Vol. 2 No. 3 (2025)

ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

STUDIES ON LACTOBACILLUS SUBTILIS AND LACTOBACILLUS PLANTARUM TO INCREASE THE AMYLASE ACTIVITY IN SILVER FISH

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ARTICLE INFO:

Keywords:
Digestive enzyme activity, Physio-chemical parameters, Aquaculture nutrition, Fish growth performance, ANOVA statistical analysis

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Article History:

Published on 18 September 2025

ABSTRACT

Lactobacillus plantarum is a significant probiotic species known for its ability to produce various active compounds. This research aimed to investigate the effects of *Lactobacillus subtilis* and *Lactobacillus plantarum* on amylase activity in *Hypophthalmichthys molitrix*. Three different diets were prepared for the experiment, labelled as Control group (T1) and two experimental groups (T2).The experimental fish were supplemented with varying concentrations of probiotics: the control group received 0% probiotics, T1 received 2.75% *Lactobacillus subtilis* and T2 received 2.45% *Lactobacillus plantarum*. Probiotics were administered twice daily. Weekly measurements of physio-chemical parameters such as water temperature, pH, dissolved oxygen, total hardness, total alkalinity, calcium and magnesium contents were recorded. At the end of each month, fish were dissected and their organs were isolated for evaluating amylase activity. The results revealed that amylase activity in T1 (30.389±1.76) increased significantly compared to T2 (28.7885±1.39) and the control group (28.22±1.25) over the duration of the trial. Among all treated groups, fish fed a diet containing 2.75% probiotics (T1) exhibited the highest

amylase activity. Statistical analysis using ANOVA and correlation methods was employed to analyse the data related to amylase activity and physio-chemical parameters. The findings demonstrated that increasing the concentration of *Lactobacillus subtilis* and *Lactobacillus plantarum* significantly enhanced the amylase activity of silverfish.

INTRODUCTION

Aquaculture has emerged as a vital economic activity in numerous countries, with on-going efforts to enhance conditions that promote infection prevention and address disease-related challenges. Bacterial diseases pose a significant threat to fish farming, often resulting in high mortality rates and substantial financial losses (Dawood *et al.* 2019) Traditionally, antibiotics have been heavily relied upon for the mitigation and treatment of bacterial diseases (Chakrabarti *et al.*2006).There are several environmental influences, including septicity, reduced oxygen amount and temperature. Freshwater fish of the family Cyprinidae, the silver carp (*Hypophthalmichthys molitrixi)* belongs to the order Cypriniformes (Mc Bryan *et al.,* 2013).

Probiotics are defined as living microorganisms that, when consumed in sufficient quantities, provide health benefits to the host. The phrases "probiotic" and "biotic" are Greek words that together indicate "for life," which is why they are often viewed as healthy microorganisms. The microbial balance of the fish gut can be improved with the help of probiotics (Legrand *et al.* 1020). Probiotics are live microbes added to the intestines of fish with food (Wang *et al.,* 2008). Intestines competing for nutrients necessary for the survival of pathogens and producing an antitoxin effect. Probiotics are also able to modulate the immune system, regulate the body's allergic response, and reduce the spread of cancer in mammals. Therefore, it is commonly referred to as friendly bacteria or healthy bacteria. It also improves water quality in aquaculture systems (Aly *et al.,* 2016).

Numerous probiotic strains, encompassing both Gram positive and Gram-negative bacteria, have been extensively studied in aquaculture as potential replacements for antibiotics (Xing *et al.,* 2014). Consequently, the integration of probiotics marks a new era in aquaculture. Notably, *Bacillus subtilis* and *Lactobacillus plantarum* are among the most commonly employed probiotics, renowned for their beneficial effects in aquaculture (Song *et al.,* 2006).

Lactic acid bacteria, particularly Lactobacilli, play a prominent role in the realm of probiotics. *Lactobacilli* encompass a diverse group of probiotic microorganisms, including various species of *Lactobacilli* and bifid bacteria. *Lactobacillus plantarum* is a significant probiotic species known for its ability to produce various active compounds (Cebeci *et al.,* 2003). Furthermore, research has highlighted the synergistic effects of combining multiple probiotic bacteria, including Lactobacillus species, in stimulating enhanced growth and immune systems in aquatic host animals (Tengjaroenkul *et al.,* 2000). *Lactobacillus plantarum* can positively impact the performance and growth of fish species. In aquatic species, the growth improvement, immune responses, and activity of antioxidant and digestive enzymes can all be improved by *Lactobacillus plantarum* and *B. subtilis* (Ringo *et al.,* 2020).

