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Effects of Solid-State Fermented (SSF) Pelleted Feed with *Lactobacillus plantarum* on *Tachysurus fulvidraco*: Growth, Digestion, Antioxidant, Immunity, Intestinal Morphology, and Microbiota

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Abstract: This study aimed to evaluate the effects of solid-state fermented commercial pelleted feed with *Lactobacillus plantarum* on growth performance, digestive physiology, antioxidant, and immune capacity, as well as morphology and microbiota in intestinal of *Tachysurus fulvidraco*. A total of 420 fish (49.96 ± 7.10 g) were randomly allocated to twelve 300 L buckets for a 60 d farming trial. The diets of three treatments were established: the untreated commercial diet (UCD), the commercial diet mixed with fermented liquid (MFLD), and the solid-state fermented commercial diet (SSFD). The results indicated that SSFD treatment had a significant positive effect on the growth performance of *T. fulvidraco*, with an increase of 15.69% to 16.57% ($p < 0.05$) compared with UCD and MFLD treatments. MFLD and SSFD treatments also showed higher total anti-oxygen capacity, catalase, and glutathione peroxidase activities in the intestine significantly compared with UCD treatment ($p < 0.05$). Furthermore, the activities of alkaline phosphatase and lysozyme activities both in the liver and intestine were significantly higher in MFLD and SSFD treatments than in UCD treatment ($p < 0.05$). The villus height in the midintestine was also greater in MFLD and SSFD treatments compared with UCD treatment ($p < 0.05$). Regarding the intestinal microbiota, the dominant bacteria in UCD treatment was *Cetobacterium*, with the highest abundance in whole intestinal segments. However, in MFLD and SSFD treatments, the abundance of *Cetobacterium* in the foreintestine significantly decreased ($p < 0.05$). In conclusion, this study elucidates that solid-state fermentation feed may not only improve the digestive capacity, antioxidant ability, immune function, and intestinal morphology of *T. fulvidraco* to enhance growth performance but also influence intestinal microbial composition. These findings provide beneficial proof for developing fermented feed of *T. fulvidraco*.

Keywords: solid-state fermentation; *Tachysurus fulvidraco*; *Lactobacillus plantarum*; growth performance

Key Contribution: *Lactobacillus plantarum* can be used for solid-state fermentation in commercial pelleted feed, enhancing the nutritional value of the feed. Solid-state fermentation feed with *Lactobacillus plantarum* can improve fish growth performance and affect the abundance of *Cetobacterium* in the foreintestine.

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1. Introduction

Probiotics are widely applied in aquaculture as biological agents [1–3]. *Lactobacillus plantarum* is a facultative anaerobe that secretes lactic acid during fermentation, and it has a positive effect on fish health improvement and disease resistance [4,5]. Probiotics can be incorporated into aquafeed in various ways, such as adding fermented liquid to pelleted feed prior to feeding [6,7] or utilizing probiotics as a feed additive mixed with other ingredients during the formula feed manufacturing process [8,9].

In recent years, the solid-state fermentation (SSF) of probiotics in feed has become an aspect of growing interest and attention in the aquaculture industry, due in large part to advances in microbiology and fermentation technology [10]. The utilization of SSF in the feed industry has been explored through two methods: fermentation of some feed ingredients [11,12] and fermentation of commercial feed [13]. Currently, SSF of a specific feed ingredient has become a new means to develop new feed sources and reduce the usage of fishmeal in feed. Relative research focuses on fermented soybean meal [14], cottonseed meal [15], and feather meal [16]. During the fermentation process, microorganisms can break down complex nutrients such as starch and fiber, which are difficult for aquatic animals to digest and absorb, into easily digestible molecules [17,18]. Additionally, SSF can degrade anti-nutritional factors (ANFs) in certain feed ingredients [15], thereby improving the efficiency of feed utilization. The fermentation process also produces substances such as short-chain fatty acids and free amino acids [19,20], which have a positive effect on feed intake and improve the palatability of feed ingredients.

However, the high temperature during the subsequent pelleting process, such as steam and extrusion [21,22], makes it difficult for probiotics used in fermented feed ingredients to survive in the feed and results in an attenuated probiotic effect. With the continuous improvement in aquafeed pelleting technology, the stability of feed in water has been greatly improved [23]; thus, the post-fermentation process is generated for commercial pelleted feed. To overcome the challenge of probiotic survival during the pelleting process, the method of fermenting feed after pelletization has gradually developed. Fermenting feed in the process after pelletization can achieve pre-digestion of complex feed and make it easier for animals to digest and absorb.

This approach also ensures the viability of probiotics in the fermented feed. However, the effects of SSF in commercial feed on growth, digestion, immunity, intestinal microbiota, and intestinal morphology of fish are still poorly understood, and more research is needed to evaluate the potential benefits and drawbacks of this technology overall.

Tachysurus fulvidraco, commonly known as yellow catfish, is a small freshwater benthic fish, which is an omnivorous fish with a preference for a carnivorous diet. There are significant differences between male and female individuals, with males growing faster and reaching larger sizes. It is widely cultivated as a major commercial species in continental China [24]. Among the various strains available, "All-male No. 1" (authenticated by the National Committee for Pedigree Seed and Improved Varieties, 2010, China) has gained considerable popularity for commercial cultivation. Under intensive aquaculture conditions, *T. fulvidraco* is highly susceptible to bacterial diseases, which can result in significant economic losses [25]. Recent research reports [25,26] on the probiotics of *T. fulvidraco* show that probiotics have a positive effect on growth performance while also enhancing the immune system and improving the composition of the intestinal microbiota, reducing the proportion of pathogenic bacteria. However, there have been relatively few studies on the effects of fermented feed on *T. fulvidraco* and even other fish species. To investigate the impact of either *Lactobacillus plantarum* fermented liquid or solid-state fermented commercial feed on *T. fulvidraco*, we established a trial design to evaluate the effects of dietary feed treated with liquid or solid-state *Lactobacillus plantarum* fermentation on growth, digestion, antioxidant, immunity, intestinal microbiota, and intestinal morphology of *T. fulvidraco*.

2. Materials and Methods

Feeding trials were conducted at the Aquatic Greenhouse of Yangzhou University (Jiangsu, China). Throughout the experiment, the Animal Care and Use Committee of Yangzhou University approved our research on *T. fulvidraco* (ethical protocol code: YZUDWSY 2017-09-06), and we took all necessary measures to reduce any potential suffering of *T. fulvidraco*.

2.1. Bacterial Strains

The bacterial strain *Lactobacillus plantarum* was obtained from Jiangsu Lvke Biotechnology Co., Ltd. in Yangzhou, Jiangsu, China. It was identified as *Lactobacillus plantarum* strain Sourdough_B8 (GenBank accession MG754609) through 16S rDNA sequencing, with a sequence homology of 99%. The *Lactobacillus plantarum* strain was cultured in MRS medium at 37 °C until it reached the logarithmic phase of growth, which was determined by measuring the optical density at 600 nm.

2.2. Fish and Experimental Diet

Juvenile all-male *T. fulvidraco* were obtained from Yangzhou Hongsheng Aquatic Technology Co., Ltd. in Yangzhou, Jiangsu, China. Prior to the trial, all fish were acclimated to a commercial diet under controlled experimental conditions for two weeks. After a 24 h period of starvation, 420 healthy *T. fulvidraco* with similar sizes (initial mean body weight = 49.96 ± 7.10 g, initial mean body length = 13.97 ± 0.80 cm) were randomly divided into 12 cylindrical buckets (300 L), with 35 fish per bucket (the landing density was about 116 ind/m^3 or 5.8 kg/m^3) and four replicates for each treatment.

