Effects Of Probiotics (Saccharomyces Cerevisiae) And Phytobiotics (Zingiber Officinale) On Growth Performance Of Labeo Rohita Fish

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Abstract
With the intensification and pollution of aquatic environments, fish species have faced various health challenges leading to population declines. To enhance fish meat quality, this study investigates the impact of dietary supplementation with probiotic Saccharomyces cerevisiae, and phytobiotics Zingiber officinale, both individually and in combination, on the growth and serum biochemical parameters of Labeo rohita fingerlings. Over 60 days, fingerlings were provided with four distinct diets: a control diet with no supplements, a diet with 5% yeast, a diet with 5% ginger, and a diet with a combination of 2.5% yeast and 2.5% ginger. The results revealed that the combination of probiotics and phytobiotics (yeast+ginger) significantly improved several key growth performance indicators. These included a notable increase in percentage weight gain, percentage length gain, average daily weight gain, average daily length gain, specific growth rate, total protein levels, and protein efficiency rate compared to the control group ($p < 0.05$). Additionally, the survival rate remained at 100% for all groups under investigation. Furthermore, the study found that the supplementation with probiotics and phytobiotics led to a significant decrease in the levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase compared to the unsupplemented group. This indicates enhanced liver function and overall fish health. These findings emphasize the potential benefits of incorporating probiotics and phytobiotics in the diets of Labeo rohita fingerlings, offering a promising approach to mitigate the impact of intensified aquaculture and pollution on fish populations.

Keywords: Fish, Labeo rohita, Probiotics, Phytobiotics, Growth

Introduction

As the demand for food increases and the taste of human become versatile, quality foods are needed to minimize the burden in the food sector. Aquaculture is sharing the burden by producing fish or other species. It is expanding day by day to overcome the high demand for food by producing fish at the commercial level (Ganguly, Dora et al. 2013). Among all seafood fish is the most adible species rich in nutrients and minerals (O'Dea, Lagisz et al. 2019). Fish production is decreasing day by day in the intensification culture system due to weak immunity, and bacterial or viral infections (Mountzouris, Tsirtsikos et al. 2007). This production can be increased by adding various supplements in the feed known as feed additives to enhance their immunity. These are small live organisms or production other than feed materials added to the feed or mixed in the water to enhance the immunity and digestibility of fish. Feed additives in aquaculture serve various purposes including enhancing feed effects, improving animal product quality, and mitigating environmental impact. They encompass probiotics, prebiotics, Synbiotics, antibiotics, phytobiotics, phytogenics, vitamins, organic acids, and oils. Among all these feed additives probiotics and phytobiotics are extensively used (Lawrence, Lawrence et al. 2013). Saccharomyces cerevisiae, a naturally occurring yeast (probiotics), is used in aquaculture as a dietary supplement. It contains immunostimulants such as β-glucans and oligosaccharides, enhancing the immune response and promoting fish growth. This fermented product includes various beneficial components but rarely contains living cells (El-Nobi, Hassanin et al. 2021). Zingiber officinalis (ginger) is a locally available cheap and commercially cultivated phytobiotic worldwide. Gingerol is the most abundant and active component found in ginger. It has a large number of medicinal properties like cardioprotective activity, antimicrobial activity, antioxidant properties, anti-proliferative, neuroprotective, hepatoprotective activity, and antibiotic activity (Banerjee and Ray 2017). Few reports have been studied on the combined effect of probiotics along with phytobiotics in Pakistan. Our research was conducted to assess the effect of locally available probiotics (yeast) and probiotics (ginger) to increase the growth, survival, and serum biochemical parameters of fish.

