COMPARATIVE ANALYSIS OF THE HAEMATO-BIOCHEMICAL PARAMETERS AND MEAT CHEMICAL COMPOSITION OF HEALTHY AND INFECTED CIRRHINUS MRIGALA, AND (LABEO ROHITA X CIRRHINUS MRIGALA) HYBRID WITH ABDOMINAL DROPSY DISEASE

Sadia Nazir¹, Sheeza Bano ¹, Muhammad Asghar¹, Aamna Batool², Sadaf Hameed²*

¹Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan.
²Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan

Abstract

The current study has been conducted for investigating effects of Aeromonas hydrophila infection on haematological indices of Cirrhinus mrigala, and Labeo rohita×Cirrhinus mrigala (hybrid). However, some haematological indices, including those for healthy and infected fish, were measured in two adult groups of Indian major carps. Our findings showed that infected fish had significantly lower values for red blood cells, white blood cells, neutrophil, lymphocyte, monocyte, eosinophils, basophils, hemoglobin, hematocrit, mean corpuscular volume and platelet count ($p<0.05$) as compared to healthy fish. Meat chemical analysis values were also shown significant ($p<0.05$) both in infected and healthy fish. In conclusion, our findings concluded that Aeromonas hydrophila can alter the haematology of Indian major carps to affect their health.

Keywords: Indian major carps, Abdominal dropsy, Aeromonas hydrophila, Haematology, Body composition.

I. INTRODUCTION

Pathogenic bacteria cause high mortality and huge economic losses in aquaculture industry (Wang et al., 2008). When a fish’s immunity wanes as a result of various stressors, these bacteria can cause illnesses (Briede, 2010). Around the world, fish diseases have been linked to several bacterial pathogens. Pseudomonas spp., Aeromonas spp. and Vibrio spp. are
bacteria that are particularly significant in freshwater (Zahran et al., 2016). Bacterial infections frequently have subclinical stages and show no outward symptoms. Ectoparasites, excessive ammonia, improper handling, high stocking density and feeding, and stressful circumstances are all predisposing factors that allow microorganisms to infect the host fish (Moraes & Martins, 2004).

Haematological indices are the main metrics used to evaluate the state of fish health. The haematological parameters are significantly influenced by fish age (Jamalzadeh & Ghomi, 2009), and pathogenic infection (Chen et al., 2020). Therefore, straightforward haematological tests can be used as a supplement to standard diagnostic techniques and determine how stress or other conditions have an impact on a fish’s nutritional health status (Fazio, 2019). The fundamental haematological characteristics of both healthy and infected fish haven’t been fully described, though. Additionally, there are notable interspecific variations in haematological parameters among various fish species or notable variations among different pathogenic infections. Fish haematological reference ranges are intended to gain all the necessary information about fish health. The financial losses in aquaculture can be decreased when hematological parameters are used for primary diagnosis of fish disease diagnostics and prognosis (Trinanes & Martinez-Urtaza, 2021).

Indian major carps are the dominant freshwater cultured species in the Pakistan aquaculture system and one with a very high commercial value, suffer many diseases including infectious dropsy. Dropsy is a bacterial disease and commonly applied term for coelomic (i.e., abdominal). Gram-negative, facultative anaerobic bacteria called *Aeromonas hydrophila* causes stress-related pathogenicity in both (warm and cold) water fish such as carps, catfish, and salmonids (Harikrishanan & Balasundaram, 2005).

To investigate the effects of the pathogenic bacterial strain (*Aeromonas hydrophila*) on the nutritional value and immune mechanism of the fish after infection by the bacterial pathogen, these were selected as the experimental organisms. One of the most widely farmed species in aquaculture, including Pakistan, is the Indian major carp. This study was purposed to examine the impact of *A. hydrophila* infection on this species haematological indicators. This study can help to understand the mode of the action of these bacteria in the haematological level and can aid in understanding how these bacteria act at meat chemical composition and haematological level.
II. MATERIAL AND METHODS

Fish Samples

The study was conducted at Research and Training Facilities, Department of Fisheries and Aquaculture, UVAS, Ravi Campus, Pattoki. Indian major carp (*Cirrhinus mrigala* 100±0.5 g, 26.21±0.19 cm) and (*Labeo rohita* × *Cirrhinus mrigala* 45.56±7.45 g, 14.22±0.85 cm) fingerling infected with *Aeromonas hydrophila* and healthy were collected from a carp culture UVAS pond. Samples were taken for analysis after processing at the laboratory immediately.