Gram-positive, non-motile, and catalase negative, lactobacilli possess the ability to convert different sugars into lactate and acetate. Most lactobacilli are harmless and can even act as antagonists against pathogenic bacteria. Furthermore, certain lactobacilli exhibit antimicrobial properties, such as *L. casei,* which demonstrates the capability to inhibit the growth of *Helicobacter pylori* (Mohammadian *et al.,* 2014). The *Saccharomyces cerevisiae,* Lactobacillus spp., Bacillus spp., and Lactococcus spp. are the most popular probiotics used in carp cultivation (Aly *et al.,* 2010). Major carp displayed improved innate immune responses, increased survival and growth rates and greater resistance to *Aeromonas hydropliila* infection when fed a diet containing *B.subtilis* (Kumar*et al.,* 2008).

Additionally, it has been shown that Bacillus additions influence the activity of digestive enzymes favourably and aid in lowering vibrio and total viable bacterial counts (Li *et al.,* 2009). In recent years, our understanding of *Bacillus subtilis* has evolved, shifting from considering it a

strict aerobe to recognizing its ability to function as a facultative an aerobe. This adaptability is particularly advantageous due to the remarkable tolerance of its spores to harsh environmental conditions, enabling long-term storage at ambient temperatures (Hong *et al.,* 2005).It is well-established that lactic acid bacteria (such as *Lactobacillus* spp.) and *Bacillus* spp. are widely utilized as probiotics, spanning fish and invertebrate species (Wang *et al,*2019).

Amylases can come from a variety of sources, including plants, animals and microorganisms.. Amylases are crucial enzymes involved in the breakdown of complex polysaccharides, such as starch and glycogen, into smaller sugar units. Amylases of microbial origin had nearly totally changed chemically hydrolyzing of starch in the starch processing sector (Sharma*et al.,* 2010). Two sub-types of amylases are endoamylases and exoamylases. Endoamylases produce straight and non- straight Oligosaccharides with variable chain lengths as a result of the random hydrolysis that they catalyse inside the starch molecule. Exoamylases active sequentially produces brief end products from the non-reducing end (Biswas *et al.,*2013).Numerous fish species have had digestive amylase found throughout their entire GI tract (Fernandez *et al.,* 2001).

METHODOLOGY

Experimental Design

The aim of this study was to investigate the impact of different doses of probiotics, specifically *Lactobacillus subtilis* and *Lactobacillus plantarum* on the amylase activity of silver carp. The fish were randomly assigned to two groups: a control group and a group fed with probiotics. Throughout the experiment, both groups were closely monitored twice a day. The water in the aquariums was exchanged daily at a rate of 40%, and the water quality parameters were carefully maintained to meet the requirements of the fish. At the end of each month, the amylase activity was measured and analyzed.

Pre-Treatments

Prior to the experiment, a two-week acclimation period was provided for the fish in glass aquaria. The experimental setup consisted of aquaria with dimensions of 51 cm in length, 11 cm in width, and 56 cm in depth. Each group had three replicates, and the aquaria were filled with 50 litre of aerated tap water, which was free from chlorination. To ensure the elimination of any pathogenic organisms, all aquaria were thoroughly sun-dried and treated with CaO (calcium oxide).

During Experiment

After a two-week acclimatization period, the fish were randomly distributed into individual aquariums. The control group received a regular feed throughout the entire trial period, while two other groups (T1 and T2) were fed with varying doses of commercial probiotics for a duration of 12 weeks. Weekly monitoring of amylase activity, as well as various growth performance indicators such as average weight, total length, feed conversion efficiency, specific growth rate, and feed intake, was carried out. The parameters of water quality were assessed using the methods outlined in the A.P.H.A (2005) guidelines.

Evaluation of the survival rate of the fish.

