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Clinico – Haematological Response to Experimental Infection of *Escherichia coli* O157:H7 Strain in *Clarias gariepinus*

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ABSTRACT

Escherichia coli O157:H7 is an important causative agent of severe gastrointestinal disease in humans. This study therefore aimed at elucidating more information on the pathogenesis and haematological response of *C. gariepinus* experimentally infected with *E. coli* O157:H7. The test fish consist of 100 clinically healthy *C. gariepinus* (body weight 110±3.0g). Three concentrations- 10^2, 10^4 and 10^6 cfu/ml of *E. coli* O157:H7 were inoculated intramuscularly into the fish. Blood samples of the test fish were collected at intervals of 48,96,168,336 and 504 hours. The blood and plasma biochemical parameters were analysed using standard methods. The pathogen in the harvested tissue increased from 2.372log10cfu/g to 5.079log10cfu/g by the 72hr incubation but a decline to 4.4log10cfu/g was observed by 216hrs incubation. The significant increase at ($P<0.05$) 48 and 96hours observed in enzymes AST, ALT and ALP suggest liver damage while increase recorded in the level of creatinine and BUN of the test fish is an indication of kidney problem. This study revealed that the general physiology of the fish was adversely affected even at low concentration. Following the trend of the results, it can be inferred that *C. gariepinus*, a hardy fish species, has a strong immune response which should further be investigated.

Keywords: haematology,*Clarias gariepinus*, *Escherichia coli*,plasma biochemistry.

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INTRODUCTION

African catfish, *C. gariepinus i*s one of the most suitable species for aquaculture in Africa and has been considered to hold a great promise for fish farming especially in Nigeria.

Escherichia.coli comprises a group of bacteria found in the intestine of humans, animals and birds. *Escherichia.coli* 0157:H7 strain produces potent toxins and can cause food borne poisons to persons transmitted disease after ingestion of very low numbers of microorganism. Since the first recognized outbreak of food borne disease caused by *E.coli* 0157:H7 in 1982 [1],the serovar has become increasingly prominent, as a cause of serious illness in many countries around the world. Particular characteristics of this *E. coli* strain makes this group a much more serious public health concern.Information on the pathogenesis of most fish borne bacteria infections, in particular *E. coli* O157:H7 infection is scarce. *E. coli* are a group of gram-negative rod-shaped facultative anaerobes that are motile by peritrichous flagella [2]. *E.coli* species are commonly classified by their virulence properties, mechanisms of pathogenicity, clinical syndromes, and O and H serotype [3]. *E.coli* O157:H7 is classified by its serotype, O157:H7, because it expresses the 157th somatic (O) antigen and the 7th flagella (H) antigen [4]. It is an important causative agent of severe gastrointestinal disease in humans, including hemorrhagic colitis. The pathogen causes the majority of sporadic and multiperson outbreaks of bloody diarrhoea in the U.S. *E.coli* O157:H7 is also responsible for the majority of cases of hemolytic uremic syndrome (HUS), a major cause of acute renal failure in children [5;6].According to [7],the major source of food borne *E.coli* 0157:H7 disease is undercooked ground beef, roast beef, roast chicken, raw milk, pasteurized milk and water.

In Nigeria, fish and meat bought from open market are been housed in the same nylon bag and preserved together in the freezer. *E.coli* can survive refrigeration or freezer storage according to [6]. Inconsistency in electricity supply in Nigeria, a developing country, makes the situation worse. The fridge and freezer ends up becoming an incubator for the *E.coli* strain. *E.coli* from meat ends up contaminating the fish eventually.Improperly cooked fish predisposes consumers to the disease. This strain has been shown to be transferable from animal to animal, animal to man, from man to man on food and from person to person through close contact or food (6).

Relatively little information is available on the clinico-haematological changes in experimental fish with this disease. This study therefore aims at elucidating more information on the pathogenesis and haematological response of *C. gariepinus* experimentally infected with *E. coli* O157:H7.

MATERIALS AND METHODS

Fish and experimental conditions

Hundred clinically healthy *C. gariepinus* (body weight 110$\pm$3.0g) were brought from the University of Ibadan, fish farm, Nigeria and transported to the laboratory. They were

acclimatized for two weeks during which they were fed with CHI Coppens ® 4mm feed. The fish were subjected to photoperiod of 12 hours light and 12 hours darkness. The fish were starved for 24 hours before the commencement of the experiment. To evaluate the effects of these serial dilutions on *C. gariepinus* post juvenile fish, twelve test fish were stocked per 60 litres tank [8] and each treatment were replicated thrice while untreated concentrations served as control.

Bacterial Strain and culture conditions

A laboratory stock culture of an isolate of *E. coli* O157:H7 stored at -20^{o}C was used in this study. This isolate was previously isolated from a meat table at Ibadan municipal abattoir, Nigeria. Preliminary identification of the isolate was based on morphological characteristics, aerobic fermentation on sorbitol MacConkey and procedures as described by [9]. Definitive identification of the isolate as *E. coli* O157:H7 was done using *E. coli* O157:H7 antiserum (Difco laboratories). An aliquot of the culture was then inoculated on MacConkey agar and incubated for 24hrs at 37^{o}C. The strain was subsequently purified at least thrice under the same conditions. A serial dilution of 1ml of an overnight broth culture of the pure isolate was made in 0.1% sterile peptone water to obtain concentrations of 10^2, 10^4 and 10^6 colony forming units per milliliter (cfu/ml).

Administration of inoculum

Three concentrations: 10^2, 10^4 and 10^6 cfu/ml of *E. coli* O157:H7 were inoculated intramuscularly into the test fish. Clinical signs and gross changes were observed throughout the experiment. Trend in bacteriological, haematological and plasma biochemical parameters were also observed.

