Haematological studies of Catla catla fish (Hamilton,1822) infected with Aeromonas hydrophila and Flavobacterium columnare.

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Abstract: The live freshwater *Catla catla* fish species were collected from Perupally of Khammam district of Telangana state.The present investigation deals with hematological variables in *Catla catla* infected by different pathogens such as *Aeromonas hydrophila* and *Flavobacterium columnare*. Each blood sample was examined for whole blood examination. It consists of Total Leucocyte count(WBC), Total Erythrocyte count(RBC), Haemoglobin content(Hb) Haematocrit(PCV), Leucocyte different count and blood indices such as MCV, MCH, MCHC. In haematological findings RBC, WBC, Hb, PCV parameters showed significant differences ($P<0.05$) with control. Significant difference was not observed in Erythrocyte indices between control and infected group.

Key words: RBC, WBC, Hb, Erythrocyte indices.

Introduction: Fish and fish related aquaculture research activities plays a major role in shaping the economic prosperity of India where a major part of the population is dependent on agriculture and farm based products for their livelihood. To carry out a fruitful aquaculture practice, maintenance of fish health is of prime concern as it has direct effect on the annual yield. Among various other factors governing fish production. Fish diseases have been gaining a great deal of importance with rapidly expanding of aquaculture and fish farming industry. Bacterial diseases are among the most important causes of economic losses in cultured fishes. *Aeromonas spp., Pseudomonas fluorescens, Vibrio anguillarum, Flavobacterium columnare , Edwardsiella tarda, Streptococcus spp.* and *Enterococcus sp.* are commonly found in aquaculture facilities (Joseph et al 1994,Plumb et al.,).They are often sub-clinical and without apparent signs. Under predisposing factors such as poor water quality, high ammonia as a result of high stocking density and feeding, ectoparasites, inadequate handling and stressful conditions, such microorganisms then found a portal of entry into the fish host (Moraes and Martins, 2004).

Haematological parameters are very important in determining health and physiological status of the fish (Clauss et al., 2008.). In addition, these parameters reflect the changes in the organism correctly and play an important role in the detection of disease and metabolism of fish living in different ecological environments (Cengizler and Şahan, 2000). This parameter is an important tool of diagnosis that reveals the state of health of the fish .Because of the variability of these clinical signs, the diagnosis of this disease when based solely on the clinical presentation of the fish is highly unreliable and can be economically disastrous to the fish producer. (Blaxhall, 1972;Rehulka,2002;Martins et al.,2004.).

Materials and methods:Hundred freshwater *Catla catla* infected with different bacterial infections were collected irrespective of age, sex and size from Perupally of Khammam district. Isolation and Identification of bacteria from infected *Catla Catla* fish was performed by

method of Obi and Krakowiaka (1983) and Bergey's manual of systematic bacteriology by Holt et al (1994). Blood samples were collected from caudal peduncle of both control and infected live fishes by dissecting in a tray separately. The blood was collected in EDTA tubes for further analysis .Red blood corpuscles (RBC) and White blood corpuscles (WBC) were estimated by Haemocytometer method (Rusia and Sood, 1992).Haemoglobin (Hb) was estimated by Haemoglobinometer according to Dacie JV and Levis S.M 1964), and Haematocrit (Hct) was estimated by Wintrobe tube method (wintrobe *et al*,1981). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using below equations (Dorafshan et., al 2008).

MCV (fl/cell) = PCV (%) ×10 cubic microns/RBC (10^6cells/cubic mm).

MCH (pg/cell) = Hb (g/dl) × 10 cubic microns/RBC (10^6cells/cubic mm).

MCHC (g/dl) = Hb (g/dl) ×100ml/PCV (%).

Results: The results of hematological parameters-Total red blood cell count(RBC),White blood cell count(WBC), Hemoglobin(Hb), Packed cell volume(PCV), Mean corpuscle volume(MCV), Mean corpuscle hemoglobin(MCH), Meancorpuscle haemo globin concentration(MCHC) in control and infected *catla catla* fish are presented in table 1.

S.NO	BLOOD PARAMETER	CONTROL	INFECTED
1	RBC	4.716±0.764	3.841±0.665*
2	WBC	9.033±1.658	13.733±2.290*
3	Hb	12.383±1.528	7.166±1.575*
4	PCV	46.333±3.932	33.166±1.834*
5	MCV	98.975±6.958	118.186±34.668NS
6	MCH	26.869±4.914	25.049±5.527NS
7	MCHC	26.904±3.260	21.803±6.020NS

All the results are expressed as Mean±Standard deviation. The variations in control and infected group were calculated by t-test.Comparision between control and infected group showed significant results *P<0.05, NS-not significant.

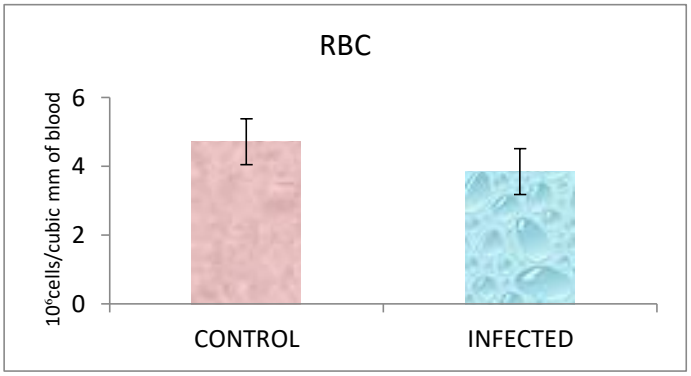


Figure1:Mean RBC (10^6cells/cu mm)in blood of fish *catla catla* both control and infected.

