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IMMUNO-STIMULATING ACTIONS OF GARLIC (*ALLIUM SATIVUM*) ON HAEMATOLO-IMMUNOLOGICAL PARAMETERS,INTESTINAL MICRO-FLORA AND GROWTH PERFORMANCE IN *LABEO ROHITA* (ROHU)

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Abstract
Vaccination and use of other chemotherapeutic agents in fishes is not valued to perform because of its residualeffects and method of delivery. It has turned out as essential to use such cheap chemically active compounds that are produced naturally, have not any side effects or residual effect on fish immunity and are easy to deliver. With the rising and commercialization of aqua-culture production, contagious diseases and occasional increase in death are major causes of heavy loss in aqua-culture enterprises. The effects of any disease in fish depend upon some specific types of factors. These Factors are categorized as; diseases and sources of these disease-causing agents, environmental stress, the level or degree of environmental stress upon the fish being effect, physical appearance and physiological condition and genetic resistance of host fish against all the manipulations cause by disease. The aim of conducted study was to determine changes in immunological parameters gut micro-flora of *Labeo rohita* (Rohu) against different supplementary doses of dietary garlic. Fishes were divided into different groups (control group, feed on garlic free feed and treatment groups-1, 2 and 3 feed on diet with inclusion of 5g, 10g and 15g garlic). Duration of the research trials was 90 days. Results of the present examination signified that the utilization of dietarygarlic supplementation at different levels in *Labeo rohita* (Rohu) feed significantly ($P \leq O.O5$) enhanced all parameters regarding growth, development and improved the ability to utilize feed more effectively. FCR, SGR, FW and FI parameters were also observed significantly increased in all groups that received garlic supplemented feed as compare to control group. Results of hematological changes demonstrated significant increase ($P \leq 0.05$) in RBCs, WBCs, Hb, PCV levels. Serums bactericidal activities and lysozyme activities were observed remarkably higher in all fish fed on garlic-additive feedings as

compared to the control group. Highest values of bactericidal activities and least viable colony count were prominently evident in each treatment group which received garlic supplementations in feed as compared to the control group. The comprehensive study regarding the effects of garlic on different parameters of growth, haematology, immunity and gut microbial load suggested the use of garlic supplements and residues in fish diet to support higher and consistence aqua-culture production with minimum losses due to unexpected break out of diseases and environmental stresses.

Key words: Vaccination, Chemotherapeutic Agents, Immunity Haematology, Lysozyme, Microbial Load

1.INTRODUCTION

Aqua-culture is the cultivation of aquatic organism as fish, plants, molluscs and shellfish with positive intervention during raising procedure toward the enhancement of outcomes that include, consistent stocking, sustainability, artificial feeding in addition with assurance of safety from predators, parasitic and pathogenic organisms. The farming may have individual or corporate ownership for the stock being cultivated (FAO). Fish is a quite nutritive and prosperous resource of animal proteins. To accomplish and provide a constant distribution of protein rich food source, the aqua-culture zone is increasing unpredictably at a mean compounded rate of 9.2% yearly in a constant line as compared with 1.4 % for capture fisheries practices and a couple of 0.8% for livestock and terrestrial based farmed meat production structures since 1970 (Cristea *et al*. 2012). For the improvement and extension of aqua-culture and to obtain most encouraging yields from accessible assets of fresh water, it has turned into basic steps to propose and utilize artificial or synthetic nutritive feed through which fish grow quickly to acquire higher or maximum weight within precise short time framework (Bhosale *et al*. 2010). With the spontaneous development and improvement in man's living style, marine life is facing threat for declination due to the activities of overfishing, contamination in water bodies, environment destruction and global warming. As a result, to overcome these effects aqua-culture industry is expanding rapidly worldwide with expectations for improved and enhanced annual food production. This development in aqua-culture has also prompted the broad utilization of intensive culture practices (Li and Galtin, 2004). Generally, health linked problems, particularly diseases, are the key issues in both natural and artificially cultivated fish farming. These issues are also directly connected to environmental and stress factors. Stressors can be intense or chronic depending on its effects on fish (Boyd and Schmittou, 1999). Usually, these Stressors classified as natural factors, chemical factors and physical factors. Higher densities of the stocking fish can probably create issues related to pathogens, andthis activity can also help to enhance genetic determination in mutant pathogens that is more harmful (Owenset *et al*, 2003). Each kind of fish differs in their resistance against infections on account of their genetic makeup and past experience with pathogens. This method is natural that helps in the advancement of defensive mechanism of organisms. Natural stressors, particularly infections and microorganisms, have been perceived such as etiological agents responsible for diseases in comprehensive fish species (Brunt and Austin, 2005). The effects of any disease infish depend upon some specific types of factors. These Factors are categorized as; diseases and sources of these disease causing agents, environmental stress, the level or degree of environmental stress upon the fish being effect, physical appearance and physiological condition and genetic resistance of host fish against all the manipulations cause by disease (Cipriano *et al*. 2001). The genetic resistance in each kind of fish species is different, depends upon their physiology. Inclusive species of bacterial pathogens at present have discovered in association with number of serious disease in fish (Fyzul and Austin, 2015).

