Blood serum analysis in Channa Marulius after application of the isolated Probiotic bacteria and development in immunology

Article *in* Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University · October 2021

CITATIONS
0

READS
54

3 authors:

Abhaysinh Rameshrao Deshmukh
Rajarshi Shahu College, Latur
6 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Vishwas S Shembekar
10 PUBLICATIONS **11** CITATIONS
SEE PROFILE

Datta Ashok Nalle
Rajarshi Shahu College (AUTONOMOUS), Latur
30 PUBLICATIONS **1** CITATION
SEE PROFILE

Blood serum analysis in *Channa Marulius* after application of the isolated Probiotic bacteria and development in immunology

Abhaysinh R. Deshmukh *, Vishwas S. Shembekar, Datta A.Nalle****

Department of Zoology & Fishery Science, Rajarshi Shahu Mahavidyalaya (Autonomous) Latur (Maharashtra) India.

Abstract: In present investigation in the results obtained evidently confirms for utilization of it as probiotics. the culturable microorganisms was isolated from the intestinal content of fingerlings of *Channa marulius* as well as from adult fishes. Five experimental diets with containing varying concentration of *lactobacillus acidophilus* (probiotic) (0.25,0.5,0.70 and 100%) were formulated using soybean as the protein source. The fish were exposed to their respective diet for 4h during each ration. In the end of experiments serum immunological parameters were checked. The serum albumin and serum globulin content was significantly higher in all the treatment groups compared to control. Significantly higher serum lysozyme activity was observed in LBD-1 group (fed with diet containing 0.25 probiotic (LBD1)) as compared to other treatment groups and control. the concluding remarks of the present investigation was all the probiotic treated group showed significantly better growth parameters as compared to control. However there was no significant difference between the treatments. Fishes fed with diet supplemented by *Lactobacillus acidophilus* Showed significantly better immune response as compared to other treatment groups and control.

The percentage survival of fishes of the entire probiotic treated group after challenging with *Aeromonas Sp* was significantly higher than the control, however in between the treatment groups there was no significant difference in the survival rate.

Index terms: *Channa marulius, blood serum,probiotic, lactobacillus acidophilus*

1. Introduction:

Various diseases in fishes due to bacteria cause a huge nuisance for aquaculture farming, and hence there is heavy economic loss as well as cause health risks for the

consumer who going to eat these diseased fishes [1] Numerous bacterial species have been recognized as pathogenic, causing fish diseases. Several studies have shown that *Aeromonas* affects both fish and sea fruit [2] and causes different diseases. For instance, furunculosis is caused by *A. salmonicida*. Hemorrhagic septicemia and ulcer were as well attributed to *Aeromonas spp.* [3] *Aeromonas spp* is a ubiquitous gram-negative, rod-shaped bacterium that causes the disease, "Motile *Aeromonas* Septicemia (MAS)", "Hemorrhagic Septicemia," "Ulcer Disease," or "Red-Sore Disease" in fish. The key factor in connection with *Aeromonas spp* infection in fish is that this is a zoonotic disease, i.e., the disease which can be spread from animals to man and vice versa. Healthy individuals exposed to this bacterium are not very likely to be infected. However, People with poor immuno-competence or immuno-deficiency such as very young, the elderly, or those with other diseases are at the highest risk. Hence, controlling the infection is the need of the hour, and many measures have been taken in recent years to control and treat the infection.

Recently, there has been an increasing practice of managing bacterial fish diseases by using probiotic strains to control populations of potential pathogens, either by competitive inhibition, enhancement of fish immunity or by the microbial enhancement of the environment. They are usually incorporated into the fish feed. Keeping all such factors in mind, in this study, an attempt was made to evaluate the immunity improvement after probiotic (*lactobacillus acidophilus*). Isolated from the gut of *Channa marulius* against *Aeromonas spp.* Infection.

2 Materials and Methods

2.1: Sample collection:

Healthy *Channa marulius* fingerlings were collected from Godavari River Nanded District (MS) (19.1383° N, 77.3210° E). All the fishes were transported to the wet laboratory and fishes were randomly divided into stock in the circular plastic tank (200 L) filled with fresh water with continuous aeration. During this period, the fishes were fed with commercial pellets (5% body weight) twice a day. Fishes were acclimatized to these conditions for 10 days before the experiments. During the experiment, the water temperature was maintained at 28°C±0.6, pH 8.1±0.5, salinity

28±3 ppt, and dissolved oxygen concentration were 5.7±0.8 (mg/l). The ammonia and nitrite content in the water was maintained at the permissible levels.

