fermentation

MDPI

Article

Diet Formulated with Rice Bran Fermented by *Rhizopus oryzae* and *Saccharomyces cerevisiae*: Impacts on Zootechnical Performance and Intestinal Gene Expression in Zebrafish (*Danio rerio*)

Gabriela Lenz [1], Rejane Macedo Martins [2], Jade Riet [3], Raíza dos Santos Azevedo [3], Arthur Cardoso [3], Bruna Félix da Silva Nornberg [3], Martín Bessonart [4], Larisa Magnone [4], Luis Fernando Fernandes Marins [3], Iuri Salim Abou Anni [3], Tatiane Penteado Gonçalves [3], Anelise Christ-Ribeiro [2] and Marcelo Borges Tesser [1,*]

[1] Laboratory of Nutrition of Aquatic Organisms, Institute of Oceanography (IO), Federal University of Rio Grande (FURG), Rua do Hotel, 2—Querência, Rio Grande 96210-030, RS, Brazil; gabrielaoceanologa@gmail.com
[2] Food Chemistry Laboratory, Federal University of Rio Grande (FURG), Av. Itália, Km 8, Rio Grande 96203-900, RS, Brazil; rejane.macedo@hotmail.com (R.M.M.); anelise.christ@hotmail.com (A.C.-R.)
[3] LEGENE—Research Group in Genetic Engineering and Biotechnology, Laboratory of Molecular Biology, Institute of Biological Sciences, Federal University of Rio Grande (FURG), Av. Itália, Km 8, Rio Grande 96203-900, RS, Brazil; jaderiet@hotmail.com (J.R.); raizasazevedo@gmail.com (R.d.S.A.); arthurcassio94@gmail.com (A.C.); brunanornberg@gmail.com (B.F.d.S.N.); luf@furg.br (L.F.F.M.); iuri.salim@furg.br (I.S.A.A.); tatiwok@gmail.com (T.P.G.)
[4] Institute of Ecology and Environmental Sciences, Faculty of Sciences, University of the Republic (UDELAR), Montevideo 11200, Uruguay; martinb@fcien.edu.uy (M.B.); larisa@fcien.edu.uy (L.M.)
* Correspondence: marcelotesser@furg.br

Abstract

The growing demand for aquaculture has driven the search for sustainable practices and utilization of agro-industrial residues. Brown rice bran, an abundant and low-cost by-product, has emerged as a promising raw material. This dissertation aimed to evaluate solid-state fermentation (SSF) of rice bran using the fungus *Rhizopus oryzae* and the yeast *Saccharomyces cerevisiae* with the goal of improving its nutritional value for use in diets formulated for zebrafish (*Danio rerio*). Proximate composition analyses revealed the strong biotransformation potential of *Rhizopus oryzae*. Fermentation with this fungus resulted in a significant 36.45% increase in protein content, a 51.62% increase in total polyphenols, and a 13.7% reduction in lipid content. In an in vivo experiment, zebrafish fed a diet containing rice bran fermented by *R. oryzae* showed the best zootechnical performance, with higher weight gain, specific growth rate, and improved feed conversion. Gene expression analysis showed a significant difference only for *glut6*, which is related to glucose transport. In summary, the fermentation of brown rice bran with *Rhizopus oryzae* represents an effective strategy to enhance its nutritional profile, establishing it as a viable alternative for the formulation of more sustainable and efficient diets in aquaculture.

Keywords: animal nutrition; agro-industrial by-product; proximate composition; *Rhizopus oryzae*; *Saccharomyces cerevisiae*; *Danio rerio*; rice fermentation; solid-state Fermentation

check for updates

Academic Editor: Tomas García-Cayuela

Received: 2 August 2025
Revised: 23 September 2025
Accepted: 29 September 2025
Published: 30 September 2025

Citation: Lenz, G.; Martins, R.M.; Riet, J.; Azevedo, R.d.S.; Cardoso, A.; Nornberg, B.F.d.S.; Bessonart, M.; Magnone, L.; Marins, L.F.F.; Anni, I.S.A.; et al. Diet Formulated with Rice Bran Fermented by *Rhizopus oryzae* and *Saccharomyces cerevisiae*: Impacts on Zootechnical Performance and Intestinal Gene Expression in Zebrafish (*Danio rerio*). *Fermentation* **2025**, *11*, 567. https://doi.org/10.3390/fermentation11100567

1. Introduction

Aquaculture has recently experienced accelerated growth, becoming an important productive activity worldwide [1]. This growth is supported by the formulation of appropriate

diets for each species, with adequate nutritional factors to reach their full potential. These diets were long based on fishmeal; however, besides its high cost and limited availability, fishmeal is insufficient to meet the increasing dietary demands of aquaculture [2–4]. As a result, there is a growing search for alternative feed ingredients, particularly agro-industrial by-products, which are low-cost and widely available [5]. According to the Agricultural Market Information System [6], rice (*Oryza sativa*) is the third most cultivated and consumed cereal in the world. Its production for the 2025/26 cycle is expected to reach an all-time high of 551.47 million tons. Rice processing generates the polished grain and a series of by-products, such as rice polish, broken rice, rice husk (RH), full-fat rice bran (FFRB), and, after oil extraction, defatted rice bran (DRB) [7]. It is estimated that global annual production of rice bran reaches 76 million tons [8], representing about 13% of total polished rice production. This by-product has various applications and is widely used in animal feed formulations for poultry, swine, and cattle [9]. Bran-based feed is included in different diets due to its diverse nutritional profile, rich in lipids, proteins, minerals, and carbohydrates, as well as containing B-complex, E, and K vitamins, phenolic compounds, tocopherols, and γ-oryzanol [10,11].

However, the use of rice bran in diets for aquatic vertebrates, such as fish, is limited due to the presence of antinutritional factors [12]. Studies indicate that rice bran contains phytic acid, which reduces mineral bioavailability, has low protein digestibility, and a high concentration of lipids with enzymatic activity (lipase, lipoxygenase, and peroxidase) that promote rancidity, in addition to instability during storage. Nevertheless, studies report that one way to increase the availability of nutrients in raw materials is through fermentation processes, which involve the use of microorganisms to promote transformations resulting from their metabolic activity [7,13]. Among the microorganisms used for this purpose, the fungus *Rhizopus oryzae* and the yeast *Saccharomyces cerevisiae* stand out [7,14]. These microorganisms are known for their fermentative capabilities and for promoting the biotransformation of compounds, enriching the substrate with essential nutrients such as amino acids, vitamins, and organic acids, and for reducing antinutritional factors [13]. The fungus *R. oryzae* is an important filamentous microorganism of great interest, as it is Generally Recognized As Safe (GRAS), meaning it is not toxigenic [15]. Notably, fermentation by this fungus activates endogenous phytases, reducing phytic acid content and increasing the protein content of rice bran [7]. Baker's yeast, *S. cerevisiae*, stands out as an excellent protein source, not only for its ability to synthesize proteins and other compounds, but also for its non-pathogenic nature, which allows its use in both human food and feed formulations [16]. *S. cerevisiae* offers significant advantages as a biotransformation agent, including the simplicity of its cultivation technique, which is widely known, its GRAS classification [17], and its contribution to the production of B-complex vitamins, improved protein profile, and reduced fiber content, resulting in a more digestible and nutritious ingredient for animal feeding [13,14].

The use of *Danio rerio* as an experimental model to test diets with fermented rice bran represents an effective approach to investigate intestinal effects of alternative ingredients through gene expression analyses, as this species is well-established in aquatic physiology and biotechnology, with a well-annotated genome, accessible genetic tools, low maintenance cost, and well-characterized reproductive and metabolic responses. Moreover, from a translational perspective, the knowledge gained can be adapted and applied to economically important aquaculture species, such as tilapia or pacu [18]. Furthermore, intestinal health is a central component in evaluating the nutritional quality of alternative feed ingredients for aquatic organisms. The intestinal epithelium serves as an immunological barrier and is directly involved in nutrient digestion and absorption, being sensitive to dietary changes. The expression of genes related to inflammatory processes (*il1b*, *ifng1*), antioxi-

dant activity (*sod1*, *cat*), and nutrient transport, such as peptides (*pept1*), has been widely used as a molecular tool to monitor physiological changes in fish [19,20]. Thus, the use of *R. oryzae* and *S. cerevisiae* in the fermentation of rice bran for animal feed production may not only add value to an agricultural by-product but also contribute to the development of healthier and more nutritious diets for fish, with positive impacts on the sustainability of aquaculture. Therefore, this study aims to evaluate the effects of fermenting full-fat rice bran with *R. oryzae* and *S. cerevisiae* on its nutritional composition and its impact on the zootechnical performance and gene expression in zebrafish (*D. rerio*).

