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Effects of dietary probiotics and probiotics combined with enzymes on growth performance of rainbow trout (*Oncorhynchus mykiss*)

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Abstract
Rainbow trout (*Oncorhynchus mykiss*) is a popular aquaculture species, often faces health and growth challenges due to high stocking density. To address these issues, the present study focused on evaluating the impact of dietary supplementation on the growth performance of rainbow trout using probiotics as well as probiotics combined with enzymes. Four groups of trout fry, each with an average stocking size of 1.423±0.002 g, 1.412±0.096 g, 1.451±0.136 g and 1.477±0.118 g, were individually placed in experimental tanks names T_1, T_2, T_3, and T_4, respectively. T_1 received a supplement of 6 gm. of Dige pro and 2 gm. of gel per kg feed. T_2 received a supplement of 6 gm. Multizymes and 2 gm. of gel per kg feed and T_3 received a supplement of 3 gm. Dige pro, 3 gm. Multizyme and 2 gm. of gel per kg feed. At the end of the 90-day period, the findings showed that dietary probiotics of 6 gm. Multizyme-p and 2 gm. Gel per kg feed (T_2) showed significantly improved growth performance in terms of weight gain and survival rate compared to both the control group and other treatments.

Keywords: Rainbow trout, commercial probiotics, dietary supplementation, growth performance

1. Introduction

Aquaculture is viewed as an important food security for a growing global human population and has rapidly developed due to intensified culture method [1]. As aquaculture practices has been shifted to an intensive culture system to meet the global demand of fish food consumption, disease outbreaks are being increasingly recognized as a significant constraint of aquaculture production and trade which ultimately affects the economic development of the sector in many countries [2]. Fish diseases are caused by a number of pathogens, such as parasites, bacteria and viruses. Of these, bacteria are the leading pathogen in commercial aquaculture. Antibiotics are the most widely used agents in the treatment of fish diseases. However, their use can result in potential environmental and food contamination, as well as in the development and/or dissemination of pathogens resistance [3].

Rainbow trout, *Oncorhynchus mykiss* is widely cultured fish species around the world. Highly stocking density adversely affects the health, survival, growth performance as well as product quality of rainbow trout [4]. As a result, there is a growing emphasis on improving fish health and growth through novel approaches, such as the use of multi-strain probiotics and probiotics with enzymes [5]. This method provides a broader spectrum of beneficial microorganism impact and synergistic interactions that can boost fish and productivity [6, 7].

The development of probiotics as an alternative or complementary of chemotherapy and vaccination to prevent fish diseases has been shown to be of great interest. Probiotics can be utilized individually or in various combinations, taking forms such as multi-strain probiotics combined with plant extracts, probiotics mixed with yeast extracts and probiotics with enzymes [8]. The use of live microorganisms can enhance the disease resistance of fish and shrimp by suppressing the pathogens, improving growth and improve immune response [2]. In the aquaculture industry, there is an increasing emphasis on the use of plant-based protein as a replacement for fish meal, in order to promote sustainability. The plant-based proteins used as substitutes for fish meal in aquaculture may contain non-starch polysaccharide compound (NSP) that are not easily digested by fish.

These anti-nutritional compound have the potential to impede the digestion process [9]. In order to mitigate the deleterious effects of anti-nutritional factors and NSP in plant-based diets for fish in aquaculture, the supplementation of exogenous enzymes has been proven to be effective. This approach improves the utilization of nutrients by improving the digestibility of carbohydrates, protein, and minerals. As a result, fish growth and health improve [10]. In the present study, we investigated the impact of administering commercial probiotics, as well as probiotic combined with enzymes, on the growth and mortality rate of rainbow trout.

2. Materials and Methods

2.1 Fish rearing

The research was carried out at the Rainbow Trout Fishery Research Station in Dhunche, Rasuwa. The rainbow trout used for the research were obtained from the same research hatchery. Each raceway used in the study had dimensions of 120 × 75 × 55 cm and was filled with water to a depth of 30 cm. The average stocking size for each treatment is presented in Table (1). To ensure optimal conditions, each raceway was aerated with recirculating freshwater and maintained at a water temperature of 14.5±1.8 °C. The dissolved oxygen level was maintained between 8.2-8.5 mg/L, and the pH level was kept at 8. The light period followed a schedule of 12 hours of light and 12 hours of darkness (12L: 12D).