In latest years, with the rising and commercialization of aqua-culture production, contagious diseases and occasional increase in death are major causes of heavy loss in aqua-culture enterprises (Naylor and Burke, 2005).In this going era, there is potential in development and expansion of aqua-culture enterprises and along thisdevelopment the alternative existence of many ordinary and contagious diseases has led to large financial and economic losses. While dealing in Fisheries and

Aqua-culture practices the main ailment causative agents are typically micro-organisms such as virus, bacteria, protozoan and some other parasites. To manipulate diseases, useof the antibiotics at extensive level and numerous other artificial chemical substances have led to drug residue and resistant in pathogens (Brown, 1989). To avoid these situations one method needed to adopt is the addition of naturally produced new consistent ingredients as supplements into fish diets to enhance better feed utilizations and to acknowledge conventional situations for fish to flourish and maintenance of the enterprises (Fernández-Navarro *etal*. 2006). A more efficient health management may cause lessen malfunction and instability within the production chain of aqua-culture and can improve the economics of aqua-culture operations. In aqua-culture, use of antibiotics, chemotherapeutics remedy and vaccines have been extensively criticized for their unanticipated negative influences. Fish like all higher vertebrates have similar type of immune system that is consist of two basic and vital components.One is the innate system of defense and the other second is acquired system also known as specific defense system of body. Innate defense system is known as nonspecific defense systems of body have vital importance (Maurilio, 2011) and serve as primary defense line against pathogens and other foreign proteins (Narnaware *et al*. 1994). This system is consisting of series of cellular and humoral components (macrophages, monocytes, granulocytes, agranulocytes and lysozymes) that circulate in body via blood. Mainstream of these components is the derivatives of White Blood Cells (Magnadóttir, 2006). In aqua-culture, addition of immune stimulants is one among the most valued methods for the strengthened of non-specific defense system in fishes (Raa *et al.* 1992). Innate immunity is the main barrier against pathogen, and play vital role against the development of disease and the foundation of other contaminations. On the innate immune system and it parameters in fish, massive research has been accomplished (Magnadóttir, 2006). There are many physical and biological parameters of fish that share common character as that of other vertebrates. The others parameters of innate immune system of fish such as; cellular parameters are also common in fish and vertebrates. This parameter is highly associated with specific cellular receptors. Plants occupying therapeutic values have been utilized into customary frameworks for the treatment of numerous infections and diseases (Bhadauria *et al.* 2002). Metabolites supplementation delivered by means of plants are naturally synthetic compounds of higher operational values which have diverse chemotherapeutic capacities. They also include bactericidal and antimicrobial capacities (Purohit and Mathur, 1999). Fish *Labeo rohita*, locally known as (Rohu) stands among one of the most crucial meal fish belongs to family cyprinidae. It is regarded for their precise nutrition values and supreme growth responses that typically rely upon environmental factors as well as supplementary feed modifications. Rohu (*Labeo rohita*) includes into the group of fishes usually referred to as Indian Major Carp's. Garlic is scientifically known as (*Allium sativum*). It belongs to a wide plant family *Liliaceae* within genus *Allium*. Garlic has a wide spectrum of distant antiviral, bactericidal, antifungal, and anti-protozoal actions. Garlic is known to exist among the earliest experienced and medically important herbal plant but observations and identification regarding its approach of activities are quite recent in discovery. Thus, in aqua- culture operations, optimized doses of garlic are now strongly authorized. The applications of garlic preparations in aqua-culture are very helpful in curing numerous fish associated diseases. The aims of the study conducted is to asses and determines impacts of garlic (*Allium sativum*) additive, supplemented feed on the innate immunity parameters (hemato-immunological parameters), and fluctuations in intestinal micro-flora and growth responses of *Labeo rohita* (Rohu).

2. MATERIALS AND METHODS

2.1. Experimental fish species and Fish culture facility

Rohu (*Labeo rohita*) were used as experimental fish species. The experiment was arranged to conduct at the fish hatchery, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus. Fingerlings of Rohu (*Labeo rohita*) with average similar size and body weight (20 g) were obtained from Punjab Fisheries department hatchery, Manga mandi, District Qasur. The fish were carried and transported to experimental site (department of fisheries and

aquaculture, hatchery) with the help of synthetic polythene bags. Bagswere filled with sufficient amount of oxygen for transportation of fish. At hatchery, fish were transferred to circular tanks and for overnight left undisturbed. Before the beginning of the investigations, fingerlings were adjusted to the exploratory framework after 10 days acclimation.

2.2. Experimental Conditions and Experimental design

The experimental trials were conducted into cemented, rectangular tanks; each tank was divided into three compartments. Each rectangular tank was supplied with continuous aeration, constant and continuous supply of water. After acclimation of 10 days, groups of ten fish of similar size and weight were selected and randomly placedinto each part of rectangular tanks with three replicates for each treatment, labeled as, Treatment-1, Treatment-2 Treatment-3 and Control group respectively. Experimental design followed was completely randomized design (RCBD).

2.3. Water quality parameters and Feed Formulation:

Standard water quality parameters were adjusted into rectangular tanks. Parameters like ammonia concentration, total alkalinity, nitrate, and nitrite were determined according to Boyd, (1979). Ammonia and Nitrite were measured on weekly basis, while water temperature Oxygen and pH were recorded daily in each rectangular tanks using multi-meter (HANNA HI98194). Garlic (*A. sativum*) was obtained from local market. Garlic bulbs were dried in oven and grounded into small fine grains using electric grinder. These fine grains were blended precisely with other ingredients of fish feed at different concentrations to accomplish four distinctive diets as 5 g, 10 g and 15g of garlic in every kg of feed (Allah and Muhammad, 2012). Water was added and homogeneity of the all ingredients was assured by mechanical and gradual mixing of all ingredients for 10 minutes. The control group was given garlic free feed.

Proximate composition of the formulated diet will be 30% crude protein.