Three experimental diets were designed: an untreated commercial diet (UCD, control treatment), a commercial diet mixed with *Lactobacillus plantarum* fermented liquid (MFLD treatment), and a solid-state *Lactobacillus plantarum* fermented commercial diet (SSFD treatment). The untreated commercial diet was manufactured by Yangzhou Hongda Feed Co., Ltd., in Yangzhou, Jiangsu, China, and the initial pH value was 5.62. The ball-like expanded pellet feed size was 3.5 mm in diameter. Ingredients and composition of the commercial diets are shown in Table 1.

Table 1. Ingredients and composition of experimental commercial diets formulation.

Ingredients	Composition (%)
Wheat flour	22.50
Rice bran	5.00
Soybean meal	14.00
Rapeseed meal	8.00
Cottonseed meal	6.00
Corn gluten meal	6.00
Fish meal	30.00
Squid paste	2.00
Soybean oil	2.00
Fish oil	0.50
Soybean lecithin	1.50
Monocalcium phosphate	1.00
Mineral–vitamin premix	1.50
Total	100.00

Note: The formula of the mineral–vitamin premix involves commercial secrets and is not disclosed.

The preparation process of experimental diets can be referred to in the schematic diagram presented in Figure 1. To prepare the *Lactobacillus plantarum* fermented liquid, 5 mL of activated *Lactobacillus plantarum* were transferred to a sealed plastic bottle containing 25 g of MRS medium, 100 g of brown sugar, and 5 kg of water, fermented the mixture at 37 °C for 72 h. After fermentation, the fermented liquid was tested containing 1.82×10^{10} colony-forming units (CFUs) per milliliter of *Lactobacillus plantarum*, and the final pH value was 3.61. MFLD was blended with 1% of the *Lactobacillus plantarum* fermented liquid to UCD half an hour prior to feeding, and the pH value of the mixture was 5.03. The SSFD treatment was produced by solid-state fermentation with UCD as the substrate in which the *Lactobacillus plantarum* was added and adjusted to an initial concentration of 1×10^6 CFU/g, then the ratio of substrate to water as 2:1 (g/mL) was mixed for better fermentation. The mixture was then placed in a polyethylene bag with a one-way air valve and fermented for one week at a temperature of 37 °C. After fermentation, the diet was

tested containing 2.20 × 10^8 CFU/g of *Lactobacillus plantarum*, and the final pH value was 5.26. Throughout the experimental period, the untreated commercial diet, fermented liquid, and SSFD were stored at 4 °C. The nutrient composition of UCD and SSFD is presented in Table 2. It should be noted that the nutrient composition of MFLD referred to UCD due to the same commercial diet feeding with 1% fermented liquid addition (only 0.025% dry basis).

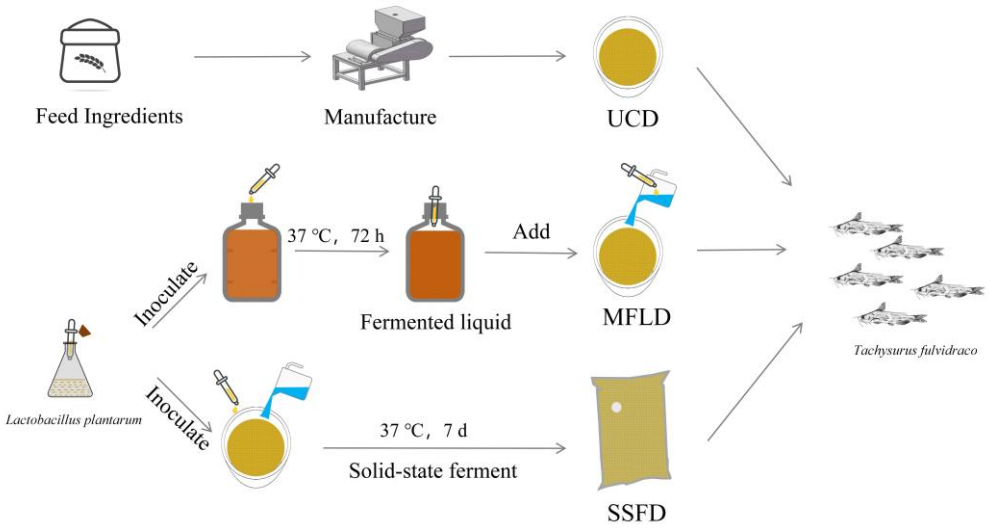

Figure 1. Experimental diet preparation process.

Table 2. Proximate compositions and amino acid profiles of the experimental diets.

	UCD	SSFD	UCD (DM)	SSFD (DM)
Proximate composition (%)				
Moisture	8.28	27.68	-	-
Crude protein	40.34	34.34	43.98	47.48
Crude lipid	8.88	7.64	9.03	10.56
Ash	9.77	7.69	10.65	10.63
Amino acid profile (%)				
EAAs				
Arg	2.70	2.01	2.95	2.77
His	1.30	0.98	1.41	1.36
Ile	1.92	1.49	2.09	2.07
Leu	3.05	2.42	3.33	3.35
Lys	2.72	2.06	2.97	2.86
Met	0.91	0.65	0.99	0.90
Phe	2.29	1.83	2.49	2.53
Thr	1.63	1.27	1.78	1.76
Val	2.04	1.57	2.22	2.17
NEAAs				
Ala	2.08	1.64	2.27	2.27
Asp	3.35	2.47	3.66	3.41
Cys	0.72	0.65	0.79	0.89
Glu	6.14	4.76	6.69	6.58
Gly	1.96	1.67	2.14	2.31
Pro	2.09	1.75	2.28	2.42
Ser	1.91	1.43	2.08	1.98
Tyr	0.44	0.33	0.48	0.45
TAAs	37.24	28.99	40.60	40.09

Note: UCD, untreated commercial diet (control treatment); SSFD, solid-state fermented diet; DM, dry matter basis; EAAs, essential amino acids; NEAAs, non-essential amino acids; TAAs: total amino acids (without Trp).

2.3. Fish Farming and Sampling

The trial was conducted in an indoor recirculatory aquaculture system (RAS, Haisheng, Shanghai, China). RAS consists of farming tanks, circulating water pumps, aerators, microfilters, biochemical purification tanks, ultraviolet disinfection lamps, aerated tanks, and water quality monitoring equipment. Water quality parameters, including temperature (28.0 ± 1.1 °C), dissolved oxygen (above 6.0 mg/L), pH (7.84 ± 0.22), ammonia nitrogen (below 0.1 mg/L), and nitrite nitrogen (below 0.01 mg/L) were monitored daily.

Fish were hand-fed at 1.5% dry matter feed of the body weight twice daily (08:00, 18:00) for 60 days under the same ingestion amount in dry matter level among treatments during the feeding trial. During the experimental period, feed intake was recorded. The leftover feed was removed daily and dried in a hot-air oven to assess the feed conversion ratio (FCR).

At the end of the 60-day feeding test, all fish per bucket were weighed and euthanized with 60 mg/L MS-222 to minimize stress after 48 h fasting. Following the principle of random sampling, five fish per bucket were individually weighed, measured for body length, and dissected for visceral weight. The other three fish in each bucket were collected (without anti-coagulant) for a caudal vein blood sample (1–2 mL), and then three tissue samples (liver, intestine, and stomach) were dissected for enzyme activity analysis individually. These samples for enzyme activity analysis were rapidly placed in liquid nitrogen for quick freezing. At the same time, the blood samples were centrifuged at 1500 g and 4 °C for 10 min to obtain serum. The serum and enzyme activity tissue samples were stored at −80 °C until analysis. The whole body (without gill and visceral tissue) of three fish per bucket and the muscle (without skin and intraperitoneally mucosa tissues) of three fish per bucket were sampled under −20 °C to take proximate analysis subsequently. The intestine of two fish per bucket was collected and fixed with neutral formalin for morphology observation. Under sterile conditions, the entire intestine was dissected and classified into three sections based on variations in diameter: foreintestine (thicker section), midintestine (moderate thickness section), and hindintestine (thinner section) [27], which were stored at −80 °C for further analysis of intestinal microbial diversity.

