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Effect of Partial Feed Deprivation on Serum Liver Enzymes' Activities and Hepatic Histoarchitecture in *Clarias gariepinus* (African Catfish)

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Abstract

The aim of this study was to evaluate serum liver enzymes' activities and hepatic histoarchitecture in partially feed-deprived *Clarias gariepinus* (African Catfish). A total of forty-eight (48) active, live and apparently normal catfish randomly placed into two groups were used for this study. Fish in control group (labeled B) were fed with 4% of their body weight twice daily while fish in the feed-deprived group (labeled A) were fed with 25% of that quantity fed to the control group. Blood and liver samples were obtained at intervals of 7 days for 28 days for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and for histopathology respectively. Results revealed significantly higher ($P < 0.05$) values for ALT, AST and ALP in the feed-deprived group (A) compared to the control group (B). On histopathology, liver sections of feed-deprived fish showed lipid vacuolation and this became accentuated over the period and most prominent on day 28. The study has demonstrated that starvation in catfish produced liver damage reflected by increase in serum activities of these liver enzymes.

Keywords: Liver enzymes; Histoarchitecture; *Clarias gariepinus*; Starvation; Lipid vacuolation.

1. Introduction

Fishes, like other animals, are affected by lack of adequate feed supply, and this will surely manifest in them as either retarded growth rate, loss of weight and even shape among others [1]. Many fish species exhibit extraordinary resilience to prolonged starvation, but the reasons for that and the mechanisms employed during these periods are poorly understood [2]. The liver in fishes, just like in other animals, is a very good pointer to many abnormalities because of its diverse functions and indispensability in many metabolic/biochemical processes in the body system [3]. A number of tests have been devised for detection of alterations in liver function. The liver function test makes use of serum analysis of some liver specific enzymes and others that albeit produced by other organs in the body, but much of it is by the liver. The serum activities of these enzymes are measured to widely ascertain the functional status of the liver and invariably that of the animal patient [4]. Alanine amino transferase (ALT) and aspartate amino transferase (AST) are some of the liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathological changes, and can be assessed within a shorter time. However, Oluah [5], noted that increased or decreases in the activities of ALT and AST indicated tissue damage in liver, kidney, muscle and gill. Starvation response in animals is a set of adaptive biochemical and physiological changes that reduce metabolism in response to a lack of food [6]. In this study, activities of serum liver enzymes and liver histologic changes of *Clarias gariepinus* (African Catfish) were evaluated to establish the role of partial feed deprivation in causing liver damages.

2. Materials and Methods

2.1. Ethical Considerations

The use of fish in this study was approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC).

2.2. Experimental Animals

Fourty-eight (48) active, live *Clarias gariepinus* (African Catfish) of about 300-400 grams body weight raised in concrete ponds were used for the investigation. They were randomly placed in to groups A (feed-deprived) and B (control) of 24 each, both of which were acclimatized for 24 hours before onset of experiment.

The control group (B), was fed twice daily with 3-4% of their body weight, average of 12-16 grams of 6mm extruded floating pellets of Multifeed®, while the experimental group (A) was fed once daily 1/4 the quantity given to the control groups, an average of 3-4 grams of the same feed type, as recommended by Craig and Helfrich [7].

Water was changed routinely at intervals of 3 days throughout the period of experiment. Samples were obtained at intervals of 7 days for a period of 28 days and a total of 6 fish (3 from each group) were humanely euthanized during each sampling.

2.3. Blood Sampling and Analysis

Blood samples were collected from each fish with a 2ml syringe and 23G needles using the caudal vein into labeled sterile plain sample bottle. The harvested sera were transferred into labeled sample bottles for ALT, AST and ALP analysis using the method of the test of Confidence (Reckon Diagnostics P. Ltd.).

2.4. Postmortem Examinations

Each fish was humanely euthanized and the organs were examined. The liver was examined grossly for changes and sections were collected in 10% buffered neutral formalin for histopathology using standard histological techniques [8].