2. Materials and Methods

2.1. Source of Inputs

Full-fat rice bran was obtained from a rice processing plant located in the southern region of Rio Grande do Sul State, Brazil. The filamentous fungus *Rhizopus oryzae* CCT 7560 was acquired from the André Tosello Tropical Foundation Culture Collection. *Saccharomyces cerevisiae* was purchased from local commerce (Fleischmann's Dry Yeast, AB Brasil Indústria e Comércio de Alimentos Ltda., Jundiaí, SP, Brazil) and used as the source of cells.

2.2. Solid-State Fermentation (SSF) of Rice Bran by Rhizopus oryzae

The fermentation process was carried out at the Mycotoxin Laboratory of the School of Chemistry and Food at FURG using full-fat rice bran as the substrate. *R. oryzae* CCT 7560 cultures were maintained on Potato Dextrose Agar (PDA) at 4 °C, and spores were incubated for 7 days at 30 °C. For biomass generation, a standardized methodology [21] was used, which consisted of adding a nutrient solution (KH_2PO_4, $MgSO_4$, NH_2CONH_2) and a spore suspension at an initial concentration of 4×10^6 spores per gram of bran. The moisture content of the medium was adjusted to approximately 50% by the addition of sterile water. The fermentation lasted 96 h at a constant temperature of 30 °C, and the obtained product was immediately frozen at −20 °C. Subsequently, the biomass was lyophilized for 48 h, ground, and then stored in a freezer until the experimental diets were formulared.

2.3. Fermentation of Rice Bran by Saccharomyces cerevisiae

For yeast fermentation, full-fat rice bran was spread in a 1 cm layer in tray bioreactors (29 cm × 17 cm × 5.5 cm) and sterilized. Subsequently, previously hydrated *Saccharomyces cerevisiae* yeast (3% *w*/*w*), was added, and the moisture content of the substrate was adjusted to 30% by adding sterile water. This process was conducted in an incubator with air circulation at 30 °C. A biomass aliquot was collected after 96 h of fermentation, ground, and stored in a freezer for later use in the formulation of experimental diets [22].

2.4. Proximate Composition of the Brans

The proximate composition analyses followed the methodologies described by the AOAC [23], as detailed in Table 1. Moisture content was determined by the gravimetric method using an oven (Marconi™ MA-035/3, Piracicaba, Brazil) at 105 °C for 5 h. Ash content was quantified by muffle furnace incineration (Marconi™ MA-385, Piracicaba, Brazil) at 600 °C for 5 h. Crude protein was determined by the micro Kjeldahl method (Marconi™ MA-036, Piracicaba, Brazil), while crude fiber was analyzed according to the method described by Silva and Queiroz [24]. Lipids were quantified by cold extraction following the Bligh and Dyer method [25].

Table 1. Formulation, proximate composition, and total polyphenol content of the experimental diets.

Ingredients (%)	Treatments		
	Control	**Yeast**	**Fungus**
Fish meal	32	32	32
Soybean meal	15	15	15
cornflour	12	12	12
Rice bran	25	0	0
Rice bran fermented by yeast	0	25	0
Rice bran fermented by fungus	0	0	25
Casein	10	10	10
Gelatin	2	2	2
Premix mineral and vitamin	2	2	2
Cellulose	2	2	2
TOTAL	100	100	100
Total polyphenol content (mg/g)	3.55 ± 0.02	3.72 ± 0.01	4.56 ± 0.05
Crude protein (%)	42.15 ± 0.27	44.16 ± 0.83	47.13 ± 0.67
Lipids (%)	10.4 ± 0.78	11.22 ± 0.42	10.03 ± 0.59
Humidity (%)	1.03 ± 0.05	4.31 ± 0.14	2.02 ± 0.02
Ash (%)	13.49 ± 0.10	13.35 ± 0.24	13.84 ± 0.25

Treatments: Control: Diet formulated with non-fermented rice bran; Yeast: Diet formulated with rice bran fermented with the yeast *Saccharomyces cerevisiae*; Fungus: Diet formulated with rice bran fermented with the fungus *Rhizopus oryzae*. Results are expressed as the mean ± standard deviation (n = 3).

Total polyphenols were quantified using the Folin–Ciocalteu colorimetric method, as described by Singleton and Rossi [26], with some modifications. Samples were reacted with Folin–Ciocalteu reagent and sodium carbonate (Na_2CO_3), and absorbance was measured with a spectrophotometer at 765 nm after the established reaction time. Results were expressed as gallic acid equivalents ($C_7H_6O_5$) in mg/g, based on a standard calibration curve.

2.5. Experimental Diet Formulation

Experimental diets were prepared at the Aquatic Organisms Nutrition Laboratory (LANOA). The ingredients were ground, sieved, and weighed in the appropriate proportions. Subsequently, water was added, and the mixture was homogenized manually. Finally, the resulting dough was pelleted using a meat grinder (Metalúrgica 9000, PC-22l, Piracicaba, Brazil), dried in an oven (Marconi, MA035, Piracicaba, Brazil) at 60 °C for 24 h, and stored in a freezer at −18 °C until use. Proximate composition analyses of the diets were performed according to AOAC [23] for moisture, protein, and ash, and Bligh and Dyer [25] for lipids. The total polyphenols content in the diets were measured according to Singleton and Rossi [26]. The formulation, proximate composition, and total polyphenol content of the diets are shown in Table 1.

2.6. Quantification of Fatty Acids (FA) in the Diets

Lipids were extracted from the samples as described by Folch et al. [27] and transesterified using sulfuric acid (1%) in methanol [28]. Butylated hydroxytoluene (BHT) (50 mg L^{-1}) was used to prevent fatty acid oxidation. The samples were incubated at 40 °C for 16 h in a nitrogen atmosphere. Subsequently, a hexane/ether solution (1:1 *v*/*v*) was used to extract the fatty acids, and potassium bicarbonate ($KHCO_3$) (20 g L^{-1}) was used to wash the hexane/ether solution. Finally, the fatty acids were dried for 24 h, diluted in chloroform at 30 mg mL^{-1}, and stored under nitrogen atmosphere at −20 °C until chromatographic analysis.

Fatty acids were quantified using a gas chromatograph (Hewlett Packard 5890, Wilmington, NC, USA) equipped with a fused silica capillary column (30 m × 0.32 mm internal

diameter; Supelco, Bellefonte, PA, USA). Nitrogen was used as the carrier gas, and injections were performed in the split mode. The injector and detector temperatures were both set at 250 °C. The initial temperature was 180 °C for 10 min, then increased to 212 °C at a rate of 2.5 °C/min and held at this temperature for 13 min. Chromatogram data processing was performed using Chromatography Station for Windows software (version CSW 1.7, Data Apex, Prague, Czech Republic).

2.7. Experimental Design

The biological assay was approved by the Animal Use Ethics Committee (CEUA) under protocol number 23116.007908/2024-28. The experiment consisted of three treatments: Control (diet containing non-fermented rice bran), Yeast (diet containing rice bran fermented with *Saccharomyces cerevisiae*), and Fungus (diet containing rice bran fermented with *Rhizopus oryzae*). Each group was assigned to a recirculating system and stocked with 60 fish, with an initial average weight of 0.09 ± 0.02 g for the control treatment, 0.09 ± 0.01 g for the yeast treatment, and 0.09 ± 0.008 g for the fungus treatment. The fish were randomly distributed into six aquaria (10 L) following the recommendations of [29], with a stocking density of 1 fish/L, totaling 10 fish per tank. For 47 days, fish were fed to apparent satiation twice daily.

