BMC Microbiology

RESEARCH Open Access

Evaluation of the synbiotic effects of *Saccharomyces cerevisiae* and mushroom extract on the growth performance, digestive enzyme activity, and immune status of zebrafish *danio rerio*

Seyedeh Sedigheh Hosseini[1,2*], Mohammad Sudaagar[3], Hamideh Zakariaee[3], Hamed Paknejad[3], Kartik Baruah[4†] and Parisa Norouzitalab[4†]

Abstract

Background The quest for candidate probiotics and prebiotics to develop novel synbiotics for sustainable and profitable fish farming remains a major focus for various stakeholders. In this study, we examined the effects of combining two fungal probiotics, *Saccharomyces cerevisiae* and *Aspergillus niger* with extracts of Jerusalem artichoke and white button mushroom to develop a synbiotic formulation to improve the growth and health status of zebrafish (*Danio rerio*). An initial in vitro study determined the most effective synbiotic combination, which was then tested in a 60-day in vivo nutritional trial using zebrafish (80 ± 1.0 mg) as a model animal. Four experimental diets were prepared: a control diet (basal diet), a prebiotic diet with 100% selected mushroom extract, a probiotic diet with 10^7 CFU of *S. cerevisiae*/g of diet, and a synbiotic diet with 10^7 CFU of *S. cerevisiae*/g of diet and 100% mushroom extract. As readouts, growth performance, survival, digestive enzyme activity and innate immune responses were evaluated.

Results In vitro results showed that the *S. cerevisiae* cultured in a medium containing 100% mushroom extract exhibited the maximum specific growth rate and shortest doubling time. In the in vivo test with zebrafish, feeding them with a synbiotic diet, developed with *S. cerevisiae* and mushroom extract, led to a significant improvement in the growth performance of zebrafish ($P < 0.05$). The group of zebrafish fed with the synbiotic diet showed significantly higher levels of digestive enzyme activity and immune responses compared to the control group ($P < 0.05$).

Conclusion Taken together, these results indicated that the combination of *S. cerevisiae* and mushroom extract forms an effective synbiotic, capable of enhancing growth performance and immune response in zebrafish.

Keywords Probiotic, Growth, Immunity, Natural extract, Funal probiotics, Zebrafish

†Kartik Baruah and Parisa Norouzitalab these authors shared the senior authorship.

*Correspondence:
Seyedeh Sedigheh Hosseini
Hosseini.s@goums.ac.ir

Full list of author information is available at the end of the article

Background

Research into the gut microbiota of farmed fish dates back to the early half of the 20th century but more recently, interest in this area has grown at a significant rate coinciding with the expansion of the aquaculture industry. Several lines of evidence suggest that a well-functioning gut with a balanced microbiota helps fish obtain essential nutrients necessary for growth and development [1–3]. The gut-associated microbiota consists of a diverse community of microorganisms, including bacteria, fungi, and protozoa, and they play important roles in various gut functions, such as digestion, metabolism, and immune regulation and protection against pathogens [4]. Imbalances in the gut microbiome, known as dysbiosis, caused by factors like antibiotics, poor diet, stress, or other factors, can result in the onset of poor growth and health, and the occurrence of diseases [5–7]. Thus, maintaining a well-functioning gut and a balanced gut microbiome are becoming a major focus of study [8, 9]. To promote gut health in fish, several strategies have been implemented over the past few years. One such promising approach is the use of synbiotic formulations combination prebiotics and probiotics that work synergistically to promote a healthy gut microbiome and enhance the overall performance of the organisms [10–13]. In farmed aquatic organisms, there is evidence suggesting the positive effects of synbiotics on the microbial communities in the fish gut, thereby causing beneficial effects on the health and growth performances of the fish, e.g., by averting the adverse effects caused by the infection stress, and by elevating the activities of the digestive enzymes, which eventually contribute to improved feed utilization and growth performances [14–19]. As a source of probiotics in the synbiotic formulation, a wide range of Gram-negative and Gram-positive bacteria have been examined and some were successfully used in the development of the formulation [20–22].

Recently, there has been a burgeoning interest in incorporating fungal-based probiotics in synbiotic formulations for farmed aquatic animals, which is driven by their multifaceted benefits [23]. From promoting a balanced gut microbial environment that aids in the digestion and absorption of nutrients and boosting immunity to offering a sustainable and natural alternative to unsustainable chemotherapeutics, fungal probiotics present a promising avenue for improving the growth and health performances in aquaculture species [24]. Fungal-based probiotics were shown to improve growth performance, feed efficiency, immune responses, and disease resistance in both farmed fish and shrimps, indicating their potential effectiveness as prophylactic agents against future diseases [25–27]. For instance, in whiteleg shrimp (*Penaeus vannamei*) feeding probiotic *Aspergillus niger* at a dose of 1.0–1.5 g/kg of diet for 56 days resulted in a significant improvement in growth indices, immunity, and gut microbiota [28]. This ultimately led to increased resistance of the shrimp against the pathogenic bacterium *Vibrio parahemolyticus* [28]. In another study carried out on common carp (*Cyprinus carpio*) feeding a diet supplemented with *A. niger* for 60 days markedly improved the growth, immunity, digestive enzyme activity, and hematological indices of the fish [29]. Overall, the research on fungi-based probiotics highlights the potential benefits of using them as an important supplement for improving gut health and performance in farmed fish [30–32].

Prebiotics are non-digestible oligosaccharides that selectively stimulate the growth and activity of beneficial microorganisms in the gut [33]. The metabolic byproducts produced by these microorganisms can have positive effects on the host's health [34, 35]. The most studied prebiotics in fish include inulin, mannan oligosaccharides, fructooligosaccharides, galactooligosaccharides, and nano-oligosaccharides [36–41]. These types of prebiotic components are naturally present in various plant and microbial species, such as Jerusalem artichoke, mushrooms, cereals, leeks, asparagus, garlic, onion, and banana [42–45]. It is noteworthy to mention that the utilization of food-grade prebiotic compounds obtained from natural sources as functional feed additives in the aquafeed industry is constrained by the expenses associated with the extraction and isolation procedures. To tackle the issue of high production costs and minimize the waste generated from the isolation and extraction process, there has been a growing emphasis on investigating the direct utilization of raw extracts as potential sources of natural prebiotics [45]. Such natural prebiotics have been shown to serve as good substrates for fermented microorganisms, such as bacteria (e.g. *Lactobacillus* sp) and yeast [46–51]. In a previous study, we examined the effects of combining extracts from the Jerusalem artichokes (*Helianthus tuberosus*) and button mushrooms (*Agaricus bisporus*) with two different strains of bacterial probiotics (*Lactobacillus acidophilus* and *L. delbrueckii subsp. Bulgaricus*) on farmed fishes. The main aim of the study was to develop a synbiotic formulation that could improve the growth, survival, and reproductive performances of the fish [45]. We used zebrafish (*D. rerio*) as a model organism to conduct the study. The results showed that the combination of *L. acidophilus* or *L. bulgaricus* with mushroom extract caused positive effects on the growth and reproductive performances of the zebrafish [45]. In the current study, we used two fungal probiotic strains: *Saccharomyces cerevisiae* (ATCC-2601) and *Aspergillus niger* (ATCC-1004), to examine their interaction with the natural prebiotics, specifically Jerusalem artichoke and white button mushroom. We conducted an in vitro study to evaluate the ability of the

extract to promote the growth of these two probiotic strains. Subsequently, we identified the most promising prebiotic candidate and combined it with each probiotic strain to explore potential synbiotic combinations. To verify the efficacy of the synbiotic preparations, we performed an in vivo experiment using zebrafish as the model organism. Our main focus was to evaluate growth traits, survival rates, and immune status as measurable indicators of the synbiotic effects. Through the analysis of these outcomess, we aimed to determine the impact of the synbiotic combination on the overall health and performance of the zebrafish.

Materials and methods

Fungal strains and Inoculum preparation

Two fungal strains, *Saccharomyces cerevisiae* (ATCC-2601) and *Aspergillus niger* (ATCC-1004) were obtained from Persian Type Culture Collection, Tehran, Iran. Stock cultures were prepared by mixing a pure culture of the lyophilized strain, grown in Sabouraud Dextrose (SD) Broth medium (Merck, Darmstadt, Germany) for a maximum duration of 72 h as previously described [52]. Briefly, stock cultures were separately inoculated in flasks containing the mentioned medium and incubated on a shaker incubator (800 x g) at 30ºC for 72 h. Inoculums were prepared by inoculating the 1 ml of fungus strain in 100 ml of SD broth (1% v/v) followed by incubation at 30 °C for a maximum duration of 72 h. First, the pH of the culture medium for *A. niger* and *S. cerevisiae* was adjusted to 4 and 5, respectively using 1 N H_2S0_4 [52]. The media were then sterilized by autoclaving at 121^0C for 15 min. For inoculation, we used a fresh volume of each microorganism.

Fungal growth analysis

The growth of *S. cerevisiae* and *A. niger* was monitored by measuring the optical cell density using a UV/Visible spectrophotometer at 600 nm (Shimidzo, Japan) [53]. The measured values were plotted on standard growth curves. The maximum specific growth rate during the exponential growth phase was calculated following the equation of Kask et al. [54]:

$\mu\ (t\text{-}t_0) = Ln\ N\text{-}LnN_0$.

where t=time, N=optical density at the end of the exponential growth phase (t), N_0=optical density at the beginning of the exponential growth phase (t_0), μ=specific growth rate constant (h^{-1}).

The doubling time was determined by the equation: $T_d = Ln2/\mu_{max,}$ where, μ_{max} - maximum specific growth rate, t_d- doubling time.

Preparation of natural extracts as prebiotic

The natural ingredients, Jerusalem artichokes (*H. tuberosus*; hereafter referred to as JA) and white button mushrooms (*A. bisporus*; hereafter referred to as WBM) were procured from a private company in Iran (WBM: Dorrin company Tehran, Iran. and JA: Sanmive company, Tehran, Iran). Extracts prepared from these ingredients were used as sources of prebiotics. The preparation of the extracts was carried out following the procedure previously optimized by Zakariaee et al. [45]. Firstly, the ingredients were washed, dried, and sliced into small pieces. Subsequently, they were suspended in 0.5% (w/v) citric acid solution for 15 min to prevent browning. The ingredients were then further dried in an oven at 50˚C for 48 h. The dried samples were ground in a grinder. The extraction process was performed using the soaking method described by Harborne [55]. Briefly, 100 g of each dry powder was mixed with 1 L of distilled water and the mixture was kept in the dark for 48 h. After that, the resulting mixture was subjected to centrifugation (10 000 x *g* at 4˚C, 15 min) and the resulting precipitate was discarded [56]. Finally, the supernatant was filtered using a 0.22 μm syringe filter and used for preparing the desired concentration of prebiotics.

In vitro analysis of prebiotic properties of extracts

JA and WBM extracts were added to the glucose-free SD broth/agar medium at different concentrations (2, 6.25, 12.5, 25, 50, 75, and 100%). The SD broth/agar medium supplemented with glucose (SDG) as a carbon source served as a positive control. The glucose-free SD media (Mirmedia, Iran) containing JA (SDJ) or WBM (SDM) was used to cultivate the probiotic strains. The *S. cerevisiae* and *A. niger* were inoculated in 50 ml of the SDJ or SDM broth at a concentration of 10^7 CFUs/ml [47]. The pH of the media was adjusted to 4.5-5.0. The solutions were incubated on shaker (Incu-Shaker™ 10 L, Korea) at 30 ˚C for 120 h for SC and 160 h for AN under aerobic conditions. After incubation, the cell count was determined using a spectrophotometer (Shimidzo, Japan) by measuring the optical density at 600 nm [45, 52]. A schematic representation of in vitro experimental design is shown in Fig. 1A.

Determination of viable *S. cerevisiae* cells

To determine the number of viable cells in CFU/ml, the samples were subjected to serial dilutions from 10^1 to 10^6 CFU/ml and plated onto SD agar supplemented with the most effective concentration of prebiotic extracts obtained from the in vitro tests. Volumes of 100, 50, 25 and 10 μL were evenly spread on SD agar plates. The Petri dishes were then incubated at 30 °C for 72 h, with daily monitoring for signs of growth. Once visible growth was detected, the colonies were counted as previously described [57].

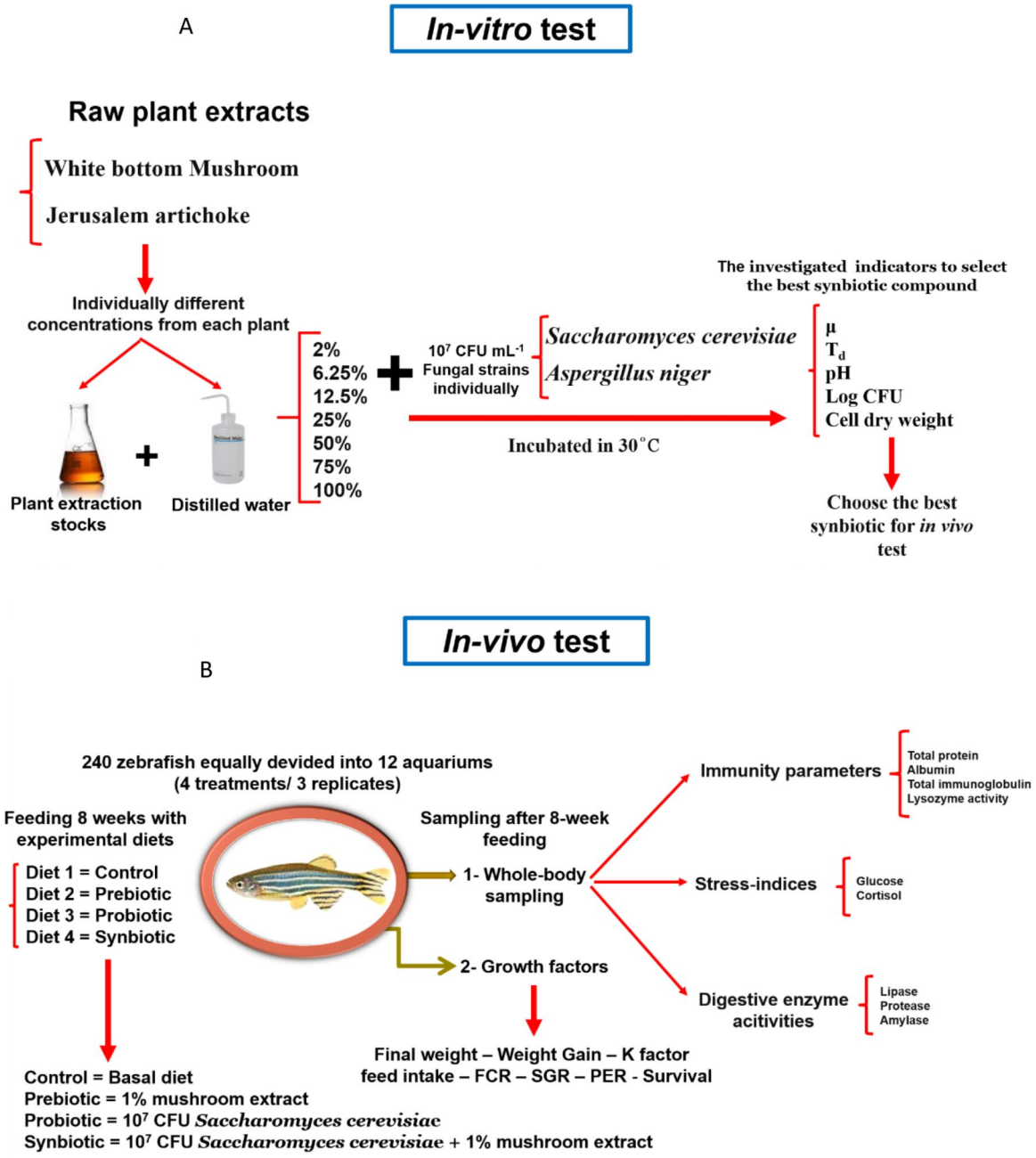

Fig. 1 Growth of Saccharomyces cerevisiae and Aspergillus niger in presence of mushroom or artichoke extract. Growth curve of A. niger and S. cerevisiae in medium containing mushroom or artichoke extract (2, 6.25, 12.5, 25, 50, 75 and 100%) as the only carbon source. A and B: growth of the two fungal strains in presence of artichoke extract. C and D: growth of the two fungal strains in presence of mushroom extract. Data are the mean of three replicates (n = 3). Positive control: Sabouraud dextrose broth medium with the standard amount of glucose; negative control 1: Free glucose Sabouraud dextrose broth medium containing a concentration of 100% of plant extract without inoculation fungi; negative control 2: Free glucose and plant extract (as a prebiotic) Sabouraud dextrose broth with fungi