2.5. Data Analysis

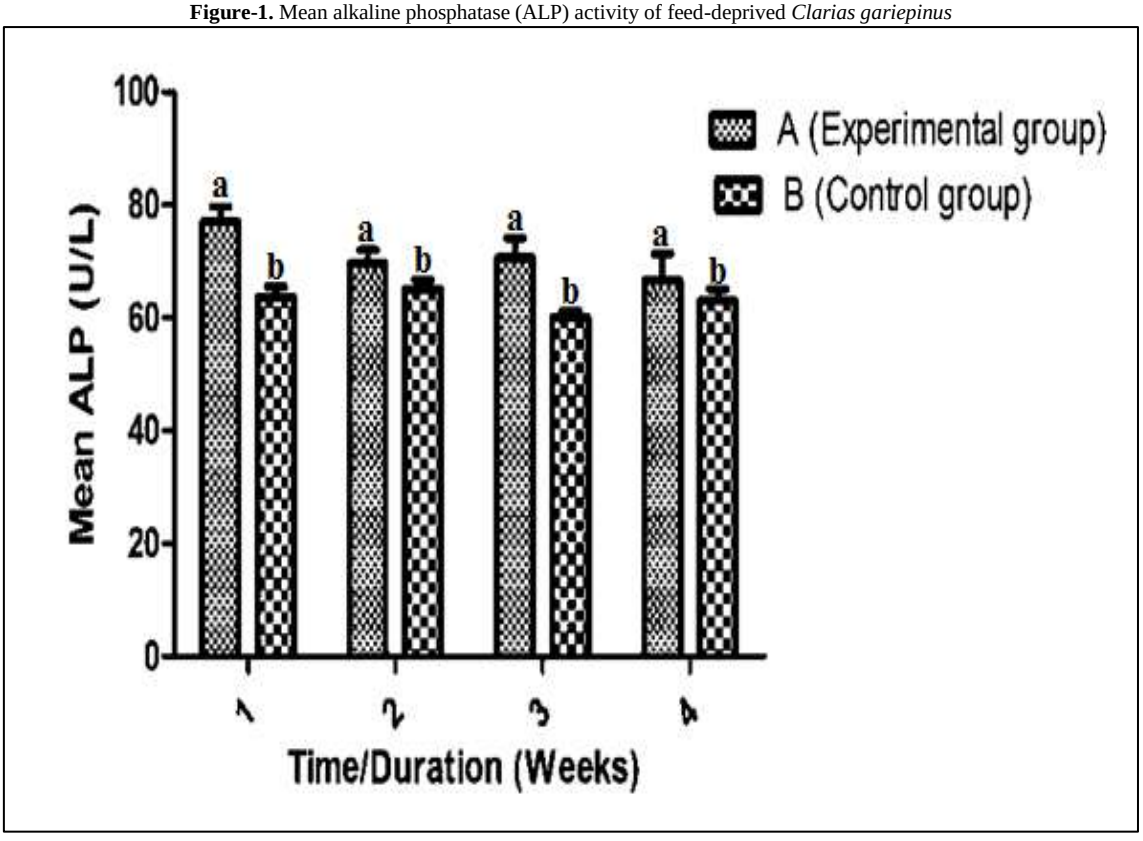
The values obtained were expressed as mean ± SEM and tabulated. These were subjected to two-tailed unpaired student t-Test, using GraphPad prism version 5.0 for levels of significance. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Liver Enzymes Activity

