EFFICACY OF PROBIOTICS SUPPLEMENTATION ON GROWTH PERFORMANCE, IMMUNOLOGICAL AND HAEMATOLOGICAL PARAMETERS OF *LABEO ROHITA*

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2022

RESEARCH COMPLETION CERTIFICATE

It is certified that Eisha Umer, Jamila Fatima, Maryam Tariq, and Momna Khalid of BS Hons. (Session-2018-2022) Department of Zoology have carried out this research Project entitled **Efficacy of probiotic supplementation on growth performance, immunological and hematological parameters of *Labeo Rohita*** under my supervision.

It is assured that this research work is original and not yet been published anywhere else. "All changes suggested by examiner during defense are incorporated in this final copy".

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ABSTRACT

To access water soluble probiotic supplementation mixture of *Saccharomyces cerevisiae, Bacillus subtilis, Bacillus licheniformis, Trichoderma long brachium* on growth, hematological and immunological parameters of *Labeo rohita*. Fish is divided into two groups, control and experimental. Control group fed only with feed. Experimental group fed with diet + water soluble probiotics. After 60 days there was significant increase in growth of fishes, protein efficiency ratio (PER) and feed conversion ratio (FCR).

In evaluating hematological parameters includes hemoglobin (Hb), hematocrit (Ht) Red bloodcell count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular hemoglobin concentration (MCHC) and Mean Corpuscular hemoglobin (MCH),. As compared to control (2.81 ± 0.03) the red blood cell count (RBC) was significantly higher at treatment group (3.67 ± 0.22) for 60 days and ($P < 0.05$) which were highly significant. The highest hemoglobin percent was recorded at experimental group for 60 days (5.88 ± 0.05) and ($P < 0.05$), whereas the lowest was in the control group (5.45±0.04).The experimental group had the highest Mean Corpuscular hemoglobin concentration values (22.87 ± 0.06) and ($P < 0.05$) which was highly significant than the control group which have the lowest value (19.76 ± 0.04) for 60 days.

The immunological parameters include leucocytes count and serum lysozyme activity. The total amount of leucocytes in the fish diet with experimental group was substantially higher ($P < 0.05$) than the fish in the control group. Similarly, the experimental group (7.89 ± 0.05 U ml^{1}) had considerably higher serum lysozyme activity than the control group (4.90 ±0.05 U ml^{1}). The results showed that water soluble probiotics exert a significant influence on survival, growth, hematological, and immunological parameters of *Labeo rohita.*

TABLE OF CONTENT

LIST OF ABBREVATIONS

ABBREVATION	FULL FORM
FCR	feed conversion ratio
PER	protein efficiency ratio
Ht	hematocrit
Hb	hemoglobin
RBC	Red blood cell count
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular hemoglobin
MCHC	Mean Corpuscular hemoglobin concentration
SRG%	the percentage increase in body weight per fish
Ln WT	the natural log of weight at time
Ln Wt	the natural log of initial weight
T	time
T	Initial time
Ln	natural logarithm
WBC	White blood cells

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Labeo rohita is one of the aquaculture freshwater species of the Carp family. This species is widely cultured in Bangladesh as well as other Asian countries such as India, Myanmar, Nepal, Pakistan, and Vietnam [1]. Its culture practice has been expanded because of its high production rate, nutritional significance, good taste, and market acceptability. However, it has a high mortality rate (70–80%) and a slow growth rate throughout the early phases of development. These constraints limit the availability of high-quality seed and the expansion of this species production, resulting in a financial loss [2].

Fishes are the major sources of protein, vitamins, minerals, and a low quantity of fats. Fish have ahigh level of output on a commercial and industrial scale. In many countries, better quality fish are cultured in fish farming systems, but in others, poor growth, disease outbreak and low survival of fish due to harmful pathogens combined with an unhygienic aquatic environment can be a source of decrease in this supply chain. In the aquaculture sector, pathogenic bacteria and viruses cause substantial mortality and significant economic losses. Antibiotics and vaccines are employed in aquaculture systems to control the high incidence of harmful bacteria and viruses. Antibiotic resistance is caused by overuse and misuse of antibiotics, according to various researches [3]. Probiotics are being used in new aquaculture procedures to reduce harmful bacteria outbreaks. Probiotics in aquaculture have been demonstrated to boost the appetite and bio-growth performance of farmed species in a sustainable and environmentally friendly way [4]. Probiotics also have been shown to lower harmful antimicrobial compounds, particularly antibiotics.

Probiotics are bacteria that help to prevent the body against disease. Probiotics are feed supplements that help improve the equilibrium of gut bacteria, according to Fuller (1992). Microbes have a large and crucial impact on aquaculture since their activities affect water quality parameters and disease control [5]. Probiotics can boost nutritional competition and antibacterial substance synthesis, making them extremely useful in the fight against fish infections. Disease concerns in aquaculture necessitate the use of microorganisms as probiotics and antibiotic alternatives [6]. The results of the research revealed that probiotics used as antibiotics can help fish

develop faster. In a trial, it was discovered that mixing three distinct kinds of bacteria, such as *Bacillus subtilis, Lactococcus lactis,* and *Saccharomyces cerevisiae*, into the *L. rohita* diet increased growth performances and nutritional utilization [7].

Many microbial strains are employed as antibiotics in aquaculture. Antibiotics from *Enterococcus spp., Bicdobacterium spp., Bacillus spp., Saccharomyces spp., Vibrio spp., Lactobacillus spp.,* and *Bicdobacterium subtilis* are commonly used in aquaculture for bacteriotherapy [8]. Following antibiotic treatment, a sufficient dose of *B. subtilis* should be consumed to restore normal microflora. Probiotic strains are utilized in the diet of grow-out fish to improve development characteristics [9]. The use of appropriate probiotics in the aquaculture industry enhanced intestinal microbial balance and feed absorption, resulting in higher growth rates and lower FCR (feed conversion ratio) during the cultural period. To boost feed utilization, growth rate, and bacterial infection management, Bacillus species have been employed in fish feed [10].

Hence, in present study we investigated the effect of mixture of probiotics *Bacillus spp, Saccharomyces cerevisiae* and *Lactobacillus subtilis* on growth, hematological and immunological parameters.*Saccharomyces* cerevisiae has been utilized as an antioxidant, antidiabetic, anti-inflammatory,neuroprotective, immunological booster, antimalarial agent.

Bacillus subtilis benefits include improved digestive health, lipid metabolism and immune system function. The benefits of soil-based probiotics aren't limited to the gastrointestinal tract. Bacillus subtilis and other probiotics found in soil are harmless. These probiotics significantly enhance the growth performance of Rohu as well as improve the hematological and immunological parameters. Therefore, in future the findings of this study will be useful in the commercial cultivation of fish employing probiotics.

RATIONALE

Labeo rohita is one of the aquaculture freshwater species of the Carp family. Its culture practice has been expanded because of its high production rate, nutritional significance, good taste, and market acceptability. Probiotics in aquaculture have demonstrated to enhance appetite and bio-growth performance of farmed species in an environmentally friendly and sustainable way, in addition to reducing toxic antimicrobial compounds, particularly antibiotics. Mixture of probiotics *Bacillus spp*, *Saccharomyces cerevisiae* and *Lactobacillus subtilis* have a great impact on growth,hematological and immunological parameters. These probiotics significantly enhance the growth performance of Rohu as well as improve the hematological and immunological parameters. Therefore, in future the findings of this study will be useful in the commercial cultivation of fish employing probiotics as little to no work has been done on this topic before.

AIMS AND OBJECTIVE

The aims of the research will be to:

1. To check the efficacy of probiotics on the growth of fish fry and fingerlings.

2. To check the effect of probiotics on hematological parameters.

3. To check the immune response by detecting different immunological parameters.

CHAPTER 2

REVIEW OF LITERATURE

The studied shows the efficacy of water probiotics on the growth performance and gut microflora in rohu *Labeo rohita*. The fish was divided into four experimental groups in which Group 1 is the control group with control basal diet, Group 2 with basal diet with water probiotics, Group 3 with 1g/kg feed probiotics with basal diet and the last group of fish Group 4 with the combination of water probiotics and feed probiotics with basal diet. This experiment is done for 60 days. Every month, the body weight, specific growth rate, feed consumption ratio, and protein efficiency were measured, along with the separation of pre and post microflora. The result showed the increase in body weights, protein consumption, feed conversion ratio and specific growth rate when compared with control group. The pre isolation of microflora is compared with post probiotic isolation from the gut content it shows the increase in post probiotics isolation and water probiotics, feed probiotics and combination of probiotics were significant. This study found that a combination of probiotics improves immune response by regulating growth performance and gut probiotics. The study showed that more and larger intervention studies are needed to determine the efficacy of probiotics in treating fish diseases in specific investigations. This research shed light on prospective probiotics mechanisms of action in fish gut microbiota toward enhanced health through probiotic efficacy assessment [11].

In other experimental studies the fingerlings of rohu fish were taken in four tanks with different feeds and probiotics. In tank 1 commercial pelleted feed was added and it is the control tank and in tank 2 feed with *Lactobacillus sporogenes*, tank 3 feed with *Saccharomyces cerevisiae*, and *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* are both present at in tank 4. The study took place in cement tanks for 120 days, with probiotics and no probiotics being supplied at 15-day intervals. After Every 15 days, samples were taken to assess weight increase percent, specific growth rate percent, feed efficiency ratio,total plate counts and feed conversion ratio The average starting mass of the fish in each tanks stayed around 44 g, however when feeding with probiotic feed, the weight grew to 150.78±0.68 gm, 176.13±0.75g, and 183±0.91g in tank 2, tank 3, and tank 4, respectively, compared to 102.05±0.99g in control group. Except for the control tank, all the tanks had probiotic microorganisms after 15 days. After feeding them over the next

15 days, the total plate count of the probiotic bacteria was 0 in tank 1 and tank 2. While the total plate count of probiotic microbes in tank 1 and tank 2 was 0, *Saccharomyces cerevisiae* was 2.38±0.02 x105 CFU/g in tank 3, *Lactobacillus sporogenes* was 0 and *Saccharomyces cerevisiae* was 2.70 ±0.008 x105 CFU/g in tank 4, it was discovered that the expansion of tank 4 was mostly attributable to the production of *Saccharomyces cerevisiae* colonies. After 15 days, they had colonized the gut. The results showed the difference in weight increase percentage between treatment groups was determined to be highly significant ($P < 0.05$). T4 had the greatest weight gain, whereas T1 had the smallest. Staining, catalase, methyl red, starch, fructose, and lactose were all positive for *Lactobacillus sporogenes*, however indole and nitrate reduction were both negative. *Saccharomyces cerevisiae* is a nonmotile yeast that is positive for fructose and starch, but negative for nitrate reduction and lactose [12].

In this article six iso-nitrogenous and iso-caloric diets without and with probiotics supplementationnamely Tank 1 with Basal feed without probiotics was the control group, tank 2 with Basal feed +*Bacillus subtilis* and *Lactococcus lactis*, tank 3 with Basal feed + *Lactococcus lactis* and *Saccharomyces cerevisiae*, tank 4 with Basal feed + *B. subtilis* and *S. cerevisiae*, tank 5 with Basalfeed + *B. subtilis*, L. In triplicate tanks, these meals *Labeo rohita* seedlings were fed at 6.0 ± 0.06 g for 60 days (30 fish per tank). In all probiotic-supplemented diets, the probiotic concentration was constant at 1011 cfu kg feed. After 60 days of culture, the fish fed a mixture of three probioticsin equal percentages in tank five had greater ($P < 0.05$) growth, protein efficiency ratio, nutrient retention, and digestibility, as well as a lower ($P > 0.05$) feed conversion ratio than the other treatment groups. On the 15th and 30th days after sampling, the entire population of heterotrophic bacteria in the colon of the tank 3, tank 4, and tank 5 groups was significantly lower than the original value. Apart from tank 6, gut colonization of respective probiotics delivered through meals was similarly improved up to 30 days of fish culture before remaining constant. The article showedhow probiotics affect fish growth and nutritional usage. Analysis of diverse digestive enzyme activity supported the growth data. To better understand how different probiotics interact, their gastrointestinal tract colonization was studied [13].

