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Research Article

Some Haemo-biochemical Changes Induced by Gastrointestinal Parasites of Cultured and Wild African Catfish in Zaria, Kaduna, Nigerian

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ABSTRACT

Keywords: Haematological, biochemical, gastrointestinal parasites, *Clarias gariepinus* The present study investigated the occurrence of gastrointestinal (GIT) parasites and their relationship with some haemato-biochemical parameters in wild and cultured catfish (Clarias gariepinus) in Zaria and its Environs, Nigeria. A total of two hundred and forty (240) African catfish (*Clarias gariepinus*) were sampled from cultured ponds and some water bodies around Zaria. About 2 ml of blood was collected from the caudal vein of each catfish and dispensed into EDTA and plain sample bottles for haematological and serum biochemical analysis, respectively. After blood sample collection, the catfish were dissected longitudinally to examine for parasites (GIT) grossly and using Simple flotation technique for parasite eggs and oocysts of Helminthes and protozoans. Modified Ziehl Neelson staining technique was further performed on oocysts suspected to be *Cryptosporidium*. Percentage distributions of the various parasites include: Crytosporidium oocysts, Eimeria oocysts and nematodes were 86.2%, 48.4% and 40% in the wild; as against 13.8%, 51.6% and 60% in cultured Clarias gariepinus, respectively. The PCV, RBC, and Hb were significantly lower (p<0.05) in the infected (28.0 \pm 0.331%, 3.4 \pm 0.592 x 10^{12} /L, 8.3 ± 0.174 g/dl) compare with the uninfected *Clarias gariepinus* (33.3 ± 0.229%, $3.9 \pm 0.050 \times 10^{12}$ /L, 10.5 ± 0.110 g/dl), respectively. However, the WBC was significantly higher (p = 0.041) in the infected (4.5 \pm 0.047 x 10⁹/L) when compared to the uninfected $(3.9 \pm 0.017 \times 10^9/L)$ Clarias gariepinus species. Also, there were statistically significant increases (p<0.05) in the mean values of AST, ALT, and ALP in the infected (82.2 \pm 1.037 U/L, 95.8 \pm 1.209 U/L, 68.8 \pm 1.180 U/L) when compared with the uninfected (60.7 \pm 0.741 U/L, 68.9 \pm 0.844 U/L, 47.1 \pm 0.759 U/L) Clarias gariepinus, respectively. The results of present study overserved that helminth parasites induce haematobiochemical changes.

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INTRODUCTION

Fish provides a comparatively cheap source of animal protein for man and his livestock and attention is now being focused on fish production, both from natural water and aquaculture (Komatsu and Kitanishi, 2015). Fish as smoked, dried, or fresh is highly priced in Nigeria. It contributes 22% of animal protein supply in sub-Sahara Africa and 40% of animal protein consumption in Nigeria (Omeji et al., 2022). Fish as common important source of protein do contain lipids, minerals, oils, and vitamins. The most common fish available in Nigeria are the catfish species of C. gariepinus and C. angularis (Kawe et al., 2016). The Clarias gariepinus from the family Clariidae is among the most important tropical catfish species for aquaculture in West Africa. The wide geographical spread, high growth rate and the resistance to handling and stress has made C. gariepinus well valued in a wide number of African countries (Enas et al., 2013).

The demand for fish as a source of protein increases as the human population grows (Nnabuchi et al., 2015). To increase fish supply as protein source, there has been tremendous increase in the development of fish farming (Skelton and Teugel, 1992). The growth rate in both fresh and saltwater fishing in Nigeria increased at an average rate of 7.4 percent in the 1960s (FAO, 2016) to 18% in 2020 (Odioko and Becer, 2022). However, Fish industry has been plagued by the problems which include overcrowding, poor environmental conditions and pollution which often results in reduced immunity of fish and higher susceptibility to parasites and diseases (Murray, 2005; Al-Murjan and Abdullahi, 2008).

Haemato-biochemical changes are used as indicators of organisms' responses to stressors and their health status under such conditions (Anyeni *et al.*, 2022). Generally, haematological tests are used to establish normal health status and to diagnose diseases caused by

various factors: viz heavy metals, environmental stress, parasitic infections, genotoxic effect of pollutants, nutrition, and pollution in human and veterinary science (Fedato et al., 2010). Haematological parameters act as physiological indicators to changing external environments (Caruso et al., 2005) because of their relationship with energetic (metabolic levels), respiratory (haemoglobin) and defense mechanisms (leukocyte levels) (Oriakpono et al., 2012). They also provide an integrated measure of the health status of an organism, which over time manifests in changes in weight (Yaji et al., 2007). Hence, the aim of this research was to study some of the haemato-biochemical changes induced by gastrointestinal parasites of Clarias gariepinus in both cultured and wild, which could be used in diseases diagnosis and in guiding the implementation of the treatment or preventive measures.

