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Efficacy of *Aeromonas hydrophila* Vaccine on Biochemical Parameters of Freshwater Fish *Channa striatus*

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Abstract: The experimental fish *Channa striatus* was divided as control and experimental groups. To find out the efficacy of *Aeromonas hydrophila* vaccine, the fish were infected with Gram negative Bacterial Strain *Aeromonas hydrophila* with a dosage of 0.1 ml. The samples of blood were collected on the 1st, 3rd, 5th, 7th and 14th day intervals. The serum was evaluated for the biochemical parameters such as Protein, Albumin and Globulin, ratio of Albumin and Globulin and blood glucose level. SDS PAGE analysis was made to find out different protein fractions in the blood sample of fish which was treated earlier with *Aeromonas hydrophila*. Another set of fish was treated with heat killed and formalin (chemically) killed attenuated vaccine of *A. hydrophila*. Then the vaccinated fish was post challenged by infected with *A. hydrophila* to find out the efficacy of the vaccine. The results were tabulated and statistically analyzed. The present investigation revealed that the administration of vaccines could enhance the fish defense mechanisms. However, the heat killed (HK) vaccine showed great impact on improving the health condition of fish by enhancing its resistance capacity against pathogenic bacteria.

Keywords: *Aeromonas hydrophila*, Vaccine, Biochemical parameters, SDS PAGE

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Introduction

Aquaculture represents one of the fast growing food producing sectors of the world and aims to increase productivity of fish which is a major source of palatable protein. Stephen and

Ananthraja (2006) revealed that large scale mortalities of fish were found in aquaculture due to microbial and parasitic infections which have been resulted from retaining high dense stock of

fish or environmental stress. Among various kinds of diseases, the Epizootic Ulcerative Syndrome (EUS) is a very common disease found in fishes. Sniesko (1974) found that infectious disease occurs when a susceptible host is exposed to virulent pathogen under stress. From the descriptions of fish diseases in the early scientific literature Otte (1963) speculated that septicemia infections in fish caused by motile aeromonads were common throughout Europe during the Middle Ages.

Aeromonas hydrophila is a motile, gram negative bacillus. This bacterium is an opportunistic, free living and may infect many species of freshwater and brackish water fishes (Elliot and Schotts, 1980). Among various kinds of bacteria identified as pathogens, *A. hydrophila* is considered to be one of the foremost economically important pathogen, because of its infection can lead to growth reduction and unmarketable appearance of infected fish (Na-Nakron *et al.*, 1995). Heat or formalin inactivated bacterin vaccine introduced either through injection or immersion also provides protection against *A. hydrophila* (Chandran *et al.*, 2002).

Haematopoietic and lymphoid tissues are the basis of the immunologic response in animals. The component of serum includes albumin, lipoproteins, alpha, beta and gamma globulins, serum enzymes and antibodies which are used as parameters in the diagnosis of fish diseases (Finn and Nielsen, 1971).

The prime objectives of this present study were designed to determine the pathogenicity of bacteria to host fish and preventing the fish population susceptible to microbial pathogen. Hence, vaccine was administered to the fish for detecting the efficacy of the prepared vaccine by challenging the fish against *A. hydrophila*. This study provides necessary steps to be undertaken to improve the health condition of economically important fish fauna.

Materials and Methods

The healthy striped murels *Channa striatus* (20±5

cm; 230 ± 20 g) were collected from in and around Melapalayam, Tirunelveli District, and Tamil Nadu, India. The live fish were transported to the laboratory and they were allowed to acclimatize to laboratory condition and fed with commercial balanced feed supplemented with chopped meat for 2 weeks prior to experiments. The water was cleaned every third day by siphoning off two-thirds of water and replacing it with freshwater. The fish were maintained at a stocking density of ten fish in each container.

Microbial Technique:

Collection and Preparation of Bacterial Antigen:

Aeromonas hydrophila was commercially procured from IMTEC, MMTC in Chandigarh. In order to prepare the bacterins, each bacterial isolate was inoculated separately into tryptic soy broth (TSB) and incubated for 24 h at 25°C. The test culture was allowed to grow in 100 ml broth for 24 h at 37°C. The entire culture was centrifuged at 10,000 rpm for 15 min. The bacterial pellet was washed in phosphate buffer saline (PBS) and again the pellet was resuspended in the same buffer solution.