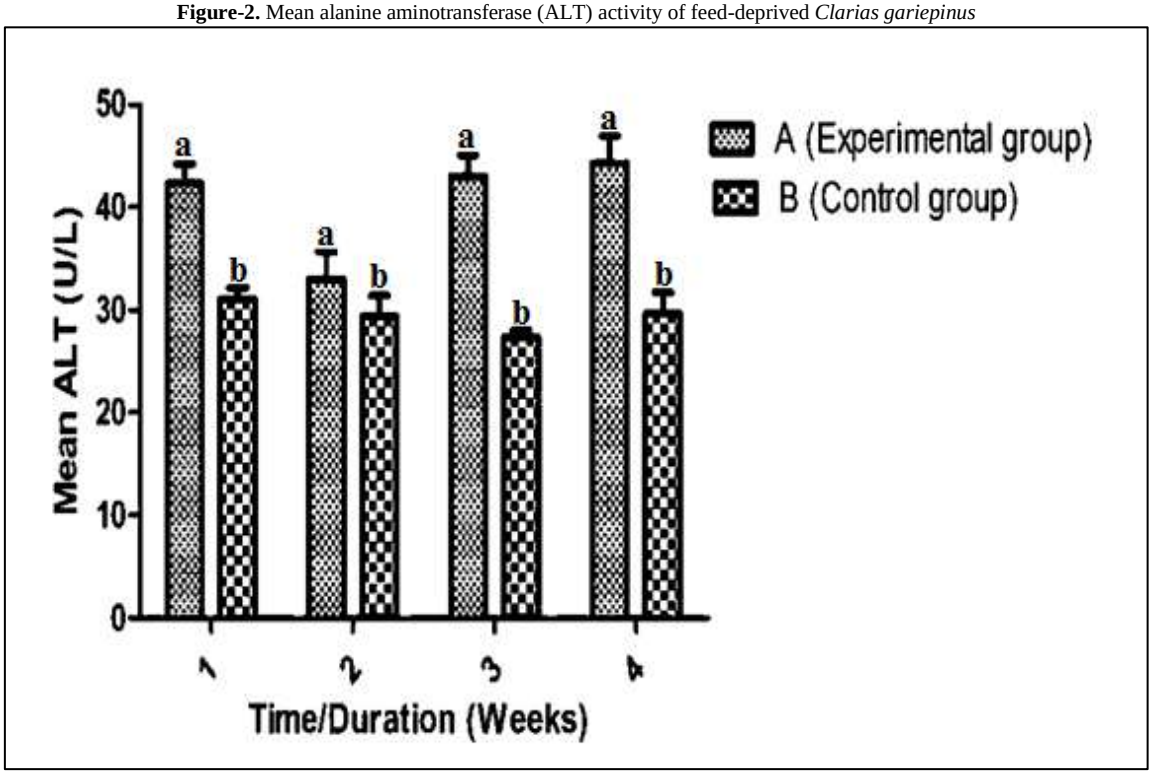
The mean ALP serum activities in the feed deprived *C. gariepinus* group as at the first week of the experiment was 77.000 ± 2.646 U/L. This was significantly ($P < 0.05$) higher than the value (63.667 ± 1.856 U/L) recorded for the control group during the same week (Fig. 4.1). Similarly, the mean ALP serum levels of the experimental group was significantly ($P < 0.05$) higher at the end of the second (69.667 ± 2.333 U/L), third (70.667 ± 3.480 U/L) and fourth (66.667 ± 4.631 U/L) weeks compared to the control group with values of (65.000 ± 1.732 U/L), (60.000 ± 1.155 U/L) and (63.000 ± 2.082 U/L) respectively (Figure 1).

Figure-1. Mean alkaline phosphatase (ALP) activity of feed-deprived *Clarias gariepinus*



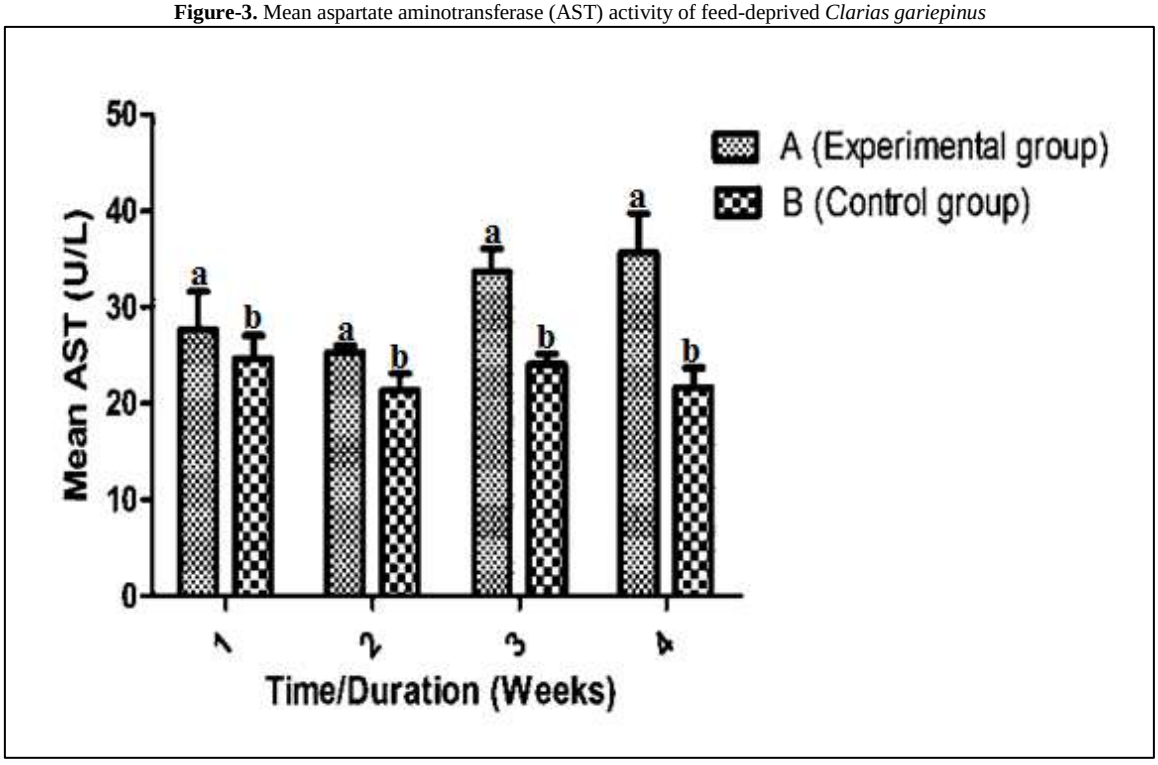
After the first week of feed deprivation, mean serum ALT activities of *C. gariepinus* was 42.333 ± 1.856 U/L, being significantly ($P < 0.05$) higher than that of the control group (31.000 ± 1.155 U/L). In the second week, the values decreased insignificantly in both groups but differed significantly between the experimental and control groups (Figure 2). While the mean values of the enzyme increased thereafter, reading 43.000 ± 2.082 U/L in the third week and 44.333 ± 2.603 U/L at the end of the fourth week of feed deprivation, the control group had decrease in mean values which were also significantly lower ($P < 0.05$) than in the experimental group (Figure 2).

