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Haemato-biochemical Parameters in African Catfish Experimentally Infected with Single and Mixed *Escherichia coli* and *Salmonella gallinarium*

Anagor, T.A[1], Chah, k.F[2]., Omeje, V.O[1*]. and Anene, B.M[1]

[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

ABSTRACT

Clarias gariepinus is an important food fish in Sub-Saharan Africa yet there are dearth of information on the effect of *Escherichia coli* and *Salmonella gallinarium* on the haematology and clinical chemistry of the species. However, the importance of haematology and clinical chemistry in the diagnosis of infections in fish cannot be overemphasized. The study was designed to determine the heamato-biochemical alterations that occur in African Catfish experimentally infected with single and mixed *E. coli* and *S. gallinarium* strains. Clinical isolates were used for the infections as follows: group A (E.coli only), group B (*S. gallinarium* only), group C (*E.coli* and *S.gallinarium*) and group D served as uninfected control. Fishes in the experimental groups were infected by immersing them in water containing $1x10^8$ colony forming units/ml of each test isolate. Blood samples were collected 1 and 2 weeks post infection from the four groups for haematology and serum biochemistry analysis. There was a significant decrease ($p<0.05$) in RBC in group B (1.93±0.18) when compared to groups A (2.31±0.08) and C (2.28±0.14) and uninfected group D (2.22±0.13). There was also a significant decrease ($p<0.05$) in PCV of group B (21.17±0.60) when compared with groups A (22.33±0.33), C (23.00±0.29) and D (23.50±0.87). There was no significant difference ($p>0.05$) in the haemoglobin concentration and WBC count among the groups. There was no significant difference in creatinine, total protein and blood urea nitrogen unlike Alanine amino transferase and bilirubin that significantly increased ($p<0.05$) two weeks post infection. This study has shown that the presence of *E. coli* and *S. gallinarium* in pond can induce some changes in the blood parameters and serum enzymes of fish.
KEY WORDS: *Escherichia coli, Salmonella gallinarium*, *Clarias gariepinus*, haematology

INTRODUCTION

The African catfish (*Clarias gariepinus*) which is an ideal aquaculture species in sub-Saharan Africa thrives in diverse environments. It is hardy and adaptable principally as a result of its air breathing ability, feeds on variety of food under diverse conditions and is able to breed under captive conditions when induced. It is highly resistant to disease and its potential for intensive culture with relatively poor water quality[1] made it the choice for this study. Cultured and even feral fishes are prone to diseases such as salmonellosis when they are infested with parasites especially in faecal polluted waters[2]. Invasion of fish muscle due to the breakage of immunological barrier of fish by pathogens is likely to occur when the fish are raised in pond with faecal coliforms (*Escherichia coli* and *Salmonella gallinarium*) of greater than 10^3 /ml in pond water[3]. Blood parameters have been commonly used to observe and follow fish health, since variations in blood tissues of fish are caused by environmental stress[4], malnutrition[5], gender[6], seasonal difference and breeding[7]. Often, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish due to their close association with their environment. [8]noted that studies on fish blood gives the possibility that fish blood will reveal conditions within the fish long before there is outward manifestation of diseases. The present study therefore seeks to investigate the haemato-biochemical parameters associated with *S. gallinarium* and *E. coli* infection (either as a single or mixed infection) in African catfish (*C. gariepinus*).

MATERIALS AND METHOD

One hundred and sixty apparently healthy 6-weeks-old *Clarias gariepinus* post fingerlings were used for the study. They were acclimatized for 2 weeks during which period 2mm fish basal diet was provided twice daily[9]. The water

