

Use of yacon (*Smallanthus sonchifolius*) juice byproduct as an additive in fish diets: Response of growth performance, antioxidant status, and disease resistance juvenile black rockfish (*Sebastes schlegelii*) to different feeding strategies

Jin Choi

National Institute of Fisheries Science

Hwa Yong Oh

Gyeongsang National University

Tae Hoon Lee

Gyeongsang National University

Gyu Jin Lee

Gyeongsang National University

Hee Sung Kim (✉ bluesonn@gnu.ac.kr)

Gyeongsang National University

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Abstract

Yacon juice byproduct (YJB) is regarded waste, but it contains bioactive compounds and can be used as an additive in fish feed. This study evaluated the effects of feeding strategy of YJB on the growth performance, digestive and antioxidant capacity, and disease resistance of rockfish. 480 juvenile rockfish (15.5 ± 0.01 g) were distributed into tanks. Four different YJB feeding strategies were evaluated in triplicate: feeding with the basal diet continuously (control, T0), feeding with YJB continuously (T1), feeding with YJB for 1 day and the basal diet for the next day (T2), and feeding with YJB for 1 day and the basal diet for the following 2 days (T3). At the end of 8 week feeding experiment, T1 and T2 groups exhibited enhancement in growth performance compared with T0 and T3 groups. Intestinal digestive enzyme activity was higher in T1 and T2 than in T0 and T3. Lysozyme and antioxidant capacity were higher all YJB feeding regimens than the control treatment. The survival rates in all YJB treatment groups after the challenge with *Streptococcus iniae* were enhanced. In conclusion, offering YJB at day-to-day intervals is recommended to improve growth performance, digestive and antioxidant status, and disease resistance.

Introduction

The provision of functional feed additives is becoming an increasingly common alternative method for increasing aquaculture production and preventing disease (Faggio et al. 2015; Guardiola et al. 2016; Hoseinifar et al. 2018; Dawood et al. 2019; Elumalai et al. 2021). In particular, phytogenic nutraceutical-based feeds are regarded as important for fish farming due to their functions in enhancing growth performance, feed digestion, immunity, and resistance to biotic and abiotic stresses (Reverter et al. 2014; Suttili et al. 2018; Elumalai et al. 2020; Paray et al. 2021; Dawood et al. 2022). Plant-based feed additives (essential oils, plant extracts, and plant food industry byproducts) are superior to other additives owing to their high levels of organic substances, which have no impact on fish and human health and do not disrupt the environment or ecology (Ahmadifar et al. 2021; Tsiplakou et al. 2021).

Plant byproducts, a plant-based feed additive, are mainly derived from the vegetables and fruits used to manufacture some products, such as juice, jam, and canned food (Leyva-López et al. 2020). Byproducts from the agricultural industry have garnered considerable attention because they pose a constant threat as environmental contaminants and are a significant operational issue for the food sector (Tsiplakou et al. 2021). Nonetheless, byproducts from the agricultural industry, such as the pomaces of apple, tomato, citrus, and grape, might be a source of potentially useful substances that could be utilized as functional supplements in animal feed across the food chain (Beigh et al. 2015; Davies et al. 2020; Painsi et al. 2021; Tsiplakou et al. 2021). The use of plant byproducts in organic livestock has been allowed recently (Schmidt, 2019). In aquatic animal culture, the above feeding approach converts byproducts or waste from plant food processing into high-value seafood and contributes to the lowering of the feed-to-food competition ratio in the aquatic animal production chain, which has important technical consequences for the entire food chain. Several studies have investigated the possibility of using plant food processing byproducts in aquafeeds (Baldișsera et al. 2019; Harikrishnan et al. 2021; Dawood et al. 2022). Frosi et al.

(2021) reported that plant byproducts from food processing contain several active nutrients, such as polyphenols, flavonoids, tannins, anthocyanins, pigments, essential oils, minerals, fatty acids, and bioactive peptides. These bioactive chemicals contribute to a variety of pharmacological actions that help activate the antioxidative, immunological, and antistress responses of aquatic animals (Ben-Othman et al. 2020; Leyva-López et al. 2020). Plant byproducts from food processing are also abundant in polysaccharides, which are considered prebiotic supplements necessary for enhancing intestinal microbial balance, digestibility, and local intestinal immunity (Samavat et al. 2019; Ahmadniaye Motlagh et al. 2020).

Yacon (*Smallanthus sonchifolius*) is a plant with tuberous roots that is native to the Andes and widely distributed in South America (Marques et al. 2020). It is recognized as a functional food primarily because of its fructan components (fructo-oligosaccharides [FOS] and inulin), phenolic compounds, and flavonoids (Ojansivu et al. 2011). The advantages of yacon have attracted attention worldwide (Delgado et al. 2012) because consumers are increasingly becoming interested in foods that provide health benefits beyond nutrition. In producing countries, yacon roots are used to make a variety of products, including flour, dehydrated products, slices or chips, tea (dry leaves), juices, purees, and sweeteners (Delgado et al. 2012). In Korea, yacon is either usually consumed fresh or processed into juice (Yan et al. 2019). A substantial amount of pomace is produced during the production of yacon juice. Nevertheless, most of the by-products generated during the production of yacon juice were typically regarded as trash and are intended for disposal.

However, Oh et al. (2023) reported that yacon juice byproduct (YJB) contains 9.8 g/100 g FOS, 270.3 gallic acid mg/100 g total phenolics, and 364.8 gallic acid mg/100 g flavonoids. On the basis of these components, the supplementation of 2.5 g/kg YJB in black rockfish (*Sebastes schlegelii*) feed improved growth performance, digestive activity, antioxidant status, and nonspecific immunity. Lee et al. (2021) also showed that YJB improved growth performance, antioxidant capacity, and disease resistance against *Vibrio anguillarum* as a functional phytofeed additive for the *S. schlegelii* diet.

S. schlegelii is considered as a commercially important cultured marine species in East Asian countries, including China, Japan, and Korea (Woo, 2022; Xu et al. 2022). Intensive culture systems, which are common in *S. schlegelii* farming, frequently induce stressful conditions that reduce fish growth and well-being. Feeding costs account for approximately two-thirds of the overall cost of fish farming worldwide. The application of excessive feed additives, in addition to the expense of regular production, decreases the profitability of fish culture. Furthermore, the continuous application of functional feed additives, such as phyto-additives, probiotics, and prebiotics, increases the expense of the fish-culturing process, and finding a balance between the positive benefits and the desired outcome is highly suggested (Amphan et al. 2019; Dawood et al. 2019; Tachibana et al. 2020). Amphan et al. (2019) reported that feeding Nile tilapia (*Oreochromis niloticus*) every other 2 weeks or every 4 days is the optimal choice for immunostimulant administration. Dawood et al. (2019) stated that *Aspergillus oryzae* administered every 2 days enhanced the growth of fish and advised supplying this supplement with day-to-day intervals to enhance the growth performance, digestive enzyme activity, intestinal histomorphology, and blood health

of *O. niloticus*. Developing an optimal feeding strategy with low cost and high efficiency based on the type of additive, target fish species, and required dietary dose is necessary. Hence, examining the efficiency of feed additive administration via various feeding strategies is of increasing importance. However, the effects of phyto-additive feeding frequency on the performance of *S. schlegelii* are unknown. Thus, this study aims to investigate the effect of YJB feeding regime on the growth performance, intestinal digestive enzyme activity, antioxidant enzyme activity, and resistance against *Streptococcus iniae* in *S. schlegelii*.