A commercially available pellet diet with a protein content of 40%, fulfilling the nutritional requirements of rainbow trout, was used. The pellet diet underwent grinding in a blender to obtain a powdered form, followed by sieving through a 50-mesh sieve to eliminate larger particles. Probiotic supplements, including Dige Pro containing *lactobacillus*, *bifidobacterium* and *streptococcus*, as well as Multezyme containing probiotics and enzymes (xylanase, protease, amylase, lipase), were incorporated into the diets. The feed was meticulously mixed with probiotics and a 2 g gel to ensure proper binding with the pellets. Subsequently, the diets were air-dried for 24 hours. The experiment used a commercially available diet as the control group, while the treatment groups (T_1, T_2, and T_3) received diets with different levels of probiotics and probiotics combined with enzymes for a duration of 90 days. The composition of each diet can be found in Table 1. The fish were fed twice daily, at 09:00 and 16:00 hours, with a feed rate equivalent to 3% of their body weight. All fish were individually weighed on a weekly basis, recording data on fish body weight and feed intake. Mortality rates were recorded on a daily basis. The experiment followed a complete randomized design with three replicates.

Table 1: Composition of ingredients in different diets for T_1, T_2, T_3, and T_4

Ingredients	T_1 (Kg)	T_2 (Kg)	T_3 (Kg)	T_4 (Kg) (Control)
Dried Anchovy	17.75	17.75	17.75	17.75
Soya Chokar	2.75	2.75	2.75	2.75
Wheat	2.75	2.75	2.75	2.75
Rice bran	1.25	1.25	1.25	1.25
Mineral	0.25	0.25	0.25	0.25
Vitamin	0.25	0.25	0.25	0.25
Probiotics with gel	(6 gm Dige-pro + 2 gm Gel)/ kg feed	(6 gm Multizyme + 2 gm Gel)/ kg feed	(3 gm Dige pro + 3 gm Multizyme+ 2 gm Gel)/kg feed	0

Table 2: Average stocking sizes (in grams) for different treatment

Treatment	Stocking size (gram)
T_1	1.423±0.002
T_2	1.412±0.096
T_3	1.451±0.136
T_4	1.477±0.118

2.2 Growth performances

During the study, various growth factors were assessed, which included final weight, daily weight gain, specific growth rate (SGR) and survival rate.

2.3 Statistical analysis

One-way analysis of variance (ANOVA, R-stat) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by LSD test. All tests used a significance level of $p<0.05$. Data are reported as means± standard errors.

3. Results and Discussion

3.1 Fish growth performance and Survival rates

During a 90-day period, the impact of dietary probiotics and probiotic plus enzymes on the growth performance of rainbow trout was examined. Table 3 presents the total stocked weight, total harvested weight, survivability, daily weight gain rate, and specific growth rate of fish among different treatment groups. The results revealed that the final weight, daily weight gain, and specific growth rate were significantly higher in the T_2 treatment group when compared to both the control group and other treatment groups. Additionally, the survival rates were determined as 77.77%, 71.42%, 66.66%, and 71.11% for T_1, T_2, T_3, and the control group, respectively. Similarly, mean harvest weight was significantly higher in T_2 as compared with remaining treatment T_1, T_2 and T_4 ($p<0.05$). The highest mean harvested weight was observed in treatment T_2 (8.26±0.07 g) followed by treatment T_1 (8.07±0.06 g) and T_4 (8.07±0.13g) which were not significantly different with each other ($p>0.05$). Conversely, the lowest mean harvest weight was recorded in treatment T_3 (8.06±0.06 g). The daily growth rate (DGR) was seen highest in T_2 (0.092 g/day) which was significantly different with treatment T_1 (0.090 g/day), T_3 (0.090 g/day) and T_4 (0.090 g/day) ($p<0.05$). Similarly the highest specific growth rate (SGR) was seen in T_2 (7.62), which is significantly different with the other treatment T_1 (7.41), T_3 (7.38) and T_4 (7.43) ($p<0.05$).

Table 3: Mean Value of growth parameter of rainbow trout during the experimental period of 90 days

Growth and Production Parameter	Unit	Treatment			
		T_1	T_2	T_3	T_4
Total Stocked Number	No	315	315	315	315
Total stocked wt.	(g)	441	437.85	447.30	437.85
Mean Stock wt.	(g/fish)	1.40±0.15	1.39±0.16	1.42±0.12	1.39±0.13
Total harvested wt.	G	1977.5	1857.75	1692.60	1808.43
Mean harvested wt.	g/fish	$8.07±0.06^b$	$8.26±0.07^a$	$8.06±0.06^b$	$8.07±0.13^b$
Daily weight gain	(g/fish/day)	0.090^b	0.092^a	0.090^b	0.090^b
Survival rate	(%)	77.77	71.42	66.66	71.11
Specific growth rate	%	7.41^b	7.62^a	7.38^b	7.43^b