Figure-2. Mean alanine aminotransferase (ALT) activity of feed-deprived *Clarias gariepinus*



The mean activities of serum AST on day 7 of feed deprivation was 27.667 ± 3.930 U/L (Figure 3). This was significantly ($P < 0.05$) higher than that of the control group (24.667 ± 2.404 U/L). It was observed that the mean serum levels of this enzyme increased and was continuously higher in the experimental than control groups during the period of investigation. It was also observed that the values of the enzyme rather decreased in the control group (Figure 3).

Figure-3. Mean aspartate aminotransferase (AST) activity of feed-deprived *Clarias gariepinus*



3.2. Gross Pathology and Histopathology

The livers in the control group were apparently normal in size and colour, being reddish brown to dark brown colouration (Figure 4). The livers of feed deprived group showed yellowish discolouration (fatty change), which became so marked on days 21 and 28 (Figure 5).

The hepatic parenchyma was apparently normal on day 7 of partial deprivation, but on day 14, a significant and prominent lipid vacuolation of hepatic cells was observed (Figure 6). On day 28, there were hardly any normal hepatocytes seen on the section. The vacuoles were larger and distorted the architecture, making the section to

appear as an adipose tissue (Figure 8). The livers from the control groups had normal tissue architecture throughout the period of the investigation (Figure 7).

Figure-4. Photograph of an apparently normal liver of *Clarias gariepinus* from control group

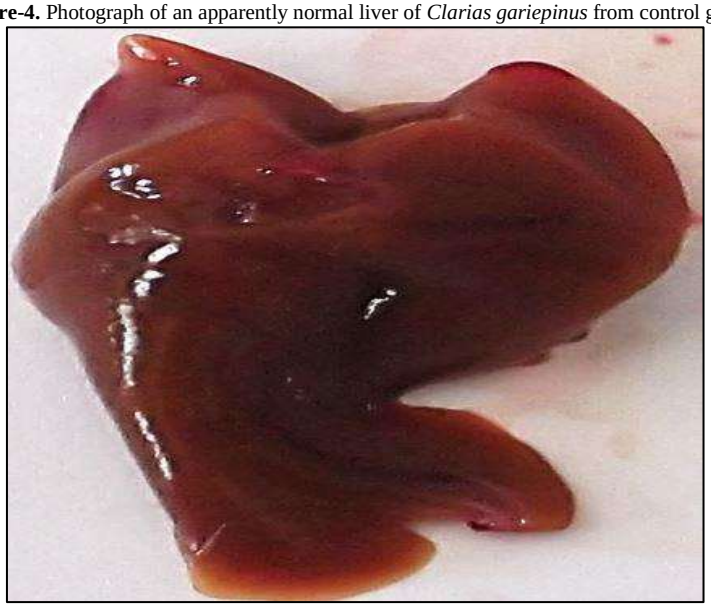


Figure-5. Photograph of liver from feed deprived *Clarias gariepinus.* Note the fatty change (diffused yellowish discolouration after 28 days of feed deprivation