The experiment was conducted in a recirculating system equipped with physical and biological filters, UV light and heater. To ensure water quality throughout the experiment, physicochemical parameters, such as pH, salinity, and conductivity were measured twice a week using a multiparameter probe (Akso, São Leopoldo, Brazil). Nitrite and total ammonia were analyzed on the same day using commercial kits (Labcon, Alcon, Camboriú, Brazil). The photoperiod was set to 12 h of light and 12 h of darkness. Water temperature was maintained at 25 °C and pH was maintained at 6.8. When necessary, pH corrections, cleaning, and siphoning of the aquariums were performed.

2.8. Biometry

Every 15 days, the animals were subjected to weighing measurements to evaluate the zootechnical performance in response to the experimental diets. For weighing measurements, fish were anesthetized by immersion in buffered tricaine methanesulfonate (MS-222, Sigma-Aldrich, Darmstadt, Germany), pH 7.2, at a dose of 100 mg·L^{-1}. Subsequently, they were individually weighed on a precision scale and measured in lateral recumbency by using a digital caliper to obtain the total length. After biometry, fish were returned to their respective tanks after confirming recovery of consciousness and swimming movements.

At the end of the experiment, all animals were subjected to a 24 h fasting period to empty the digestive tract. Subsequently, the final biometrics were performed, followed by euthanasia by immersion in tricaine at a dose of 400 mg·L^{-1}. Four individuals were randomly selected from each aquarium for intestinal tissue collection, totaling 24 fish per experimental group. The intestinal tissues were preserved in TRIzol reagent (Invitrogen, São Paulo, Brazil) and stored in an ultrafreezer at −80 °C until use in molecular analyses. The zootechnical performance was assessed using the following indices:

$$Weight\ Gain\ (WG, g) = Final\ Weight\ (FW, g) - Initial\ Weight\ (IW, g), \quad (1)$$

$$Survival\ (\%) = \left(\frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish}\right) \times 100, \quad (2)$$

$$Specific\ Growth\ Rate\ (SGR, \%) = \left[\frac{(lnFW - lnIW)}{number\ of\ days}\right] \times 100, \quad (3)$$

$$Feed\ Conversion\ Ratio\ (FCR, \frac{g}{g}) = \frac{Feed\ of\ fered\ (\mathrm{g})}{WG\ (\mathrm{g})}, \quad (4)$$

$$Condition\ Factor\ (CF) = \frac{Body\ Weight\ (\mathrm{mg})}{[Total\ Length\ (\mathrm{mm})^3]} \quad (5)$$

2.9. Total RNA Extraction and Complementary DNA (cDNA) Synthesis

Four individuals were randomly selected per aquarium for intestinal tissue collection, totaling 24 fish per experimental group. Total RNA extraction was performed using TRIzol reagent (Invitrogen, Brazil), following the manufacturer's protocol. To reduce individual variability and maintain statistical power, samples were pooled into groups of four intestines randomly selected within each treatment, resulting in six pools per experimental group (n = 6). The extracted RNA was treated with DNase I (Amplification Grade, Invitrogen, Brazil) to eliminate possible genomic DNA contamination. RNA quality and concentration were assessed by spectrophotometry (BioDrop, Isogen Life Science, BV, Veldzigt, The Netherlands) and RNAhile integrity was verified by 1% agarose gel electrophoresis. Subsequently, complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, São Paulo, Brazil), according to the manufacturer's instructions.

2.10. Quantitative PCR (qPCR)

To investigate the effects of the three experimental diets on zebrafish, the gene expression associated with different physiological processes was evaluated. The analyzed genes included (i) peptide transport—PepT1a (*slc15a2*) and PepT1b (*slc15a1b*); (ii) pro-inflammatory response—interferon gamma 1 (*ifng*) and interleukin 1 beta (*il-1b*); (iii) antioxidant response—superoxide dismutase 1 (*sod1*) and catalase (*cat*); and (iv) glucose transport—*glut2* and *glut6*. Gene expression quantification was performed by real-time quantitative PCR (qPCR) using the PowerUP SYBR Green Master Mix kit (Applied Biosystems), with the following thermal protocol: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s.

For relative expression normalization, the reference genes ef1α and rpl13a, which encode eukaryotic elongation factor 1 alpha and ribosomal protein L13 alpha, respectively, were used. Data analysis was conducted using the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen [30]. All the procedures were performed according to the manufacturer's recommendations, and the primer sequences used are listed in Table 2.

Table 2. Target genes and primer sequences used in the qPCR analysis.

Gene	Sequence (5′–3′)	Efficiency (%)	GenBank Accession
slc15a2	F: cacagccggagaagtcatgt R: gaacggatttcatgctcgcc	98	NM_001039828.1
slc15a1b	F: gcatctacgcaaagcagagc R: atgagggcaaccaccatgag	98	NM_198064.1
ifng1	F: acgcttgcaaaggattgggttgg R: acacagcctggcaagtgcagg	99	NM001020793
il-1b	F: ccacgtatgcgtcgcccagt R: gggcaacaggccaggtacagg	100	NM212844
sod1	F: caccgtctatttcaatcaagagg R: agaatgttggcctgacaaagtta	114	BC055516
cat	F: aacaacacccccatcttctttat R: atgtgtgtctgggtaggagaaaa	103	BC051626

Table 2. *Cont.*

Gene	Sequence (5′–3′)	Efficiency (%)	GenBank Accession
glut2	F: ttaacaggcacgctcgctct R: ttcatgctctgtgccatttcc	98	NM001042721
glut6	F: tttggcctgattttgccgtg R: gtggtaacgtggagaggtcg	98	XM684000
ef1α	F: caaaattggaggtattggaactgtac R: tcaacagacttgacctcagtggtt	99	NM131263
rpl13a	F: tctggaggactgtaagaggtatgc R: agacgcacaatcttgagagcag	99	NM212784

F: forward; R: reverse.

2.11. Statistical Analysis

Data were subjected to the Shapiro–Wilk test for normality and the Bartlett test for homogeneity of variances. When assumptions of normality and homoscedasticity were met, one-way analysis of variance (ANOVA) was applied to compare the experimental groups. Tukey's post hoc test was performed for multiple comparisons when significant differences were detected ($p < 0.05$). The diet fatty acid composition was analyzed by the Mann—Whitney test. The results are presented as mean ± standard deviation.

3. Results

3.1. Proximate Composition of Rice Bran

The results showed that rice bran fermented with *Rhizopus oryzae* presented higher crude protein content (21.26 ± 0.52%) compared to the control (15.58 ± 0.15%) and the bran fermented with *Saccharomyces cerevisiae* (16.18 ± 0.62%). A higher crude fiber content was also observed in the fungal fermentation treatment (16.43 ± 0.68%) compared to the control (6.81 ± 0.43%) and yeast treatment (10.03 ± 0.99%). The total polyphenol concentration was greater in the *R. oryzae*-fermented bran (9.24 ± 0.02 mg/g) compared to the control (4.47 ± 0.01 mg/g) and yeast-fermented bran (3.17 ± 0.05 mg/g). In contrast, lipid content was lower after fungal fermentation (15.46 ± 0.64%) compared to the control (17.91 ± 0.79%) and yeast treatment (17.20 ± 1.73%). These results indicate that fermentation with *R. oryzae* improves the nutritional and functional properties of rice bran (Table 3).

Table 3. Proximate composition (%) and total polyphenol content of the rice bran used in the experimental diets.

Component	Control	Yeast	Fungus
Ash %	9.48 ± 0.13 b	8.68 ± 0.09 a	9.65 ± 0.10 b
Crude protein %	15.58 ± 0.15 c	16.18 ± 0.62 bc	21.26 ± 0.52 a
Lipids %	17.91 ± 0.79 b	17.20 ± 1.73 ab	15.46 ± 0.64 a
Crude fiber %	6.81 ± 0.43 c	10.03 ± 0.99 b	16.43 ± 0.68 a
Total polyphenol content (mg/g)	4.47 ± 0.01 c	3.17 ± 0.05 b	9.24 ± 0.02 a

Treatments: Control: Non-fermented rice bran; Yeast: Rice bran fermented with the yeast *Saccharomyces cerevisiae*; Fungus: Rice bran fermented with the fungus *Rhizopus oryzae*. Means followed by different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$). Values are expressed on a dry matter basis and presented as mean ± standard deviation (n = 3).

3.2. Fatty Acids

The fatty acid compositions of the diets are presented in Table 4. A progressive reduction in the levels of saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids was observed in the diets containing rice bran fermented by *S. cerevisiae* and *R. oryzae* compared to the control diet.

