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BIOCHEMICAL CHANGES IN THE JUVENILES OF AFRICAN CATFISH (*CLARIASGARIEPINUS*) DUE TO STARVATION USING BIO-ASSAY METHOD

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ABSTRACT

The increase in human population and reports of large numbers of people, malnourished or starving (especially in the developing countries) has made the need for food production a major worldwide issue of concern. Therefore, this study is aimed at finding out the changes in the haematological and serum biochemical parameters induced by starvation in African catfish (*Clariasgariepinus)*.The experiment was carried out in two plastic experimental tanks (50x34cm) for 3 weeks in the Fisheries Laboratory, of the Department Of Animal and Environmental Biology, University of Port Harcourt, Nigeria. Blood samples were collected from caudal blood vessels, blood was separated in two portions; one portion was mixed with anticoagulant while another portion of sample was centrifuged without anticoagulant for serum separation. Haematology, biochemical indices and blood serum results from the experiment were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 2006 version 15.0. In the starved fish (21 days) a significant reduction in the hemoglobin and hematocrite content was observed. It was observed that the starvation induced a significant decrease in protein, creatinine, triglycerides, enzymes like Serum glutamate oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate, Transaminase (SGPT) and electrolyte sodium whereas glucose and Blood Urine Nitrogen (BUN) increased after starvation in the sample organism. The changes in environmental factors such as deprivation of food causes stress on the fish which may bring about disturbance in the blood parameters and thereby affecting the survival of the fish. Starvation induces different responses on blood biochemical level, depending on how long the starvation lasts, therefore, proper feeding of farmed fish should be practiced by farmers to reduce fish mortality, increase fish production and enhance hunger eradication

KEYWORDS: Clariasgariepinus, Haematological, Biochemical indices, Starvation

INTRODUCTION

Fish is known worldwide as a very important component of human diet because of its high nutritive value and significance in improving human health (Ladu, 2001). It contributes significantly to the survival and wellbeing of a large number of the people around the world. Fish is an important source of essential nutrients which includes; protein, lipids, vitamins and minerals (Tsado *et al.,* 2012). Starvation has been reported by the NIR, (2006) to induce numerous psychological and biochemical changes in fish. Long term starvation has been reported to cause stress in fishes, how they respond to this stress

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Vol.1 No.1; 2020

conditions depends on the species NI R, (2006). It was observed by Froese and Pauly. (2014)that there was a reduction of egg laying ability in tilapia, due to starvation for 14days, also the males were unable to mate, as there was a drastic reduction in courtship behaviors. Starvation has been reported to orchestrate a wide range of haematological changes in fish NIR, (2006), these changes which may include but not limited to alteration in body metabolism, blood glucose and serum levels, electrolyte concentration level etc, all these blood parameters when altered can cause severe and lethal effect on fish. Significant biochemical changes were observed in fish by Careymost and Lawson (1973), when they fed the African Catfish (*Clariasgariepinus*) with different fish feeds and insect's larvae; this indicated that the type of fish feed used in culturing influences the biochemical composition of the fish. Long term starvation can cause death of fish; as likewise all organisms NIR, (2006). Changes in fish habitat were reported to cause different impact on European eel (*Anguilla anguilla*) by Caruso *et al*., (2010).They reported that European eel (*Anguilla anguilla*) exposed to lead and other heavy metals accumulated a high amount of these metals in their tissues thus, causing biochemical changes and impairing other vital activities like, finding mate and food in. Habitat changes have also been implicated in orchestrating electrolyte imbalance by Careymost and Lawson, (1973).This research was aimed at studying and documenting the biochemical changes in juveniles of African catfish (*Clariasgariepinus*) due to starvation using bio-assay method.

MATERIALS AND METHODS

Study Design

The experiment was carried out in two plastic experimental tanks (50x34cm) for 3 weeks in the Fisheries Laboratory of the Department Of Animal and Environmental Biology, University of Port Harcourt, Nigeria. The water level in each tank was maintained at volume of 35 litres throughout the study period. The treatment tank was labeled TT (meaning treatment tank), while the starved tank was labeled ST (which implies starved tank) Water in each tank was replaced every three meanwhile, each tank was well aerated.

Experimental Procedure and Feeding Trials

The experimental diet used in this study was the coppens feed. Each treatment and starved tank had a mean initial body weight of 7.39±0.02g. The fish were acclimatized for fourteen days before the experiment and fed at 3% body weight daily.

Preparation of Experimental Diets

The mean proximate composition of the experimental diet was 40.00±0.01% crude protein, 15.90±0.04% ether extract, 15.70±0.02% ash, 7.40±0.08% moisture, and 20.90±0.01% Nitrogen Free Extract. The feed (coopens) also contains some other nutritional requirement necessary for fish development.

ISSN: 2582-6271

Vol.1 No.1; 2020

Blood Parameters

Blood samples were collected from caudal blood vessels, blood was separated in two portions, one portion was mixed with anticoagulant another portion of sample was centrifuged without anticoagulant for serum separation.