2.4. Growth Performance Parameters

Growth performance parameters were computed following the completion of the trial according to the following formulae:

$$\text{Survival Rate (SR, \%)} = \frac{\text{final number of } T.\ fulvidraco}{\text{initial number of } T.\ fulvidraco} \times 100$$

$$\text{Weight Gain Rate (WGR, \%)} = \frac{\text{final body weight (g)} - \text{initial body weight (g)}}{\text{initial body weight (g)}} \times 100$$

$$\text{Specific Growth Rate (SGR, \%/d)} = \frac{\text{Ln final body weight (g)} - \text{Ln initial body weight (g)}}{\text{culturing period (d)}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{dry matter weight of feed intake (g)}}{\text{final body weight (g)} - \text{initial body weight (g)}}$$

$$\text{Condition Factor (CF, g/cm}^3\text{)} = \frac{\text{final weight (g)}}{\text{final length}^3\ (\text{cm}^3)}$$

$$\text{Viscerosomatic Index (VSI, \%)} = \frac{\text{viscera weight (g)}}{\text{fish body weight (g)}} \times 100$$

2.5. Serum Biochemistry Analyses

Serum samples were detected by the Yangzhou Centre for Disease Control and Prevention (Yangzhou, Jiangsu, China) through a Hitachi 7600 automatic biochemical analyzer (Tokyo, Japan).

2.6. Nutrient Composition

The experimental diets, whole fish body, and muscle samples were analyzed for composition according to the procedures established by the Association of Official Analytical Chemists [28]. Briefly, whole fish body and muscle samples were dried at 105 °C by a constant temperature drying oven (Jinghong DHG-9240A, Shanghai, China) until constant weight to estimate the moisture content. Crude protein was measured using the Kjeldahl method by determining nitrogen (FOSS Kjeltec 8400, Hilleroed, Denmark), and the percentage of protein was calculated as total nitrogen × 6.25. Crude lipid was determined by petroleum ether extraction using a G100 Automated Filter-Bag Fat Extractor (Sonnen, Shanghai, China). Ash was determined by incineration in a furnace (Shanghai Experimental Instrument Factory SX2, Shanghai, China) at 550 ± 25 °C until constant weight. Amino acid contents of samples were analyzed by high-performance liquid chromatography (HPLC, Agilent 1220 Infinity, Agilent Technologies, Santa Clara, CA, USA) after acid digestion with hydrochloric acid. A Venusil AA column (4.6 mm × 250 mm, 5 μm, Agilent Technologies, USA) was applied for this HPLC. The modified methods for amino acid analysis were previously described by Dong et al. [29] and involved a 24 h acid digestion with 6 M HCl at 110 °C.

2.7. Enzyme Activity Analyses

Liver, stomach, and intestine samples were homogenized separately. The trypsin activity, chymotrypsin activity, pepsin activity, lipase activity, amylase activity, alkaline phosphatase (AKP, A059-1-1) activity, acid phosphatase (ACP, A060-1-1) activity, lysozyme (LZM, A050-1-1) activity, superoxide dismutase (SOD, A001-1-2) activity, catalase (CAT, A007-1-1) activity, total anti-oxygen capacity (TAOC, A015-1-2) activity, glutathione peroxidase (GSH-Px, A005-1-2) activity, and malonaldehyde (MDA, A003-1-2) content of each sample were measured by means of colorimetric methods with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The specific wavelengths and reagents used in the assays were as specified by the manufacturer. The manufacturer's instructions were followed carefully, including any specific protocols or procedures. A UV spectrophotometer (MAPADA UV-1100, Shanghai, China) was used for enzyme activity determination.

2.8. Intestinal Morphology Parameters

The intestinal samples fixed in 10% neutral buffered formalin were removed and divided into three segments: foreintestine, midintestine, and hindintestine. The intestinal samples were dehydrated in a graded series of ethanol and embedded in paraffin wax after treatment with xylene to remove residual water. Then, 7 μm thick sections were cut under a microtome (Leica RM2016, Wetzlar, German), stained with hematoxylin–eosin (HE), and sealed with neutral resin adhesive. The intestinal thickness and villus height were photographed and measured through an optical microscope (Olympus BX53, Tokyo, Japan) with a magnification of 40×. Villus height was defined as the distance between the tip of the villus and the base of the crypt.

2.9. Intestinal Microbial Diversity

Intestine, solid-state fermented feed, and liquid-state fermented liquid samples were collected and stored in dry ice before being delivered to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for microbial analysis. The microorganisms in the samples were detected by performing the Illumina miseq sequencing method, which involved sequencing the 16S rDNA gene to identify bacterial communities.

2.10. Statistical Analysis

The experimental data were analyzed by running SPSS 18.0 software (SPSS Inc., New York, NY, USA). One-way ANOVA was applied to compare the means of three or more treatments, followed by Duncan's multiple range test to identify significant differences between treatments. Normality and homogeneity of variances were checked to ensure that the data met the assumptions of the statistical tests used. The confidence interval was set at 95%, and statistical significance was set at $p < 0.05$. Descriptive statistics were reported as mean ± standard deviation (SD). The data on intestinal microbial diversity were analyzed relying on the Majorbio Cloud Platform (www.majorbio.com, accessed on 14 December 2022), a reliable and established online platform for biological data analysis.

3. Results

3.1. Growth Performance

The growth performance data are presented in Table 3. The SR ranged from 95.72% to 99.29% and did not show a significant difference among treatments ($p > 0.05$). The FBW, WGR, and SGR in SSFD treatment were significantly higher than those in UCD treatment ($p < 0.05$). The FCR in SSFD treatment was significantly lower than that in UCD treatment ($p < 0.05$). There were no significant differences in CF and VSI among treatments ($p > 0.05$).

Table 3. Growth performance of *T. fulvidraco* fed the experimental diets for 60 days.

	UCD	MFLD	SSFD
IBW (g)	49.96 ± 0.05	49.96 ± 0.07	49.96 ± 0.06
FBW (g)	95.59 ± 0.78 ^{a}	95.75 ± 0.74 ^{a}	102.89 ± 1.35 ^{b}
SR (%)	95.72 ± 3.69	97.84 ± 1.44	99.29 ± 1.43
WGR (%)	91.07 ± 1.56 ^{a}	91.73 ± 1.49 ^{a}	106.15 ± 2.70 ^{b}
SGR (%/d)	1.08 ± 0.01 ^{a}	1.08 ± 0.02 ^{a}	1.21 ± 0.03 ^{b}
FCR (%)	1.52 ± 0.03 ^{b}	1.51 ± 0.03 ^{b}	1.31 ± 0.03 ^{a}
CF (g/cm^3)	1.75 ± 0.14	1.82 ± 0.14	1.76 ± 0.15
VSI (%)	10.58 ± 1.14	9.60 ± 1.32	10.07 ± 1.09

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet; IBW, initial body weight; FBW, final body weight; SR, survival rate; WGR, weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; CF, condition factor; VSI, viscerosomatic index. Values in the same rows with different superscript letters are significantly different ($p < 0.05$), while values in the same rows with the same or without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.2. Serum Biochemistry Analyses

The serum biochemical data are presented in Table 4. The AST activity in the serum of MFLD treatment was lower than that in UCD treatment ($p < 0.05$). The lower values with regard to total protein, globulin, and cholesterol and higher creatinine were detected in fish fed SSFD, compared with UCD ($p < 0.05$). The activity of ALP in the serum of both MFLD and SSFD treatments significantly increased than that in UCD ($p < 0.05$), but the content of triglyceride in the serum of MFLD and SSFD treatments decreased ($p < 0.05$). The content of blood urea nitrogen in the serum was significantly lower in MFLD treatment than in UCD treatment ($p < 0.05$). There were no significant differences in ALT and albumin among treatments ($p > 0.05$).