Microbial Serial Dilution:

The sample was serially diluted until it came to 30-300 colony forming units (CFU) on the culture plates. A serial dilution technique was adopted which enabled to get countable colonies on the samples. Typical dilutions are 10-fold dilutions; each dilution is 1/10th the concentration of the previous dilution. The samples were diluted by using standard volume of 9 ml of distilled water as diluents in a series of 10² to 10¹⁰ CFU of *Aeromonas hydrophila* was used for short experimental studies to note the serological parameters and the experimental fish were introduced. 0.1 ml of each dilution was injected intraperitoneally and the total viable count (TVC) in the injected samples was taken by plating onto nutrient agar. Mortality rate was observed from 18 h to 7 days and the degree of virulence (LD50) was identified in the dilution resulted in 50% mortalities of experimental animal.

Preparation of Vaccine:

Formalin (chemically) killed vaccine (FK/CK) and Heat killed (HK) vaccines were prepared from *A. hydrophila* by the procedure described by Baxa (1988). Formalin (37w/v) was added to the broth culture at a final concentration of 0.5%v/v and left for 48 h at room temperature. The heat-killed vaccine was prepared by heating the broth culture for 30 min at 100°C. After that the bacterins were tested for their sterility (free from the living cells) by antibiotic sensitivity test.

Experimental Design:

The selected dose of 10^5 CFU dilutions of *Aeromonas hydrophila* was taken for serological studies to find out the changes in biochemical parameters in the experimental fish. The treatments consisted of unvaccinated and vaccinated fish. The vaccination was done by injecting (0.0l ml g⁻¹ fish) the selected dose group. A minimum of ten uniform sized fish were kept in captivity. Experimental and control fish were kept over a period of 14 days for evaluating the changes in serological parameters. Observation was carried out to find out the rate of infestation in the experimental fish initially and after 1st day, 3rd day, 5th day, 7th day and 14th day.

Biochemical Analysis:

Serum Separation:

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

Serum total Protein Estimation:

The total serum protein was estimated by Biuret method. 0.01 ml of serum was added to 1 ml Biuret reagent, mixed well and let stand for 3 min and the optical density was read on Spectrophotometer at 545 nm wave length. The standard was prepared by adding 0.01 ml of total

protein standard with 1 ml of Biuret reagent. The amount was calculated using following formula:

$$\text{g Total Protein/dl} = \frac{\text{Sample OD}}{\text{Standard OD}} \times 5$$

Serum Albumin Estimation:

The serum albumin content was estimated by BCG method. 0.01 ml of serum was added to 1 ml of BCG Reagent, mixed well and allowed to stand for 5 min at room temperature and the optical density was read on a Spectrophotometer at 630 nm wave length. The standard was prepared by adding 0.01 ml of Albumin standard with 1 ml of BCG reagent. The amount was calculated using following formula:

$$\text{g Albumin/dl} = \frac{\text{Sample OD}}{\text{Standard OD}} \times 5$$

Serum Globulin Estimation:

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 (A/G ratio):

Albumin globulin ratio was calculated by dividing the albumin concentration with globulin concentration.

$$\text{A/G ratio} = \frac{\text{Albumin}}{\text{Globulin}}$$

Blood Glucose:

The blood glucose was estimated by GOD-POD method using Glucometer. One drop of fresh blood was allowed to flood the Glucocard 01 strip and the reading was noted from the glucometer directly. The results obtained were statistically analysed using the following formulae:

$$\% \text{ Change} = \frac{\text{Experimental} - \text{Control}}{\text{Control}} \times 100$$

Statistical analysis:

The results obtained for blood parameters were analyzed statistically by One way ANOVA using SPSS (16) Software.

SDS-PAGE analysis:

The serum proteins of all group fishes were electrophoretically separated by SDS- PAGE and the Electrophoretogram was analysed densitometrically. The basic methodology adopted was as described by Laemmli *et al.* (1970) with some modifications. Standardization of the technique was performed as the percentage of separating gel is a critical parameter in all electrophoretic separations of different proteins in the sample. Separating gels of 12.5%, 11.5% and 11% were tried to choose an ideal percentage, which gives a better electrophoretic separation.

The separating gel components were mixed gently and poured into the prepared cassette. Few drops of butanol were over layered to prevent meniscus formation and the gel was left undisturbed to set for 30 min. After polymerization of the separating gel, the overlaying butanol was removed and the cassette was washed with double distilled water and dried. The prepared stacking gel mixture was then poured over the separating gel. The comb was placed in the stacking gel and allowed to set for 30 min.