Table 2.1. Feed ingredients

Ingredients (gm**.)**	**T1 (**5g garlic**)**	**T2 (**10g garlic**)**	**T3 (**15g garlic**)**	**Control (**garlic free**)**
Soybean Meal	15	15	15	15
Canola Meal	14	14	14	14
Fish Meal	14	14	14	14
Maize Gluten	22	22	22	22
Rice Polish	30	25	20	35
Garlic	5.0	10	15	00
Total	**100**	**100**	**100**	**100**

2.4. Feeding strategies

Feed was given to fish twice in a day at 8:OO to 9:00 A.M and 4:OO to 5:00 P.M respectively, according to3% body weight of fish. The feed quantity was re-manipulated according to net weight gain.

2.5. Growth Trials

To determine growth parameters like, increase in weight, total body length, feed conversions ratio, feed consumption and survival percentage of each treatment (control group, feed on garlic free feed and treatment groups-1, 2 and 3 feed on diet with inclusion of 5g, 10g and 15g garlic) was monitor on fortnight basis for 90 days. Total increase in body length, weight, feed conversions ratio and growth rate was measured according to Recker (1975) and Castell and Tiews (1980).

- **Total gain in weight** (g) = Final weight (g) of body - initial weight (g)
- **FCR** = weight of dry feed / fish weight gain.

2.6. Hemato- immunological parameters

On the end of trial, four individuals from replicates of each treatment group were selected for further

study of hemato-immunological parameters and intestinal micro-flora. For the analysis of hemato-immunological parameters(White Blood Cell or leukocytes count, Lymphocyte, basophils, Monocyte, Eosinophil, thrombocytes, serum proteincontent (globulin and albumin), Phagocytic cells and Serum lysozyme activity) samples of blood were obtained and collected with sterile syringes from caudal vein puncture. The samples obtained were stored into sterile vacuette tubes coated with heparin or EDTA that functions as anticoagulant to avoid clotting of blood. Hemato- immunological parameters were determined through complete blood count (CBC) analyzer.

2.7. Determination of Lysozymes activity

For lysozymes activities assay, turbid metric technique was followed as proposed and described by (Parry et al. 1965). 2 ml of a suspension of microorganisms (Pseudomonas aeruginosa), obtained in the course examination of gutquantitative analysis was added into blood Serum (100µl) and mixed with O.O5 M sodium phosphate buffer solutionof (pH 6.2). The examination of lysozymes activities was carried out at 25ºC and absorbance of light was estimated on spectrophotometer (IEMECO U2020) at 530 nm wavelength after various times intervals. Unit of lysozymes activity was characterized as decreased in absorbance of O.OO1/min (Sahu et al. 2007).

2.8. Serum's Bactericidal activities

For determination of serums bactericidal activities the procedure described by (Kajita et al. 1990) was followed. 100µlof bacterial suspension and 100µl blood serum (for each individual treatment) were mixed thoroughly. The mixture was allowed to incubate at 25ºC for one hour. After incubation 100µl of mixture (serum bacteria) was diluted at the ratio of 1:10 into sodium phosphate buffer (0.05 M) maintained at pH 6.2 and again incubation was followed on nutrient agar petri dishes at 25ºC for one day. After 24 hours incubation, numbers of colonies from eachtreatment and control group were observed with the help of colony counter. The same procedure was followed to determine serum bactericidal activity in control group.

2.9. Granulocytes/ Agranulocytes Analysis as immunity indicator

For the morphological identification of leukocytes, lymphocytes, thrombocytes, basophils, Monocyte, and Eosinophil, anticoagulant agent (heparin or EDTA) and blood sample was slightly shacked and allowed for mixing into sterile vacuette tubes. On a glass slide, 1 drop of blood was placed and mixed with a drop of Leishman‟s stain for the staining of blood. The mixture was covered by cover slip and was observed under microscope by 60X magnification. Quantitative analysis of parameters such as lymphocytes, thrombocytes, basophils, Monocyte, Eosinophil, serum protein content (globulin and albumin), was determined through complete blood count (CBC) test.

2.10. Determination of intestinal micro-flora

2.10.1. Quantitative analysis of intestinal bacterial flora

For determination of intestinal micro-flora, three samples were collected from each experimental group for Quantitative analysis of the bacterial flora in the intestine was determined by the examination of intestine. Body cavity of the fish was opened and intestine was removed aseptically to prevent any contamination. Intestine was thanweighed and grounded finely through sterile scissors. To precede further chopping, intestine was smashed in a sterilemortar-pestle.

2.11. For quantitative study

1.0g finely ground intestine of fish was diluted in 9.0 ml 0.2% peptone water and followed by series of dilutions. Standard plate count (SPC) technique was used for quantitative analysis of fish intestinal micro-flora. In Plate CountTechnique, agar medium was used as culture medium. The quantitative difference between colonies growth on agar medium was determine with the help of digital colony counter.

2.12. Data analysis

Effects of garlic supplemented feed on different parameters such as, growth performances, hemato-immunologicalparameters and gut micro-flora was analyze using One Way Analysis of Variance (ANOVA). The significant difference among different treatments was compared by Duncan‟s multiple range test (DMRT) using SAS version
9.1. at 5% Significance level.

3- Results

3.1. Growth performance

Growth perimeters such as increase in average wet weight and average increase in total length were recorded on fortnight bases. The data presented in (table 4.1) indicated the average values for all the growth parameters of treatment groups and the control group, recorded forthrightly. The data clearly showed significance difference for all parameters among all treatments. The increase in body weight was significantly higher in all treatments groups. The maximum weight gain among all treatments was observed with the mean value of (51.48±0.21) in treatment-3, while minimum weight gain was recorded in control group (43.17±1.25) after the experimental duration of 3 months.The same trend was observed regarding total increase in body length. The increase in body length was recorded significantly higher in treatments groups on the end of the research trail. Results also specified the fish which were fed on garlic supplemented feed represented significant increase in weight of fish. In treatments, the fish that were fed on 15g garlic per kg feed (treatment-3) presented highest length gain (31.07±2.71). However, minimum increase in length was observed in control group (23.92± 1.60). Similarly, (Fig 4.1.) also showed the overall growthperformance of the fishes in all treatment groups and control group. The bars clearly presented the maximum weight gain and length gain in treatment-3 as compared to other treatment groups as well as the control group.