***Corresponding Author:** Omeje, V.O., Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. Email: okonkwo.omeje@unn.edu.ng

was changed once in 2 days by gradual removal and addition to ensure adequate oxygenation. Three to four colonies of *Escherichia coli* and *Salmonella gallinarium* strains were homogenized separately in sterile phosphate buffered saline and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard (equivalent to $1x10^8$ colony forming units/ml). There were four experimental treatment groups (A, B, C and D) with four replicates of 10 fish each. Fish in groups A, B and C were infected by immersing them in appropriate tank containing 1ml/litre of bacterial inoculum as follows: group A (*Escherichia coli* only), group B (*Salmonella gallinarium* only), group C (*Escherichia coli* and *Salmonella gallinarium*) and group D served as uninfected control. Three fishes were randomly selected on the day of stocking, week one and weeks two post infection from each replicate for haematology and serum biochemistry. Universal bottles treated with ethylene diamine tetra acetic acid (EDTA) were used for blood collection for haematological determination. Blood collection was done by severing the caudal vein. Collected blood was well mixed with the anticoagulant and the following haematological studies carried out to measure the packed cell volume (PCV), haemoglobin concentration (Hb), erythrocyte count, leukocyte count and differential WBC count[10]. The blood counts were determined by the haemocytometer method[11]. The serum alanine aminotransferase activity was determined by the Reitman-Frankel colorimetric method for the in-vitro determination of ALT in serum or plasma using ALT test kit[12], total proteins was determined by the direct Biuret method[13], Serum urea nitrogen was determined by the modified Berthelot-Searcy method[14]. The serum creatinine was determined using the modified Jaffe method[15] and total serum bilirubin was determined following the Jendrassik-Grof method[16]. Data obtained from the tests were compared using one way analysis of variance (ANOVA). Variant means were separated using Duncan Multiple range test. Significant difference was accepted at ($p < 0.05$).

RESULTS

There was significant ($p<0.05$) reduction in PCV and RBC two weeks post infection in fish in group B when compared with fish in groups A and D respectively. However there was no significant difference *($p\geq 0.05$)* in haemoglobin concentration and white blood cell among the groups two weeks post infection as shown in (Table 3 and 4). The absolute neutrophil, lymphocyte and eosinophil did not differ significantly *($p > 0.05$)* but there was a significant difference ($P>0.05$) in the absolute monocyte count in group C one week post infection when compared to group D as shown in(Tables 5, 6, 7, and 8). Mean ALT (41.23±0.76) significantly ($p<0.05$) increased in group B when compared with group D (39.78±0.33) two weeks post infection. There was also an increase in mean bilirubin in groups A (2.16±0.25) and B (1.80±0.07) when compared with group D (1.15±0.15) by two weeks post infection. Group C was comparable to groups B and D but was significantly lower than group A. There was no significant difference ($p > 0.05$) in the mean creatinine, mean total protein and blood urea nitrogen (Tables 9, 10 and 11)

DISCUSSION

In this study, African catfish experimentally infected with *Escherichia coli* and *Salmonella gallinarium* was investigated in order to evaluate the effect of these organisms in their haemato- biochemical parameters. Changes in blood parameters are known to occur in fishes under disease, agitation or nutritive stress[17, 18]. The results of this study have shown the capability of these organisms to induce different changes in haematological parameters. The results of haematology were in agreement with the work of[19] who reported that haematological parameters are crucial and serve as a possible indicator of physiological or pathological changes in diseases investigation and fishery management. The low PCV and low RBC count recorded from the *Salmonella gallinarium* infected fish may be due to endotoxins produced by the organism which may lead to lysis of the red blood cell thereby leading to anaemia. The result is in concurrence with earlier work of[20] who reported a significant decrease in RBC and PCV in *Clarias gariepinus* and *Heterobranchus bidorsalis* exposed to some bacterial disease. Physiologically, haemoglobin is crucial to the survival of fish, being directly related to the oxygen binding capacity of blood. However, the range observed in this study (from 8.45±0.37 to 9.14±0.46 g/dl) may not have a deleterious effect on African catfish, given that the values are within the normal range recorded for African catfish[21, 22]. The white blood cell, eosinophils, neutrophils and lymphocytes did not differ significantly. This is probably due to level of severity of the infection in the fish.