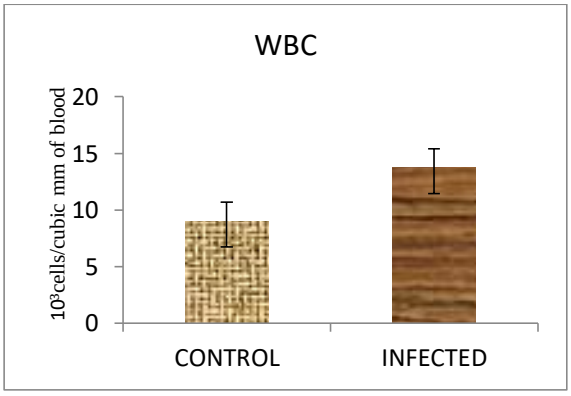


Figure 2:Mean WBC(10^3cells/cu mm)in blood of fish *Catla catla* both control and infected.

Figure 3:Mean Haemoglobin(gm/dl)in blood of fish *Catla catla* both control and infected.

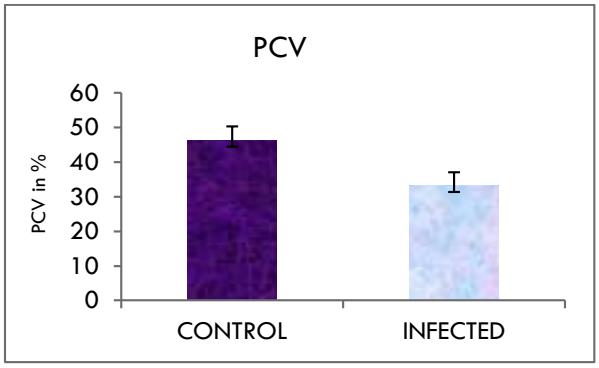


Figure 4:Mean PCV(%) in blood of fish *Catla catla* both control and infected.

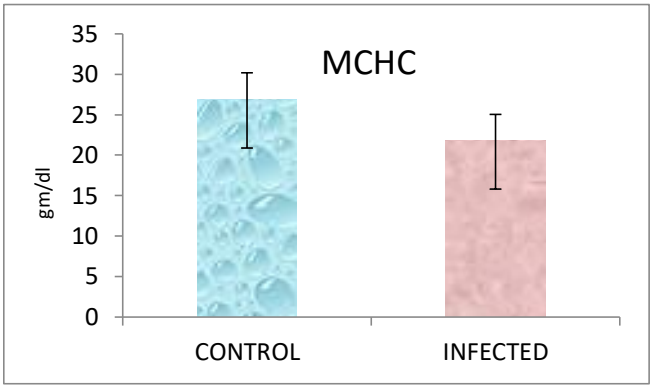


Figure 7:Mean MCHC(gm/dl) in blood of fish *Catla catla* both control and infected. .

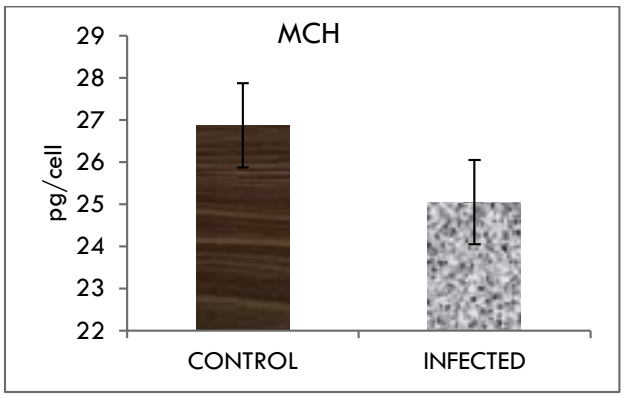


Figure 5: Mean MCH (Pg/cell)in blood of fish *Catla catla* both control and infected.

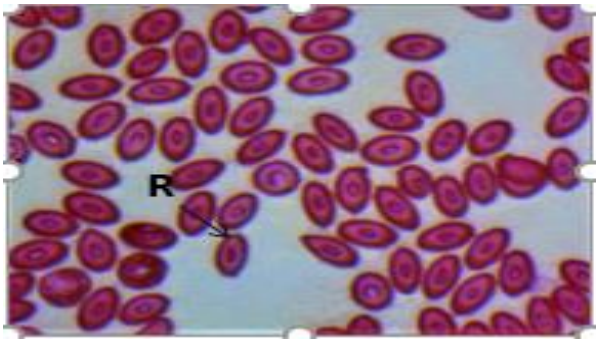


Figure 8:Blood cells of normal Catla catla fish May-Grunwald Giemsa stain 100X.R-RBC normal in size and shape.