Table 4. Effect of different diets on serum biochemistry parameters in *T. fulvidraco*.

	UCD	MFLD	SSFD
ALT (U/L)	27.00 ± 2.24	28.86 ± 3.83	29.21 ± 1.04
AST (U/L)	500.57 ± 54.30 ^{b}	453.07 ± 22.96 ^{a}	485.93 ± 17.45 ^{ab}
Total protein (g/L)	42.15 ± 1.90 ^{b}	42.27 ± 1.04 ^{b}	35.52 ± 1.42 ^{a}
Albumin (g/L)	10.06 ± 0.18	9.99 ± 0.36	10.12 ± 1.23
Globulin (g/L)	32.09 ± 1.78 ^{b}	32.28 ± 1.23 ^{b}	25.40 ± 1.41 ^{a}
AKP (U/L)	19.79 ± 0.57 ^{a}	21.07 ± 0.53 ^{b}	23.13 ± 0.99 ^{c}
Creatinine (μmol/L)	30.93 ± 2.23 ^{a}	31.71 ± 1.82 ^{a}	43.29 ± 5.68 ^{b}
Blood urea nitrogen (mmol/L)	1.37 ± 0.13 ^{b}	0.73 ± 0.46 ^{a}	1.56 ± 0.11 ^{b}
Cholesterol (mmol/L)	6.22 ± 0.20 ^{b}	6.16 ± 0.75 ^{b}	5.11 ± 0.18 ^{a}
Triglyceride (mmol/L)	6.85 ± 0.24 ^{c}	6.38 ± 0.28 ^{b}	5.31 ± 0.47 ^{a}

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet; ALT, alanine transaminase; AST, aspartate transaminase; AKP, alkaline phosphatase. Values in the same rows with different superscript letters are significantly different ($p < 0.05$), while values in the same rows with the same or without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.3. Nutrient Composition of Whole Body and Muscle

The nutrient composition data of the whole body and muscle are presented in Table 5. The moisture, crude protein, crude lipid, and ash contents of the whole body and muscle showed no significant differences among treatments ($p > 0.05$).

Table 5. Effect of different diets on nutrient composition of whole body and muscle in *T. fulvidraco*. (fresh matter).

	UCD	MFLD	SSFD
Whole body			
Moisture (%)	69.89 ± 1.53	71.74 ± 2.82	70.83 ± 2.14
Crude protein (%)	17.24 ± 0.74	16.80 ± 0.86	17.41 ± 0.60
Crude lipid (%)	7.06 ± 1.00	6.57 ± 1.33	6.30 ± 1.17
Ash (%)	6.10 ± 0.85	5.30 ± 0.62	5.67 ± 0.35
Muscle			
Moisture (%)	76.94 ± 1.03	75.37 ± 2.17	76.30 ± 1.80
Crude protein (%)	17.18 ± 0.44	17.37 ± 0.77	17.60 ± 0.76
Crude lipid (%)	4.35 ± 1.12	5.87 ± 2.01	5.87 ± 2.01
Ash (%)	1.20 ± 0.11	1.27 ± 0.07	1.19 ± 0.05

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet. The whole body excluded gill and visceral tissue. The muscle excluded skin and intraperitoneally mucosa tissues. Values in the same rows without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.4. Digestive Enzyme Activities

The digestive enzyme activity data are presented in Table 6. In the stomach, the pepsin activity in SSFD treatment was significantly higher than that in UCD treatment ($p < 0.05$). MFLD and SSFD treatments had significantly decreased trypsin activity in the intestine and increased trypsin activity in the liver compared with UCD treatments ($p < 0.05$). Compared with UCD treatment, both the chymotrypsin activity in the intestine of SSFD treatment and the amylase activity in the intestine of SSFD treatment significantly increased ($p < 0.05$). *T. fulvidraco* fed with MFLD and SSFD presented higher lipase activity in the intestine compared with those fed with UCD ($p < 0.05$). No significant differences with respect to lipase and amylase activities in the liver were observed among treatments ($p > 0.05$).

Table 6. Effect of different diets on digestive enzyme activities in *T. fulvidraco*.

	UCD	MFLD	SSFD
Stomach			
Pepsin (U/mgprot)	13.28 ± 0.11 [a]	14.69 ± 2.54 [ab]	17.14 ± 1.42 [b]
Intestine			
Trypsin (U/mgprot)	28,454.95 ± 558.29 [c]	13,629.95 ± 606.69 [a]	23,219.47 ± 1292.86 [b]
Chymotrypsin (U/mgprot)	1.06 ± 0.16 [a]	1.34 ± 0.16 [b]	0.79 ± 0.04 [a]
Lipase (U/gprot)	200.26 ± 22.70 [a]	243.46 ± 7.23 [b]	227.42 ± 5.06 [b]
Amylase (U/mgprot)	8.09 ± 0.44 [a]	8.33 ± 0.31 [a]	9.90 ± 0.30 [b]
Liver			
Trypsin (U/mgprot)	2776.89 ± 64.24 [a]	3505.12 ± 192.83 [b]	3407.36 ± 119.16 [b]
Lipase (U/gprot)	36.39 ± 2.12	37.20 ± 2.88	40.50 ± 3.41
Amylase (U/mgprot)	0.58 ± 0.04	0.64 ± 0.03	0.63 ± 0.04

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet. Values in the same rows with different superscript letters are significantly different ($p < 0.05$), while values in the same rows with the same or without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.5. Antioxidant Enzyme Activities

The antioxidant enzyme activity data are presented in Table 7. In the liver, the TAOC, CAT, and GSH-Px activities of the MFLD treatment were significantly elevated than those of the other treatments ($p < 0.05$). Compared with UCD treatment, MFLD and SSFD displayed lower levels of MDA in the liver ($p < 0.05$). Compared with UCD treatment, MFLD and SSFD treatments had significantly higher levels of MDA in the intestine ($p < 0.05$). However, in the intestine, the TAOC, CAT, and GSH-Px activities both in MFLD and SSFD treatments were significantly higher than those in UCD treatment ($p < 0.05$). There were no significant differences in SOD activity of the liver and intestine among treatments ($p > 0.05$).

Table 7. Effect of different diets on antioxidant enzyme activities in *T. fulvidraco*.