MATERIALS AND METHODS Study Location

The research was conducted at the Department Veterinary Medicine. Ahmadu Bello of University Zaria, Kaduna State, Nigeria. Zaria is located within the Northern Guinea Savannah Zone of Northwestern Nigeria. It lies between latitude 7° to 11° N and longitude 7° 44 E. It has an average rainfall between 1,000 to 1, 250 mm and an average temperature between 17 °C to 33 ⁰C and vegetation made of predominantly trees and grasses, while the main economic activity is agriculture. It has a land area of 300^2 km and a human population of about 408, 000 (Okolo and Makanjuola, 2021). It has a climate that is divided into two seasons, a rainy season between May to October and the dry season between Novembers to April, with a cold dry spell between Novembers to February (Harmatan).

Sample Size

The sample size was determined based on the formula by Thrusfield (2008). According to the work of Oniye *et al.*, (2004), a prevalence of

19.17% was obtained for helminth infection of Catfish in Zaria, and this prevalence was used for the calculation of sample size and 5% absolute precision, and 95% confidence interval were used. Therefore, the calculated sample size was arrived at as follows:

$$\frac{1}{d^{2}} \frac{1}{d^{2}}$$
n = sample size
p = prevalence (19.17%) (Oniye *et al.*, 2004)
d = desired absolute precision (0.05)
Z = Standard normal deviate for 95% confidence level (1.96)
q = 1 - p

$$\frac{1.96^{2} \times 0.1917 \times (1 - 0.1917)}{0.05^{2}} = \frac{3.84 \times 0.1917 \times 0.8083 = 0.5950}{0.0025} = 234$$

However, a total of 240 catfish (120 each of specifically *Clarias gariepinus* wild and cultured fish) were sampled to increased precision.

Sources of catfish

 Z^2 pg

n =

Two hundred and forty (240) *live Clarias gariepinus* were procured and used for this study. The 240 were sourced from local fishermen and commercial fish farms in Zaria (at n=120 for each, respectively). The fish were transported to the Helminthology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria in a 25-litre plastic container containing water.

Blood Sample Collection

About 2 ml of blood sample were obtained from the catfish via caudal vein as described by Kori-Siakpere *et al.* (2005). Blood samples collected were properly labelled as infected and noninfected based on the presence of parasites in dissected fish. From the 2 ml of the blood sample, 1 ml was dispensed into heparinized tubes while the remaining 1 ml was collected into non heparinized tubes for haematological and biochemical analyses, respectively. The non-heparinized blood samples were centrifuged at 3000 rpm for 10 minutes to extract serum.

Dissection of Catfish for Parasites Examination

For easy handling prior to dissection, the fish were immobilized by cervical dislocation. A longitudinal slit on the ventral surface of the abdomen from the anus to a point level with the pectoral fins was made using a surgical blade. The viscera were removed and separated into oesophagus, stomach, and intestine. Each of the sections were placed into separate Petri dishes containing 0.6% saline before examination for the presence of parasites under a dissecting microscope. Fish that shows microscopic presence of parasites (*Crytosporidium oocysts, Eimeria oocytes and* Nematodes) were considered infected.

Identification of Parasites

For the identification of the parasites, nematodes were cleared with lactophenol while the cestode and trematodes were stained overnight with a weak Ehrlich's haematoxylin solution and passed through graduated concentrations of alcohol (30, 50, 70, 90% and absolute) for 45 min to dehydrate, and cleared in methylsalicylate. The parasites were then covered and mounted on a slide in Canada balsam and viewed using Olympus Microscope under high power magnification (x40). The parasites were identified to species level using appropriate keys according to Pouder et al. (2011). All parasites recovered were recorded accordingly.

Simple flotation technique

From 2 g of intestinal contents in a centrifuge tube, 5 ml of flotation medium with specific gravity of 1.18-1.2 was added. The suspended sample was sieved into a test tube using gauze. To obtained the desired amount of sediment (8 ml of sample formalin mixture), it was further sieved through 2 layers of gauze into a conical 15 ml centrifuge tube. Then 0.85% Nacl was added, and centrifuged for 10 mins at 500 x g. The supernatant was collected in a test-tube and suspended in 2 ml of Zinc sulfate. And then centrifuge for 2 mins at 500 xg, allowed to stopped and settle on its own with the tube still in the centrifuge. This was followed by taking 2 drops using a pipette and placed on a clean glass slide and viewed under X10 objective. Oocysts seen were identified with the aid of an Atlas Encyclopedia of Parasitology Photographs and Manual of Parasitology (Levine, et al., 1985).

