EVALUATION OF IMMUNOMODULATORY AND GROWTH PROMOTING POTENTIAL OF β-GLUCAN IN *LABEO ROHITA*

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ABSTRACT

Fish is the cheapest source of good Protein, hence there is a good scope for in-land Aquaculture. Growth and disease resistance are directly related to good health. Immunostimulants are substances that improve the disease resistance potential by activating and strengthening the non-specific and or specific immunity thereby reducing the susceptibility of the organism to infections. The present work is aimed to evaluate the growth promoting and immunostimulating potential of β-glucan in the fish *Labeo rohita*. Three different concentrations of β-glucan (250mg, 500mg and 1000mg) incorporated diet are fed to the experimental fish for 40 days. Growth, haematological, serum biochemical and immunological parameters were analysed at the end of the experimental period. Significant improvement in specific growth rate, total leucocyte and lymphocyte was observed in 250mg glucan fed fishes, whereas haemoglobin, serum protein, globulin concentrations were significantly higher in 500mg glucan diet group fishes. The immunological parameters like agglutinating antibody titre, bactericidal activity, lysosomal concentration and myeloperoxidase activity had tremendously improved in 500mg glucan diet fed fishes. No significant changes were observed in blood cholesterol and blood glucose level. Glucan induced improvement in blood cellular changes can be correlated to improved serum biochemical and immunological changes. Thus incorporation of β-glucan in diet strengthens both cellular and humoral immunity.

Key words: β-glucan, Immunostimulant, *Labeo rohita*, Haematology, Serum biochemistry

INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world. A great proportion of this production comes from the developing world (91.2% in 2000), currently all major aquaculture producing countries are in Asia. They are China, India, Japan, Indonesia, Thailand, Bangladesh and Vietnam (Currie 2014). Besides Industrialization, urbanization, deforestation, mining etc, Environmental degradation and increasing land and water scarcity

are the greatest threats to inland fish production. In fish farming, production always depends on ecosystem management and disease management.

Ecosystem management mainly focuses on water quality, soil quality and stocking density, adverse changes in them induces 'Physiological stress' to the fish, which weakens the immune system of the fish making them more susceptible to infection which may either bring out mortality or morbidity (decreased growth), both decreases the production. The loss of fish production from infectious diseases accounts for 60% of all diseased (Mishra 2010).

Labeo rohita, the Indian Major Carp, is one of the most preferred species in the Indian subcontinent, which contributes about 35% of the total carp production (FAO 2001). Development of an economical alternate to accelerate the growth and to maintain the health status of this species is of prime importance for sustainable carp culture. The present trend of intensification of aquaculture is a major concern for the outbreak of disease as fishes are more prone to stress and subsequent infection by pathogen.

An Immunostimulant is a chemical, drug, synthetic or natural substance that elevates the non-specific defence mechanism or the specific immune response against those infectious agents (viruses, bacteria, fungi, and parasites), producing subclinical disease without risks of toxicity, carcinogenicity or tissue residues. They help to hasten the maturation of non-specific and specific immunity in young susceptible animals. They promote a greater and more effective sustained immune response. Application or use of immunostimulants is more common in animal husbandry and human health care. But in aquaculture the concept of immunostimulants is of recent origin and it is mainly to replace the use of antibiotics (Shodhganga-Inflibnet). Immunostimulants may directly initiate activation of the innate defense mechanisms acting on receptors and triggering intracellular gene activation that may result in production of antimicrobial molecules (Bricknell and Dalmo 2005).

The Immunostimulant substances may be of bacterial derivatives, yeast derivatives, algal derivatives, synthetic compounds, animal extracts, vitamins, hormones and herbal extracts. The Immunostimulants can be administered either as injection or added with the feed in small doses. Plenty of reports are there that oral administration along with feed is also equally effective. Immunostimulants are effective against controlling bacteria, virus and parasites (Debtanu Barman *et al* – Aquafind.com).