Materials and methods

Fish and experimental diets

Juvenile *S. schlegelii* were obtained from a commercial hatchery (Namhae-gun, Gyeongsangnam-do, Korea). The present experiment was carried out at the Marine Bio-Education and Research Center, Gyeongsang National University (Tongyeong, Gyeongsangnam-do, Korea). Fish were kept for 2 weeks in a 1.5-ton round polyethylene tank and fed with commercial extruded pellets (Jeil Feed Co., Haman, Gyeongsangnam-do, Korea; 52% crude protein and 10% crude lipids) prior to the feeding trial. After the completion of acclimatization, 480 juvenile rockfish with an initial average weight of 15.5 ± 0.01 g (means $\pm$ SD) were randomly assigned to 12 tanks (water: 250 L) with 3 replicates per group (40 fish per tank). The tanks had a water flow rate of 2.7 L/min.

Fresh yacon was purchased from a local market in Daegu, Korea, and then transported to a private Health Juice store (Youngjin, Daegu, Korea) to produce the YJB. Before being air-dried at room temperature, yacon was washed with tap water to remove soil. The dry yacon was ground with a grinder (WP3500A, WONPOOL, Gwangju, Korea) before being pressed with a juice extractor (KR-70, Koryeo, Daegu, Korea). The YJB was then transported to the laboratory, where it was dried at 20°C using an agricultural product dryer (KED-M07D1, Kiturami Co. Ltd., Seoul, Korea) and ground using a domestic blender. The YJB was kept at 4°C before preparing the experimental diets.

The basal diet was formulated (Table 1). The YJB diet was supplemented with dry YJB powder at the dose of 2.5 g/kg (Table 1). The dose of YJB was set in reference to studies on juvenile black rockfish (Oh et al. 2023). All dry ingredients were mechanically combined well into a homogenous mixture to formulate the experimental diets. Then, fish and soybean oils and distilled water were added to the mixture to achieve a uniform texture appropriate for pelleting (3.0–4.0 mm pellets) by using a chopper (3.0 mm diameter, SL Machinery, Incheon, Korea). The obtained pellets were then dried at 20°C in an agricultural product dryer (KED-M07D1, Kiturami Co. Ltd., Seoul, Korea) for 48 h. Then, the experimental diets were stored at – 20°C until use.

Table 1
Experimental diet formulation (g/kg, dry matter basis)

Experimental diets		
	Basal diet	YJB
Jackmackerel meal	520	520
Fermented soybean meal	120	120
Wheat flour	255	252.5
YJB ^a	0	2.5
Fish oil	40	40
Soybean oil	40	40
Vitamin premix ^b	10	10
Mineral premix ^c	10	10
Choline	5	5
<i>Proximate composition (g/kg)</i>		
Dry matter	932	932
Crude protein	478	480
Crude lipid	133	132
Ash	91	96
^a YJB (yacon juice byproduct) supplied by Youngjin Health Food Store (Daegu, Korea).		
^b Vitamin premix contained the following contents diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL- α -tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; <i>p</i> -aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.		
^c Mineral premix contained the following ingredients (g/kg mix): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 80.0; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 370.0; KCl, 130.0; ferric citrate, 40.0; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, 0.15; KI, 0.15; $\text{Na}_2\text{Se}_2\text{O}_3$, 0.01; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 2.0; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1.0.		

Feeding trial

Two sets of the experimental diets (basal diet and YJB-supplemented diet) were formulated. Four groups were designed with different feeding strategies, wherein the first group was fed the basal diet continuously (T0), the second group was fed the YJB diet continuously (T1), the third group was fed the

YJB diet for 1 day and the basal diet the next day (T2), and the fourth group was fed the YJB diet for 1 day and the basal diet for the next 2 days (T3) for 8 weeks. The fish were hand fed twice a day at 08:00 and 17:00. Fecal matter was removed, and the amount of feed consumed by the fish in each tank was recorded daily. Water quality parameters were monitored daily throughout the feeding trial. Water temperature, dissolved oxygen, and salinity were measured by using a YSI-Pro20 (YSI Inc., Yellow Springs, OH, USA). The average rearing water characteristics during the trial were as follows: water temperature of $22.9 \pm 0.90^{\circ}\text{C}$, dissolved oxygen of $7.6 \pm 0.34 \text{ mg/L}$, and salinity of $31.49 \pm 0.24 \text{ psu}$.

Growth performance and feed utilization

The length and weight of all the fish in each tank were individually measured at the end of the feeding trial. For this purpose, all fish were fasted for 24 h and anesthetized by using 150 ppm tricaine methanesulfonate MS-222 (Sigma-Aldrich, USA). Finally, growth performance and feed utilization were calculated in accordance with the following formulas:

Survival (SR, %) = (number of fish at the end of the trial/number of fish at the beginning of the trial) $\times$ 100

Weight gain (WG, g/fish) = final body weight – initial body weight

Specific growth rate (SGR, %/day) = $[\ln \text{ final weight of fish} - \ln \text{ initial weight of fish} / \text{days of feeding}] \times 100$

Feed consumption (FC, g/fish) = total dry feed intake/number of surviving fish

Feed efficiency (FE) = WG of fish/feed consumed

Protein efficiency ratio (PER) = WG of fish/protein consumed

Condition factor (CF) = Fish weight $\times$ 100/total length³

Blood sampling

Blood samples were taken from the caudal veins of 5 fish per tank (15 fish per group) by using a heparin-coated syringe and centrifuged in a microtube centrifuge at $2000 \times g$ for 10 min. Plasma samples were collected and stored at -80°C until biochemical assays and antioxidant enzyme analysis. For serum collection, blood was collected from the caudal vein of another 3 fish per tank by syringes without anticoagulant and allowed to clot for 30 min, centrifuged in a microtube centrifuge at $3000 \times g$ for 5 min and then stored at -80°C for lysozyme activity analysis.

Proximate whole-body composition

To analyze the chemical composition, a homogenized paste was prepared by finely chopping and processing the whole bodies of 5 fish sampled from each tank. The Kjeldahl digestion method was used to determine the crude protein ($\text{N} \times 6.25$) content using a KD310–A–1015 KjelROC Analyzer (OPSIS Liquid LINE, Sweden). The Soxhlet extraction method to evaluate the crude lipid composition, using a

Soxtec extractor (ST 243 Soxtec™; FOSS, Hillerod, Denmark). The moisture and ash contents were analyzed through oven drying at a temperature of 105°C for 24 hours and using a muffle furnace at a temperature of 600°C for 4 hours, respectively.

Plasma biochemical indices

An automatic chemistry system (Fuji Dri-Chem NX500i; Fujifilm, Tokyo, Japan) to determine different plasma biochemical parameters, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (T-CHO), total protein (TP), and glucose (GLU).