After the gel got solidified, the comb was removed without distorting the shape of the well. The electrode buffer was added to the tanks and the electrodes were then connected to the power pack.

Sample application and electrophoresis:

Serum stored at -20°C was brought to room temperature. 10 µl of the sample was mixed with 90 µl of distilled water. 50 µl of this mixture was then mixed with 50 µl of sample buffer with SDS and boiled for 1 min. 10 µl of protein molecular weight marker was mixed with 60 µl of sample with 2% SDS and boiled for 1 min. The prepared samples were applied into the wells of the stacking gel and layered with running buffer in order to avoid disturbance to the sample. A constant voltage of 60 volts was applied until the dye front crossed the stacking gel and it was increased to 140 volts and electrophoresis was

continued until the dye front reached the bottom of the gel.

Staining the gels:

Immediately after the completion of electrophoresis, the gels were carefully separated from the trays, transferred into plastic trays and washed in tap water to remove excess SOS. After staining the gels for 2 h in Coomassie Brilliant Blue R 250, the excess stains were washed off and the gels were immersed in destaining solution.

Determination of Molecular Weight:

Molecular weight of standards used as molecular markers for SDS PAGE were 205000, 97400, 66000, 43000, 29000, 20100, 14300, 6500, 3000 Daltons. Rf values of the standard markers were calculated by using the following formula:

Distance moved by the solute Rf (Relative Front) =
Distance moved by the dye

The Rf values, molecular weight and the amount of the protein present in each protein fraction was analyzed using densitometer.

Results

In the present study the sample of blood was collected periodically on the 1st, 3rd, 5th, 7th and 14th day from the experimental fish *Channa striatus* to evaluate the biochemical parameters when the fish was exposed to 10⁻⁵ CFU dose of *Aeromonas hydrophila*.

Biochemical parameters:

The biochemical parameters such as serum total protein, Albumin, Globulin, Albumin and Globulin ratio (A/G), and blood glucose were determined in *Channa striatus* exposed to *A. hydrophila* bacterin and compared with the control (Table 1).

The mean value of serum protein, globulin and blood glucose showed decreasing trend in the *Aeromonas* infected group except on the 1st day and both 1st and 3rd day for blood glucose (Table 1). Likewise the albumin values also decreased in the later part of the experimental period but

Table 1: Biochemical parameters of control and *Aeromonas* infected fish *Channa striatus*