Blood Biochemical Parameters

Total Serum Proteins (TP) was measured by using the modified Biuret method, end point assay as described by Lawrence, (1986), serum glucose determined by (GOD-POD) Glucose oxidase – peroxidase, end point and assay method, Blood urea nitrogen (BUN)was determined by modified Berthelot method, cholesterol was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method, HDL was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method, LDL was determined by Friedewald's equation

$$LDL = Total\ cholesterol - \frac{Triglycerides}{5} - HDL\ cholesterol$$

Triglycerides (TG) were determined by (GPO-PAP) glycerol-3-phosphate oxidase - phenol aminophenazone end point assay method. Creatinine was determined by modified Jaffe's method Kinetic test without deproteinisation according to the Jaffe's method. Serum glutamate oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) activity was assayed following modified International Federation for Clinical Chemistry (IFCC) method using commercial kit. Sodium and Potassium: are determined by colorimetric method. Calcium is determined by Modified Arsenazo method.

Statistical Analysis

Haematology, biochemical indices and blood serum resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 15.0).

RESULTS

Results of blood serum component levels in starved and fed Clariasgariepinus

The blood protein results obtained from the present day study indicate a value of 5.82mg/l for the fed group while 3.99mg/l was observed in the starved group. 48.23mg/l of glucose was observed in the fed group, while 75.51mg/l was observed in the starved group. 302.96mg/l of triglycerides was observed in the fed group, while 275.65mg/l was recorded in the starved group. The cholesterol level of the fed

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Vol.1 No.1; 2020

group was observed to be 253.5mg/l and 253.76 for the starved. Creatinine was observed to be 2.44mg/l and 0.56mg/l for the fed and starved group respectively. The result is shown in Table 1.

Results for Haematological Parameters of Fed and Starved Clariasgariepinus

The hematological results indicated a value of 8.70g/dl hemoglobin in the fed group and 6.77g/dl for the starved group. 21.5% Haematocrite was observed in the starved group and 18.33% for the fed group (Table 2).

Table 1: Blood serum component levels in starved and fedClariasgariepinus

Parameters (mg/l)	Fed group	Starved group
Protein	5.82 ± 0.79	3.99 ± 1.47 **
Glucose	48.23 ±8.87	75.51 ± 3.20***
Triglycerides	302.96 ± 65.09	275.65±149.41**
Cholesterol	253.5 ± 29.21	253.76 ± 74.67^{NS}
Creatinine	2.44 ± 0.37	0.56 ± 0.26***

* Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

Table 2: Results forHaematological Parameters of Fed and Starved*Clariasgariepinus*

Parameters	Fed group	Starved group
Haemoglobin (Hb) g/dl	8.70 ± 0.74	6.77 ± 1.35**
Haematocrite (Hct) %	21.5 ± 2.21	18.33 ±3.88**

Each value is expressed as mean ± SD, N = 6.

NS = Not significant, * = significant P = < 0.05, ** = significant P = < 0.01, *** = significant = P < 0.001

Results for enzymes Parameters for Fed and Starved *Clariasgariepinus*

6.97mg/l and 39.16mg/l of Blood Urine Nitrogen were observed in the fed and starved group respectively. HDL of the fed group was 67.32 while the starved group was 20.32. The LDL was observed to be 41.54 and 178.13 for the fed and starved groups respectively. Serum glutamate oxaloacetate Transaminase (SGOT) was observed to be 15.65 and 1.87 for the fed and starved groups respectively. Serum Glutamate Pyruvate Transaminase (SGPT) was observed to be 16.94 and 6.64 for the starved and fed groups respectively. ALP was observed to be 59.55 and 58.89 respectively for the starved and fed groups (Table 3).

Results for electrolyte concentration for fed and Starved *Clariasgariepinus*

ISSN: 2582-6271

Vol.1 No.1; 2020

Sodium (Na) ion concentration was observed to be 74.13 mg/l and 17.14mg/l respectively. In the fed and starved group. Potassium (K) ion concentration was observed to be 14.9mg/l and 16.61mg/l respectively. Calcium (Ca) ion concentration was observed to be 9.01mg/land 9.32mg/l respectively (Table 4)

Table 3: Results for enzymes Parameters for Fed and Starved*Clariasgariepinus*

Parameters	Fed group	Starved group
BUN	6.97 ± 0.59	39.16 ± 10.38***
HDL	67.32 ± 18.45	20.35 ± 4.54***
LDL	41.54 ± 12.38	178.13 ± 48.28***
SGOT	15.65 ± 0.69	1.87 ± 1.21***
SGPT	16.94 ± 0.26	6.64 ± 3.48***
ALP	59.55 ± 6.64	58.89 ± 7.50^{NS}

Each value is expressed as mean ± SD, N = 6.

NS = Not significant, * = significant P = < 0.05, ** = significant P = < 0.01, *** = significant = P < 0.001

Table 4: Results for electrolyte concentration for fed and starved Fed and Starved *Clariasgariepinus*

Parameters	Fed group	Starved group
Sodium (Na)	74.13 ± 13.07	17.14 ± 3.26***
Potassium (K)	14.96 ± 1.59	16.61 ± 1.35^{NS}
Calcium (Ca)	9.01 ± 0.68	9.32 ± 1.36^{NS}

Each value is expressed as mean ± SD, N = 6.