2.2: Experimental groups:

The different experimental groups were shown in table 1. The fishes were fed with prepared pelleted diets at a rate of 5% of the bodyweight every day. Two times feeding trials were implemented the first feeding trial was feed at 9 AM and 6 PM. Ingredients of the present diet were shown in table 1.

2.3: Serum collection:

For serum collection, another three fishes from each tub were anesthetized and blood was collected without anticoagulant and allowed to clot for 2 hours followed by the collection of straw-colored serum with a micropipette and stored at -20^0C until use. The serum was used for the estimation of serum protein, albumin, serum lysozyme activity and serum bactericidal activity.

2.3 A: Serum proteins:

Serum total protein was estimated using total protein kit (Biuret method) and albumin estimated using the albumin kit (BCG dye-binding method) of Merck Specialities Pvt Ltd., Mumbai. Globulin was calculated by subtracting the albumin values from total protein.

Globulin (g%) = total protein(g%) - albumin(g%)

A/G ratio was calculated by dividing albumin values by globulin values.

2.3 B: Serum lysozyme activity:

Serum lysozyme activity was measured by the turbidimetric assay of Parry [4] with the microplate adaptation of Hutchinson and Manning. For this 0.03% lyophilized *Micrococcus lysodeikticus* in 0.05 M, sodium phosphate buffer (pH 6.2) was used as a substrate. Serum (0.01ml) was added to 0.25 ml of bacterial suspension

in a microplate and the reduction in the absorbance at 490 nm was determined after 0.5 and 4.5 min incubation at 22^0C using a microplate reader. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min^{-1}.

2.3 C: Serum bactericidal activity:

Serum bactericidal activity was analyzed following Rainger [5] For this bacterial culture of *Aeromonas hydrophila* was centrifuged and the pellet was washed and suspended in phosphate-buffered saline (PBS). The optical density of the bacterial suspension was adjusted to 0.65 at 540 nm. This suspension was then serially diluted 1:10 with PBS five times. The diluted *A. hydrophila* suspension (2ml) was incubated with 0.02ml of serum in a micro vial for 1hr at 37°C. In the control, PBS replaced the serum. After incubation, the number of viable bacteria was determined by counting the colonies grown on a nutrient agar plate for 24 hr at 37^0C.

2.4: Challenge study with *Aeromonas spp.*

Aeromonas spp. was grown on nutrient broth for 24 hours at 28°C. After 24 hours the bacterial culture was centrifuged at 3000 rpm for 10 minutes. The pellet was washed three times in PBS (pH – 7.4) and resuspended in PBS and the final concentration was adjusted to 1.6×10^7 CFU ml^{-1}. After 8 weeks experimental period, fishes in all the experimental groups were injected intraperitoneally with 0.2ml of *Aeromonas spp.* suspension. The mortality of fishes in all groups was observed for 7 days. The relative percentage of survival was calculated using the following formula.

$$\text{Relative percentage survival} = \frac{\text{No. of survived fish after challenge}}{\text{No. of fish injected with bacteria}} \text{ X } 100$$

2.5: Statistical analysis:

The comparison between all the treatments and control was done by one way ANOVA at a 5% probability level.

3: RESULTS:

3.1- I: Serum total protein against *Aeromonas spp.:*

The serum total protein content of *Channa marulius* fingerlings of different experimental groups is presented in Table 2 and 1. The serum total protein content of different experimental groups showed a significant difference (P≤0.05) between the treatments and the control. The higher total protein content (2.71± 0.05) was found in the B3 group fishes which were fed with the diet 3 containing probiotic 0.70 *lactobacillus spp.* and the control group was with the lower protein content (1.61 ± 0.03).

3.1- II: Serum albumin:

The effect of different probiotic treatments on the serum albumin content of *L. rohita* fingerlings is presented in Table 2 and FIGURE 2. There was no significant difference (P≥0.05) in the serum albumin content of different treatment groups. But the serum albumin content of all the treatment groups was significantly higher (P≤0.05) than that of the control group fishes (0.82 ± 0.02). The higher value (1.8 ± 0.03) was observed in the B3 group fishes.