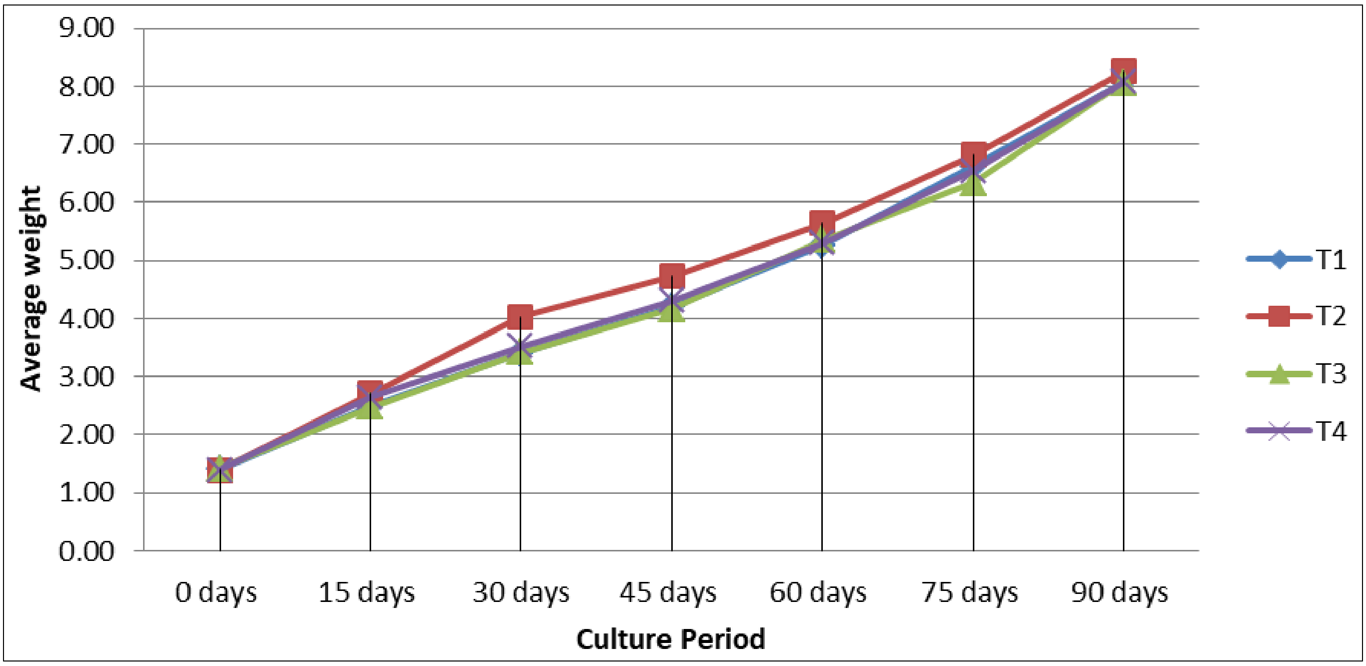

Fig 1: Growth parameter of rainbow trout during the experimental period of 90 days

In this study, Rainbow trout were fed a diet enriched with either a single probiotic, a combination of several probiotics, or a probiotic blend comprising enzymes. The current study found that including both probiotics and exogenous enzymes in the food considerably increased the growth rate of T_2, as shown by increases in specific growth rate and daily weight gain across the culture period. Adeoye *et al*., (2016) [11] and Ghodrati *et al*., (2021) [12] supported these findings by demonstrating similar positive effects of commercial probiotics mixed with enzymes such as phytase, xylase, protease, amylase, cellulose, and lipase on tilapia and Siberian Sturgeon growth. The improved growth performance could be related to the probiotics' ability to create enzymes that aid in fiber digestion. These enzymes collaborate with the fish's own digestive enzymes as well as external enzymes to boost the availability of appropriate chemicals for probiotic effect [13]. The presence of non-starch polysaccharide compounds (NSP) in the gut can increase viscosity, causing detrimental effects on digesta viscosity, the mucus layer, and gut morphology. These changes adversely affect the digestion rate and nutrient absorption, leading to impaired function of internal organs and reduced growth performance. The presence of enzymes, on the other hands, serves to mitigate the negative impacts of anti-nutritional factors and NSP, thereby minimizing these adverse effects [14]. In addition, these enzymes positively impact the gut flora by enhancing digestibility, allowing for better nutrient absorption and assimilation, resulting in increased fish growth performance [15]. Multizyme is a probiotic and enzyme blend that includes enzymes such as amylase, xylanase, phytase, lipase, protease, and cellulose. Enzyme supplementation has the ability to reduce the effects of anti-nutritional components while increasing the uptake of nutrients [16, 17]. Considering that aquatic species lack enzymes like xylanase, phytase including xylanase in their diet improves nutrient utilization by breaking down non-starch polysaccharides, resulting in higher feed efficiency [18, 19]. Furthermore, the use of phytase in aquafeed might boost nutritional value by hydrolyzing indigestible organic phosphorus into free form. This process supplies necessary nutrients for the growth of intestinal microbiota, enhancing overall gut health and consumption of nutrients in aquatic organisms [20].