Haematological indices

To collect the blood, fish were chosen. Syringes were used to take blood from vaccutainers that already contained the anticoagulant (EDTA). The following parameters were calculated by using fingerling blood sample: red blood cells, white blood cells, neutrophil, lymphocyte, monocyte, eosinophils, basophils, hemoglobin, hematocrit, mean corpuscular volume, and platelet count by using an automated hematology analyzer (MEK6550).

Proximate composition analysis

Proximate tests of fingerling fish meat include moisture, ash, fat, protein, and fiber contents following the protocol of (AOAC, 2006). In a summary, all the samples were oven-dried 105°C for determination of their moisture content; crude protein was estimated with Kjeldahl method by acid digestion. Crude fat was estimated using Soxhlet apparatus through ether extraction method. For the determination of crude ash, samples were burnt for one day at 105°C. Water quality parameters such as, dissolved oxygen (DO)=5.76-6.89 mgL⁻¹, temperature= 28.76-30.25°C, water total ammonia= 0.19-0.3 mg L⁻¹; pH= 8.09-8.43) were kept constant (Model 55, YSI Inc, Yellow Springs Ohio, USA).

Statistical data

Data was obtained through the statistical analysis by using SAS 9.1 version through one-way analysis of variance (ANOVA) by applying Duncan’s Multiple Range Test. The data is
presented as mean ± standard deviation. The results were taken as statistically significant at \( p < 0.05 \).

**Fig. 1**: Indian major carps after infecting with *Aeromonas hydrophila*: **A** Healthy *Cirrhinus mrigala*; **B** infected *Cirrhinus mrigala* with swollen abdomen; **C** dissected the diseased *Labeo rohita*\( \times \) *Cirrhinus mrigala* (hybrid) with dropsy.

### III. RESULTS

#### Haematology indices

Table 1 displays the results of comparing the haematological values of healthy versus infected *Cirrhinus mrigala* and *Labeo rohita*\( \times \) *Cirrhinus mrigala* (hybrid). Both infected fish had lower values of HB, HCT, RBCs, MCV, WBCs, NEUTR, LYMPH, MONO, EO, BASO, and PLT than healthy fish \( (p < 0.05) \). Hemoglobin (Hb) contents also appeared to show a decreasing trend in infected fish and in healthy groups it increased slightly.
Table 1: Haematology of *Cirrhinus mrigala* and *C. mrigala x Labeo rohita* (hybrid).

| Variables          | Infected *C. mrigala* | Healthy *C. mrigala* | Healthy *C. mrigala x Labeorohita* (hybrid) | Infected *C. mrigala x Labeorohita* (hybrid) | P value |
|--------------------|-----------------------|----------------------|---------------------------------------------|---------------------------------------------|---------|
| HGB, g/dL          | 3.28±0.06a            | 6.75±0.44c           | 7.93±0.52d                                 | 4.27±0.61b                                  | 0.000   |
| HCT, %             | 25.4±0.38a            | 32.5±0.15c           | 34.4±0.34d                                 | 27.3±1.30b                                  | 0.000   |
| RBC, 10^6/μL       | 1.05±0.01a            | 2.05±0.01b           | 2.10±0.02c                                 | 1.05±0.01a                                  | 0.000   |
| MCV, fL            | 48.7±0.80b            | 55.8±1.81c           | 54.1±1.60c                                 | 45.1±1.60a                                  | 0.000   |
| WBC, 10^3/μL       | 45.8±2.53a            | 53.6±1.33b           | 55.0±2.33b                                 | 47.4±0.50a                                  | 0.001   |
| NEUTR, %           | 4.67±0.29a            | 5.30±0.06a           | 6.22±0.57b                                 | 5.26±0.12a                                  | 0.003   |
| LYM, %             | 78.2±1.51a            | 86.1±1.46b           | 87.4±2.13b                                 | 80.3±1.67a                                  | 0.000   |
| MONO, %            | 1.43±0.09a            | 2.36±0.07c           | 2.43±0.12c                                 | 1.75±0.10b                                  | 0.000   |
| EO, %              | 1.34±0.11a            | 2.11±0.06b           | 2.09±0.06b                                 | 1.30±0.16a                                  | 0.000   |
| BASO, %            | 5.36±0.04a            | 6.78±0.04c           | 7.53±0.44d                                 | 6.01±0.28b                                  | 0.000   |
| PLT, 10^6/μL       | 1.11±0.02b            | 2.16±0.05c           | 2.12±0.03c                                 | 1.02±0.00a                                  | 0.000   |