3.1- III: Serum globulin:

The effect of different probiotic treatments on the serum globulin content of *L. rohita* fingerlings is presented in Table 2 and FIG.3. The serum globulin content was significantly higher (P≤0.05) in all the treated groups compared to control. The higher content (1.58 ± 0.02) was recorded in B3 group fishes. The lowest serum globulin content (0.70^{a}±0.03) was observed in the control group fishes.

3.1- IV: Serum lysozyme activity:

The effect of different probiotic treatments on the serum lysozyme activity of *Channa marulius* fingerlings is presented in Table 2 and FIG.4. Significantly higher (P≤0.05) lysozyme activity was observed in B1 (149 ± 11.35). Lowest serum lysozyme activity was observed in the control group (84.4 ± 11.21), but it was not significantly different (P≥0.05) from the other two treatment groups.

3.1- V: Serum bactericidal activity:

The effect of different probiotic treatments on the serum bactericidal activity of *L. rohita* fingerlings are presented in Table 2 and FIG-5. Significantly lower (P≤0.05) colony count was observed in the B3 group fishes (391± 8.7).

3.1- VI: A/G Ratio:

The serum A/G Ratio of *Channa marulius* fingerlings is presented in Table 2 and FIG.3.18. The A/G Ratio of B3 (0.64 ± 0.04) and B2 (0.59 ± 0.03) group fishes were significantly lower than B1 (1.06±0.04) and control group (1.09^{b}±0.04) fishes.

3.2: Percentage survival after challenge study:

The percentage survival of *Channa marulius* fingerlings of different experimental groups after challenging with *A. hydrophila.* is graphically represented in FIG.3.25. The percentage survival of fishes of the entire probiotic treated group was significantly higher (P≤0.05) than the control. There was no significant difference (P≥0.05) between the probiotic treated groups. The higher survival was in the B3 group (80.95 ± 2.4), followed by B1 (78.57 ± 4.1), B2, B3 (71.23 ± 4.1) and control (50 ± 4.1) respectively.

4: DISCUSSION:

The serum total protein content of *Channa marulius* fingerlings of different experimental groups showed a significant difference between the treatments and the control. The higher total protein content was found to be in the B3 group (fed with a diet containing 0.70 probiotics (LBD3). The control group was observed to be with the lower protein content. There was no significant difference in the serum albumin content of *Channa marulius* fingerlings of different treatment groups. But the serum albumin content of the control group fishes was found to be significantly lower than that of all the treatment groups. The serum globulin content was significantly higher in all the treated groups compared to control. The A/G Ratio of the B3 and B3 group fishes were significantly higher than B1 and control group fishes. The serum total protein, albumin and globulin content were observed to be higher in the group that was fed with a diet containing 0.70 probiotics (LBD3), compared to other treatment groups. Nayak et al., (2007) observed increased serum protein and globulin content

and a lower A/G ratio in *L. rohita* treated with *B. subtilis*. The increases in serum protein, albumin and globulin levels are thought to be associated with a stronger innate immune response of fish (Wiegertjes et al., 1996).

According to Magnodottir (2006) lysozyme is an important component in the immune system of fish. It is bactericidal by hydrolyzing bacterial cell wall peptidoglycans resulting in bacteriolysis and it is also known to act as an opsonin. In the present study, significantly higher serum lysozyme activity was observed in the B1 group (fed with diet containing 0.25 probiotic (LBD1)) and lower values were observed in the control group. Other findings support the higher lysozyme activity induced by Bacillus strains. Dietary administration of Bacillus strains significantly enhanced the serum lysozyme activity of *Oncorhynchus mykiss (*Merrifield et al., 2009, Fyzul et al., 2007). In *E. coioides*, increased serum lysozyme activity was observed when fed with *B. pumillus* or *B. clausii* containing diets (Sun et al., 2010).