	UCD	MFLD	SSFD
Liver			
SOD (U/mgprot)	728.97 ± 2.82	696.39 ± 37.95	712.96 ± 17.40
MDA (nmol/mgprot)	2.92 ± 0.10 [b]	2.37 ± 0.14 [a]	2.25 ± 0.08 [a]
TAOC (U/mgprot)	2.63 ± 0.02 [a]	2.98 ± 0.32 [b]	2.47 ± 0.09 [a]
CAT (U/mgprot)	7.18 ± 0.14 [a]	7.74 ± 0.13 [b]	7.22 ± 0.23 [a]
GSH-Px (U/mgprot)	13.12 ± 0.67 [a]	18.39 ± 2.12 [b]	11.25 ± 0.11 [a]
Intestine			
SOD (U/mgprot)	136.33 ± 5.08	145.82 ± 45.13	128.76 ± 3.53
MDA (nmol/mgprot)	2.89 ± 0.07 [a]	4.13 ± 0.20 [c]	3.50 ± 0.23 [b]
TAOC (U/mgprot)	1.67 ± 0.06 [a]	1.90 ± 0.04 [b]	2.19 ± 0.13 [c]
CAT (U/mgprot)	2.39 ± 0.15 [a]	2.69 ± 0.03 [b]	2.50 ± 0.01 [a]
GSH-Px (U/mgprot)	80.15 ± 3.80 [a]	123.99 ± 5.82 [c]	100.43 ± 1.09 [b]

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet; SOD, superoxide dismutase; MDA, malondialdehyde; TAOC, total anti-oxygen capacity; CAT, catalase; GSH-Px, glutathione peroxidase. Values in the same rows with different superscript letters are significantly different ($p < 0.05$), while values in the same rows with the same or without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.6. Non-Specific Immune Enzyme Activities

The non-specific immune enzyme activity data are presented in Table 8. In the liver and intestine, the AKP and LZM activities both in MFLD and SSFD treatments were significantly promoted than those in UCD treatment ($p < 0.05$). The ACP activity in the liver and intestine showed no significant differences among treatments ($p > 0.05$).

Table 8. Effect of different diets on non-specific immune enzyme activities in *T. fulvidraco*.

	UCD	MFLD	SSFD
Liver			
AKP (U/gprot)	7.24 ± 0.29 [a]	9.21 ± 0.66 [b]	9.59 ± 0.45 [b]
ACP (U/gprot)	15.83 ± 0.68	14.40 ± 1.12	16.67 ± 2.86
LZM (U/mgprot)	14.67 ± 1.09 [a]	19.87 ± 1.53 [b]	24.92 ± 0.23 [c]
Intestine			
AKP (U/gprot)	14.01 ± 0.60 [a]	21.11 ± 1.89 [b]	31.41 ± 2.35 [c]
ACP (U/gprot)	19.08 ± 0.99	19.74 ± 0.02	20.73 ± 1.88
LZM (U/mgprot)	17.12 ± 2.09 [a]	64.34 ± 2.41 [c]	33.17 ± 0.26 [b]

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet; AKP, alkaline phosphatase; ACP, acid phosphatase; LZM, lysozyme. Values in the same rows with different superscript letters are significantly different ($p < 0.05$), while values in the same rows with the same or without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.7. Intestinal Morphology

The intestinal morphology data are presented in Table 9 and Figure S1. There were no significant differences with regards to intestinal thickness both in the foreintestine and midintestine among treatments ($p < 0.05$), but the intestinal thickness of the hindintestine in SSFD treatment was significantly greater than that of the others ($p < 0.05$). In the foreintestine, villus height in SSFD treatment was significantly higher than that in the other treatments ($p < 0.05$). In the midintestine, the villus height in MFLD and SSFD treatments was significantly higher than that in UCD treatment ($p < 0.05$). In the hindintestine, there were no significant differences in villus height among treatments ($p < 0.05$).

Table 9. Effect of different diets on intestinal morphology in *T. fulvidraco*.

	UCD	MFLD	SSFD
Foreintestine			
Intestinal thickness (μm)	267.21 ± 43.97	257.39 ± 49.17	253.71 ± 49.07
Villus height (μm)	710.74 ± 103.61 [a]	700.56 ± 75.69 [a]	865.64 ± 167.56 [b]
Midintestine			
Intestinal thickness (μm)	241.47 ± 9.08	220.32 ± 23.97	250.12 ± 42.84
Villus height (μm)	364.21 ± 33.90 [a]	443.99 ± 41.06 [b]	452.85 ± 42.65 [b]
Hindintestine			
Intestinal thickness (μm)	210.14 ± 26.81 [a]	191.45 ± 22.27 [a]	237.49 ± 21.07 [b]
Villus height (μm)	285.83 ± 30.32	292.01 ± 15.21	287.75 ± 34.42

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet. Values in the same rows with different superscript letters are significantly different ($p < 0.05$), while values in the same rows with the same or without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

3.8. Intestinal Microbial Diversity and Feed Microbial Composition

The intestinal microbial diversity alpha data are presented in Table 10. In different intestinal segments, there were no significant changes in microbial alpha diversity among treatments ($p > 0.05$).

Both the intestine and feed microbial composition information are presented in Figure 2 and Tables S1 and S2. In the intestine of *T. fulvidraco*, the dominant microbiota at the phylum level were classified as *Firmicutes*, *Fusobacteriota*, *Proteobacteria*, *Bacteroidota*, and *Acidobacteriota*, respectively. At the genus level, the dominant microbiota were classified as *Cetobacterium*, norank_f_*Barnesiellaceae*, *Ralstonia*, *Turicibacter*, *Acinetobacter*, and 15 other genera, respectively. In the solid-state fermented feed and *Lactobacillus plantarum* fermented liquid, the common dominant microbiota belonged to *Firmicutes* at the phylum level and *Lactobacillus* at the genus level. In the foreintestine, the relative abundance of *Proteobacteria* in MFLD and SSFD treatments significantly increased (22.65~35.28%) compared with UCD treatment, while the relative abundance of *Fusobacteriota* in MFLD and SSFD treatments

significantly decreased (25.83~27.65%) compared with UCD treatment ($p < 0.05$). Moreover, compared with UCD treatment, the relative abundance of *Acidobacteriota* in MFLD treatment significantly increased ($p < 0.05$). In the midintestine, only the relative abundance of *Firmicutes* in MFLD treatment significantly increased (15.62%) ($p < 0.05$). In the hindintestine, there were no apparent changes in relation to the composition of dominant microbiota at the phylum level among treatments ($p > 0.05$). In the foreintestine, the relative abundance of *Cetobacterium* both in MFLD and SSFD treatments significantly decreased (25.63~27.69%) compared with UCD treatment ($p < 0.05$). The relative abundance of *Ralstonia*, *Geobacillus*, and *Lactobacillus* in the foreintestine of SSFD treatment significantly increased compared with UCD treatment ($p < 0.05$). *Rhodococcus* in the foreintestine of MFLD treatment significantly increased compared with UCD treatment ($p < 0.05$). In the midintestine, the relative abundance of *Acinetobacter*, *Geobacillus*, and *Lactobacillus* in MFLD treatment significantly increased compared with UCD treatment ($p < 0.05$). In the hindintestine, there were no significant differences with respect to the composition of dominant microbiota at the genus level among treatments ($p > 0.05$). Principal component analysis (PCA) showed that the microbial beta diversity of UCD and SSFD treatments in the foreintestine were far away from each other and had no overlapping area, while UCD and MFLD in the foreintestine had few overlapping areas, there were many overlapping areas observed in PCA analysis of midintestine and hindintestine among treatments.

Table 10. Effect of different diets on intestinal microbial alpha diversity evaluation in *T. fulvidraco*.