Haematological and plasma biochemical parameters measurement

Blood samples of the test fish were collected at intervals of 48, 96, 168, 336 and 504 hours .The blood samples were collected in heparinized bottles by inserting a syringe needle at the ventral midline just posterior to the anal fin and to continue the insertion at 45^{0} angle until it penetrate the caudal vessels lying between adjacent hermal arches [10].They were centrifuged at 3000G for 5 minutes with the aid of Millipore Personal Centrifuge and the plasma was separated and analyzed. Packed Cell Volume (PCV) and haemoglobin (Hb) concentration were analysed immediately after collection.Red blood cells (RBC) and white blood cells (WBC) were counted by Neubauer's improved haemocytometer using Hyem's and Turk's solution as a diluting fluid respectively.The plasma was analysed for Triglyceride according to [11], Alanine aminotransferase(ALT),Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) ,Cholesterol according to [12] and Total protein according to [13].

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Statistical Analysis

All the data generated were analysed using SAS package and Duncan Multiple Range test was used to separate the means.

RESULTS

Clinical signs and gross changes

The fish became lethargic and disoriented in their swimming in the first 24 hours. At 48 hours, they started hanging and the rates of hanging were dose dependent. Abscess was formed at 48 hours in fish exposed to 10^6 while it took longer time for the abscess to show in the other two concentrations. Swimming ability and the frequency at which feed is been consumed decreased in fish exposed to 10^6 when compared with the rest.

Haematology and plasma biochemistry

The results of the haematology and plasma biochemistry are presented in figures 1-17.

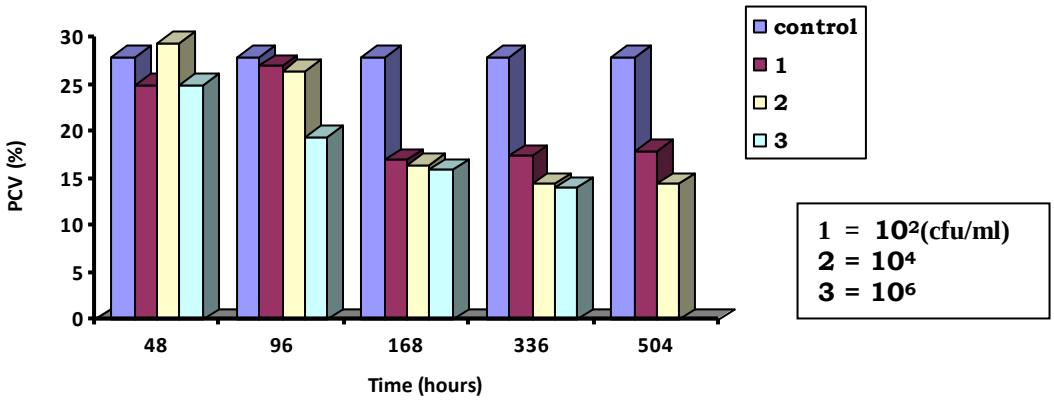

Fig. 1: Variations in PCV of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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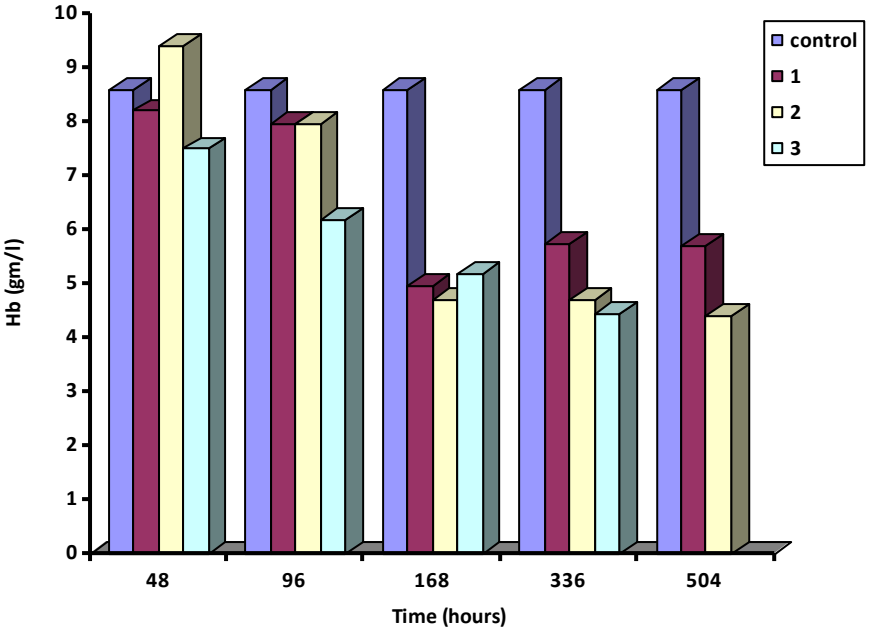