The higher serum bactericidal activity was observed in B3 group fishes (fed with a diet containing 0.70 probiotics (LBD3)) compared to other treatment groups and control. Aly et al., (2008a) observed high serum bactericidal activity when *Oreochromis niloticus* was treated with a mixture of the two bacteria *Bacillus subtilis* and *Lactobacillus acidophilus*. The higher bactericidal activity may indicate the efficiency of *L. casei* to control the pathogenic bacteria. Misra et al., (2006) mentioned that the serum bactericidal activity was always higher in fish injected with β-glucan, compared to control.

It can be concluded that significantly higher erythrocyte count, haemoglobin content, haematocrit value, leukocyte count and respiratory burst activity were observed in *L. casei* treated group as compared to other treatment groups, and control showed significantly lower values. The number of adherent neutrophils was significantly higher in all the treated groups than the control. But there was no difference between the treatments. Significantly higher total protein content was found in the B3 (fed with L. casei supplemented diet). The serum albumin and serum globulin content were significantly higher in all the treatment groups compared to control. Significantly higher serum lysozyme activity was observed in the B1 group fed with diet containing 0.25 probiotic (LBD1)) as compared to other treatment groups and control.

4.2: Percentage Survival after Challenge Study:

The percentage survival of fishes of the entire probiotic treated group after challenging with *Aeromonas spp. and Vibrio spp.* was observed to be significantly higher than the control, however, in between the treatment groups, there was no significant difference in the survival rate. This may be due to the protective effect of the probiotic bacteria against the pathogenic *Aeromonas spp*. Similar results were obtained by Aly et al., (2008a) when he evaluated the probiotic activity of two bacteria (*Bacillus subtilis* and *Lactobacillus acidophilus*) by its effect on the immune response of Nile tilapia (*Oreochromis niloticus*), and the in-vitro antimicrobial assay showed that *Bacillus subtilis* and *Lactobacillus acidophilus* inhibited the growth of *A. hydrophila*. Increased survival was also noticed by Gildberg (1997) when Atlantic cod fry fed on dry feed containing lactic acid bacteria (*Carnobacterium divergens*) was exposed to a virulent strain of *Vibrio anguillarum*.

References

1. Lau, S. K., P. C. Woo, R. Y. Fan, R. C. Lee, J. L. Teng and K. Y. Yuen, 2007. Seasonal and tissue distribution of Laribacter Hong kongenisis a novel bacterium associated with gastroenteritis in retail fresh water fish in Hong Kong. Int. J. Food Microbiol., 113(1): 22-22.
2. Paniagua, C., O. Rivero, J. Anguita and G. Naharro, 1990. Pathogenecity factors and virulence for rainbow trout (Salmo gairdneri) of motile Aeromonas ssp isolated from a river. J. Clin. Microbiol., 28: 350-355.
3. Dierckens, K. R., J. Vandenberghe, L. Beladjal, G. Huys, J. Mertens and J. Swings, 1998. Aeromonas hydrophila causes „black diseases" in fairy shrimps (Anostraca crustacean). J. Fish Dis., 21: 113-119
4. Parry R.M., Chandan R.C. & Shahani K.M. 1965 Arapid and sensitive assay of muramidase.ExperimentalBiology and Medicine (N.Y.)119, 384–386
5. G. Rainger, A. Rowley, 1993 Antibacterial activity in the serum and mucus of rainbow trout, Oncorhynchus mykiss, following immunization with Aeromonas salmonicida Fish Shellfish Immunol., 3 , pp. 475-48
6. Nayak, S., Swain, P., and Mukherjee, S. 2007. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, Labeo rohita (Ham.). Fish Shellfish Immun. 23, 892–896.
7. Wiegertjes G.F. , Stet R.J.M. , Parmentier H.K. , Vas Muiswinkel W.B. 1996 Immunogenetics of disease resistance in fish: a comparable approach Dev. Comp. Immunol., 20 , pp. 365-381.