Parameters	Days	Control	<i>Aeromonas</i> injected	HK vaccinated	HK post challenged	FK vaccinated	FK post challenged
Protein(g/dl)	1	2.2±0.02 ^{ab}	2.6±0.006 ^b	2.2±0.01 ^a	2.3±0.005 ^a	2.1±0.0005 ^a	2.2±0.05 ^a
	3	2.3±0.01 ^{ab}	2.1±0.03 ^{ab}	2.2±0.02 ^a	2.5±0.005 ^{ab}	2.3±0.03 ^a	2.5±0.07 ^{ab}
	5	2.0±0.02 ^{ab}	1.9±0.007 ^{ab}	2.5±0.02 ^{ab}	2.6±0.02 ^{ab}	2.5±0.012 ^{ab}	2.7.0±0.03 ^{ab}
	7	2.5±0.006 ^{ab}	1.5±0.01 ^b	2.7±0.01 ^{ab}	3.1±0.03 ^b	2.8±0.01 ^b	2.8±0.02 ^{ab}
	14	2.6±0.007 ^b	1.2±0.002 ^a	2.9±0.01 ^b	2.6±0.006 ^{ab}	3±0.012 ^b	3.0±0.011 ^b
Albumin(g/dl)	1	1.0±0.004 ^b	1.2±0.001 ^b	1.0±0.002 ^a	1.0±0.007 ^a	0.9±0.002 ^a	1.0±0.003 ^a
	3	1.0±0.003 ^b	1.0±0.006 ^b	1.0±0.003 ^a	1.1±0.001 ^{ab}	1.0±0.003 ^a	1.1±0.004 ^{ab}
	5	0.9±0.002 ^{ab}	0.9±0.003 ^{ab}	1.1±0.003 ^{ab}	1.2±0.005 ^b	1.1±0.005 ^{ab}	1.2±0.002 ^b
	7	1.2±0.002 ^b	0.6±0.007 ^a	1.0±0.001 ^a	1.3±0.003 ^b	1.2±0.004 ^{ab}	1.2±0.004 ^b
	14	1.2±0.001 ^b	0.4±0.003 ^a	1.2±0.002 ^{ab}	1.2±0.006 ^b	1.3±0.003 ^b	1.3±0.005 ^b
Globulin(g/dl)	1	1.2±0.003 ^{ab}	1.4±0.003 ^b	1.2±0.003 ^a	1.3±0.004 ^{ab}	1.2±0.004 ^a	1.2±0.005 ^a
	3	1.3±0.005 ^b	1.1±0.002 ^{ab}	1.2±0.002 ^a	1.4±0.005 ^{ab}	1.3±0.006 ^{ac}	1.4±0.003 ^{ab}
	5	1.1±0.002 ^{ab}	1.0±0.001 ^{ab}	1.4±0.002 ^{ab}	1.4±0.005 ^{ab}	1.4±0.002 ^{ab}	1.5±0.004 ^{ab}
	7	1.3±0.002 ^b	0.9±0.001 ^a	1.1±0.001 ^a	1.8±0.002 ^b	1.6±0.004 ^b	1.6±0.005 ^b
	14	1.4±0.004 ^b	0.7±0.004 ^a	1.7±0.004 ^c	1.4±0.004 ^{ab}	1.7±0.002 ^c	1.7±0.006 ^b
A/G ratio	1	0.83±0.002 ^{ab}	0.85±0.003 ^{ab}	0.83±0.003 ^b	0.76±0.006 ^b	0.75±0.005 ^{ab}	0.83±0.004 ^c
	3	0.76±0.004 ^{ab}	0.9±0.002 ^b	0.83±0.002 ^b	0.78±0.004 ^b	0.76±0.005 ^{ab}	0.78±0.002 ^b
	5	0.81±0.002 ^{ab}	0.9±0.001 ^b	0.78±0.002 ^{ab}	0.85±0.005 ^c	0.71±0.002 ^{ab}	0.8±0.004 ^c
	7	0.92±0.001 ^b	0.66±0.003 ^a	0.9±0.001 ^b	0.72±0.002 ^b	0.75±0.003 ^{ab}	0.75±0.004 ^b
	14	0.66±0.003 ^a	0.57±0.004 ^a	0.58±0.003 ^a	0.85±0.005 ^c	0.76±0.002 ^{ab}	0.76±0.005 ^b
Blood glucose (mg/dl)	1	12.5±0.9 ^{ab}	13.3±0.11 ^b	14.5±0.03 ^c	13.8±1.03 ^b	14.0±1.2 ^c	13.2±0.98 ^{ab}
	3	12.0±0.92 ^{ab}	12.3±0.32 ^{ab}	13.5±0.13 ^{bc}	12.9±0.8 ^{ab}	13.5±0.9b ^c	13.0±1.1 ^{ab}
	5	12.5±0.54 ^{ab}	11.2±0.7 ^a	12.9±0.12 ^b	12.8±0.9 ^{ab}	12.4±0.95 ^{ab}	12.7±0.93 ^{ab}
	7	13.4±0.5 ^b	11.1±0.5 ^a	13.3±0.14 ^{bc}	12.5±0.76 ^a	12.6±1.1 ^{ab}	12.9±1.02 ^{ab}
	14	13.2±0.6 ^b	10.4±0.33 ^a	13.5±0.2 ^{bc}	13±1.1 ^{ab}	12.9±1.1b ^c	14.2±1.06 ^b

Each value represents Mean±SD; Means with same superscript in the same column is not statistically significant

slightly increased on the 1st day and no changes found between controls and infected on 3rd and 5th day. The A/G ratio elevated on the 1st, 3rd and 5th day but it was less on the 7th and 14th day of experimental period.

The mean values of protein in the HK vaccinated group showed increasing trend from 5th day up to the 14th day of experimental period as compared with the control whereas the values of albumin and globulin showed fluctuation between the control and the HK vaccinated group (Table 1). The value of A/G ratio declined from 5th day up to the 14th day of experimental period. But on the contrary the blood glucose value elevated in the beginning and showed fluctuation in the later part of experiment. Not much significant variation was found between the control and the HK vaccine administered fish.