Some probiotics have been shown to affect the factors of fish growth and microbial flora like O. niloticus in previous studies. The fish were fed three different diets and were evenly distributed among three tanks. The fish were fed a food supplemented with dead *Saccharomyces cerevisiae*

yeast in the first tank, a feed supplemented with *Bacillus Saccharomyces cerevisiae* yeast in the second tank, and a basic diet in the third or last tank. It was discovered that the fish in different tanks had improved in growth characteristics such as feed conservation ratio, protein efficiency ratio and body weight after roughly six weeks. Different formulas developed by Annet in 1985 and De Silvia and Anderson in 1995 are used to calculate these improvements. The findings revealed that the second group failed to re-isolate some harmful germs, whereas the first group had no effect at this time. On diverse solid medium, colonies with different morphological features were found in the *Oreochromis niloticus* intestinal tract before and after the use of probiotic-supplemented meals. Pure colonies were distinguished by biochemical parameters that indicated the absence or presence of other bacterial flora. The presence of *E. coli, Klebsiella, Salmonella spp* and *Pseudomonas fluorescens* was discovered during the pre-treatment bacterial identification according to this (Marzouk MS, Moustafa MM, Mohamed N) [14].

In other research, the consequence of oral probiotics on the productivity, growth and persistence of Nile tilapia from eight different brackish water ponds was investigated. The average body weight of tilapia in these locations is 0.15 g, and they are stocked at a density of 5 Nos/m^2, with pond salinity ranging from 10 to 16 ppt. The fish are fed a 28 % protein feed with three types of probiotics added: Safegut in tank 1 (T1), Zymetin in tank 2 (T2), and probio-aqua in tank 3. (T3). The control tank (T4), on the other hand, is not treated with probiotics. After 105 days, the T3 therapy, which included probio-aqua supplemented with diet, had the maximum production and survival, while T4 had the lowermost, but there was no major difference between the treatments. The conclusion showed that probiotics supplemented with feed had no influence on brackish water environment, although oral probiotics have a good effect on tilapia fish growth, survival, and production but have no effect on overall brackish water pond productivity [15].

The effects of marketed human probiotics and antibiotics supplemented in diet on the growth, survival, illness resistance, and intestinal microbial flora of two species of ornamental fish, Goldfish (*Carassius auratus*) and swordtail (*Carassius auratus*), were reported in a recent article (*Xiphophorus helleri*). In *C. auratus,* food conversion ratio,specific growth rate and total body weight did not differ drastically across treatments, but in *X. helleri,* there was a major difference. In all treatments, antibiotic-resistant bacteria in fish guts increased within a few days, especially in antibiotic-fed fish. The development of antibiotic resistance among the bacterialflora of the fish

gut was reduced in probiotic fed groups of these ornamental fishes. The findings demonstrated that human probiotics have a varying influence on ornamental fish growth, survival, and disease resistance, and that generating such an effect across species is difficult [16].

In this previous article the present study to check whether management of single stress probiotic for the duration of early reading could enhance the survival and increase growth of rohu, *Labeo rohita.* The experiment lasted seventy days, and two hundred post larvae (average moist body weight, 0.60 ± 0.05 mg) were divided into two groups: control and treated (n=two hundred post larvae /fiberglass tank). Except for the addition of probiotic, Geotrichum candidum, QAUGC01 in the raising water of the dealt with group, both groups obtained equivalent feed. After task with *Staphylococcus aureus*, larvae raised in the presence of probiotic showed significantly better ($P<0.05$) survival rate, advanced increase performance (weight benefit and particular increase rate), improved intestinal protease, amylase, and cellulase activity, and drastically lower mortality ($P = 0.003$). Furthermore, proximate composition revealed considerably higher crude protein values along with significantly lower residue matter material ranges in muscle of fry fed on probiotic treatment. This study is the first to document the favorable effects of a single regionally remote strain of *Geotrichum candidum*as a probiotic on *L. rohita* adolescent stages, and it demonstrates an economically realistic way to boost fish productivity [17].

In a study conducted, the effect of probiotics supplementation (*Bacillus subtilis, Lactobacillus plantarum*, or a combination of both) and yeast (*Saccharomyces cerevisiae*) on Rohu's immunological response was investigated. Two experiments were conducted to study the effect of different probiotics. In one experiment, *Labeo rohita* (Rohu) were fed with *Bacillus subtilus* denoted as D1, *Lactobacillus plantarum* denoted as D2, mixture of both denoted as D3 and yeast denoted as D4 Fish have been served a basic nutrition with no probiotics in the control experiment. All the experiments in which fish were fed with probiotics showed increased acid phosphatase activity, lysozyme activity and increased amount of total immunoglobins in blood samples which were collected from fish as compared to control experiments in which no probiotics were given. In second experiment tilapia were fed with three concentration of *Lactobacillus plantarum* (105,107,109 CFU/grams) (D1, D2 and D3) respectively for 60 days. Fish fed with D2 diet showed increased phagocytic activity, while fish fed with D3 diet showed increased phosphatase acid activity. Diet with both D2 and D3 showed high lysozyme activity. Fish fed with diet D1 and D3 showed increased amount of total immunoglobins. According to the results, using probiotics in

fish diets stimulates the immunological response of the fish. Probiotics increase immunoglobin levels, which promotes fish performance and health. To improve the gut health of fish, these probiotics could be included as dietary supplements in commercial fish feed or in the form of bacterial biofilm [18].

The effect of the probiotic *Enterococcus faecium* on *Labeo rohita* development, hematologic factors, and non-specific immune response was investigated in this study (Rohu). The aquaculture business is growing at a rapid pace. The pursuit of better husbandry conditions in intensive aquaculture is ongoing. Probiotics have been shown to improve fish output by changing gut microbial balance, strengthening the immune system, then decreasing the antibiotic use. Systematic use of probiotics along with fish defense remains a contentious issue. The effects of consuming Rohu with probiotics on *Aeromonas hydrophila* were investigated. The following feeding tactics were evaluated: constant feeding along with simply a basic food, continuous feeding with a probiotic enhanced diet, and 7-day and 14-day pulse administration feeding. Examine the pace of growth, as well as the immune system and metabolic indicators. Burst respiratory analysis revealed the P14 approach. If used for 14 days, probiotic (*Enterococcus faecim*) promotes fish growth and immune system, and if used constantly, it effectively protects the fish [19].

The effect of two bacteria, *Bacillus amyloliquefaciens* (BA) and *Lactobacillus sp.* from dairy yoghurt (DY), on growth, feed conversion ratio (FCR), and hematological and immunological parameters was investigated in a prior study. For 99 days, *Labeo rohita* (Rohu) were stocked in $0.42m^3$ tanks at $67m^{-3}$ and fed two probiotics mixing diets and a control diet, followed by a 61day baseline diet. FCR and mean weight showed no significant differences after probiotic feeding. The BA fish, on the other hand, grew faster and had a higher FCR after 61 days on the usual diet than the control diet. The dairy yoghurt bacteria group grew the slowest and had the lowest FCR score. The probiotic feeding group had higher serum lysozyme activity, total immunoglobins, and head-kidney superoxide dismutase than the control diet group. Even when the probiotic was removed, the BA-fed fish had the dominating gut microbiota. The probiotic strain isolated from dairy yoghurt did not survive in the intestines for long. These findings demonstrate that BA therapy improves FCR, growth, and other immunological parameters [20].

The aim of the previous study was to see how effective yeast (*Saccharomyces cerevisiae*) as a probiotic was at improving the intestinal microbiota of Rohu fish. Improved gut architecture and resistance can be attributed to improved intestinal flora. The varied levels (0.15%, 0.30%, 0.45 %, 0.60%, 0.75%, and 1%) of yeast indicated as SC1, SC2, SC3, SC4, SC5, SC6) were investigated for 60 days in this study. As compared to the control trial, the results of the experiment reveal a better influence on gut microbiota ($P<0.05$). SC4 therapy had the highest count of gut bacteria. Various bacteria strains were isolated and identified using a variety of methods. It can be observed that including 0.60 percent yeast in the Rohu fish's diet improves the intestinal microbiota. The treatment group had a higher bacterial count than the control group, indicating that yeast had a beneficial effect [21].

The most popular fish in South Asia is the Rohu (*Labeo rohita*). Due to a low survival rate during nursing, it had to reduce its farming. To prove that probiotics help, several types of dosages of two multi strain probiotics have being investigated by the larvae (days 8-38), juvenile (days 38-68), and adult (days 68-98) stages. *Bacillus subtilis* as well as yeast made up the first probiotics (P1). *Lactobacilli* and yeast made up the second probiotic (P2). The researchers looked at different doses of the P1 and P2 diets, as well as a control diet made up of mustard oil cake, rice bran, wheat flour, and fish powder. Over control group, standard and extreme doses of P1 improved hatchling endurance by 14.4 percent and 16.6 percent, correspondingly. Standard and elevated doses of P2 diet increased survival by 22.1 percent and 22.3 percent, correspondingly, when associated to the control. Probiotics have helpful as well as important benefits during Rohu hatchling and fry nursing before the age of 68 days, but not after that [22].

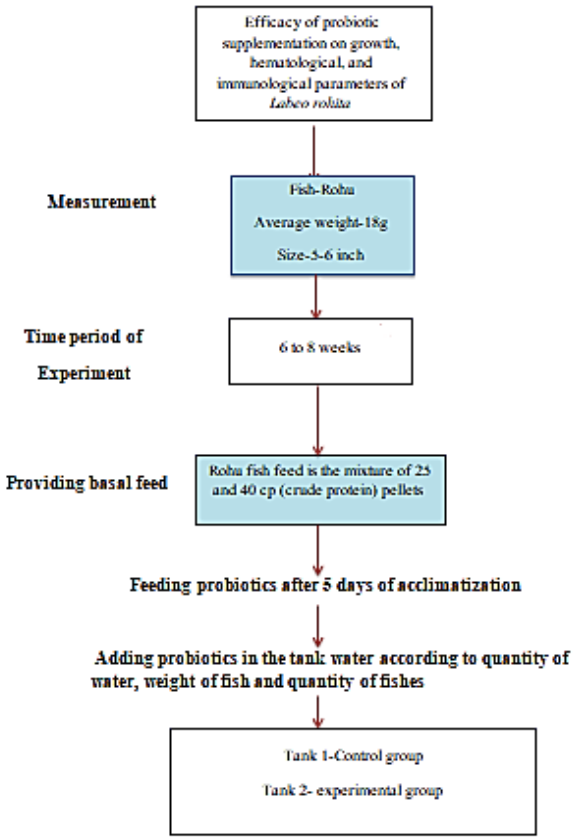
The aim of this assessment was to see how probiotics affected the development and biochemical execution of rohu fingerlings in freshwater. Probiotics were used to complete the dietary assessment, endurance rate, and development of new fish water *Labeo rohita* fingerlings fed with false foods and supplements such as Calcium, Starch, and Sardine oil. *Bacillus subtilis* + *Lactobacillus rhamnoses* diet-E3, basal with Antibiotics diet-E2, and only basal eating regimen in the control - E1 are the three groups' basal eating regimens with probiotics. Weight gain, Explicit development rates, Percent endurance, Biochemical examination, Microbial investigation, and Antibacterial movement of probiotics were all observed ($P<0.05$) during trail tests. When compared to control deities-E1, higher in probiotics counts calories (Calcium)- E3 consolidated

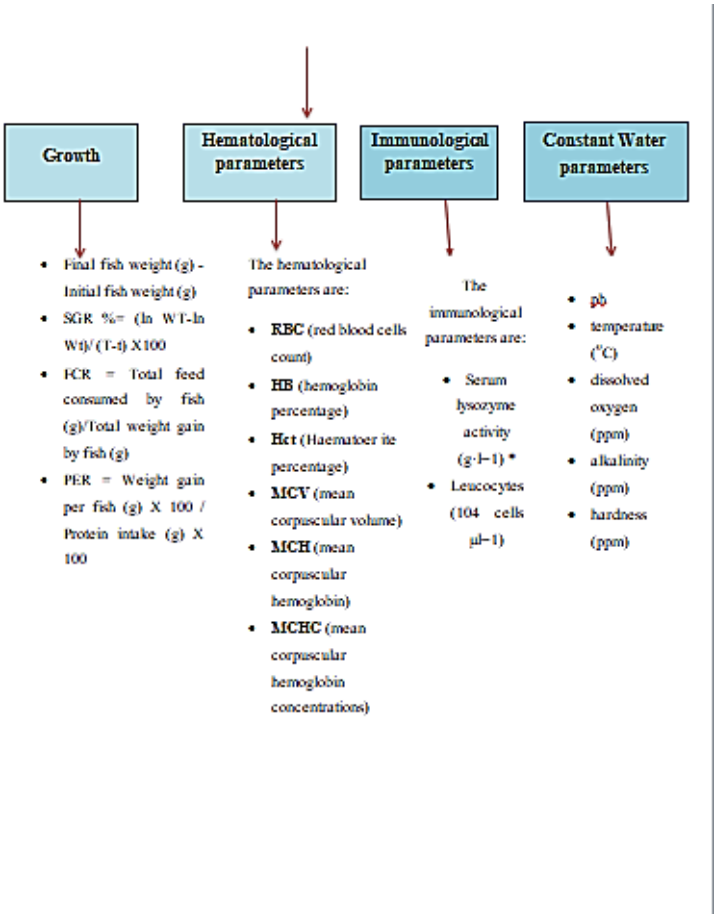
diet took care of fish followed by other test bunches. The FCR values were also better with dietE2 compared to Control diet-E1, demonstrating the fishes' efficient utilization of feed. According to the current study, probiotic with starch was the optimum feed focus nutrition for freshwater fish *Labeo rohita* [23].