These observations and measurements were conducted on a weekly basis to assess the impact of the different probiotic doses on the growth and overall performance of the fish.

The SGR of fish can be calculated as:

$$\text{Specific growth rate} = \frac{\text{In (Final weight) - In (initial weight)}}{\text{Experimental periods in days}} \times 100$$

Feed conversion ratio

$$\text{Feed conversion ratio} = \frac{\text{Feed provided}}{\text{Rise in body weight}} \times 100$$

Percentage survival %

One of the most important factors in aquaculture was survival. The following formula was used to calculate the survival rate:

$$\% \text{ survival} = \frac{\text{Initial no. of fish stocked mortality}}{\text{Initial no. of fish}} \times 100$$

Diet formation

All the ingredients were finely ground and mixed with water to create a paste. An extruder was then utilized to shape the paste into pellets for feeding. The fish were provided with the designated treatments twice a day, specifically from 8-9 am and 4-5 pm, throughout the three-month trial period.

Table 1: Composition (^{0}/0) of experimental diet

Ingredients	Treatment-T1 Control Diet	Treatment-T2 Probiotics= 2.75g	Treatment-T3 Probiotics= 2.45g
Fish	300	189.76	165.55

meal (g/kg)			
Rice polish (g/kg)	139	210.22	190.10
Soya oil (g/kg)	1 1	12.12	10.09
Gluten (g/kg)	150	179.44s	154.45
Premix (g/kg)	20	20.11	15.99
Probiotic s (g/kg)		2.75	2.45

Determination of physio-chemical parameters

Weekly water samples were collected from the aquariums, and various physiochemical parameters were analyzed. The HANNA HI-9146 electronic meter was employed to accurately measure and record water temperature and dissolved oxygen levels. Additionally, pH and electrical conductivity were measured using the digital meter from the laboratory, specifically the WTW meter.

Total Ammonia

To measure the total ammonia concentration in a 10 ml sample of pond water, the following procedure was followed. The water sample was transferred to a beaker, and while continuously swirling, 1-2 drops of Na-K titrate solution were added. After that, 0.06 ml of Nessler's reagent was introduced as an indicator, and the mixture was allowed to stand for 15 minutes. Using a spectrophotometer, the ammonia concentration was determined at a wavelength of 420 nm and with a light path length of 1 cm. The concentration of ammonia was calculated by comparing the sample values to the standards within the nitrogen range, using a blank reagent for assessment.

Carbon dioxide (Co_2)

In order to calculate the concentration of $_{C02}$, a water sample was placed in a beaker, and a solution of sodium carbonate was prepared in a burette. To monitor the end point of the reaction, phenolphthalein indicator was added. The sodium carbonate solution was used to titrate a known volume of water, where the sodium carbonate reacted with the free $_{C02}$ present in the water, resulting in the formation of sodium bicarbonate. The completion of the titration was indicated by the pH of the solution reaching 8.3, as observed by the colour change of the phenolphthalein indicator from purple to a less intense shade. The concentration of Co_2 was determined using a specific formula.

$$Co_2\,(mg/L) = \frac{\text{Volume of sodium carbonate used}}{\text{Volume of sample (ml)}} \times 100$$

Total alkalinity

To ensure the preservation of sample integrity, plastic bottles were utilized to collect samples for total alkalinity testing. Methyl orange was employed as an indicator in the titration process. A sub sample of 50 ml of water was placed in an Erlenmeyer flask, to which 0.1 ml of methyl orange indicator was added. The mixture was then titrated against 0.10 N standard sulphuric acid until reaching the endpoint, indicated by a slight orange colour change. To calculate the total alkalinity, the following formula was applied:

$$\text{Total alkalinity} = \frac{\text{Volume of acid used}}{\text{Volume of the sample (mL)}}$$

Total hardness

Total hardness was determined through a titration method. Initially, a buffer solution was added to the water sample to increase its pH. The buffered sample was then placed in an Erlenmeyer flask, and after thorough mixing with 10 stirs, 0.1 ml of EBT indicator was introduced. The titration process involved the addition of EDTA solution with a normality of 0.1, carefully titrating until the mixture turned blue. The volume of EDTA solution used during the titration was recorded.