NS = Not significant, * = significant P = < 0.05, ** = significant P = < 0.01, *** = significant = P < 0.001

DISCUSSION

This study was undertaken to evaluate the response of fish to short term exposure to starvation causing stress which was assessed by analyzing different hematological and blood biochemical parameters. The knowledge of the hematological and blood biochemical adopted by the fish Clariasgariepinus to face different type of stress conditions may have important practical implications in culturing this species. It may help to prevent the probable damage to fish health resulting from these conditions. It is also well known that the homeostatic system enables fish to face the effect of environmental changes (temperature, salinity, oxygen, food availability), but the responses to environmental stressors can vary

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Vol.1 No.1; 2020

greatly according to the fish species, the developmental stage and the individual characteristics in addition to the type of stress and its duration (Pottingeret al., 1992). Starvation affects the normal body metabolism and prolonged starvation may even cause death of animal (Joshi, 1973). A decline in various body constituents of fish, following experimental starvation have been reported by various authors considering starvation as a chronic stress condition, the response of fish to stress involves activation of the neuro – endocrine system, by releasing the stress related hormones in the blood as a primary response followed by hematological and biochemical changes as a secondary response which includes growth inhibition, impaired reproduction and immune response in many fish species (Reddy et al., 1995; Pascualet al., 2003).

In the starved fish, a significant reduction in the hemoglobin and hematocrite was observed. Conflicting results exists however in the scientific literature concerning the effect of starvation on blood hemoglobin content and hematocrite value. Increase or decrease in hematocrite on exposure to starvation has been reported in earlier studies, recently Caruso et al., (2010) reported for the European eel (Anguilla anguilla) and found the reduction in the hemoglobin and hematocrite. Starvation is known to induce different responses on blood glucose level, depending on how long the starvation lasts, as well as on the species-specific differences in the metabolism and its regulation. The present study shows that the concentration of serum glucose increased significantly during starvation, suggesting that starved fish were able to maintain their value of glycaemia by enhancing gluconeogenesis, similar to the results obtained by Hung et al. 1997. The good gluconeogenic capability in the starved fish,suggested their adaptive response to food shortage by means of mobilization of non carbohydrate sources in order to preserve their glucose homeostasis.

The amount of protein, glucose and glycogen also decreased as the period of starvation increased (Letcher et al., 1996). Tripati and Verma, (2003) found decrease in rate of protein in the fresh water cat fish Clariasbatrachus and suggested that the rate of protein synthesis were also could result from reduced protein synthesis capacity brought about by reduction in the concentration of ribosome. In the present study a significant reduction in the serum total protein was observed in the fish. Love in 1980 demonstrated and suggested that, during prolonged starvation, the fish use protein as an energy source via, gluconeogenesis. Liver function would likely result in a decrease of urea production as these pathways are energetically expensive. However the increasing urea content in plasma is likely an indicator of failing gill osmoregulatory capability (Wood et al., 2003). In the present study the elevated BUN indicate failure of gill osmoregulatory function due to starvation and leading to osmoregulatory stress. However, the levels of blood urea nitrogen (BUN) are related to the protein content (Asper et al., 1990). Excessive protein metabolism can be demonstrated by plasma chemistry caused by starvation (increases in blood urea nitrogen [BUN]), breakdowns in lipid metabolic regulation (free fatty acid and ketone bodies) and carbohydrate regulation (glucose), all of which are altered in pinnipeds that are

ISSN: 2582-6271

Vol.1 No.1; 2020

fasting for extended periods or have begun to starve (Castelini and Rea, 1992). In the present study blood urea nitrogen was found to be elevated and creatinine levels were not changed significantly after starvation in the fish. The cholesterol is an important component of cell membrane and functions as a precursor of the synthesis of sexual hormones. In the present study the C.gariepinus was found to be same in the serum cholesterol levels in the starved fish as compared to the fed fish this may be due to shorter period of exposure of starvation. The decrease in Triglycerides (TG) ratio was observed in the fish, after starvation for two weeks. The decrease in the blood Triglycerides level observed allows presuming that lipolysis during starvation probably was the major source of energy (Hung et al., 1997) particularly during first two weeks of starvation. The HDL levels were decreased and LDL levels were increased in the starved fish as compared to fed fish. A significant difference found in the HDL and LDL for the carp Cyprinuscarpio exposed to starvation has also been reported. The decrease in the blood serum protein level was accompanied by a significant ($p < 0.001$) reduction in SGOT and SGPT activity, which pointed out to a slowed-down rate of amino acid transformations via transamination indicated that the starvation causes damage either in the hepatopancreas or in the muscles, as their activity is evidenced in the carp, Cyprinuscarpio. (Friedrich and Stepanowska, 2000). Similarly a significant reduction in the enzymes SGOT and SGPT was observed in the fish in this study. In the present study the alkaline phosphatase activity was reduced in the fish C.gariepinus during starvation.

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ISSN: 2582-6271

Vol.1 No.1; 2020

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