CHAPTER 3

METHODOLOGY

STUDY PLAN





Material and Method

Experimental Designs

The study was carried out in the Research department of Zoology, Kinnaird College Lahore. The experiment was conducted in the Zoology Laboratory of Kinnaird for 60 days. The *Labeo Rohita* fish were collected from Anjum Fish Farm in Chunian district Kasur in Pakistan. The fishes collected from farm were alive and transported to the laboratory in polyethylene containers. The experiment was conducted for 6 to 8 weeks. The fishes were fed with basal diets and with probiotics**.** The length of the fingerlings was 14-15 cm and weight was 17-19 grams**.** The Rohu fish was treated with 1% salt for 5 to 7 minutes and then these were subsequently acclimatized to laboratory settings for five days. Fish were fed feed during the acclimation period. When fishes arrived, they were placed in tanks for rearing and the fishes were placed in them for five days so that they can adapt the environment around them. The fish were divided into two tanks. The tank 1 contains control group of fishes which were feed with simple feed and the tank 2 contains experimental fishes which were feed with simple feed and probiotics [24].

Feed

The feed used in the experiment was mixture of hi-tech feed 40 cp (crude protein) and 25 cp (crude protein). The feed was present in pallet form which was grinded to make a powder form feed which can be fed to the fishes. The feed was made up of fish meal, corn cake, and soya cake etc. It contains high nutrients for fish growth. The diameter of the pellets is between 2mm-8mm [25].

Probiotics

The probiotics used for the experiment was the commercially available mixture of different bacteria which help to increase the immunity of fish. The probiotics was DIGEST 54 PLUS. It was a water and soil probiotic with enzymes that improves the pond water carrying capacity and promotes growth of fish, thus favored the natural productivity. The probiotics was the mixture of:

- Processed grain by-product
- *Yucca schidigera* plant extract
- Yeast cultures (*Saccharomyces cerevisiae*)

- Dried *bacillus subtilis* fermentation product
- Dried *bacillus licheniformis* fermentation product
- Dried *Trichoderma longibrachiatum* fermentation extract

Dosage

The probiotics dosage was used as per recommended. The dose of probiotics used for week one and 2 was 3.57g per 500 L water. After two weeks 1g probiotics was used per 500 L water and for rest of the trail for week four, five, six, seven, and eight the dosage of probiotics used was 0.3428g [25].

Sampling of fish

The Rohu fish was collected from Anjum fish farm Chuniya using fishing net. Length and Weight of fishes were measured separately to obtain the health of fish and their growth. The weight and length of fish were documented with the help of weight balance and measuring scale. After initial 15 days, the fishes were caught with the help of using net from each experimental treatment and then these fishes were used for hematological analysis [26].

Sampling of blood

The samples of blood were collected during the experimental period of 15-, 30-, 45-, and 60-days interval. By Cardiac puncture blood were strained with the help of syringes like hypodermic needles. The blood collected from fish were transferred into EDTA tubes of about 1.5 ml capacities and then stored in refrigerator till further examination [26].

Trial

After two days the rohu fish was feed with feed and probiotics and were divided into two tanks. T1 was control and T2 was experimental. The initial length and weight readings were noted, and blood samples were taken from both control and experimental fishes after dissection to check its hematological parameters. After initial 15 days the growth was observed in the weight and length of the fish. The other parameters such as hematological parameters were observed after dissecting fishes and getting blood by puncturing the heart of fishes taken from control and experimental

groups. After another fifteen days the weight and length were again measured to observe the growth in the fishes and blood samples were taken from both control and experimental fishes by heart puncturing method to check its hematological parameters. After 6 weeks or at 45 days the readings of length and weight were measured, and blood samples were taken from the dissected fish. On the 60th day of trail or after 8 weeks the final analyses were prepared by the weight and length of the fishes and the blood samples were taken for getting the hematological parameters.

Growth Parameter

The Rohu fish is placed in control and experimental conditions. The weight gain of fish can be calculated by the formula as given by Annet in 1985.

Final fish weight (g) - Initial fish weight (g)

It is also determined that the percentage increases in weight per fish each day utilizing this equation.

SGR %= (ln WT-lnWt)/ (T-t) X100

S GR percent denotes the percentage increase in body weight per fish, Ln WT denotes the natural log of weight at time T, Ln Wt denotes the natural log of initial weight, T denotes time, t denotes initial time, and Ln denotes natural logarithm. The formula for calculating the feed conversation ratio (FCR) is as follows:

FCR = Total feed consumed by fish (g)/Total weight gain by fish (g)

Hematological parameter

Every 15th day, blood samples were obtained from the fish. A 25-gauge needle and a 1ml syringe were used to extract the samples from the caudal vein. Hematocrit, hemoglobin (Hb), RBCs, WBCs, and serum lysozyme activity were measured. The action of lysozyme in the serum will be assessed using an agarose gel cell lysis assay, as described by Schltz (1987). To determine the Mean corpuscular hemoglobin concentration, The MCHC formula is used to calculate mean corpuscular hemoglobin MCH and mean cell volume MCV [27].

MCHC = Hb\ PCV x 100

MCV = PCV\ RBC x 10

MCH = Hb\ RBC x 10

Procedure

Place 0.38 ml of the fluid used to dilute white blood cells (WBC) in a clean, dry, and grease-free test tube. Add 0.02 ml or 20 l of blood specimen to the tube holding the dilution solution using a micropipette or WBC pipette. Prepare your Hemocytometer / Neubauer's Chamber by carefully mixing for a few minutes.Remove the Neubauer's chamber / Hemocytometer from its case and clean it with a swabor a piece of gauze. Similarly, thoroughly clean the cover glass and set it over the grooved portion of the hemocytometer Fill the WBC pipette halfway with the Diluted Specimen, thoroughly mix it, and then remove1-2 drips before charging the chamber. To hang the next drop of fluid, softly press the rubber tubeof the WBC pipette. Make a 45° angle by placing the pipette's tip and hanging drop on the edge of the coverslip. Allow capillary action to fill the chamber with a small volume of fluid from the pipette. The chamber should not contain any air bubbles and should not be overloaded.

White blood cells counting under microscope

Focus the ruling with the 10x Objective lens, and then count the white blood cells in the four huge corner squareswith the 40x Objective lens, as indicated before. The cells on the lower and right lines of the four corner squares are counted, but not the cells on the opposite line. Count the cells that are on the "L" line, which should be on the right and lower lines or, in the case of marginal cells, the left and higher lines.

Calculations

After counting the cells under the microscope, we know how many WBC are in each of the four corner squares. Consider the number of cells to be 'N'. The volume of fluid inside the chamber is now the product of the area and depth of the Hemocytometer / Neubauer's chamber.

4 x 1 x 1x 1/10 = 2/5

The depth of the Hemocytometer is 0.1 or 1/10 mm, as mentioned in the Hemocytometer's brief description above. To compute the Total White Blood Cell Count, apply the following formula: 2/5 mm3 includes = N Dilution.

Then, 1 mm3 consists of = N 20 5/2

N 50 / mm3 = total WBC count

Water quality Parameters

Temperature (25.7-26.3), pH (7.4-7.6), dissolved oxygen was determined to be in the range during the experiment. The quality of the water was measured twice daily, between 06:00 am and 14:30am in Environmental lab. Every 15 days, the other parameters were examined. Throughout the trial, all water quality metrics were determined to be within the optimal range for fish rearing [28].

Immunological parameter

Leukocytes and Serum Lysozyme Activity was one by following procedure to collect the serum, from each tank experimental a control three fishes were taken. Blood was drain without the use of any anticoagulant and collected in an EDTA vacutainer tube. Tubes were kept in upright position for about 2-4 h at room temperature, allow the blood to block. Then the blood samples were centrifuge at 3000 g for 5-6 minutes. Serum from all sample were collected in dried Eppendorf and used to detect serum lysozyme activity.Leucocyte counts and serum lysozyme activity were assessed using established procedures that are detailed elsewhere (Rawling et al2009; Merrifield et al. 2010c). According to Doumas et al. (1971), total serum albumin was measured using the Bromocresol Green method and total blood protein was evaluated using the Biuret test.

Statistical Analysis:

Independent t test was used to assess data of hematological and immunological parameters using SPSS 22.0 software (SPSS Inc.)

The data was presented as mean ± standard error (SE), and the P-value <0.05 was considered as statistically significant.

CHAPTER 4

RESULT AND DISCUSSION

WATER QUALITY PARAMETERS

During experimental period Water quality parameter were calculated in Zoology laboratory of Kinnaird College day by day which was represented in Table (1). Water quality parameters were obtained at temperature (27.81–30.6°C), dissolved oxygen (6.22-7.52ppm) Alkalinity (130.2–161.0 ppm), total hardness (490-512ppm), during the whole testing period some parameters are analyzed every 15 days.

Table no 01: Water Quality parameter of Rohu fed with experimental diet for 60 days

Sr.no	**pH**	**Temperature °C**	**Dissolved oxygen(ppm)**	**Alkalinity (ppm)**	**Hardness (ppm)**
Minimum	7.16	27.81	6.22	130.2	150.2
Maximum	8.05	30.6	7.52	161.0	220.4
Mean	7.60	29.2	6.87	145.6	185.3

GROWTH PARAMETER

RESULTS:

Growth parameters, such as increase in net weight of fishes (net weight gain in control group was 15.97g ± 0.86 and in experimental group was 21.53g ±1.265,), feed conversion rate (FCR) in controlgroup was 3.34g and in experimental group was 2.536g, protein efficiency ratio (PER) in control group was 0.89g and in experimental group was 1.331g and survival rate were recorded in experimental group was 89 % and control group was 70% on a regular basis for two weeks [31].

DISCUSSION

In contrast with this recent research paper the effects of dietary probiotics on the growth function of *Labeo rohita* under laboratory testing was shown the growth parameter, weight gain was significantly higher in experimental (1.89 ± 0.30) and all other treatments except control group. Similarly, in our trial experimental groups was higher value than control group (net weight gain in control group was 15.97g ± 0.86 and in experimental group was 21.53g±1.265,)

In the case of Specific Growth Rate (% per day), experimental group 1(0.58 ±0.06) and experimental group 2 (0.52 ± 0.13) was significantly higher than controls. Similarly in our trial Specific Growth Rate (SGR) value were also higher in experimental group (0.6g) than control group (0.5 g). On the other hand, protein efficiency ratio (PER) in control group was (0.89g) and in experimental group was (1.331g) Feed Conversion Ratio in probiotic group of this paper shows (1.97 ± 0.27) for control and Feed Conversion Ratio obtained from experimental (2.22 ± 0.56) is higher than control. The same results of feed conversion ratio in our experimental group 2.526 g were significantly higher than control group 3.34 g [31].