β-Glucans are high molecular-weight substances composed of glucose as building blocks, usually isolated from cell walls of bacteria, mushrooms, algae, cereal grains, yeast and fungi. Pharmacologically, they are classified as biological response modifiers (BRM). The common feature of immunomodulatory glucans is a chain of glucose residues linked by β-1.3-linkages, also called beta-glucans. Of the different β-glucans, the products known as β-1.3/1.6-glucans derived from baker's yeast, are suggested to be the most potent immune-system enhancers. β-1.3/1.6-glucans is characterized by side-chains attached to the backbone that radiate outward like branches on a tree. The primary structure of the β-1,3/1,6-glucan is determinant for its immune-enhancing ability (Zekovic and Kwiatowski 2005).

Glucan specific receptors are present on the surface of many cells especially on monocytes, macrophages, neutrophils and natural killer cells. When the glucans binds to glucan receptors all immune functions are improved, including diapedesis, phagocytosis, degranulation, initiating signal transduction pathways and induce gene expression synthesizing the immune related products, release of certain cytokines (Intercellular hormones), interferons to process the antigens. The cytokines also stimulates the formation of new white blood cells to enhance the immunity (Meena *et al* 2013).

Hematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the hematological characteristics (Gabriel *et al* 2004).

The present study is aimed to evaluate the growth promoting and immune enhancing potential of β-Glucans in the fish *Labeo rohita,* through haematological, serological and immunological analysis.

MATERIALS AND METHODS

Collection and Rearing

The test fish (50±5 gram weight) was purchased from Sabari fish farm, Kallidaikurichi, Tirunelveli and transported to the laboratory in plastic containers containing oxygenated water. They were acclimated to the laboratory condition (Water temperature 29±1^{0}C, pH 7.2±0.1, dissolved oxygen 5.84±0.042 ml/l and Salinity 0.127±0.023 ppt) and fed with Rice bran and Groundnut oil cake *adlibitum* for 15 days. The aquarium water was changed daily in order to maintain the fishes in healthy state. The diseased and dead fishes

were discarded immediately. Tap water was used for the present study in all the experiments in which the fishes were pre-acclimatized.

Feed Preparation

The β-Glucan (1-3/1-6 Glucan) purchased from Source Naturals.INC, P.O.Box 2118, Santa Cruz, CA 95062 (www.Source naturals.com) was used in this experiment. The control and balanced diet were prepared by mixing rice bran (10g), wheat bran (10g), Soya flour (23g), fish meal (24g), Ground nut oilcake (23g), tapioca flour (10g) and vitamin and mineral premix (2g). The above ingredients are mixed well with water and sterilized in pressure cooker for 30 minutes, cooled and required amount of the β-glucan (C diet = 0 g, G1 diet = 250mg, G2 = 500mg and G3 = 1000mg) and vitamin & mineral premix were added, made into dove consistency by adding sunflower oil, prepared into the form of noodles which were then shade dried and broken into small appropriate sized pieces. They were packed in air tight containers and stored in the refrigerator.

Experimental Design

The fishes were divided into four groups each with 50 fishes. Group-1 received control diet (C), Group-2 received 0.25% β-glucan incorporated diet (G-1), Group-3 was fed with 0.5% β-glucan supplemented diet (G-2), Group-4 received 1% β-glucan incorporated diet (G-3). The fishes were fed *adlibitum* daily twice (Morning and Evening) for 40 days. The fishes were weighed on day one and at the end of 40^{th} day. The whole experiment was run in triplicate.

Collection of Blood

At the end of 40 days feeding trial, 10 fishes from each group was collected very gently using a small dip net and transferred to separate plastic containers and anesthetized. Immobilized fishes were collected for blood sampling. The blood was collected from caudal vein and or from the heart directly using 1ml insulin syringe. The blood was collected in non-heparinized Eppendorf tubes for the collection of the serum and in heparinized tubes for routine haematological studies.

Haematological Analysis

The total Erythrocyte counts, total leucocyte counts and differential leucocyte counts were performed by the method prescribed by Wilhelm Schaperclaus 1991. The total thrombocyte counts, Erythrocytic indices like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated as prescribed by Mukherjee 2002. Haemoglobin content was determined by Cyanmethaemoglobin method, using the reagent HEMOCOR-D. using colorimeter. Blood smears were stained with Giemsa stain to enumerate differential leucocyte count.