Lysozyme and antioxidant enzyme activities

A turbidimetric assay was performed in accordance with Lange et al. (2001) to measure serum lysozyme activity. In short, lysozyme activity was measured by adding 100 µL of test blood to a 1.9 mL suspension of *Micrococcus lysodeikticus* (0.2 mg/mL; Sigma, St. Louis, MO, USA) in 0.05 M sodium phosphate buffer (pH 6.2). At 25°C, reactions took place, and a spectrophotometer (Thermo Fisher Scientific, Tewksbury, MA, USA) was used to measure absorbance at 530 nm between 0 and 60 min. As the lysozyme activity unit, the amount of enzyme required to generate a 0.001/min reduction in absorbance was considered.

The activities of superoxide dismutase (SOD) and catalase (CAT) and the concentration of glutathione (GSH) in plasma samples were determined by using a commercial kit (Cayman's Assay Kit, Cayman Chemical, Ann Arbor, MI) in accordance with instruction of manual. Using a spectrophotometer (Thermo Scientific MULTISKAN GO, Vantaa, Finland), absorbance was measured.

Digestive enzyme measurements

The intestines of 5 fish per tank were dissected to obtain samples. These samples were then homogenized in a solution of ice-cold 0.86% physiological saline at a ratio of 10 volumes to weight. In an ice bath, the homogenization process was carried out using a TissueLyser II (QIAGEN, Netherlands). The resulting mixture was then centrifuged at 13,000 rpm for 10 min at 4°C to obtain the supernatant. The enzymatic activities of amylase, trypsin, and lipase were assessed using a commercially available kit (Abcam, UK), following the guidelines stated in the supplementary manual.

S. iniae challenge

Twenty fish were randomly selected from each tank to conduct a challenge test. The chosen fish were subsequently redistributed into the tanks. The *S. iniae* (FP5024) strain was obtained from the Korean Culture Collection of Aquatic Microorganisms, National Institute of Fisheries Science (Busan, Korea). The fish were subjected to artificial infection through intraperitoneal injection using a 0.1 mL pathogenic *S. iniae* culture suspension with a concentration of 5.0×10^6 CFU/mL. The water temperature was consistently held at $20.5 \pm 0.15^\circ\text{C}$ (mean $\pm$ SD), and the concentrations of dissolved oxygen were at 7.1 ± 0.24 mg/L. The daily survival rate of fish was documented over 12 days post-infection. Fish mortality was recorded at 12-hour intervals throughout the observation period.

Statistical analyses

The data were presented as the mean $\pm$ standard error. Before analysis, all percentage data were arcsine converted. The homogeneity of variances among treatments was tested using Levene's test. One-way analysis of variance (ANOVA) and Tukey's HSD multiple range tests to assess the mean differences among various groups, with a significance level of $P < 0.05$. Fish survival during the 12-days post-observation period after artificial *S. iniae* injection was analyzed using Kaplan–Meier survival curve, Log-rank, and Wilcoxon tests. The statistical analyses were conducted using the SPSS version 27.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Growth performance

The growth and feed utilization indices of juvenile rockfish subjected to different feeding strategies with the dietary supplementation of YJB are shown in Table 2. After the 8-week feeding trial, SR did not differ among treatments ($P > 0.05$). However, final body weight (FBW), WG, and SGR in the T1 and T2 groups had significantly ($P < 0.05$) enhanced compared with those in the T0 and T3 groups. Furthermore, the FC, FE, and PER in the T1 and T2 groups had significantly ($P < 0.05$) improved compared with those in the T0 and T3 groups. No significant differences in CF, VSI, and HSI were observed among all groups.

Table 2

Growth performance of juvenile rockfish under different feeding regimens with the YJB-supplemented diet

	Experimental diets				<i>P</i> -value
	T0	T1	T2	T3	
IBW (g)	15.5 ± 0.02 ^a	15.5 ± 0.01 ^a	15.5 ± 0.03 ^a	15.5 ± 0.01 ^a	0.441
FBW (g)	37.3 ± 0.35 ^a	40.9 ± 0.27 ^c	39.7 ± 0.14 ^b	38.0 ± 0.08 ^a	0.001
SR (%)	98.3 ± 1.67 ^a	98.3 ± 1.67 ^a	100.0 ± 0.00 ^a	98.3 ± 1.67 ^a	0.802
WG (g/fish)	21.8 ± 0.34 ^a	25.4 ± 0.28 ^c	24.2 ± 0.13 ^b	22.5 ± 0.08 ^a	0.001
SGR (%/day)	1.79 ± 0.019 ^a	1.98 ± 0.015 ^c	1.92 ± 0.007 ^b	1.83 ± 0.004 ^a	0.001
CF	1.70 ± 0.036 ^a	1.74 ± 0.033 ^a	1.72 ± 0.021 ^a	1.72 ± 0.011 ^a	0.695
VSI (%)	10.45 ± 0.101 ^a	10.48 ± 0.202 ^a	10.50 ± 0.35 ^a	10.51 ± 0.096 ^a	0.988
HSI (%)	2.69 ± 0.044 ^a	2.72 ± 0.010 ^a	2.73 ± 0.073 ^a	2.71 ± 0.026 ^a	0.911
FC (g/fish)	24.0 ± 0.64 ^a	27.1 ± 0.80 ^b	25.7 ± 0.19 ^b	24.4 ± 0.31 ^a	0.008
FE	0.92 ± 0.004 ^a	0.95 ± 0.004 ^b	0.94 ± 0.002 ^b	0.92 ± 0.004 ^a	0.046
PER	1.89 ± 0.024 ^a	1.96 ± 0.040 ^b	1.96 ± 0.004 ^b	1.92 ± 0.025 ^a	0.011
Values (means of triplicate ± SE) in the same row sharing different superscript letters are significantly different at $P < 0.05$. Abbreviations: IBW, initial body weight; FBW, final body weight; SR, survival; WG, weight gain; SGR, specific growth rate; CF, condition factor; VSI, viscerosomatic index; HSI, hematosomatic index; FC, feed consumption; FE, feed efficiency; PER, Protein efficiency ratio.					

Proximate composition of whole-body and plasma biochemical parameters

Table 3 presents the effects of different feeding strategies with the dietary supplementation of YJB on the chemical whole-body composition and plasma biochemical indices of fish are shown in Table 3. No significant difference was detected in the moisture, crude protein, crude lipid, ash, AST, ALT, T-CHO, TP, and GLU of fish between treatments ($P > 0.05$).