Materials and Methods

Experimental site and design

Labeo rohita (Rohu) fingerlings were acquired from Central fish seed Manawaan, Lahore. The fingerlings were acclimatized to the experimental conditions in glass aquaria at Fish Nutrition Laboratory, Fisheries Research & Training Institute, Lahore for 15 days. During this period the fingerlings was fed on basal diet. After acclimatization, fingerlings were randomly distributed into four treatment groups, each group has 90 fish (30 fish/ replicate/ group) with an average body weight and length of (18.36 and 10. 22) respectively. Yeast (Safari instant dry yeast) and ginger (National ginger powder) powder were purchased from commercial store, Lahore. Fingerlings were fed at the rate of 5% of their wet body weight twice a day. The table 1 shows the preparation of diet for fishes.

Proximate composition of basal diet:
The proximate composition of basal diet showed percentage protein contents (25.27%), lipids (8.64%), ash (17.21%) and moisture (11.15%) (Latif, Faheem et al. 2020).

Table 1: Preparation of experimental diet

Groups	**Group 1(control group)**	**Group 2**	**Group 3**	**Group 4**
Diet	Commercial feed (without any supplement	Commercial feed + probiotics (yeast) 5%	Commercial feed + phytobiotics (ginger) 5%	Commercial feed + probiotics + phytobiotics 2.5% each

Fish sampling
Fish sampling was done on fortnightly basis (5fish/replicates/group) basis and ration size was adjusted. At the end of the experiment (60 days) final body weight and length was recorded. At the end of the experiment blood sample were taken from the caudal peduncle of the fish (5fish/group) using syringe. Blood was immediately transferred into (serum tube) and stored at 20 c until to obtain the serum. Serum was used for performing different biochemical assays (AST, ALT, ALP and total protein).

Statistical analysis
The obtained data was checked for normality using Kolmogorov Smirnov test. One way (ANOVA) was conducted to compare the difference among the means followed by a post-hoc test (Tukey honest). The data was considered significant at ($p \leq 0.0.5$). All analysis was performed using GraphPad Prism8.At the end of this feeding trial final fish length and weight was recorded (5 fish/replicate/group) several growth parameters such as (% WG, %LG, SGR, ADLG, ADWG) was calculated by following(Abraham, Babu et al. 2007, Goda, El-Haroun et al. 2007, Latif, Faheem et al. 2020, Ogueji, Iheanacho et al. 2020). ALT, ALP and AST activity in serum was determined according to the method of (Young, Russell et al. 2007) using biosystem reagent kits commercially available in chemical company.

Results
At the start of experiment, the average initial body weight and length of Rohu fingerlings was 18.48±0.13 and 10.17±0.123 respectively. At the end of the experiment the average final weight of fish under treatment 1 was 24.36±0.059. Fish under treatments 2, 3 and 4 have an average final body weight of 27.1±0.089, 28.47±0.077, 29.37±0.071 respectively. The obtained values of percentage weight gain (% WG), percentage length gain (%LG) specific growth rate (SGR %g/day), average daily weight gain (ADWG), average daily length gain (ADLG), condition factor (CF), hepatosomatic index (HIS) and protein efficiency ratio (PER) for all the studied treatment groups are shown in table. The table 2 shows the growth performance of Rohu (*L. rohita*) fingerlings fed with diets supplemented with Yeast (probiotic) Ginger (phytobiotic) alone and in combination (Yeast + Ginger) for 60 days

