 2020). Additionally, the relative abundance of *Pantoea* was increased significantly in the T23 supplemented groups. *Pantoea* is a Gram-negative bacterium and many strains of this genus, such as *Pantoea agglomerans*, are beneficial to fish health by improving the immune responses and gut morphology (Amenyogbe et al., 2022).

ACE and Chao1 Indexes reflect species abundance while Shannon and Simpson indexes are used to measure species diversity. The present results indicated that supplementation of 0.5T23 decreased the species abundance and diversity in the gut microbiota of Nile tilapia, compared with both the control and the low-level T23 groups. PCoA analysis indicated that the diversity of gut microbiota in T23 groups was significantly different from the control group. In sum, these results suggested that low levels (0.05, 0.1 and 0.2) of the fermented product of T23 promoted the abundance of beneficial bacteria, thereby modifying the gut microbiota homeostasis of Nile tilapia positively.

5. Conclusion

Collectively, we evaluated the probiotic effects of solid state fermented product of autochthonous *B. velezensis* T23 in GIFT tilapia growth performance, liver and intestine health, and gut microbiota profile. The results indicated that solid state fermentation product of dietary T23 was effectively alleviated liver inflammation and oxidative stress induced by excessive lipid accumulation and played a significant role in improving the structural integrity, intestinal barrier, and immune responses of the intestine of the fish. Furthermore, T23 improved the gut microbiota structure and diversity of Nile tilapia by promoting the growth of beneficial intestinal bacteria and hindering the growth of potentially harmful bacteria species. On the other hand, an excessive solid state fermentation product of T23 supplementation in the diet of tilapia reduced the abundance and diversity of gut bacteria and altered negatively. Based on the results supplementation of 0.2 g/kg of T23 to the basal diet could be the appropriate dose for tilapia liver and gut health and gut microbiota homeostasis which boosts the production and profitability of Nile tilapia farming.

CRediT authorship contribution statement

Zhen Zhang: Validation, Resources, Methodology, Conceptualization. **Zhigang Zhou:** Supervision, Project administration, Funding acquisition, Conceptualization. **Chao Ran:** Resources, Conceptualization. **Yalin Yang:** Resources, Conceptualization. **Qianwen Ding:** Methodology, Data curation. **Yuanyuan Yao:** Visualization, Conceptualization. **Einar Ringø:** Supervision, Conceptualization. **Tsegay Teame:** Writing – review & editing. **Qiang Hao:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Rolf Erik Olsen:** Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Data availability

Data will be made available on request.

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