Table 3

Proximate composition (% wet weight basis) and blood biochemical parameters of juvenile rockfish under different feeding strategies with the YJB-supplemented diet

	Experimental diets				<i>P</i> -value
	T0	T1	T2	T3	
Moisture (%)	70.0 ± 0.13 ^a	69.8 ± 0.21 ^a	70.6 ± 0.21 ^a	69.9 ± 0.12 ^a	0.059
Crude protein (%)	14.8 ± 0.12 ^a	14.9 ± 0.15 ^a	15.0 ± 0.26 ^a	15.1 ± 0.41 ^a	0.797
Crude lipid (%)	8.4 ± 0.12 ^a	8.3 ± 0.12 ^a	8.6 ± 0.36 ^a	8.5 ± 0.06 ^a	0.746
Ash (%)	4.2 ± 0.17 ^a	4.2 ± 0.03 ^a	4.1 ± 0.17 ^a	4.1 ± 0.12 ^a	0.896
AST (U/L)	38.7 ± 3.18 ^a	34.3 ± 4.33 ^a	36.3 ± 3.53 ^a	32.3 ± 7.42 ^a	0.821
ALT (U/L)	22.7 ± 2.85 ^a	23.7 ± 2.40 ^a	24.0 ± 0.58 ^a	26.3 ± 0.67 ^a	0.601
T-CHO (mg/dL)	171.7 ± 3.93 ^a	173.0 ± 4.62 ^a	179.7 ± 7.62 ^a	172.0 ± 3.79 ^a	0.681
TP (g/dL)	4.2 ± 0.32 ^a	4.3 ± 0.41 ^a	4.9 ± 0.26 ^a	4.2 ± 0.35 ^a	0.421
GLU (mg/dL)	113.7 ± 5.36 ^a	111.0 ± 5.00 ^a	110.3 ± 3.53 ^a	117.0 ± 4.93 ^a	0.753
Values (means of triplicate ± SE) in the same column sharing different superscript letters are significantly different (<i>P</i> < 0.05).					
Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-CHO, total cholesterol; TP, total protein; GLU, glucose.					

Lysozyme and antioxidant enzyme activities

The lysozyme, SOD, and CAT activities and GSH content of rockfish under each treatment are presented in Table 4. The serum lysozyme activity in T1 group, followed by that in the T2 and T3 groups, was significantly higher (*P* < 0.05). The plasma SOD and GSH in the T1 and T2 groups, followed by those in the T3 group, were significantly (*P* < 0.05) higher than in T0 group. Among the parameters assessed in this study, lysozyme, SOD, and GSH were lowest in the T0 group (*P* < 0.05). CAT activity in the T1, T2, and T3 groups had significantly (*P* < 0.05) increased compared with that in the control group (T0) without any differences among different YJB feeding regimens.

Table 4

Serum lysozyme activity and antioxidant status of juvenile rockfish under different feeding regimens with the YJB-supplemented diet

	Experimental diets				<i>P</i> -value
	T0	T1	T2	T3	
Lysozyme (U/mL)	1.34 ± 0.019 ^a	1.63 ± 0.022 ^c	1.47 ± 0.010 ^b	1.49 ± 0.046 ^b	0.000
SOD (U/mL)	1.50 ± 0.065 ^a	2.43 ± 0.065 ^c	2.38 ± 0.056 ^c	1.68 ± 0.035 ^b	0.000
CAT (nmol/min/mL)	315.4 ± 3.26 ^a	342.6 ± 3.36 ^b	335.4 ± 4.21 ^b	334.4 ± 3.84 ^b	0.004
GSH (μM)	2.39 ± 0.342 ^a	5.82 ± 0.642 ^c	5.73 ± 0.690 ^c	3.73 ± 0.316 ^b	0.004
Values (means of triplicate ± SE) in the same column sharing different superscript letters are significantly different (<i>P</i> < 0.05).					
Abbreviations: SOD, superoxide dismutase; CAT, catalase; GSH, glutathione.					

Intestinal digestive enzyme activities

The effects of different feeding strategies with the dietary supplementation of YJB on the activities of intestinal digestive enzymes, including amylase, trypsin, and lipase, are presented in Table 5. All digestive enzyme activities were significantly (*P* < 0.05) higher in the T1 and T2 groups than in the T0 and T3 groups.

Table 5

Digestive enzyme activities of juvenile rockfish under different feeding regimens with the YJB-supplemented diet

	Experimental diets				<i>P</i> -value
	T0	T1	T2	T3	
Amylase (U/L)	32.2 ± 1.83 ^a	46.9 ± 2.20 ^b	49.4 ± 1.68 ^b	32.4 ± 1.60 ^a	0.000
Trypsin (U/L)	33.0 ± 3.26 ^a	55.8 ± 2.30 ^b	54.0 ± 3.08 ^b	39.8 ± 3.76 ^a	0.002
Lipase (U/L)	48.2 ± 2.51 ^a	76.2 ± 9.08 ^b	67.9 ± 2.29 ^b	50.3 ± 4.23 ^a	0.015
Values (means of triplicate ± SE) in the same column sharing different superscript letters are significantly different (<i>P</i> < 0.05).					

Challenge test

Figure 1 shows the survival rate of fish artificially infected with *S. iniae* 12 days after infection. The survival rates of fish fed with YJB and challenged with *S. iniae* were significantly (*P* < 0.05) higher than

those of the control group. Fish fed with a continuous supply of YJB (T2 group) showed the highest survival rate among all the tested groups.

Discussion

The effect of the T1 (fed with YJB continuously) and T2 treatments (fed for 1 day with YJB and with the basal diet on the following day) on growth (FBW, WG, and SGR), feed utilization (FC, FE, and PER), and digestive enzyme activity showed significant difference from that of the T0 treatment (fed with the basal diet continuously). FOS, a prebiotic present in YJB, is implicated in the digestion, absorption, and metabolism of essential nutrients in aquatic animals (Ringø et al. 2010; Soleimani et al. 2012; Zhang et al. 2014; Poolsawat et al. 2020; Pérez-Jiménez et al. 2022). The growth performance of snout bream (*Megalobrama amblycephala*) administered 4 g/kg FOS discontinuously was better than that of the control group fed the basal diet continuously but was not significantly different from that of fish fed 4 g/kg FOS continuously (Zhang et al. 2014). Dimitroglou et al. (2009) and Zhang et al. (2014) indicated that the growth performance of fish is directly related to intestinal enzyme activities and the length and quantity of the intestinal villus, which may aid digestive and absorption processes in the intestine. Bai et al. (2010) showed that white shrimp (*Litopenaeus vannamei*) fed with dietary β -glucan for 2 days followed by the basal diet for 5 days exhibited the highest specific growth rate. However, the T3 treatment (feeding for 1 day with YJB and for the next 2 days with the basal diet) showed no significant difference from the T0 treatment (feeding with the basal diet continuously). Additionally, Merrifield et al. (2010) and Tachibana et al. (2020) demonstrated that the feeding strategy involving the delivery of feed additives to fish may induce distinct growth responses. The differences in growth performance depending on the feeding strategy of feed additives can be attributed to several factors, including the duration of the additive's effect, dietary additives concentration and physiological requirements of fish species. However, given the lack of relevant studies, the exact mechanism underlying this process is unclear and requires additional investigation.