Table 2: Growth performance of Rohu (*L. rohita*) fish

Parameters/ Groups	**Control (T1)**	**Dietary Supplements** **Yeast (T2)**	 **Ginger (T3)**	 **Yeast + Ginger (T4)**
Initial weight (g/fish)	18.48±0.138	18.21±0.208	18.45±0.232	18.30±0.203
Initial Length (cm)	10.17±0.123	10.28±0.203	10.24±0.0214	10.19±0.0204
Final weight (g/fish)	24.36±0.095	27.1±0.089	28.47±0.077	29.37±0.071
Final Length (cm)	12.04±0.016	12.66±0.026	12.85±0.022	13.2±0.022
%WG	31.87±0.059	51.47±0.0737*	54.65±0.0441*	60.70±0.0421**
%LG	18.61±0.174	24.12±0.172	26.72±0.188	30.81±0.140*
SGR (%g/day)	0.4373±0.0645	0.6563±0.0784*	0.7131±0.0492*	0.7795±0.0442***
ADWG	0.8404±0.135	1.269±0.157	1.431±0.112	1.581±0.107*
ADLG	0.2675±0.024	0.3400±0.048	0.3729±0.049	0.4300±0.050
CF (g/cm^3)	1.442±0.101	1.423±0.130	1.391±0.083	1.331±0.090
HIS (g)	1.031±0.046	0.9652±0.051	0.9218±0.028	0.8986±0.035
PER	0.2179±0.035	0.3290±0.040	0.3710±0.029*	0.4099±0.027***
Survival rate (%)	100	100	100	100

Experimental values are expressed as mean± standard error of mean (n=15). Values with the * in the same row are statistically different *(P* < 0.05).

Effect of dietary supplements on (%WG), (%LG), (SGR) and (ADWG) of fish

There was a significant increase in the percentage body weight gain (%WG) of Rohu fingerlings fed on probiotic and phytobiotic supplemented diets either alone or in combination when compared to the control group. There was a significant increase in the percentage body length gain of rohu fingerlings fed on probiotic (yeast) and phytobiotic (ginger) supplemented diets when administered alone. However, a significance in the percentage body length gain was only noted for combination group (T4, yeast +ginger) when compared to the control group. There was a significant increase in specific growth rate of Rohu when fed on diet supplemented with probiotic (yeast) and phytobiotic (ginger) either alone or in combination when compared to the control group (T4, T3, T2 vs T1). A significant increase in average daily weight gain was noted for all the supplemented groups over un-supplemented ones. However, only fish under treatment T3, T4 showed significant increase in their average daily weight gain. The table 3 shows the effects of diet supplemented with yeast (probiotics), ginger (phytobiotics) alone and in combination (yeast+ginger) on percentage weight gain (%WG), (%LG), (SGR) and (ADWG) of *L. rohita* fingerlings

Table 2: Indicates the effect of diet in L. rohita

Groups/ Parameter	Percentage weight gain (%WG) Mean ± Stdev	Percentage length gain (%LG) Mean ± Stdev	Specific growth rate (SGR) Mean ± Stdev	Average daily weight gain (ADWG) Mean ± Stdev
Control (T1)	31.87 ± 0.059	18.61 ± 0.174	0.4373 ± .0645	0.8404 ± 0.135
Yeast (T2)	51.47±0.0737*	24.12±0.172	0.6563±0.0784*	1.269±0.157
Ginger (T3)	54.65±0.0441*	26.72±0.188	0.7131±0.0492*	1.431±0.112
Yeast + Ginger (T4)	60.70±0.0421**	30.81±0.14*	0.7795±0.0442*	1.581±0.107*

Mean ± Stdev (Mean and Standard Deviation); * Indicates significance, ** Indicates highly significance

Effect of dietary supplements on (ADLG), (CF), (HSI) and (PER) of fish

There was an increase in average daily length gain (ADLG) of fish fed on supplemented diets. However, only combination groups T4 (yeast+ginger) showed a significant increase in ADLG when compared to the control group ($p < 0.05$). An improved condition factor (CF) of fish was noted for supplemented groups when compared to control group. A decreased in hepatosomatic index (HSI) of fish fed on supplemented diets probiotic (yeast), Phytobiotic (ginger) alone and in combination was noted when compared to the control group. A significant increase in protein efficiency rate (PER) was noted for ginger (T3) alone and in combination (T4) groups when compared to the control group (T1) $p \leq 0.05$. The table 3 show the Effects of diet supplemented with yeast (probiotics), ginger (phytobiotics) alone and in combination (yeast+ginger) on (ADLG), (CF). (HSI), and (PER) of *L. rohita* fingerlings.

Table 3: Effects of different diet in *L. rohita* fingerlings.