	UCD	**MFLD**	**SSFD**
Foreintestine			
Sobs	209.75 ± 68.55	258.25 ± 83.48	193.50 ± 53.24
Ace	2.60 ± 0.97	3.58 ± 0.55	3.07 ± 0.42
Chao	0.21 ± 0.17	0.09 ± 0.02	0.13 ± 0.05
Shannon	224.74 ± 52.02	260.36 ± 85.60	196.48 ± 55.75
Simpson	224.48 ± 51.63	263.65 ± 90.28	197.06 ± 55.93
Coverage	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Midintestine			
Sobs	231.00 ± 89.14	257.00 ± 84.20	222.25 ± 34.20
Ace	2.22 ± 1.12	3.43 ± 0.53	2.89 ± 0.25
Chao	0.32 ± 0.30	0.10 ± 0.02	0.16 ± 0.03
Shannon	241.61 ± 77.56	260.43 ± 85.69	225.95 ± 34.19
Simpson	239.75 ± 81.28	264.70 ± 89.87	234.43 ± 33.99
Coverage	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Hindintestine			
Sobs	152.25 ± 78.81	77.00 ± 39.23	155.00 ± 66.07
Ace	1.64 ± 1.05	1.29 ± 0.28	1.73 ± 0.86
Chao	0.44 ± 0.32	0.39 ± 0.09	0.37 ± 0.23
Shannon	193.67 ± 30.94	123.12 ± 52.97	190.94 ± 44.47
Simpson	179.72 ± 51.91	108.09 ± 40.26	178.12 ± 60.69
Coverage	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

Note: UCD, untreated commercial diet (control treatment); MFLD, the commercial diet mixed with fermented liquid; SSFD, solid-state fermented diet. Values in the same rows without superscript letters are not significantly different ($p > 0.05$). The values presented were average ± standard deviation (n = 4).

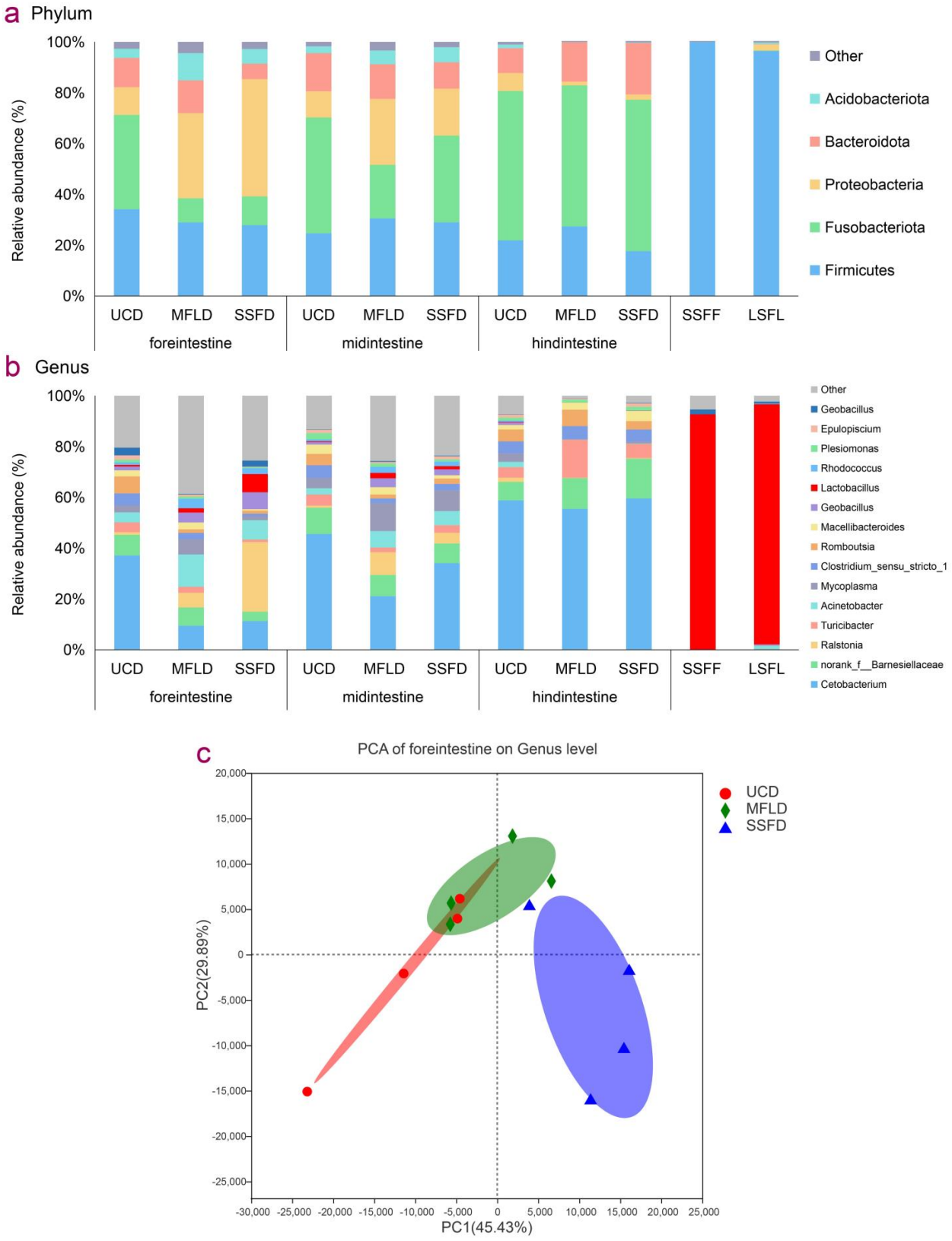

Figure 2. *Cont*.