Digestive enzymes are a combination of enzymes found in the intestines of animals and are responsible for breaking down macromolecules into their smaller forms to increase the metabolism of macromolecules (Assan et al. 2022). Trypsin, lipase, and amylase are the major digestive enzymes produced by fish for feed digestion and absorption. Additionally, the body's metabolism may increase if the levels of these enzymes rise (Dawood et al. 2014). A previous study demonstrated that in fish, YJB may stimulate digestive enzyme activity to stimulate appetite (Oh et al. 2023). In particular, FOS in YJB has been demonstrated to be highly helpful in boosting digestive enzyme activity in fish (Xu et al. 2009; Soleimani et al. 2012; Abasubong et al. 2022; Wang et al. 2022). In addition, the proteases, amylases, and lipases released by FOS may complement the shortage of digestive enzymes in fish, enhance the digested product content in chyme, and activate intestinal chemoreceptors to induce the production of endogenous digestive enzymes (Soleimani et al. 2012). In this work, the digestive enzyme activity in the T2 and T3 groups was significantly enhanced relative to that in the T0 group, thus indicating that the administration of YJB at 1-day intervals increased growth and feed utilization by enhancing the activity of intestinal digestive enzymes in juvenile rockfish.

In this study, different YJB feeding regimens did not appear to affect the body composition (moisture, crude protein, crude lipid, and ash) of juvenile rockfish. These results indicated that any YJB feeding regimen did not appear to inhibit nutrient absorption. Similar to this study, previous works showed that the type and dosage of the supplementation of yacon products, including YJB, had no effect on the body composition of fish (Lee et al. 2021; Yin et al. 2021; Oh et al. 2023).

Blood biochemical parameters can be employed as physiological biomarkers of the improvements in fish health caused by the inclusion of functional feed additives (Faggio et al. 2014; Van Doan et al. 2017; Gobi et al. 2018). However, in this study, juvenile black rockfish under different feeding YJB regimens had normal plasma biochemical parameters. These results revealed that the fish were in good health and that YJB had no negative effects on the blood biochemical indices of juvenile black rockfish (Lee et al. 2021; Oh et al. 2023).

Lysozyme, an essential component of the body's non-specific immunity, could dissolve Gram-positive bacteria and kill Gram-negative bacteria with cell walls that have been eliminated by the body (Magnadóttir, 2006; Chiu et al. 2010). In this study, the serum lysozyme activity of rockfish under all YJB feeding regimens was considerably higher than that of the fish fed the basal diet, hence increasing resistance against *S. iniae* bacterial infection. Lee et al. (2021) and Oh et al. (2023) reported that supplementing the feed of juvenile black rockfish with YJB enhanced serum lysozyme activity and then decreased mortality rates after challenge with Gram-positive (*Vibrio anguillarum*) and Gram-negative (*S. iniae*) bacteria. In addition, fish treated with FOS are well known to show enhanced immunity (Song et al. 2014). Zhang et al. (2014) demonstrated that dietary FOS exerted beneficial effects on the non-specific immune responses of snout bream, as indicated by the significantly greater immunological parameters (including serum lysozyme) in FOS-fed fish. These results were comparable with the findings described in this study, demonstrating that a suitable feeding frequency of YJB might boost the non-specific immunity of rockfish.

SOD is an essential antioxidant enzyme in the body. It can remove O^{2-} free radicals and transform O^{2-} into H_2O_2 . It is destroyed by CAT and GSH, hence maintaining a lower state of equilibrium for free radicals, to prevent oxidative damage to functional macromolecules (Martínez-Álvarez et al. 2005; Li et al. 2007). This study revealed that consistent with the non-specific response (lysozyme activity), plasma SOD, CAT, and GSH activities were all enhanced by the application of the YJB feeding regimen. These results indicated that any YJB feeding strategy enhanced the antioxidant enzyme activity of the fish. Similarly, FOS might enhance the antioxidant capacity of juvenile blunt snout bream (Zhang et al. 2014), as supported by the fact that antioxidant enzymes can scavenge reactive oxygen species (ROS) and lipid peroxidation products, thereby protecting cells and tissues from oxidative damage (Li et al. 2007). In addition, polyphenols exhibit antioxidant capabilities and have the ability to impede the production of oxygen anions while also scavenging free radicals (Cicerale et al. 2010; Hamden et al. 2010). Similarly, the inclusion of olive polyphenols in the diet of juvenile Asian sea bass (*Lates Calcarifer*) resulted in enhancements to their nonspecific immune system, as seen by increased lysozyme activity, total immune globulin level, and alternative complement pathway activity and the antioxidant capacity of these sea

bass juveniles was improved, as indicated by elevated activities of catalase (CAT), glutathione reductase, and glutathione S-transferase (GST) (Ahmadi et al. 2022). Previous studies have demonstrated that plant flavonoids, such those present in ferns (Wang et al. 2017a, 2017b), orange peel (Chen et al. 2017), and *Allium mongolicum* Regel (Li et al. 2019), exhibit a strong non-specific immune response and antioxidant activity.

Cumulative mortality is a crucial measure used to assess the health of cultured organisms and the influence of immune stimulants (Xiaolong et al. 2020). At the end of this experiment, the cumulative mortality of the juvenile rockfish in all groups fed with YJB was significantly lower than that in the control group, thus indicating that when given at a reasonable feeding frequency, YJB acts as an activator that effectively improves the cellular and humoral immune functions of fish. This improvement, in turn, results in significantly increased resistance against *S. iniae* infection. Other previous studies ascribed this enhanced disease resistance to the ability of FOS to improve host defense while also stimulating the immune system by increasing immunoglobulin M production and cytokine regulation (Lomax et al. 2009). Li et al. (2019) reported that the increased immunological parameters in groups supplemented with plant flavonoids indicated that the immune factors protecting fish from *Aeromonas hydrophila* infection were enhanced. These results may indicate that the different feeding strategy of YJB supplementation enhances the non-specific immune response (lysozyme and antioxidant enzyme activities), which may be related to the increased resistance of juvenile black rockfish to pathogenic *S. iniae*.

Conclusion

In conclusion, YJB offered every 2 days ameliorated the non-specific immune response and antioxidant status of juvenile black rockfish. YJB should be supplied with day-to-day intervals to improve the growth performance, digestive enzyme activity, and blood health of fish. Feeding with the basal diet for 1 day followed by feeding with the 2.5 g/kg YJB diet for 1 day was the most suitable feeding regimen for juvenile black rockfish culture given its economic cost-saving effect.

Abbreviations

IBW
initial body weight
FBW
final body weight
SR
survival
WG
weight gain
SGR
specific growth rate

CF
condition factor
VSI
viscerosomatic index
HSI
hematosomatic index
FC
feed consumption
FE
feed efficiency
PER
Protein efficiency ratio.

Declarations

Ethical approval All experiments were performed following the guidelines of the International Animal Care and Use Committee of Gyeongsang National University, Korea (approval no. GNU-211230-E0107).