8. Magnadottir B. 2006. Innate immunity of fish (overview). Fish Shellfish Immunol 20: 137-151.
9. Merrifield, D. L., G. Bradley, R. T. M. Baker and S. J. Davies, 2009. Probiotic applications for rainbow trout (Oncorhynchus mykiss Walbaum) II. Effects on growth performance, feed utilization, intestinal micro organisams and related health criteria post antibiotic treatment. Aquac. Nutr., doi:10.1111/j.1325-2095.2009.00288.x.
10. Newaj-Fyzul, A., A. A. Adesiyun, A. Mutani, A. Ramsubhag, J. Brunt and B. Austin, 2007. Bacillus subtilis AB1 controls Aeromonas infection in rainbow trout, Oncorhynchus mykiss (Walbaum). Appl. Microbiol., 103 : 1299-1702.
11. Sun Y.Z., Yang H.L., Ma R.L. & Lin W.Y. 2010 Pro-biotic applications of two dominant gut Bacillus strains with antagonistic activity improved the growth performance and immune responses of grouper Epinepheluscoioides. Fish and Shellfish Immunology29, 803–809.
12. Aly, S. M., M. F. Mohamed and G. John, 2008a. Effect of probiotics on the survival, growth and challenge infection in Tilapia nilotica (Oreochromis niloticus). Aquac. Res., 39: 247- 252.
13. Misra CK, Das BK, Mukherjee SC, Meher PK 2006 The immunomodulatory effects of tuftsin on the non-specific immune system of Indian Major car, Labeo rohita. Fish Shellfish Immunol 20:728–738
14. Gildberg, A. and Mikkelsen, H. 1998. Effects of supplementing the feed to Atlantic cod (Gadus morhua) fry with lactic acid bacteria and immunostimulating peptides during a challenge trial with Vibrio anguillarum. Aquaculture 167: 103-113.

Authors

Author – **Abhaysinh R. Deshmukh**: M.Sc. PhD, Assistant Professor.
Author – **Vishwas S. Shembekar**, M.Sc. PhD, Professor and ex. Head
Author: **Datta A. Nalle**. M.Sc. PhD, Pursuing, Assistant Professor.
All Authors are from Department of Zoology & Fishery Science,
Rajarshi Shahu Mahavidyalaya (Autonomous) Latur (Maharashtra) India.

TABLE 1: Ingredient content of five experimental diets with different levels of Probiotics (g 100g^{-1} of diet).

Ingredient	Diets				
	LBDO (control)	LBD1	LBD2	LBD3	LBD4
Groundnut oil cake	60.00	60.00	60.00	60.00	60.00
Rice bran	3.20	2.95	2.70	2.45	2.20
Wheat flour	3.20	3.20	3.20	3.20	3.20
Soybean	25.60	25.60	25.60	25.60	25.60
Chromic oxide (Cr_2O_3)	1.00	1.00	1.00	1.00	1.00
Beef Extract powder	7.00	7.00	7.00	7.00	7.00
Probiotics	-	0.25	0.50	0.70	1.00

TABLE 2: Various Serum parameters of *Channa marulius* fingerlings against *Aeromonas spp.*

Experimental groups	Serum parameters					
	Serum total protein[1]	Serum albumin[2]	Serum globulin[3]	Serum lysozyme activity[4]	Serum Bacteriacidal Activity[5]	A/G Ratio
B1	2.10b±0.01	1.05b±0.04	1.04b±0.01	149b±11.35	420±12.6	1.06±0.04
B2	2.59c±0.03	1.00b±0.02	1.56c±0.02	98.8a±8.74	436b±8.9	0.59a±0.03
B3	2.71d±0.05	1.08b±0.03	1.58c±0.02	94.82a±11.25	391a±8.7	0.64a±0.04
B4	2.56c±0.05	1.03b±0.02	1.57c±0.04	97.5a±4.70	438b±9.2	0.60a±0.05
B0	1.61a±0.03	0.82a±0.02	0.70a±0.03	84.4a±11.21	483c±4.20	1.09b±0.04

The mean values bearing different superscript differ significantly (P≤0.05).

[1](gdL^{-1}), [2] (gdL^{-1}), [3](gdL^{-1}), [4](U min^{-1}mg^{-1}protein), [5]colony count.

Table 3: Percentage of survival after challenge study with Pathogens.