Serum biochemical Analysis

The non heparinized blood was collected in a clot activator Eppendorf tubes and allowed to clot at room temperature, then the tube was centrifuged for 10 minutes at 3000rpm to separate the serum. The supernatant serum was carefully separated and stored in a refrigerator.

The total serum protein was estimated by Biuret Method using total protein test kit purchased from Jeev Diagnostics Private limited, Chennai. The serum albumin content was estimated by BCG method using Albumin Test Kit purchased from Jeev Diagnostics Private Limited, Chennai.

The globulin concentration of the serum was estimated by subtracting serum albumin from serum total protein**.** (Serum Globulin = Total serum protein – serum albumin**.** Albumin globulin ratio was calculated by dividing the albumin concentration with globulin concentration.The serum Cholesterol content was estimated by CHOD-POD method using Cholesterol Test Kit purchased from Jeev Diagnostics Private Limited, Chennai. The blood glucose was estimated by GOD-POD method using Glucometer (Arkray, Japan).

Immunological Analysis

Agglutinating antibody titer was determined with a microagglutination assay using each antigen independently following the method of Klesius *et al* (2000) with some modifications. Serum lysozyme was determined by turbidometric assay by the method of Sankaran and Shanto (1972) with some modifications. Serum Bactericidal Activity was performed by the method prescribed by Kajita *et al* (1990). Myeloperoxidase content of serum was determined as described by Quadi and Rath (1997) and partially modified by Sahoo *et al* (2005).

Statistical Analysis

The results obtained were statistically analysed using the following formulae

$$Mean = \frac{\sum x}{N}$$

Where $\sum x$ = Total summation of the samples

N= Total Number of samples

$$\text{Standard Deviation} = \sqrt{\frac{\sum(x - \bar{x})^2}{N - 1}}$$

where $\sum(x - \bar{x})^2$ = The total squared deviation of each score from mean

N = Total Number of samples

The results were analysed statistically by One way Anova (Post hoc –Duncan) using SPSS (16) software**.**

Growth Parameters

Growth Parameters were calculated using the following formulae

Mean body weight gain = Final weight (g) – Initial weight (g)

$$PercentWeightGain = \frac{\text{weight gain (g)}}{\text{Initial weight (g)}} \text{x } 100$$

$$SpecificGrowthRate\ (\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Time (days)}} \text{x } 100$$

RESULTS AND DISCUSSION

Labeo rohita fishes were divided into 4 groups each in triplicate were fed with different feeds (C = control, G-1 = 0.25% Glucan incorporated feed, M-2 = 0.5% Glucan incorporated feed and G-3 = 1% Glucan incorporated feed) for 40 days. At the end of the experimental duration, growth, haematological, serum biochemical and immunological parameters were analysed to evaluate the feed induced changes. The results were tabulated and statistically treated.

Growth Parameters

Highly significant elevation in mean weight gain, % weight gain and specific growth rate was observed in fishes fed with G-1 and G-2 feed and a decline in growth was observed in G-3 group when compared with control. When compared to all groups higher growth promoting potential was observed in G-1 group (Table-1). The improved fish growth and feed utilization may be because of the palatability or attractiveness of the diets, which in turn cause increased feed intake, further the immunostimulant may inhibit the pathogens present in the digestive tract, may enhance the population of beneficial microorganisms, and/or may enhance the microbial enzyme activity that consequently improves the feed digestibility and nutrient absorption (Mohsen Abdel-Tawwab *et al* 2010).

Erythrocytic Parameters

Decline in total erythrocyte count (TEC) with increase in glucan concentration was recorded; however the levels were higher in G-1 and G-2 groups when compared with control counterparts. In contrast to total erythrocytes, haemoglobin recorded a highly significant increase in all glucan fed groups and was highest at G-2. Similarly Haematocrit (Ht) level remained high in all the experimental groups especially significantly higher in G-1 group, this can be attributed to increased levels of erythrocytes, platelets and leucocytes in the same group (Table-2).