Calcium

The calcium concentration in the water sample was determined using the standardized method recommended by A.P.H.A. (American Public Health Association). Initially, a 50 ml sample of water was taken and its pH was adjusted to a range of 12 to 13 by carefully adding the appropriate volume of NaOH solution with a normality of I After thorough mixing, a single drop of ammonium purporate indicator was added to the sample. The titration process involved slowly adding EDTA solution with a normality of 0.01 while continuously stirring the sample until a purple tint, indicating the endpoint, was achieved.

Determination of enzyme activity

Ten individuals from each species were selected for analysis. The fish were humanely euthanized by delivering a blow to the head. The total weight of each fish was recorded. Subsequently, the specimens were dissected, and the digestive tracts

were carefully removed from each fish on a cutting board placed on ice. The contents of the digestive tracts were extracted by applying gentle pressure. The activity of a-amylase, an enzyme, was then measured in the gut tissue immediately. Each tissue sample was individually ground in a pre-chilled homogenizer made of ground glass, while maintaining a low temperature with the help of ice. A buffer solution of 50 mM Tris (pH 7.2) was added in a ratio of 9 parts to 1 part tissue. After centrifugation, the supernatants (liquid portion) were carefully separated and stored in a refrigerator for later use in the assays.

Assay a-Amylase

Procedure

In the determination of amylase activity, starch was employed as the substrate following the method described by Bernfeld in 1955. A volume of I ml of properly diluted gut extract was combined with 1 ml of a 1% starch substrate solution. The starch substrate consisted of I g of soluble starch and 0.0067 M NaCl in 100 ml of a 0.02 M NaH2P04 buffer with a pH of 6.9. The mixture was incubated at 37^{o}c for 3 minutes. To stop the reaction, 2 ml of 3,5-dinitrosalicylic acid reagent was added. The resulting solution was then heated in boiling water for 5 minutes, followed by cooling. Subsequently, 20 ml of distilled water was added. The absorbance of the solution at 540 nm was measured, and a standard curve was established using maltose dissolved in distilled water at concentrations ranging from 0.1 to 1.0 mg ml-I. This standard curve enabled the conversion of absorbance readings into maltose units. The specific activity of amylase was defined as the production of 1 mg of maltose per minute per mg of protein at 37 ^{0}C. The amount of soluble protein present in the gut extracts was determined using the Lowry method, as described by Lowry et al. in 1951. In this method, 0.1 ml of the gut extract sample or a standard protein solution was mixed with 0.1 ml of 2 N NaOH. The mixture was hydrolyzed in a boiling water bath at 100^{0}C for 10 minutes, and then cooled to room temperature. Following this, 1 ml of a freshly mixed complex-forming reagent was added to the solution. After 10 minutes, 0.1 ml of Folin reagent was introduced and mixed using a vortex mixer. The absorbance of the resulting solution was read at 550 nm after 30 minutes

Statistical analysis

The statistical calculation of amylase activity was performed using a factorial design, utilizing analysis of variance (ANOVA). To determine the rankings of the groups, a post hoc test known as Tukey's test was conducted using SPSS software. Additionally, correlation analysis was employed to assess the relationships between the physio-chemical parameters.

RESULTS

Amylase activity of silver fish

The amylase activity was evaluated in the intestines of *Hypopthalmicthys molitrix* after being exposed to different concentrations (0%, 2.75%, and 2.45%) of probiotics *L. subtilis* and *L. plantarum* over a three-month trial period. Amylase activity in the treated groups, results of the analysis of variance, indicating significant variations (13<0.05) among the treatments (control, 0%, 2.75%, and 2.45%) as well as the duration (1st, 2nd, and 3rd month).

In the control group, the amylase activity was measured as 28.02075±1.768159. In the first month, when fish were exposed to a concentration of 2.75% probiotics (treatment Tl), the amylase activity increased to 28.97325±1.52739. With each subsequent month, the amylase activity showed a gradual increase, reaching 30.389±1.76 in the second month and further increasing in the third month. For fish exposed to a concentration of 2.45% probiotics, the amylase activity remained moderate, falling between the T1 group and the control group, with a measurement of 28.7885±1.394354. In the second month, there was a slight increase in amylase activity (28.88±1.40), but it did not surpass the levels observed in the T1 group. Similarly, in the final month of the trial, the amylase activity increased, but it did not exceed that of the T1 group. In the control group, the minimum amylase activity was observed at 28.22±1.25. Comparing the T1 group to the duration of exposure, the amylase activity followed the trend of 3rd month > 2nd month > 1st month. The amylase activity was significantly higher in the T1 group, where fish were treated with a 2.75% concentration of probiotics, compared to the T2 group with 2.45% probiotic supplementation, and the control group.