Table 3.1. Results of statistical analysis for weight gain and length increase in *Labeo rohita* in treatment groups and control group after experimental trials.

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	Initial length (cm)	Final length (cm)	Length gain (cm)
Control	14.90±2.55$_b$	43.17±1.25$_b$	28.27±0.03$_b$	10.95±0.24$_b$	23.92± 1.60$_b$	12.9±0.03$_b$
Treatment 1	14.60±2.54$_b$	44.34±0.83$_b$	29.74±0.11$_b$	11.25±0.15$_a$	24.45±2.81$_b$	13.2±0.21$_b$
Treatment 2	15.17±2.61$_a$	45.00±1.24$_b$	29.83±0.6$_b$	11.30±0.02$_a$	27.60±1.43$_b$	16.3±0.11$_b$
Treatment 3	14.70±2.52$_b$	51.48±0.21$_a$	36.78±0.09$_a$	11.02±0.28$_a$	36.07±2.71$_a$	20.05±1.01$_a$

Note: Data with different superscript letters significantly differs from each other at $p<0.05$.

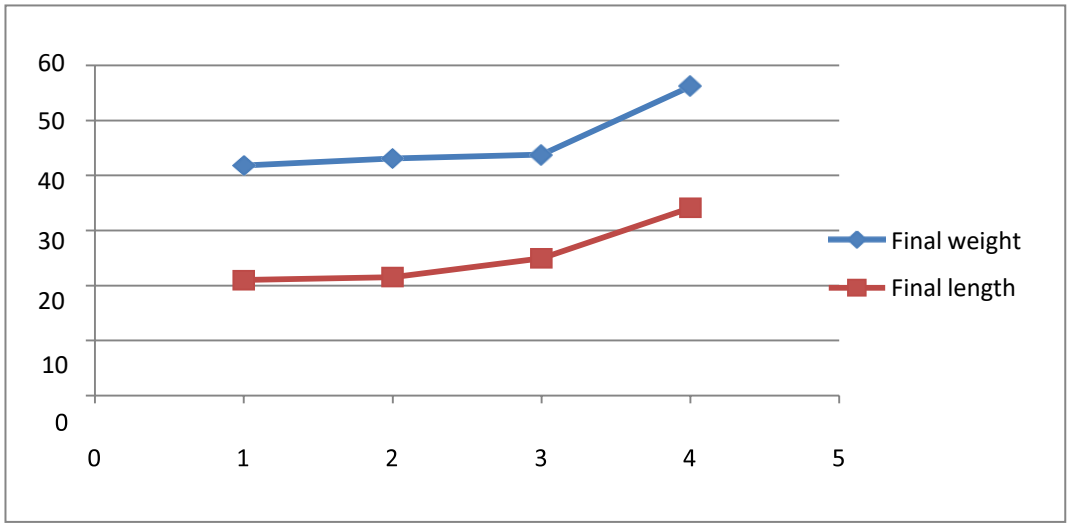

Figure 3.3. Graph showed significant increase in bodyweight and length as the garlic concentration increased. Each line represents (Mean ± SE) of the treatment groups and the control group.

3.2. Feed utilization:

The obtained values of FCR, FCE and F.I are showed in (table 4.2). The data indicated significant difference for all parameters among all treatments when comparison was made with the control group. The maximum values of FCR, FCE and F.I among all treatments were observed with the mean value of (3.20 ±1.11) in treatment-3 and minimum value recorded in control group (1.85 ±0.09). On the other hand, the maximum values of FCE and F.I among all treatments was observed in treatment-3 with the mean value (0.31 ±0.11 and 2.94 ±0.49). While, the minimum values recorded in control group (0.53 ±0.01and 1.64 ±0.32) respectively. The difference among these parameters in treatment groups and control group presented dependency on different doses of garlic supplementations.

Table 3.2. Results of statistical analysis of feed utilization abilities of *Labeo rohita* observed in treatment groups and the control group during course of study.

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
FCR	3.20 ±1.11 a	2. 56 ±1.03 $_b$	2.42 ±O.11 $_b$	1.85 ±0.09 c
FCE	0.53 ±0.O1 $_a$	O.41 ±O.07 $_b$	O.38 ±0.O1 $_b$	O.31 ± O.11 $_b$
F.I	1.64 ±0.32$_b$	2.41 ±0.47 $_a$	2.69 ±0.78 $_a$	2.94 ±0.49 $_a$

Note: Data with different superscript letters significantly differs from each otherat $p<0.05$.

3.3. Physio-chemical parameters

The physio-chemical parameters were monitored and recorded throughout the entire study period. The DO, pH, Salinity and temperature were recorded regularly on daily basis. Results of physio-chemical parameters are shown in(table 4.3). Both maximum and minimum values were recorded during the study period. The maximum value of DOrecorded was (5.31ppm) while, the least value of DO observed was (5.38ppm). Similarly, maximum and least valuesof pH (max 8.01ppt, min 8.07ppt), salinity (similar values (1.01) was observed as minimum and maximum) and temperature (max 29.3°C, min 30.7°C) was recorded respectively.

Table 3.3. Mean values for water quality parameters during the research trials.