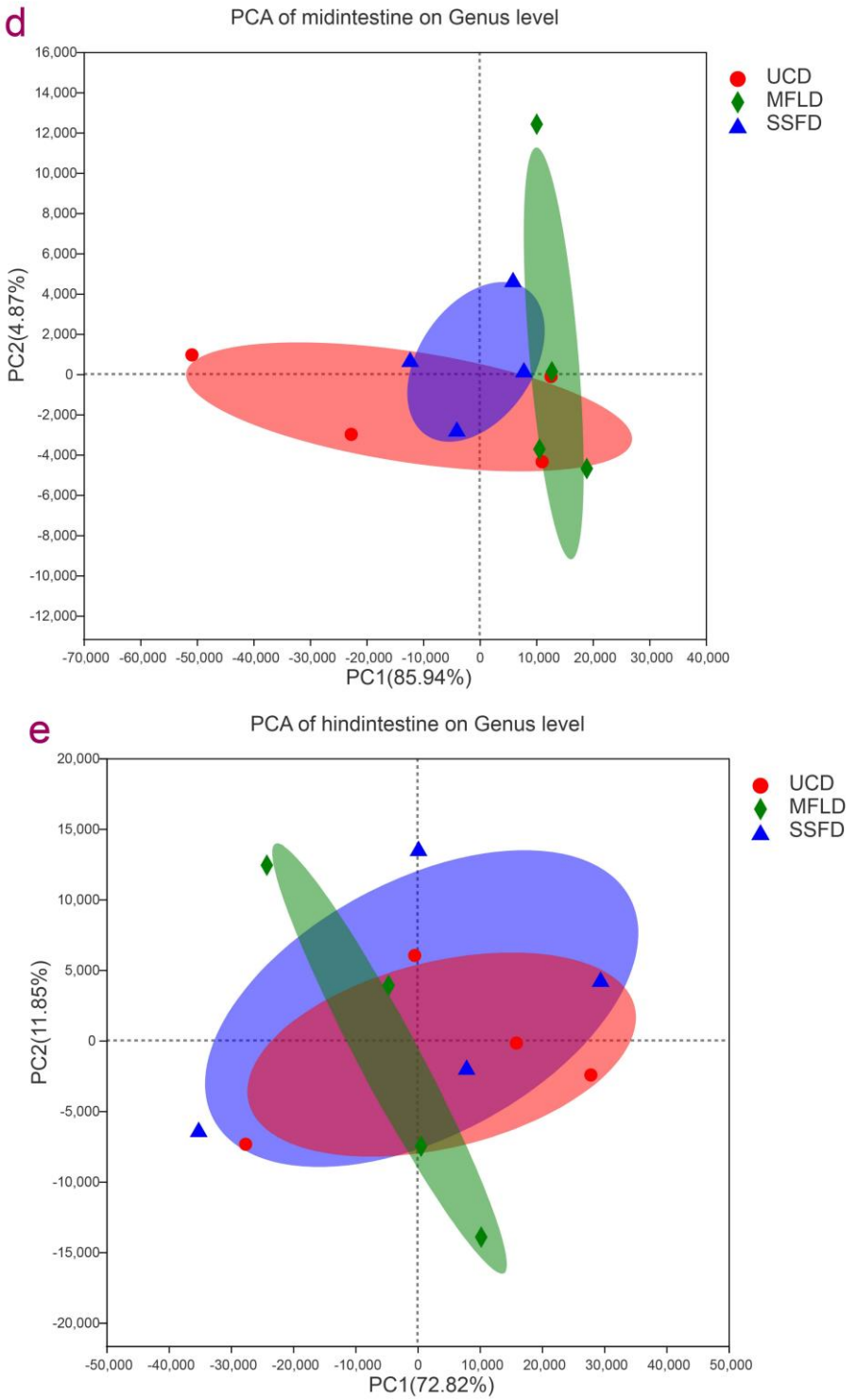

Figure 2. (**a**) Relative abundance of microbiota on phylum level at the different samples (n = 4). (**b**) Relative abundance of microbiota on genus level at the different samples (n = 4). (**c**) PAC of foreintestine on genus level (n = 4). (**d**) PAC of midintestine on genus level (n = 4). (**e**) PAC of midintestine on genus level (n = 4). In (2.c)~(2.e), ellipses with different colors represent the degree of dispersion and directionality in different treatments, respectively. In (2.a)~(2.e), UCD means untreated commercial diet (control treatment); MFLD means the commercial diet mixed with fermented liquid; SSFD means solid-state fermented diet; and SSFF and LSFL represent solid-state fermented feed in SSFD and liquid-state fermented liquid in MFLD, respectively.

4. Discussion

Biological solid-state fermented feed, as a kind of new complete fermentation formula pelleted feed which is different from fermenting feedstuff, is produced by incorporating probiotics into formula pelleted feed for a short-term fermentation. In contrast to the direct addition of fermentation liquid and microbial powders to feed, solid-state fermented feed has a prominent advantage in elevating microbial quantity and viability. These advantages make it possible with potential extensive applications in the aquafeed industry. As popularly used probiotics, lactic acid bacteria (LAB), whether employed in solid-state or liquid-state fermentation, have attracted much attention [30]. *Lactobacillus plantarum*, in particular, has remarkable adaptability and metabolic diversity [31]. Previous studies have demonstrated the impact of nutrient composition upon fermented soybean meal with *Lactobacillus plantarum*, resulting in a reduction in starch content and an increase in crude protein content following fermentation [32,33]. These results provide further evidence that the nutritional composition of the diet in the solid-state fermented feed has a higher protein and lipid content compared to the untreated commercial diet.

In general, the process of biological solid-state fermentation on feedstuff could generate a range of beneficial compounds, such as organic acids, enzymes, vitamins, peptides, and unknown growth factors, through the degradation of complex nutrients and anti-nutritional factors [34], which will facilitate digestion and absorption of feed, improve feed absorption efficiency and growth performance in animals [35–37]. For instance, adding 40 g/kg of fermented soybean meal to the feed can effectively improve the growth performance of juvenile largemouth bass [38]. The positive effects were also achieved by our experiment when *T. fulvidraco* was fed with solid-state fermentation pelleted feed (SSFD) compared to UCD. Moreover, enhanced digestive enzymatic activity was observed in SSFD, which was in accordance with the improved growth performance. That means solid-state fermentation may alter feed digestibility beneficially by boosting digestive enzyme activity, leading to optimal digestive physiology conditions for better assimilation. Furthermore, the improved histology of intestinal villus height enhanced nutrient absorption. Undoubtedly, digestive enzymes play key roles in the digestive absorption of fish. In the current experiment, the activity of digestive enzymes such as stomach pepsin, intestinal lipase, and intestinal lipase amylase from the SSFD treatment was significantly increased, contributing to the high digestibility of SSFDs. However, it is noteworthy that a decrease in intestinal trypsin activity was observed in the MFLD and SSFD treatments, and this phenomenon did not have a negative impact on the growth performance in SSFD treatment. In fact, digestive enzymes usually present dynamic changes in different tissues upon ingestion and starvation [39]. Specifically, during the fasting period, in the MFLD and SSFD treatments, the lower activity of intestinal trypsin could potentially be attributed to the influence of lower dietary pH [40]; interestingly, a higher level of trypsin activity in the liver was also observed. Following re-intake, the higher activity of trypsin would be secreted into the intestine from the liver to match the biophysiological function for better digestion; this phenomenon was confirmed by our additional experimental data that the trypsin activity in the intestine maintained a high level within 6 h after feeding (data not yet published). The morphology of intestinal tissue is crucial for nutrient absorption in fish. In SSFD treatment fed with solid-state fermented feed, the villus height of the foreintestine and midintestine was significantly increased. Similarly, in MFLD treatment with the addition of fermented liquid, an increase in villus height was observed in the midintestine. This suggests that *T. fulvidraco* undertakes nutrient absorption at the foreintestine and midintestine majorly. Previous studies have realized that LAB use sugar as a growth factor to produce metabolites such as short-chain fatty acids (SCFAs) during fermentation, which play prebiotic functions. Some studies demonstrated that prebiotics or their metabolites may simulate intestinal villus development [41,42]. For instance, fermented soybean meal could promote the height of fish villus [43]. However, no improvement in growth performance was observed in MFLD treatment, possibly due to the partial loss of fermented liquid in water, resulting in an insufficient dosage of LAB. Research has found that a high dosage of LAB is needed to

improve the growth performance of fish [44]. This phenomenon suggests that solid-state fermented feed may have apparent advantages in achieving higher quantity and viability probiotics than feed just mixed with fermented liquid, resulting in a better growth effect.