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Abasubong KP, Li XF, Adjoumani JJY, Jiang GZ, Desouky HE, Liu WB (2022) Effects of dietary xylooligosaccharide prebiotic supplementation on growth, antioxidant and intestinal immune-related genes expression in common carp *Cyprinus carpio* fed a high-fat diet. J Anim Physiol Anim Nutr 106:403-418. <https://doi.org/10.1111/jpn.13669>
2. Ahmadniaye Motlagh H, Javadmanesh A, Safari O (2020) Improvement of non-specific immunity, growth, and activity of digestive enzymes in *Carassius auratus* as a result of apple cider vinegar administration to diet. Fish Physiol Biochem 46:1387-1395.
3. Ahmadifar E, Fallah HP, Yousefi M, Dawood MAO, Hoseinifar SH, Adineh H, Yilmaz S, Paolucci M, Doan HV (2021) The Gene Regulatory Roles of Herbal Extracts on the Growth, Immune System, and Reproduction of Fish. Animals 11:2167. <https://doi.org/10.3390/ani11082167>
4. Amphan S, Unajak S, Printrakoon C, Areechon N (2019) Feeding-regimen of β -glucan to enhance innate immunity and disease resistance of Nile tilapia, *Oreochromis niloticus* Linn., against

- Aeromonas hydrophila* and *Flavobacterium columnare*. Fish Shellfish Immunol 87:120-128. <https://doi.org/10.1016/j.fsi.2018.12.062>
5. Assan D, Kuebutornye FKA, Hlordzi V, Chen H, Mraz J, Mustapha UF, Abarike ED (2022) Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: a mini review. Comp Biochem Physiol B Biochem Mol Biol 257:110653. <https://doi.org/10.1016/j.cbpb.2021.110653>
 6. Bai N, Zhang W, Mai K, Wang X, Xu W, Ma H (2010) Effects of discontinuous administration of β -glucan and glycyrrhizin on the growth and immunity of white shrimp *Litopenaeus vannamei*. Aquaculture 306:218-224. <https://doi.org/10.1016/j.aquaculture.2010.06.017>
 7. Baldissera MD, Souza CF, Descovi SN, Verdi CM, Zeppenfeld CC, da Silva AS, Baldisserotto B (2019) Grape pomace flour ameliorates *Pseudomonas aeruginosa*-induced bioenergetic dysfunction in gills of grass carp. Aquaculture 506:359-366. <https://doi.org/10.1016/j.aquaculture.2019.03.065>
 8. Beigh YA, Ganai AM, Ahmad HA (2015) Utilisation of apple pomace as livestock feed: A review. Indian Journal of Small Ruminants 21:165-179.
 9. Ben-Othman S, Jöodu I, Bhat R (2020) Bioactives from agri-food wastes: Present insights and future challenges. Molecules 25:510. <https://doi.org/10.3390/molecules25030510>
 10. Chiu CH, Cheng CH, Gua WR, Guu YK, Cheng W (2010) Dietary administration of the probiotic, *Saccharomyces cerevisiae* P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. Fish Shellfish Immunol 29:1053-1059. <https://doi.org/10.1016/j.fsi.2010.08.019>
 11. Davies SJ, Guroy D, Hassaan MS, El-Ajnaf SM, El-Haroun E (2020) Evaluation of co-fermented apple-pomace, molasses and formic acid generated sardine based fish silages as fishmeal substitutes in diets for juvenile European sea bass (*Dicentrarchus labrax*) production. Aquaculture 521:735087. <https://doi.org/10.1016/j.aquaculture.2020.735087>
 12. Dawood MAO, Dakar AE, Mohsen M, Abdelraouf E, Koshio S, Ishikawa M, Yokoyama S (2014) Effects of Using Exogenous Digestive Enzymes or Natural Enhancer Mixture on Growth, Feed Utilization, and Body Composition of Rabbitfish, *Siganus rivulatus*. J Agric Sci Technol B 4:180-187.
 13. Dawood MA, Eweedah NM, Moustafa Moustafa E, Shahin MG (2019) Effects of feeding regimen of dietary *Aspergillus oryzae* on the growth performance, intestinal morphometry and blood profile of Nile tilapia (*Oreochromis niloticus*). Aquac Nutr 25:1063-1072. <https://doi.org/10.1111/anu.12923>
 14. Dawood MA, El Basuini MF, Yilmaz S, Abdel-Latif HM, Alagawany M, Kari ZA, Van Doan H (2022) Exploring the roles of dietary herbal essential oils in aquaculture: A review. Animals 12:823. [10.3390/ani12070823](https://doi.org/10.3390/ani12070823)
 15. Delgado GTC, Thomé R, Gabriel DL, Tamashiro WM, Pastore GM (2012) Yacon (*Smallanthus sonchifolius*)-derived fructooligosaccharides improves the immune parameters in the mouse. Nutr Res 32:884-892. <https://doi.org/10.1016/j.nutres.2012.09.012>
 16. Dimitroglou A, Merrifield DL, Moate R, Davies SJ, Spring P, Sweetman J, Bradley G (2009) Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut

- morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Anim Sci 87:3226-3234.
<https://doi.org/10.2527/jas.2008-1428>
17. Elumalai P, Kurian A, Lakshmi S, Faggio C, Esteban MA, Ringø E (2020) Herbal immunomodulators in aquaculture. Reviews in Fisheries Science and Aquaculture 29: 33-57.
<https://doi.org/10.1080/23308249.2020.1779651>
 18. Elumalai P, Kurian A, Lakshmi S, Musthafa MS, Ringo E, Faggio C (2021) Effect of *Leucas Aspera* Against *Aeromonas Hydrophila* in Nile Tilapia (*Oreochromis Niloticus*): Immunity and Gene Expression Evaluation. Turk J Fish Aquat Sci 22. <https://doi.org/10.4194/TRJFAS19802>
 19. Faggio C, Fedele G, Arfuso F, Panzera M, Fazio F (2014) Haematological and biochemical response of *Mugil cephalus* after acclimation to captivity. Cah Biol Mar 55:31-36.
 20. Faggio C, Fazio F, Marafioti S, Arfuso F, Piccione G (2015) Oral administration of Gum Arabic: effects on haematological parameters and oxidative stress markers in *Mugil cephalus*. AquaDocs 14:60-72.
<http://hdl.handle.net/1834/11832>
 21. Frosi I, Montagna I, Colomb R, Milanese C, Papetti A (2021) Recovery of Chlorogenic Acids from Agri-Food Wastes: Updates on Green Extraction Techniques. Molecules 26:4515.
<https://doi.org/10.3390/molecules26154515>
 22. Gobi N, Vaseeharan B, Chen JC, Rekha R, Vijayakumar S, Anjugam M, Iswarya A (2018) Dietary supplementation of probiotic *Bacillus licheniformis* Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*. Fish Shellfish Immunol 74:501-508.
<https://doi.org/10.1016/j.fsi.2017.12.066>
 23. Guardiola FA, Porcino C, Cerezuela R, Cuesta A, Faggio C, Esteban MA (2016). Impact of data palm fruits extracts and probiotic enriched diet on antioxidant status, innate immune response and immune-related gene expression of European seabass (*Dicentrarchus labrax*). Fish Shellfish Immunol 52:298-308. <https://doi.org/10.1016/j.fsi.2016.03.152>
 24. Harikrishnan R, Devi G, Balasundaram C, Van Doan H, Jaturasitha S, Saravanan K, Ringø E (2021) Impact of cinnamaldehyde on innate immunity and immune gene expression in *Channa striatus* against *Aphanomyces invadans*. Fish Shellfish Immunol 117:1-16.
<https://doi.org/10.1016/j.fsi.2021.07.009>
 25. Hoseinifar SH, Sun Y, Wang A, Zhou Z (2018) Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front Microbiol 9:2429.
<https://doi.org/10.3389/fmicb.2018.02429>
 26. Lange S, Guðmundsdóttir BK, Magnadóttir B (2001) Humoral immune parameters of cultured Atlantic halibut (*Hippoglossus hippoglossus* L.). Fish Shellfish Immunol 11:523-535.
<https://doi.org/10.1006/fsim.2000.0333>
 27. Lee DY, Lee CH, Kim KD, Lim HJ, Kim HS (2021) Effects of diet supplementation with plant juice processing by-products on juvenile black rockfish (*Sebastes schlegelii*) growth performance, feed