In our study, we fed fish with mixture of probiotics, as well as various growths weight gain parameters, SGR, FCR and PER were determined. At the end of the test period treatment groups receiving a diet rich in probiotics revealed a significant increase in body weight gain, growth rate (SGR), and protein efficiency (PER) rate represented a relationship between body weights. And a significant decrease in feeds conversion ratio (FCR) compared to the control group [31].

Fish have a profound effect on the health and well-being of most of the world's population. This study was designed to promote the different characteristics of Rohu’s probiotics from observing active growth and their impact on the body's response to fish infected with pathogenic bacteria. Probiotic has a high nutritional value. A certain growth rate (SGR) takes about the same W.G pattern where Rohu in the experimental group has a much higher SGR compared to the control group. This was also true of the protein efficiency ratio (PER) measure; Rohu in the experimental group treated with foods with an additional probiotic exceeded the control group value. This is consistent with previous findings by various authors [32]. Jafaryan [33] also reported that probiotic (*Bacillus*) - additional foods significantly increase the length,weight and specific growth rate of fish.[33].

Rohu's nutrient feed conversion rate (FCR) in basic diets was higher than in those receiving additional probiotics that showed a positive aspect of a probiotic diet. The best FCR values recognized by a diet containing the added probiotics suggest that the probiotics enhances feed intake, this means that the probiotics used can reduce the quantity of feed needed for animal growth which could lead to a reduction in production costs. Different probiotics increase the development and use of specific fish nutrients in different ways, accordingto Noh and Bogut [34].

The results of PER have shown that supplementation with probiotics significantly improves the utilization of Rohu protein. It is reported that, the amount of food-borne bacteria affects the amount of bacteria in the digestive tract. [35].The beneficial effects of probiotics such as Immuno- booster have already been tested in a few freshwater fish *Labeo rohita*. According to certain reports, digestive organs are reportedly more sensitive to the nutritional content of food and produce quick changes in the activity of digestive enzymes, which in turn affect fish health and growth. [36].Additionally, bacteria liberate free amino acids and proteases from their products, which might improve an animal's nutritional status. [37]. It has been proven that including a variety of probiotics in the diet improves fish performance. It may be due to changes in diet (FCR), specific growth rate (SGR), consumption (PER) and weight gain (W.G) significant decrease in intestinal THB levels and better adherence / introduction of probiotic micro flora in the gut.

In our study, a high level of adhesion added to food may be responsible for improved growth and consumption of fish nutrients. Many reports indicate that many probiotics exert their effect by using the colon to manage and release a few growth-promoting nutrients [38]. The results propose that probiotic living microorganisms may be synthesized while forming, nutritious carp food withits good growth and nutrient uptake.

EXPERIMENTAL GROUP

Table no 02: Growth of Rohu fed with experimental diet for 60 days

Sr.no	initial weight	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	net weight gain
01	14	15.5	17.5	19.5	21	22.5	25	29.5	34.5	20.5
02	13	14	15.5	17.5	19.5	21	24	28.5	32.5	19.5
03	18	20.5	22	24.6	26.5	29	32.5	36	39.5	21.5
04	19	21.5	23	25.5	28	30.5	34	37.5	41	22
05	20	22.5	25	27	29.5	32	35.5	39	42	22
06	18.5	21	23.5	26	28.5	31	34.5	38	41	22.5
07	17	19.5	22	24	26.5	29	32.5	36	39.5	22.5
08	18.6	21.2	23.7	26.2	28.7	31.2	34.7	38.2	41.7	23.1
09	11	13.5	16	18	20.5	23	26.5	30	33	22
10	12.5	15	17.5	20	22.5	25	28.5	32	35.5	23
11	12	14.5	17	19.5	22	24.5	28	31.5	35	23
12	17.9	20.4	22.9	25.4	27.4	29.9	33.4	36.9	40.4	22.5
13	19.9	22.4	24.9	27.4	29.9	32.4	35.9	39.4	42.9	23
14	18.5	20	21.5	24	25.5	28	31	33.5	37	18.5
15	16	18.5	20	22.5	24	26.5	29.5	33	36	20
16	15.5	18	20.5	22	23.5	26	29.5	33	36.5	21
17	14.6	17.1	18.6	21.1	23.6	25.1	27.6	30.6	34.1	19.5
18	20.5	23	25.3	27.8	29.3	31.8	35.3	38.8	42.3	21.8
19	17.5	20	22.5	25	26.5	29	32.5	36	39.5	22
20	11.9	14.4	16.9	19.4	21.9	24.4	27.9	31.4	34.9	23
21	16.6	19.9	21.6	23.1	25.6	28.1	31.6	35.1	38.6	22
22	17.4	19.9	22.4	24.9	27.4	28.9	34.9	38.4	41.9	24.5
23	14.4	16.9	19.4	21.9	24.4	26.9	30.4	33.9	37.4	23
24	18	20.5	23	25.5	28	30.5	34	37.5	41	23
25	20.6	23.1	25.6	28.1	30.6	32.1	35.6	39.1	42.6	22
26	18.5	20	21.5	24	26.5	28	31.5	35	38.5	20
27	20	22.5	24	26.5	28	30.5	34	37.5	41	21
28	21	22.5	25	26.5	29	30.5	34	37	40.5	19.5
29	17	19.5	21	23.5	25	27.5	31	34.5	38	21
30	20.5	23	24.5	27	28.5	31	34.5	37.5	41	20.5
31	17.8	20.3	22.8	25.3	26.8	29.3	32.3	35.8	39.3	21.5
32	16.3	18.8	20.3	22.8	24.3	26.8	30.8	33.8	37.3	21
33	19.4	21.9	23.4	25.9	27.4	29.9	33.4	37	40.5	21.1
34	12.8	15.3	17.8	19.3	21.8	23.3	26.8	30.3	33.8	21
35	18.7	21.2	22.7	25.2	27.7	29.2	32.7	36.2	39.7	21
36	19.8	22.3	24.8	26.3	28.8	31.3	34.8	38.3	41.8	22
37	20.3	22.8	24.3	26.8	29.3	30.8	34.3	37.8	41.3	21

38	19.2	21.7	24.2	26.7	29.2	30.7	34.5	38	41.6	22.4
39	16.6	19.1	21.6	23.1	25.6	27.1	30.6	34.5	38	21.4
40	15.3	17.8	19.3	21.8	24.3	26.8	30.3	34	37.9	22.6
41	13.2	15.7	17.2	18.7	20.2	21.7	24.7	27.7	31.2	18
42	16.3	18.8	21.3	23.8	26.3	27.8	31.3	34.8	38.3	22
43	18.9	21.4	23.9	26.4	27.9	30.4	33.9	37.4	40.4	21.5
44	15.7	18.2	19.7	22.2	23.7	26.2	29.7	33.2	36.7	21
45	20.8	23.3	25.8	28.3	30.8	33.3	36.8	40.3	43.8	23
46	21.3	23.8	26.3	28.2	30.7	32.3	35.9	39.4	42.4	21.1
47	20.5	23	25.5	27	29.5	31	34.5	38	41.5	21
48	15.6	18.1	19.7	22.3	24.8	26.6	30.1	33.6	37.1	21.5
49	19.2	21.7	23.6	26.1	28	30.5	34	37	40.5	21.3
50	12.9	15.4	17.9	20.4	22.9	25.4	27.9	30.9	33.9	
51	19.9	22.4	24.9	27.4	29.9	33.4	36.9	40.4		
52	17.5	20	22.5	25	27.5	31	34.5			
53	14.7	17.2	19.7	22.2	24.7	28.2				
54	12.9	15.4	17.9	20.4	22.9					
55	19.6	22.1	24.6	27.1	19.6					
56	13.5	16	18.5	21						
57	11.7	14.2	16.7							
58	15.8	18.3								
59	18.5	21								
60	16.3									
	=17.3±2.8								=38.82±3.055	=21.53±1.265

CONTROL GROUP

Table no 03: Growth of Rohu fed with Control diet for 60 days

Sr.no	Initial weight	1st we Ek	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	net weight gain
01	21.2	22.5	24	25.5	27.4	28.9	30.4	33.4	36.9	15.7
02	20	22.1	23.6	25.2	27.2	29.1	30.1	33.1	36.6	16.6
03	16.2	18	19.8	21.7	23.5	25.5	27	30	33.5	17.3
04	14.5	16	17.5	19	20.5	22	23.5	27	30.5	16
05	15.2	17.4	18.3	19.8	21.3	23.2	24.7	27.7	31.2	16
06	19.3	21.9	23.4	25.4	27.6	28.4	29.2	32.7	35.7	16.4
07	13.6	15.2	17.3	19	20.5	21.4	22.9	25.9	29.4	15.8
08	19.4	20.8	22.3	23.8	25.3	27.2	28.7	31.7	34.7	15.3
09	16.4	17.5	18.8	20	22	23.5	25	28	31.5	15.1
10	11.5	12.5	14	15.5	17	18.5	20	23	26	14.5
11	18.3	19.7	21.3	22.9	24.4	25.9	27.4	30.4	33.9	15.6
12	17.6	19.5	21	22.5	24.4	26.8	28.5	31.5	35	17.4
13	14.9	16.4	17.9	19.4	20.9	22.4	25.4	28.9	31.9	17
14	12	13.5	15	16.5	18	19.5	22.5	26		
15	13	14.5	16	17.5	19	20.5	24			
16	15	16.5	18	19.5	21	22.5	25			
17	14.4	15.9	17.4	18.9	20.4	23.4				
18	15	16.5	18	19.5	21					
19	19	20.5	22	23.5						
20	16.6	18.1	19.6							
	=16.3±±2.764								=32.91±3.135	=15.9±0.86

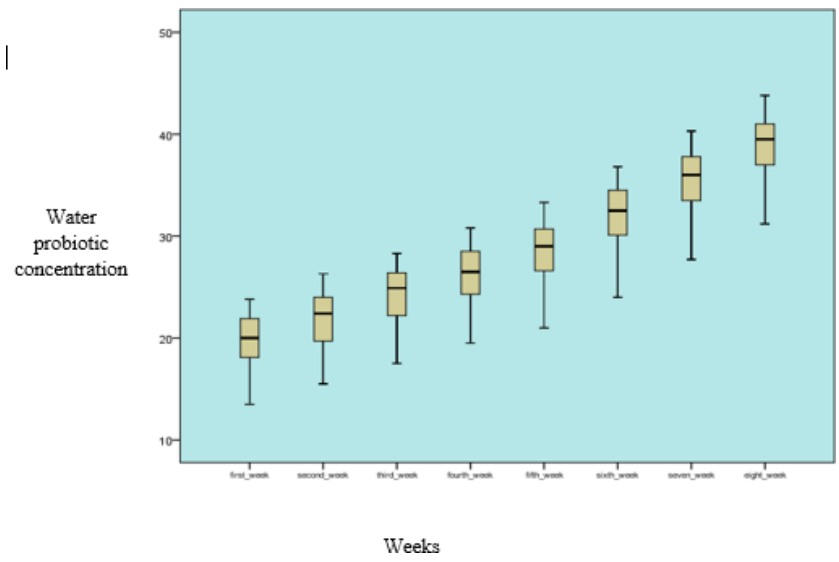


Figure 4.1: Box plot shows growth of Rohu with Control diet for 60 days trial

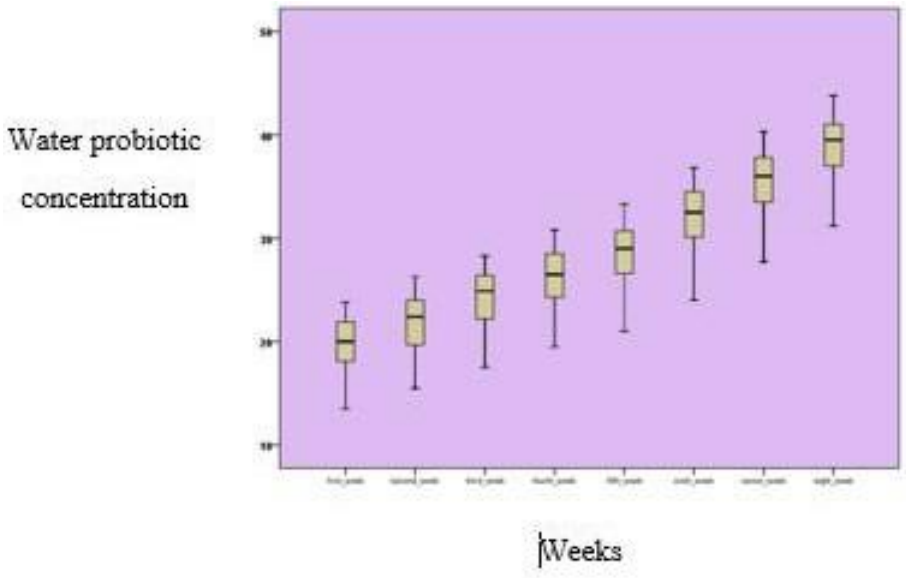


Figure 4.2: Box plot shows growth of Rohu with experimental diet for 60 days trial