Month 1

Treatment	Mean	Overall mean
	30.300 a	28.7885±1.394354
	29.860 a	
0	28.207a	

	26.787a	
2.75	30.973a	28.02075±1.768159
2.75	27.783a	
2.75	26.820a	
2.75	26.507a	
2.45	30.343a	28.97325±1.152739
2.45	29.393a	
2.45	28.100a	
2.45	27.167a	

Table 2: Comparison of amylase mean activities of *H. molitrix*, after exposure to different concentrations of probiotic Treatment of *Lactobacillus subtilis* and *Lactobacillus plantarum* percentages:

Control group = 0%
Treatment two (T1) = 2.75%
Treatment three (T2) =2.45%

In month one the mean values slightly different from each other, which means that amylase activity of experimental groups which was fed with different concentrations of *Lactobacillus plantarum* and *Lactobacillus subtilis* slightly increased than the control group.

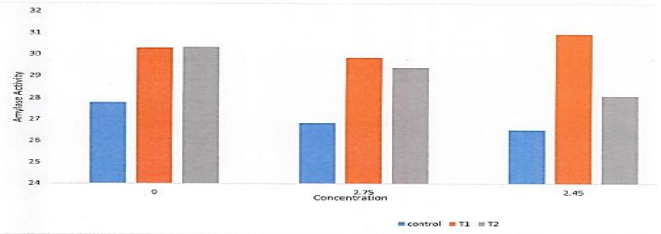

Fig. 1: Amylase activity of *H. molitrix* in month one exposed to *L. subtilis* and *L. plantarum*

Concentration = (0%)
Treatment T1 = (2.75%)
Treatment T2 = (2.45%)

Month 2

Treatment	Mean	Overall mean
	30.293a	29.6435±0.826932
0	30.107a	
0	29.947a	
0	28.227a	
2.75	31.627a	29.10175±1.63378
2.75	29.127a	
2.75	28.550a	
2.75	27.103a	
2.45	29.140a	28.14175±1.344215
2.45	28.857a	
2.45	28.743a	
2.45	25.827a	

Table 3: Comparison of amylase mean activities of *H. molitrix*, after exposure to different concentrations of probiotic

Treatment of *L. subtilis* and *L. plantarum* percentages:
Control group = 0%
Treatment two (T1) = 2.75%
Treatment three (T2) = 2.45%

In month two the mean values significantly different from each other, which means that amylase activity of experimental groups which was fed with different concentrations of *L. plantarum* and *L. subtilis* increased than the control group. T1 group showed highest amylase activity with 2.75% of probiotic concentration than other two groups.

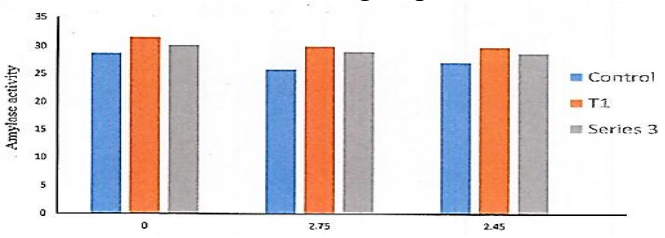

Fig. 2: Amylase activity of *H. molitrix* in month one exposed to *L. subtilis* and *L. plantarum*

Concentration = (0%)
Treatment T1 = (2.75%)
Treatment T2 = (2.45%)

Month 3

Treatment	Mean	Homogeneous group	Overall mean
	34.797		29.7225± 3.11285
	29.150	ABCD	
	28.600	ABCD	
	26.343	CD	
2.75	33.330	AB	28.77825±3. 327011
2.75	29.303	ABCD	
2.75	28.520	BCD	
2.75	23.960	D	
2.45	30.927	ABC	29.421±1. 136379
2.45	30.097	ABCD	
2.45	28.503	BCD	
2.45	28.157	BCD	