RBC, red blood cells; WBC, white blood cells; NEUTR, Neutrophil; LYM, Lymphocyte; MONO, Monocyte; EO, eosinophils; BASO, basophils; MCV, mean corpuscular volume; HGB, hemoglobin; HCT, hematocrit; PLT, platelet count.

**Meat chemical composition**

The chemical analysis of the infected versus healthy *Cirrhinus mrigala* and *Labeo rohita x Cirrhinus mrigala* (hybrid) are given in Table 2. Changes in the values of protein, fat, ash, crude fiber, and moisture were all significant (*p*<0.05).

Table 2: Proximate analysis of healthy and infected fishes (% dry basis).

| Parameters | Healthy *C. mrigala* | Infected *C. mrigala* | Healthy *C. mrigala x Labeorohita* (hybrid) | Infected *C. mrigala x Labeorohita* (hybrid) | P value |
|------------|----------------------|-----------------------|---------------------------------------------|---------------------------------------------|---------|
| Ash Contents | 18.35±0.04a         | 19.14±0.27b           | 19.17±0.05b                                 | 18.45±0.39a                                 | 0.052   |
The Journal of Microbiology and Molecular Genetics (JMMG)
Vol. 3, No. 2 (2022), pp. 24-32

|                | Crude protein % | Crude fat %   | Crude fiber % | Moisture %   |
|----------------|-----------------|---------------|---------------|--------------|
|                | 55.65±0.02a     | 58.02±0.60b   | 0.46±0.01a    | 12.29±0.07c  |
|                | 58.77±0.79b     | 8.60±0.21b    | 0.92±0.04b    | 11.32±0.13b  |
|                | 54.85±0.77a     | 7.68±0.33a    | 0.84±0.07b    | 11.07±0.01a  |
|                | 0.009           | 0.037         | 0.005         | 0.000        |

|                | 0.51±0.09a      | 12.29±0.04c   |

IV. DISCUSSION

Dropsy is a persistent issue with fishes all over the world. The diseased fish used in this study had hemorrhagic lesions on their skin and eye, as well as slow movement, loss of balance, and an enlarged abdomen. Other researchers have described similar sign and as well as the outcomes connected to the mixed bacterial infection (Molnar & Csaba, 2005 and Foysal et al., 2011).

It is well known that aquatic animals, including fish, can use haematological factors as biomarkers of health status. The high fish stocking rate in intensive aquaculture systems consequently increases the risk of disease. A bacterium called Aeromonas hydrophila has detrimental effects on fish health in cultured medium. Numerous studies have been done up to this point on the histopathological effects of this bacteria on fish. However, information regarding their effects on hematological indices is scarce. In the current study, healthy and infected fishes were used to examine the blood parameters, including Hb and RBCs.

According to the findings, infected fish had lower Hb, Htc and RBC values than fish that were in good health. Infected common carp, cyprinus carpio, with monogenean parasites have been shown to have decreases in these factors in the previous study (Shah et al., 2009). WBC was employed for infection. According to Fazio et al. (2013), a healthy S. schlegelii has WBC count higher than that of a Gobiusniger; but lower than that of Sparus aurata.

Current results showed that all chemical composition of Indian major carps showed significant differences between the dry contents, fat, ash, and protein contents of healthy and infected fish. Celik et al. (2005) reported similar results in Sander lucioperca. Trbovic et al. (2013) reported similar data for the proximate composition of carp on influence of diet.
CONCLUSION

In conclusion, our findings demonstrated that Aeromonas hydrophila spp. dropsy infection affects Indian major carp’s health through a change in meat chemical composition and haematology.