Regarding the long-chain polyunsaturated fatty acids (PUFAs) of the n3 and n6 series, which are essential for fish, the n6 content was higher in the control diet (1.38 ± 0.01), with a decrease in the *S. cerevisiae* (1.15 ± 0.05) and *R. oryzae* (1.02 ± 0.01) treatments. The n3 fatty acids showed comparable levels among the treatments, with a slightly higher n3 HUFA value in the *R. oryzae* group (0.53 ± 0.02). The n6/n3 ratio, an important indicator of lipid balance, was lower in *R. oryzae* treatment (1.64 ± 0.03).

The EPA/DHA ratio remained close to 1.3 across all treatments, suggesting a balanced proportion between these two highly unsaturated long-chain fatty acids, which are crucial for fish development and health.

Table 4. Fatty acid composition (mg/g) of the different diets containing rice bran fermented with fungus or yeast.

Fatty Acids	Control	Yeast	Fungus
8:00	0.08 ± 0.06 ac	0.06 ± 0.01 ab	0.09 ± 0.01 c
10:0	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.00
11:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12:0	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
13:0	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
14:0	1.33 ± 0.02 a	1.27 ± 0.07 a	1.40 ± 0.01 b
14:1n-5	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
15:0	0.25 ± 0.01 a	0.24 ± 0.02 a	0.27 ± 0.01 b
15:1n-5	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.00
16:0	14.12 ± 0.02 a	12.36 ± 0.59 b	11.62 ± 0.06 c
16:1n-9	0.23 ± 0.00 a	0.22 ± 0.03 a	0.28 ± 0.01 b
16:1n-7	2.83 ± 0.02 a	2.80 ± 0.13 a	3.05 ± 0.01 b
16:1n-5	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
16:2	0.13 ± 0.00	0.13 ± 0.01	0.15 ± 0.00
16:2n-4	0.03 ± 0.00	0.02 ±0.02	0.05 ± 0.00
17:0	0.30 ± 0.01 a	0.29 ± 0.01 a	0.32 ±0.02 b
17:1n-7	0.17 ± 0.00	0.17 ± 0.00	0.20 ± 0.02
16:3n-4	0.15 ± 0.00	0.15 ± 0.00	0.16 ± 0.01
16:4	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.00
18:0	2.75 ± 0.01 a	2.62 ± 0.13 b	2.79 ± 0.02 a
18:1n-11	0.04 ± 0.01	0.03 ± 0.00	0.06 ± 0.00
18:1n-9	18.66 ± 0.12 a	16.38 ± 0.76 b	15.41 ± 0.13 c
18:1n-7	1.39 ± 0.02 a	1.31 ± 0.06 b	1.44 ± 0.00 c
18:1n-5	0.06 ± 0.00	0.05 ± 0.01	0.06 ± 0.00
18:2n-6	12.55 ± 0.07 a	10.25 ± 0.44 b	8.57 ± 0.05 c
18:2n-4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:3n-6	0.07 ± 0.00	0.09 ± 0.01	0.33 ± 0.02
18:3n-4	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
18:3n-3	0.94 ± 0.01 a	0.82 ± 0.07 b	0.69 ± 0.01c
18:4n-3	0.17 ± 0.00	0.17 ± 0.00	0.19 ± 0.00
18:4n1	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
20:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:1n-11	0.26 ± 0.01 a	0.25 ± 0.00 a	0.28 ± 0.00 b
20:1n-9	0.62 ± 0.02 a	0.58 ± 0.01 b	0.65 ± 0.00 c
20:1n-7	0.12 ± 0.01	0.12 ± 0.01	0.14 ± 0.00
20:2n-9	0.16 ± 0.00	0.15 ± 0.01	0.14 ± 0.00
21:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:3n-6	0.10 ± 0.00	0.10 ± 0.02	0.10 ± 0.02
20:4n-6 (ARA)	0.55 ± 0.01 a	0.56 ± 0.02 a	0.61 ± 0.01 b
20:3n-3	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00
20:4n-3	0.12 ± 0.01 a	0.11 ± 0.01 a	0.13 ± 0.01 b
22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:5n-3 (EPA)	1.82 ± 0.01 a	1.76 ± 0.10 a	1.95 ± 0.05 b
22:1n-11	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
22:1n-9	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 0.00

Table 4. *Cont.*

Fatty Acids	Control	Yeast	Fungus
22:1n-7	0.00 ± 0.01	0.07 ± 0.01	0.06 ± 0.00
22:2n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
21:5n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
23:0	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.00
22:4n-6	0.18 ± 0.00	0.17 ± 0.01	0.19 ± 0.01
22:5 n-6	0.20 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
24:0	0.25 ± 0.01 a	0.24 ± 0.00 b	0.30 ± 0.01 c
22:5n-3 (DPA)	0.57 ± 0.01 a	0.56 ± 0.02 a	0.63 ± 0.01 b
24:1n-9	0.11 ± 0.00 a	0.12 ± 0.00 a	2.63 ± 0.10 b
22:6n-3 (DHA)	2.33 ± 0.03 a	2.26 ± 0.13 a	2.62 ± 0.10 b
SAFA	19.22 ± 0.09 a	17.21 ± 0.84 b	16.90 ± 0.06 b
MUFA	24.99 ± 0.04 a	22.50 ± 1.03 b	22.22 ± 0.09 b
PUFA	20.23 ± 0.01 a	17.65 ± 0.88 b	16.92 ± 0.11 b
n-9	19.77 ± 0.08 a	17.45 ± 0.81 b	16.64 ± 0.11 c
n-6	13.81 ± 0.05 a	11.50 ± 0.49 b	10.19 ± 0.07 c
n-3	5.95 ± 0.05 a	5.68 ± 0.33a	6.21 ± 0.19 b
n-3 HUFA	4.84 ± 0.06 a	4.70 ± 0.26 a	5.33 ± 0.17 b
n-6/n-3	2.32 ± 0.03 a	2.03 ± 0.03 b	1.64 ± 0.06 c
DHA/EPA	1.28 ± 0.01 a	1.28 ± 0.00 a	1.34 ± 0.02 c

Treatments: Control: Diet formulated with non-fermented rice bran; Yeast: Diet formulated with rice bran fermented with the yeast *Saccharomyces cerevisiae*; Fungus: Diet formulated with rice bran fermented with the fungus *Rhizopus oryzae*. SAFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids (n = 3). Means followed by different letters in the same row indicate significant differences according to Mann–Whitney test ($p < 0.05$).

3.3. Zootechnical Performance

The results show significant differences in the evaluated zootechnical parameters (Table 5). The group fed rice bran fermented by *Rhizopus oryzae* exhibited the best zootechnical performance, with the highest weight gain (0.127 ± 0.02 g) and the highest specific growth rate (1.78 ± 0.27%), which differed significantly from the other treatments ($p < 0.05$).

Table 5. Effects of the diets on the zootechnical performance parameters of *D. rerio* fed experimental diets for 47 days.

	Control	Yeast	Fungus
IW (g)	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.008
FW (g)	0.17 ± 0.037 b	0.18 ± 0.02 ab	0.22 ± 0.02 a
WG (g)	0.075 ± 0.01 b	0.091 ± 0.02 b	0.127 ± 0.02 a
SGR (%)	1.30 ± 0.16 b	1.40 ± 0.20 b	1.78 ± 0.27 a
S (%)	100	100	100
FCR (g/g)	1.09 ± 0.10 b	0.82 ± 0.18 a	0.71 ± 0.09 a
CF	1.84 ± 0.11 a	1.64 ± 0.08 b	1.82 ± 0.05 a

Treatments: Control: Diet formulated with non-fermented rice bran; Yeast: Diet formulated with rice bran fermented by *Saccharomyces cerevisiae*; Fungus: Diet formulated with rice bran fermented by *Rhizopus oryzae*. IW: Initial weight; FW: Final weight; WG: Weight gain; SGR: Specific growth rate; S: Survival; FCR: Feed Conversion Ratio; CF: Condition factor. Means followed by different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$). Data are expressed as the mean ± standard deviation (n = 6).