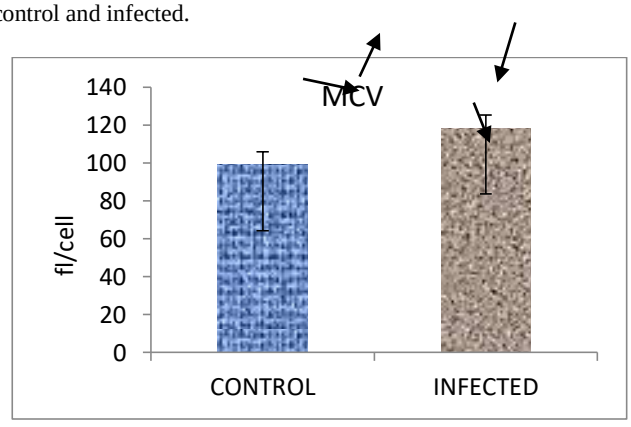


Figure 6: Mean MCV(fl/cell) in blood of fish *Catla catla* both control and infected.

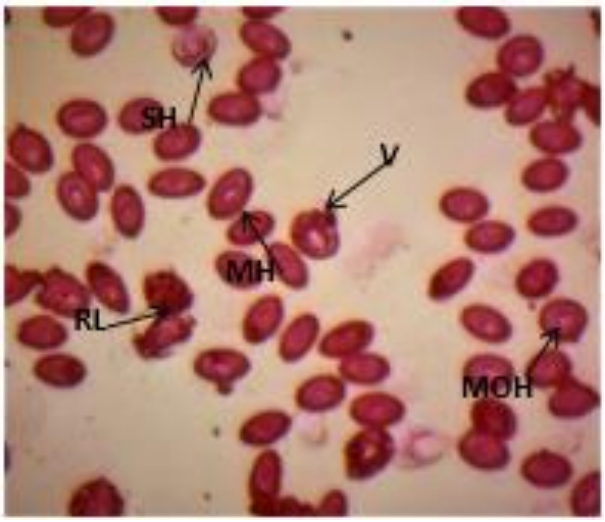
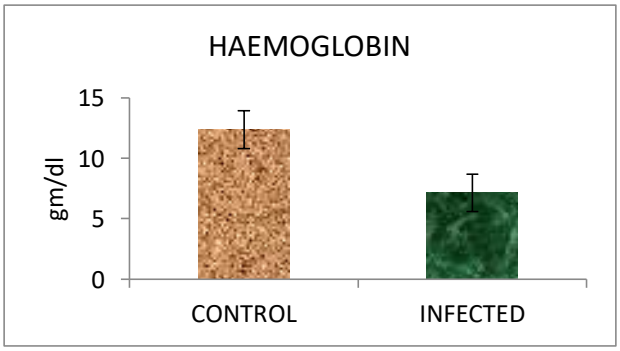


Figure 9:Blood cells of infected catla catla fish May-Grunwald Giemsa stain 100X.MH-mild hypochromia,MOH-moderate hypochromia,SH-severe hypochromia,RL-reactive lymphocyte,V-vacuolization in cytoplasm.



The total RBC count is expressed as 10^6cells/cubic millimeter of blood.The total RBC count showed significant changes in blood of fish infected with

Aeromonas hydrophila and *Flavobacterium columnare.The* total RBC value of control group fishes was calculated as(4.716±0.764).The total RBC values decreased slightly in bacterial infected fish(3.841±0.665)(Table 1, figure 1).

The WBC values are expressed as 10^3cells/cubic millimeter of blood.The total WBC count showed significant changes in blood of fish infected with *Aeromonas hydrophila* and *Flavobacterium columnare.The* total value of control group fishes was calculated as(9.033±1.658).The total WBC values increased significantly in bacterial infected fish(13.733±2.290)(Table 1,figure 2).

The Haemoglobin values are expressed in gm %.The haemoglobin value of control group was recorded as(12.383±1.528).The haemoglobin value decreased significantly in infected group(7.166±1.575)(Table 1,figure 3).

PCV is expressed as %.The PCV of control was found to be (46.333±3.932).The PCV value decreased significantly in infected group(33.166±1.834)(Table 1,figure 4).

The erythrocyte indices MCV is represented as fl/cell.The MCV of control group obtained was (98.975±6.958).MCV of the infected was group increased (118.186±34.668)(Table 1,figure 5).

MCH is expressed as Pg/cell.The MCH of control group obtained was (26.869±4.914).The MCH value decreased slightly in infected group (25.049±5.527)(Table 1,figure 6).

The erythrocyte indices MCHC is expressed in g/dl. MCHC of control group is recorded as (26.904±3.260).MCHC slightly decreased in the infected group (21.803±6.020)(Table 1,figure 7).

Erythrocytes or the red blood corpuscles are the major cellular elements found in the blood. The observations revealed that the structure of normal red cell or normocyte varies in shape from being oval or elliptical to biconcave disc or round (Fig. 8 & 9).