Determination of *A. niger* cell dry weight

Due to the complete coverage of the culture plate surface by colonies of *A. niger*, it was difficult to accurately count the individual surface colonies. Therefore, an alternative and complementary method, measuring the dry weight of cells, was employed to determine the growth as an alternative to CFU/ml [52]. For *A. niger*, cells were collected by centrifugation at 4000 *g* for 30 min, followed by filtration using Whatman filter No. 1. The filtered solution was then washed with distilled water to remove any residual substrates and subsequently dried in the hot air oven at 70 °C. The weight of the filter paper was

Table 1 Chemical components of the using diet for the experiment (blue line/Italy)

Chemical composition		(%)
Dry matter		0.5
Crude protein		60
Crude lipid		17
Ash		10.5
Mineral premix	**%**	
Na total		0.5
Ca total		1.5
P total		1.8
Vitamin premix	**in per kg of feed**	
A		1000 UI
D_3		1000 UI
E		300 mg/kg

measured before filtering the solution, and after drying, the combined weight of the filter paper and cells was measured. The cell dry weight was calculated as follows: Cell dry weight = weight of filter paper and cell after drying – weight of filter paper only, as previously described [52].

Final pH of the culture medium

The final pH of the resulting fermented broth was measured using pH meter (Mettler- Toledo AG, 8603 Schwerzenbach, Switzerland) [58, 59].

Diet preparation

A commercial diet sourced from the Blue Line Company (Italy, Almenno San Bartolomeo) was used as the basal diet, and it served as the basis for the experimental diets. The proximate composition of the basal diet is mentioned in Table 1. Based on the outcome from the in vitro studies (see Fig. 1A), we selected only *S. cerevisiae* and designed four experimental diets: Diet 1 served as the control diet, Diet 2 consisted of the basal diet supplemented with 1% WBM extract, Diet 3 included the basal diet supplemented with *S. cerevisiae* at a concentration of 10^7 CFU/g of diet, and Diet 4 combined the basal diet with *S. cerevisiae* at 10^7 CFU/g of diet along with 1% WBM extract. The supplements were carefully sprayed onto the feed, manually mixed, and each experimental diet was coated with 5% gelatin. To avoid any potential effects of gelatin, the control diet was also coated with 5% gelatin. Subsequently, all diets were dried at room temperature (25 °C) in a clean environment and stored at 4 °C until use. The diets were prepared every week throughout the feeding trial, and random samples of diets containing probiotics were analyzed for microbial viability by culturing in SD broth/agar, following the methodology described by Zakariaee et al. [45]. Briefly, 1 g of experimental diets was cultured in 100 ml of standard broth media (SD) and incubated at 30 °C for 72 h. Subsequently, a known volume of the culture was transferred to SD-agar-based media and cultured under the same conditions. Once visible growth was observed, the colonies were verified based on visual observation under microscope and were counted following standard procedure [45, 60].

Table 2 Water quality parameters during the experiments

Parameters	Temperature (°C)	pH	Total hardness (mg L^{-1})	Dissolved oxygen (mg L^{-1})	light-dark cycles (h)
Range	25 ± 2	7 ± 0.2	300 ± 10	7.1 ± 0.9	14 – 10

Experimental animals and design

Zebrafish (*Danio rerio*) larvae were obtained from a private farm in Gorgan, Golestan province, Iran. The larvae were acclimatized to the experimental conditional for a period of two weeks. Throughout this acclimatization phase, the larvae were fed *ad libitum* with a basal diet. Regular monitoring was conducted to assess the health and performance of the larvae. No mortality was recorded during the acclimatization period. After the two-week acclimatization, a total of 240 larvae, regardless of gender, with an average initial weight and length of 80 ± 1.0 mg and 16.0 ± 0.3 mm, respectively, were randomly divided into four experimental groups (diets 1 to 4 as mentioned above). Each group was maintained in three replicates, with 20 fish stocked in an aquarium (12 aquaria with a capacity of 60 L, containing 30 L of water). For details of the experimental design, please refer to Fig. 1B. Continuous aeration was provided to all the aquaria throughout the culture period. The larvae were fed three times per day (08:00, 12:30 and 18:00 h) at a rate of 5% of the body weight for 60 days [37, 60–62]. Approximately 25% of the water in each tank was replaced after removing uneaten feed and fecal matter, which was siphoned daily. The experimental conditions were conducted following the specifications outlined in Table 2 [45, 63]. No mortality was observed during the feeding period. The handling and maintenance of the zebrafish larvae adhered to the ethical guidelines for in vivo experiments set forth by the Golestan University of Medical Sciences. This accreditation complies with accepted national and international ethical norms and principles for biomedical research, as well as the guidelines and protocols of the Ministry of Health and Medical Education of the Islamic Republic of Iran (Approval ID: IR.GOUMS.REC.1397.262). The schematic representation of in vivo experimental design is presented in Fig. 1B.

Sample collection and growth analysis

At the end of the feeding trial, the growth and survival of the fish were measured with precision levels of 0.001 g

and 1 mm, respectively [45], and later calculated the following standard formulae [64, 65]:

Weight gain percentage (%) = (W_2 (mg) -W_1 (mg)) / W_1 (mg) × 100.

Specific growth rate (% day^{-1}) = (LnW_2-LnW_1) × 100/t (day).

Food conversion ratio=feed intake (mg)/weight gain (mg).

Condition or K factor (mg/mm^3)=final weight (mg) / final length $(mm)^3$×100.

Protein efficiency ratio = (body weight gain (mg) /protein intake (mg).

Survival rate (%)=number of fish survived after 60 days /initial number of fish stocked × 100.

Where W_2, W_1 and t represent final weight (mg), initial weight (mg) and the trial period (day), respectively.

Following a period of 60 days, fish in each tank that were not fed for 24 h, were randomly sampled (10 fish per aquarium /30 fish per treatment) and immediately anesthetized using a solution of 200 mg of clove powder dissolved in 1 L of water [45]. Either whole fish or entire intestine was sampled as described in the sections below for further analysis.

Intestinal digestive enzyme activity

The colorimetric measurement for the activities of intestinal digestive enzymes, namely protease, lipase and amylase, was conducted following the protocol described by Ashouri et al. [66]. To perform this analysis, the entire intestine was collected from nine fish (three fish per replicate per treatment) that were starved for 24 h. The fish were transferred to the microbiology laboratory, where they were euthanized with 500 mg L^{-1} clove powder and dissected [45]. The intestine was removed, rinsed using distilled water, dried with paper towels, and homogenized with 30 g of tissue in 70 mL of distilled water using a homogenizer (Digital Disperser, IKA's ULTRA-TURRAX, Germany). Subsequently, the samples were centrifuged at 10,000 *g* for 25 min at 4 °C [63]. The resulting supernatant was then immersed in liquid nitrogen and stored at -80 °C until further analysis [66].

The activities of amylase, lipase, and protease were measured using starch, α-naphthyl caprylate, and azocasein as substrates, respectively. Measurements were made using a UV spectrophotometer (Shimidzo, Japan) at specific OD: 550 nm for amylase, 540 nm for lipase, and 366 nm for protease [63, 66]. The specific enzyme activity was expressed as enzyme unit (U) per gram of protein (g $protein^{-1}$).

Whole-body sample preparation

To prepare whole-body samples, we followed the previously described method [67]. Briefly, a total of 9 fish (3 fish per replicate per treatment) were randomly collected and anesthetized as described above. The entire body of each fish was homogenized in a sterile falcon tube containing 25mM Tris−HCl buffer (pH 7.2). The protocol described by Pedroso et al. [68] was employed for the subsequent measurements. The homogenized fish samples were centrifuged at 4000 *g* at 4 °C for 15 min. The resulting supernatant was carefully collected, divided into smaller aliquots, transferred into clean sterile microtubes, and stored at -80 °C for further analysis of metabolic enzyme activity, stress indicators and immunoglobulin levels (Ig) [69, 70].

Whole-body metabolic enzyme activity

The activities of metabolic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the whole body were evaluated using commercial kits (Paadco, Tehran, Iran) according to the manufacturer´s instructions. The absorption readings were obtained using a spectrophotometer (Shimidzo, Japan) at an OD of 340 nm for AST and ALT, and 405 nm for ALP.

Stress-related indices

Whole-body cortisol level was measured using a commercial ELISA kit (Pars Azmoon, Tehran, Iran) following the protocol provided by the manufacturer. Glucose levels were measured using commercial kits (Pars Azmoon, Tehran, Iran) through a single-point method using the glucose-hexokinase enzyme assay according to the manufacturer´s instructions [71].

Immune indices

Four immune-related molecules, namely total protein, albumin, immunoglobulin, and lysozyme were analyzed to assess the immune response. The total protein concentration was determined using the Bradford method, with bovine albumin as the standard [72]. The albumin levels in the whole-body samples were measured at acidic pH using the Bromocresol Green reagent [73]. Total immunoglobulin levels (Ig) were measured following the method described by Siwicki et al. [74]. Briefly, the total protein level was measured using the aforementioned method, and then the immunoglobulin molecules were estimated by adding 12% polyethylene glycol solution to the samples. After centrifugation, the protein level was measured again using the Bradford method. The difference in protein content was considered as the Ig content.

The lysozyme activity was determined by adding 50 µL of whole-body sample to a 2 mL suspension of lysozyme-sensitive bacterium, *Micrococcus lysodeikticus*, suspension, for lysis. The reaction was carried out at room temperature (25 °C), and absorbance was measured using US spectrophotometer at 450 nm after 0.25 min and 5 min [75].

Statistical analysis

The data were checked for normality and homoscedasticity requirements as necessary by employing Levene´s tests, using the statistical software Statistical Package for the Social Sciences (SPSS) version 16.0. The data that satisfied those requirements were then further analyzed by employing a one-way analysis of variance (ANOVA). The differences between the means of treatments were determined using Duncan's multiple range tests at $P<0.05$ significance level.

Results

Impact of the natural extracts on the growth dynamics of the fungal probiotic

The growth rate of probiotics, *S. cerevisiae* and *A. niger*, varied over time when cultured in the different experimental media containing either artichoke or mushroom extract (0, 2, 6.25, 12.5, 25, 50, 75, or 100%) (Fig. 2A and D). Negative control groups, lacking substrate as a carbon source, showed no growth. However, when the glucose-free medium was supplemented with JA or WBM extract as substrate, a marked increase in the growth of both probiotics was observed. The maximum improvement in the growth of *S. cerevisiae* and *A. niger* was recorded in the medium containing 100% artichoke or mushroom extract. In the subsequent test, we therefore used this dose.

Impact of the natural extracts on the specific growthrate, doubling time of the fungal probiotics, and the pHof the culture media

This analysis was conducted to compare the two extracts and determine which one had the most significant impact on promoting the specific growth rate and doubling time, of the fungal probiotics, *S. cerevisiae* and *A. niger.* We have used 100% JA and WBM extract separately as the sole carbon source. These doses were chosen based on their optimal performances in the growth dynamic studies. As shown in Fig. 3A, S. *cerevisiae* had a significantly higher specific growth rate compared to *A. niger* in a medium containing 100% mushroom or artichoke extract ($P<0.05$). Moreover, in the 100% mushroom extract, the specific growth rate of *S. cerevisiae* was significantly higher than that in the 100% artichoke extract.

The doubling time of *S. cerevisiae* in a medium containing either 100% mushroom or artichoke extract was significantly shorter than that of *A. niger* cultured in the same extract concentration ($P<0.05$; Fig. 3B). *S. cerevisiae* cultured in the medium containing 100% mushroom extract showed the lowest doubling time among the different experimental groups.

The pH of the SD medium supplemented with mushroom extract and cultured with *S. cerevisiae* was significantly low. In contrast, the SD medium containing artichoke extract and cultured with *A. niger* had the highest pH, which was not significantly different from the medium containing mushroom extract and *A. niger* (Fig. 3C).

Considering that production time determines the value of a product, *S. cerevisiae* and mushroom extract (100%) were chosen to develop a synbiotic formulation and conduct validation studies with zebrafish.

Viability of *S. cerevisiae* cells

The viability of the *S. cerevisiae* cells in the synbiotic formulation containing 100% Jerusalem artichoke and mushroom extracts as the only carbon source was determined by measuring the colonies grown for approximately 200 h. The maximum number of *S. cerevisiae* live cells was recorded 72 h after inoculation. At 192 h after inoculation, no living cells were observed on the culture medium containing 100% Jerusalem artichoke or 100% mushroom extracts (Fig. 4A).

Cell dry weight

The highest amount of cell dry weight of *A. niger* was noted at 240 h after inoculation, which was more than 1 mg/ml (Fig. 4B).