Serial No.	Parameter	Mean±SD	Minimum	Maximum
1.	**pH**	8.03±0.01	8.01	8.07
2.	**DO (ppm)**	5.32±O.01	5.31	5.38
3.	**Salinity (ppt)**	1.01±8.93	1.01	1.01
4.	**Temperature (°C)**	28.71±1.43	29.3	30.7

3.4. Haematological parameter index

The results and outcomes of haematological analysis are displayed in (table 4.4). Results revealed significant increase in red blood cells (RCB‟s) of fish in all treatment groups received garlic supplemented feed. The number of white blood cells was also found expressively increased in all treatment groups in contrast to the group kept as control. Highest count of RCB‟s was observed in treatment-3 which was given 15g per kg garlic i supplementationin feed ($0.81\pm0.010\times10^6/\mu L$) as compare to control group ($0.67\pm0.020\times10^6/\mu L$). Similar significantly increases ($p<0.05$) in (WBC) count ($4.38\pm0.083\times10^3\mu L$) was also observed in treatment-3, which was given 15g per kg garlic supplementation in feed over control group ($4.15\pm0.020\times10^3\mu L$). The results also demonstrated that the percentage ofhaemoglobin (Hb) also remarkably observed higher ($p<0.05$) in all groups of treatment. However, the treatment-2and treatment-3 groups, that were given 10g and 15g per kg garlic supplemented feeding diet showed to have particular higher raise in haemoglobin (Hb) percentage (3.79±0.087 and 3.89±0.002) as compare to control group (3.56±0.083). Same increasing tread was found for HCT%, MCV%, MCH%, MCHC%, whereas this prominent increase observed with increasing the supplementation of garlic in fish diet in three month experimental period.

Table 3.4. Evaluation of blood parameters of experimental fish (*Labeo rohita*) after 3 monthsstudy duration.

Parameters	Control	Treatment 1	Treatment 2	Treatment 3	Significancelevel
WBC×10³µL	4.15±0.020 $_a$	4.20±0.017 $_a$	4.27±0.057 $_a$	4.38±0.083 $_a$	**P<0.05**
RCB×10⁶/µL	0.67±0.020 $_a$	0.72±0.011 $_a$	0.77±0.015 $_a$	0.81±0.010 $_a$	**P<0.05**
Hb%	3.56±0.083 $_a$	3.68±0.040 $_a$	3.79±0.087 $_a$	3.89±0.002 $_a$	**P<0.05**
HCT%	8.97±0.036 $_a$	9.060±0.069 $_a$	9.21±0.075 $_a$	9.29±0.010 $_a$	**P<0.05**
MCV%	120.54±0.770 $_a$	121.69±0.305**a**	122.39±0.288 $_a$	122.68±0.050 $_a$	**P<0.05**
MCH%	31.15±0.762 $_a$	32.15±0.070 $_a$	32.89±0.642 $_a$	33.90±0.200 $_a$	**P<0.05**
MCHC%	20.46±0.585 $_a$	21.34±0.190 $_a$	22.07±0.825 $_a$	23.09±0.008 $_a$	**P<0.**O**5**

WBC= White blood cells, **RCB=** Red blood cells, **HB%=** Haemoglobin, **HCT%=** hematocrit, **MCV%=** mean corpuscular volume, **MCH%=** mean corpuscular hemoglobin, **MCHC%=** Mean corpuscular Haemoglobin concentration.

Note: Data with different superscript letters significantly differs from each other at p<0.05.

3.5. Haemato-immunological parameters

Garlic supplementation at different levels of concentration greatly affected the blood confine immunological parameters. Garlic supplementation highly manipulated the numbers of monocytes, neutrophils, eosinophil, basophils lymphocytes and thrombocytes. Significantly, there was observed higher increase (p<O.O5) in theseimmunity parameters in every treatment groups in comparison to the control group individuals. Higher numbers of monocytes, neutrophils, eosinophil, basophils lymphocytes and thrombocytes were disclosed in treatment groupsthat were fed with 15g and 10g per kg garlic supplemented feed. The increase in these parameters was observed following garlic supplementations in fish feed (Table 4.5).

Table 3.5. Statistical analysis of Haemato-immunological parameters after the course of experimental study.

Parameters	Control	Treatment 1	Treatment 2	Treatment 3
Lymphocytes %	23.07±0.652 $_a$	24.10±0.370 $_a$	24.40±0.271 $_a$	25.73±0.030 $_a$
Monocytes %	13.96±0.575 $_a$	14.82±0.263 $_a$	15.55±0.586 $_a$	16.33±0.031 $_a$
Neutrophils %	53.83±0.173 $_a$	54.46±0.416 $_a$	55.45±0.530 $_a$	56.12±0.005 $_a$
Thrombocytes %	9.69±0.378 $_a$	10.72±0.868 $_a$	12.10±0.543 $_a$	12.82±0.005 $_a$
Eosinophils (103/µL)	0.58±0.012 $_a$	0.61±0.012 $_a$	0.74±0.091 $_a$	0.86±0.000 $_a$
Basophils (103/µL)	0.47±0.005 $_a$	0.49±0.011 $_a$	0.52±0.011 $_a$	0.55±0.002 $_a$

The results indicated prominent increase in all haemato-immunological parameters in each treatment group as compare to control group. The increasing trend followed the concentration of garlic supplementation in fish diet. The results are shown as (Mean ± SD) at Significance level p<0.05

3.6. Lysozymes activity

Results of serum lysozymes activities are shown in (table 4.6), also revealed significantly higher upturn ($p < 0.05$) in lysozymic activities in blood serums of all samples from treatment groups that were nourished on garlic supplemented feed as compared to the control group. Within treatments Fish which were given15g and 10g garlic supplementation per kg feed gave significantly higher lysozyme activity ratio (3.65±0.007, 5.88±0.028, 6.31±0.014, 6.34±0.035, 6.61±0.021) and (6.9±0.021) at the time intervals (5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, 55 and 65 minutes) as compared to control group (3.00±0.035, 5.23±0.028, 5.66±0.042, 5.9±0.021, 6.03±0.035) and (6.23±0.014) respectively. Similarly, (Fig.4.22) also showed represented the similar significant difference of lysozymes activities between treatments groups and the control group.