3.2 Water quality parameter

In aquaculture, the health of the fish is significantly impacted by the water quality parameters. During the period of experiment, critical water quality parameters like average temperature, dissolve oxygen and pH are within the acceptable range which are presented in following figures.

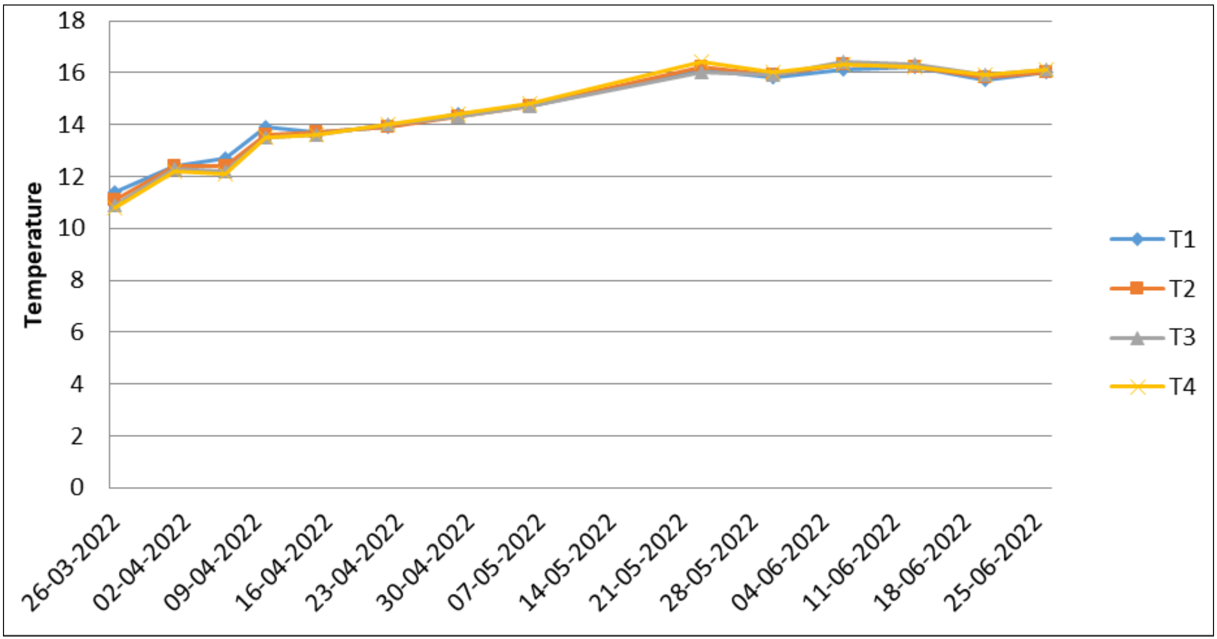

Fig 2: Water Temperature and its variation during Culture Period.