The survival rate was 100% in all the groups, indicating suitable cultivation conditions. Regarding feed conversion, the highest value was observed in the *Rhizopus* group (0.71 ± 0.09 g/g). The condition factor of the fish was similar between the Control and *Rhizopus* groups, but lower in the yeast group.

3.4. Gene Expression

The results obtained for the expression of PepT1a (*slc15a2*), PepT1b (*slc15a1b*), *ifng1*, *il1b*, *sod1*, *cat*, and *glut2* were not significantly different (Figure 1a–g).

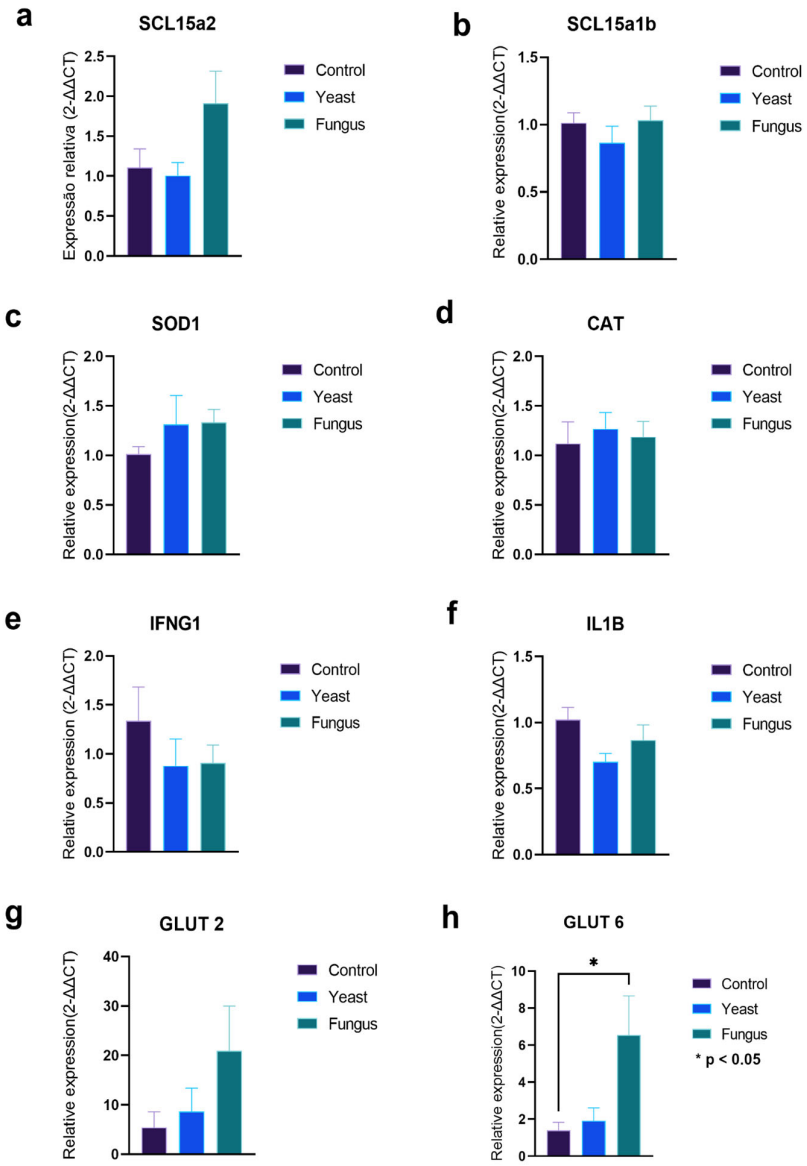

Figure 1. (**a**,**b**) Relative expression of genes related to peptide transport, PepT1a (*slc15a2*) and PepT1b (*slc15a1b*). (**c**,**d**) Relative expression of genes related to antioxidant response—superoxide dismutase 1 (*sod1*) and catalase (*cat*). (**e**,**f**) Relative expression of genes related to pro-inflammatory response, interferon gamma 1 (*ifng*) and interleukin 1 beta (*il-1b*). (**g**,**h**) Relative expression of genes related to glucose transport, *glut2* and *glut6*. The bars represent the mean expression of each gene per treatment with standard error. Treatments showing statistically significant differences are indicated by asterisk (n = 24).

The expression of the *glut6* gene was significantly higher in the group treated with *R. oryzae* than in the Control and Yeast groups (Figure 1h). This indicated that the diet fermented with the fungus positively modulated the expression of *glut6*, a glucose transporter potentially associated with cellular uptake processes or metabolic responses.

4. Discussion

4.1. Bioconversion of Macromolecules in Rice Bran

The increase in protein content observed in rice bran in the present study, especially in the *Rhizopus oryzae* treatment (21.26% vs. 15.58% in the control), is fundamentally attributed to the synthesis of nitrogen-rich microbial biomass during Solid-State Fermentation (SSF) and the preferential consumption of non-protein components. The superiority of *Rhizopus*

over yeast (*Saccharomyces cerevisiae*, 16.18%) in increasing protein content highlights its robust bioconversion capacity in the SSF environment, corroborating previous findings [31]. For aquaculture, this protein enhancement is highly relevant, since alternative ingredients with improved protein quality are crucial to reducing the dependence on more expensive and limited resources, such as fishmeal. This nutritional improvement resulted in better zootechnical performance in *Danio rerio*, as demonstrated by the increase in weight gain and specific growth rate. This benefit extends to other commercially valuable species, such as tilapia and shrimp, in which dietary protein optimization is a cornerstone of productivity and sustainability [5,32,33].

In line with the fungal mechanism of catabolizing carbon sources for energy and anabolism, the reduction in lipid content observed in rice bran fermented by *Rhizopus* (from 17.91% to 15.46%, a 13.7% reduction) can be attributed to the lipolytic activity of its enzymes [34]. Although this reduction is more moderate than some reported in the literature [35], it reflects lipid modulation that can positively impact feed stability and the fatty acid profile available to fish. The balance of polyunsaturated fatty acids, especially those of the n-3 series, is vital for fish development and health [36], and fermentation may optimize the bioavailability of these essential nutrients, as suggested by the lipid profile of the tested diets.

Beyond macronutrient bioconversion, fermentation with *R. oryzae* promoted a remarkable increase in total polyphenol content (from 4.47 mg g^{-1} in the control to 9.24 mg g^{-1}), reinforcing the potential of this process to release and synthesize bioactive phenolic compounds [18,37]. These compounds, known for their antioxidant and anti-inflammatory properties, play an important functional role. The high polyphenol content in the feed may explain the absence of *sod1* and *cat* expression, since these compounds exhibit direct antioxidant properties. In other words, if fish received a polyphenol-rich diet, the internal oxidative pressure may have been so low that there was no need to activate these endogenous antioxidant genes. Thus, the presence of polyphenols contributes to overall stress resistance and health maintenance—an increasingly desirable benefit in intensive aquaculture diets, where oxidative stress can be a major challenge [38,39].

Additionally, rice bran fermentation resulted in an increase in crude fiber content, with the *R. oryzae* treatment showing the highest value (16.43%). Interestingly, this increase did not negatively affect the growth of *Danio rerio*, suggesting that fiber properties were modified by microbial action. This modified fiber may act as a prebiotic, stimulating beneficial intestinal microbiota and promoting the production of short-chain fatty acids that nourish enterocytes and contribute to intestinal barrier integrity. This mechanism, fundamental for digestive health, is relevant to various aquatic species and may explain part of the improvement in zootechnical performance observed [13,40]. Microbiota modulation may also influence the expression of nutrient transporter genes, such as *glut6*, whose expression was significantly increased in the *R. oryzae* group, indicating greater activation of glucose uptake and transport, evidence of improved metabolic efficiency [41,42].

4.2. Characterization of the Dietary Lipid Profile

Lipid profile analysis of the diets revealed interesting variations among the treatments. A reduction in saturated fatty acids (SAFA) was observed in the diets with fermented bran compared with the control diet. Massarolo [43] also observed a reduction in saturated fatty acids (SAFA). However, the most significant difference was observed at the level of highly unsaturated fatty acids, where the *Rhizopus* diet showed higher levels of polyunsaturated fatty acids, especially of the n3 series and, in particular, n3 HUFA, which seems to enhance the contribution of these fatty acids derived from fishmeal. This point is particularly important because it is well known that fish depend on these sources of fatty acids and on

the beneficial effects that even small increases, such as the one observed, can have on most species, both marine and freshwater [36].