In the FK vaccinated group the value of protein

increased from the 5th day up to the 14th day of experimental period but it was less on the 1st day and similar to that of control on 3rd day. The value of albumin increased on 5th and 14th day but same values as to that of control on 3rd and 7th day; and reduced on the 1st day. The value of globulin was elevated from 5th day up to the day of 14th day but no change was found on 1st and 3rd day (Table 1). A/G ratio showed fluctuation but the value of blood glucose increased in the beginning and showed decreasing trend from day 5th up to the 14th day of experiment. The values of serum total protein, albumin and globulin were slightly fluctuated between the HK vaccinated and the FK vaccinated but slightly increased in later part of experimental period. Not much significant variation was found in the mean values of serum protein, albumin and globulin between HK and FK vaccinated groups. The A/G ratio was found to have slightly higher in HK vaccinated than the FK

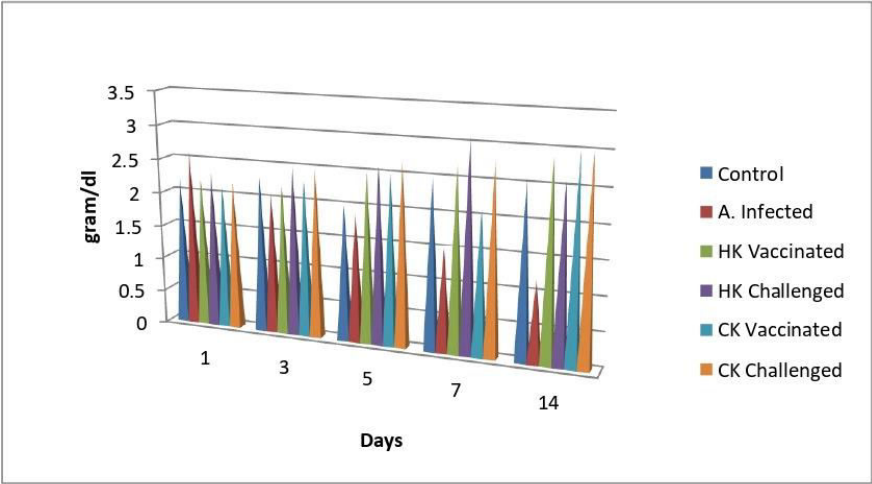


Fig. 1: Serum protein.

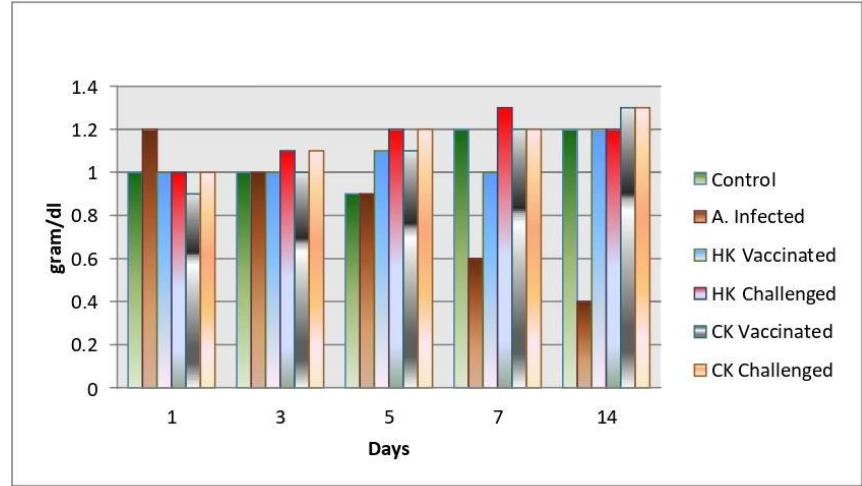


Fig. 2: Serum albumin.