Table 3.6. Statistical analysis of lysozymic activity after the course of experimental study, against differentconcentration of *Allium sativum*. Control group was given garlic free feed.

Time Intervals	Control Group	Treatment-1	Treatment-2	Treatment-3
15 minutes	3.00±0.035 $_a$	3.21±0.042 $_a$	3.44±0.035 $_a$	3.65±0.007 $_a$
25 minutes	5.23±0.028 $_a$	5.44±0.014 $_a$	5.67±0.021 $_a$	5.88±0.028 $_a$
35 minutes	5.66±0.042 $_a$	5.87±0.035 $_a$	6.1±0.028 $_a$	6.31±0.014 $_a$
45 minutes	5.9±0.021 $_a$	6.11±0.021 $_a$	6.23±0.042 $_a$	6.34±0.035 $_a$
55 minutes	6.03±0.035 $_a$	6.24±0.02 $_a$	6.47±0.03 $_a$	6.61±0.021 $_a$
65 minutes	6.23±0.014 $_a$	6.44±0.014 $_a$	6.67±0.014 $_a$	6.9±0.021 $_a$

Values of lysozymes activity were noticed higher in treatment 3 (15g garlic feed supplementation) when compared to the control group (garlic free diet). In time relation, maximum activity (6.9±0.021) was observedat 65 minutes as compare to the control group (6.23±0.014). The lysozyme activity was observed directly proportioned to the garlic concentration and the time intervals. The values are shown (Mean ± SD) at Significance level p <O.O5

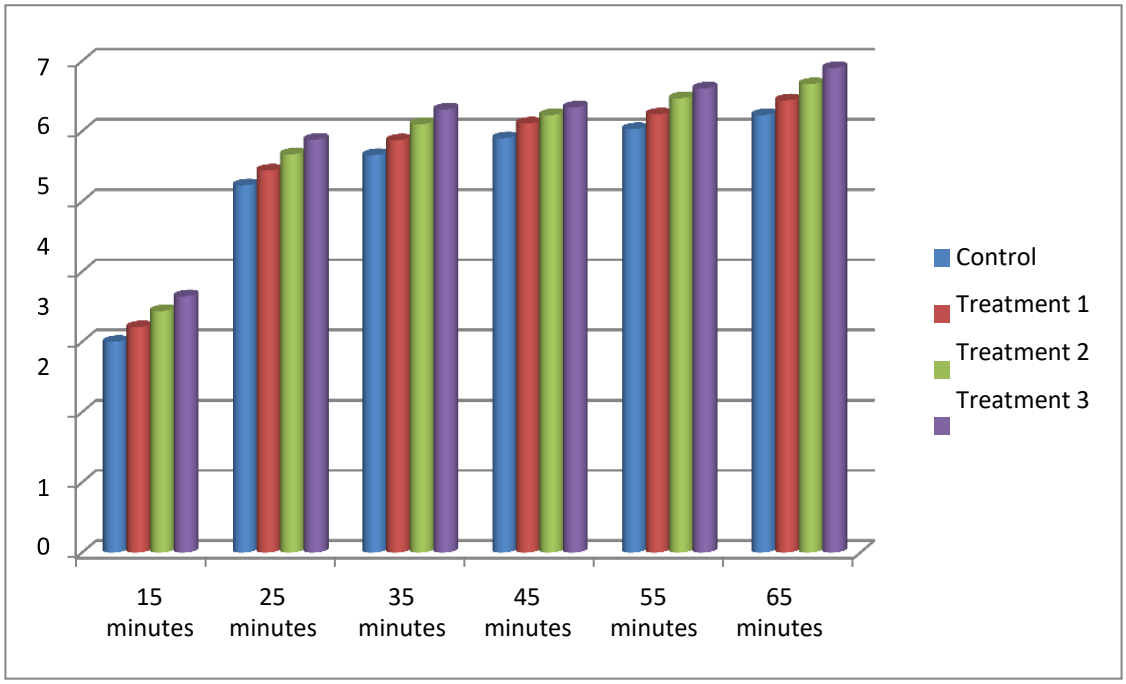

Figure 3.2. Graph similarly showed directly proportional relationship among garlic concentration, time period and lysozymes activity. Each bar represents (Mean ± SE) of the treatment groups and the controlgroup.

3.7. Serum bactericidal activities

The results of serums bactericidal activities are shown in (table 4.7). Serums bactericidal activities were observed considerably higher in all individual fish fed on garlic-additive feed diet as compared with the control group. Highestvalues of bactericidal activities and lower viable colony count were apparent discovered in all the treatment groups which received feed with garlic supplementations as compared to the control group. Among treatments Highest bactericidal activity and lower viable colony count (36±11.00) was observed in treatment-3, that were given 15 g/kggarlic supplemented feed. While, lower bactericidal activity highest viable colony count was examined in control group that were given diet free of garlic contents. The differences in results among the groups fed on garlic-additive feed were observed statistically significant ($p<0.05$). Similarly, (Fig. 4.23) also showed represented the similar significant difference of serum bactericidal activity among the treatment groups and the control group.

Table 3.7. Statistical analysis of Serum bactericidal activity after course of experimentalstudy.