Table 4: Comparison of amylase mean activities of *H. molitrix*, after exposure to different concentrations of probiotics

Treatment of *L. subtilis* and *L. plantarum* percentages:
Control group = 0%
Treatment two (T1) = 2.75%
Treatment three (T2) = 2.45%

In third month the mean values significantly different from each other, which means that amylase activity of experimental groups which was fed with different concentrations of *L. plantarum* and *L. subtilis* increased than the control group. T1 and T2 group showed highest amylase activity with 2.75% and 2.45% of probiotic concentration than other two groups.

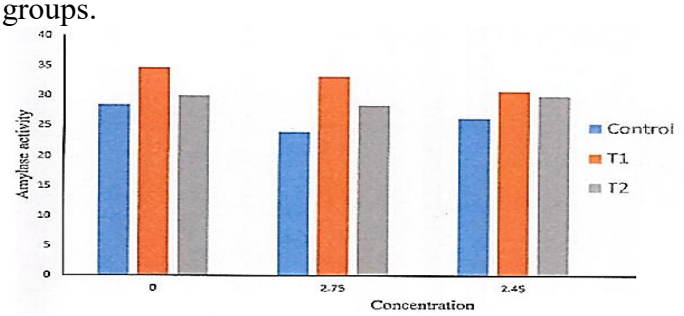

Fig. 3: Amylase activity of *H. molitrix* in month one exposed to *L. subtilis* and *L. plantarum*

Concentration = (0%)
Treatment T1 = (2.75%)
Treatment T2 = (2.45%)

Physio-chemical parameters of test media

During the investigation, the physio-chemical parameters of the test media were analyzed in relation to the supplementation of different concentrations of probiotics (0%, 2.75%, and 2.45%) in *H. Molitrix.* The trial was conducted at a constant temperature of 26^0 C, with a total hardness of 254 mg/L-I and a pH of 7.5. Throughout the experiment, the concentration of dissolved oxygen decreased, while the content of total ammonia increased with higher probiotic concentrations, specifically at 2.75%.

In the control group of *H. Molitrix* at the beginning of the experiment, the dissolved oxygen content was higher (6.14±0.075 mg/L-I), but it reached its minimum value by the end of the trial (6.14±0.064 mg/L-I). The variations in carbon dioxide content were minimal in the control group. The range of total ammonia content was between (1.218±0.09 mg/L-I) and (1285±0.15 mg/L-I), respectively.

The control group exhibited the minimum calcium content (24.756±1.93 mg/L-I), and the maximum value of calcium content was observed at the end of the experiment (23.59±2.05 mg/L-I). As calcium content decreased, the magnesium content increased significantly, while a decrease in magnesium did not affect the calcium content. The values of calcium content were as follows: (41.646±2.49 mg/L-I) and (40.48. +5.19 mg/L-I), respectively.

By utilizing correlation techniques, the physio-chemical parameters were analyzed. The results indicated a positive correlation between dissolved oxygen, magnesium, and calcium with temperature, while they were negatively correlated with total ammonia, pH, carbon dioxide, and total hardness. Additionally, magnesium, calcium, and carbon dioxide showed a negative correlation with pH. Total hardness, ammonia, and dissolved oxygen exhibited a positive correlation. Dissolved oxygen, magnesium, and calcium had a negative correlation. Carbon dioxide had a negative correlation, while magnesium and calcium had a positive correlation. Total ammonia showed a positive correlation with magnesium and carbon dioxide, while it had a negative correlation with dissolved oxygen and calcium. Calcium exhibited a negative correlation with carbon dioxide and a positive correlation with magnesium.

Magnesium showed a negative correlation with carbon dioxide.