Fig. 2: Variations in Hb of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

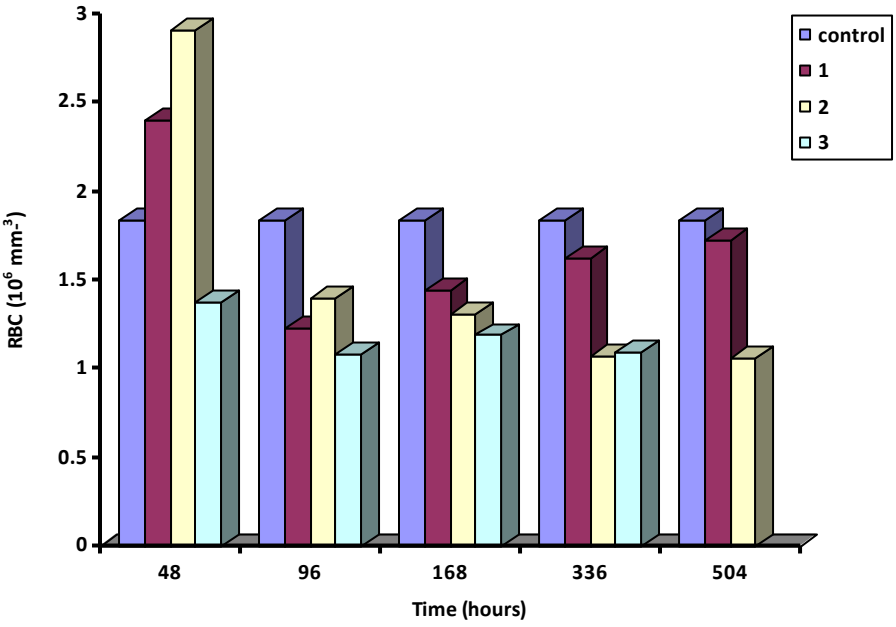

Fig. 3: Variations in RBC of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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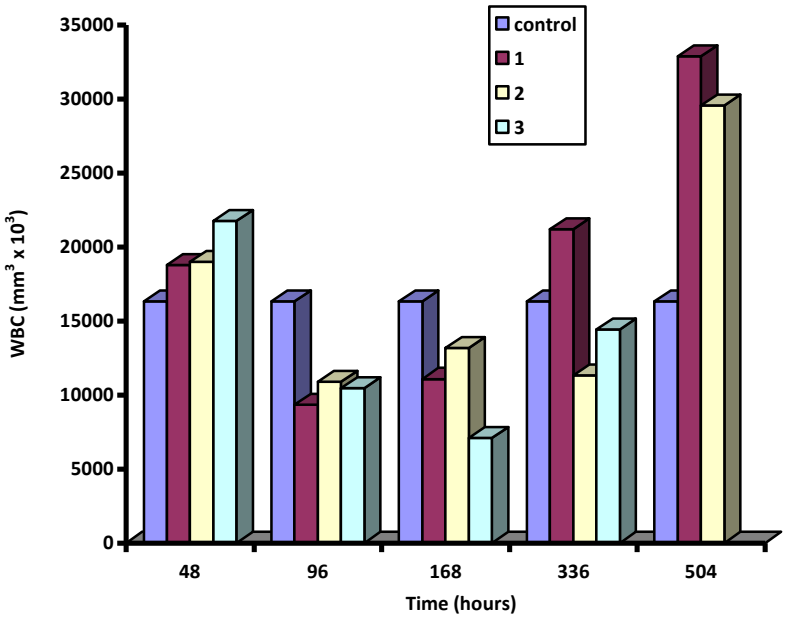

Fig. 4: Variations in WBC of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

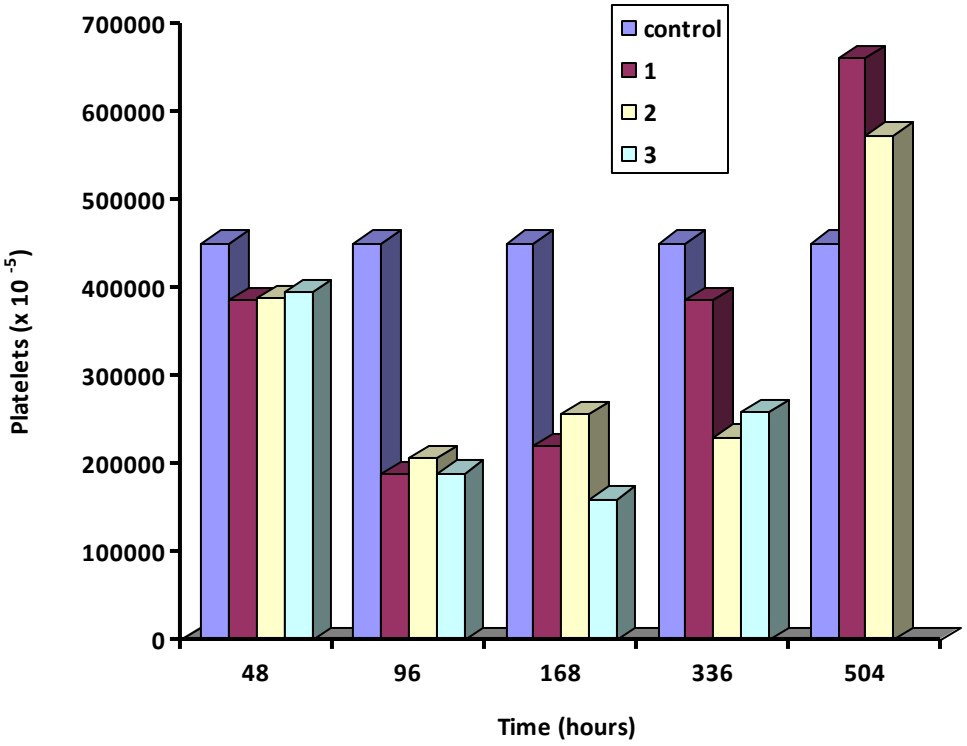

Fig. 5: Variations in platelet of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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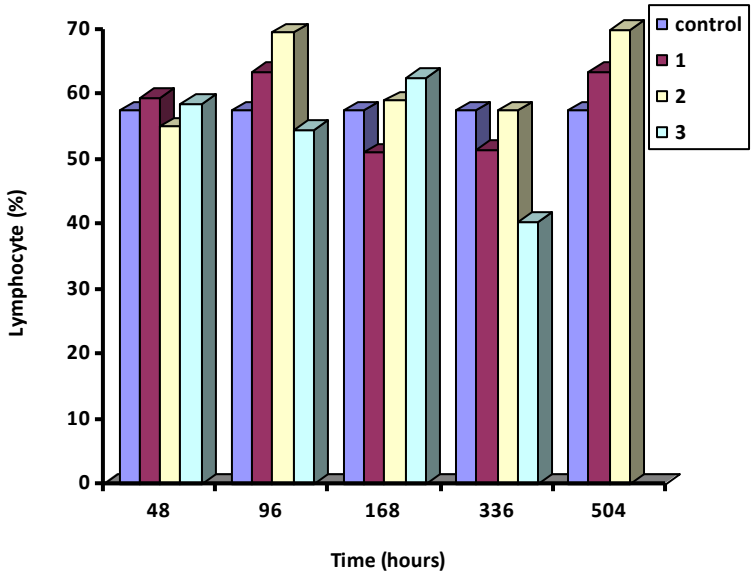