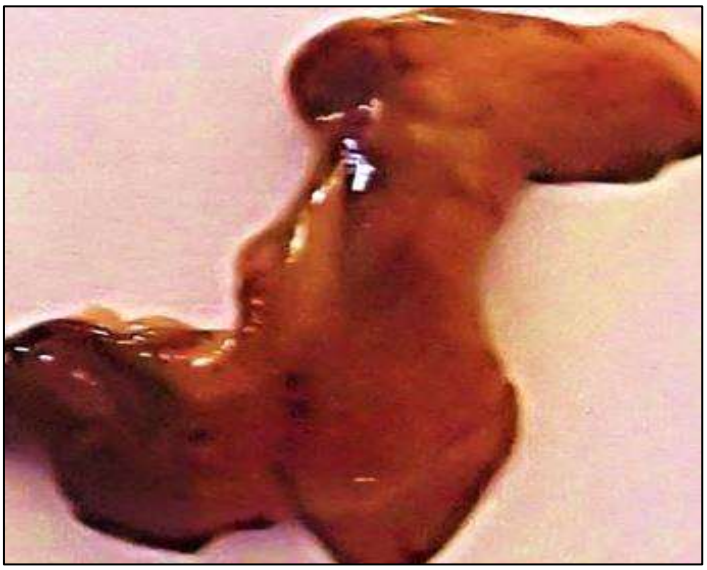


Figure-6. Photomicrograph of liver of *Clarias gariepinu,* after 14 days of feed deprivation. Note the numerous fat vacuoles (arrows) (H & E x 400)

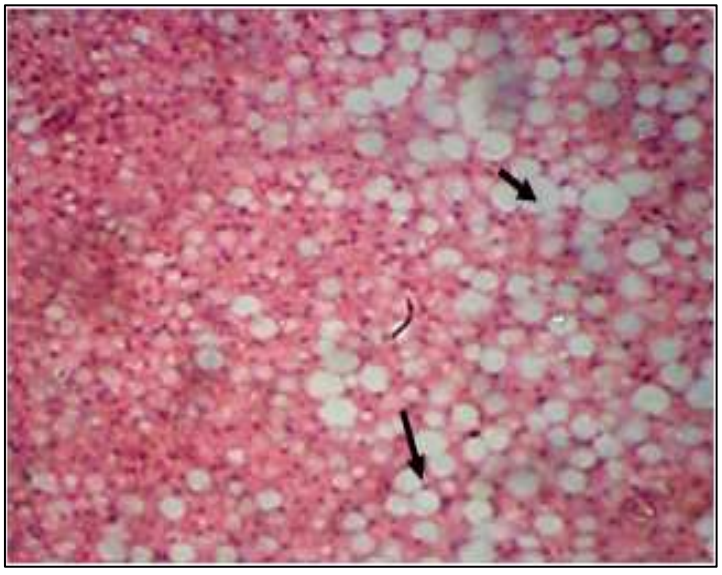


Figure-7. Photomicrograph of liver from a control *Clarias gariepinus* after 14 days of normal feeding showing normal tissue architecture H & E x 400