Discussion: The results of the present study revealed that RBC count significantly decreased in co-infected fish when compared to control fish group. The study of RBC count in combination with poor Hb mobilization from the spleen to other haemopoeitic organs resulted in decreased Hb and RBC levels due to hypochromic microcytic anemia of the fish infected with *Aeromonas hydrophila.*(Scott and Rogers 1981). The distinct decreased in the level of haemoglobin observed could induce anaemic condition in fry mortality syndrome in Iran,it clearly suggests that a hemodilution mechanism has occurred. The bacterial infection induce extravasation of blood and reduction of haemo-synthesis which in turn fails the hematopoietic tissue,to release the blood cell. Corresponding to the decrease of RBC count due to the bacterial infection, the hemoglobin percentage also exhibited a similar decrease in fishes. The level of Hb, values of PCV, MCH, MCHC and the number of RBC were significantly decreased in *Channa striatus* fish injected with *Aeromonas hydrophila*(Haniffa et al., 2011). The MCV gives an indication of the status or size of the erythrocytes and reflects an abnormal or normal cell division during erythropoiesis. The MCHC is a superior indicator of erythrocytes swelling (Wepener et al., 1992). The significant decrease in the MCHC in this study was an indication of erythrocytes swelling and/or due to a decrease in haemoglobin synthesis. Buckley et al. (1976) reported that prolonged reduction in hemoglobin content was deleterious to oxygen transport and any blood dyspraxia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants or infections.The bacterial infection influences the malfunctioning of hematopoietic system. WBC are the suspicious cells of the body .According to Douglass and Jane 2010, their levels have implications for immune response and the ability of the animal to fight infection. Increase in WBC count affords protection against infections caused by microbial agents.In carp experimentally infected with *Aeromonas hydrophila*, Harikrishnan

et al. (2003) have related increased WBC counts, confirming these results.

Statistics: The statistical analysis was done using IBM SPSS software.The values are expressed as mean±standard deviation(SD) and the data were analysed using students t-test.

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Conclusion: From the present investigation it can be concluded that *Aeromonas hydrophila* and *Flavobacterium columnare* are pathogenic to fish *Catla catla*.Bacterial infections cause variable degree of physiological adversities in the host fish and resulting effect can be evaluated in the blood components of the infected fish.The assessment of this blood parameters has always been helpful in detecting infection related diseases.

References:1.Blaxhall, P.C. & Daisley, K.W. (1973). Routine haematological methods for use with fish blood. Journal of Fish Biology, 5(6), 771781.

2.Buckley, J.A., Whitmore, C.M. and Matsuda, R.I., 1976. Changes in blood chemistry and blood cell morphology in coho salmon, Oncorhynchus kisutch following exposure to sublethal levels of total residual chlorine in municipal wastewater. Journal of Fish Research Board of Canada, 33:776-782.

3.Cengizler, İ. & Şahan, A. (2000). Seyhan Baraj Gölü ve Seyhan Nehrin de yaşayan aynalısazan (Cyprinus carpio, Linnaeus, 1758)' larda bazı kan parametrelerinin belirlenmesi. Turkish Journal of Veterinary and Animal Sciences, 24, 205-214.

4.Clauss, T.M., Alistair, D.M. & Arnold, J.E. (2008). Hematologic disorders of fish. Veterinary Clinics of North America: Exotic Animal Practice, 11(3), 445-462.

5.Douglass JW, Jane KW (eds.) (2010): Schalm s Veterinary Hematology. John Wiley and Sons, Blackwell Publishing Ltd. 1232.

6.Haniffa MA, Mydeen AK (2010). Hematological changes in Channa striatus Experimentally Infected by Aeromonas hydrophila. Bioresearch Bulletin, 4, 246-253.

7.Harikrishnan, R., Nisha, R. M., & Balasundaram, C. (2003). Hematological and biochemical parameters in common carp, Cyprinus carpio, following herbal treatment for Aeromonas hydrophila infection. Aquaculture,221(1), 41–50.

8.Joseph S and Carnahan. 1994. The isolation, identification and systematic of the motile Aeromonas species. Annual Review of the fish Diseases 4:315-343.

9. Martins ML, Tavares -Dias M, Fujimoto RY, Onaka EM and Nomura DT. 2004a. Haematological alterations of Leporinusmacro cephalus (Osteichthyes: Anostomidae) naturally infected by Goezialeporini (Nematoda: Anisakidae) in fish pond. Arq. Bras. Med. Vet. Zoot, 56, 5, p. 640-646.

10.Řehulka J (2002). Aeromonas causes severe skin lesions in rainbow trout (Oncorhynchus mykiss): clinical pathology, haematology, and biochemistry. Acta Veterinaria Brno, 71(3), 351-360.

11. Řehulka J, Minařík B (2007). Blood parameters in brook trout Salvelinus fontinalis (Mitchill, 1815), affected by columnaris disease. Aquaculture Research. 38(11), 1182-1197.

12.Plumb, J. A.(1981):Relationship of water quality and infectious diseases in cultured channel catfish, In"Fish pathogens and environment in European polyculture"(ed. by J. Olah, K. Molnar and Z. Jeney). Szarvas, pp.290-303.

13.Sovlo, A. and Nikinmaa, M., 1981. The swelling of erythrocytes in relation to the oxygen affinity of the blood of the rainbow trout Salmo

gairdnerl Richardson. In: Picketing AD (ed.) Stress and fish Academic Press, London, UK. pp.103-119.

14. Scott, A.L. and Rogers, W.A. 1981. Hematologicaleffects of prolonged sublethal hypoxia on channelcatfish Ictalurus punctatus (Rafinesque). J FishBiol. 18, 591-601.

15.Wepener, V., Van Vuren, J.H.J., and Du Preez, H.H., 1992. Effect of manganese and iron at a neutral and acidic pH on the hematology of the banded tilapia (Tilapia sparrmanii). Bulletin of Environmental Contamination and Toxicology, 49(4):613-619.

Evaluation Of Biochemical Parameters Of Catla Catla Co-Infected With Aeromonas Hydrophila And Flavobacterium Columnare.