The erythrocytic Indices like Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) increased significantly, indicating the accommodation of more haemoglobin per erythrocyte.

Leucocytic Parameters

Increase in the population of thrombocytes was not much statistically significant in the glucan fed fishes, and the total leucocyte counts were significantly higher only in G-1 group. In differential leucocyte counts, neutrophils of G-1 exhibited a significant decrease and monocytes increased significantly. The lymphocyte populations were remarkably higher in G-1 group but not statistically significant. No significant changes were observed in eosinophil and basophil populations (Table-3).

Hematocrit, haemoglobin and the erythrocytic concentration values indicate the oxygen carrying capacity and serve as indices of the aerobic capabilities of the fish. Leucocytes and thrombocytes are considered as important parameters to evaluate both the fish's state of health and their immune system (Tavares-Dias *et al* 2004).

The present study reveals an increase in cellular immunological indicators such as erythrocytes, leucocytes and thrombocytes in the experimental fish may be due to the increase in the levels of immunity which in turn could be due to the action of the glucan present in the diets.

Serum Biochemical Parameters

The serum protein, Albumin and globulin levels increased significantly in all the glucan feed fed fishes and the increase was dose dependent in protein and globulin levels. Significant to highly significant decline in albumin/globulin ratio in all glucan feed fed fishes explains an elevated globulin levels when compared to their control counterparts. No significant alterations were observed in cholesterol and blood glucose levels (Table-4).

There is a close relationship between the level of protein synthesis in liver tissue and plasma protein pools, total protein levels in plasma may be elevated due to the increased levels of protein synthesis in liver tissue. The increase in plasma protein results when anabolic processes exceed catabolic ones, and reserve protein is produced in greater quantity to meet increased metabolic requirements of the fish (Helmy *et al* 1974).

Serum protein includes various humoral elements of the non-specific immune system and increase in serum total protein, globulin and albumin are likely to be a result of the enhancement of the non-specific immune response of fishes (Citarasu *et al* 2006).

Immunological Parameters

The agglutination titer performed with the serum of control and glucan feed fed fishes demonstrated that the serum of glucan fed fishes had the potential to agglutinate with the bacteria even at 26 times dilution which is an indication of highly improved immune response. Similarly, the serum bactericidal activity and myeloperoxidase activity had increased significantly in all glucan fed fish groups. The concentration of lysozyme had increased tremendously in all the glucan fed fish groups indicating glucan's potential in improving the immune response (Figure. 1to 4). However, the improvement in immunological parameters was significant only in G-1 and G-2.

The important molecules like lysozyme, myeloperoxidases, superoxides, acute-phase proteins, interferons, complement, properdin, lysins and agglutinins, are some of the important innate immune parameters and have often been used as indicators of aquatic stress

response and disease resistance (Shailesh Saurabh and Sahoo 2008). Measurement of myeloperoxidase activity is an indicator of phagocytic and neutrophil activities in non-specific immune response (Weeks and Warinner 1986), the presence of protective proteins in fish blood can be evaluated by serum bactericidal activity and this is an important tool to analyze the innate immune system (Biller-Takahashi *et al* 2013). Significant increase in the Serum lysozyme concentration and Myeloperoxidase activity in the Glucan diet fed fishes indicates significant improvement in their stress response and disease resistance capabilities. Similar results were observed by the following researchers.

EL-Boshy *et al* (2008) fed Tilapia (Injected with Aflotoxin B1) dietary Aflotoxin B1 (200µg/kg) and/or β1-3 glucan (0.1%) individually or combinedly for 3 weeks and analysed haematological, biochemical and immunological parameters. They have observed an increase in total erythrocytes, haemoglobin, total leucocytes, lymphocytes, packed cell volume, total serum protein, superoxide anion production, bactericidal activity, lysozyme concentration, neutrophil glass adhesion, lymphocyte transformation index and macrophage phagocytic index on β1-3 glucan diet fed fishes. Thus feeding β1-3 glucan can enhance the non-specific immunity not only in healthy but also in immunocompromised (with AFB1) Tilapia.