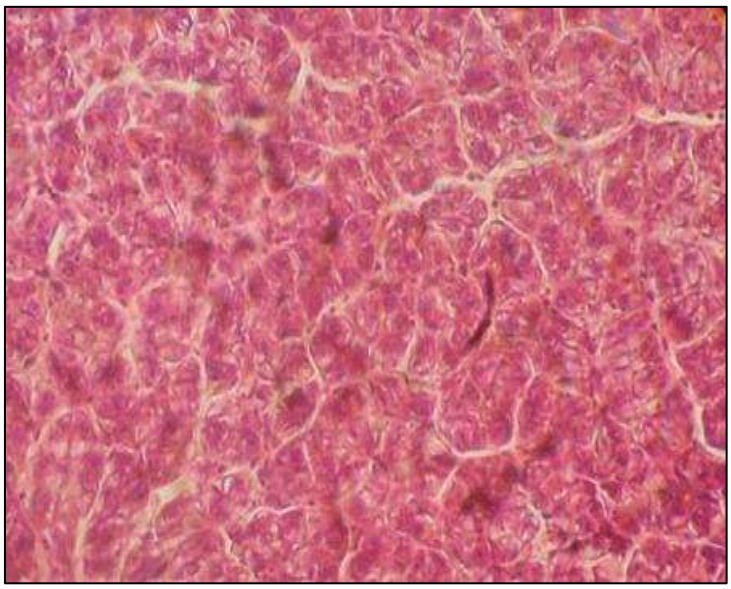
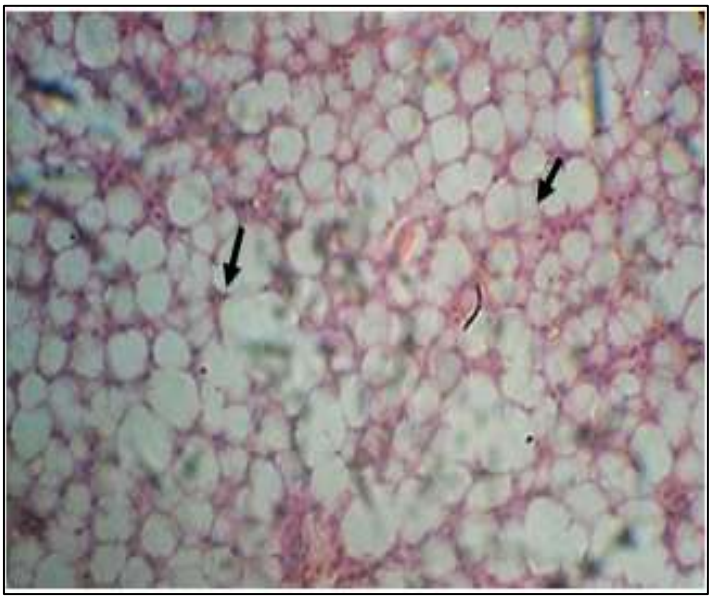


Figure-8. Photomicrograph of liver of *Clarias gariepinus*, after 28 days of feed deprivation. Note the extensive lipid (large sized and multiple) vacuoles (arrows) H & E x 400



4. Discussion

The ALT and AST are non-plasma specific enzymes that are localized in cells of liver, heart, gills, kidneys, muscles and other organs; and their presence in the serum may give specific information about organ dysfunction [9, 10]. In this study, the serum activities of AST, ALT and ALP in the feed deprived catfish were significantly elevated compared to those of the control group. This was evident at different phases of the study. These however, were not absolute elevations when compared to the normal clinical chemistry values. A decrease in the activity of lactate dehydrogenase in both liver and muscles as a function of starvation has been reported in *Heteropneustes fossilis* [11]. The results also perfectly correlates with the gross and histologic features of the livers examined in the study, in that in the experimental group with higher serum enzyme levels, the gross appearance was yellow, while the histology showed varied levels of lipid vacuoles in hepatocytes. This proportionally agrees with the duration of feed deprivation but may not be categorically established based on the gross or microscopic features of the liver , since a specialized staining technique is required to reveal the extent of lipid infiltration and that of glycogen. Wolf and Wolfe [12], had noted that it is difficult to determine the extent to which lipid vacuolization should be considered excessive and potentially deleterious. Unfortunately, there are no universally applicable criteria for the diagnosis of hepatic lipidosis. Therefore, diagnosis of fatty change in fish liver via microscopic analysis of the sectioned liver is difficult and requires a lot of patience and expertise, coupled with special stain for fat. The observed glycogen vacuoles in the hepatocytes of control group indicates storage of extra energy reserves, which is expected in properly fed fishes, and agrees with findings of Wolf and Wolfe [12], that glycogen deposition is presumably due to imbalances in energy intake and expenditure caused by artificial feeding and housing conditions. Also, glycogen and protein have been reported to be decreased following starvation in freshwater fish *Channa punctatus* [4]. The highest

degree of relative enzyme elevations was recorded in the activities on day 28 of feed deprivation, thus indicating significant liver damage.

5. Conclusions

In this study, a varied degree of starvation in *Clarias gariepinus* accounted for a proportional liver damage that led to increase in serum activities of AST, ALT and ALP. These serum enzymes activities gave a true reflection of the hepatic integrity and are therefore the biomarkers to consider in liver damage. Hence, the catalytic influence of various factors such as inhibitors and activators, during pathological and stressful conditions such as starvation reflect damages to biological tissues which can be assessed by alterations in enzyme activities.

Supplementary Materials: N/A

Author Contributions: Conceptualization, review and editing, SJS; methodology, data analyses, original draft preparation, BSA and OO. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: N/A

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Conflict of Interest

The authors declare no conflict of interest.

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