A *Aeromonas spp.*

Experimental Groups	*Aeromonas spp.*
B0	48.10±5.2
B1	82.50±3.2
B2	80.57±3.1
B3	85.90±1.4
B4	75.40±3.2
B4	72.40±3.2

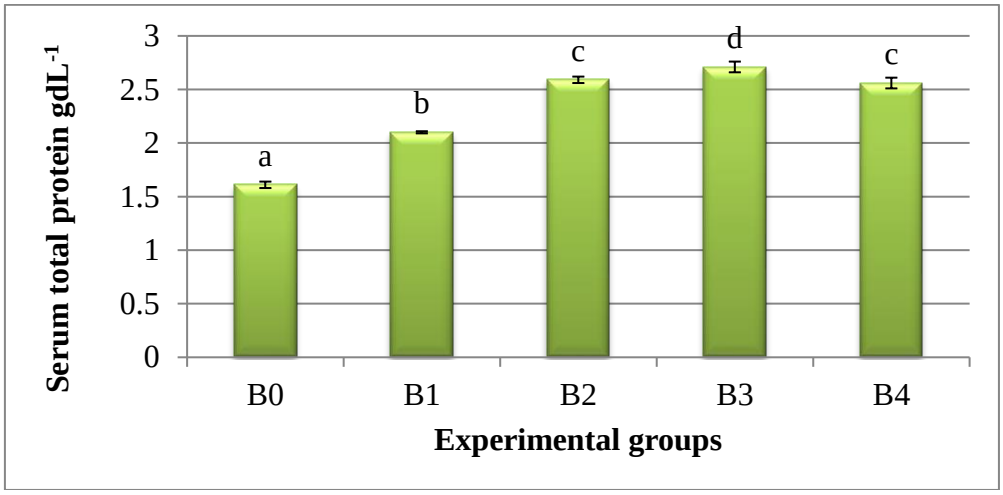


FIG 1: Serum total protein content (gdL^{-}) of *Channa marulius* fingerlings of different experimental groups against *Aeromonas spp*.

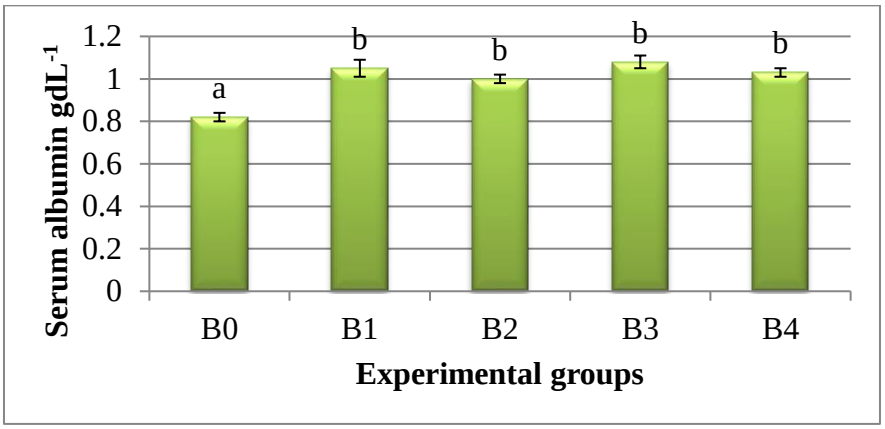


FIGURE 2: Serum albumin and globulin content (gdL^{-1}) of *Channa marulius* fingerlings of different experimental groups against *Aeromonas spp*.