Number of bacterialcolonies	Control	Treatment 1	Treatment 2	Treatment 3
	89±12.00	70±09.00	52±13.00	36±11.00

The numbers of bacterial colonies were found maximum in control group (89±12.00) as compare to the treatment groups. Least number of colonies was found in treatment-3 with 15g per kg garlic concentration. The results are shown in (Mean±SD) at Significance level p <O.O5.

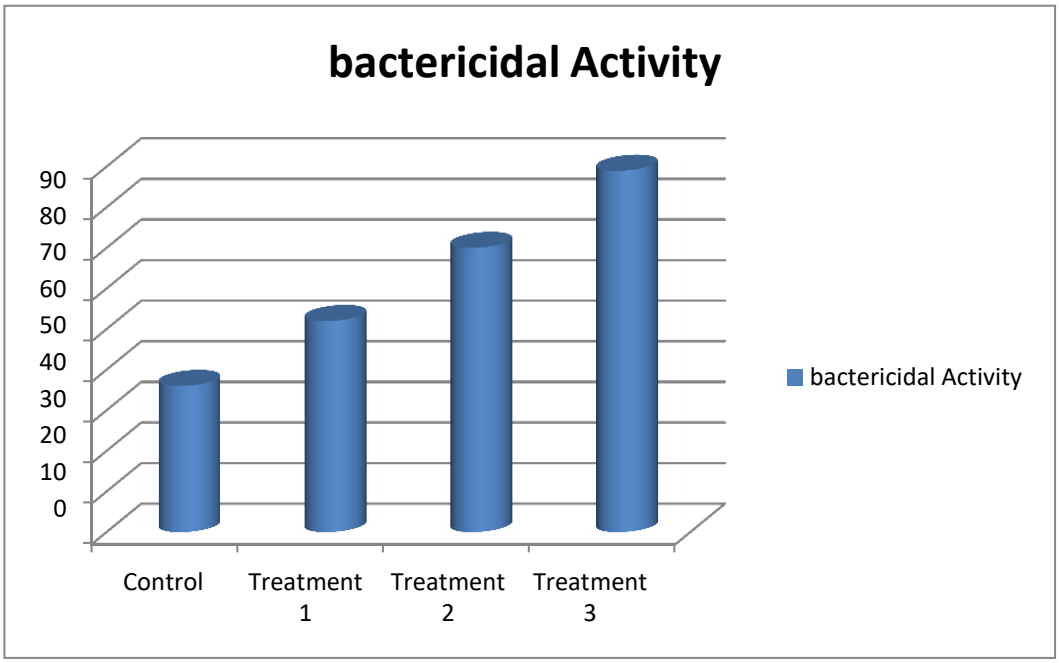

Figure 3.3. Graph shows significant increase in bactericidal activity with the increasing garlic concentration.The increasing bactericidal activity trend pointed out prominent decrease in bacterial colonies. Each bar represents (Mean ± SE) of the treatment groups and the control group.

3.8.Gut micro flora

Gut microbes have vital roles in assessment of health conditions and disease in the host‟s. For the obvious reasons, microorganisms are present in large concentrations, closely associated with the host intestine and interact with the host health parameters. It has been widely demonstrated in animals that gut micro-biota are involved in maturation and regulation of host immunity and gut functions. In the present study, micro-biota in intestine of sampled fish from each treatment was examined and quantitatively compared with control group (Table 4.8). Micro-biota of control group was observed significantly different from treatment all treatment groups. There were observed maximum no.of bacterial colonies present in the intestine of the fish samples taken from the control group as compared all other treatments.

Table 4.8. Quantitative analysis of gut micro-flora of *Labeo rohita* after garlic supplemented feeding. Control group was given garlic free feed.

Treatments	Number of colonies in Gut contents(cfu/ml)
Control	1.80×10^4
Treatment 1	1.03×10^4
Treatment 2	0.87×10^4
Treatment 3	0.31×10^4

The numbers of bacterial colonies were found maximum in control group (1.80 x 104) as compare to the treatment groups. Least number of colonies was found in treatment-3 with 15g per kg garlic concentration (0.31 x 104). The results are shown (Mean ± SD) at Significance level p <O.O5

4- DISCUSSION

Activities to regularize the utilization of natural resources and their derivative products like; natural plants that are of medicinal importance and exhibit curative may generally be accepted as feed supplementary substances to upgrade effectiveness of feed utilization. This utilization of natural substances as feed additives can be helpful to promote productive outcomes of animals (Mohamed *et al*. 2003).

(Amagase and Milner, 1993) described that the optimistic and constructive outcomes in fish development and growth parameters recommended to the biologically and chemically active ingredient of garlic that consists on sulfurcontaining compounds. These compounds are allin (present in enclosed garlic clove), diallyl sulphides and allicin (present in disclosed garlic clove). Additionally, allicin gives the characteristic strong flavor to garlic. In the majority aquatic animals, allicin has massive stimulatory impact on the olfactory system (Lee and Gao, 2O12). Alternatively, sulfur containing compounds that garlic contains are thought to have dynamic anti-microbial agent that enhance resistance and thus initiate development and boost-up growth and development (EL-Afify, 1997)

Sivam (2OO1) also proposed the improvement in growth and development by mean of garlic may be because of its antimicrobial and anti-hypertensive capacities that specify the enhancement of developmental implementations and feed utilization capabilities. Results of the present examination signified that the utilization of dietary garlic supplementation at different levels in *Labeo rohita* (Rohu) feed significantly ($P \leq O.O5$) enhanced all parameters regarding growth, development and improved the ability to utilize feed more effectively. The difference in all these parameters was remarkable in all treatments groups when compared to the control group. The disclosures of the present study are in accordance with those consequences acquired by (Shalaby *et al.* 2006) and (Khattab *et al.* 2004).