Impact of the prebiotic formulation on the growth performance and survival of zebrafish

The effects of the experimental diets on the growth, feeding efficiency and survival of the fish were evaluated after a 60-day feeding trial, and the results are shown in Fig. 5. At the start of the feeding trial, the fish in all experimental groups had a weight range of 80 and 81 mg, and the length ranged between 16.2 and 16.8 mm, with no significant differences observed among them (Fig. 5A and C). At the end of the trial, there was no significant difference in terms of length among the fish in different experimental groups (Fig. 5B). However, the experimental diets showed significant improvements ($P<0.05$) in final weight, weight gain, specific growth rate (SGR), food conversion ratio (FCR), and protein efficiency rate (PER) compared to the control group (Fig. 5; D to H). Feeding the larvae with mushroom extract or *S. cerevisiae* resulted in a weight gain increase of 35.2% and 39.7%, receptively, compared to those fed with the control diet (Fig. 5E). The synbiotic diet further significantly increased the weight gain ($P<0.05$) by 70.8% compared to the control, and by 31.1% and 35.5% compared to larvae fed with mushroom extract or *S. cerevisiae*-supplemented diet, respectively (Fig. 5E). While there was no significant ($P>0.05$) difference in feed intake among the various experimental groups (Fig. 5I), larvae fed with the test diets exhibited a significant ($P<0.05$) increase in SGR and a reduction in FCR. The most prominent effect was observed with the synbiotic diet, which increased the

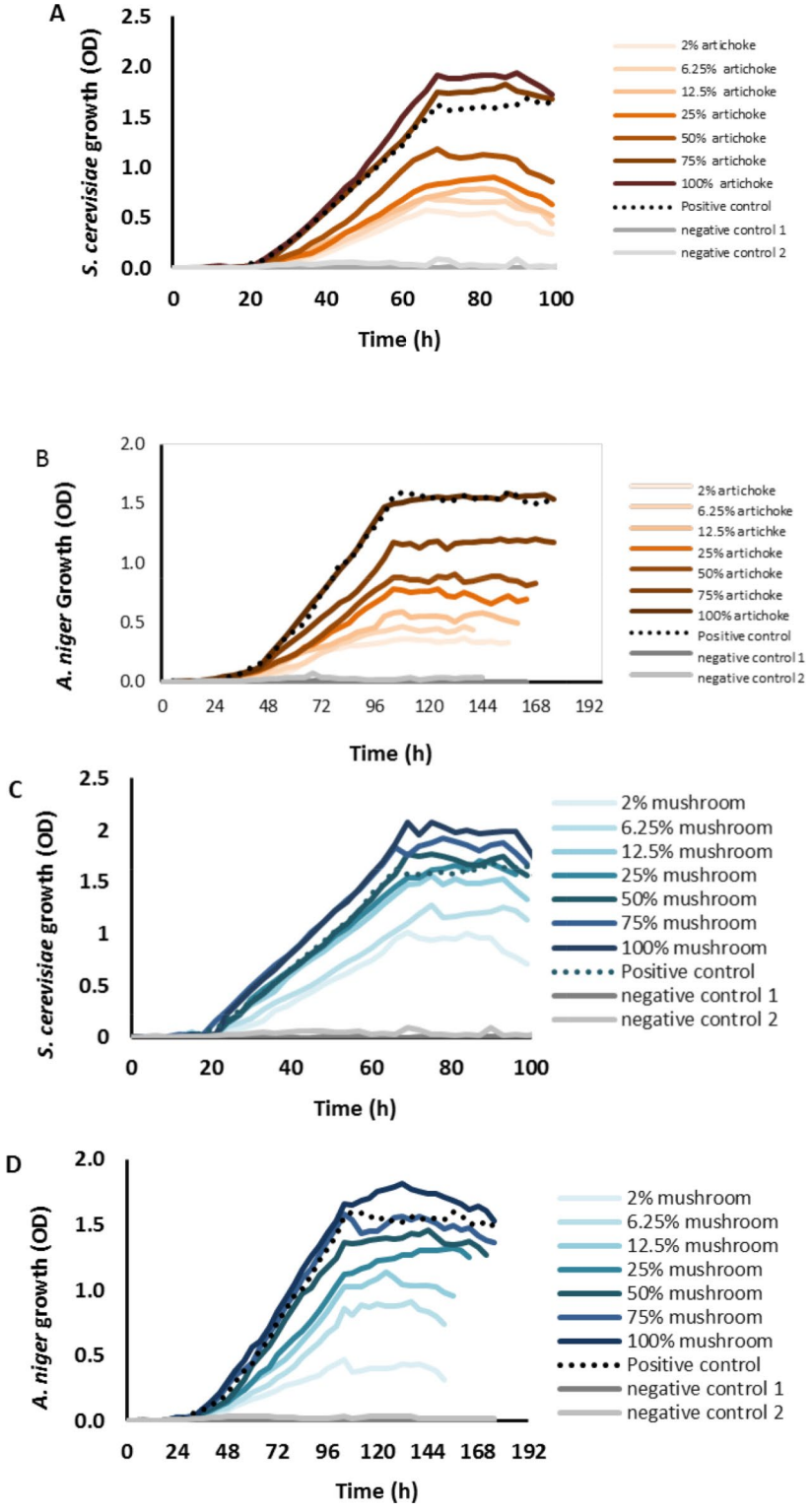

Fig. 2 Comparison of (A) μ max (h^{-1}) and (B) doubling time (h) and (C) final pH of the synbionts in presence of plant extract. *A. niger* and *S. cerevisiae* were culture in 100% of mushroom and artichoke extracts. Data are the mean of three replicates ± SE. Different letters display significant difference in each column ($P < 0.05$)

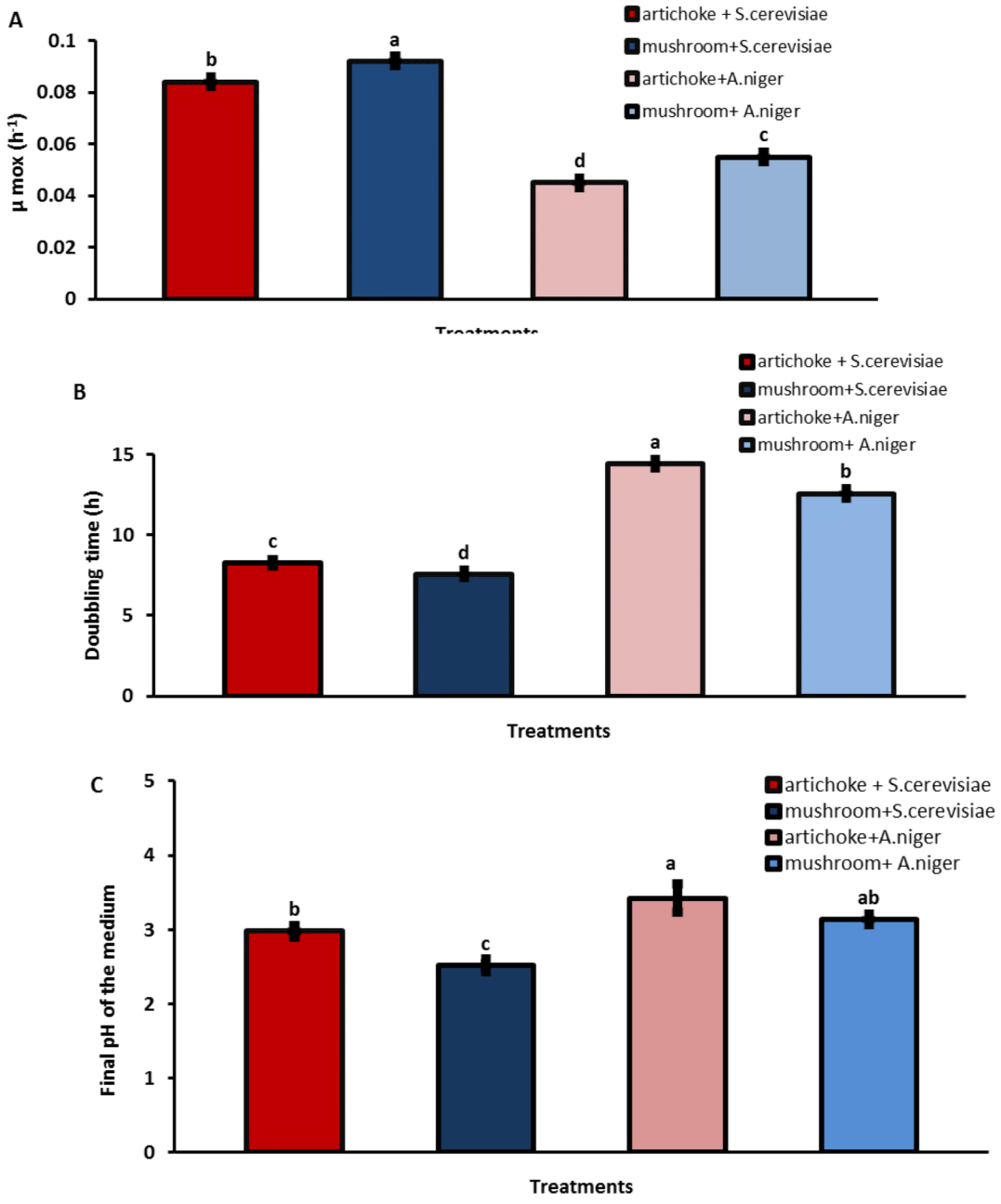

Fig. 3 Growth curve of synbionts in presence of artichoke or mushroom extract. (A) Growth of *S. cerevisiae* in medium containing 100% Jerusalem artichoke or mushroom extracts as an only carbon source. For these symbionts, visible colonies could be counted (B) Growth of *A. niger* in medium containing 100% Jerusalem artichoke or mushroom extracts as an only carbon source. For this experiment, colonies were not visible, therefore, dilutions series were made and dry weight was measured as an indicator for the growth of this fungus

SGR by 0.45% and reduced the FCR by 0.81% compared to the control group (Figs. 5F and 5G). A similar trend was observed for the PER of the larvae: groups fed with diets supplemented with mushroom extract, *S. cerevisiae*, and the synbiotic formulation significantly increased PER by 0.20, 0.22, and 0.40%, respectively, compared to the control group (Fig. 5H). No mortality was recorded during the feeding period, and the condition factor of the fish remained the same among the different experimental groups (Fig. 5J and K).

Impact of the prebiotic formulation on the activity of digestive enzymes

The activity of digestive enzymes was notably influenced by the experimental diets, as shown in Fig. 6. The zebrafish group that was fed with the synbiotic diet displayed a

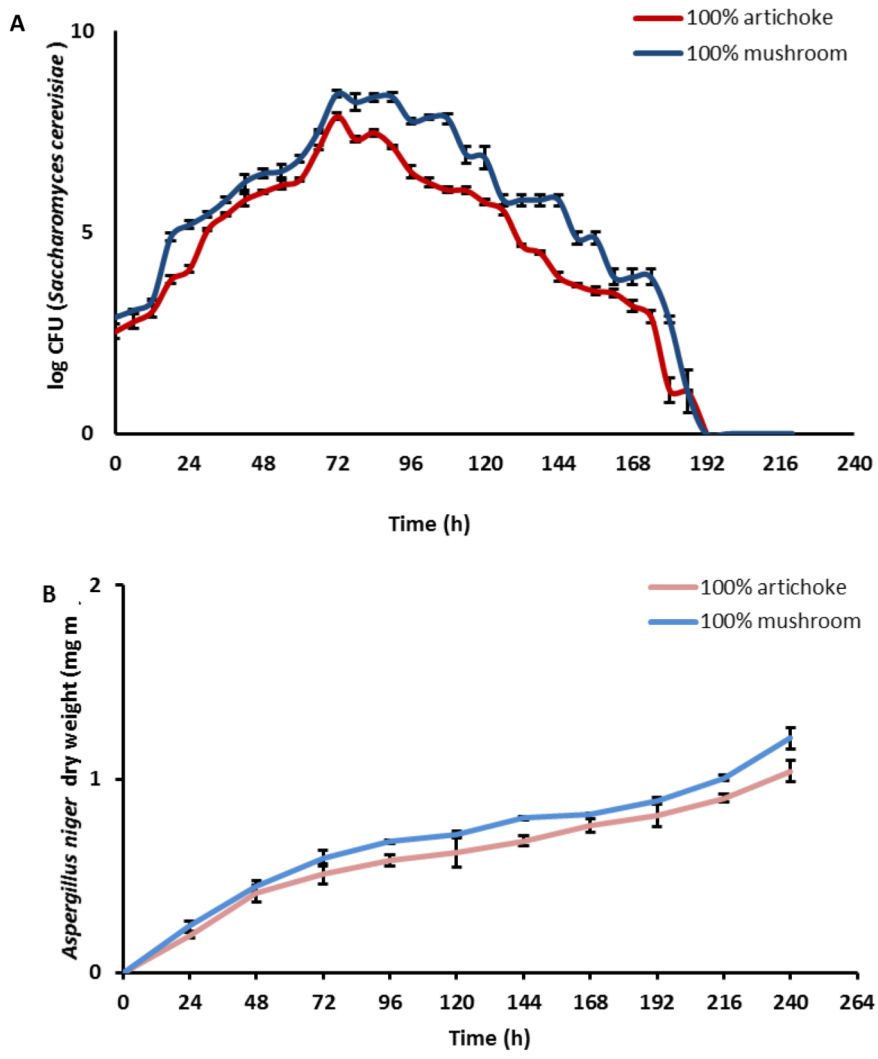

Fig. 4 Growth performance and survival of zebrafish (*Danio rario*) fed with different supplementation diets for 60 days. (A) Initial length (mm), (B) final length (mm), (C) Initial body weight (mg), (D) final body weight (mg), (E) Weight gain percentage (%), (F) Specific growth rate (% day^{-1}), (G) Feed conversion ratio, (H) Protein efficiency ratio, (I) Feed intake (g), (J) K Factor (mg mm^3), K) Survival (%). Bars with different letters represent significant differences among groups (Duncan's test, $P < 0.05$)

significantly higher level of amylase activity. The amylase activity in the synbiotic-fed group was 1.5-fold higher than that of the control group. However, there was no significant difference in amylase activity between the control group and the groups that received a diet supplemented with either mushroom extract or *S. cerevisiae* ($P > 0.05$; Fig. 6A).

The protease enzymes exhibited significantly higher activity in all the groups that were fed the test diets ($P < 0.05$; Fig. 6B). The group that received the synbiotic diet showed the highest protease activity. Compared to the control group, the experimental groups that received a diet supplemented with mushroom extract, *S. cerevisiae*, or the synbiotic had 1.7, 1.4 and 1.9-times higher protease activity, respectively, compared to the control group ($P < 0.05$). A comparable pattern was recorded in the lipase activity (Fig. 6C). The group fed with synbiotic showed the highest level of lipase activity, surpassing the control group. The group that was fed a diet supplemented with *S. cerevisiae* had the second highest lipase activity. In comparison to the control group, the lipase activity was 1.7 times higher in the *S. cerevisiae* fed group and 1.7 times higher in the synbiotic-fed group. The mushroom-fed group displayed a 1.3-fold increase in lipase activity compared to the control group.