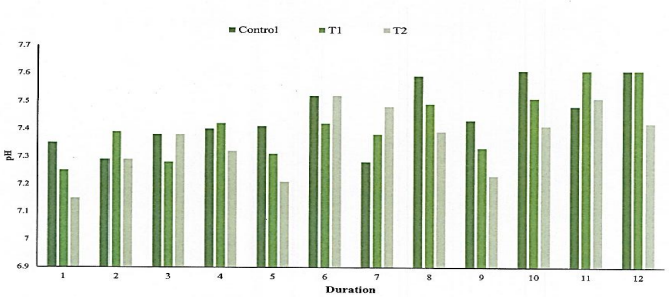

Fig. 4: Mean pH of test media at different concentrations of probiotics for *H. molitrix*

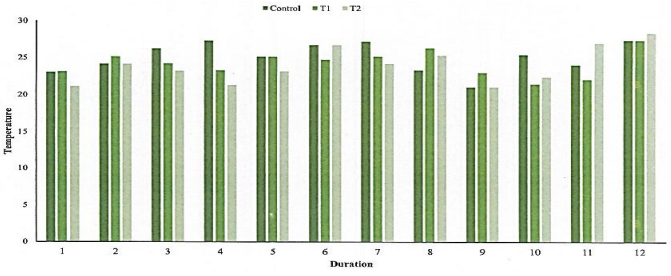

Fig. 5: Mean Temprature (^{0}C) of test media at different concentrations of probiotics for *H. molitrix*

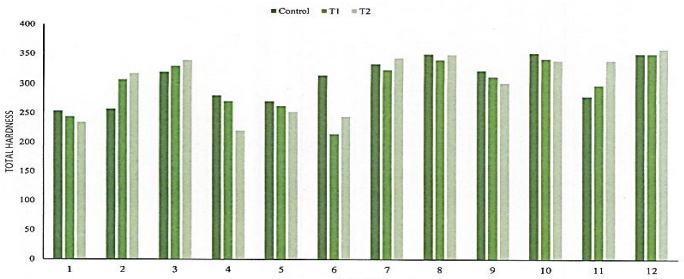

Fig. 6: Mean total hardness (mg/L) of media at different concentrations of probiotics for probiotics for *H. molitrics*

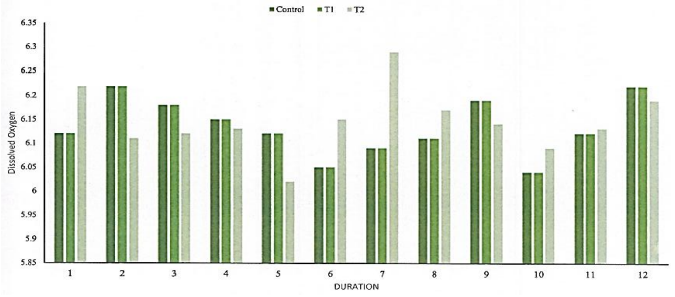

Fig. 7: Mean Dissolved oxygen (mg/L) of test media at different concentrations of probiotics for *H. molitrics*

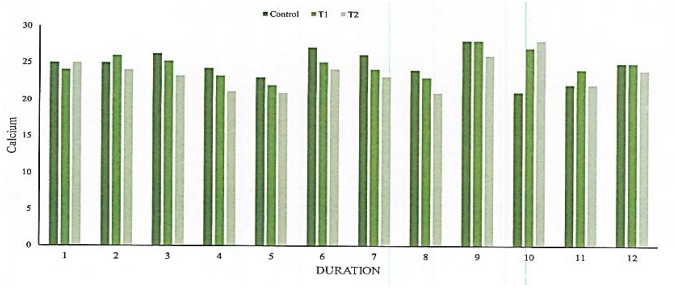

Fig. 8: Mean total calcium (mg/L) of test media at different concentrations of probiotics for *H. molitrics*

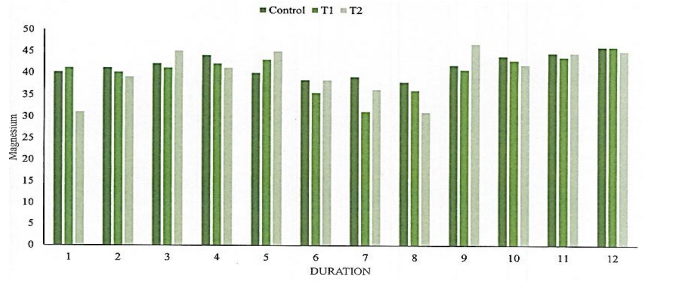

Fig. 9: Mean Magnesium (mg/L) of test media at different concentrations of probiotics for *H. molitrics*