Fig. 6: Variations in lymphocyte of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

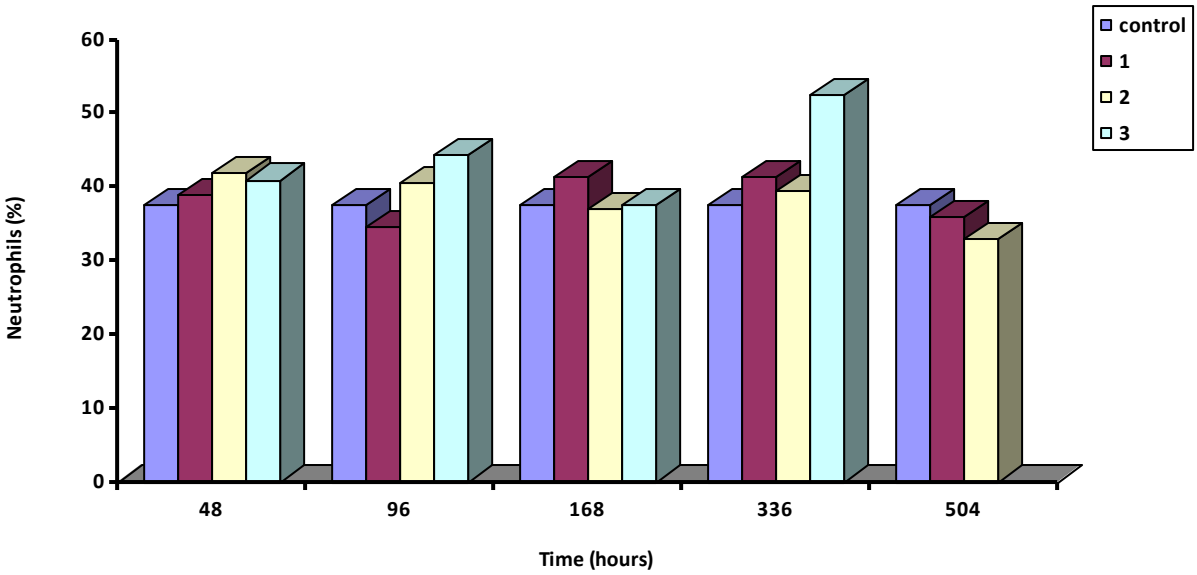

Fig. 7: Variations in neutrophils of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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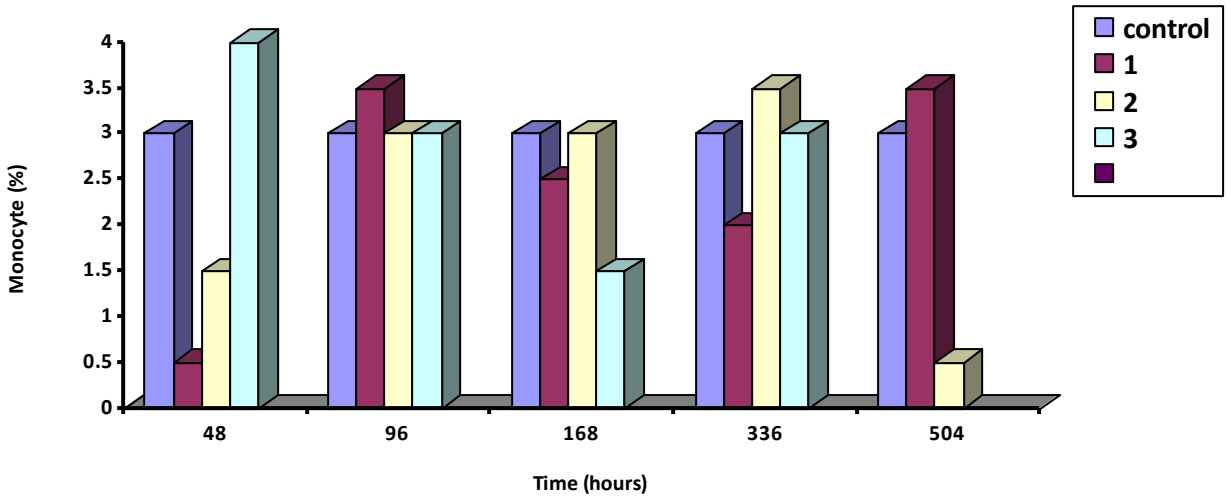

Fig. 8: Variations in monocyte of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