Groups/ Parameter	Average daily length gain (ADLG) Mean ± Stdev	Condition factor (CF) Mean ± Stdev	Hepatosomatic index (HSI) Mean ± Stdev	Protein efficiency rate (PER) Mean ± Stdev
Control (T1)	0.2675 ± 0.024	1.442 ± 0.101	1.031± 0.046	0.2179± 0.035
Yeast (T2)	0.3400±0.048	1.423±0.130	0.9652±0.051	0.3290±0.040
Ginger (T3)	0.3729±0.049	1.391±0.083	0.9218±0.028	0.3710±0.029*
Yeast+ginger (T4)	0.4300±0.050*	1.331±0.090	0.8986±0.035	0.4099±0.027***

Mean ± Stdev (Mean and Standard Deviation); * Indicates significance; ** Indicates highly significance; ***Indicates highly significance

Effect of dietary supplements on (AST), (ALT), (ALP) and Protein level of fish:

A significant decrease in the level of serum aspartate aminotransferase (AST) was noted for fish fed on supplemented diets either alone (yeast, ginger) or in combination (yeast +ginger) ($p < 0.05$). Level of alanine aminotransferase (ALT) in the serum of rohu fed on diet supplemented with yeast (T2), ginger (T3) alone and in combination (yeast +ginger) was significantly lowered when compared to the control group (un-supplemented group, T1). There was a significant decrease in the serum alkaline phosphatase level (ALP) of rahu fed on diet supplemented with ginger alone (T3) and in combination (yeast+ginger) when compared to control group (T1) i.e., ($p \leq 0.05$). A significant increase in total protein level was recorded for rohu fed on diet supplemented with (yeast, ginger) alone and in combination (T4) when compared to the control group (T1) $p < 0.05$. Experimental values are expressed as mean± standard error of mean (n=5). Values with different asterisk in the same column are statistically different *(P* < 0.05). the table 4 shows the different dietary supplements and different levels in rohu fish.

Table 4: Effects of various diets in *L. rohita* fingerlings.

Groups/ Parameter	Aspartate aminotransferase Level (U/L)	Alanine aminotransferase Level (U/L)	Alkaline Phosphatase Level (U/L)	Total Protein Level (g dL^{-1})
Control (T1)	248.3±0.194	31.67±0.296	88.33±0.578	0.936±0.136
Yeast (T2)	177.8±0.590*	15.70±0.204**	66.2±0.577	1.653±0.278
Ginger (T3)	181.7±0.102*	18.07±0.136*	49.9±0.288**	1.480±0.252
Yeast+ Ginger (T4)	163.0±0.469**	20.47±0.264*	54.33±0.317**	2.843±0.098*

Mean ± Stdev (Mean and Standard Deviation); * Indicates significance; ** Indicates highly significance

Water quality parameters:

The average values for studied physical and chemical water quality parameters were temperature 33.3^0C, dissolve oxygen 5ppm, Carbonate alkalinity 32 (mg/L), total alkalinity 380 (mg/L), calcium hardness 80 (mg/L), total hardness 156 (mg/L), chloride 10.7 (mg/L), electrical conductivity 612 (µS/cm), total dissolve solids 520 (mg/L), salanity 0.5 (ppt). The table 5 shows the physicochemical parameters of water.

Table 5: Physicochemical parameter of water

Water parameters	Concentrations
Temperature (ºC)	26.4
Dissolve oxygen (ppm)	5
PH	8.4
Carbonate alkalinity (mg/L)	32
Total alkalinity (mg/L)	380
Calcium hardness (mg/L/0	80
Total hardness (mg/L)	156
Chloride (mg/L)	10.7
Electrical conductivity (µS/cm)	612
Total dissolved solids (mg/L)	520
Salinity (ppt)	0.5