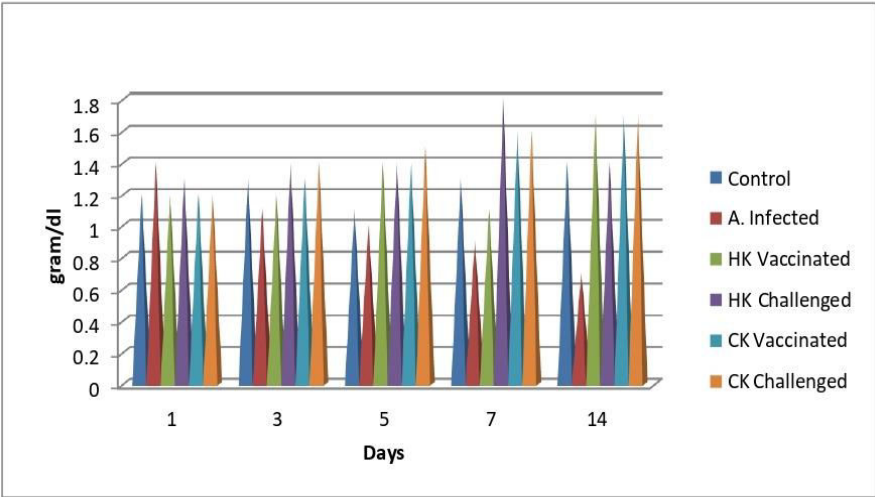


Fig. 3: Serum globulin.

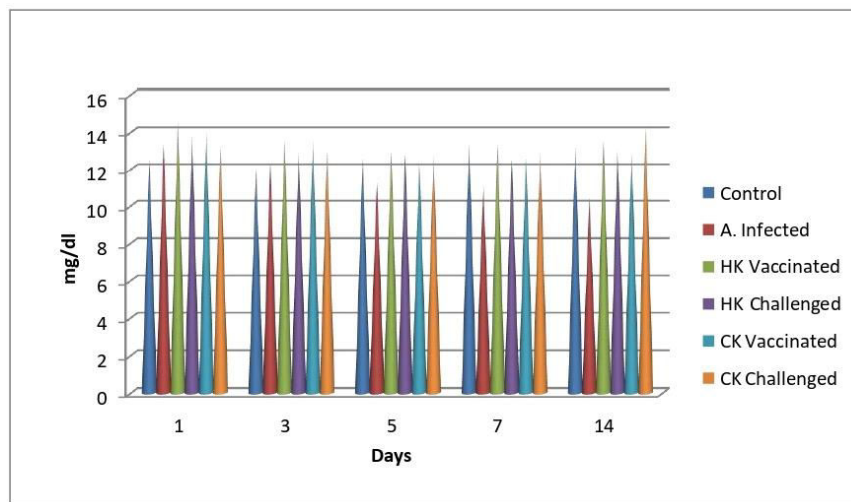


Fig. 4: Blood glucose.

but it was less particularly on the 14th day. Likewise the blood glucose level was also higher in HK vaccinated than the FK vaccinated groups. The values of all parameters were not much deviated from each other and reported to have values close to that of control. But no significant changes between HK and FK vaccinated group.

When the HK vaccinated group challenged with *A. hydrophila* the mean value of protein in challenged group showed declining trend from the 1st to 7th day and slightly increased on the 14th day. The values of albumin and globulin showed increasing trend in the challenged group. The value of A/G was less in the beginning part of the experiment but later it was higher in the challenged than the control group. The blood glucose value slightly declined in the challenged group from the 1st day up to the end of the experiment. When the comparison made between the FK vaccinated and challenged all parameters showed to have fluctuation and only a slight differences were found between the two groups. The mean value of protein, albumin, globulin and A/G ratio were slightly high in the FK challenged group and the blood glucose value showed fluctuation between two groups.

The values of protein, albumin and globulin were higher in the FK challenged group and but only a slight variation was found between HK and

FK challenged groups. But HK group showed the highest range of total protein value 3.1 ± 0.003 . Similarly the globulin value was 1.8 ± 0.002 in HK challenged but it was about 1.7 ± 0.006 in FK challenged fish. A/G also showed high value 0.85 ± 0.005 in the HK challenged compared with the FK challenged fish. Similarly the blood glucose values were also higher in the HK challenged than the FK challenged groups.

The overall comparison of tabulated mean values of Biochemical parameters such as total protein, Albumin, Globulin and blood glucose value of all groups are illustrated in Figures 1-4.

SDS-PAGE Analysis:

Serum consists of albumin, various forms of globulins such as alfa (α), Beta (β) and gamma (γ). The serological analysis was made in *Channa striatus* and these components are used as important parameters to recognize the health status of the fish. The Electrophoretic study was carried out in both control and experimental fish groups. The photographic picture of the electropherogram is given in Figure 5.