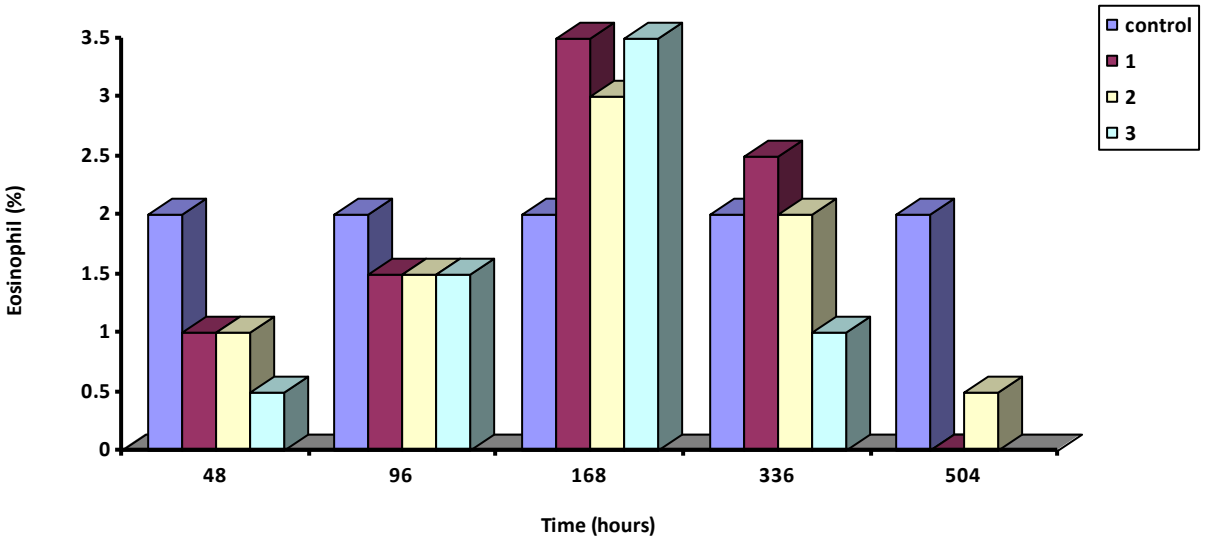

Fig. 9: Variations in Eosinophil of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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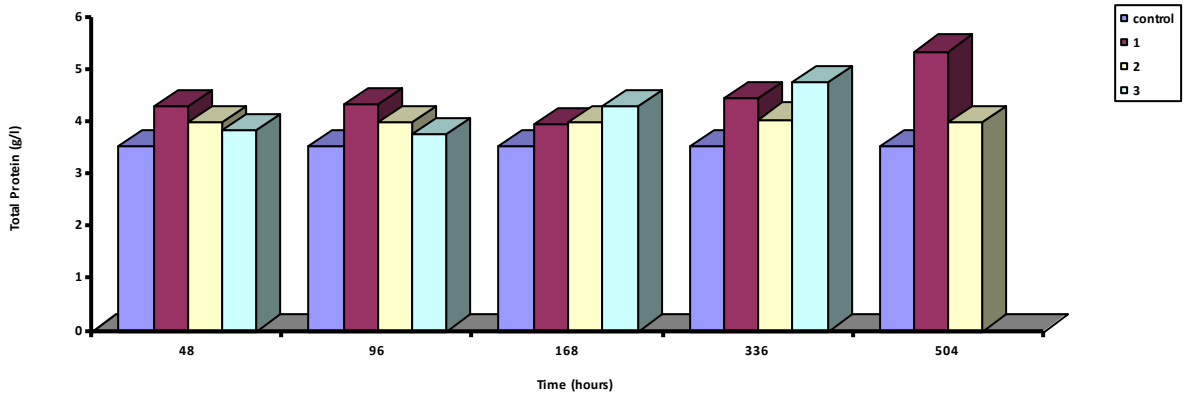

Fig. 10: Variations in total protein of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