Impact of the prebiotic formulation on the whole-body metabolic enzyme activity and stress indicators

Feeding of diets supplemented with mushroom extract and *S. cerevisiae*, alone or in combination (i.e. synbiotic formulation) did not cause any significant effect on the activity of alkaline phosphatase (Fig. 7A), aspartate

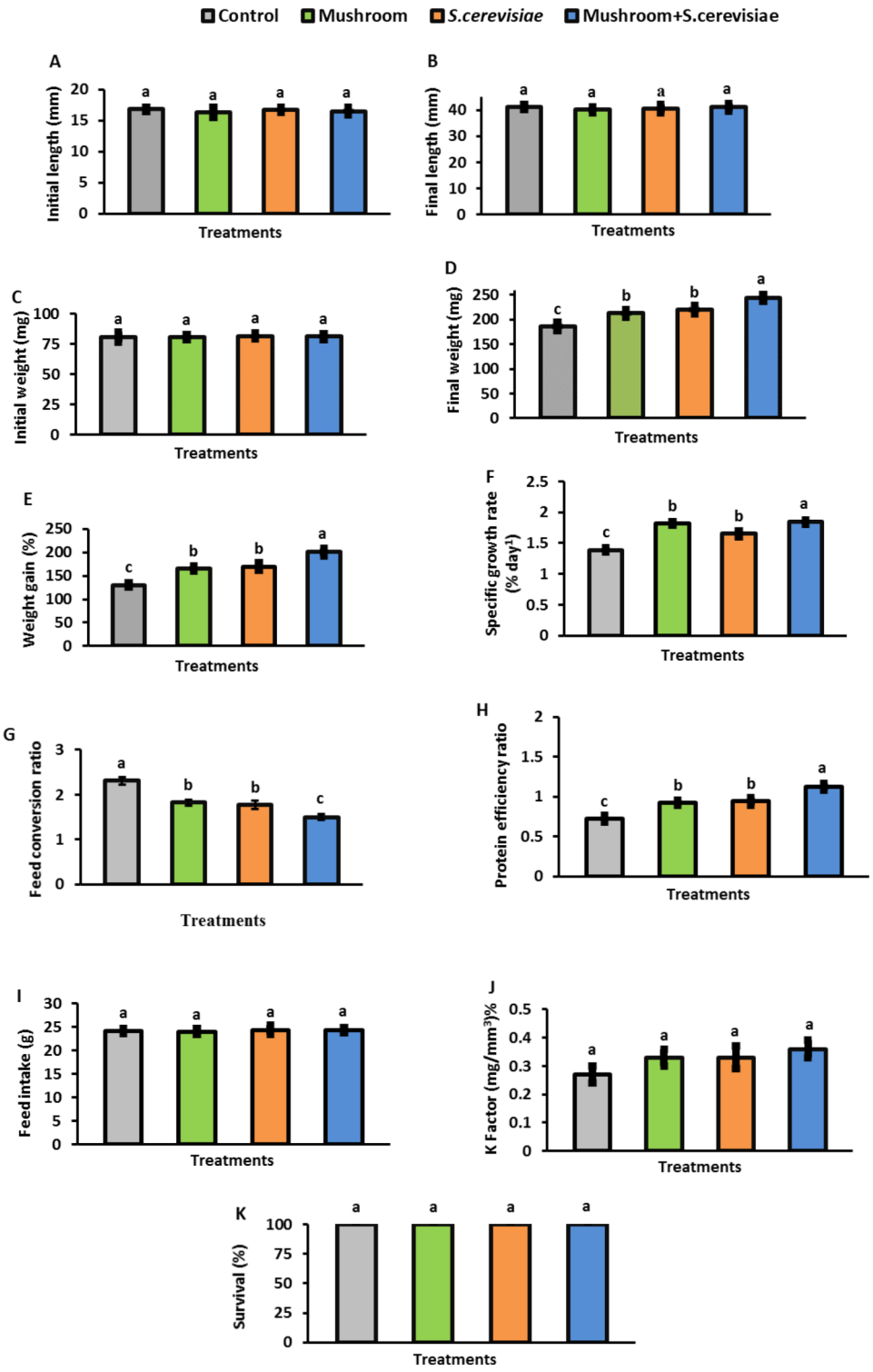

Fig. 5 Digestive enzyme activity of zebrafish fed with different diets for 60 days. The group fed no feed supplement was maintained as control; (A) Amylase (U g protein^{-1}), (B) Protease (U g protein^{-1}). B) Lipase (U g protein^{-1}). Bars with different letters represent significant differences among groups (Duncan's test, $P < 0.05$)

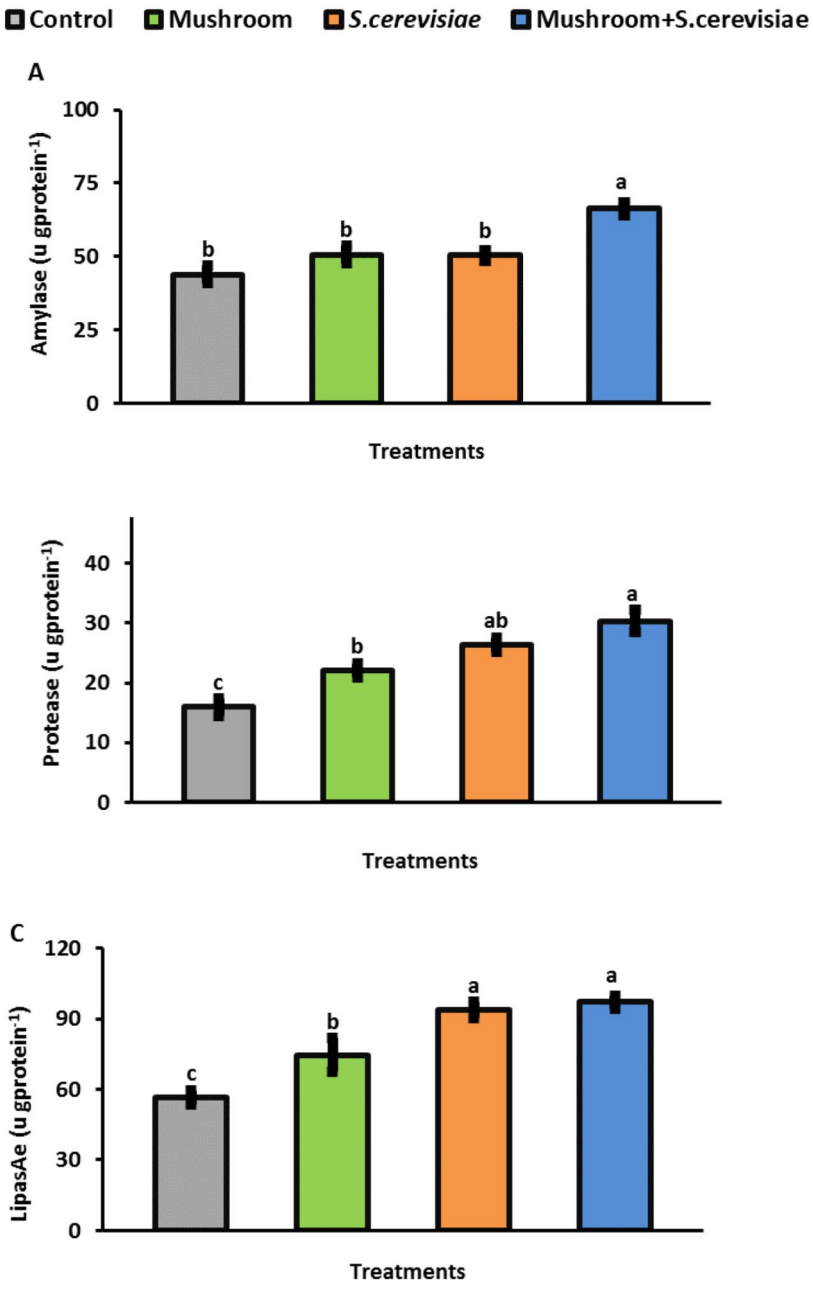

Fig. 6 Metabolic enzymes in zebrafish fed with different diets for 60 days. The group fed with no feed supplement was maintained as control; (A) ALP (U L^{-1}), (B) ALT (U L^{-1}), (C) AST (U L^{-1}). Bars with different letters represent significant differences among groups (Duncan's test, $P < 0.05$)

aminotransferase (Fig. 7B), and alanine aminotransferase enzymes (Fig. 7C). Likewise, the levels of glucose and cortisol, measured at the whole-body level, remained unchanged in response to the feeding of the experimental diets (Fig. 8).

Impact of the prebiotic formulation on the immune responses in zebrafish

Feeding zebrafish larvae with a diet supplemented with mushroom extract and *S. cerevisiae* alone significantly increased the levels of total protein (Fig. 9A) and lysozyme (Fig. 9B) compared to the control. However, the increase in the levels of these readouts was less prominent compared to the group fed with the synbiotic diet. The group fed a diet supplemented with the synbiotic formulation exhibited a significantly higher level of total protein compared to the control group (2.2-fold increase), while the groups fed with a diet with either mushroom extract or *S. cerevisiae* as a supplement showed a 1.3-fold higher protein level than the control

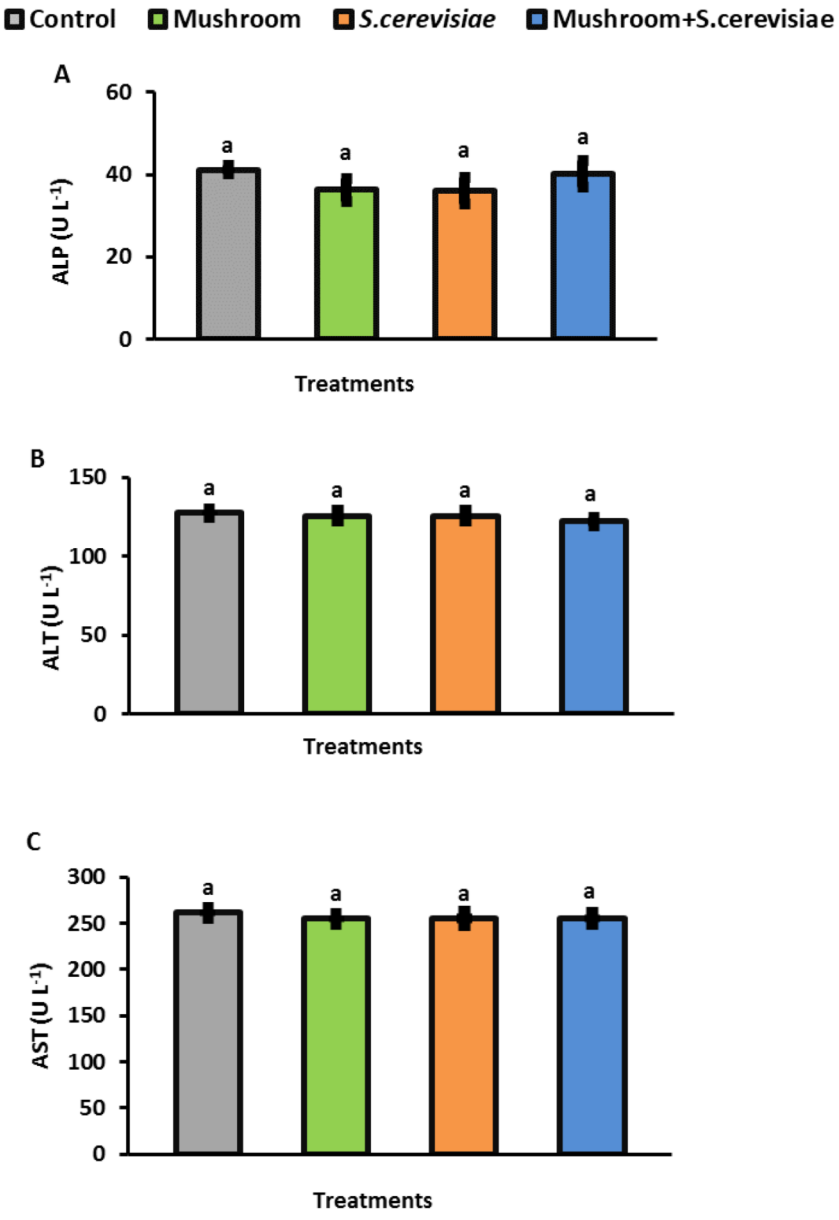

Fig. 7 Stressed indicators of zebrafish fed with different diets for 60 days. A group without site supplement was maintained as control; (A) Glucose (mg mL^{-1}), (B) Cortisol (µg mL^{-1}). Bars with different letters represent significant differences among groups (Duncan's test, $P < 0.05$)

group. There was no significant difference in total protein levels between the group fed a diet with mushroom extract and those fed a diet with *S. cerevisiae* ($P > 0.05$). Additionally, the synbiotic-fed group showed the highest level of lysozyme among all the experimental groups.

The levels of immunoglobulin and albumin in the groups fed with dietary mushroom extract did not significantly differ from the control group (Fig. 9C and D). However, the group fed with dietary *S. cerevisiae* had a significantly higher level of albumin than the control group. There was no significant effect of dietary *S. cerevisiae* on the total immunoglobulin level. However, feeding the larvae with a dietary synbiotic resulted in a significant increase in both immunoglobulin and albumin levels, with the highest increment observed among all the experimental groups.