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Abstract

The main core objective of the current study is to evaluate various biochemical parameters such as Total Carbohydrates, Total Glycogen, Total Proteins, Free amino acids and Total Lipids in various tissues of control and infected *catla catla* fish with *Aeromonas Hydrophila* and *Flavobacterium columnare.* Tissue samples from gill,liver,kidney,muscle and spleen were isolated and the bacteria was identified by morphological and biochemical characterisitics.The observed changes in these parameters may provide valuable information about adverse effect of bacterial infection in aquatic organisms.

Keywords: Isolation, Identification, Carbohydrates, Organs.

1. Introduction

The inland fisheries resources of India are noted as much for their heterogeneity in composition as for as their opulent productive potential. India is endowed with a vast expanse of open inland waters in the form of rivers, canals, estuaries, lagoons, reservoirs, lakes, ponds, tanks etc. In recent years traditional aquaculture has turned into a science based economic and commercial activity involving heavy inputs and therefore, diseases of all kinds are known to occur on an increasingly large scale. However, fish mortality is not the only criterion to evaluate the effect of fish disease. Even the morbidity which leads to weight losses and poor growth in surviving fish contributes substantial losses to the farmers (Mishra SS,Das R 2017). With increasing intensification of fish culture we are faced with an increasing number of recognized infectious diseases due to ever changing environment. Therefore, research on the pathogenesis and pathology of these diseases, their prevention and control has become essentially required.(Fish diseases-Erwin Amlacher 2005). Disease is a prime agent affecting fish mortality, especially when fishes are young. Disease can also be particularly problematic when pathogens and parasites carried by introduced species affect native species (Rohde,Klaus 2005). Diseases of fishes are caused by various types of microorganisms like Virus, Bacteria, Fungi, Protozoa etc.Innumerable diseases are caused in fishes due to bacterial pathogens and several of them are reported in Indian literature. Some of the important bacterial pathogens are *Aeromonas Hydrophila, Aeromonas Salmonicida, Psuedomonas fluorescens, Flexibacter Columnaris, Edwardsiella tarda* which have been identified as the most commonly encountered agents in fish diseases.(Diseases of fish – C.Van Duijn-2000)

Co-infections are very common in nature and occur when hosts are infected by two or more different pathogens either by simultaneous or secondary infections so that two or more infectious agents are active together in the same host.Co-infections affect has still received limited scrutiny in aquatic animals like fish and available data on this subject is still scare.During co-infections, pathogens can compete with each other for resources or target sites inside the same host..

1.1 *Aeromonas Hydrophila* Disease Characteristics

Aeromonas hydrophila is a heterotrophic, Gram-negative, rod shaped bacterium mostly found in regions with a warm atmosphere. This bacterium can be found in fresh or salt water.

Aeromonas hydrophila causes ulcers, tail decay, impaired swimming and hemorrhagic septicemia. Hemorrhagic septicaemia causes sores that lead to scale shedding, hemorrhages in the gills and butt-centric territory, ulcers, exophthalmia, and abdominal swelling.

1.2 *Flavobacterium Columnaris* Disease Characteristics

Flavobacterium columnare is a thin Gram-negative polar bacterium of the genus Flavobacterium. The name gets from the manner by which the living being develops in rhizoid columnar arrangements. *Flavobacterium columnare* is the reason for columnaris disease, a genuine condition influencing various freshwater fish species everywhere throughout the world. Heretofore, truth be told, extremely rare data is accessible on the pathogenesis of this bacterial disease, making it hard to embrace a preventive way to deal with this pathogen.

2. MATERIALS AND METHODS:

Procurement of fishes:

Fishes were divided into two groups for biochemical analysis.

Group 1: Fish grown in controlled and uninfected environment through various aquaculture techniques.This group is free from *Aeromonas hydrophila* and *Flavobacterium columnare* infection.

Group 2:Fish captured from natural water sources from Perupally pond of Khammam district located in the state of Telangana in Indian Province.This group is infected with both the bacteria *Aeromonas hydrophila* and *Flavobacterium columnare*.

Isolation and identification of bacteria:

Tissue sample from each organ like Gill, Liver, Kidney, Spleen and Muscle was dissected out from both the specimen 1 and specimen 2 for analysis.50mg of each dissected tissue is homogenized in 10ml of distilled water.1ml aliquot volume was measured in a clean, dry, sterile test tube containing 9ml of distilled water giving a 1:10 dilution .Serial dilution was performed from 1 to 10 test tubes. The swabs were inoculated on nutrient agar and incubated at 37 degree centigrade for 24 hours. The isolated bacterial colonies were identified on the basis of their morphological and biochemical characters. These cultures were subjected to various biochemical tests and gram staining, using Bergey's manual of systematic bacteriology.

Analysis of Biochemical Parameters:

Total carbohydrate and total glycogen was estimated by the method of Nicholas et al(1956).The protein content of the tissues was estimated by following Lowry's method (1951).Estimation of free amino acids was done by the method given by Moore and Stein(1954). The total lipids were extracted from the tissues, by following the method of Folsch et al., (1957).