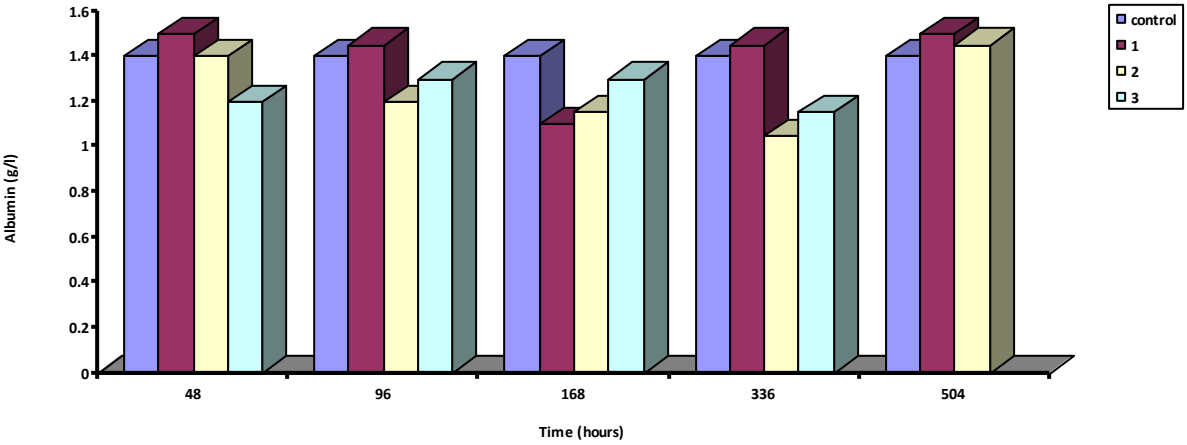

Fig. 11: Variations in albumin of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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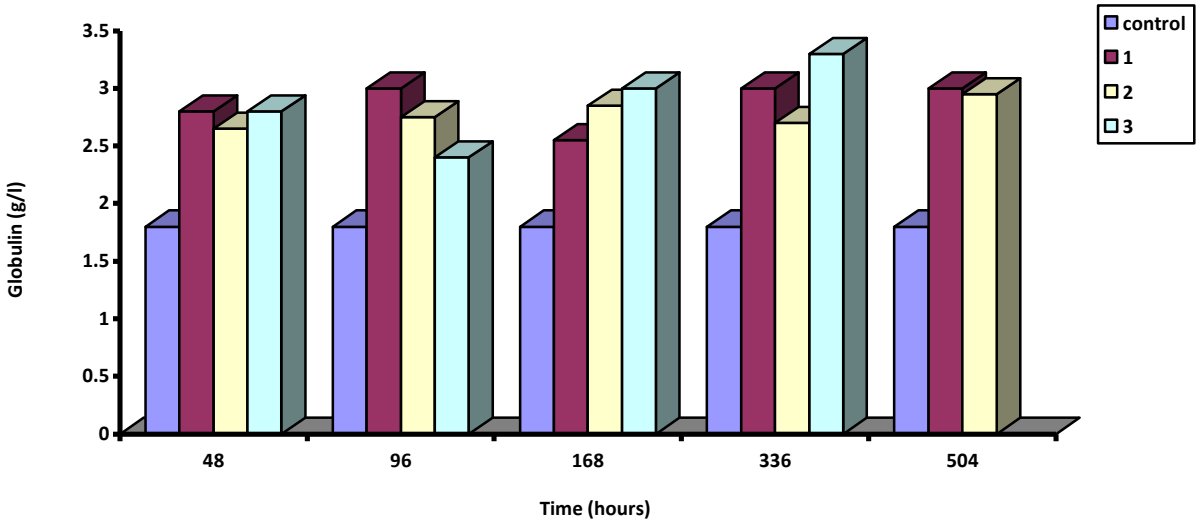

Fig. 12: Variations in globulin of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

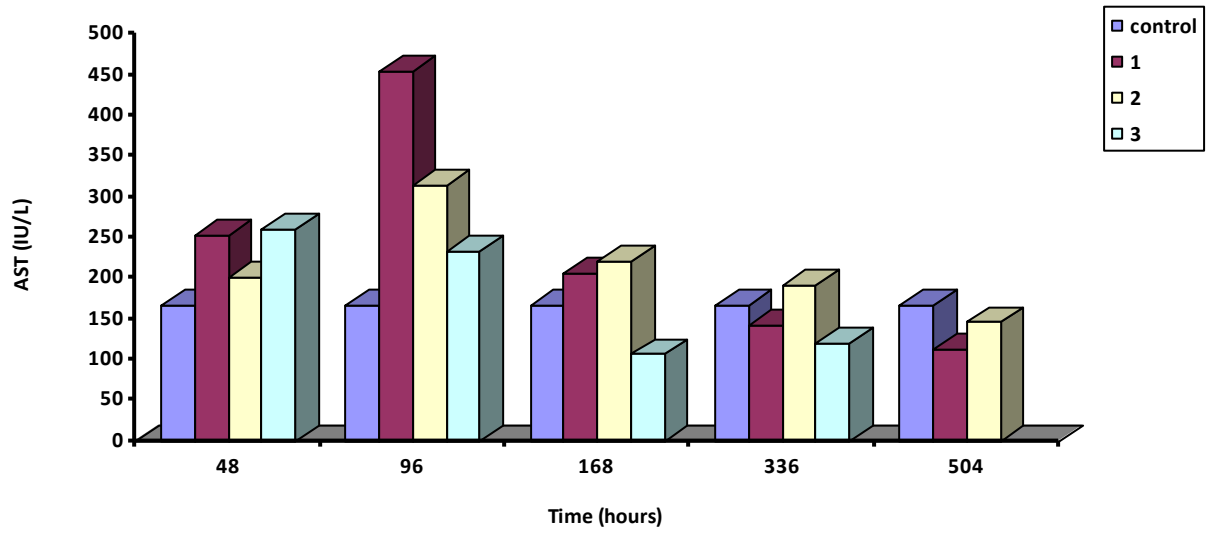

Fig. 13: Variations in AST of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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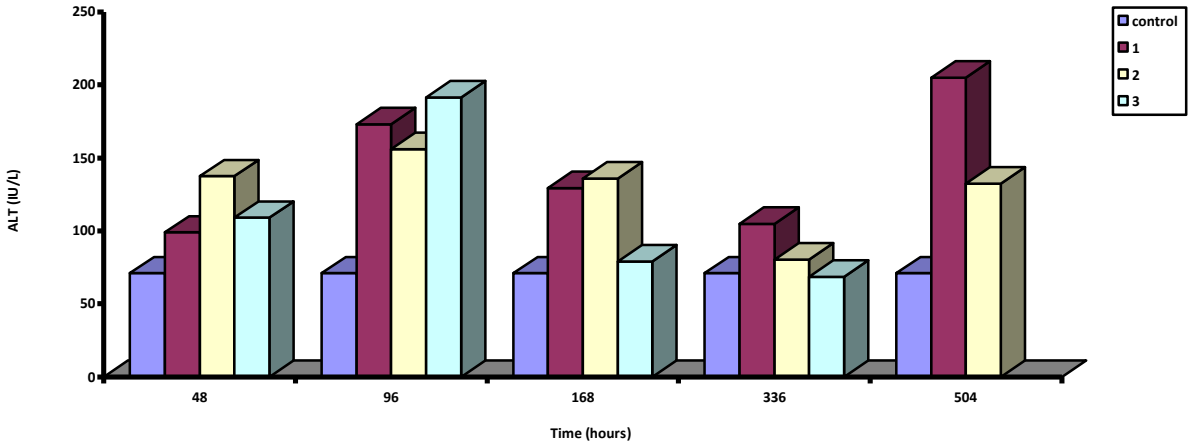

Fig. 14: Variations in ALT of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