LAB are considered probiotics due to their beneficial effects on the host's health. The adaptive mechanisms of LAB promote interactions with the host and directly enhance beneficial effects on the host's physiology and immunology [45]. During solid-state and liquid-state fermentation processes, LAB are extensively preserved, and their metabolic products are accumulated in large quantities. Long-term exposure to *Lactobacillus plantarum* and its metabolic products can significantly improve the immune function and antioxidant ability of *T. fulvidraco*. Non-specific immune enzyme activity, such as AKP, ACP, and LZM, has been widely used as indices of the immunity of fish in numerous studies [46]. The present results about AKP, ACP, and LZM activities in multiple tissues and organs, including serum, liver, and intestine, confirm that the benefit role of LAB elevated the immunity of *T. fulvidraco* in the MFLD and SSFD treatments. This finding was consistent with the results of an immune response experiment conducted by Wang et al. [7] on *T. fulvidraco* under a high-fat diet, where they evaluated the effect of noni (*Morinda Citrifolia*) fermentation juice on the immune response. It is worth noting that after LAB treatment, the activity of lysozyme in the intestine significantly increased, effectively enhancing the immune capacity of the intestine, especially in the MFLD treatment. This finding is consistent with the results of studies [47,48], indicating that lysozyme is elicited by different immunostimulating substances and acts as an integral component of aquatic animal antibacterial defense mechanisms. Oxidative stress is harmful to fish; previous studies have shown that the antioxidant defense mechanisms of fish in aquaculture are insufficient, highlighting the need to strengthen the antioxidant system of fish in commercial aquaculture [49]. CAT, SOD, and GSH-Px are important antioxidant enzymes, and these can reduce oxidative stress [24,46]. MDA, a lipid oxidation stress product, is often negatively correlated with antioxidant levels [49]. Research has demonstrated that LAB can induce an elevation in antioxidant enzyme activity in various tissues of different animal species [50]. Similar results were captured in the current experiment that feeding *T. fulvidraco* with LAB, either fermented liquid or solid-state fermented feed, could raise the activity of antioxidant enzymes in the intestine and liver to a certain extent. It is worth mentioning that a contradictory phenomenon has been observed in MFLD and SSFD treatments, with a decrease in MDA in the liver and an increase in MDA in the intestine. Previous studies have shown that the microbiota may play a role in the association between diet and intestinal oxidative stress [51]. Considering the situation of the intestinal microbiota in this study, we speculate that this phenomenon may be related to changes in the intestinal microbiota. The specific reasons need further research. These phenomena suggest that feed treated with either solid-state fermented or fermented liquid mixture may improve the health of *T. fulvidraco* by strengthening the immune and antioxidant properties of the intestine and liver.

The rebuilding of intestinal microbiota through probiotics has long been a topic of interest, as healthy, stable intestinal microbiota is important for fish to absorb nutrients and resist foreign pathogens [52,53]. In this study, we aimed to investigate the effect of *Lactobacillus plantarum* fermented feed and fermented liquid on the intestinal microbiota of *T. fulvidraco* by observing the microbiota in different segments of the intestine. *Firmicutes*, *Fusobacteriota*, *Proteobacteria*, and *Bacteroidota* were the four dominant phyla in the intestine, regardless of diet and intestinal segment, which was consistent with previous research about *T. fulvidraco* [26,54]. Among these dominant phyla, *Proteobacteria* are well adapted to survive in the fish intestine and aquatic environment, and they contribute to significant host functions such as nutrition [55]. We observed that the relative abundance of *Proteobacteria* in MFLD and SSFD treatments apparently increased in the foreintestine, which may be beneficial for nutrient utilization of *T. fulvidraco*. Additionally, previous studies have identified *Cetobacterium*, which belongs to the *Fusobacteriota*, as the dominant genus in the intestine of *T. fulvidraco* [54,56]. Similar results were found in current research; nevertheless,

the *Cetobacterium* was distributed not only widespread throughout the entire intestine but also majorly in the hindintestine with a particularly high ratio of 50% above, which may be attributed to its anaerobic feature. Some studies have suggested that *Cetobacterium* is a potentially beneficial microbiota for fish [57,58]. Most studies proposed that the proportion of *Cetobacterium* in intestinal microbiota increased after some probiotics were applied, including *Bacillus* and *Enterococcus* [26,59,60]. Interestingly, the opposite result was observed with a significant decrease in the *Cetobacterium* ratio in the foreintestine when feeding a diet fermented with *Lactobacillus plantarum*, which was consistent with Standen's study showed that in tilapia fed a mixture of multiple probiotics, including *Lactobacillus reuteri* and *Enterococcus faecium*, the percentage of *Cetobacterium* decreased from 13.80% to 0.02% [61]. Further research revealed that *Cetobacterium* is an acid-sensitive microbiota; presumably, the significant decrease in *Cetobacterium* in the foreintestine of MFLD and SSFD treatments is due to the acidic feed affecting survival. These phenomena suggest that hindintestine would be preferential colonization for *Cetobacterium*. On the contrary, *Lactobacillus plantarum* did not colonize well in the intestine under the current experiment referring to the ratio of *Lactobacillus plantarum* in different intestine segments, probably attributed to the non-fish-derived LAB, which does not become permanent settlement in the intestine or just as transient flora. The analysis of microbial beta diversity indicated that the microbiota composition in different intestine segments was apparently changed only in the foreintestine of SSFD treatment, suggesting that *Lactobacillus plantarum* mainly acts on the anterior intestine. However, there is currently limited information available regarding the co-cultivation relationship and interactions between *Lactobacillus plantarum* and *Cetobacterium*, and corresponding research is needed to investigate this further. In addition to *Lactobacillus* and *Cetobacterium*, the genera *Bacillus*, *Acinetobacter*, and *Ralstonia* have been identified as beneficial bacterial groups in the fish intestine [62]. In this study, these bacterial genera were also observed in the experimental samples, and a noteworthy increase in the relative abundance of *Ralstonia* was observed in the foreintestine of SSFD treatment. The common pathogens of *T. fulvidraco* include *Edwardsiella*, *Pseudomonas*, *Aeromonas*, and *Vibrio* [63,64]. In this experiment, none of these pathogens were found to be dominant in the intestinal microbiota of *T. fulvidraco*, which may suggest that the health status of *T. fulvidraco* in this experiment is generally good.

5. Conclusions

This study provides evidence that solid-state fermented pelleted feed with *Lactobacillus plantarum* can promote the growth performance of *T. fulvidraco* by elevating the activity of multiple digestive enzymes and the immune and antioxidant capacity, as well as improving the morphology both in foreintestine and midintestine. Furthermore, SSFD can apparently change the microbiota composition in the foreintestine but not in the hindintestine. The dominant microbiota will be altered correspondingly among different intestinal segments while feeding with SSFD. In summary, solid-state fermented feed with *Lactobacillus plantarum* has advantages in improving the growth performance and intestinal biological function of *T. fulvidraco*, which could be popularized widely and prospectively in *T. fulvidraco* farming.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fishes9010018/s1, Table S1: Effect of different diets on relative abundance of intestinal microbiota on phylum level in *T. fulvidraco*; Table S2: Effect of different diets on relative abundance of intestinal microbiota on genus level in *T. fulvidraco*; Figure S1: Histologically observed intestine of *T. fulvidraco* fed with experiment diets.

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Institutional Review Board Statement: Throughout the experiment, the Animal Care and Use Committee of Yangzhou University approved our research on *T. fulvidraco* (ethical protocol code: YZUDWSY 2017-09-06), and we took all necessary measures to reduce any potential suffering of *T. fulvidraco*.

Data Availability Statement: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

Abbreviations

The following abbreviations are used in this manuscript:

AKP	alkaline phosphatase
ALT	alanine transaminase
ANFs	anti-nutritional factors
AST	aspartate transaminase
CAT	catalase
CF	condition factor
CFU	colony-forming unit
DM	dry matter
EAAs	essential amino acids
FBW	final body weight
FCR	feed conversion ratio
FCR	feed conversion ratio
GSH-Px	glutathione peroxidase
HE	hematoxylin–eosin
IBW	initial body weight
LAB	lactic acid bacteria
LZM	lysozyme
MDA	malonaldehyde
MFLD	commercial diet mixed with *Lactobacillus plantarum* fermented liquid
NEAAs	non-essential amino acids
PCA	principal component analysis
RAS	recirculatory aquaculture system
SCFAs	short-chain fatty acids
SD	standard deviation
SGR	specific growth rate
SOD	superoxide dismutase
SR	survival rate
SSF	solid-state fermented
SSFD	solid-state *Lactobacillus plantarum* fermented commercial diet
TAAs	total amino acids
TAOC	total anti-oxygen capacity
UCD	untreated commercial diet
VSI	viscerosomatic index
WGR	weight gain rate

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