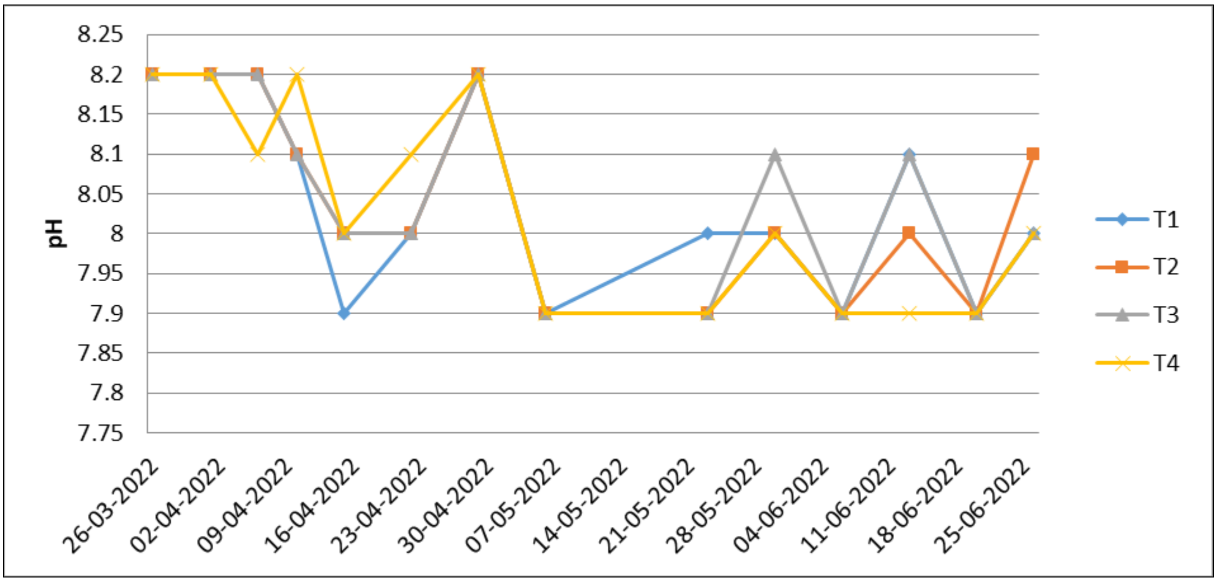

Fig 3: pH and its variation during Culture Period

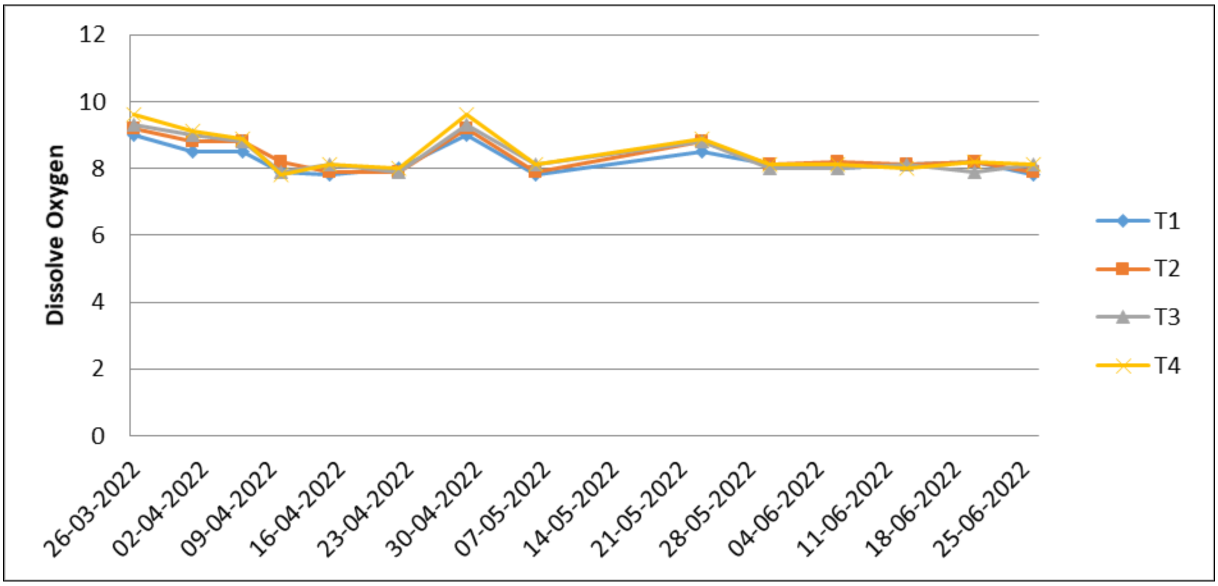

Fig 4: Dissolve Oxygen and its variation during Cultural Period

4. Conclusion

The current study's findings imply that introducing Multizyme, a probiotic and enzyme blend, at a concentration of 6 gm/kg of feed in a regular commercial diet can boost growth rates as well as enhance aquaculture health. It suggests that including probiotics in fish feeding strategies could provide further benefits in aquaculture. More research is needed, however, to fully understand the direct effects of probiotics and the precise processes underlying the reported enhancement in growth performance among the treated fish. Future research should look into hematological parameters, digestive enzyme activity, intestinal anatomy, and the chemical content of the fish.

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