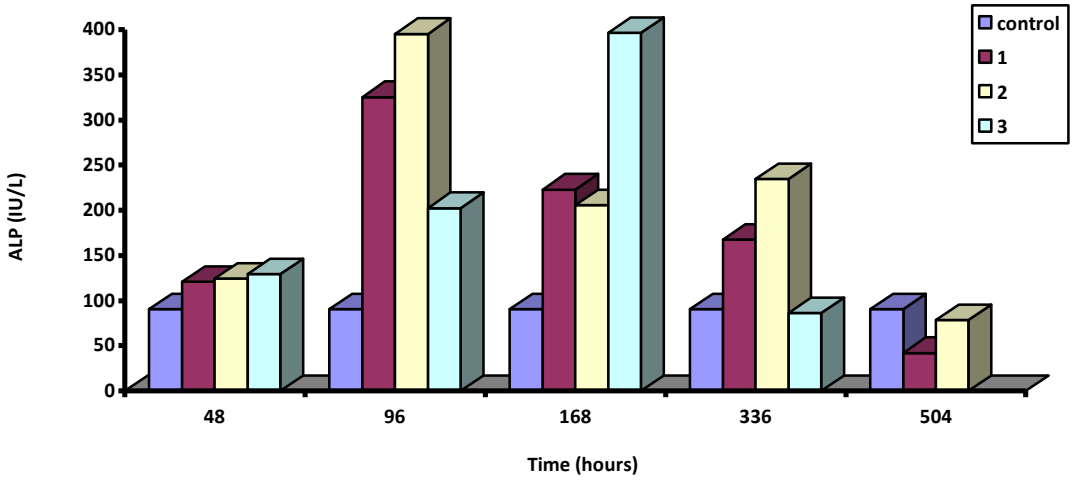

Fig. 15: Variations in ALP of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

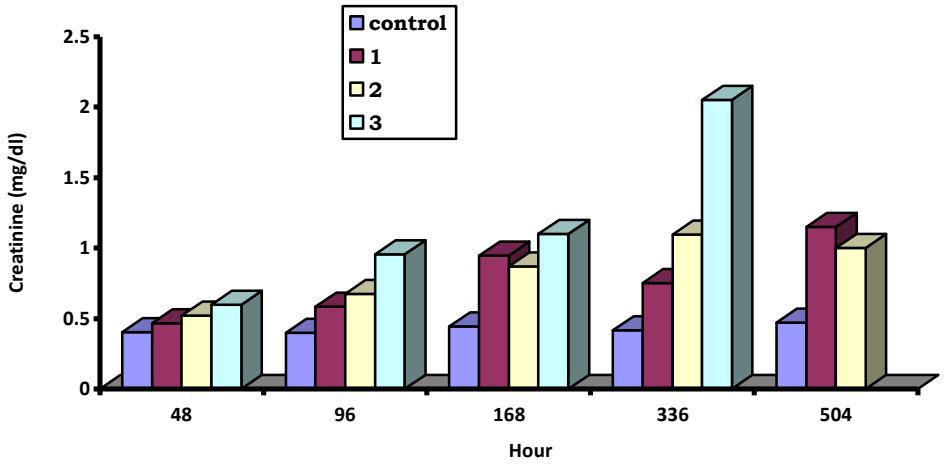

Fig. 16: Variations in creatinine of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

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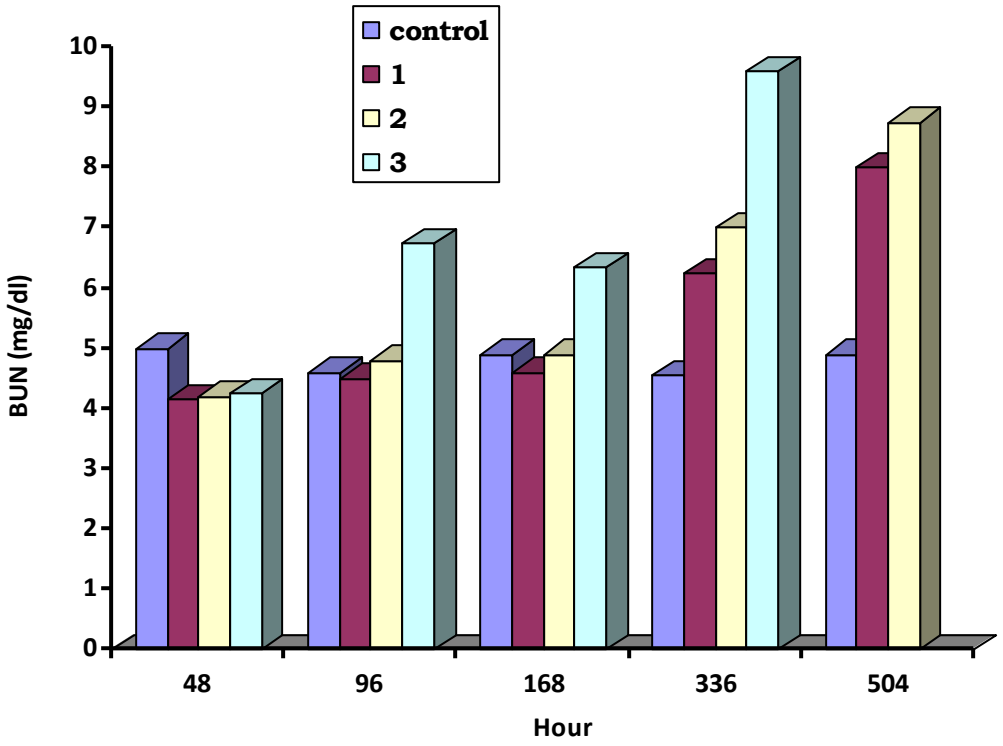

Fig. 17: Variations in BUN of *C. gariepinus* on exposure to varying concentrations of *E. coli* 0157: H7 strain