- utilization, non-specific immunity, and disease resistance against *Vibrio harveyi*. *Aquac Rep* 21:100831. <https://doi.org/10.1016/j.aqrep.2021.100831>
28. Leyva-López N, Lizárraga-Velázquez CE, Hernández C, Sánchez-Gutiérrez EY (2020) Exploitation of Agro-Industrial Waste as Potential Source of bioactive Compounds for Aquaculture. *Foods* 9:843. <https://doi.org/10.3390/foods9070843>
29. Li YM, Chan HYE, Huang Y, Chen ZY (2007) Green tea catechins upregulate superoxide dismutase and catalase in fruit flies. *Mol Nutr Food Res* 51:546-554. <https://doi.org/10.1002/mnfr.200600238>
30. Lomax AP, Calder PC (2009) Probiotics, Immune Function, Infection and Inflammation: A Review of the Evidence from Studies Conducted in Humans. *Current Pharmaceutical Design* 15:1428-1518. <https://doi.org/10.2174/138161209788168155>
31. Magnadóttir B (2006) Innate immunity of fish (overview). *Fish Shellfish Immunol* 20:137-151. <https://doi.org/10.1016/j.fsi.2004.09.006>
32. Marques C, Wojeicchowski J, Cardoso T, Mafra MR, Daltoé MLM, Masson ML (2020) Lactobionic acid as a suitable food preservative for yacon juice. *Innov Food Sci Emerg Technol* 64. <https://doi.org/10.1016/j.ifset.2020.102400>
33. Martínez-Álvarez RM, Morales AE, Sanz A (2005) Antioxidant Defenses in Fish: Biotic and Abiotic Factors. *Rev Fish Biol Fish* 15:75-88.
34. Merrifield DL, Bradley G, Baker RTM, Davies SJ (2010) Probiotic applications for rainbow trout I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. *Aquaculture* 16:504-510.
35. Oh HW, Lee TH, Lee CH, Lee DY, Sohn MY, Kwon RW, Kim JG, Kim HS (2023) Effects of by-products from producing yacon (*Smallanthus sonchifolius*) juice as feed additive on growth performance, digestive enzyme activity, antioxidant status, related gene expression, and disease resistance against *Streptococcus iniae* in juvenile black rockfish (*Sebastes schlegelii*). *Aquaculture* 569. <https://doi.org/10.1016/j.aquaculture.2023.739383>
36. Ojansivu I, Ferreira CL, Salminen S (2011) Yacon, a new source of prebiotic oligosaccharides with a history of safe use. *Trends Food Sci Technol* 22:40-46. <https://doi.org/10.1016/j.tifs.2010.11.005>
37. Paini J, Benedetti V, Ail SS, Castaldi MJ, Baratieri M, Patuzzi F (2021) Valorization of wastes from the food production industry: A review towards an integrated agri-food processing biorefinery. *Waste Biomass Valorization* 1-20.
38. Paray BA, El-Basuini MF, Alagawany M, Albeshr MF, Farah MA, Dawood MA (2021) *Yucca schidigera* usage for healthy aquatic animals: Potential roles for sustainability. *Animals* 11:93. <https://doi.org/10.3390/ani11010093>
39. Pérez-Jiménez GM, Peña-Marín ES, Maytorena-Verdugo CI, Sepúlveda-Quiroz CA, Jiménez-Martínez LD, De la Rosa-García S, Alvarez-Villagomez CS (2022) Incorporation of Fructooligosaccharides in Diets Influence Growth Performance, Digestive Enzyme Activity, and Expression of Intestinal Barrier Function Genes in Tropical Gar (*Atractosteus tropicus*) Larvae. *Fishes* 7:137. <https://doi.org/10.3390/fishes7030137>

40. Poolsawat L, Li X, Yang H, Yang P, Kabir Chowdhury MA, Yusuf A, Leng X (2020) The potentials of fructooligosaccharide on growth, feed utilization, immune and antioxidant parameters, microbial community and disease resistance of tilapia (*Oreochromis niloticus* × *O. aureus*). *Aquac Res* 51:4430-4442. <https://doi.org/10.1111/are.14786>
41. Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture* 433:50-61. <https://doi.org/10.1016/j.aquaculture.2014.05.048>
42. Ringø E, Olsen RE, Gifstad TØ, Dalmo RA, Amlund H, Hemre GI, Bakke AM (2010) Prebiotics in aquaculture: a review. *Aquac Nutr* 16:117-136. <https://doi.org/10.1111/j.1365-2095.2009.00731.x>
43. Samavat Z, Mehrgan MS, Jamili S, Soltani M, Shekarabi SPH (2019) Determination of grapefruit (*Citrus paradisi*) peel extract bio-active substances and its application in Caspian white fish (*Rutilus Frisii kutum*) diet: Growth, haemato-biochemical parameters and intestinal morphology. *Aquac Res* 50:2496-2504. <https://doi.org/10.1111/are.14204>
44. Schmidt H, Regulation (EU) 2018/848: The New EU Organic Food Law. *European Food and Feed Law Review*. 2019, 14, 15.
45. Soleimani N, Hoseinifar SH, Merrifield DL, Barati M, Abadi ZH (2012) Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. *Fish Shellfish Immunol* 32:316-321. <https://doi.org/10.1016/j.fsi.2011.11.023>
46. Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E (2014) Prebiotics as immunostimulants in aquaculture: a review. *Fish Shellfish Immunol* 40:40-48. <https://doi.org/10.1016/j.fsi.2014.06.016>
47. Suttili FJ, Gatlin III DM, Heinzmann BM, Baldisserotto B (2018) Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Rev Aquac* 10:716-726. <https://doi.org/10.1111/raq.12197>
48. Tachibana L, Telli GS, de Carla Dias D, Goncalves GS, Ishikawa CM, Cavalcante RB, Ranzani-Paiva MJT (2020) Effect of feeding strategy of probiotic *Enterococcus faecium* on growth performance, hematologic, biochemical parameters and non-specific immune response of Nile tilapia. *Aquac Rep* 16:100277. <https://doi.org/10.1016/j.aqrep.2020.100277>
49. Tsiplakou E, Pitino R, Manuelian CL, Simoni M, Mitsiopoulou C, Marchi MD, Righi (2021) Plant Feed Additives as Natural Alternatives to the Use of Synthetic Antioxidant Vitamins in Livestock Animal Products Yield, Quality, and Oxidative Status: A Review. *Antioxidants* 10:780. <https://doi.org/10.3390/antiox10050780>
50. Van Doan H, Hoseinifar SH, Dawood MA, Chitmanat C, Tayyamath K (2017) Effects of *Cordyceps militaris* spent mushroom substrate and *Lactobacillus plantarum* on mucosal, serum immunology and growth performance of Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol* 70:87-94. <https://doi.org/10.1016/j.fsi.2017.09.002>
51. Wang W, Wang Y, Duan Y, Meng Z, An X, Qi J (2022) Regulation of wheat bran feruloyl oligosaccharides in the intestinal antioxidative capacity of rats associated with the p38/JNK-Nrf2