4.3. Zootechnical Performance

After a 47-day experimental period, fish fed diets fermented with *Rhizopus oryzae* and *Saccharomyces cerevisiae* showed significant improvements in zootechnical performance compared with the control group. The groups treated with fermented diets exhibited higher final weights: 0.22 ± 0.02 g in the *R. oryzae* group and 0.18 ± 0.02 g in the *S. cerevisiae* group, compared to 0.17 ± 0.037 g in the control. Weight gain was also higher in the *R. oryzae* group (0.127 ± 0.02 g) than in the control group (0.075 ± 0.01 g), representing an increase of 69.33%, followed by the yeast group (0.091 ± 0.02 g). Similarly, the specific growth rate (SGR) was significantly higher in the fungal group (1.78 ± 0.27%), followed by the yeast group (1.40 ± 0.20%), compared to the control (1.30 ± 0.16%), corresponding to a 36.92% increase in the *R. oryzae* group.

Survival was 100% for all treatments, indicating suitable cultivation conditions during the experiment. Regarding feed conversion (FC), the *R. oryzae* group showed the best index (0.71 ± 0.09), followed by the yeast group (0.82 ± 0.18) and control (1.09 ± 0.10), demonstrating greater efficiency in diet utilization. The condition factor was similar between the control and *R. oryzae* groups, whereas the *S. cerevisiae* group had a lower value. These performance improvements may be attributed to the beneficial effects of fermentation, which acts as an effective strategy to increase protein content, improve palatability and digestibility of rice bran, and reduce antinutritional factors such as phytic acid and tannins, especially when carried out by filamentous fungi [7,13,31]. In addition, this process promotes the release of metabolites with potential functional effects, among which stand out organic acids (such as lactic and acetic acids), bioactive peptides, phenolic compounds, and molecules with antioxidant activity [44,45]. These compounds may contribute to improved nutrient digestibility, modulation of the intestinal microbiota, and promotion of gut health in fish, which helps to explain, at least in part, the superior zootechnical performance observed [46].

In this context, the increase in fiber content resulting from fermentation, particularly in the *R. oryzae* treatment, emerges as another relevant factor to explain the improvement in zootechnical performance. Although carnivorous fish have a limited capacity to utilize complex carbohydrates, the structural modification of fibers during fermentation may have enhanced their prebiotic effect, stimulating the proliferation of lactic acid bacteria (LAB) in the intestinal tract [46].

Studies have shown that microorganisms used for fermentation can modulate the intestinal microbiota of fish, with the most common changes observed after the administration of fermented ingredients being an increase in LAB concentration, while reducing the proliferation of pathogenic bacteria [46]. In a study with Atlantic salmon fed fermented sunflower meal, a significant increase in *Lactiplantibacillus* and *Lactobacillaceae* was observed [47]. Juvenile turbot (*Scophthalmus maximus*) fed soybean meal fermented with *Enterococcus faecium* showed a significant increase in *Lactobacillus* and the anti-inflammatory bacterium *Faecalibaculum* [48]. This is likely due to the acidifying effects of fermented meals in the intestine, which create ideal conditions for LAB colonization [49]. In addition, a study by Wang et al. [50] found that the solid-state fermentation of rice bran and soybean meal with *Bacillus subtilis* increased the abundance of gut health-promoting bacteria *Fusobacteriota* and *Cetobacterium* in zebrafish (*Danio rerio*).

Furthermore, studies report that the fermentation of these fibers by the microbiota results in the production of short-chain fatty acids, which exert a trophic effect on enterocytes, strengthen the intestinal barrier, and modulate metabolic pathways related to energy

utilization efficiency [51,52]. This scenario may partially explain the significant increase in *glut6* gene expression in the group treated with *R. oryzae*, suggesting the activation of a compensatory mechanism of greater glucose uptake in response to the higher availability of fermentable carbohydrates. Thus, the interaction among diet composition, intestinal microbiota activity, and gene expression regulation emerges as a robust explanatory axis for the superior zootechnical performance observed. The functional impact of fermented fiber transcends its quantitative effect on proximate composition, involving a cascade of metabolic and physiological processes of high adaptive relevance.

4.4. Gene Expression Analysis

Analysis of intestinal gene expression using real-time PCR provided important molecular insights, reaffirming the benefits of diets containing fermented rice bran. Notably, the diet treated with the fungus *Rhizopus oryzae* promoted a significant increase in the expression of the gene *glut6*, glucose transporter. This finding suggests a greater activation of glucose uptake and transport, contributing to the observed metabolic efficiency. Although *glut6* is primarily associated with glucose transport, its ability to mediate the transport of other hexoses, such as fructose and mannose [41], supports the role of fermentation in the release and utilization of simple sugars [42], optimizing the modulation of gene expression related to carbohydrate metabolism in fish intestines. Conversely, the expression of the gene *glut2* showed no statistically significant differences among the groups.

No significant differences were observed in the expression of peptide transporters between treatments. Peptide transporters play an essential role in the absorption of di- and tripeptides in fish intestines and are modulated by various factors such as diet composition, nutritional status, and environmental conditions [53]. Their regulation may be species-specific and highly dependent on the physiological context.

For example, in tilapia, Con et al. [54] demonstrated that the expression and localization of peptide transporters along the intestine vary according to environmental salinity, demonstrating the fine and multifactorial control of gene expression. In the present study, the lack of a significant effect of fermented diets on gene expression of these transporters suggests that although fermentation may alter nutrient availability, these changes might not have been sufficient to induce measurable transcriptional regulation of peptide transporter genes in *Danio rerio* under the experimental conditions adopted.

The evaluation of pro-inflammatory gene expression of *il1b* and *ifng1* aimed to verify the possible immunological effects of diets formulated with fermented rice bran, and the results showed no significant differences in the expression of these genes compared to the control group, which can be positively interpreted as indicating that the tested ingredients did not promote intestinal inflammation or excessive immune response activation.

The gene *il1b* is a classical inflammatory marker in fish and is related to neutrophil activation, cytokine production, and cellular recruitment to inflammation sites [55]. The *ifng1* gene encodes interferon gamma, a pro-inflammatory cytokine essential for the adaptive immune response, particularly in macrophage activation and Th1 response modulation [19]. The absence of induction of these genes suggests that the fermented residues did not trigger a detectable inflammatory response, thus being immunologically safe.

According to Coronado et al. [19], diet can directly modulate the intestinal environment of zebrafish and influence inflammatory gene expression. However, the impact strongly depends on diet composition, presence of antinutritional factors, associated microbiota, and intestinal mucosal integrity. Thus, the results of this study indicate that diets with fermented rice bran are nutritionally safe, as they do not induce differential expression of *il1b* and *ifng1*, genes associated with intestinal inflammation.

Antioxidant enzymes *sod1* and *cat* are essential components of the defense system against reactive oxygen species (ROS), which are generally modulated by environmental, inflammatory, or nutritional stress [38]. However, the lack of differential regulation of *sod1* and *cat* genes among groups indicates that diets formulated with fermented rice bran were well tolerated by the animals, without inducing ROS accumulation or triggering transcriptional activation of the antioxidant response.

These findings partially contrast with those of Zhang et al. [20], who observed increased antioxidant activity and expression of oxidative stress-related genes in juvenile coho salmon (*Oncorhynchus kisutch*) fed fermented soybean meal. Therefore, the maintenance of basal expression of *sod1* and *cat* in different groups indicated that the inclusion of fermented rice bran, both by *S. cerevisiae* and *R. oryzae*, did not compromise the oxidative balance in *Danio rerio*. These results are positive from the nutritional and physiological viewpoints, suggesting that these ingredients can be used without the risk of oxidative stress induction in fish maintained under normal rearing conditions.

The results demonstrated the efficiency of rice bran fermentation and its application in an aquatic vertebrate, with emphasis on the group treated with *R. oryzae* compared to other experimental groups. These findings indicate the potential of the composition and bioavailability of nutrients in fermented diets.