Modified Ziehl Neelson staining technique

For confirmation of Cryptosporidium oocysts, positive oocysts were further subjected to modified Ziehl Neelsen stain as described by Lynne and Garcia (1999). 1 to 2 drops of intestinal content were lightly smeared on a glass slide and allowed to air-dry. The slide was placed on a heating block (70°C) for 5 minutes, and then transferred into a staining rack. This was followed by flooding it with carbol fucshin, before over flaming using Bunsen burner which was stopped as soon as the stain began to steam. It was allowed to dry and stained again without heating, followed by rinsing with water. Decolorization was done with 5% sulphuric acid for 30 seconds, rinsed and drained with water. The slide was then flooded with methylene blue for 1 min, rinsed with water, stained and air dried and thereafter examined with the oil objective.

Evaluation of Haematological Parameters

Packed cell volume (PCV) was determined by the Microhaematocrit centrifugation technique as described by Brar *et al.* (2014). After gently mixing the blood sample in a vacutainer tube. The blood was drawn upto 75% of the Microhaematocrit capillary tube by capillary action and the opposite end of the tube carefully sealed with plasticine. The capillary tubes were placed, with the closed end outwards, in a Microhaematocrit centrifuge (Hawksley and Sons Limited, England) and spun at 194.81 gpm for 5 min. Then the proportion of cells in total volume of blood was measured and recorded as a percentage using the Hawskley[®] Microhaematocrit Reader.

Haemoglobin concentration measured was spectrophotometrically by the cyanmethaemoglobin method as described by *et al.* (2014). Five mililitre Brar of haemiglobincyanide (HICN) (Drabkin) solution were measured using a 5 ml syringe into plastic test tubes. Twenty microliter (20 ul) of blood was measured using a micropipette and added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 x g for 15 minutes to separate the empty RBC from interfering with the reading. The supernatant was separated into a sample bottle. The mixture was absorbed into the haemoglobin meter (XF-C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dl as the haemoglobin concentration.

Serum Biochemical Evaluation

Serum transaminases including: Aspartate aminotransferase (AST), Alanine Alkaline aminotransferase (ALT) and determined using phosphatase (ALP) were Randox reagent according kits. to manufacturer's instruction.

Statistical Analysis

All the data obtained were subjected to statistical analysis including the calculation of the means and standard error of the means. Data between groups were evaluated using student t-test and values of P < 0.05 were considered significant **PESULTS**

RESULTS

Finding from this study showed that wild animals had higher percentage of infection (61.3%) than cultured (38.7%) *Clarias gariepinus*. Percentage distributions of the various parasites include; Crytosporidium oocysts, Eimeria oocysts and nematodes having 86.2%, 48.4% and 40% in the wild; while 13.8%, 51.6% and 60% in cultured Clarias gariepinus, respectively (Table 1).

Based on infection, the PCV, RBC, and Hb were significantly (p<0.05) lower in the infected Clarias species (28.0 \pm 0.331%, 3.4 \pm 0.592 x 10^{12} /L, 8.3 ± 0.174 g/dl) when compare with the uninfected *Clarias* species $(33.3 \pm 0.229\%, 3.9 \pm$ 0.050×10^{12} /L, 10.5 ± 0.110 g/dl), respectively.

However, the WBC was significantly higher (p = 0.041) in the infected *Clarias* species (4.5 \pm 0.047×10^9 /L) when compared to the uninfected $(3.9 \pm 0.017 \text{ x } 10^9/\text{L})$ (Table 2).

The infected Clarias gariepinus had significantly (p<0.05) higher mean serum AST, ALT and ALP (82.2 ± 1.037 U/L, 95.8 ± 1.209 U/L, 68.8 ± 1.180 U/L) when compared with the uninfected (60.7 \pm 0.741 U/L, 68.9 \pm 0.844 U/L, 47.1 ± 0.759 U/L respectively) (Table 3).

Table 1: Occurrence of Gastrointestinal	parasites in <i>Clarias gariepinus</i> Based on Types of Parasites
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Parasites					
Source	Cryptosporidium oocysts (%)	Eimeria oocysts (%)	Nematodes (%)	Total (%)	
Wild	25 (86.2) ^a	15 (48.4)	6 (40)	$46(61.3)^{a}$	
Cultured	$4(13.8)^{b}$	16 (51.6)	9 (60)	29 (38.7) ^b	
Total	29 (100)	31 (100)	15 (100)	75 (100)	
a vs h = 0	001 (significant)				

a vs b = 0.001 (significant)

Parameters Heath		h Status	P value
	Infected	Non-infected	
Packed Cell Volume (%)	28.0 ± 0.331	33.3 ± 0.229	0.021*
Red Blood Cell count (x $10^{12}/L$)	3.4 ± 0.592	3.9 ± 0.050	0.003*
White Blood Cell count (x $10^{9}/L$)	4.5 ± 0.047	3.9 ± 0.017	0.041*
Haemoglobin concentration (g/dl)	8.3 ± 0.174	10.5 ± 0.110	0.011*
*n < 0.05 (significant)			

p<0.05 (significant)