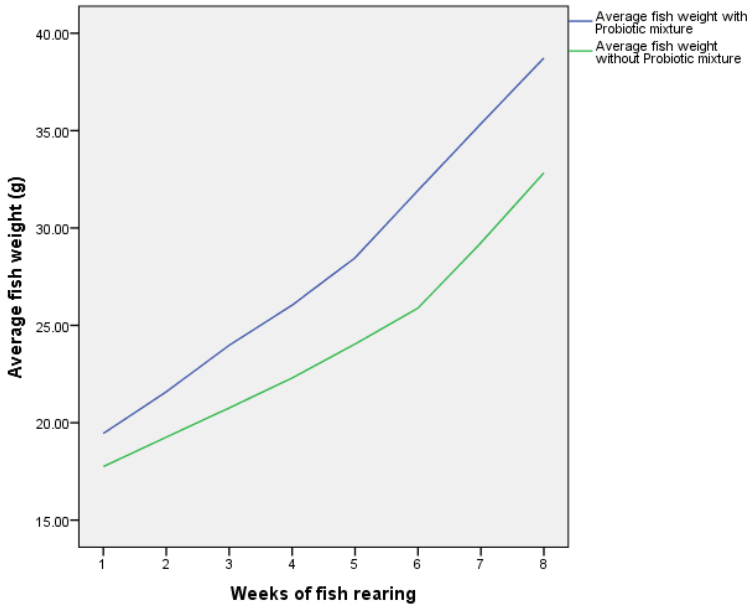


Figure 4.3: Line graph shows growth of Rohu with probiotics and without probiotics

Table no 04: Growth parameter of Rohu fed with control and experimental diet for 60 days.

Growth parameters	Control group (g)	Experimental group (g)
Initial mean weight(g)	16.39±2.764	17.30±2.821
Final mean weight (g)	32.91± 3.135	38.82±3.055
Specific growth rate SGR (g)	0.5 g	0.6 g
Feed conversion ratio FCR (g)	3.34 g	2.526 g
Protein efficiency ratio PER (g)	0.84 g	1.331 g
Survival rate %	70%	89 %

HEMATOLOGICAL PARAMETERS

RESULTS

Results shows that hematological parameters of *Labeo rohita* in experimental group fed with probiotic that was a mixture of *Yucca schidigera* plant, yeast culture, *Bacillus subtilus* fermentation product, *Bacillus licheniformis* fermentation product, and *Trichoderma longibrachium* fermentation product and control group fed with feed only. Throughout the experiment, blood samples were taken at, 15, 30, 45, and 60-day intervals. When compared to control (2.81 ± 0.03) the red blood cell count was significantly higher at experimental group (3.67 ± 0.22) for 60 days and ($P < 0.05$) were considered highly significant the highest Hemoglobin (Hb) percent was recorded at experimental group for 60 days (5.88 ± 0.05) and ($P < 0.05$), whereas the lowest was in the control group (5.43 ± 0.03). The greatest value of hematocrit (Hct) percent was seen in experimental group for 60 days (32.18 ±0.08) as compared to the control group (29.63 ± 0.05). MeanCorpuscular Volume (MCV), Mean Corpuscular hemoglobin concentration (MCHC) Mean Corpuscular hemoglobin (MCH), values were calculated; the minimum Mean Corpuscular Volume (MCV) values were observed in experimental group (154.24 ± 0.04), while the maximum values were recorded in the control group (188.32 ± 0.04), and Mean Corpuscular hemoglobin (MCH) maximum values were recorded in the control group (36.33 ± 0.04) and minimum in experimental group (31.23 ± 0.03). The experimental group had the highest Mean Corpuscular hemoglobin concentration (MCHC) values (22.87 ± 0.06) and was significantly greater and ($P < 0.05$), whereas the control group had the lowest (19.76 ± 0.05) for 60 days.

DISCUSSION

Hematology is an important parameter to be examined when evaluating the quality of a fish diet. The hematocrit (Ht) and hemoglobin (Hb) levels are the most prominent blood parameters regularly impacted by diet.

In this research probiotics Labeo rohita was treated with water soluble probiotics (for first 2 weeks was 3.57g, after two weeks 1g and for rest of the trail of eight weeks the dosage of probiotics used was 0.3428g per 500 L water and the results showed that they had a favorable influence on hematological indices. Hemoglobin (Hb), Red blood cell count (RBCs,) levels were highest in fish fed a diet enrich with probiotics. As compared to fish fed a control diet, fish fed a diet enrich with

probiotics showed a considerable ((P <0.05)) increase in Hct levels. The goal of this study was to see how the probiotic affected the blood parameters of the fish Labeo rohita. The probiotics effect on Labeo's hematological parameters was found to be beneficial.

In another study the value of RBC and Hb values in experimental group 0.955 ± 0.155a, 4.7 ± 0.2a and (P <0.05) were significantly higher than control 0.315 ± 0.045b, 2.05 ±0.35b. In the case of MCV, the highest value was found in experimental group 136.25 ± 10.25a .m in addition, a very high amount of MCH was recorded in experimental groups was higher 69.55 ± 2.95a than control 55.65 ±1.95 [39][40]

Positive result of probiotics on hematological parameters in tilapia had been reported also but in the other hand, [39] *Oreochromis niloticus* fed a diet enriched with *Bacillus subtilis* [40] or *Pediococcus acidilactici* exhibited some difference (but not significant) in Hemoglobin and Hematocrit levels between the control and fish fed a probiotic-enriched diet [40][41].

Fish fed a probiotic-supplemented diet had the highest amounts of hemoglobin, red blood cells, and white blood cells.When compared to fish fed the control diet, both groups fed the dietenriched with dead Saccharomyces cerevisiae yeast and both live B. subtilis and S. cerevisiae showed significant increases in Ht levels. Hb content in rainbow trout (*Oncorhynchus mykiss*) fedvarious amounts of probiotic was substantially different from the control [43][44].

Probiotics had a good influence on Labeo's hematological parameters, as evidenced by a considerable increase in Hb %, HCT %,RBC count and red cell indices such MCV, MCH, and MCHC in the tables. These results could be explained that the probiotics administeredenhanced blood parameter levels due to hematopoietic stimulation. These findings verified the result of [45].

Table no 5: Triplicates values of hematological parameters of *Labeo rohita* in control and experimental groups.

Sr.no	Hematological Parameters	15th days		30th days		45th days		60th days	
		Exp	Control	Exp	Control	Exp	Control	Exp	Control
1	RBC (x10^6 µl-1)	2.10 2.18 2.25	2.06 2.09 2.13	2.7 2.78 2.85	2.29 2.31 2.35	2.98 3.07 3.13	2.59 2.64 2.69	3.45 3.67 3.9	2.78 2.81 2.85
2	Hb (g/dl)	5.19 5.21 5.25	5.02 5.05 5.09	5.35 5.39 5.44	5.11 5.13 5.15	5.70 5.78 5.80	5.19 5.22 5.24	5.83 5.90 5.93	5.39 5.42 5.48
3	Hct (x 10^3 µl-1)	29.78 29.84 29.87	27.61 27.65 27.69	30.19 30.24 30.27	28.11 28.15 28.19	31.18 31.26 31.31	28.29 28.32 28.36	32.10 32.20 32.26	29.58 29.64 29.69
4	MCV (fl)	136.9 137.2 137.35	144.20 144.25 144.28	138.39 138.41 138.49	156.39 156.43 156.49	140.75 140.80 140.85	167.20 167.26 167.29	154.20 154.24 154.28	188.28 188.32 188.37
5	MCH (pg)	29.19 29.21 29.24	29.17 29.21 29.26	30.37 30.4 30.45	32.37 32.42 32.46	31.10 31.13 31.18	34.16 34.21 34.27	31.20 31.23 31.26	36.30 36.33 36.38
6	MCHC (g/dl)	19.21 19.24 19.27	19.15 19.21 19.24	20.3 20.33 20.36	19.30 19.36 19.40	21.80 21.85 21.90	19.44 19.46 19.51	22.81 22.88 22.94	19.70 19.78 19.80

Table no 6: Mean values of hematological parameters of *Labeo rohita* in control and experimental groups.

Sr. no	Hematological Parameters	15th days		30th days		45th days		60th days	
		Exp	Control	Exp	Control	Exp	Control	Exp	Control
1	RBC ($x10^6$ µl-1)	2.17±0.07	2.09 ± 0.03	2.7± 0.07	2.31 ± 0.03	3.06±0.07	2.64 ± 0.05	3.67± 0.22	2.81 ± 0.03
2	Hb (g/dl)	5.21±0.03	5.05 ± 0.03	5.39 ±0.04	5.13 ± 0.02	5.76±0.05	5.21 ± 0.02	5.88± 0.05	5.43± 0.04
3	Hct (x 10^3 µl-1)	29.83± 0.04	27.65± 0.04	30.23±0.04	28.15± 0.04	31.25± 0.06	28.32±0.03	32.18 ±0.08	29.63± 0.05
4	MCV (fl)	137.15 ±0.22	144.24 ± 0.04	138.43±0.05	156.43±0.05	140.8± 0.05	167.25± 0.04	154.24±0.04	188.32± 0.04
5	MCH (pg)	29.21± 0.02	29.21± 0.04	30.40±0.04	32.41± 0.04	31.13± 0.04	34.21±0.05	31.23 ±0.03	36.33± 0.04
6	MCHC (g/dl)	19.24± 0.03	19.2± 0.04	20.33±0.03	19.35± 0.05	21.85± 0.05	19.47± 0.03	22.87 ±0.06	19.76± 0.05

- **Values expressed in triplicates and mean ± standard deviation**

RBC: Red Blood Cell count, Hb: Hemoglobin percentage, Hct: Hematocrit percentage, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular hemoglobin, MCHC: Mean Corpuscular hemoglobin concentration,

IMMUNOLOGICAL PARAMETERS

RESULT

According to the immunological parameters, the total amount of leucocytes in the fish diet with probiotics ($24.27 \pm 0.84 \times 10^6$ cells $\mu l^{-}1$) and ($P < 0.05$) was substantially higher than the fish in the control group ($6.7 \pm 0.30 \times 10^6$ cells μl^{-1}). The probiotic group had considerably higher serum lysozyme activity (7.89 ± 0.05 U ml^{-1}) and ($P < 0.05$) than the control group (4.90 ± 0.05 U ml^{-1}) and the results were shown in table 9.

DISCUSSION

In the case of improving antimicrobial resistance, probiotics are gaining popularity as environmentally friendly immunobooster for aquatic animals and as a substitute to antibiotics. Much research has focused on the use of *Bacillus spp.* and / or *Lactobacillus spp.* such as probiotics, with only a small amount of effort made to assess the favorable effects of probiotics combination in aquatic animals.

The parameters of immunology represent the health of fish, nutrients, and living conditions, and are affected by numerous factors such as age, size, diet,species and cultural environment.