RESULTS AND DISCUSSION: The coinfected fish with *Aeromonas hydrophila* and *Flavobacterium columnare* showed the following result in gill,liver, muscle, kidney and spleen when compared to control group.The results of the present study showed that the biochemical parameters viz total carbohydrates total glycogen total lipids free amino acids and total lipids were disrupted in infected fish. The alterations were tissue specific and hence can be used as an important indicator of pathology in fish. This type of study provides useful information about the

adverse effects of infection on aquatic biota especially fish,which constitutes important food source for human consumption. Hence it is important to monitor the occurrence of infection as these cause adverse effect on the health of fish.The total carbohydrate content was found to be highest in liver(114.35±14.95) followed by muscle, kidney, gill, spleen in control group. The concentration of total carbohydrate decreased significantly in liver(92.23±7.86) of bacterial coinfected group ,when compared to control group(Table 1&figure 1).The total glycogen content was highest in liver(39.10±0.50) of control group when compared to infected(27.48±5.65) followed by muscle, kidney, gill, spleen

(Table 2 &figure 2).The maximum value of protein content was found in muscle(146.63±3.65) of control group,it showed a significant decrease in muscle(115.78±3.74) of infected group(Table 3&figure 3). Free amino acids estimated were highest in the muscle tissue(21.66±1.78) of control group those that of the infected(7.16±0.55) group (Table 4 & figure 4).Total lipid content was high in the control liver(171.48±13.18) when compared to infected liver tissue(134.01±6.33) followed by muscle, gill, spleen, kidney(table 5 & figure 5). Discussion: The results obtained from the present study showed that the values of total carbohydrates, total glycogen, total proteins, free amino acids in all the five organs-gill, liver, muscle, kidney and spleen decreased significantly in co infected group of fish. Exposure to any kind of infection results in stress which ultimately results in reduction of total carbohydrate content in various tissues. Under stressful conditions, carbohydrate reserves are depleted in order to meet energy requirement by all tissues.(Arasta et al.,)Under stress conditions energy demands are met by increased glycogenolysis which leads to decrease in tissue glycogen.(Wasserman et al.,). Dietary proteins are the source of essential amino acids and provide nitrogen for the synthesis of non-essential amino acids. Proteins in the body tissues are built using about 23 amino acids. Of these, 10 are essential amino acids which must be supplied in the fish diet. Proteins or amino acids are necessary for maintenance, growth, reproduction and for the replacement of depleted tissues. A deficiency of essential amino acids may lead to poor utilization of dietary protein, and may result in growth retardation, poor live weight gain, and low feed efficiency. In severe cases, amino acid deficiency lowers resistance to diseases and impairs the effectiveness of the immune response mechanism. Deficiencies of specific amino acids may also elicit clinical signs. For example, experiments have shown that tryptophan deficient fish become scoliotic, showing a characteristic curvature of the spine (Kloppel and Post 1975) and a methionine deficiency is one cause of lens cataracts (Poston et al. 1977)

The nutritionally active components of dietary lipids are fatty acids. Fish and mammals appear to be unable to synthesize fatty acids that are unsaturated in the w-3 or w-6 positions unless a suitable precursor is supplied in the diet. Thus, the lipid component of the diet must provide an adequate amount of essential fatty acids for growth as well as for required dietary fuel. . Therefore, sufficient amounts of essential fatty acids (w-3 fatty acids or longer chain members of these series) must be included in the dietary lipids. One percent linolenic acid (18:3w3) in the diet is required by rainbow trout to avoid such deficiency signs as loss of pigmentation, fin erosion, cardiac myopathy, fatty infiltration of the liver,and shock syndrome (Castel et al. 1972).

Table 1:Total Carbohydrate (mg/gm wet tissue) in gill, muscle, liver, kidney, spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

S.NO	TISSUE	CONTROL	INFECTED
1	GILL	21.466±0.943	15.433±2.775*
2	MUSCLE	82.583±1.060	72.266±1.898*
3	LIVER	114.350±14.951	92.233±7.863*
4	KIDNEY	33.133±3.820	20.383±1.254*
5	SPLEEN	16.350±1.046	9.466±3.707*

Values are expressed as Mean±standard deviation.* indicates P<0.05.

Table 2:

Total Glycogen (mg/gm wet tissue)in gill, muscle, liver, kidney, spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

Values are expressed as Mean±standard deviation.* indicates P<0.05

S NO	TISSUE	CONTROL	INFECTED
1	GILL	9.033±0.686	3.616±1.4038*
2	MUSCLE	26.433±1.504	17.733±1.676*
3	LIVER	39.10±0.509	27.483±5.659*
4	KIDNEY	11.116±1.683	6.416±0.646*
5	SPLEEN	10.416±0.79	7.383±1.01*

Table 3:

Total Proteins (mg/gm wet tissue) in gill,muscle,liver,kidney,spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

S.NO	TISSUE	CONTROL	INFECTED
1	GILL	110.36±3.41	94.10±2.46*
2	LIVER	92.51±1.25	77.0±3.22*
3	MUSCLE	146.63±3.65	115.78±3.74*
4	KIDNEY	107.41±6.11	78.31±4.08*
5	SPLEEN	79.16±2.24	57.66±4.40*

Values are expressed as Mean±standard deviation.* indicates P<0.05

Table 4:Free aminoacids (mg/gm wet tissue)in gill,muscle,liver,kidney,spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