Table 3: Biochemical Parameters of Clarias species Based on Health Status

Parameters	Health	Health Status	
	Infected	Non-infected	
Aspartate amino transferase (U/L)	82.2 ± 1.037	60.7 ± 0.741	0.015*
Alanine amino transferase (U/L)	95.8 ± 1.209	68.9 ± 0.844	0.001*
Alkaline phosphatase (U/L)	68.8 ± 1.180	47.1 ± 0.759	0.001*

*p<0.05 (significant)

DISCUSSION

The helminth parasites identified in this study comprised of 2 groups: namely Nematoda (Procamallanus laevionchus and Capillaria spp) and protozoans (Cryptosporidium and Eimeria oocysts). The observed occurrence rate of 61.3% for gastrointestinal parasites in wild and 38.7% in cultured catfish in the study area was

considerably high when compared to the rate of 19% reported by Onive et al. (2004) in Zaria, and Akinsanya and Otubanjo, (2006) in Lagos. The observed high levels of gastrointestinal parasites in the wild catfish than in cultured catfish may be attributed to the fact that the fish in the wild are allowed to fed for themselves during which the likelihood of them being

exposed to parasitism is higher when compared to cultured fish, where feed is provided under a controlled environment.

It is also important to note that blood is a good bio-indicator of the health of an organism. It also acts as a pathological reflector of the whole body. Hence, haematological parameters are important in diagnosing the functional status of fish. Thus, the reduction in RBC count, haemoglobin concentration and packed cell volume in infected fish in this study may be because of parasitic infection that often leads to anaemia (Martins et al., 2004). Furthermore, the parasites simply might have acted as stressors and during the primary stages of stress; the PCV was altered due to the release of catecholamine (an enzyme) which mobilizes RBCs to swell because of fluid entry into the intracellular compartment (Chocchia et al., 1982). Similar results were recorded by Hassen (2002) and Ismail (2003) in Clarias gariepinus naturally infected with T.mukasai. Lofty et al. (2003) and Omran and El-Kholony (2003) also reported that the severe anaemia that may be responsible to the reduction of RBC, PCV and Hb of the infected fish maybe because of chronic liver inflammation which causes depression of erythrogenesis. On the other hand, this study recorded significant elevation in WBC of the infected fish when compared to those of the uninfected fish. And this according to Lebola et al. (2001) and Hassen, (2002), the increase in WBCs count might have occurred as a pathological response since WBCs play a major role during infestation by stimulating the haemopoietic tissues and immune system to produce antibodies and chemical substances which work as defense agent against parasite infection. The finding also agrees with previous report by Pinky et al. (2012).

The observed significant higher activity of serum AST, ALT and ALP in the infected catfish than those in uninfected catfish agrees

with the earlier result of Younis, (1999) who reported that AST, ALT and Urea increased significantly in O. niloticus infected with external protozoa and monogenetic trematodes. Osman et al. (2009) also reported that serum AST and ALT enzyme activities, creatinine and Urea values were increased in Trichodina infected Clarias gariepinus. Mahmoud et al. (2011) more so reported that the serum AST, ALT, creatinine, and urea values were elevated in infected O. niloticus and Clarias gariepinus. The increase in the activity of AST and ALT in the serum of the infected fish revealed that the parasites might have had effects on the tissues parenchymous and/or skeletal musculature which probably may have altered the permeability and integrity of cell organelle (Egbu, 2011). Thus, allowing more of these enzymes to leak into the blood circulation.

Finding from our studies revealed that the presence of Cryptosporidium oocysts, Eimeria oocysts and nematode (infection) in both cultured and wild African catfish is associated with decreased in PCV, RBC and Hb (28.0 \pm 0.331%, $3.4 \pm 0.592 \times 10^{12}$ /L, 8.3 ± 0.174 g/dl) when compare with the uninfected Clarias gariepinus (33.3 \pm 0.229%, 3.9 \pm 0.050 x 10¹²/L, 10.5 ± 0.110 g/dl), respectively. However, the WBC was significantly higher (p = 0.041) in the infected Clarias gariepinus (4.5 ± 0.047 x 10^{9} /L) when compared to the uninfected (3.9 ± 0.017 x $10^{9}/L$). The levels of transaminases (AST, ALT and ALP) were also were significantly higher (p<0.05) in the infected Clarias gariepinus (82.2 \pm 1.037 U/L, 95.8 \pm $1.209 \text{ U/L}, 68.8 \pm 1.180 \text{ U/L})$ when compared with the uninfected (60.7 \pm 0.741 U/L, 68.9 \pm $0.844 \text{ U/L}, 47.1 \pm 0.759 \text{ U/L}$), respectively. It is therefore recommended that strict biosecurity measures and routine deworming should be ensured in the management of cultured African catfish for improve growth and health.

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