The most important effect of probiotics is that it alters the immune system, giving the host better protection against pathogens [46].

When mixture of probiotics was used to feed the fingerlings, the chances of survival with A. hydrophile was much higher than control fish. The findings are consistent with previous results of the supply of probiotics in *Catla catla, Oreochromis niloticus,* and *Anguilla japonica.* The findings suggest that immunostimulants such as probiotics can improve host health by stimulating immune function and altering intestinal microflora while producing compounds that reduce the actions of pathogenic microorganisms [47][48].

The probiotics have been shown in experiments to develop specific components of the immune system indirectly, allowing disease to be eliminated easily. Probiotic supplementation has been shown to improve lysozyme activity in fish serum [49].

In this research study the value of leucocytes was significantly higher at experimental group (24.27 ± 0.84 × 10^6 cells $\mu l^{-}1$) and ($P < 0.05$) which were significant than the control group (6.7 ± 0.30× 10^6 cells μl^{-1})

In other study the value of leucocytes count was significantly higher at experimental group 30.8± 4.1 than the control group 7.6 ± 1.4.

It was found that Nile tilapia fed with diet enrich with probiotics had a remarkable increase in serum lysozyme activity. The increase levels of leucocytes and serum lysozyme was detected in this study suggests that Rohu's nonspecific immune system has been stimulated, but a more thorough examination of the immunological response is required to confirm this [50][51].

Probiotics have been shown to increase hemopoesis and produce nonspecific immunity in fish, according to studies.

Table no 07: Immunological parameters of *Labeo rohita* fed with experimental diet for 60 days

- **Values expressed as means ± standard deviation**

Immunological parameters	**Control**		**Probiotics**	
	Triplicates	**Mean**	**Triplicates**	**Mean**
Leucocytes(10^4 cells μl^{-1})	**7.12** **6.51** **6.73**	**6.7±0.30**	**24.31** **25.10** **23.42**	**24.27±0.84**
Serum lysozyme activity (U·ml ^{1}) †	**4.90** **4.85** **4.96**	**4.90±0.05**	**7.95** **7.84** **7.90**	**7.89±0.05**

CONCLUSION

In the dietary supplementation of probiotics (Yucc*a schidigera plant, yeast culture, Bacillus subtilus* fermentation product, *Bacillus licheniformis* fermentation product, and *Trichoderma long brachium* fermentation product) were very beneficial for aquaculture industry. *Bacillus* provides beneficial effects in many ways including enhancing growth, suppress irritation anddisease attacks by importation of the immune system, promoting nutrition, and improving theaquatic environment for growth and reproduction of fish *Labeo rohita*. *Bacillus* species can increase the Growth Rate (SGR) level once lower the Feed Conversion Ratio (FCR) of fish tothe desired level. The *bacillus* can grow Fecundity, survival rate, Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin (Hb) in the blood, resistance to stress, and resistance to disease. The study concluded that *B. subtilis* as an addition to the fish diet may improve the growth rate by a favorable economic situation.

CHAPTER 5

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CHAPTER 6

ANNEXURE

Figure no 01: Fish Feed from Chuniya Fish Farm

Figure no 02: Fish Fingerlings in Control Group

Figure no 03: Fish Fingerlings in Control Group

Figure no 04: Fish Fingerling in Experimental group

Figure no 05: Fish Fingerling in Experimental group

Figure no 06: Weight measuring of Fish after completing the trial of 15thdays

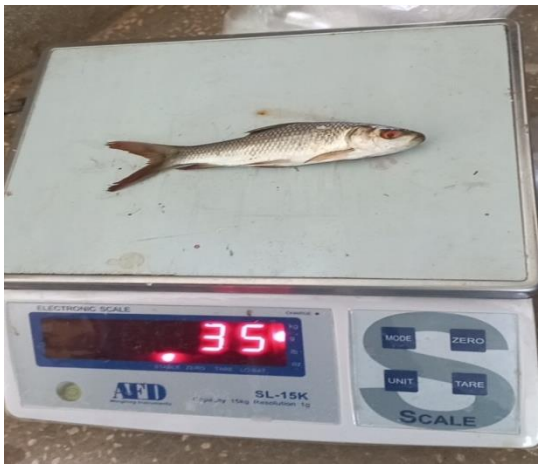


Figure no 07: Also Measure the length of the fish during trial 30th days

Figure no 08: Sampling of Fish after completing the trial period of 15th

Figure no 09: Death rate during the first trial period

Figure no 10: Death rate during the 2nd trial

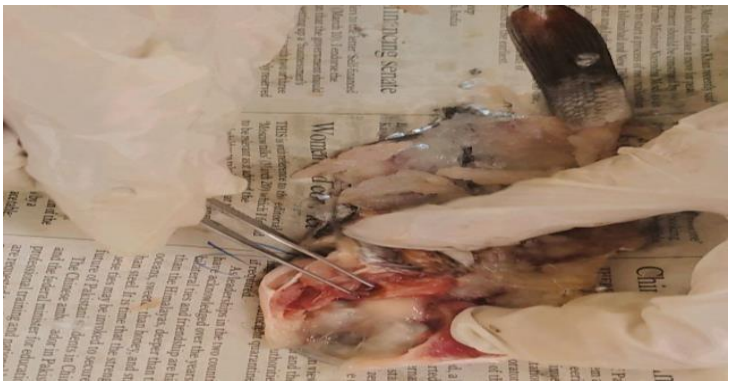


Figure no 11: Sample collection by dissecting the fish

STATISTICAL ANALYSS OF HAEMATOLOGICALPARAMETERS

Red blood cell count (RBC)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Control RBC	.164	12	.200*	.904	12	.178
Experimental RBC	.129	12	.200*	.958	12	.759

*. This is a lower bound of the true significance.

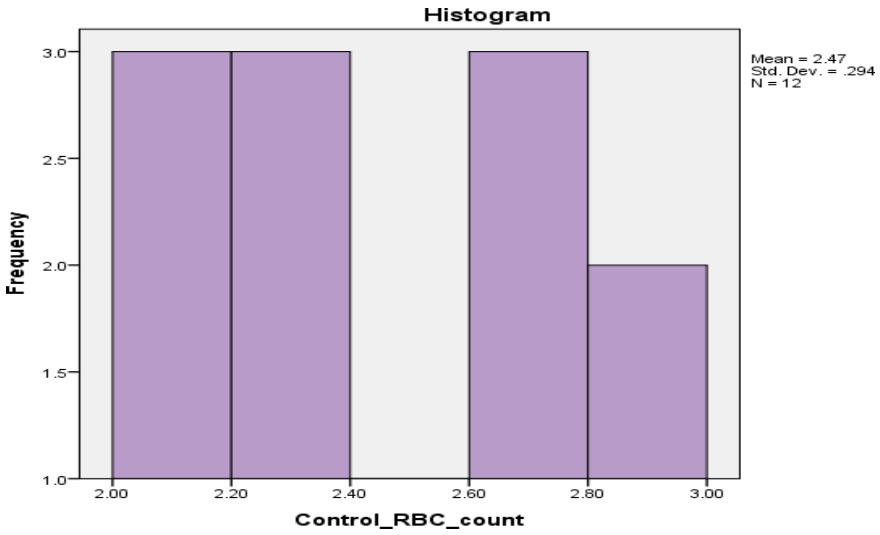


Figure no 12: Control group of Red Blood Cell count

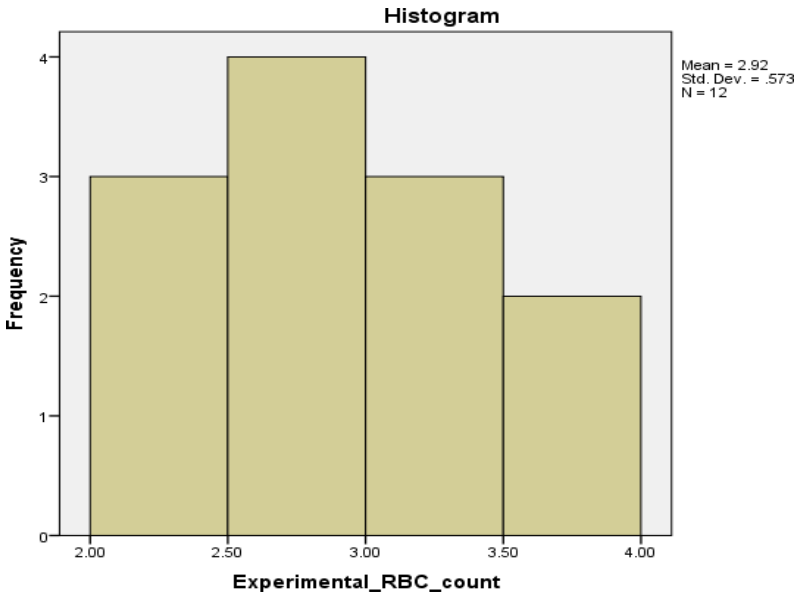


Figure no 13: Experimental group of Red Blood Cell count

T-Test

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
RBC_count	experimental	12	2.9217	.57333	.16551
	control	12	2.4658	.29358	.08475

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
RBC_count	Equal variances assumed	3.265	.084	2.451	22	.023	.45583	.18594	.07021	.84145
	Equal variances not assumed			2.451	16.398	.026	.45583	.18594	.06243	.84924

Hemoglobin level (Hb)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experimental_Hb	.192	12	.200*	.878	12	.084
Control_Hb	.164	12	.200*	.916	12	.252

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

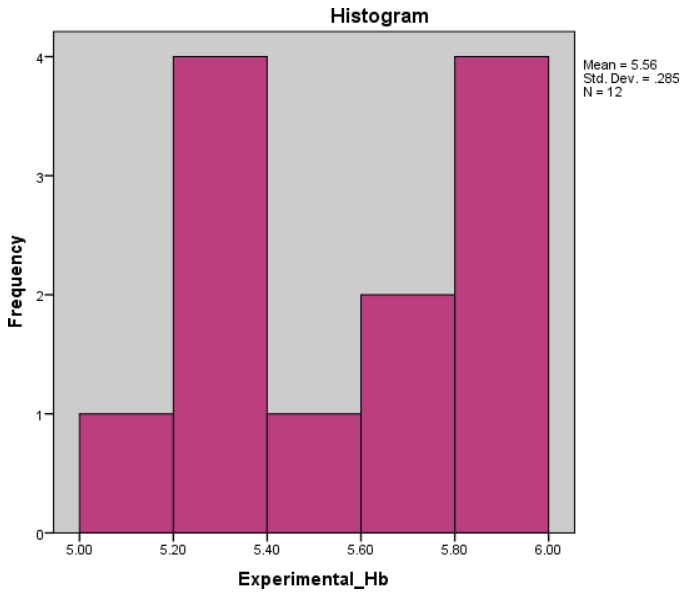


Figure no 14: Histogram graph of Hemoglobin level (Hb) in Experimental group of Rohu

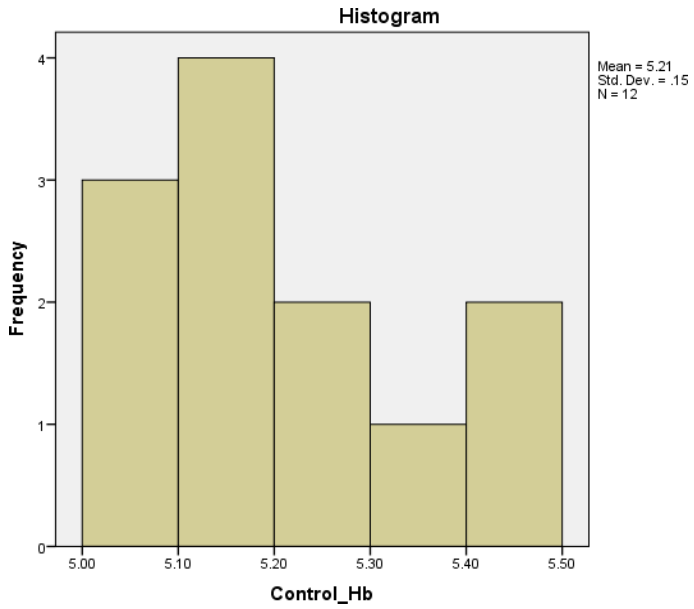


Figure no 15: Histogram graph of Hemoglobin level (Hb) in Control group of Rohu

T-Test

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
Hb	experimental	12	5.5642	.28503	.08228
	control	12	5.2075	.14980	.04324