Discussion

The impact of probiotics has undergone extensive examination across various aquatic animals. S. cerevisiae, in particular, has been acknowledged for its potential benefits in certain fish species. Reports indicate enhanced growth in Oreochromis niloticus (Lara-Flores, Olvera-Novoa et al. 2003, Asadi Rad, Zakeri et al. 2012), Onchorhynchus mykiss (Pooramini, Kamali et al. 2009), Epinephelus coioides (Chiu, Cheng et al. 2010) Channa striatus (Dhanaraj and Haniffa 2011), and Acipenser persicus (Iranshahi, Faramarzi et al. 2011) when fed with S. cerevisiae. Present study was conducted on (Oreochromis niloticus) and showed increase in percentage body weight gain, percentage body length gain, specific growth rate, average daily weight gain, and average daily length gain and protein efficiency rate. An improved condition factor of fish was noted for supplemented groups when compared to control group. Diet: 4 considered the best containing 2.5% ginger and 2.5% yeast for Rahu fingerlings.

Body weight gain of Nile tilapia, (Oreochromis niloticus), was tripled after feeding trail of Saccharomyces cerevisiae these results are in agreement with our study (Ozorio, Kopecka-Pilarczyk et al. 2016). When 0.5%, 1% and 2% yeast per food was given to Three Spot Cichlid, Cichlasoma trimaculatum an increase in fish body weight gain, fish body length gain, average daily growth rate was observe. These results also supports our findings (Mohammadi, Ghasemzadeh-Mohammadi et al. 2013). Our results are also in agreement with (Gültepe, Acar et al. 2014, Nofouzi, Aghapour et al. 2017, Jahanjoo, Yahyavi et al. 2018), they reported that dietary ginger increased percentage weight gain and percentage length gain of fish feed with supplemented diets either alone in supplemented groups.

Significant differences in HSI were observed among the experimental treatments, indicating a substantial contribution of the liver to lipid deposition in the three-spot cichlid. Morphological parameters such as HSI play a crucial role in identifying potential liver alterations and diseases. Additionally, CF has been employed to evaluate the overall condition of fish (Van der Oost, Beyer et al. 2003). HSI is closely linked to the liver's energy reserves and the nutritional status of the fish (Pyle, Rajotte et al. 2005). Notably, in this study, CF remained unaffected by dietary treatments, a finding consistent with previous research (Pooramini, Kamali et al. 2009, Kafilzadeh, Mousavi et al. 2013). In the present study, a notable surge in percentage body weight gain (%WG), percentage body length gain, and percentage body length gain in rohu fish was exclusively observed in the combination group (T4, yeast + ginger) compared to the control group. Additionally, a significant increase in average daily weight gain was noted across all supplemented groups compared to the unsupplemented ones. The groups receiving ginger alone (T3) and in combination (T4) displayed an increase in average daily length gain, improved condition factor, a decrease in hepatosomatic index, and a significant increase in protein efficiency rate when compared to the control group.

Furthermore, this study observed a noteworthy reduction in the serum aspartate aminotransferase level, a lower level of alanine aminotransferase, and a significant decrease in serum alkaline phosphatase (ALP) levels in rahu subjected to diets supplemented with ginger alone (T3) and in combination with yeast+ginger, as compared to the control group (T1). Many research article are available on the effect of probiotics and phytobiotics separately purpose of our finding is to check out the combine effect of locally available probiotics and phytobiotics on the growth performance, average daily weight gain, average daily length gain, percentage weight gain, percentage length gain, and survival rate of fingerlings under semi intensive culture system.

In summary, the findings of this study under the specified experimental conditions highlight the enhanced growth and improved food utilization in fish achieved through the utilization of probiotics (S. cerevisiae) and phytobiotics (Zingiber officinale). The inclusion of probiotics and phytobiotics in the diets of fingerlings presents a promising strategy to alleviate the repercussions of intensified aquaculture and pollution on fish populations. From a practical standpoint, the use of probiotics has the potential to reduce the required food quantity for animal growth, proving to be a cost-effective approach.

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