Author Contributions: Conceptualization, G.L.; methodology, G.L., B.F.d.S.N., L.F.F.M., A.C.-R. and M.B.T.; validation, G.L., M.B.T. and A.C.-R.; formal analysis, G.L.; investigation, G.L., R.M.M., J.R., R.d.S.A., A.C., B.F.d.S.N., M.B., L.M., I.S.A.A. and T.P.G.; writing—original draft preparation, G.L., A.C.-R. and M.B.T.; writing—review and editing, G.L., A.C.-R., L.F.F.M., M.B. and M.B.T.; supervision, A.C.-R. and M.B.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Brazilian Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Education Personnel (CAPES, code 001).

Institutional Review Board Statement: The study was conducted in accordance with the Animal Use Ethics Committee (CEUA) under protocol number 23116.007908/2024-28 (approval date: 31 July 2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors thanks Marcelo B. Tesser and Luis Fernando Marins receive a productivity research fellowship from CNPq (305112/2023-6; 307304/2022-1) respectively.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO. *The State of World Fisheries and Aquaculture 2024*; FAO: Rome, Italy, 2024. Available online: https://www.fao.org/publications (accessed on 11 June 2025).
2. *OECD-FAO Agricultural Outlook 2025–2034, Chapter 7: Fish and Other Aquatic Products*; OECD Publishing: Paris, France; FAO: Rome, Italy, 2025. [CrossRef]
3. Al Khawli, F.; Martí-Quijal, F.J.; Ferrer, E.; Ruiz, M.J.; Berrada, H.; Gavahian, M.; Barba, F.J.; de la Fuente, B. Aquaculture and Its By-Products as a Source of Nutrients and Bioactive Compounds. *Adv. Food Nutr. Res.* **2020**, *92*, 1–33. [CrossRef]
4. Lenz, G.; Ayres, T.S.M.; Tesser, M.B.; Christ-Ribeiro, A. Rice bran as an agro-industrial by-product in aquaculture: Types, properties, and applications. *Trop. Anim. Health Prod.* **2025**, *57*, 402. [CrossRef]
5. He, Y.; Guo, X.; Tan, B.; Dong, X.; Yang, Q.; Liu, H.; Zhang, S.; Chi, S. Replacing fish meal with fermented rice protein in diets for hybrid groupers (*Epinephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂): Effects on growth, digestive and absorption capacities, inflammatory-related gene expression, and intestinal microbiota. *Aquac. Rep.* **2021**, *19*, 100603. [CrossRef]

6. AMIS—Agricultural Market Information System. *AMIS Market Monitor, No. 129, June 2025*; AMIS Secretariat: Rome, Italy, 2025. Available online: https://www.amis-outlook.org (accessed on 11 June 2025).
7. Christ-Ribeiro, A. Fermentation process in the availability of nutrients in rice bran. *Res. Rev. J. Microbiol. Biotechnol.* **2017**, *6*, 45–52.
8. Kahlon, T.S. Chapter 14. Rice bran: Production, composition, functionality and food applications, physiological benefits. In *Fiber Ingredients: Food Applications and Health Benefits*; Cho, S.S., Samuel, P., Eds.; CRC Press: Boca Raton, FL, USA, 2009; pp. 305–321.
9. Manlapig, J.; Matsui, H. Production and utilization of fermented rice bran as animal feed. *Anim. Sci. J.* **2025**, *96*, e70037. [CrossRef]
10. Bhatnagar, A.S.; Prabhakar, D.S.; Prasanth Kumar, P.K.; Raja Rajan, R.G.; Gopala Krishna, A.G. Processing of commercial rice bran for the production of fat and nutraceutical rich rice brokens, rice germ and pure bran. *LWT-Food Sci. Technol.* **2014**, *58*, 306–311. [CrossRef]
11. IAFFD—International Aquaculture Feed Formulation Database. Available online: https://www.iaffd.com/ (accessed on 16 June 2025).
12. Pastore, S.C.G.; Gaiotto, J.R.; Ribeiro, F.A.S.; Nunes, A.J.P. Boas práticas de fabricação e formulação de rações para peixes. In *NutriAqua: Nutrição e Alimentação de Espécies de Interesse Para a Aquicultura Brasileira*; Fracalossi, D.M., Cyrino, J.E.P., Eds.; Sociedade Brasileira de Aquicultura e Biologia Aquática: Florianópolis, Brazil, 2016; Volume 1, Chapter 16; pp. 295–346.
13. Feddern, V.; Furlong, E.B.; Soares, L.A.S. Effects of fermentation on the physicochemical and nutritional properties of rice bran. *Ciência E Tecnol. Aliment.* **2007**, *27*, 800–804. [CrossRef]
14. Magalhães, A.P.L.; Teixeira, A.Z.A. Análises organolépticas e proteica da fermentação do farelo de arroz com a levedura *Saccharomyces cerevisiae*. *Rev. Ibero-Am. Humanidades Ciências E Educ.* **2020**, *6*, 9. [CrossRef]
15. Londoño-Hernandez, L.; Ruiz, H.A.; Ramírez, C.; Ascacio, J.A.; Rodríguez-Herrera, R.; Aguilar, C.N. Fungal detoxification of coffee pulp by solid-state fermentation. *Biocatal. Agric. Biotechnol.* **2020**, *23*, 101467. [CrossRef]
16. Rodrigues, A.M.; Sant'Anna, E.S. Effect of sodium chloride on protein production (*Saccharomyces cerevisiae*) in semi-solid fermentation. *Ciência E Tecnol. Aliment.* **2001**, *21*, 57–62. [CrossRef]
17. Aquarone, E.; Borzani, W.; Schmidell Netto, W.; Lima, U.D.A. *Biotecnologia Industrial—Biotecnologia na Produção de Alimentos*; Edgard Blücher: São Paulo, Brazil, 2001; p. 523.
18. Liu, N.; Wang, Y.; An, X.; Qi, J. Study on the enhancement of antioxidant properties of rice bran using mixed-bacteria solid-state fermentation. *Fermentation* **2022**, *8*, 212. [CrossRef]
19. Coronado, M.; Solis, C.J.; Hernandez, P.P.; Feijóo, C.G. Dietary modulation of intestinal inflamemation in zebrafish: Insights into the cellular and molecular mechanisms. *Front. Immunol.* **2019**, *10*, 610. [CrossRef]
20. Zhang, Q.; Li, F.; Guo, M.; Qin, M.; Wang, J.; Yu, H.; Xu, J.; Liu, Y.; Tong, T. Growth performance, antioxidant and immunity capacity were significantly affected by feeding fermented soybean meal in juvenile coho salmon (*Oncorhynchus kisutch*). *Animals* **2023**, *13*, 945. [CrossRef]
21. Christ-Ribeiro, A.; Graça, C.S.; Kupski, L.; Badiale-Furlong, E.; Souza-Soares, L.A. Cytotoxicity, antifungal and antimycotoxin effects of phenolic compounds from fermented rice bran and *Spirulina* sp. *Process Biochem. Process Biochem.* **2019**, *80*, 190–196. [CrossRef]
22. Christ-Ribeiro, A.; da Silva Graça, C.; Massarolo, K.C.; Jaeschke, D.P.; de Souza Soares, L.A. Production of high-value-added biomass by *Saccharomyces cerevisiae* using lignocellulosic substrate. *Fermentation* **2025**, *11*, 257. [CrossRef]
23. AOAC—Association of Official Analytical Chemists. *Official Methods of Analysis*, 18th ed.; AOAC: Gaithersburg, MD, USA, 2007.
24. Silva, D.J.; Queiroz, A.C. *Análise de Alimentos: Métodos Químicos e Biológicos*, 3rd ed.; Universidade Federal de Viçosa: Viçosa, MG, Brazil, 2006; p. 235.
25. Bligh, E.C.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef]
26. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
27. Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
28. Christie, W.W. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*; Pergamon Press: Oxford, UK, 1982.
29. Schneider, A.C.R.; Maurer, R.L.; Matte, U.; Porawski, M.; Schaefer, P.G.; dos Santos, J.L.; da Silveira, T.R. Implementação de um novo modelo de experimentação animal—Zebrafish. *Clin. Biomed. Res.* **2009**, *29*, 100–103.
30. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef]
31. Oliveira, M.D.S.; Feddern, V.; Kupski, L.; Cipolatti, E.P.; Badiale-Furlong, E.; de Souza-Soares, L.A. Physico-chemical characterization of fermented rice bran biomass. *CyTA-J. Food* **2010**, *8*, 229–236. [CrossRef]
32. Zhang, Q.; Guo, M.; Li, F.; Qin, M.; Yang, Q.; Yu, H.; Xu, J.; Liu, Y.; Tong, T. Evaluation of fermented soybean meal to replace a portion fish meal on growth performance, antioxidant capacity, immunity, and mTOR signaling pathway of coho salmon (*Oncorhynchus kisutch*). *Aquac. Nutr.* **2023**, *2023*, 2558173. [CrossRef]