Visual observation of the electropherograms appeared as bands but they were expressed as lane 1, lane 2, lane 3, lane 4, lane 5, and lane 6 which represented as control, *Aeromonas* infected, heat killed vaccinated, heat killed challenged, chemically killed vaccinated and chemically killed

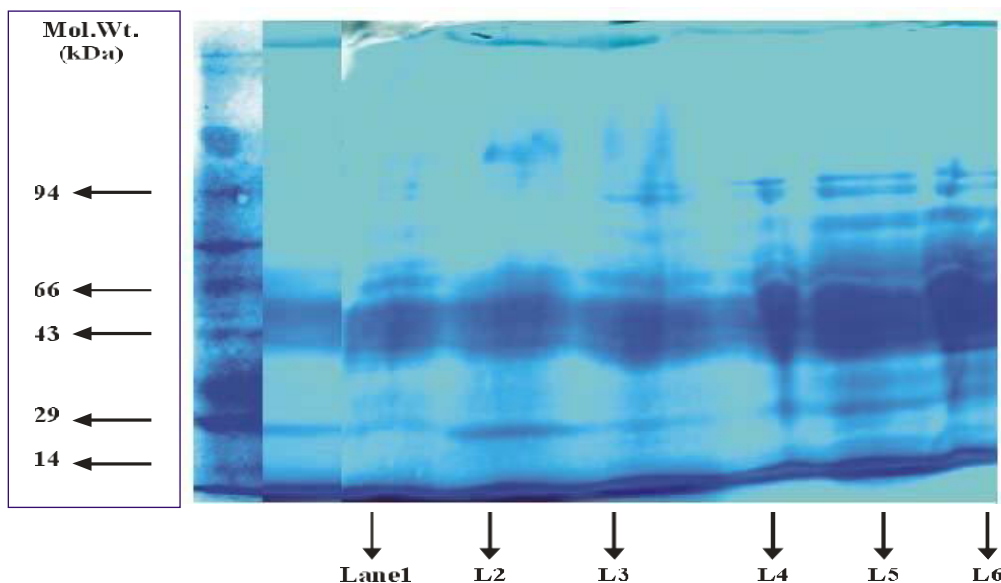


Fig. 5: Protein profile of the experimental and control serum of *Channa striatus*. Lane 1 – Control; Lane 2 - *Aeromonas* injected; Lane 3 - Heat Killed (HK) vaccinated; Lane 4 - HK vaccinated post challenged with *Aeromonas*; Lane 5 – Formalin killed (FK) vaccinated; Lane 6 - FK vaccinated challenged with *Aeromonas*.

challenged with *Aeromonas*, respectively. It indicated the production of humoral antibodies in the sera on the 5th day of experimental period.

When the serum was analysed electrophoretically for the presence of different protein fractions, *Aeromonas hydrophila* (lane 2) showed 29 kDa proteins as prominent one, whereas these were absent or less stained band in the control. Higher molecular weight protein 80 kDa was absent in the *Aeromonas hydrophila* treated whereas these were very prominent in all the experimental and control serum.

Figure 5 shows SDS – PAGE patterns of serum proteins profile of control and experimental fish *C. striatus* exposed to 10⁻⁵ CFU doses of *Aeromonas hydrophila* on 5th day and it was compared with the Molecular Weight Marker (MWM).

Discussion

As the aquaculture industry expands, tools to monitor the health status of fish using standardized and inexpensive methods are needed. Evaluation of hematological parameters facilitate early detection of infectious disease and

identification of sub-lethal conditions affecting production performance. This will contribute to more specific, timely and effective disease management in future.

Infections caused by members of the genus *Aeromonas*, which have a relatively high antibiotic resistance, are among the most common bacterial diseases of fish (Saavedra *et al.*, 2004). *A. hydrophila* is one of the most common aerobic heterotrophic bacteria in freshwater and occasionally in the marine environment affecting fish (Lilley *et al.*, 1997; Cipriano *et al.*, 2001; Pianetti *et al.*, 2005). The organism has been associated with a range of diseases in fish and humans; the latter via contaminated food or drink (Handfield *et al.*, 1996) or sometimes by direct contact with contaminated water.

In the present study *A. hydrophila* was proved to be pathogenic after the fish was injected with doses of *A. hydrophila*. 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). Serum albumin not only maintains osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues but also acts as plasma carrier protein to transport steroid hormones, hemin, fatty acids and also compounds like drugs (Asadi *et al.*, 2012).