JayaKumari and Sahoo (2006) fed different groups of catfish (*Clarias batrachus*) with different immunostimulant incorporated feeds, *ie*, β 1-3-glucan (0.1%), Levamisole (50mg/kg), Lactoferrin (100mg/kg) and vitamin C (500 mg/kg) for 30 days. Each group was further divided into two groups, one is immune compromised by cyclophosphamide 3 doses (200mg/kg) and the other was non-immuno compromised. Fishes of all the groups were vaccinated on the first day with formalin killed *Aeromonas hydrophila*. After the experimental period the antibody titre and survival rate was found to be enhanced in both healthy and immune compromised fishes. The immunostimulants were also graded based on their immunostimulant potential as β Glucan > Levamisole > Lactoferrin >Vitamin C.

Sahoo and Mukherjee (2001) fed β-1,3 glucan (0.1%) incorporated diet for 7 days to healthy and aflatoxin B1-induced immunocompromised *Labeo rohita* in a 60 day trial and observed specific and non-specific immune responses. A marked rise in specific immune response parameters (bacterial agglutination titre, haemagglutination and haemolysin titre) and non specific immune parameters (phagocytic ratio, phagocytic index and serum bactericidal activity) were observed. Further increased survival against *Aeromonas*

hydrophila infection was observed in glucan fed fishes. Thus feeding glucan has increased both specific and non-specific immunity against *A.hydrophila*.

CONCLUSION

In the present work it was observed that incorporation of β-Glucan in fish feed has significantly elevated the growth, haemoglobin, packed cell volume, MCV, MCH, MCHC, total leucocytes, serum protein, albumin, globulin, especially at 0.25% level. It was also recorded that incorporation of glucan has significantly increased the agglutination titre, serum bactericidal activity, Lysozyme concentration and myeloperoxidase activity. From the present study it was observed that the humoral and cellular immunity has strengthened in fishes fed with glucan incorporated diet. Thus it can be concluded that *β* Glucan is a potent growth promoter and a potential Immunostimulant to fish.

REFERENCES

Biller-Takahashi J D, Takahashi L S, Pilarski F, Sebastiao F A & Urbinati E C, Serum bactericidal activity as indicator of innate immunity in pacu *Piaractus mesopotamicus* (Holmberg 1887), *Arg Bras Med Vet Zootec*, 65(6) (2013) 1745.

Bricknell I & Dalmo R A, The use of immunostimulants in fish larval aquaculture, *Fish Shellfish Immunol,* 19 (2005) 457.

Citarasu T, Sivaram, Immanuel G, Rout N & Murugan V, Influence of selected Indian Immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp *Penaeus monodon* with reference to haematological, biochemical and immunological changes, *Fish shellfish Immunol*, 21 (2006) 372.

Currie, FAO-*The growing Importance of Aquaculture in Food Security* – Gulfood, Dubai, 24 Feb, 2014.

Debtanu Barman, Vikash Kumar, Suvra Roy,.Sanjit Singh A, Debolina Majumder, Abhay Kumar Atom Arun Singh, The Role of Immunostimulants In Indian Aquaculture. http://aquafind.com/articles/Immnostimulants-In-Indian-Aquaculture.php

El-Boshy M E, EL-Ashram A M M & Nadia A Abd EL-Ghany, Effect of dietary 1,3-Glucan on immunomodulation on diseased *Oreochromis niloticus* experimentally infected

with aflatoxin B_1, 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt,12-14 October, 2008.

FAO, *Yearbook on Fishery Statistics*, Rome (Italy), 2001.

Gabriel U U, Ezeri G N O& Opabunmi O O, Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822), *Afr J Biotechnol*, 3 (2004) 463.

Helmy A M, Badawi H K & El-Bishry, Seasonal variations in the protein composition of blood serum of *Anguilla vulgaris* and *Mugil cephalus*, *Bull Inst Oceano & Fish A R E*, 4 (1974) 367.

Jaya Kumari & Sahoo P.K, Dietary beta-1,3 glucan potentiates innate immunity and disease resistance of Asian catfish, *Clarias batrachus* (L.). *Journal of Fish Diseases*, 29 (2006) 95.