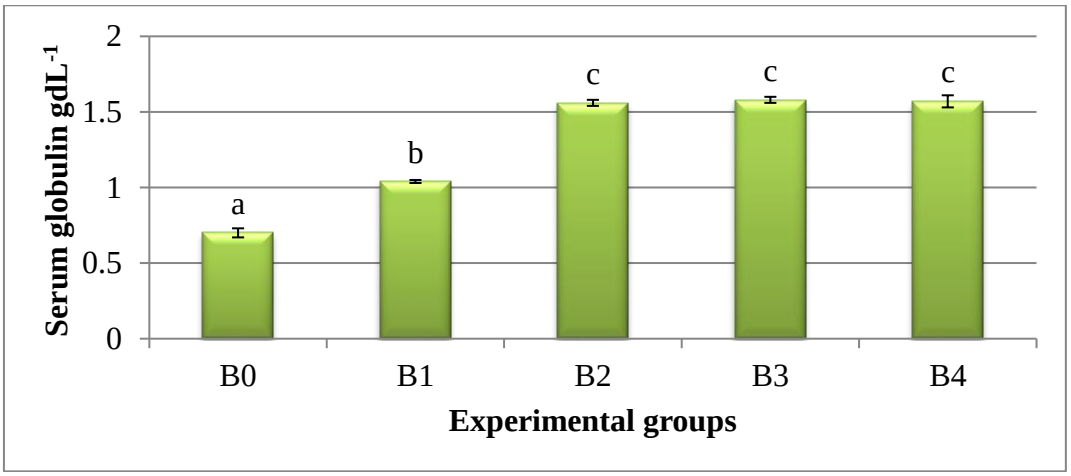


FIG.3: Percentage survivability of *Channa marulius* fingerlings of different experimental groups against *Aeromonas spp.*

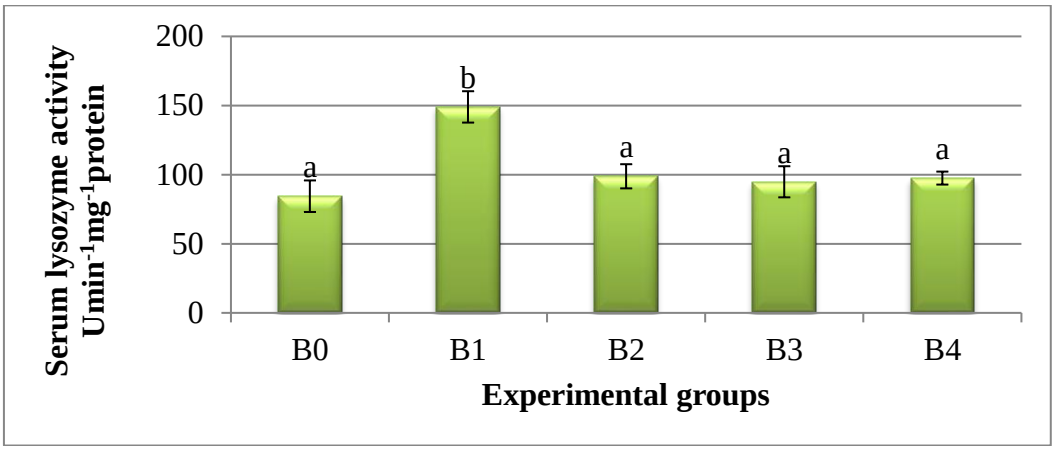


FIG.4: Serum Lysozyme activity (U $min^{-1}mg^{-1}$protein) of *Channa marulius* fingerlings of different experimental groups against *Aeromonas spp*.

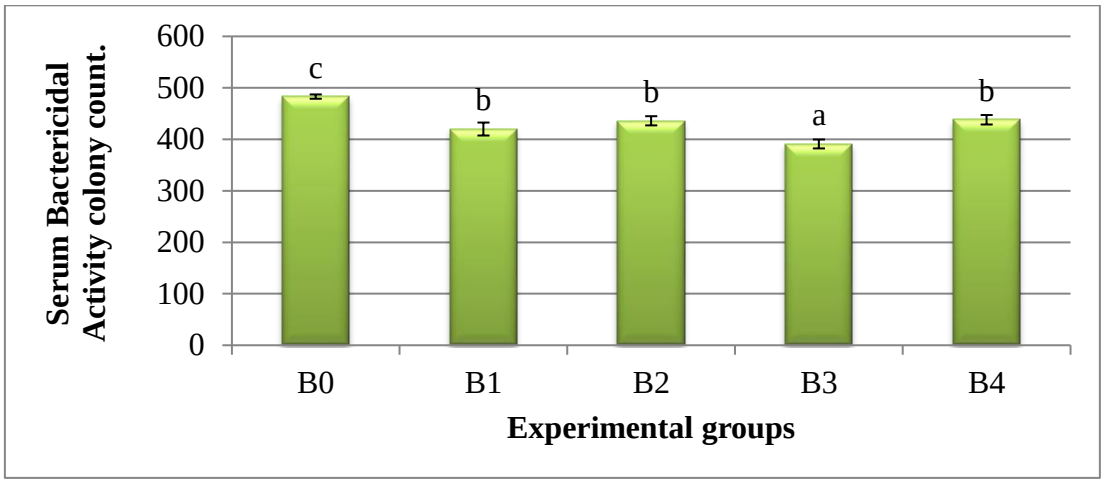


FIG.5: Serum bactericidal activity (mean colony count) of *Channa marulius* fingerlings of different experimental groups against *Aeromonas spp*.