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Hb	Equal variances assumed	15.746	.001	3.837	22	.001	.35667	.09295	.16389	.54944
	Equal variances not assumed			3.837	16.645	.001	.35667	.09295	.16023	.55310

Hematocrit level (HCT)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experiment_ Hct	.236	12	.064	.869	12	.063
Control_ Hct	.292	12	.006	.818	12	.015

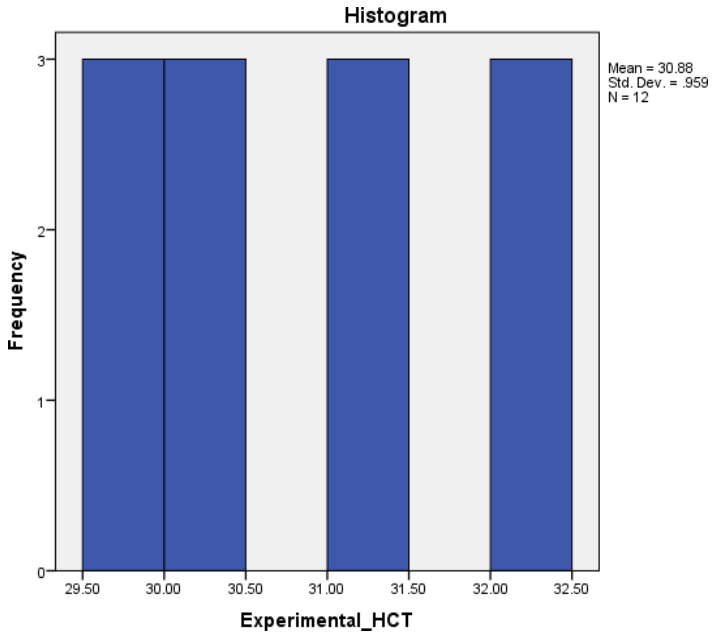


Figure no 16: Histogram graph of Hematocrit level (HCT) in Experimental group of Rohu

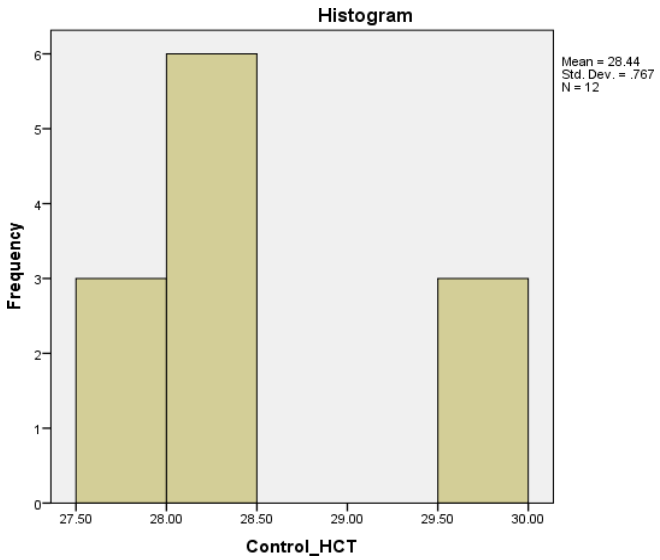


Figure no 17: Histogram graph of Hematocrit level (HCT) in Control group of Rohu

NPar Test

Mann-Whitney Test

Ranks

	group	N	Mean Rank	Sum of Ranks
HCT	control	12	6.50	78.00
	experimental	12	18.50	222.00
	Total	24		

Test Statistics

	HCT
Mann-Whitney U	.000
Wilcoxon W	78.000
Z	-4.157
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]

a. Grouping Variable: group

b. Not corrected for ties.

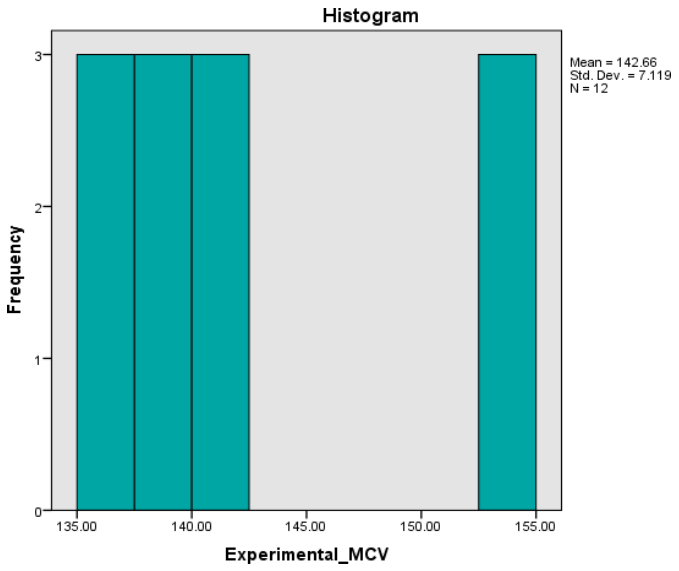
Mean Corpuscular Volume (MCV)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experimental_ MCV	.350	12	.000	.704	12	.001
Control_ MCV	.174	12	.200*	.866	12	.058

This is a lower bound of the true significance.

a. Lilliefors Significance Correction



Figurer no 18: Histogram graph of Mean Corpuscular Volume (MCV) inExperimental group of Rohu

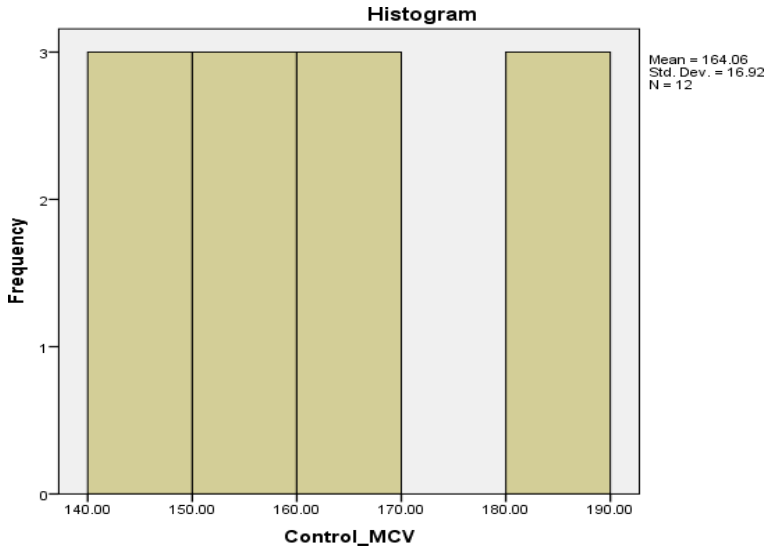


Figure no 19: Histogram graph of Mean Corpuscular Volume (MCV) in controlgroup of Rohu

NPar Tests

Mann-Whitney Test

Ranks

	group	N	Mean Rank	Sum of Ranks
MCV	control	12	17.75	213.00
	experimental	12	7.25	87.00
	Total	24		

Test Statistics

	MCV
Mann-Whitney U	9.000
Wilcoxon W	87.000
Z	-3.637
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]

a. Grouping Variable: group

b. Not corrected for ties.

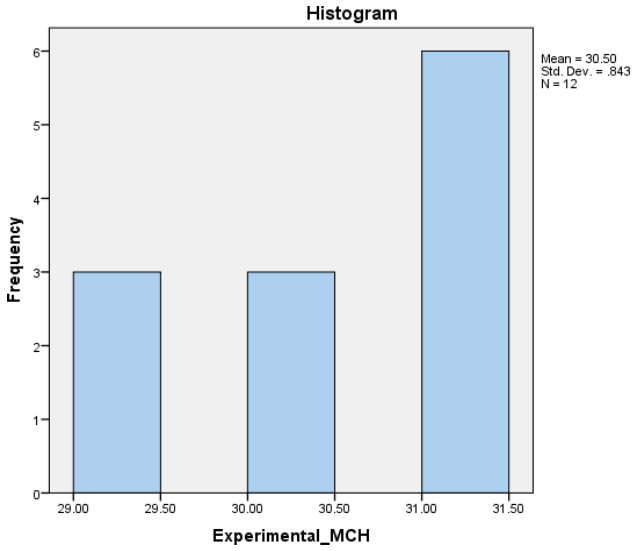
Mean Corpuscular hemoglobin (MCH)

Tests of Normality

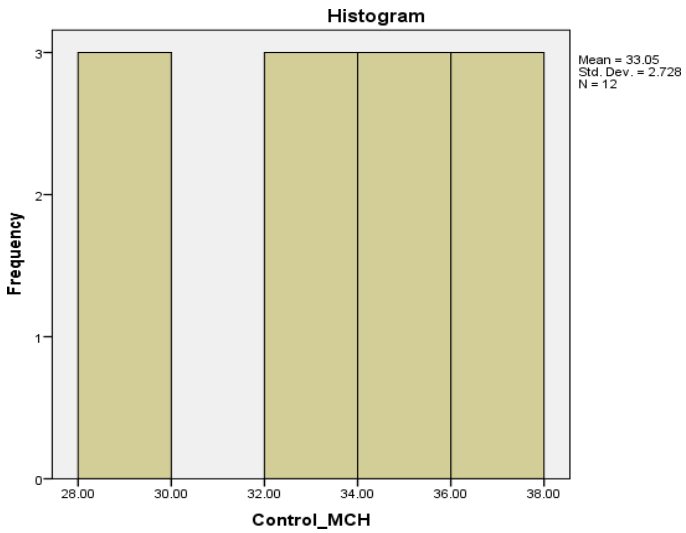
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experimental_ MCH	.263	12	.022	.787	12	.007
Control_ MCH	.167	12	.200*	.880	12	.087

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction



Figurer no 20: Histogram graph of Mean Corpuscular hemoglobin (MCH) in Experimental group of Rohu



Figurer no 21: Histogram graph of Mean Corpuscular hemoglobin (MCH) in Control group of Rohu

NPar Tests

Mann-Whitney Test

Ranks

	group	N	Mean Rank	Sum of Ranks
MCH	control	12	15.88	190.50
	Experimental	12	9.13	109.50
	Total	24		

Test Statistics

	MCH
Mann-Whitney U	31.500
Wilcoxon W	109.500
Z	-2.339
Asymp. Sig. (2-tailed)	.019
Exact Sig. [2*(1-tailed Sig.)]	.017[b]

a. Grouping Variable: group

b. Not corrected for ties.