In the same concern, Soltan and El-Laithy (2008) also provided detailed study that also pointed the enhancement of parameters like; FCR, SGR, FW and FI in fish *O. niloticus* that revealed significant increase with expanding level of Allium sativum concentration in feed. Furthermore, Aly *et al*. (2OO8) also reported the same significant results of *Allium sativum* supplementation in *O. niloticus* feed. They used 10g and 20g per kg feed garlic concentrations in eight months study duration. Aly and Mohamed (2010) express similar effects of *Allium sativum* supplementations in *O. niloticus* feed. FCR, SGR, FW and FI parameters were also observed significantly increased in all groups that received garlic supplemented feed as compare to control group.

Another approach by Abdel-Hakim *et al.* (2010) also revealed the most striking growth and development linked parameters within the groups that were given garlic supplementation in basal diet at concentration of 3g per kg. The additions of garlic supplementation at the concentration of 5g per kg verify the best results of feed utilizing parameters. Thus, the control group in comparison did not show any significant results. Conversely, Ndong and Fall (2007) also accounted the negative impacts. They did not found any remarkable improvements in growth of hybrid tilapia fish that were fed on 0.5% and1% garlic supplemented feed for two to four weeks. However, the control group reflected significant growth as compare to treatment groups.

To determine physiological changes and pathogenic load in fish, screening of hematological qualities changes is alsoused as a vital tool which can be used up as useful and responsive index (Xiaoyun*et al.,* 2009). Results of the presentinvestigations also demonstrated the addition of different level of garlic concentrations enhanced several blood parameters significantly ($P \leq 0.05$). RBCs, WBCs, Hb, PCV level were found increased in all treatment groups in a smooth pattern parallel to the concentration of garlic in feed. Whereas, these parameters were evidence non-significant in the control group.

The results observed are also in concurrence with and contrasted the similar findings of Shalaby *et al.* (2006). Martins *et al.* (2002). They also illustrated in their results and concluded that the accumulation of *Allium sativum* into feed of fish positively enhanced WBCs numbers, Hb content, and immunity linked blood parameters; leucocytes, thrombocytes. Furthermore, similar

increase in leucocytes count, lysozyme activity, phagocytic activity and respiratory burst was recorded in hybrid tilapia after feeding them on basal diet mixed with garlic at 0.5g per kg level. The experimental study was arranged and conducted by Ndong and Fall (2007).

Moreover, Nwabueze (2012) study also notified altogether significant increase in *Clarias gariepinus* parameters; RBC‟s count, PCV and Hb content. These results were achieved after the feeding of the experimental fish *Clarias gariepinus* on garlic supplemented feed at the ration of 5g garlic per kg feed. The promising outcomes of hematological and blood biochemical parameters in current examinational study confer rise in the immunity and resistance response in fish. Moreover, this increase in immunity directly influenced the physical and health conditions of fish. This observable fact reflected the better improvement in productivity, growth, feed utilizing and higher survival rate of fish. Where, *Allium sativum* has several important components that play vital role in the stimulation of invulnerable immunity framework. These components also affect the capacity and function of organs identified to have direct relation with blood cell development; bone marrow, spleen and thymus (Jeorg and Lee, 1998). The antioxidant properties of *A. sativum* are accounted for defensive nature against gastrointestinal infections due to presence of sulfur derived compound alliin. This antioxidant property is also significant against blood clotting. The compound alliin has identified to have confined fibrinolytic action (Schulz *et al*. 2004).

Serums bactericidal activities were observed remarkably higher in all fish fed on garlic-additive feedings as compared to the control group. Highest values of bactericidal activities and least viable colony count were prominently evident in each treatment group which received garlic supplementations in feed as compared to the control group. But Highest values of bactericidal activities and lower viable colony count was observed in treatment-3, that were given 20 g/kg garlic supplemented feed. While, lower bactericidal activity highest viable colony count was observed in control group. Allyl sulfides also present in garlic *Allium sativum* increase and advance glutathione s-transferase enzyme framework, which alternatively improve the liver detoxification capacity against cancer- causing substances. *Allium satvum* also possess immunity improving exercises that incorporate synthesis and development of lymphocyte, discharge of cytokine and phagocytic activity of natural killer cell, macrophages (Kyo*et al.* 1998).
The similar comparable results regarding serums lysozyme activities were observed in blood serum of all samples from treatment groups that were fed on garlic supplemented feed. 15g and 10g garlic supplementation per kg feed gave significantly higher lysozyme activity ratio at different time intervals as compared to control group. The present study findings are in accordance with those disclosed by (Sahu *et al.* 2007). Micro biota in intestine of sampled fish from each treatment were examined and compared with control group. Micro biota of control group was different from treatments. There were present maximum numbers of disease causing bacteria in the intestine of control group compared to the treatments.

The distinctions in the results attained from past examinations and the current investigation may be because of the distinction in the conditions of examinational study as the size of fish, age and natural quality of the garlic supplementation.

5- Conclusion

In conclusion, results attained in present study recommended Garlic supplementations may be used in fish diet as a growth enhancer in *Labeo rohita* (Rohu). It is recommended that Garlic supplementation additions in cultured fish had best growth performance parameters with minimum input of expenditure and maximize output. Garlic also has vital role to inhibit the growth and occurrence of various disease causing bacterial fauna in intestineof fish. The presence of compound „allinin‟ and diallyl sulphidesvitamins in Garlic help to enhancement immunity, prevent and cure numerous diseases. The biologically active compound „allicin‟ found to present in crushed Garlic is

greatly responsible for antimicrobial properties. However, It is concluded that Garlic supplementation in fish feed improve immunity, growth and linked health conditions and parameters in fishes.

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