- signaling pathway and gut microbiota. J Sci Food Agric 102:6992-7002.
<https://doi.org/10.1002/jsfa.12061>
52. Woo SJ (2022) Effects of benzo [a] pyrene exposure on black rockfish (*Sebastes schlegelii*): EROD activity, CYP1A protein, and immunohistochemical and histopathological alterations. Environ Sci Pollut Res 29:4033-4043.
53. Xiaolong G, Caihuan K, Fucun W, Xian L, Ying L (2020) Effects of *Bacillus lincheniformis* feeding frequency on the growth, digestion and immunity of *Haliotis discus hannai*. Fish Shellfish Immunol 96:1-12. <https://doi.org/10.1016/j.fsi.2019.11.038>
54. Xu B, Wang Y, Li J, Lin Q (2009) Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). Fish Physiol Biochem 35:351-357.
55. Xu X, Wang X, Liu Q, Qi X, Zhou L, Liu H, Li J (2022) New insights on folliculogenesis and follicular placentation in marine viviparous fish black rockfish (*Sebastes schlegelii*). Gene 827:146444. <https://doi.org/10.1016/j.gene.2022.146444>
56. Yan MR, Welch R, Rush EC, Xiang X, Wang X (2019) A Sustainable Wholesome Foodstuff; Health Effects and Potential Dietotherapy Applications of Yacon. Nutrients 11:2632. <https://doi.org/10.3390/nu11112632>
57. Yin LL, Cho SH, Park SC, Kwon MG (2021) Ethanol extracts of yacon and ginger in diet of juvenile olive flounder (*Paralichthys olivaceus*): effect on growth, feed utilization, body composition, plasma chemistry and challenge test against *Vibrio anguillarum*. Turk J Fish Aquat Sci 21:265-274. https://doi.org/10.4194/1303-2712-v21_6_01
58. Zhang CN, Li XF, Jiang GZ, Zhang DD, Tian HY, Li JY, Liu WB (2014) Effects of dietary fructooligosaccharide levels and feeding modes on growth, immune responses, antioxidant capability and disease resistance of blunt snout bream (*Megalobrama amblycephala*). Fish Shellfish Immunol 41:560-569. <https://doi.org/10.1016/j.fsi.2014.10.005>

Figures

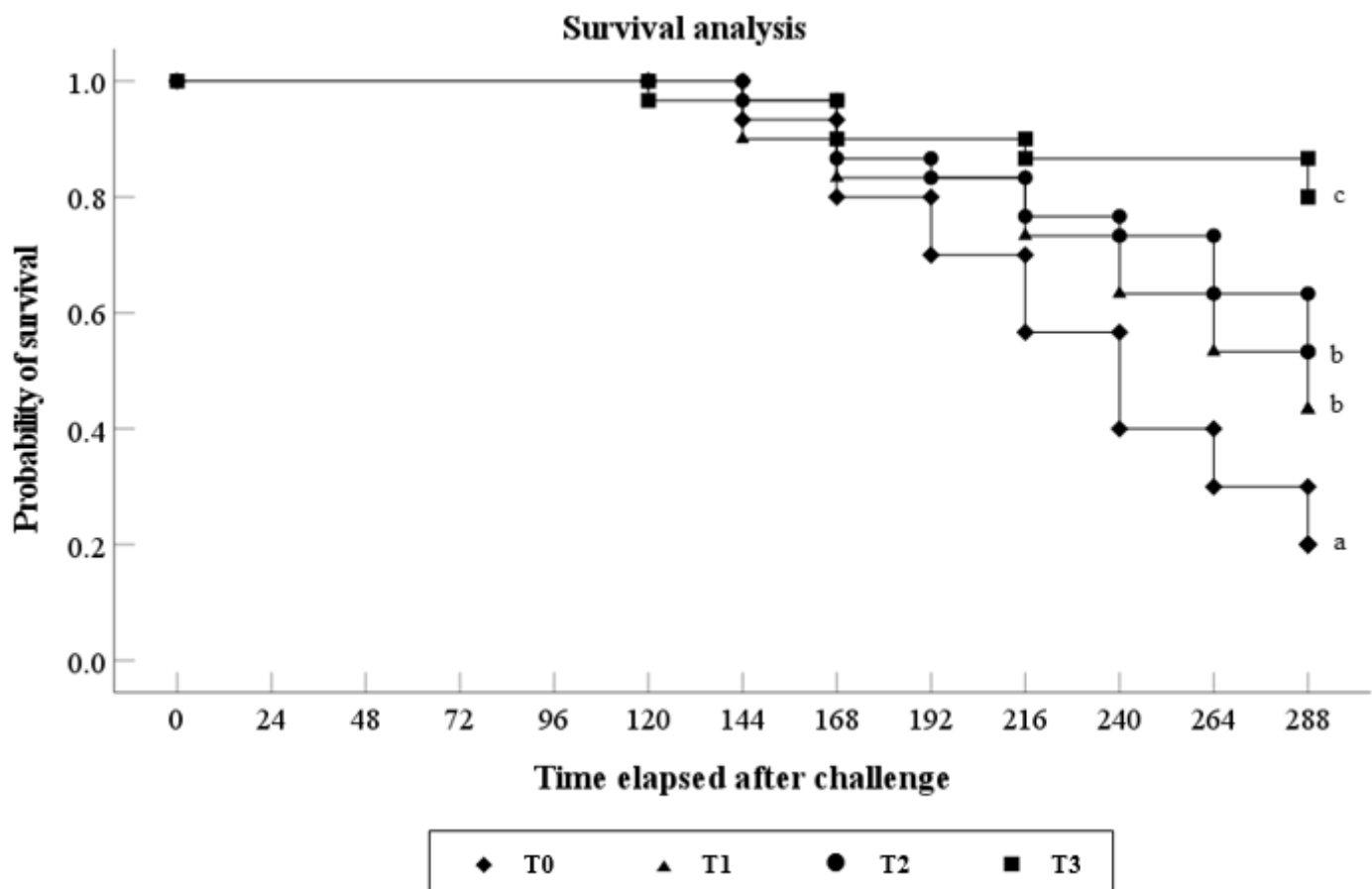


Figure 1

Survival of juvenile black rockfish under different feeding regimens with the YJB-supplemented diet for 8 weeks, then infected by *Streptococcus iniae*. Values are mean of triplicate groups. Different lowercase letters indicate significant differences ($P < 0.001$; log-rank and Wilcoxon tests)