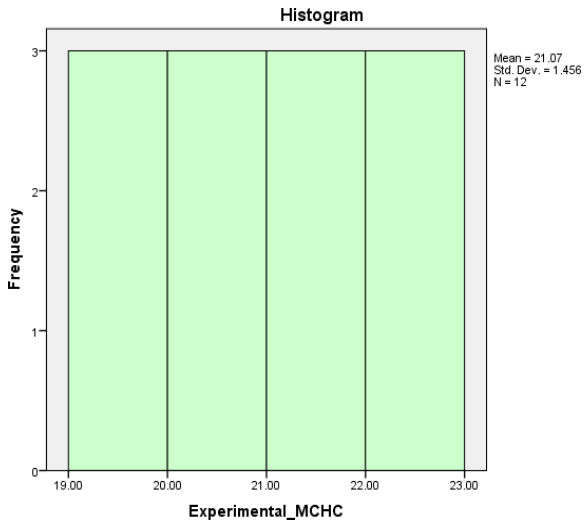
Mean Corpuscular hemoglobin concentration (MCHC)

Tests of Normality

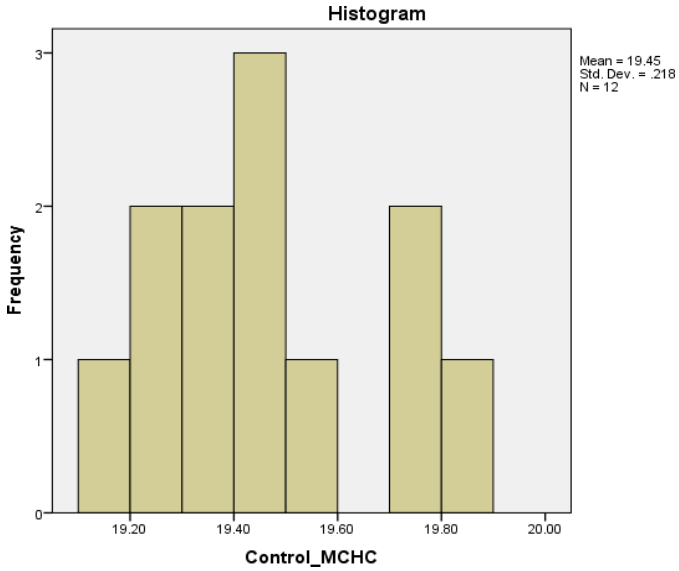
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experimental_ MCHC	.191	12	.200*	.876	12	.079
Control_ MCHC	.141	12	.200*	.930	12	.385

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction



Figurer no 22: Histogram graph of Mean Corpuscular hemoglobin concentration (MCHC) in Experimental group of Rohu



Figurer no 23: Histogram graph of Mean Corpuscular hemoglobin concentration (MCHC) in Control group of Rohu

T-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
MCHC	Experimental	12	21.0742	1.45614	.42035
	control	12	19.4458	.21790	.06290

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
MCHC	Equal variances assumed	46.493	.000	3.831	22	.001	1.62833	.42503	.74687	2.50979
	Equal variances not assumed			3.831	11.492	.003	1.62833	.42503	.69771	2.55895

STATISTICAL ANALYSIS OF IMMUNOLOGICAL PARAMETERS

Leucocyte count

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experimental_ Leucocytes	.182	3	.	.999	3	.934
Control_ Leucocytes	.239	3	.	.975	3	.695

a. Lilliefors Significance Correction

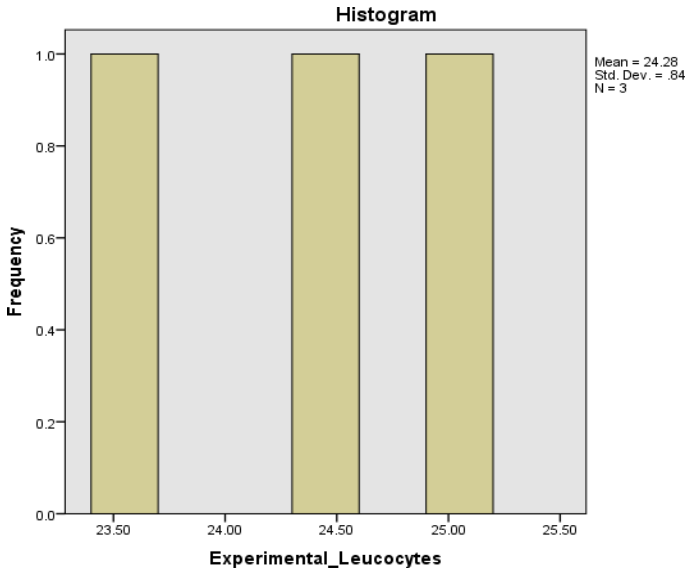


Figure no 24: Histogram graph of Leucocyte count in experimental group of Rohu

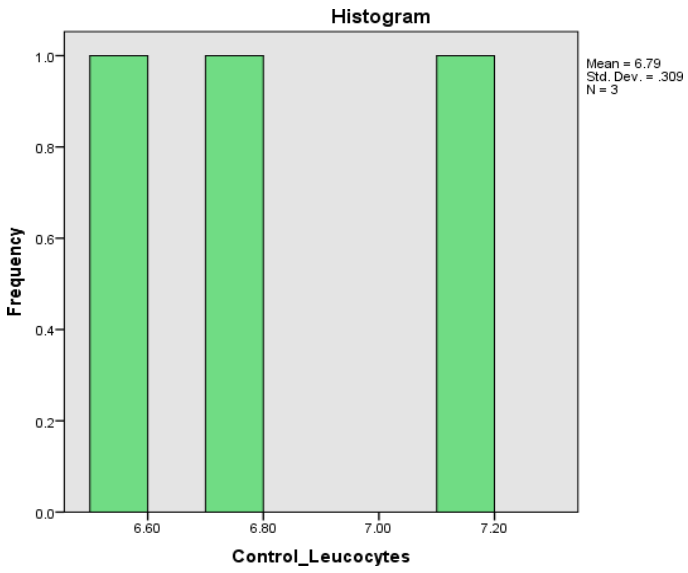


Figure no 25: Histogram graph of Leucocyte count in Control group of RohuT-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Leucocytes	Experimental	3	24.2767	.84050	.48526
	control	3	6.7867	.30892	.17836

Independent Samples Test

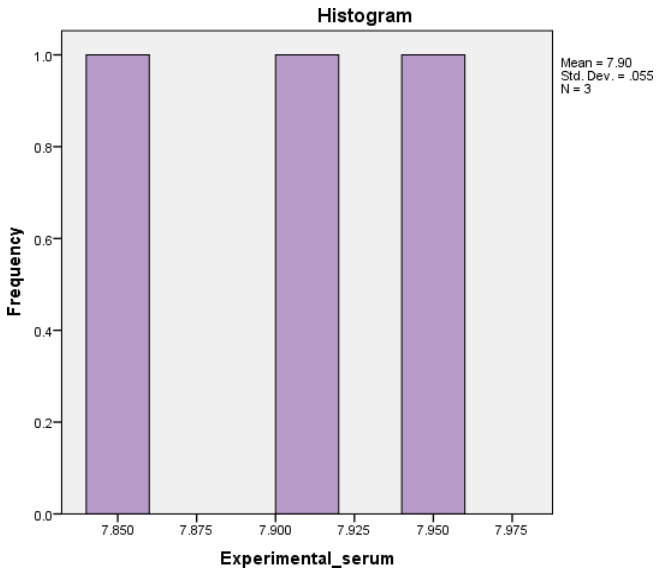
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Leucocytes	Equal variances assumed	1.531	.284	33.830	4	.000	17.49000	.51700	16.05458	18.92542
	Equal variances not assumed			33.830	2.531	.000	17.49000	.51700	15.65784	19.32216

Serum lysozyme activity

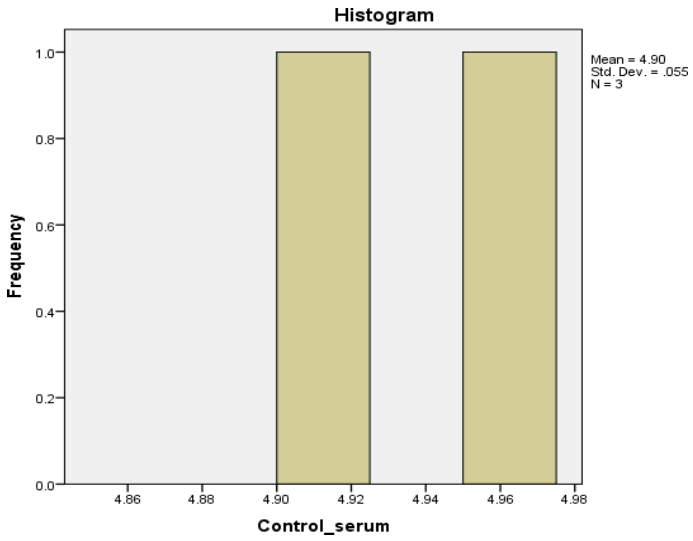
Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Experimental_ serum	.191	3	.	.997	3	.900
Control_ serum	.191	3	.	.997	3	.900

a. Lilliefors Significance Correction



Figurer no26: Histogram graph Serum lysozyme activity in Experimental group of Rohu



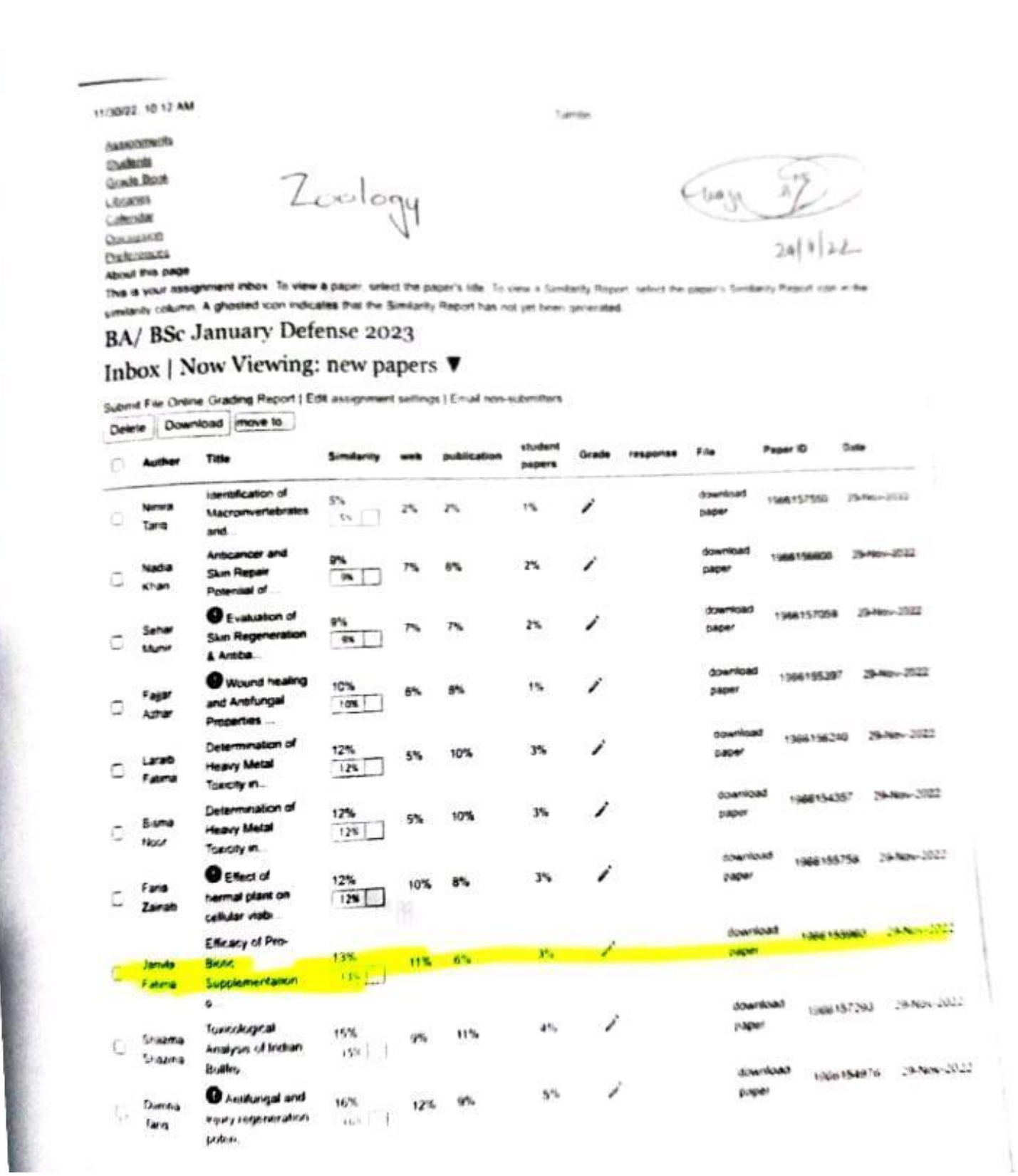
Figurer no 27: Histogram graph Serum lysozyme activity in Control group of Rohu

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Serum	1	3	7.8967	.05508	.03180
	0	3	4.9033	.05508	.03180

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Serum	Equal variances assumed	.000	1.000	66.564	4	.000	2.99333	.04497	2.86848	3.11819
	Equal variances not assumed			66.564	4.000	.000	2.99333	.04497	2.86848	3.11819



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