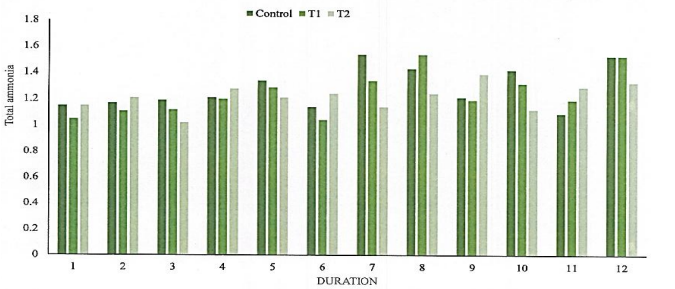

Fig. 10: Mean Total ammonia (mg/L) of test media at different concentrations of probiotics for *H. molitrics*

DISCUSSION

In this research focus was on studying the effects of *L. subtilis* and *L. plantarum* on increasing the activity of amylase in silver fish. The results showed significant changes in the amylase activity of the fish when different concentrations of probiotics were used. A comparative study was conducted on the digestive enzymes of fish with different dietary habits, specifically focusing on proteolytic and amylase activities. The study examined eight fish species and found that carps had the highest enzymatic activity in their digestive systems. The study also observed that digestive enzyme activities decreased as the incubation temperature decreased, although this trend varied depending on the carp species and tissues being examined. Our results are similar to Aslam *et al.* (2018), evaluate the effect of experimental diets on the activities of intestinal digestive enzymes of *Ctenopharyngodon idella* and *Hypophthylmichthys molitrix*.

The research compared the enzyme activity between common carp caught from the River Garma and those spawned in ponds. The results indicated that a-amylase activity was influenced by the feeding patterns of the three species. Interestingly, common carp from the ponds showed significantly higher a-amylase activity compared to those from the River Garma. The results are also compared with the study of Adorian *et al.* (2019), Effects of probiotic bacteria *Bacillus* on growth performance, digestive enzyme activity, and hematological parameters of Asian sea bass, Lates calcarifer (Bloch).

Furthermore, feeding grass carp with *Bacillus subtilis* at a concentration of 2.75% resulted in significantly increased immunity, antioxidant function, growth performance, and amylase activity. Our results are also relatable with the study of Al-Tameemi *et al*. (2010) who investigated the activity of a-amylase in three Cyprinid species with different feeding habits in Southern Iraq.

Another study focused on the influence of *Bacillus subtilis* added to the diet of common carp. The results showed that the probiotic reduced lactic acid bacteria and total viable count in the fish's intestines without affecting their growth performance, indicating a potential modulating effect on the intestinal microbiota of common carp. These findings are also comparable with the study of Horn *et al*. (2006) who determined the structure and function of the stomachless digestive system in three related species of New World silverside fishes representing herbivory, omnivory and carnivory. Additionally, a study examined the activities of digestive enzymes in different size groups and parts of the digestive tract of *Labeo rohita,* a fish species.

The researchers found that enzyme activities such as trypsin, amylase, total protease, and chymotrypsin increased with the size and mass of the fish, reaching their highest levels in a specific size group. The study also revealed that amylase activity was highest in the adjacent part of the intestine, highlighting regional variations in digestive enzyme distribution.

Conclusion

The maximum amylase value of 31.89±0.51 mg/L in the intestine of silver fish was observed in treatment T1 with a 2.75% concentration of probiotics. They also shed light on the influence of dietary habits, fish size, and tissue location on digestive enzyme activities. These findings have implications for optimizing fish farming practices, improving fish health, and enhancing overall aquaculture productivity. The experimental fish fed a supplementary diet of probiotics exhibited a significant variation (13<0.01) in amylase activity. Throughout the experiment, the amylase activity increased in treatment T1 with an increase in the duration of exposure. Treatment T2 with a 2.45% concentration of probiotics showed significant changes, while no alterations were observed in the control group. There was a significant correlation between all physio-chemical parameters and the different concentrations of *L. subtilis* and *L. plantarum.*

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