Kajita Y, Sakai M, Atsuta S & Kobayash M, The Immunostimulatory effects of levamisole on Rainbow trout, *Onchorhyncus mykiss, Fish Pathol*, 25 (1990) 93.

Klesius P H, Shoemaker G A, & Evans J J, Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular route in Tilapia (*Oreochromis niloticus*), *Aquaculture,* 188 (2000) 237

Meena D K, Das P, Kumar S, Mandal S C, Prusty A K, Singh S K, Akhtar M S, Behera B K, Kumar K, Pal A K & Mukherjee S C, Beta –Glucan: as ideal Immunostimulant in Aquaculture (A Review), *Fish Physiol Biochem,* 39(3) (2013) 431.

Mohsen Abdel-Tawwab, Mohammad H. Ahmad, Medhat E, Seden A, Saleh F & Sakr M, Use of Green Tea, *Camellia sinensis* L., in Practical diet for growth and protection of Nile Tilapia, *Oreochromis niloticus* (L) against *Aeromonas hydrophila* infection, *Journal of the World Aquaculture Society*, 41(S2) (2010) 203.

Mishra S, Fish Disease Management in Integrated Farming System, *Web med Central Parasitology*, WMC00663,1(9) (2010).

Mukherjee K L, *Medical Laboratory Technology*: *A Procedure Manual for Routine Diagnostic Tests* (Volume 1), (Tata McGraw-Hill Publishing Company Limited, New Delhi). Tenth reprint 2002.

Quade M J & Roth J A, A rapid, direct assay to measure degranulation of bovine neutrophil primary granules, *Vet Immunol Immunopathol*, 58 (1997) 239.

Sahoo P K & Mukherjee S C, Effect of dietary β-1,3 glucan on immune responses and disease resistance of healthy and aflatoxin B_1-induced immunocompromised rohu (*Labeo rohita* Hamilton), *Fish Shellfish Immunol*, 11 (2001) 683.

Sankaran K, Shanto G, On the variation in catalytic activity of lysozyme in fish, *Indian J Biochem Biophysics*, 91 (1972) 162.

Shodhganga. inflibnet, shodhaganga.inflibnet.ac.in/bitstream/10603/6977/5/05 chapter% 202. pdf.

Shailesh Saurabh & P K Sahoo, Lysozyme: an important defence molecule of fish innate immune system, *Aquaculture Research*, 39 (2008) 223.

Tavares-Dias M & Moraes F R, *Haematology in Teleost Fish*, (Sao Paulo Ribeirao Preto), 2004.

Weeks B & Warinner J E, Functional evaluation of macrophages in fish from a polluted estuary, *Vet Immunol Immunopathol*, 2 (1986) 313.

Wilhelm Schaperclaus, *Fish Diseases* Volume I, Editors W Schaperclaus, H Kulow and K Schreckenbach, (Oxonian Press Private Limited, New Delhi), 1991.

Zekovic D B & Kwiatowski, Natural and modified (1-3)-beta-D-glucans in health promotion and disease alleviation, *Crit Rev Biotechnol*, 25 (2005) 205.

Table-1. Effect of dietary β-glucan in growth parameters of *Labeo rohita* (Mean±SD)

Sl.No	Parameters	Control	G-1	G-2	G-3
1	Weight Gain	2.91^{a} ± 0.2	7.36^{c} ± 0.45	5.15^{b} ± 0.05	3.07^{a} ± 0.08
2	% Weight Gain	6.55^{a} ± 0.71	15.25^{c} ± 1.69	10.21^{b} ± 0.36	5.70^{a} ± 0.28
3	Specific Growth Rate (%)	7.28^{a} ± 0.5	18.39^{c} ± 1.15	12.88^{b} ± 0.12	7.69^{a} ± 0.21

G-1 = 0.25%β-Glucan, G-2 = 0.5%β-Glucan, G-3 = 1%β-Glucan.
Means with same superscript in the same row is not statistically significant

Table-2. Effect of dietary β-glucan in Erythrocytic parameters of *Labeo rohita* (Mean±SD)