33. Albuquerque, L.F.G. Zootechnical and Economic Performance of *Penaeus vannamei* Culture Using the Aquamimicry System. Ph.D. Thesis, Federal University of Ceará, Fortaleza, Brazil, 2019.
34. Helal, S.E.; Abdelhady, H.M.; Abou-Taleb, K.A.; Hassan, M.G.; Amer, M.M. Lipase from *Rhizopus oryzae* R1: In-depth characterization, immobilization, and evaluation in biodiesel production. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 1. [CrossRef]
35. Silveira, C.M.; Badiale-Furlong, E. Characterization of nitrogenated compounds in solid-state fermented bran. *Ciênc. Tecnol. Aliment.* **2007**, *27*, 805–811. [CrossRef]
36. Parrish, C.C. Essential Fatty Acids in Aquatic Food Webs. In *Lipids in Aquatic Ecosystems*; Arts, M.T., Brett, M.T., Kainz, M., Eds.; Springer: New York, NY, USA, 2009; pp. 309–326. [CrossRef]
37. Kupski, L.; Cipolatti, E.; Rocha, M.; Oliveira, M.S.; Souza-Soares, L.A.; Badiale-Furlong, E. Solid-state fermentation for the enrichment and extraction of proteins and antioxidant compounds in rice bran by *Rhizopus oryzae*. *Braz. Arch. Biol. Technol.* **2012**, *55*, 937–942. [CrossRef]
38. Shi, Q.; Xiong, X.; Wen, Z.-Y.; Qin, C.; Li, R.; Zhang, Z.; Gong, Q.; Wu, X. Cu/Zn Superoxide Dismutase and Catalase of Yangtze Sturgeon, *Acipenser dabryanus*: Molecular Cloning, Tissue Distribution and Response to Fasting and Refeeding. *Fishes* **2022**, *7*, 35. [CrossRef]
39. Mansoori, A.; Allaf Noveirian, H.; Hoseinifar, S.H.; Sajjadi, M.; Ashouri, G.; Imperatore, R.; Paolucci, M. Polyphenol-rich extracts enhance growth, immune function, and antioxidant defense in juvenile rainbow trout (*Oncorhynchus mykiss*). *Front. Nutr.* **2024**, *11*, 1487209. [CrossRef] [PubMed]
40. Ng'onga, L.; Amoah, K.; Chen, H.; Huang, Y.; Wang, B.; Shija, V.M.; Mpwaga, A.Y.; Fachri, M.; Cai, J.; Adjei-Boateng, D. The metabolism and antioxidant properties of probiotics and prebiotics in fish: A review. *Front. Mar. Sci.* **2025**, *12*, 1622474. [CrossRef]
41. Manolescu, A.R.; Witkowska, K.; Kinnaird, A.; Cessford, T.; Cheeseman, C. Facilitated hexose transporters: New perspectives on form and function. *Physiology* **2007**, *22*, 234–240. [CrossRef]
42. Lennartsson, P.R.; Taherzadeh, M.J.; Edebo, L. Rhizopus. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Tortorello, M.L., Eds.; Academic Press: London, UK, 2014; pp. 284–290.
43. Massarolo, J. Cultivo de *Rhizopus Oryzae* em Farelo de Arroz Integral e Seus Impactos na Fração Lipídica. Master's Thesis, Universidade Federal do Rio Grande, Rio Grande, Brasil, 2016; p. 92.
44. Nagarajan, M.; Rajasekaran, B.; Venkatachalam, K. Metabólitos microbianos em produtos alimentícios fermentados e seus benefícios potenciais. *Int. Food Res. J.* **2022**, *29*, 466–486. [CrossRef]
45. Yao, W.; Gong, Y.; Li, L.; Hu, X.; You, L. Os efeitos das fibras alimentares do farelo de arroz e do farelo de trigo na microbiota intestinal: Uma visão geral. *Food Chem. X* **2022**, *13*, 100252. [CrossRef]
46. Siddik, M.A.B.; Julien, B.B.; Islam, S.M.M.; Francis, D.S. Fermentation in Aquatic Feed Processing: Achieving Sustainability in Global Aquaculture Feed Production. *Rev. Aquac.* **2024**, *16*, 1244–1265. [CrossRef]
47. Eide, L.H.; Morales-Lange, B.; Kuiper, R.V.; Dale, O.B.; Rocha, S.D.C.; Djordjevic, B.; Øverland, M. Fermented Sunflower Meal in Diets for Atlantic Salmon under Commercial Farming Conditions Promotes Intestinal Lactic Acid Bacteria and Controls Distal Intestine Inflammation. *Aquaculture* **2024**, *595*, 741517. [CrossRef]
48. Li, C.; Zhang, B.; Liu, C.; Zhou, H.; Wang, X.; Mai, K.; He, G. Effects of Dietary Raw or *Enterococcus faecium* Fermented Soybean Meal on Growth, Antioxidant Status, Intestinal Microbiota, Morphology, and Inflammatory Responses in Turbot (*Scophthalmus maximus* L.). *Fish Shellfish Immunol.* **2020**, *100*, 261–271. [CrossRef]
49. Catalán, N.; Villasante, A.; Wacyk, J.; Ramírez, C.; Romero, J. Fermented Soybean Meal Increases Lactic Acid Bacteria in the Intestinal Microbiota of Atlantic Salmon (*Salmo salar*). *Probiotics Antimicrob. Proteins* **2018**, *10*, 566–576. [CrossRef]
50. Wang, A.; Meng, D.; Hao, Q.; Xia, R.; Zhang, Q.; Ran, C.; Yang, Y.; Li, D.; Liu, W.; Zhang, Z.; et al. Effect of Supplementation of Solid-State Fermentation Product of *Bacillus subtilis* HGcc-1 to High-Fat Diet on Growth, Hepatic Lipid Metabolism, Epidermal Mucus, Gut and Liver Health and Gut Microbiota of Zebrafish. *Aquaculture* **2022**, *560*, 738542. [CrossRef]
51. Kasubuchi, M.; Hasegawa, S.; Hiramatsu, T.; Ichimura, A.; Kimura, I. Dietary Gut Microbial Metabolites, Short-Chain Fatty Acids, and Host Metabolic Regulation. *Nutrients* **2015**, *7*, 2839–2849. [CrossRef]
52. Zhang, Y.; Liang, X.-F.; He, S.; Chen, X.; Wang, J.; Li, J.; Zhu, Q.; Zhang, Z.; Li, L.; Alam, M.S. Effects of Diet-Modulated Microbiota on the Health of Chinese Perch (*Siniperca chuatsi*) under a Carbohydrate-Rich Diet. *Front. Microbiol.* **2020**, *11*, 575102. [CrossRef]
53. Vacca, F.; Barca, A.; Gomes, A.; Mazzei, A.; Piccinni, B.; Cinquetti, R.; Del Vecchio, G.; Romano, A.; Rønnestad, I.; Bossi, E.; et al. The peptide transporter 1a of zebrafish *Danio rerio*, an emerging model in nutrigenomics and nutrition research: Molecular characterization, functional properties and expression analysis. *Genes Nutr.* **2019**, *14*, 33. [CrossRef]

54. Con, P.; Nitzan, T.; Cnaani, A. Salinity-dependent shift in the localization of three peptide transporters along the intestine of the Mozambique tilapia (*Oreochromis mossambicus*). *Front. Physiol.* **2017**, *8*, 1039. [CrossRef] [PubMed]
55. Yan, B.; Han, P.; Pan, L.; Lu, W.; Xiong, J.-W.; Zhang, M.; Zhang, W.; Li, L.; Wen, Z. Il-1β and reactive oxygen species differentially regulate neutrophil directional migration and basal random motility in a zebrafish injury–induced inflammation model. *J. Immunol.* **2014**, *192*, 5998–6008. [CrossRef] [PubMed]