Conflict of interest

We declare no conflict of interests in this work and all parties were in full agreement and in accordance with the ethos of the investigation.

Authors contributions

Equal contribution from all Authors to this work.

Data availability statement

All supporting data for this study are available upon a reasonable request.

V. REFERENCES

AOAC. 2006. Association of Official Analytical Chemists, 17th edn. method number 1125.

Briede, I. 2010. The prevalent bacterial fish diseases in fish hatcheries of Latvia. Environmental and Experimental Biology, 8:103-106.

Chen, H., Yuan, G., Su, J. and Liu, X. 2020. Hematological and immune genes responses in yellow catfish (Pelteobagrus fulvidraco) with septicemia induced by Edwardsiella ictaluri. Fish & Shellfish Immunology, 97: 531-539.

Celik, M., Diler, A. and Kucukgulmez, A. 2005. A comparison of the proximate compositions and fatty acid profiles of zander (Sander lucioperca) from two different regions and climatic conditions. Food Chemistry, 92(4):637-641.

Fazio, F. 2019. Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture, 500: 237-242.

Fazio, F., Marafioti, S., Arfuso, F., Piccione, G. and Faggio, C. 2013. Comparative study of the biochemical and haematological parameters of four wild Tyrrhenian fish species. Veterinární Medicína, 58(11): 576-581.
Fazio, F., Saoca, C., Casella, S., Fortino, G. and Piccione, G. 2015. Relationship between blood parameters and biometric indices of Sparus aurata and Dicentrarcus labrax cultured in onshore tanks. *Marine and Freshwater Behavior and Physiology, 48*(4): 289-296.

Foysal, M.J., Rahman, M.M. and Alam, M. 2011. Antibiotic sensitivity and *in vitro* antimicrobial activity of plant extracts to Pseudomonas fluorescens isolates collected from diseased fish. *International Journal of Natural Sciences, 1*(4): 82-88.

Harikrishnan, R. and Balasundaram, C. 2005. Modern trends in Aeromonas hydrophila disease management with fish. *Reviews in Fisheries Science, 13*(4): 281-320.

Jamalzadeh, H.R. and Ghomi, M.R. 2009. Hematological parameters of Caspian salmon Salmo trutta caspius associated with age and season. *Marine and Freshwater Behaviour and Physiology, 42*(1): 81-87.

Molnar, K. and Csaba, G. 2005. Sanitary management in Hungarian aquaculture. *Veterinary Research Communications, 29*(2): 143-146.

Moraes, F.R. and Martins, M.L. 2004. Favourable conditions and principal teleostean diseases in intensive fish farming. *Especial Topics in Tropical Intensive Freshwater Fish Farming. São Paulo: TecArt*, pp.343-383.

Shah, A.W., Muni, P., Mir, S.H., Sarwar, S.G. and Yousuf, A.R. 2009. Impact of helminth parasitism on fish haematology of Anchar Lake, Kashmir. *Pakistan Journal of Nutrition, 8*(1): 42-45.

Trbovic, D., Markovic, Z., Milojkovic-Opsenica, D., Petronijevic, R., Spiric, D., Djinovic-Stojanovic, J. and Spiric, A. 2013. Influence of diet on proximate composition and fatty acid profile in common carp (Cyprinus carpio). *Journal of Food Composition and Analysis, 31*(1):75-81.

Trinanes, J. and Martinez-Urtaza, J. 2021. Future scenarios of risk of Vibrio infections in a warming planet: a global mapping study. *The Lancet Planetary Health, 5*(7): 426-e435.

Wang, Y.B., Li, J.R. and Lin, J. 2008. Probiotics in aquaculture: challenges and outlook. *Aquaculture, 281*(1-4):1-4.
Zahran, E., Manning, B., Seo, J.K. and Noga, E.J. 2016. The effect of Ochratoxin A on antimicrobial polypeptide expression and resistance to water mold infection in channel catfish (Ictalurus punctatus). Fish & Shellfish Immunology, 57:60-67.