Discussion

In a previous study, we examined the two natural extracts Jerusalem artichokes and button mushrooms with two different bacterial probiotics *Lactobacillus acidophilus* and *L. bulgaricus* to develop a synbiotic formulation [45]. We examined the effect of the synbiotic on its potential to improve the growth and reproductive performances

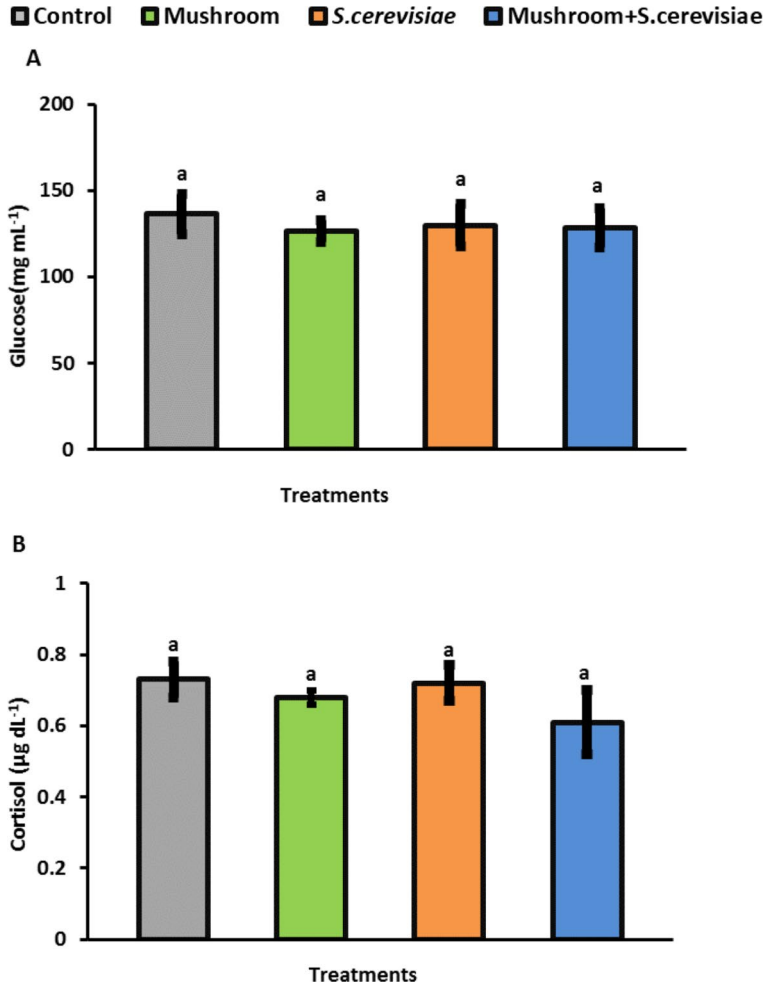

Fig. 8 Non-specific immunity responses in zebrafish fed with different diets for 60 days. A group without site supplement was maintained as control; (A) Total protein (g dL^{-1}), (B) Total immunoglobulin (g dL^{-1}), (C) Albumin (g dL^{-1}), (D) Lysozyme activity (U mg $protein^{-1}$). Bars with different letters represent significant differences among groups (Duncan's test, $P < 0.05$)

of farmed aquatic animals using zebrafish as a model organism [45]. The results showed that a synbiotic formulation, developed with the selected combination of *L. acidophilus*, *L. bulgaricus*, and 50% mushroom extract, showed a positive influence on the growth and reproductive performances of the zebrafish, suggesting that the combination of the mushroom extract with the probiotic bacteria *L. acidophilus* or *L. bulgaricus* could be a potential synbiotic for the successful production of aquaculture species. While prebiotics are commonly associated with supporting the growth of bacterial probiotics, a limited number of studies have highlighted the potential of natural extracts as prebiotics for enhancing the growth of fungal probiotics [76–78]. Building upon the findings of our earlier study [45], we carried out the present study to investigate the effectiveness of the Jerusalem or mushroom extract as a prebiotic for fungal probiotics, *S. cerevisiae* and *A. niger* to develop a synbiotic formulation. In the present study, we used fungal-based probiotics owing to their multi-functional biological activities and assessed multiple biological endpoints, such as growth traits, immune and stress biomarkers, and digestive and metabolic enzymes. These fungal species were chosen based on their availability, commercial significance, and documented evidence of positive effects on growth performance and health status in farmed animals [79–83]. At first, we used an in vitro approach to monitor the growth dynamics of the test probiotics in the presence of the different doses of the natural extracts to select interesting combination(s) for further verification under in vivo conditions. The results showed that the growth dynamics of *S. cerevisiae* was most prominent when cultured in a medium containing 100% mushroom extract as manifested by a higher growth rate and lower doubling time of this species in the presence of 100% mushroom extract compared to 100% artichoke extract. This suggests that mushroom extract exhibited stronger prebiotics effects, promoting robust growth of *S. cerevisiae.* The

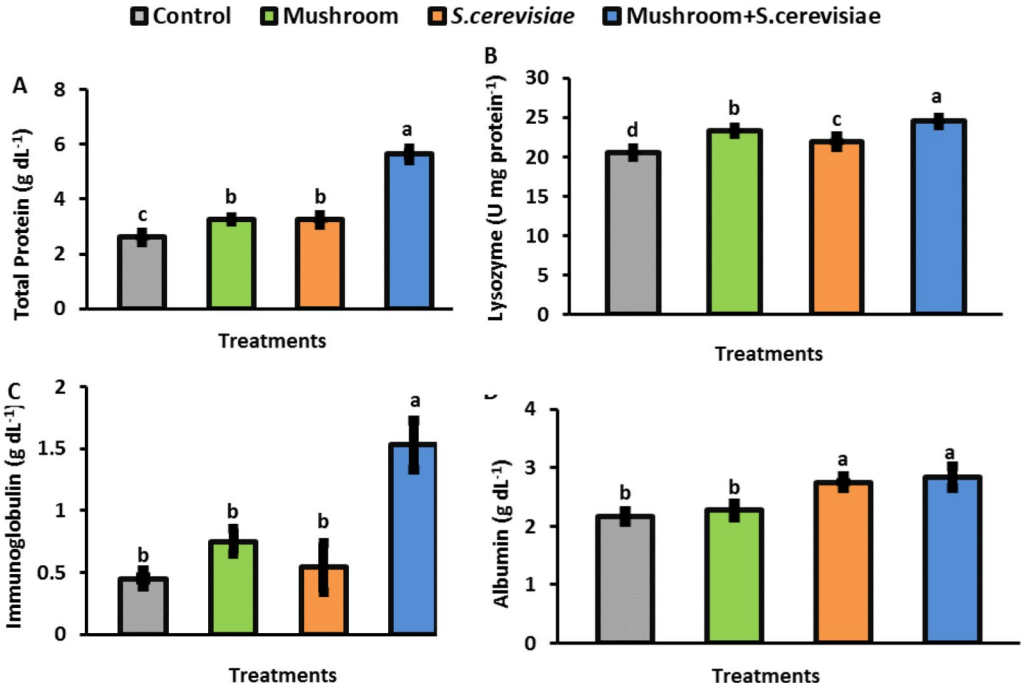

Fig. 9 Immunity responses in zebrafish fed with different diets for 60 days. A group without site supplement was maintained as control; (A) Total protein (g dL^{-1}), (B) Total immunoglobulin (g dL^{-1}), (C) Albumin (g dL^{-1}), (D) Lysozyme activity (U mg $protein^{-1}$). Bars with different letters represent significant differences among groups (Duncan's test, $P < 0.05$)

presence of varying types of fermentable fibers or other beneficial prebiotic components in mushroom extracts, such as chitin, hemicellulose, β- and α-glucans, mannans, which are preferred by *S. cerevisiae* for fermentation [51, 84–87] may be responsible for these effects. Our suggestion that *S. cerevisiae* ferments mushroom extract more efficiently is supported by the significant decrease in pH of the medium containing 100% mushroom extract, compared to the medium containing 100% artichoke extract and *A. niger*. The marked decrease in pH indicates a high level of microorganism activity and a substantial production of short-chain fatty acids, which is often considered an important indicator of carbohydrate fermentation [87–91]. Additional tests using cell count, colony-forming units, and cell dry weight confirmed that *S. cerevisiae* growth, viability and biomass production in the medium supplemented with mushroom extract were higher than those in Jerusalem extract. This confirmed that fermentation occurred, and despite a slower growth rate and fermentation process, the cell dry weights and viability were higher in the presence of mushroom extract, supporting our previous explanation that the degree of polymerization of prebiotics justifies the rate of fermentation.

Next, we proceeded to formulate a synbiotic by combining the optimized dose of the mushroom extract with *S. cerevisiae* and validated the potential positive impacts of this combination on the growth performance and overall health status of zebrafish, which served as the in vivo model organism in our study. The results suggested that the synbiotic formulated using *S. cerevisiae* and mushroom extract had a positive effect on the growth performance and feed efficiency in zebrafish. The weight gain percentage, after 60 days of feeding, of the synbiotic-fed group increased to 201%, compared to 130.2% for the control group, and 165.5% and 170% for the groups fed with either mushroom extract or *S. cerevisiae* alone, respectively. Accordingly, the synbiotic-fed group exhibited an increase in the SGR by a fold of 1.3 compared to the control. This resulted in an FCR that was significantly lower for the synbiotic-fed group. It is important to note that the synbiotic-fed group had the highest protein efficiency ratio among all the experimental groups, despite the fact that these groups received the same amount of dietary protein and had the same level of feed intake. Our findings also indicated that the condition factor (K), which serves as an indicator of the overall health of the fish, remained within the optimal range throughout the entire duration of the experiment [92, 93]. This was further reflected in the survival rates, as no significant mortality was observed in response to feeding the experimental diets. These results suggest that the 100% mushroom extract and selected *S. cerevisiae*, whether used alone or in combination, did not have any detrimental effects on the fish under our specific experimental conditions. Therefore, they can be considered safe for the farmed species for inclusion in fish feed. There are several possibilities to explain the observed growth improvement in zebrafish when fed with synbiotic-supplemented diet.

The first is the fermentation of the prebiotic components in the mushroom extract (e.g. chitin, mannans, galactans, xylans, glucans, krestin, lentinan, and hemicellulose) by the fungal probiotic, stimulating the proliferation of fungal and other beneficial microbes and inhibition of potentially pathogenic organisms in the gut. The second is the improvement of digestion and nutrient utilization by increasing enzymes produced by the microbes and host. The third is the production of vitamins and short chain fatty acids and other beneficial metabolites for the host, and the fourth is the improvement in the immune status of the fish [94–99]. The beneficial effects of mushroom extract or *S. cerevisiae* on the growth performances of farmed fishes are well documented but no information is available on the growth performances of fish fed on synbiotic formulation based on mushroom extract and *S. cerevisiae*. However, enhanced growth performance has been previously reported in rainbow trout fed on synbiotics of fungal and fructooligosaccharides [100], and in the zebrafish fed a diet supplemented with synbiotics, developed from probiotic bacteria and conventional prebiotics, such as FOS, MOS and β-glucan [41, 45, 101, 102], findings in agreement with that of our study.

We did not analyze the impacts of the synbiotic developed on the gut microbiome as well as on the production of metabolites in the gut to corroborate these factors with the observed growth performances. However, we focused on the activities of the major digestive enzymes (i.e., amylase, protease, and lipase) to substantiate our argument that the growth-promotion effects observed in zebrafish were related to the improvement in the activities of digestive enzymes. The results showed that zebrafish fed on a synbiotic-supplemented diet exhibited the highest activities of amylase, protease, and lipase enzymes. The increased activities of the enzymes might have contributed to the better digestion of dietary nutrients, such as protein and lipids [102, 103]. This in turn might have led to more efficient feed utilization and improved growth performance as observed in our study [104, 105]. The higher PER value in the synbiotic-fed group that coincides with higher protease activities in this group is a clear indication that synbiotic supplementation facilitated the process of digestion, absorption and utilization of dietary nutrients. The causes for the improvement of digestive enzyme activity could be due to: (i) creation of a healthy gastrointestinal microbial ecology by the synbiotic and/or (ii) modification in the secretion of bacterial and/or host enzymes [106]. Similar results have also been reported in earlier studies on synbiotic based on sodium alginate and *Pediococcus acidilactici* in Asian sea bass *Lates calcarifer* [66].

Alkaline phosphatase (ALP) is a phosphomonoesterase enzyme found in different tissues of fish, including the liver, intestine, kidney, gills, and bone. In fish, it plays essential functions in the processes of detoxification, nutrient digestion, absorption, and phagocytosis [107, 108]. ALP is typically used as an indicator of liver health due to its presence in liver tissues and its sensitivity to changes in liver function [109–112]. Elevated levels of ALP activity in the blood can suggest liver damage or dysfunction. Therefore, measuring ALP activity is a common diagnostic tool to assess liver health in fish. In our study, feeding zebrafish with a diet supplemented with mushroom extract or *S. cerevisiae* alone or in combination did not cause a marked change in the whole-body ALP activity when compared to the control group. This indicates that the dietary supplements have no adverse effect on the zebrafish organs and hence on the health performance of the fish.

Additionally, we also measured cortisol and glucose levels in the fish to evaluate the physiological responses and metabolic changes in the fish due to feeding synbiotics. Cortisol is a stress hormone produced by the fish in response to changes in their environmental conditions and nutritional factors [115–117]. Glucose, on the other hand, serves as a vital energy source for fish, supporting their metabolic processes and physiological functions [118, 119]. The combined analysis of cortisol and glucose levels allows to understand the impact of synbiotic feeding on fish stress levels, energy metabolism, and overall health [120, 121]. Cortisol and glucose levels were shown to be directly influenced by stressful and unfavourable conditions [122]. The release of cortisol and glucose is influenced by various factors such as food, stress, culture conditions, disease, and water quality indices [123, 124]. Our results showed that dietary synbiotics caused no effect on the cortisol and glucose levels when compared with the group fed control diet. Since cortisol and glucose are commonly used as stress indicators, the lack of changes in their levels in the treatment groups suggests that the synbiotic diet caused no nutritional stress conditions in the fish [125]. The effects of feeding synbiotics on the cortisol and glucose levels in fish have yielded mixed results. Some studies have reported no significant effects on these parameters, while others have observed increased or decreased levels of glucose and cortisol. For instance, Yu et al. [123] observed an increase in the serum glucose content in the white-legged shrimp *Litopenaeus vannamei* fed a diet supplemented with *Bacillus* sp. and medicinal herbs. On the other hand, Sadat Hoseini Madani et al. [124] reported lower plasma glucose and cortisol levels in shrimp fed probiotic belonging to *Bacillus* species. Both these findings contrast with our results. These discrepancies in the findings can be attributed to several factors like variations in experimental design, such as fish species, initial metabolic and health status of the experimental fish, feeding duration, specific combination of probiotics and prebiotics used in the synbiotic formulation, and dosage of synbiotics administered.

Total blood protein is a valuable indicator of health and stress in various organisms, including fish [126, 127]. It refers to the combined concentration of proteins in the blood, encompassing albumins, globulins, enzymes, hormones, and immunoglobulins. Our study used whole-body extract of zebrafish larvae to measure total blood protein levels indirectly. The highest total protein levels were recorded in zebrafish fed with a synbiotic diet. In agreement with our results, there were earlier reports showing positive correlation between fish immune status and the level of total serum protein in fish fed with various fungal and mushroom products alone or in combination [26]. For instance, Abdel-Tawwab et al. [128] reported increased serum total protein, albumin, and globulin were observed in Nile tilapia fed *Yucca schidigera* and fungal. However, whole-body extracts contain protein from various tissues, which may not accurately reflect total blood protein levels to indicate accurately the overall health condition of fish. We therefore carried out further analysis of the whole-body samples for albumin, total immunoglobulin and lysozyme levels - key components of the defence system in fish that contribute markedly to the immune response in fish, albeit through different mechanisms. Albumins support immune function by aiding in the transport and distribution of immune-related molecules (e.g. antibodies, immune cells), while lysozymes combat microbial pathogens by degrading their cell walls. Our study found increased albumin, total immunoglobulin levels, and lysozyme enzyme activity in zebrafish fed with synbiotics compared to the control group. These results are consistent with previous studies showing the positive effects of fungal-enriched diets on fish immunity. For example, there was a marked enhancement in lysozyme activity and total immunoglobulin level in *Cyprinus carpio*, *Oncorhynchus mykiss*, *Huso huso*, *Pagrus major*, *Sciaenops ocellatus*, *Channa striata*, and *Oreochromis niloticus* fed with diets supplemented with fungal product [129–137]. The observed increase in immune molecules in the synbiotic-fed group could be due to polysaccharides, such as β-glucan, mannan oligosaccharides, chitin in the mushroom extract as well as in the fungal *S. cerevisiae*, and the production of beneficial bioactive components by *S. cerevisiae* like vitamins, short-chain fatty acids in the gut of zebrafish. However, these are pure speculations, and warrants further research.