However there was an increase in alanine amino transferase (ALT) and bilirubin respectively in the *Salmonella gallinarium* and *E.coli* infected fish. Plasma enzymes are useful as appropriate markers of tissue (organ) damage[23]. An elevated level of plasma enzyme may suggest damage to tissue. In this study therefore, elevated levels of serum Alanine amino tranferase and bilirubin observed may suggest damage to the liver. Damage to the liver may cause poor detoxification and deamination which can lead to poor feed conversion, loss of weight and mortality. The serum creatinine, total protein and blood urea nitrogen did not differ significantly ($p < 0.05$) which is an indication that the infection had no adverse effect on the kidneys.

Heavy contamination of aquatic environment by pathogens especially *Escherichia coli* and *Salmonella gallinarium* may not necessarily result in outright mortality but could lead to several physiological dysfunctions in fish which could induce changes in their blood parameters and serum enzymes.

Table 1: Mean Packed Cell Volume (PCV %)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	$22.50^{a} \pm 0.76$	$23.00^{a} \pm 1.26$	$22.33^{a} \pm 1.01$	$22.00^{a} \pm 0.29$
1	$20.67^{a} \pm 0.88$	$20.17^{a} \pm 1.09$	$21.83^{a} \pm 0.93$	$21.67^{a} \pm 0.44$
2	$22.33^{ab} \pm 0.33$	$21.17^{b} \pm 0.60$	$23.00^{ab} \pm 0.29$	$23.50^{a} \pm 0.87$

Mean with the same superscript on the same row are not significantly different ($p > 0.05$) while those of different superscript are significantly different ($p \leq 0.05$)

Table 2: Mean Red Blood Cell Count (x 10^{6}/mm)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	$2.16^{a} \pm 0.17$	$2.02^{a} \pm 0.15$	$2.13^{a} \pm 0.10$	$1.85^{a} \pm 0.03$
1	$1.90^{a} \pm 0.08$	$1.85^{a} \pm 0.19$	$2.00^{a} \pm 0.27$	$1.79^{a} \pm 0.06$
2	$2.31^{a} \pm 0.08$	$1.93^{b} \pm 0.18$	$2.28^{ab} \pm 0.14$	$2.22^{ab} \pm 0.13$

Mean with the same superscript on the same row are not significantly different ($p > 0.05$) while those of different superscript are significantly different ($p \leq 0.05$)

Table 3: Mean Haemoglobin Concentration (g/dl)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	8.79±0.26	8.45±0.83	8.19±0.48	8.10±0.34
1	8.45±0.37	8.79±0.30	8.62±0.17	8.62±0.31
2	8.97±0.46	8.45±0.46	9.14±0.46	8.79±0.79

The mean haemoglobin concentration did not vary significantly ($p > 0.05$) among the groups.

Table 4: Mean White Blood Cell Count (x 10^{9}/l)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	11.67±0.74	12.07±0.52	12.33±0.49	11.13±0.69
1	11.35±1.47	11.35±1.24	11.64±0.83	12.73±1.58
2	13.97±0.98	10.85±0.49	13.27±2.33	12.48±1.39

The mean WBC count did not vary significantly ($p > 0.05$) among the groups.

Table 5: Mean Absolute Neutrophil Count

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	2.26±0.18	2.24±0.22	2.57±0.16	2.42±0.36
1	3.47±0.70	2.32±0.18	2.39±0.19	2.59±0.31
2	3.04±0.33	2.17±0.18	2.71±0.70	2.41±0.32

The mean absolute neutrophil count did not vary significantly ($p > 0.05$) among the groups.

Table 6: Mean Absolute Lymphocyte Count

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	9.15±0.63	9.64±0.31	9.24±0.61	8.38±0.58
1	7.53±0.66	8.79±1.17	9.07±0.86	9.57±1.26
2	10.00±0.63	8.42±0.46	10.02±1.61	9.54±1.15

The mean absolute lymphocyte count did not vary significantly ($p > 0.05$) among the groups.