The PCV, Hb and RBC values (figures 1, 2 and 3) obtained in *C.gariepinus* exposed to *E.coli* 0157:H7 decreased over the time intervals. White blood cell count (figure 4) increased initially at 48 hours but later decreased from 96 hours to 168 hours. Gradual increase was observed at 336 hours while a significant increase ($P<0.05$) was recorded at 504 hours. Decrease in platelet count (figure 5) was obtained from this study. No significant increase was observed in lymphocyte, neutrophil and albumin counts (figures 6,7 and 8) in fish exposed to varying concentrations of *E.coli* 0157:H7 strain compared with the control. A decrease in eosinophil count (figure 9) was obtained at 48 and 96 hours while a significant increase was observed at 168 hours.No significant decrease in total protein count (figure 10) was observed in fish exposed to the *E.coli* strain compared to the control.Significant increase ($P<0.05$) in globulin (figure 12) was observed in fish exposed to the *E.coli* strain throughout the experiment. Also, increase in ALP, ALT and AST (figures 13, 14 and 15) was first observed in fish infected with *E. coli* at 48 hours. The values steadily increased as from 96 hours to 504 hours while creatinine (figure 16) recorded an increase from 48 hours to 504 hours.

Microbial load

The pathogen in the harvested tissue increased from 2.372log10cfu/g to 5.079log10cfu/g by the 72hr incubation but a decline to 4.4log10cfu/g was observed by 216hrs incubation.

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DISCUSSION

The significant decrease recorded in PCV and haemoglobin level in the affected fish indicated an anaemic condition [14].As it causes anaemia in humans, so also is recorded here. This may be due to haemolysis as result of the *E.coli* strain infection [15].

Platelets play an important role in blood clotting. The condition, thrombocytopenia which is a condition in which lower than normal number of platelets in the blood was obtained in this study. This is attributed to the *E.coli* 0157:H7 strain. The increase in plasma AST up to 168hours in this work could be due to liver damage produced by the infected bacteria.ALT recorded significant increase in fish infected with *E.coli* .This is in agreement with the findings of [16] who observed a significant increase in AST and ALT in chicken infected with *E.coli*. Contrary to the observation of [17],no significant difference was recorded in total protein. Total plasma protein were insignificantly different in Guinea pigs experimentally infected with *E.coli* as documented by [18] when compared with the control. There was a significant increase in globulin and this could be associated with its ability to fight infections [19]. The significant change in albumin could be due to liver and kidney damage which could be associated with bacteria infection [20].The increase in urea and creatinine could be due to the effect of the E.coli strain on the kidneys.This result agrees with [20; 21] who reported increased creatinine and urea level in case of renal disease. [22] also recorded markedly raised Blood Urea Nitrogen(BUN) and serum creatinine in humans. Also,elevated BUN was recorded by Panda(2010) as a result of *E.coli* 0157:H7 infection in Dutch Belted and New Zealand White Rabbits suggesting renal dysfunction. This is in agreement with the report of [17] who reported an increased level of creatinine and urea after subjecting Baladi Broiler chicken to E.coli 0157:H7 infection.

According to [23], neutrophils are the first responders to bacterial infection, and pathogens have strategies to combat this response that include inhibition of chemoattractant receptors and inactivation of C5a and IL-8. In order to fight infection, neutrophils must leave the bloodstream to reach effector sites. No significant increase was observed in the neutrophil counts compared with the control. [24] who worked on the modulation of neutrophil function by a secreted Mucinase of Escherichia coli 0157:H7 reported that during infection, E.coli 0157:H7 secretes StcE, a metalloprotease that promotes the formation of attaching and effacing lesions and inhibits the complement cascade via cleavage of mucin-type glycoproteins.lt was found that StcE cleaved the mucin-like, immune cell-restricted glycoproteins CD43 and CD45 on the neutrophil surface and altered neutrophil function. Also, the use of Zebrafish embryos to model neutrophil migration revealed that StcE induced neutrophil retention in the fin after tissue wounding, suggesting severe complications caused by infection with *E.coli* 0157:H7.Therefore, neutrophil function was directly altered during infection with this *E.coli* strain.This corroborates the insignificant difference observed in this study.

The increase in White Blood Cell Count (WBCC) in the first 48 hours agrees with the findings of [25].The decrease in WBCC observed at 96 and 168hours may be due to the hardy

nature of the fish.The count began to increase again at 336hours which culminated to a significant increase at 504hours.This shows the prevailing negative effect of the E.coli strain on the test fish.

The pathogen in the harvested tissue increased from 2.372log10cfu/g to 5.079log10cfu/g by the 72hr incubation but a decline to 4.4log10cfu/g was observed by 216hrs incubation. The presence of this pathogen in tissues harvested from the dead or crucified fish carcasses acertains that this pathogen was the cause of the haematological changes observed in this study. The fact that some of the fish were still alive at the end of the experiment shows the *C. gariepinus* is a very hardy fish type.

CONCLUSION

From this study, even though total protein was not affected at acute and chronic exposure to *E. coli* O157:H7 strain, the general physiology of the fish was adversely affected even at low concentration. Following the trend of the results it can be inferred that *C. gariepinus*, a hardy fish, which is the most cultured in Nigeria has a strong immune response which should further be investigated. Also, as the occurrence of this pathogen cannot be totally eradicated, adequate care should be taken to minimize its effects.

RECOMMENDATIONS

A series of measures can be taken to minimize the risk of infection:

- maintain good fish nutrition
- avoid overcrowding of stocked fish species
- maintain good personal hygiene on the fish farm
- Always wash hands after contact with farm animals, animal faeces, and animal environments with antibacterial soaps
- In case of polyculture practice (poultry and aquaculture),disinfectant foot baths should be placed at the entrance of the poultry house
- Dripping live haul truck that travel from one farm to another should be disinfected at regular intervals
- limit visitors to the farm.

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