Conclusion

In summary, the in vitro assay demonstrated that the fungal probiont *S. cerevisiae* exhibited a preference for mushroom extract as a prebiotic. Subsequently, a synbiotic formulation was developed using the selected combination of 10^7 CFU/mL of *S. cerevisiae* and 100% mushroom extract. This synbiotic formulation showcased positive effects on the growth performances and health conditions of zebrafish, serving as an in vivo model organism. Our findings also suggest that the improvement in the growth performance was associated with a marked increase in the activities of digestive enzymes and immune responses, which possibly contribute to better nutrient utilization. Taken together, these results indicate that the combination of *S. cerevisiae* and mushroom extract holds promise as a potential synbiotic for aquaculture species. Further research is necessary to validate this formulation through on-farm nutritional trials in economically significant aquaculture species.

Abbreviations

CFU	colony-forming unit
SD	Sabouraud Dextrose
AN	Aspergillus niger
SC	Saccharomyces cerevisiae
JA	Jerusalem artichokes
WBM	white button mushroom
SDJ	The glucose-free SD media, with JA
SDM	The glucose-free SD media, with WBM
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
Ig	immunoglobulin
SGR	Specific growth rate
FCR	Food conversion ratio
PER	Protein efficiency rate

Acknowledgements

The authors are grateful to the technical staff of the Gorgan University of Agricultural Sciences and Natural Resources, Iran for their excellent technical assistance,

Author contributions

HZ conducted the experiment and executed most of the data processing and analysis. MS, SH, and HP participated in the designing of the experiments and data analysis, SH and MS guided and supervised the work. HZ, KB and PN wrote the bulk of the manuscript and critically edited by MS, SH, and HP. All authors contributed to the article and approved the submitted version.

Funding

This present study is conducted at Gorgan University of Agricultural Sciences and Natural Resources (Iran) and supported by the project grant provided by Golestan University of Medical Sciences (Iran). The authors wish to express their thanks for the financial support of the research.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval

The zebrafish larvae were kept and handled following the ethical guidelines for in vivo experiments developed by the Golestan University of Medical Sciences. This accreditation is valid within the framework of the accepted national and international ethical norms and principles for biomedical research as well as the guidelines and protocols of the Islamic Republic of Iran, Ministry of Health and Medical Education (Approval ID: IR.GOUMS.REC.1397.262).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan 4934174515, Iran

[2]Department of Laboratory Sciences, Faculty of Para-medicine, Golestan University of Medical Sciences, Gorgan 4934174515, Iran
[3]Department of Aquaculture, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan 4918943464, Iran
[4]Department of Applied Animal Science and Welfare, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Uppsala 7070, SE-750 07, Sweden

Received: 25 November 2023 / Accepted: 9 August 2024
Published online: 09 September 2024

References

1. Bengmark S. Gut microbiota, immune development and function. Pharmacol Res. 2013;69(1):87–113. https://doi.org/10.1016/j.phrs.2012.09.002.
2. Harmsen HJ, de Goffau MC. The human gut microbiota. Microbiota Hum body: Implications Health Disease. 2016;95–108. https://doi.org/10.1007/978-3-319-31248-4_7.
3. Gomaa EZ. Human gut microbiota/microbiome in health and diseases: a review. Antonie Van Leeuwenhoek. 2020;113(12):2019–40. https://doi.org/10.1007/s10482-020-01474-7.
4. Kuang T, He A, Lin Y, Huang X, Liu L, Zhou L. Comparative analysis of microbial communities associated with the L., gill, gut, and habitat of two filter-feeding fish. Aquac Res. 2020;18:100501. https://doi.org/10.1016/j.aqrep.2020.100501.
5. Kriss M, Hazleton KZ, Nusbacher NM, Martin CG, Lozupone CA. Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery. Curr Opin Microbiol. 2018;44:34–40. https://doi.org/10.1016/j.mib.2018.07.003.
6. Proffitt C, Bidkhori G, Moyes D, Shoaie S. Disease, drugs and dysbiosis: understanding microbial signatures in metabolic disease and medical interventions. Microorganisms. 2020;8(9):1381. https://doi.org/10.3390/microorganisms8091381.
7. Khor B, Snow M, Herrman E, Ray N, Mansukhani K, Patel KA, et al. Interconnections between the oral and gut microbiomes: reversal of microbial dysbiosis and the balance between systemic health and disease. Microorganisms. 2021;9(3):496. https://doi.org/10.3390/microorganisms9030496.
8. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. Genome Med. 2011;3(3):1–12. https://doi.org/10.1186/gm228.
9. Wu L, Cao Y, Liao C, Huang J, Gao F. Systematic review and meta-analysis of randomized controlled trials of simethicone for gastrointestinal endoscopic visibility. Scand J Gastroenterol. 2011;46(2):227–35. https://doi.org/10.3109/00365521.2010.525714.
10. Gurry T. Synbiotic approaches to human health and well-being. Microb Biotechnol. 2017;10(5):1070–3. https://doi.org/10.1111/1751-7915.12789.
11. Puri P, Sharma JG, Singh R. Biotherapeutic microbial supplementation for ameliorating fish health: developing trends in probiotics, prebiotics, and synbiotics use in finfish aquaculture. Anim Health Res Reviews. 2022;1–23. https://doi.org/10.1017/S1466252321000165.
12. del Valle JC, Bonadero MC, Gimenez AVF. *Saccharomyces cerevisiae* as probiotic, prebiotic, synbiotic, postbiotics and parabiotics in aquaculture: an overview. Aquaculture. 2023;739342. https://doi.org/10.1016/j.aquaculture.2023.739342.
13. Krumbeck JA, Maldonado-Gomez MX, Ramer-Tait AE, Hutkins RW. Prebiotics and synbiotics: dietary strategies for improving gut health. Curr Opin Gastroenterol. 2016;32(2):110–9. https://doi.org/10.1097/MOG.0000000000000249.
14. Huynh TG, Shiu YL, Nguyen TP, Truong QP, Chen JC, Liu CH. Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review. Fish Shellfish Immunol. 2017;64:367–82. https://doi.org/10.1016/j.fsi.2017.03.035.
15. Kumar P, Jain KK, Sardar P, Jayant M, Tok NC. Effect of dietary synbiotic on growth performance, body composition, digestive enzyme activity and gut microbiota in *Cirrhinus mrigala* (Ham.) Fingerlings. Aquacult Nutr. 2018;24:921–9. https://doi.org/10.1111/anu.12628.
16. Mohammadian T, Nasirpour M, Tabandeh MR, Mesbah M. Synbiotic effects of β-glucan, mannan oligosaccharide and *Lactobacillus casei* on growth performance, intestine enzymes activities, immune-hematological parameters and immune-related gene expression in common carp, *Cyprinus carpio*: an experimental infection with *Aeromonas hydrophila*. Aquaculture. 2019;511:634197. https://doi.org/10.1016/j.aquaculture.2019.06.011.
17. Hasyimi W, Widanarni W, Yuhana M. Growth performance and intestinal microbiota diversity in Pacific White Shrimp *Litopenaeus vannamei* Fed with a probiotic bacterium, Honey Prebiotic, and Synbiotic. Curr Microbiol. 2020;77:2982–90. https://doi.org/10.1007/s00284-020-02117-w.
18. Moustafa EM, Saad TT, Khalil RH, Dawood MAO, Lolo EE. The ameliorative role of synbiotic culture techniques application in white shrimp *(Litopenaeus vannamei)* during nursery stage. Adv Anim Veterinary Sci. 2020;8:260–77. https://doi.org/10.17582/journal.aavs/2020/8.3.260.277.
19. Kong Y, Li M, Chu G, Liu H, Shan X, Wang G, et al. The positive effects of single or conjoint administration of lactic acid bacteria on *Channa argus*: Digestive enzyme activity, antioxidant capacity, intestinal microbiota and morphology. Aquaculture. 2021;531:735852. https://doi.org/10.1016/j.aquaculture.2020.735852.
20. Nayak SK. Probiotics and immunity: a fish perspective. Fish Shellfish Immunol. 2010;29(1):2–14. https://doi.org/10.1016/j.fsi.2010.02.017.
21. Abu-Elala N, Marzouk M, Moustafa M. Use of different *Saccharomyces cerevisiae* biotic forms as immune-modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens. J Vet Sci. 2013;1(1):21–9. https://doi.org/10.1016/j.ijvsm.2013.05.001.
22. Chuang WY, Hsieh YC, Lee TT. The effects of fungal feed additives in animals: a review. Animal. 2020;10(5):805. https://doi.org/10.3390/ani10050805.
23. Gupta A, Gupta SK, Priyam M, Siddik MA, Kumar N, Mishra PK, Gupta K, Sarkar B, Sharma TR, Pattanayak A. Immunomodulation by dietary supplements: A preventive health strategy for sustainable aquaculture of tropical freshwater fish, *Labeo rohita* (Hamilton, 1822). Reviews in Aquaculture. 2021; 13(4): 2364–2394. https://doi.org/10.1111/raq.12581
24. Rohani M, Islam SM, Hossain MK, Ferdous Z, Siddik MA, Nuruzzaman M, Padeniya U, Brown C, Shahjahan M. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: upgrading growth, reproduction, immunity and disease resistance in fish. Fish Shellfish Immunol. 2022;120:569–89. https://doi.org/10.1016/j.fsi.2021.12.037.
25. Jasim SA, Abdelbasset WK, Shichiyakh RA, Al-Shawi SG, Yasin G, Jalil AT, et al. Probiotic effects of the fungi, *aspergillus Niger* on growth, immunity, haematology, intestine fungal load and digestive enzymes of the common carp, *Cyprinus carpio*. Aquac Res. 2022;53(10):3828–40. https://doi.org/10.1111/are.15890.
26. Siddik MA, Foysal MJ, Fotedar R, Francis DS, Gupta SK. Probiotic yeast *Saccharomyces cerevisiae* coupled with *Lactobacillus casei* modulates physiological performance and promotes gut microbiota in juvenile barramundi, *lates calcarifer*. Aquaculture. 2022;546:737346. https://doi.org/10.1016/j.aquaculture.2021.737346.
27. Tegegne BA, Kebede B. Probiotics, their prophylactic and therapeutic applications in human health development: a review of the literature. Heliyon. 2022;e09725. https://doi.org/10.1016/j.heliyon.2022.e09725.
28. Cheng AC, Peng X, Chen W, Tseng DY, Tan Z, Liu H, et al. Dietary probiotic *aspergillus Niger* preparation improves the growth performance, health status, and gut microbiota of white shrimp, *Penaeus vannamei*. Aquaculture. 2023;577:739988. https://doi.org/10.1016/j.aquaculture.2023.739988.
29. Jasim SA, Abdelbasset WK, Shichiyakh RA, Al-Shawi SG, Yasin G, Jalil AT, et al. Probiotic effects of the fungi, *aspergillus* Niger on growth, immunity, haematology, intestine fungal load and digestive enzymes of the common carp, *Cyprinus carpio*. Aquac Res. 2022;53(10):3828–40. https://doi.org/10.1111/are.15890.
30. Fathi MM, Ebeid TA, Al-Homidan I, Soliman NK, Abou-Emera OK. Influence of probiotic supplementation on immune response in broilers raised under hot climate. Br Poult Sci. 2017;58(5):512–6. https://doi.org/10.1080/00071668.2017.1332405.
31. Yadav AK, Tyagi A, Kumar A, Panwar S, Grover S, Saklani AC, et al. Adhesion of lactobacilli and their anti-infectivity potential. Crit Rev Food Sci Nutr. 2017;57(10):2042–56. https://doi.org/10.1080/10408398.2014.918533.
32. Lin H, Ding B, Chen L, Zhang Z, He H, Wang J, et al. The effect of *Aspergillus Niger* as a dietary supplement on blood parameters, intestinal morphology, and gut microflora in Haidong chicks reared in a high altitude environment. Veterinary World. 2020;13(10):2209. https://doi.org/10.14202/vetworld.2020.2209-2215.
33. Divyashri G, Karthik P, Murthy TK, Priyadarshini D, Reddy KR, Raghu AV, Vaidyanathan VK. Non-digestible oligosaccharides-based prebiotics to ameliorate obesity: overview of experimental evidence and future perspectives. Food Sci Biotechnol. 2023;32(14):1993–2011. https://doi.org/10.1007/s10068-023-01381-3.
34. Gibson GR, Hut1kins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: the International Scientific Association for

Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Reviews Gastroenterol Hepatol. 2017;14(8):491–502. https://doi.org/10.1038/nrgastro.2017.75.
35. Lafontaine GMF, Fish NM, Connerton IF. *In vitro* evaluation of the effects of commercial prebiotic GOS and FOS products on human colonic caco–2 cells. Nutrients. 2020;12(5):1281. https://doi.org/10.3390/nu12051281.
36. Cid García RA, Hernández Hernández LH, Carrillo Longoria JA, Fernández Araiza MA. Inclusion of yeast and/or fructooligosaccharides in diets with plant-origin protein concentrates for rainbow trout (*Oncorhynchus mykiss*) fingerlings. J World Aquaculture Soc. 2020;51(4):970–81. https://doi.org/10.1111/jwas.12661.
37. Dawood MA, Eweedah NM, Moustafa EM, Shahin MG. Synbiotic effects of *aspergillus oryzae* and β-glucan on growth and oxidative and immune responses of Nile Tilapia, *Oreochromis niloticus*. Probiotics Antimicrob Protein. 2020a;12(1):172–83. https://doi.org/10.1063/1.4975427.
38. Kishawy AT, Sewid AH, Nada HS, Kamel MA, El-Mandrawy SA, Abdelhakim T, et al. Mannanoligosaccharides as a carbon source in Biofloc boost dietary plant protein and water quality, growth, immunity and *Aeromonas hydrophila* resistance in Nile tilapia (*Oreochromis niloticus*). Animal. 2020;10:1724. https://doi.org/10.3390/ani10101724.
39. Pietrzak E, Mazurkiewicz J, Slawinska A. Innate immune responses of skin mucosa in common carp (*Cyprinus carpio*) fed a diet supplemented with galactooligosaccharides. Animal. 2020;10:438. https://doi.org/10.3390/ani10030438.
40. Zhou L, Zhang J, Yan M, Tang S, Wang X, Qin JG, et al. Inulin alleviates hypersaline-stress induced oxidative stress and dysbiosis of gut microbiota in Nile tilapia (*Oreochromis niloticus*). Aquaculture. 2020;529:735681. https://doi.org/10.1016/j.aquaculture.2020.735681.
41. Ghafarifarsani H, Hoseinifar SH, Talebi M, Yousefi M, Van Doan H, Rufchaei R, et al. Combined and singular effects of ethanolic extract of persian shallot (*Allium Hirtifolium* Boiss) and synbiotic Biomin® IMBO on growth performance, serum-and mucus-immune parameters and antioxidant defense in zebrafish (*Danio rerio*). Animal. 2021;11(10):2995. https://doi.org/10.3390/ani11102995.
42. Pimpimol T, Klahan R, Chitmanat C. The effects of garlic, banana, and onion as prebiotic supplementation on growth performances, feed utilization, and survival rate of *Anabas testudineus*. J Sci Technol 2018; 23(4).
43. Amenyogbe E, Chen G, Wang Z, Huang J, Huang B, Li H. The exploitation of probiotics, prebiotics and synbiotics in aquaculture: present study, limitations and future directions.: a review. Aquacult Int. 2020;28(3):1017–41. https://doi.org/10.1007/s10499-020-00509-0.
44. Sribounoy U, Pirarat N, Solval KM, Sathivel S, Chotiko A. Development of pelleted feed containing probiotic *Lactobacillus rhamnosus* GG and Jerusalem artichoke for Nile Tilapia and its biocompatibility studies. 3 Biotech. 2021;11(6):1–9. https://doi.org/10.1007/s13205-021-02829-1.
45. Zakariaee H, Sudagar M, Hosseini SS, Paknejad H, Baruah K. In vitro Selection of synbiotics and in vivo investigation of growth indices, reproduction performance, survival, and ovarian *Cyp19a* gene expression in zebrafish *Danio rerio* Frontiers in Microbiology. 2021; 12: 758758. https://doi.org/10.3389/fmicb.2021.758758
46. Din ARJM, Razak SA, Sabaratnam V. Effect of mushroom supplementation as a prebiotic compound in super worm based diet on growth performance of red tilapia fingerlings. Sains Malays. 2012;41(10):1197–203.
47. Van Doan H, Doolgindachbaporn S, Suksri A. Effect of lactobacillus plantarum and Jerusalem artichoke (*Helianthus tuberosus*) on growth performance, immunity, and disease resistance of Pangasius CATFISH (*Pangasius bocourti*, Sauvage. 1880). Aquacult Nutr. 2016;22(2):444–56. https://doi.org/10.1111/anu.12263.
48. Jabłońska-Ryś E, Sławińska A, Radzki W, Gustaw W. Evaluation of the potential use of probiotic strain *Lactobacillus plantarum* 299v in lactic fermentation of button mushroom fruiting bodies. Acta Scientiarum Polonorum Technologia Aliment. 2016;15(4):399–407. https://doi.org/10.17306/J.AFS.2016.4.38.
49. Solano-Aguilar GI, Lakshman S, Jang S, Beshah E, Xie Y, Sikaroodi M, et al. The effect of feeding cocoa powder and *Lactobacillus rhamnosus* on the composition and function of pig intestinal microbiome. Curr Developments Nutr. 2018;2(5):nzy011. https://doi.org/10.1093/cdn/nzy011.
50. Sawangwan T, Wansanit W, Pattani L, Noysang C. Study of prebiotic properties from edible mushroom extraction. Agric Nat Resour. 2018;52(6):519–24. https://doi.org/10.1016/j.anres.2018.11.020.
51. Sewaka M, Trullas C, Chotiko A, Rodkhum C, Chansue N, Boonanuntanasarn S, et al. Efficacy of synbiotic Jerusalem artichoke and Lactobacillus rhamnosus GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against Aeromonas veronii in juvenile red tilapia (Oreochromis spp). Fish Shellfish Immunol. 2019;86:260–8. https://doi.org/10.1016/j.fsi.2018.11.026.
52. Itelima J, Ogbonna A, Pandukur S, Egbere J, Salami A. Simultaneous saccharification and fermentation of corn cobs to bio-ethanol by co-culture of *Aspergillus Niger* and *Saccharomyces cerevisiae*. Int J Environ Sci Dev. 2013;4(2):239–42. https://doi.org/10.7763/IJESD.2013.V4.343.
53. Sinha A, Pick E. Fluorescence detection of increased reactive oxygen species levels in *Saccharomyces cerevisiae* at the diauxic shift. Reactive Oxygen Species: Methods Protocols. 2021;81–91. https://doi.org/10.1007/978-1-0716-0896-8_7.
54. Kask S, Adamberg K, Orłowski A, Vogensen FK, Møller PL, Ardö Y, et al. Physiological properties of *Lactobacillus paracasei*, *L. Danicus* and *L. Curvatus* strains isolated from Estonian semi-hard cheese. Food Res Int. 2003;36(9–10):1037–46. https://doi.org/10.1016/j.foodres.2003.08.002.
55. Harborne JB. Plant phenolics. In: Bell EA, Charlwood BV, editors. Secondary Plant products. Encyclopedia of Plant Physiology, New Series. Volume 8. New York: Springer–; 1990. pp. 329–402. https://doi.org/10.1371/journal.pone.0194932.
56. Sobye A, Knop Lund ML, Fojan P. Bioproduction of natural carotenoids by *Dietzia maris* and *Rhodococcus opacus*. Master thesis, Aalborg university, Denmark. 2018; 85. https://projekter.aau.dk/projekter/files/281299885/Thesis.pdf
57. Aberkane A, Cuenca-Estrella M, Gomez-Lopez A, Petrikkou E, Mellado E, Monzon A, et al. Comparative evaluation of two different methods of inoculum preparation for antifungal susceptibility testing of filamentous fungi. J Antimicrob Chemotherap. 2002;50(5):719–22. https://doi.org/10.1093/jac/dkf187.
58. Ilgın M, Germec M, Turhan I. Inulinase production and mathematical modeling from carob extract by using *aspergillus Niger*. Biotechnol Prog. 2020;36(1):e2919. https://doi.org/10.1002/btpr.2919.
59. Gürler HN, Germec M, Turhan I. The inhibition effect of phenol on the production of *Aspergillus Niger* inulinase and its modeling. J Food Process Preservatio. 2021;45(8):e14522. https://doi.org/10.1111/jfpp.14522.
60. Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Merrifield DL, Ringø E. In vitro selection of a synbiotic and in vivo evaluation on intestinal microbiota, performance and physiological response of rainbow trout (*Oncorhynchus mykiss*) fingerlings. Aquacult Nutr. 2017;23(1):111–8. https://doi.org/10.1111/anu.12373.
61. Singh SK, Tiwari VK, Chadha NK, Munilkumar S, Prakash C, Pawar NA. Effect of dietary synbiotic supplementation on growth, immune and physiological status of *Labeo rohita* juveniles exposed to low pH stress. Fish Shellfish Immunol. 2019;91:358–68. https://doi.org/10.1016/j.fsi.2019.05.023.
62. Akbari Nargesi E, Falahatkar B, Sajjadi MM. Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquacult Nutr. 2020;26(1):98–108. https://doi.org/10.1111/anu.12970.
63. Mohammadi Arani M, Salati AP, Safari O, Keyvanshokooh S. Dietary supplementation effects of *Pediococcus acidilactici* as probiotic on growth performance, digestive enzyme activities and immunity response in zebrafish (*Danio rerio*). Aquacult Nutr. 2019;25(4):854–61. https://doi.org/10.1111/anu.12904.
64. Abedian Amiri A, Azari Takami GH, Afsharnasab M, Razavilar V. The comparative effects of dietary supplementation with *Pediococcus acidilactici* and *Enterococcus faecium* on feed utilization, various health-related characteristics and yersiniosis in rainbow trout (*Oncorhynchus mykiss* walbaum, 1972). Iranian Journal of Fisheries Sciences. 2017; 16: 753–773. https://doi.org/10.22092/ijfs.2018.114695
65. Guo J, Bao Y, Davis R, Abebe A, Wilson AE, Davis DA. Application of meta-analysis towards understanding the effect of adding a methionine hydroxy analogue in the diet on growth performance and feed utilization of fish and shrimp. Reviews Aquaculture. 2020;12(4):2316–32. https://doi.org/10.1111/raq.12436.
66. Ashouri G, Soofiani NM, Hoseinifar SH, Jalali SAH, Morshedi V, Valinassab T, et al. Influence of dietary sodium alginate and *Pediococcus acidilactici* on liver antioxidant status, intestinal lysozyme gene expression, histomorphology, microbiota, and digestive enzymes activity, in Asian sea bass (*Lates calcarifer*) juveniles. Aquaculture. 2020;518:734638. https://doi.org/10.1016/j.aquaculture.2019.734638.
67. Rashidian G, Boldaji JT, Rainis S, Prokić MD, Faggio C. Oregano (*Origanum vulgare*) extract enhances zebrafish (*Danio rerio*) growth performance, serum and mucus innate immune responses and resistance against *Aeromonas*

hydrophila challenge. Animal. 2021;11(2):299. https://doi.org/10.3390/ani11020299.
68. Pedroso GL, Hammes TO, Escobar TD, Fracasso LB, Forgiarini LF, da Silveira TR. Blood collection for biochemical analysis in adult zebrafish. J Visualized Experiments. 2012;63:e3865. https://doi.org/10.3791/3865.
69. Ahmadifar E, Dawood MA, Moghadam MS, Sheikhzadeh N, Hoseinifar SH, Musthafa MS. Modulation of immune parameters and antioxidant defense in zebrafish (*Danio rerio*) using dietary apple cider vinegar. Aquaculture. 2019;513:734412. https://doi.org/10.1016/j.aquaculture.2019.734412.
70. Ahmadifar E, Dawood MA, Moghadam MS, Shahrestanaki AH, Van Doan H, Saad AH, et al. The effect of *Pediococcus acidilactici* MA 18/5 M on immune responses and mRNA levels of growth, antioxidant and immune-related genes in zebrafish (*Danio rerio*). Aquaculture Res. 2020;17:100374. https://doi.org/10.1016/j.fsi.2013.09.036.
71. Falahatkar B, Poursaeid S, Shakoorian M, Barton BA. Responses to handling and confinement stressors in juvenile great sturgeon *Huso huso*. J Fish Biol. 2009;75:784–96. https://doi.org/10.1111/j.1095-8649.2009.02334.x.
72. Bradford MM. A rapid sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem. 1976;72:248–54. https://doi.org/10.1016/0003-2697(76)90527-3.
73. Hedayati SA, Farsani HG, Naserabad SS, Hoseinifar SH, Van Doan H. Protective effect of dietary vitamin E on immunological and biochemical induction through silver nanoparticles (AgNPs) inclusion in diet and silver salt (AgNO3) exposure on Zebrafish (*Danio rerio*). Comparative *Biochemistry* and *Physiology* Part - C: Toxicology and Pharmacology. 2019; 222: 100–107.https://doi.org/10.1016/j.cbpc.2019.04.004
74. Siwicki AK, Vergnet C, Charlemagne J, Dunier M. Monoclonal antibodies against goldfish (*Carassius auratus*) immunoglobulin: application to the quantification of immunoglobulin and antibody-secreting cells by ELISPOT and seric immunoglobulin and antibody levels by ELISA in carp (*Cyprinus carpio*). Vet Res. 1994;25(5):458–67.
75. Yousefi S, Hoseinifar SH, Paknejad H, Hajimoradloo A. The effects of dietary supplement of galactooligosaccharide on innate immunity, immune related genes expression and growth performance in zebrafish (*Danio rerio*). Fish and shellfish immunology. 2018; 73: 192–6. https://doi.org/10.1016/j.fsi.2017.12.022
76. Jonova S, Ilgaza A, Zolovs M. The impact of inulin and a novel synbiotic (yeast *Saccharomyces cerevisiae* strain 1026 and inulin) on the development and functional state of the gastrointestinal canal of calves. Veterinary Medicine International. 2021; 2021: 1–9. https://doi.org/10.1155/2021/8848441
77. Sridhar KR, Mahadevakumar S. Fungal probiotics and Prebiotics. Fungal biotechnology prospects and avenues. Boca Raton, FL, USA: CRC; 2022. pp. 260–79. https://doi.org/10.3390/foods12102010.
78. Rousta N, Aslan M, Yesilcimen Akbas M, Ozcan F, Sar T, Taherzadeh MJ. Effects of fungal based bioactive compounds on human health. Crit Rev Food Sci Nutr. 2023;1–24. https://doi.org/10.1080/10408398.2023.2178379.
79. Mohammady EY, Aboseif AM, Soaudy MR, Ramadan EA, Hassaan MS. Appraisal of fermented wheat bran by *Saccharomyces cerevisiae* on growth, feed utilization, blood indices, intestinal and liver histology of Nile tilapia, *Oreochromis niloticus*. Aquaculture. 2023;739755. https://doi.org/10.1016/j.aquaculture.2023.739755.
80. Zhang M, Liang H, Lei Y, Zhang Y, Tan Z, Chen W, Li S, et al. *Aspergillus Niger* confers health benefits and modulates the gut microbiota of juvenile Pacific white shrimp (*Penaeus vannamei*) under farming conditions. Front Mar Sci. 2023;10:1211993. https://doi.org/10.3389/fmars.2023.1211993.
81. Ariyanto F, Nugroho RA, Aryani R, Manurung H, Rudianto R. Effect of Water Hyacinth Leaf Flour (*Eichhornia crassipes*) fermented by *Aspergillus Niger* on the growth, Survival Rate and Blood Profile of Sangkuriang Catfish (*Clarias gariepinus*). Aceh J Anim Sci. 2023;8(2):52–7. https://doi.org/10.13170/ajas.8.2.31390.
82. Said AF, Al-Taee NTT, Al-Taee SKE. Impact of *Saccharomyces cerevisiae* on Growth Performance of Carp Fish Exposed to CuSo4. In IOP Conference Series: Earth and Environmental Science. 2023; 1158 (5): 052008. IOP Publishing. https://doi.org/10.1088/1755-1315/1158/5/052008
83. Samidjan I, Rachmawati D, Dody S, Riyadi PH. Effects of various doses of *Saccharomyces cerevisiae* on the growth, survival rate, and blood profile of saline red tilapia (*Oreochromis spp.)* in the semi-intensive culture conditions. Pertanika J Sci Technol. 2023;31(1). https://doi.org/10.47836/pjst.31.1.31.
84. Zhong C, Ukowitz C, Domig KJ, Nidetzky B. Short-chain cello-oligosaccharides: intensification and scale-up of their enzymatic production and selective growth promotion among probiotic bacteria. J Agric Food Chem. 2020;68(32):8557–67. https://doi.org/10.1021/acs.jafc.0c02660.
85. Napisah H, Nuriah MS, Zarinah Z, Deli RRA. Fermentation properties and potential prebiotic activity of HCl-breadfruit resistant starch type III by *Lactobacillus plantarum* ATCC 13649 and *L. Brevis* ATCC 8287. Int Food Res J. 2020;29(1):210–22. https://doi.org/10.47836/ifrj.29.1.22.
86. Mohan K, Karthick Rajan D, Muralisankar T, Ramu Ganesan A, Marimuthu K, Sathishkumar P. The potential role of medicinal mushrooms as prebiotics in aquaculture: a review. Reviews Aquaculture. 2022;14(3):1300–32. https://doi.org/10.1111/raq.12651.
87. Fernandes A, Nair A, Kulkarni N, Todewale N, Jobby R. Exploring mushroom polysaccharides for the development of Novel Prebiotics: a review. Int J Med Mushrooms. 2023;25(2). https://doi.org/10.1615/IntJMedMushrooms.2022046837.
88. Ali MS, Elnaz M, Ladan N. Prebiotic effect of Jerusalem artichoke (*Helianthus tuberosus*) fructans on the growth performance of *Bifidobacterium bifidum* and *Escherichia coli*. Asian Pac J Trop Disease. 2016;6(5):385–9. https://doi.org/10.1016/S2222-1808(15)61053-2.
89. Prasad KN, Bondy SC. Dietary fibers and their fermented short-chain fatty acids in prevention of human diseases. Bioactive Carbohydr Diet Fibre. 2019;17:100170. https://doi.org/10.1016/j.bcdf.2018.09.001.
90. Wang M, Wichienchot S, He X, Fu X, Huang Q, Zhang B. In vitro colonic fermentation of dietary fibers: fermentation rate, short-chain fatty acid production and changes in microbiota. Trends Food Sci Technol. 2019;88:1–9. https://doi.org/10.1016/j.tifs.2019.03.005.
91. Firrman J, Liu L, Mahalak K, Tanes C, Bittinger K, Tu V, et al. The impact of environmental pH on the gut microbiota community structure and short chain fatty acid production. FEMS Microbiol Ecol. 2022;98(5):fiac038. https://doi.org/10.1093/femsec/fiac038.
92. Froese R. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J Appl Ichthyol. 2006;22:241–53. https://doi.org/10.1111/j.1439-0426.2006.00805.x.
93. Geffen R. The origin of Fulton's Condition factor— setting the record straight. Fisheries. 2006;31:236–8.
94. Hasan KN, Banerjee G. Recent studies on probiotics as beneficial mediator in aquaculture: a review. J Basic Appl Zool. 2020;81(1):1–16. https://doi.org/10.1186/s41936-020-00190-y.
95. Chen M, Fan B, Liu S, Imam KMSU, Xie Y, Wen B, et al. The in vitro effect of fibers with different degrees of polymerization on human gut bacteria. Front Microbiol. 2020;11:819. https://doi.org/10.3389/fmicb.2020.00819.
96. Hassaan MS, Mohammady EY, Soaudy MR, Elashry MA, Moustafa MM, Wassel MA, et al. Synergistic effects of *Bacillus pumilus* and exogenous protease on Nile tilapia (*Oreochromis niloticus*) growth, gut microbes, immune response and gene expression fed plant protein diet. Anim Feed Sci Technol. 2021;275:114892. https://doi.org/10.1016/j.anifeedsci.2021.114892.
97. Knipe H, Temperton B, Lange A, Bass D, Tyler CR. Probiotics and competitive exclusion of pathogens in shrimp aquaculture. Reviews Aquaculture. 2021;13(1):324–52. https://doi.org/10.1111/raq.12477.
98. Chuphal N, Singha KP, Sardar P, Sahu NP, Shamna N, Kumar V. Scope of Archaea in Fish feed: a new chapter in Aquafeed Probiotics? Probiotics Antimicrob Proteins. 2021;13(6):1668–95. https://doi.org/10.1007/s12602-021-09778-4.
99. Menberu MA, Cooksley C, Ramezanpour M, Bouras G, Wormald PJ, Psaltis AJ et al. *In vitro* and *in vivo* evaluation of probiotic properties of *Corynebacterium accolens* isolated from the human nasal cavity. Microbiological Research. 2022; 255: 126927. https://doi.org/10.1016/j.micres.2021.126927
100. Vallejo-García LC, Rodríguez-Alegría ME, López Munguía A. Enzymatic process yielding a diversity of inulin-type microbial fructooligosaccharides. J Agric Food Chem. 2019;67(37):10392–400. https://doi.org/10.1021/acs.jafc.9b03782.
101. Nekoubin H, Gharedashi E, Imanpour MR, Asgharimoghadam A. The influence of synbiotic (Biomin Imbo) on growth factors and survival rate of zebrafish (*Danio rerio*) larvae via supplementation with biomar. Global Vet. 2012;8(5):503–6. https://idosi.org/gv/GV8(5)12/13.pdf.
102. El-Haroun ER, Goda AS, Kabir Chowdhury MA. Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis Niloticus* (L). Aquac Res. 2006;37(14):1473–80. https://doi.org/10.1111/j.1365-2109.2006.01584.x.
103. Ketnawa S, Ogawa Y. Evaluation of protein digestibility of fermented soybeans and changes in biochemical characteristics of digested fractions. J Funct Foods. 2019;52:640–7. https://doi.org/10.1016/j.jff.2018.11.046.
104. Carnevali O, de Vivo L, Sulpizio R, Gioacchini G, Olivotto I, Silvi S, et al. Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene

expression. Aquaculture. 2006;258(1–4):430–8. https://doi.org/10.1016/j.aquaculture.2006.04.025.
105. Magouz FI, Dawood MA, Salem MF, Mohamed AA. The effects of fish feed supplemented with Azolla meal on the growth performance, digestive enzyme activity, and health condition of genetically-improved farmed tilapia (*Oreochromis niloticus*). Annals Anim Sci. 2020;20(3):1029–45. https://doi.org/10.2478/aoas-2020-0016.
106. Yang S-C, Chen JY, Shang HF, Cheng TY, Tsou SC, Chen JR. Effect of synbiotics on intestinal microflora and digestive enzyme activities in rats. World J Gastroenterol. 2005;11(47):7413–7. https://doi.org/10.3748/wjg.v11.i47.7413.
107. Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, Ringø E. Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol. 2014;40(1):40–8. https://doi.org/10.1016/j.fsi.2014.06.016.
108. Yao W, Li X, Zhang C, Wang J, Cai Y, Leng X. Effects of dietary synbiotics supplementation methods on growth, intestinal health, non-specific immunity and disease resistance of Pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 2021;112:46–55. https://doi.org/10.1016/j.fsi.2021.02.011.
109. Arias-de la Rosa I, Ruiz-Ponce M, Cuesta-López L, Pérez-Sánchez C, Leiva-Cepas F, Gahete MD, Navarro P, et al. Clinical features and immune mechanisms directly linked to the altered liver function in patients with rheumatoid arthritis. Eur J Intern Med. 2023;In press. https://doi.org/10.1016/j.ejim.2023.08.002.
110. Moss DW. Alkaline phosphatase isoenzymes. Clin Chem. 1982;28(10):2007–16. https://pubmed.ncbi.nlm.nih.gov/6751596/.
111. Sharma U, Pal D, Prasad R. Alkaline phosphatase: an overview. Indian J Clin Biochem. 2014;29(3):269–78. https://doi.org/10.1007/s12291-013-0408-y.
112. Fernandez NJ, Kidney BA. Alkaline phosphatase: beyond the liver. Vet Clin Pathol. 2007;36(3):223–33. https://doi.org/10.1111/j.1939-165x.2007.tb00216.x.
113. Bhardwaj S, Canlas K, Kahi C, Temkit MH, Molleston J, Ober M, et al. Hepatobiliary abnormalities and disease in cystic fibrosis: epidemiology and outcomes through adulthood. J Clin Gastroenterol. 2009;43(9):858–64. https://doi.org/10.1097/MCG.0b013e31819e8bbd.
114. Bhardwaj M, Mani S, Malarvizhi R, Sali VK, Vasanthi HR. Immunomodulatory activity of brown algae *Turbinaria ornata* derived sulfated polysaccharide on LPS induced systemic inflammation. Phytomedicine. 2021;89:153615. https://doi.org/10.1016/j.phymed.2021.153615.
115. Jerez-Cepa I, Gorissen M, Mancera JM, Ruiz-Jarabo I. What can we learn from glucocorticoid administration in fish? Effects of cortisol and dexamethasone on intermediary metabolism of gilthead seabream (Sparus aurata L). Comp Biochem Physiol Part Mol Integr Physiol. 2019;231:1–10. https://doi.org/10.1016/j.cbpa.2019.01.010.
116. Klatzkin RR, Baldassaro A, Rashid S. Physiological responses to acute stress and the drive to eat: the impact of perceived life stress. Appetite. 2019;133:393–9. https://doi.org/10.1016/j.appet.2018.11.019.
117. Thau L, Gandhi J, Sharma S, Physiology. cortisol. StatPearls. 2021. https://www.ncbi.nlm.nih.gov/books/NBK538239/
118. Enes P, Panserat S, Kaushik S, Oliva-Teles AA. Nutritional regulation of hepatic glucose metabolism in fish. Fish Physiol Biochem. 2009;35:519–39. https://doi.org/10.1007/s10695-008-9259-5.
119. Aghahosseini H, Ramazani A, Azimzadeh Asiabi P, Gouranlou F, Hosseini F, Rezaei A, Min BK, Woo Joo S. Glucose-based biofuel cells: nanotechnology as a vital science in biofuel cells performance. Nanochemistry Res. 2016;1(2):183–204. https://doi.org/10.7508/ncr.2016.02.006.
120. Stevens CH, Croft DP, Paull GC, Tyler CR. Stress and welfare in ornamental fishes: what can be learned from aquaculture? J Fish Biol. 2017;91(2):409–28. https://doi.org/10.1111/jfb.13377.
121. Odhiambo E, Angienda PO, Okoth P, Onyango D. Stocking density induced stress on plasma cortisol and whole blood glucose concentration in Nile tilapia fish (*Oreochromis niloticus*) of lake Victoria, Kenya. Int J Zool. 2020;17:1–8. https://doi.org/10.1155/2020/9395268.
122. de Freitas Souza C, Baldissera MD, Bianchini AE, da Silva EG, Mourão RHV, da Silva LVF, et al. Citral and linalool chemotypes of *Lippia alba* essential oil as anesthetics for fish: a detailed physiological analysis of side effects during anesthetic recovery in silver catfish (*Rhamdia quelen*). Fish Physiol Biochem. 2018;44(1):21–34. https://doi.org/10.1007/s10695-017-0410-z.
123. Yu MC, Li ZJ, Lin HZ, Wen GL, Ma S. Effects of dietary Bacillus and medicinal herbs on the growth, digestive enzyme activity, and serum biochemical parameters of the shrimp *Litopenaeus vannamei*. Aquacult Int. 2008;16(5):471–80. https://doi.org/10.1007/s10499-007-9159-1.
124. Sadat Hoseini Madani N, Adorian TJ, Ghafari Farsani H, Hoseinifar SH. The effects of dietary probiotic Bacilli (*Bacillus subtilis* and *Bacillus licheniformis*) on growth performance, feed efficiency, body composition and immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) postlarvae. Aquac Res. 2018;49(5):1926–33. https://doi.org/10.1111/are.13648.
125. Meshkini S, Tafi AA. Presentation and usage of immunostimulators in aquaculture. Shabestar Branch Islamic Azad Univ, 2016; 190 p. http://www.file:///C:/Users/n/Downloads/prohpylaxisinaquaculture.pdf
126. Ashouri G, Soofiani NM, Hoseinifar SH, Jalali SAH, Morshedi V, Van Doan H, et al. Combined effects of dietary low molecular weight sodium alginate and Pediococcus acidilactici MA18/5 M on growth performance, haematological and innate immune responses of Asian sea bass (Lates calcalifer) juveniles. Fish Shellfish Immunol. 2018;79:34–41. https://doi.org/10.1016/j.fsi.2018.05.009.
127. Rashidian G, Mahboub HH, Fahim A, Hefny AA, Prokić MD, Rainis S, et al. Mooseer (Allium hirtifolium) boosts growth, general health status, and resistance of rainbow trout (Oncorhynchus mykiss) against Streptococcus iniae infection. Fish Shellfish Immunol. 2022;120:360–8. https://doi.org/10.1016/j.fsi.2021.12.012.
128. Abdel-Tawwab M, Mounes HA, Shady SH, Ahmed KM. Effects of yucca, *Yucca schidigera*, extract and/or yeast, *Saccharomyces cerevisiae*, as water additives on growth, biochemical, and antioxidants/oxidant biomarkers of Nile tilapia, *Oreochromis niloticus*. Aquaculture. 2021;533:736122. https://doi.org/10.1016/j.aquaculture.2020.736122.
129. Zhou QC, Buentello JA, Gatlin DM III. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture. 2010;309(1–4):253–7. https://doi.org/10.1016/j.aquaculture.2010.09.003.
130. Hoseinifar SH, Mirvaghefi A, Merrifield DL. The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. Ellipsoideus on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). Aquaculture. 2011;318(1–2):90–4. https://doi.org/10.1016/j.aquaculture.2011.04.043.
131. Hoseinifar SH, Mirvaghefi A, Amoozegar MA, Sharifian M, Esteban MÁ. Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. Fish Shellfish Immunol. 2015;45(1):27–32. https://doi.org/10.1016/j.fsi.2015.03.029.
132. Talpur AD, Munir MB, Mary A, Hashim R. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. Aquaculture. 2014;426:14–20. https://doi.org/10.1016/j.aquaculture.2014.01.013.
133. Momeni-Moghaddam P, Keyvanshokooh S, Ziaei-Nejad S, Salati AP, Pasha-Zanoosi H. Effects of Mannan oligosaccharide supplementation on growth, some immune responses and gut lactic acid bacteria of common carp (*Cyprinus Carpio*) fingerlings. Veterinary Res Forum. 2015;6(3):239.
134. Van Doan H, Hoseinifar SH, Dawood MA, Chitmanat C, Tayyamath K. 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. 2017;70:87–94. https://doi.org/10.1016/j.fsi.2017.09.002.
135. Reda RM, Selim KM, Mahmoud R, El-Araby IE. Effect of dietary yeast nucleotide on antioxidant activity, non-specific immunity, intestinal cytokines, and disease resistance in Nile Tilapia. Fish Shellfish Immunol. 2018;80:281–90. https://doi.org/10.1016/j.fsi.2018.06.016.
136. Chen XQ, Zhao W, Xie SW, Xie JJ, Zhang ZH, Tian LX, et al. Effects of dietary hydrolyzed yeast (*Rhodotorula mucilaginosa*) on growth performance, immune response, antioxidant capacity and histomorphology of juvenile Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 2019;90:30–9. https://doi.org/10.1016/j.fsi.2019.03.068.
137. Shadrack RS, Manabu I, Yokoyama S, Koshio S, Miguel VA, Yukun Z, et al. Specific importance of low level dietary supplementation of yeast strain in red sea bream (*Pagrus major*). Annals Anim Sci. 2022;22(3):1073–85. https://doi.org/10.2478/aoas-2022-0012.

Publisher's Note