Table 7: Mean Absolute Monocyte Count

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	$0.15^{a} \pm 0.08$	$0.16^{a} \pm 0.05$	$0.21^{a} \pm 0.05$	$0.19^{a} \pm 0.05$
1	$0.22^{ab} \pm 0.06$	$0.14^{ab} \pm 0.02$	$0.11^{b} \pm 0.06$	$0.30^{a} \pm 0.06$
2	$0.19^{a} \pm 0.05$	$0.14^{a} \pm 0.07$	$0.35^{a} \pm 0.08$	$0.22^{a} \pm 0.11$

Mean with the same superscript on the same row are not significantly different ($P > 0.05$) while those of different superscript are significantly different ($P \leq 0.05$)

Table 8: Mean Absolute Eosinophil Count

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	$0.04^{a} \pm 0.04$	$0.04^{a} \pm 0.04$	$0.24^{b} \pm 0.06$	$0.15^{ab} \pm 0.04$
1	$0.20^{a} \pm 0.05$	$0.10^{a} \pm 0.05$	$0.07^{a} \pm 0.07$	$0.27^{a} \pm 0.09$
2	$0.23^{a} \pm 0.04$	$0.14^{a} \pm 0.03$	$0.13^{a} \pm 0.03$	$0.20^{a} \pm 0.03$

Mean with the same superscript on the same row are not significantly different ($P > 0.05$) while those of different superscript are significantly different ($P \leq 0.05$)

Table 9: Mean Creatinine (mg/dl)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	0.44±0.11	0.44±0.11	0.44±0.11	0.44±0.11
1	0.44±0.11	0.44±0.11	0.33±0.00	0.44±0.11
2	0.44±0.11	0.44±0.11	0.33±0.00	0.33±0.00

The mean creatinine did not vary significantly ($p > 0.05$) among the groups.

Table 10: Mean Total Protein (g/dl)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	4.13±0.44	4.30±0.25	4.07±0.41	3.97±0.89
1	3.72±0.34	3.72±0.56	3.78±0.49	3.22±0.10
2	3.91±0.61	3.85±0.12	3.62±0.36	3.80±0.44

The mean total protein did not vary significantly ($p > 0.05$) among the groups.

Table 11: Mean Bilirubin (mg/dl)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	$1.51^{a} \pm 0.12$	$1.66^{a} \pm 0.07$	$1.29^{a} \pm 0.33$	$1.08^{a} \pm 0.13$
1	$1.44^{a} \pm 0.14$	$1.44^{a} \pm 0.63$	$1.73^{a} \pm 0.25$	$1.59^{a} \pm 0.14$
2	$2.16^{c} \pm 0.25$	$1.80^{bc} \pm 0.07$	$1.59^{ab} \pm 0.14$	$1.15^{a} \pm 0.15$

Mean with the same superscript on the same row are not significantly different ($p > 0.05$) while those of different superscript are significantly different ($p \leq 0.05$)

Table 12: Mean Alanine Amino Transferase (IU/L)

Experimental Group				
Week post infection	**A**	**B**	**C**	**D**
0	$41.30^{a} \pm 1.38$	$42.87^{a} \pm 0.11$	$42.99^{a} \pm 0.19$	$42.76^{a} \pm 0.39$
1	$41.43^{a} \pm 0.76$	$41.32^{a} \pm 0.28$	$41.09^{a} \pm 0.39$	$41.17^{a} \pm 0.37$
2	$40.68^{ab} \pm 0.53$	$41.23^{b} \pm 0.30$	$40.86^{ab} \pm 0.34$	$39.78^{a} \pm 0.33$

Mean with the same superscript on the same row are not significantly different ($p > 0.05$) while those of different superscript are significantly different ($p \leq 0.05$)

Table 13: Mean Blood Urea Nitrogen (mg/dl)

Experimental Group				
Week post infection	**GROUP A**	**GROUP B**	**GROUP C**	**GROUP D**
0	2.73±1.39	3.33±1.32	4.85±1.60	2.73±0.91
1	3.94±1.51	3.33±1.32	2.73±0.91	1.82±0.91
2	3.64±1.05	3.33±1.32	2.12±0.80	2.43±0.61

The mean BUN did not vary significantly ($p > 0.05$) among the groups

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