Sl.No	Parameters	Control	G-1	G-2	G-3
1	TEC (10^6)	1.69^{a} ± 0.04	1.82^{ab} ± 0.11	1.71^{ab} ± 0.09	1.69^{a} ± 0.11
2	Haemoglobin (g%)	5.83^{a} ± 0.35	7.06^{ab} ± 0.21	8.26^{c} ± 0.32	7.53^{b} ± 0.21
3	Haematocrit (%)	15.66^{a} ± 0.57	24.33^{c} ± 1.15	18.66^{bc} ± 0.57	17.67^{ab} ± 1.52
4	MCV (µ3)	92.50^{a} ± 1.6	133.28^{c} ± 2.67	104.85^{b} ± 0.89	104.23^{b} ± 2.57
5	MCH (µgm)	34.43^{a} ± 1.28	38.73^{b} ± 1.4	48.19^{d} ± 1.37	44.56^{c} ± 1.71
6	MCHC (%)	37.22^{b} ± 1.34	29.06^{a} ± 1.01	45.95^{d} ± 1.05	42.79^{c} ± 2.59

G-1 = 0.25%β-Glucan, G-2 = 0.5%β-Glucan, G-3 = 1%β-Glucan.
Means with same superscript in the same row is not statistically significant

Table-3. Effect of dietary β-glucan in Leucocytic parameters of *Labeo rohita* (Mean±SD)

Sl.No	Parameters	Control	G-1	G-2	G-3
1	Thrombocyte (10^5)	1.19^{ab} ± 0.009	1.19^{ab} ± 0.06	1.27^{b} ± 0.11	1.28^{b} ± 0.11
2	TLC (10^4)	3.87^{a} ± 0.12	4.42^{b} ± 0.17	3.91^{a} ± 0.07	3.83^{a} ± 0.09
3	Neutrophils (%)	30.33^{ab} ± 2.51	24.33^{a} ± 3.51	30.66^{ab} ± 5.03	35.67^{b} ± 3.05
4	Eosinophils (%)	2.33^{ab} ± 0.57	3^{abc} ± 1	2.33^{ab} ± 1.15	4.33^{c} ± 0.58
5	Basophils (%)	3^{a} ± 1	1.66^{a} ± 0.47	1.66^{a} ± 0.47	2.3^{a} ± 1.15
6	Lymphocytes (%)	59.66^{b} ± 2.51	63.66^{b} ± 3.78	59.66^{b} ± 4.93	53^{a} ± 2
7	Monocytes (%)	4.66^{a} ± 1.52	7.33^{c} ± 0.15	5.66^{ab} ± 1.15	4.67^{a} ± 1.52

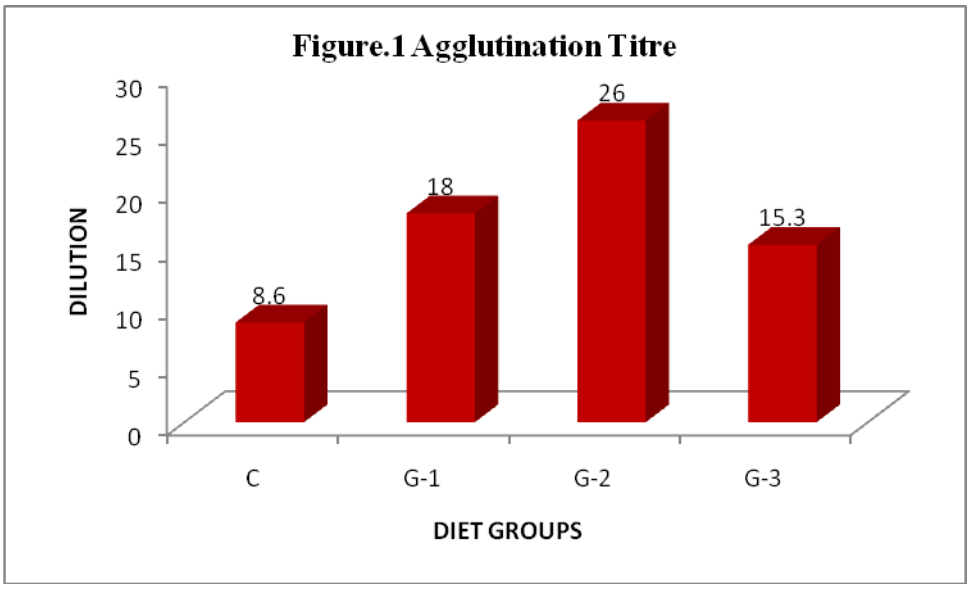
G-1 = 0.25%β-Glucan, G-2 = 0.5%β-Glucan, G-3 = 1%β-Glucan.
Means with same superscript in the same row is not statistically significant