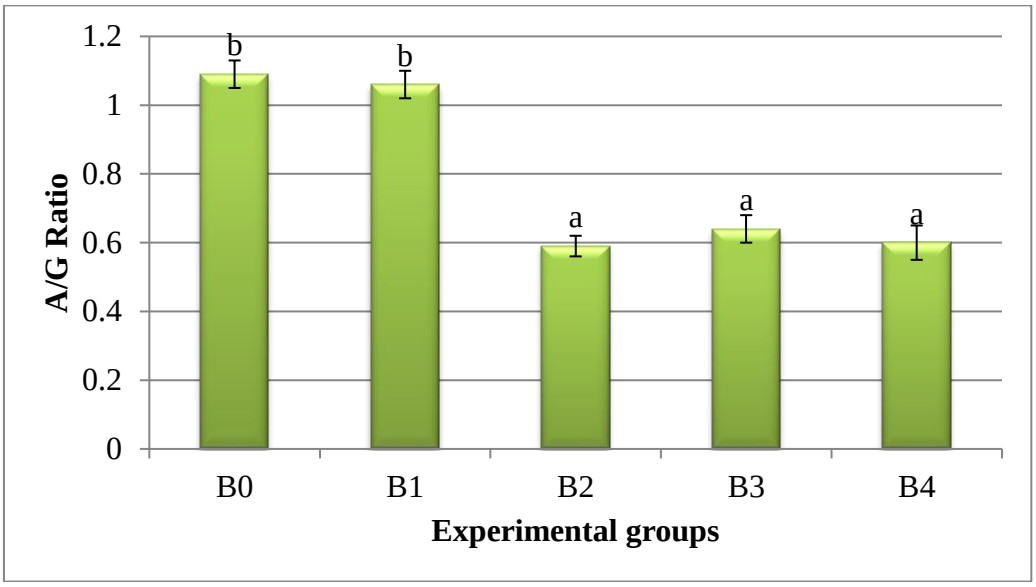
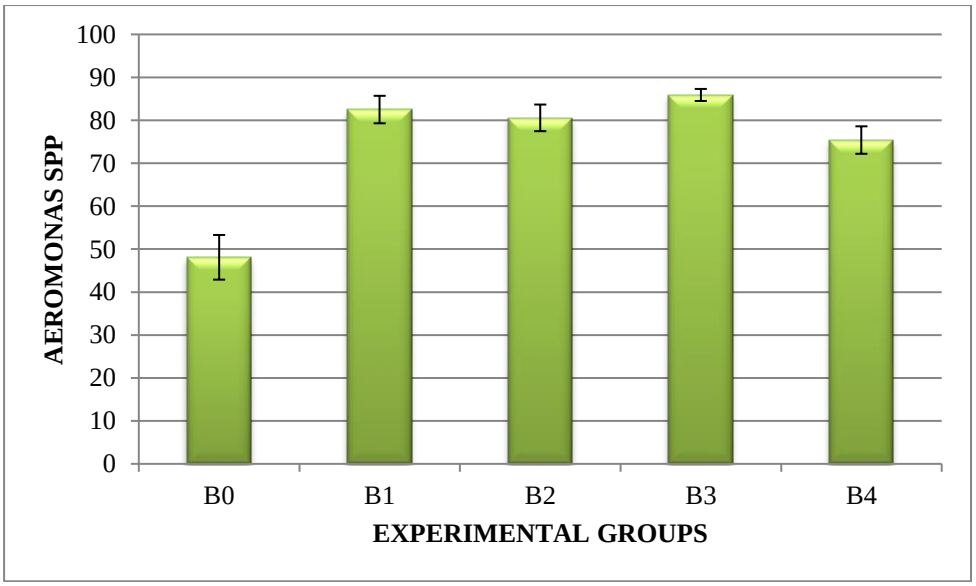


FIG.6: Serum A/G Ratio of *Channa marulius* fingerlings of different experimental groups against *Aeromonas spp*.



7 : Percentage survival after challenge study with *Aeromonas spp.*