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 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 (Halmy *et al.*, 1974).

In the present work the total protein level increased in the fish after challenged with *A. hydrophila*. Since bactericidal proteins have enhanced, the serum biochemical parameters such as protein, albumin, globulin and A/G ratio were determined to prove the increased protective protein production after a challenge. Among total serum proteins, globulins correspond to proteins present in blood responsible for the organism's defense, such as immunoglobulins, proteins of the complement activated by alternative pathways, acute phase proteins, cytokines, lysozyme, transferrin and lectins. In the present study the increase in total serum protein and globulin indicates the increase in protective proteins after the challenge and can be correlated to the serum bactericidal activity (Murray and Fletcher, 1976; Arason, 1996; Ellis 1999, 2001; Magnadottir, 2006; Maqsood *et al.*, 2009).

In the present work an increase in the total protein of vaccinated fishes was observed that which might have been caused by the increment of total serum immunoglobulins. The increase of these parameters together with the increased agglutination titer of IP vaccinated fish indicated an enhancement in the specific humoral immune response for the vaccinated fishes

(Kaattaru and Piganelli, 1996). Tu *et al.* (2010) also observed increased immunoglobulin in the serum of Nile tilapia against *A. hydrophila* between fourth and seventh week post immunization.

It has been reported that production of specific antibodies and lymphocytes responded well to the bacterial vaccines (Austin and Adams, 1996). Enhancement of protective specific immunity may be reflected as a result of change in serum protein composition. Accordance with the report of Rahim Peyghan *et al.* (2010) the results of present work also showed that in intraperitoneal injection of killed bacteria, total albumin as well as the ratio of albumin/globulins was significantly increased than the infected group. Because of the high osmotic pressure produced by albumin relatively low molecular weight and rather high concentration in the blood stream, the albumin fraction exerts more influence on plasma volume than any other plasma protein. Hence, condition of hyperalbuminemia is rarely seen except in the presence of acute dehydration and shock. Although certain pathological conditions that may lead to hypoalbuminemia in control are deficient intake of protein, deficient synthesis of albumin by liver and/or excessive breakdown or loss of albumin.

Rahim *et al.* (2014) classified the SDS-PAGE serum proteins in grass carp (*Ctenopharyngodon idella*) into 6 protein fractions such as pre-albumin, albumin, alpha-1, alpha-2, beta and gamma globulins from low to heavy molecular weight. The new protein fractions with moderate molecular weight appeared in the vaccinated fish may indicate enhancement in the humoral immunity.

On the other hand, reduced glucose concentration either before or after challenge corroborated the findings of Harikrishnan *et al.* (2003). They found an increase in the glucose concentration in infected carp treated with herbal extract. The present results confirmed the findings of Barton (2000) who have reported that glucose concentration can increase in short periods in

order to supply the energetic demand in stress situations in fact after immunization and challenge. Increased glycemia in tilapia can be used as a stress factor, according to Martins *et al.* (2004). However, in this study, glucose was stable in all fish except in those submitted to vaccination on day 7. These indices were similar to those found in other studies with healthy Nile tilapia (Okamura *et al.*, 2007).

Conclusion

The present work was carried out to find out the impact of the gram negative bacteria *A. hydrophila* on influencing the serological parameters in the economically and commercially important fish *Channa striatus*. After exposure to *Aeromonas* bacterine the health status of the fish were recognized through Biochemical and Immunological studies by treating them with both Heat killed and chemically killed vaccine and had been post challenged with *A. hydrophila*. The findings of the present study revealed that the health status of the experimental fish has enhanced through vaccination. Electrophoretic studies of the blood serum of vaccinated fish and non-vaccinated fish show variation in the protein banding patterns which indicates the production of new proteins in form of antibodies as to play a specific immune responses. When the HK and FK vaccinated fishes were post challenged with *A. hydrophila*, the vaccination has enhanced the humoral immune response of the fish against pathogenicity.

However, the HK vaccine showed great impact on the improving the health condition of fish by boosting its resistance capacity against pathogenic bacteria. Hence, the administration of vaccines could enhance the fish defense mechanisms and induce both humeral and cellular responses as observed from the present investigation. Further the identification of such vaccines could be an alternative to antibiotics and chemotherapeutic drugs.

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