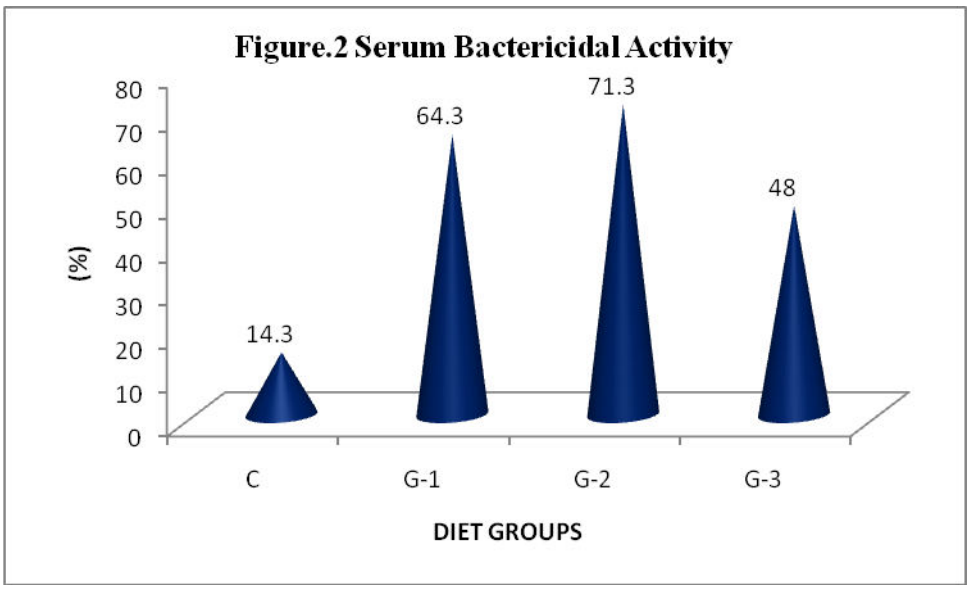
Table-4. Effect of dietary β-glucan in Serum Biochemical parameters of *Labeo rohita* (Mean±SD)

Sl.No	Parameters	Control	G-1	G-2	G-3
1	Protein (g/dl)	5.13^{a} ± 0.15	7.06^{b} ± 0.25	7.96^{c} ± 0.35	8.1^{c} ± 0.2
2	Albumin (g/dl)	2.20^{ab} ± 0.13	2.71^{bc} ± 0.2	2.75^{c} ± 0.13	2.02^{a} ± 0.19
3	Globulin (g/dl)	2.93^{a} ± 0.02	4.35^{b} ± 0.05	5.21^{c} ± 0.23	6.07^{d} ± 0.02

4	Albumin/Globulin Ratio	0.74^{d} ± 0.04	0.62^{c} ± 0.04	0.52^{b} ± 0.01	0.33^{a} ± 0.03
5	Cholesterol (mg/dl)	128^{a} ± 2.64	132.6^{a} ± 12.01	132.66^{a} ± 7.5	129^{a} ± 0.7
6	Blood Glucose (mg/dl)	93^{ab} ± 7	99.3^{bc} ± 8.08	89^{ab} ± 8.54	84^{a} ± 8

G-1 = 0.25%β-Glucan, G-2 = 0.5%β-Glucan, G-3 = 1%β-Glucan.
Means with same superscript in the same row is not statistically significant





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