S.NO	TISSUE	CONTROL	INFECTED
1	GILL	105.366±7.303	77.766±3.770*
2	MUSCLE	124.116±93.483	93.483±4.021*
3	LIVER	171.483±13.186	134.016±6.333*
4	KIDNEY	70.333±5.278	46.466±3.383*
5	SPLEEN	84.416±3.805	67.033±4.331*

Values are expressed as Mean±standard deviation.* indicates P<0.05

Table 5:

Total lipids (mg/gm wet tissue)in gill,muscle,liver,kidney,spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

S.NO	TISSUE	CONTROL	INFECTED
1	GILL	105.366±7.303	77.766±3.770*
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3	LIVER	171.483±13.186	134.016±6.333*
4	KIDNEY	70.333±5.278	46.466±3.383*
5	SPLEEN	84.416±3.805	67.033±4.331*

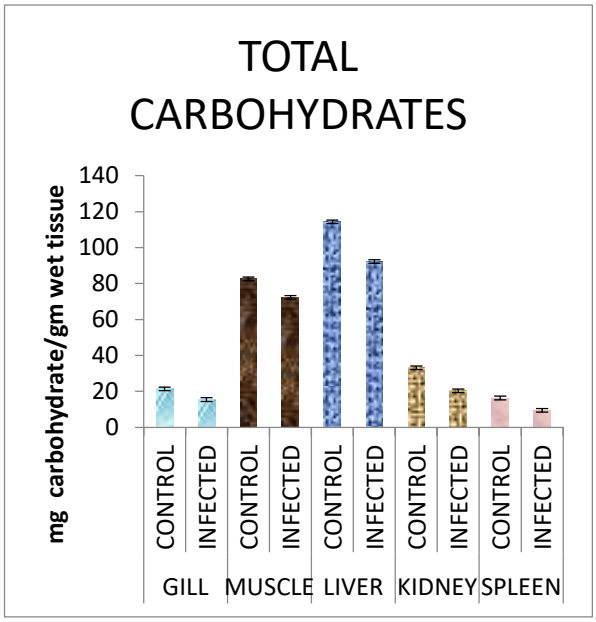

Fig 1. Total Carbohydrate (mg/gm wet tissue) in gill, muscle, liver, kidney, spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

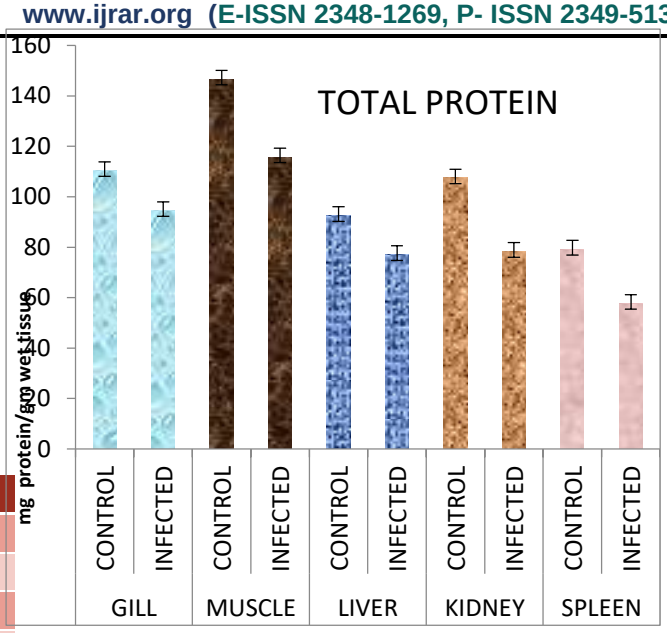

Fig.2 Total Glycogen (mg/gm wet tissue)in gill, muscle, liver, kidney, spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

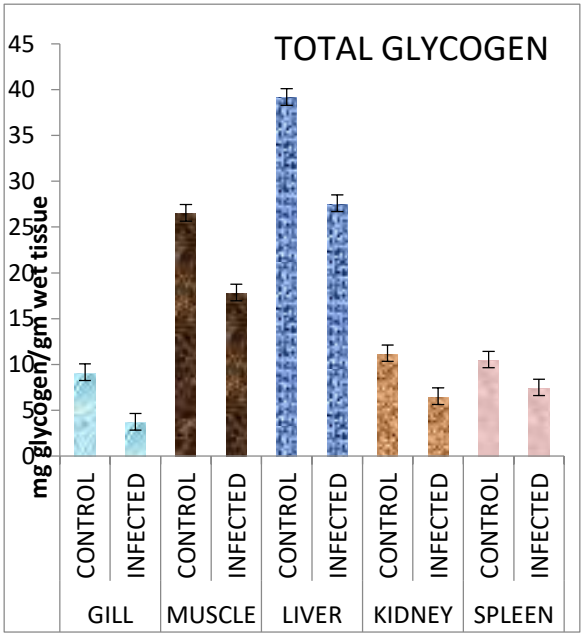

Fig 3. Total Proteins (mg/gm wet tissue) in gill,muscle,liver,kidney,spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

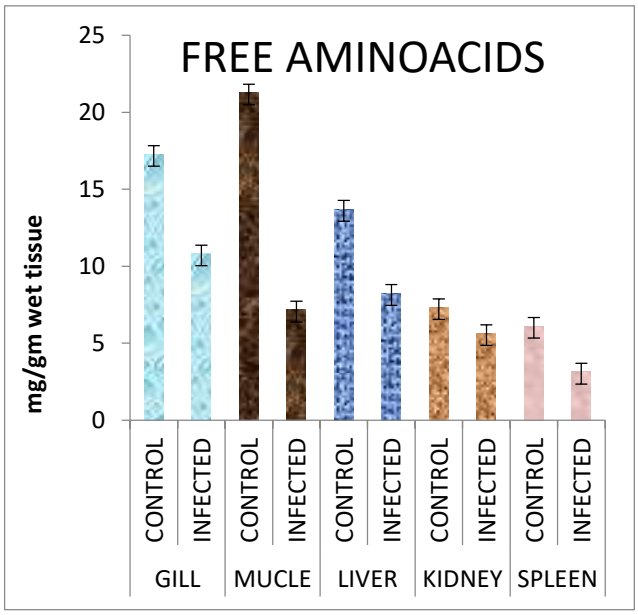

Fig 4. Free aminoacids (mg/gm wet tissue)in gill,muscle,liver,kidney,spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

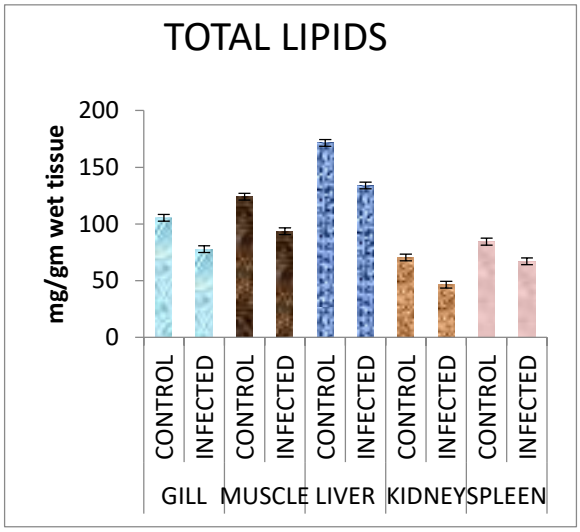

Fig 5. Total lipids (mg/gm wet tissue)in gill,muscle,liver,kidney,spleen of *catla catla* coinfected with *Aeromonas hydrophila* and *Flavobacterium columnare* bacteria.

STATISTICS: The statistical analysis system (IBM SPSS) was used to analyse the data. The values are expressed as mean±standard deviation (SD) and the data were analysed using students t-test. Difference were considered statistically significant at $P<0.05$ for all the experiments conducted in this study.

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References:

1. Acharya G, Mohanty PK. Haematological and serum biochemical parameters in different sexes of walking cat fish, *Clarias batrachus* (Linnaeus, 1758) Int J Sci Res (IJSR) 2014;3(8):1914–1917.

2. Aneja, K.R., Jain, P., Aneja, R.. A textbook of basic and applied microbiology, 1stedn. New Delhi, India: Newage International Pvt. Ltd., 349-64 (2008).

3. Austin B, Austin DA. Bacterial fish pathogens: diseases of farmed and wild fish. 4. Chichester: Praxis Publishing; 2007.

4. Balasurya LKSW. Current fish disease problems in Srilanka. In: Arthur JR, editor. Fish quarine and fish disease in South and Southeast Asia: 1986 update. Special publication No. 1. Manila: Asian Fisheries Society; 1987. pp. 36–40.

5. Experimental challenge studies in vietnamese catfish *pangasianodon hypophthalmus* (sauvage), exposed *to*

*Edwardsiella ictalur*i and *Aeromonas hydrophila* (Crumlish M, Thanh PC, 2010))

6. Concurrent infections of *Flavobacterium Columnare* and *Edwardsialla ictaluri* in striped catfish *Pangasianodon hypothalmus* in Thailand (Dong HT, Nguyen VV-2015)
7. Harikrishnan R, Nisha Rani M, Balasundaram C. Haematological and biochemical parameters in common Carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture. 2003; 221(1–4):41–50. doi: 10.1016/S0044-8486(03)00023-1.
8. Karunasagar I, Sugumar G, Karunasagar I. Virulence characters of Aeromonas spp. isolated from EUS affected fish. In: Shariff M, Arthur JR, Subasinghe RP, editors. Diseases in Asian aquaculture. Manila: Fish Health Section, Asian Fisheries Society; 1995. pp. 307–314.
9. Kavitha K, Haniffa MA*, Abdul Kader Mydeen KP, Jayasheela P. Emerging dreadful ulcer disease in threatened murrel, Channa aurantimaculata. J Res Anim Sci. 2014;2(1):087–093.
10. Bacterial infections of chinook salmon, *oncorhynchus tshawytscha* (Walbaum) returning to gamete collecting weirs in Michigan- (Loch TP, Scribnerk-2012)
11. The impact of co-infections on fish: a review(Mohammed H Kotob 2017)
12. A case of natural co-infection of tilapia lake virus and *aeromonas veronii* in a Malaysian red hybrid tilapia (*oreochromis niloticus,oreochromis mossambicus*) farm experiencing high mortality (M.N.A Amal,et al feb-2018)
13. Satheeshkumar P, Ananthan G, Senthilkumar D, Khan AB, Jeevanantham K (2012): Comparative investigation on haematological and biochemical studies on wild marine teleost fishes from Vellar estuary, southeast coast of India. Comparative Clinical Pathology 21, 275 281.