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Changes in Enzymes Activities of *Clarias Gariepinus* Brood Fish Exposed to Anaesthetics Metomidate

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Abstract *The effect of anaesthetic metomidate on the enzymes activities (Alanine transminase – ALT; Aspartate transaminase – AST; Alkaline phosphatase-ALP and Lactate dehydrogenase – LDH) in female Clarias gariepinus was investigated using different concentrations (0.00mlL^{-1} – control; 0.25; 0.75; 1.00; 2.00; 6.00; 8.00; 10.00 and 12.00mlL^{-1}) in triplicates. The results from the study indicated that the anaesthetic caused a concentration dependent significant increase ($p < 0.05$) in the four enzymes under consideration. The highest activities in all the enzymes were observed in 12.00mlL^{-1} and the lowest in the control. The results from this work therefore suggests that the anaesthetics impair enzymes activities in the fish which was more noticeable in the fish exposed to higher concentration of 8.00-12.00m/L^{-1}. Hence caution should be exercised in the application of this anaesthetics in sedation of C. gariepinus.*

Keywords: *anaesthetics, aquaculture, stress, catfish, enzymes*

1. Introduction

The intensive nature of aquaculture worldwide involves manipulation of fish. This entails some management practices such as handling, confinement, transportation and other farm management operations from the hatchery to the final commercial stage [1,2]. These procedures as crucial as they are, produce some level of disturbances [3], which can elicit a stress response leading to decreased fish performance [4] and alterations of the peripheral leucocytes distribution, heamoglobin content and red blood cell indices [5,6]. It should be noted that some very devastating effects of stress on the stock may occur during application of handling procedures in aquaculture with and without apparent warning. This then raises the question of proper monitoring of stress in fish, in order to reduce to the barest minimum the negative effects of such management procedures on fish physiology [7]. Sedation and immobilization achieved by application of anaesthetics, is seen as one of the efficient means of achieving this.

Anesthesia is generally defined as a loss of sensation through depression of the nervous system caused by an applied external agent. Anaesthetics are among important veterinary medicines employed in various hatchery operations and fish transportation. Anaesthesia is a state of unconsciousness induced in an animal by a chemical. The three levels of anaesthesia are anaesthesia (pain relief), amnesia (loss of memory) and immobilization [8]. The drugs used to achieve anesthesia usually have varying effects in each of these areas. According to Tort et al. [9], some drugs may be used individually for these purposes or in combination with other drugs to achieve full anaesthesia. The use of anaesthetics facilitates work with fish at the research level and is required for invasive studies. These included surgical preparation for physiological investigations, where the fish must be held immobile for extended periods of time [10,11].

Anaesthetics may be local or general depending on their purpose and application, also, method and administration for each anesthetics is fairly well defined [12], but the choice of anaesthetic depends on a number of factors. For example, if the maintenance of gill ventilation during an experimental procedure is desirable, then ketamine hydrochloride would be one possible anaesthetic [13]. In minimizing transportation stress, a light sedation brought about by low concentrations of an anaesthetic such as TMS buffered with sodium bicarbonate [14]. Anasthetics such as MS-222, clove oil, quinaldinesulphate, and metomidate are popularly used during production procedures in fish hatcheries process [15].

Anaesthetics act with various intensity, driving fish into general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity and reduced skeletal muscle movement [16]. Regardless of the agent, the process of anesthesia in fish develops in a similar way and runs in a progressive pattern, however, overdose of an anaesthetic or retaining the fish in an anaesthetic bath for too long leads to the fading of ventilation, hypoxia and finally respiration and cardiac collapse [17].

Onset of anaesthesia also caused changes in some enzyme activities of the fish [18]. A study by Trimmer et al., [19], demonstrated changes in some enzyme activities in fish, which included enzymes activity in the brain of male African catfish, *Clarias gariepinus*. This enzymes is involved in the biosynthesis of catecholestrogens that plays a role in the negative feedback mechanism of gonadal steroids on gonadotropin release. In an extensive study by Weber et al., [20], alterations were observed in some plasma enzymes, alanine transaminase (ALT), aspatate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) in Indian catfish, *Clarias batrachus* exposed to various levels of an anaesthetic, 2-phenoxyethanol and metomidate in the laboratory. Velisek et al., [21] associated distortion in plasma enzyme activities of rainbow trout with the effect of anaesthetic metomidate. However, there is paucity of information in the effects of metomidate anaesthetics on the enzyme activities in *Clarias gariepinus*, hence the need to carry out this work.

2. Materials and Methods

A total of 66 gravid female fish ready to spawn brood fish (mean length 60.23cm ±0.36SD and mean weight 2.640g ±1,365SD were obtained from a private fish farm, water shed, fish farms, Rumuodara, Port Harcourt, Rivers State, Nigeria and transferred in an open 100l plastic tank to the laboratory in department of Fishery and Aquatic Environment, Rivers State University of Science and Technology, Port Harcourt, Rivers State, Nigeria. They were acclimated to laboratory for a period of 10 days. After this, they were exposed to solution of anaesthetic metomidate at different concentrations (0.00 – control, 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00, and 12.00m/L^{-1}) in 30L aquaria in triplicates for a period of 1 hour, when the fish in all exposed concentration have become immobilized [22].

Blood samples for enzyme assays and transminase activities, were collected from fish in each concentration including the control. This was done by severity the caudal pendicle, with 21 Gauge hypodermic needle and 5ml disposable syringe and transferred into heparinised bottle, this was latter centrifuge at 500rpm for 5min in (Hettich Zentrifuges, ROTINA 380) to obtain blood serum samples and stored at – 20℃. ALT, AST, and ALP were determined by near-infrared particle immuno-assay detection system with synchron L x 20 PRO (Beckman Counter Inc. Fullerton, CA, USA), LDH was evaluated according to Svelger et al. [23] using kits from bio-mericux, France.

Data from the study was analyzed using one way analysis of variance (ANOVA) at 0.03% probability and differences among means was determined by Tuckeys multiple comparison test [24].

3. Results

There were no obvious signs of disease or abnormality in the physical conditions and the behaviour of the experimental fish during the experimental period. No mortality was recorded in the brood fish during the treatment. Introduction of metomidate anaesthetics in the tanks did not cause any significant change (P>0.05) in the water quality variables before and after the trial (Table 1). However, it caused a drop in the dissolved oxygen level and light rise in ammonia concentration.

Table 1. Physicochemical parameters of water in the experimental tanks during trial

Parameter	Before Trial Mean ±SD	After trial Mean ±SD
pH	6.56 ±0.39^{a}	6.60 ±0.14^{a}
DO (mg/L)	5.22 ±0.78^{a}	4.99 ±1.16^{a}
Temperature (℃)	28.17 ±0.71^{a}	28.14 ±0.32^{a}
Ammonia (mg/L)	0.38 ±0.01^{a}	0.40 ±0.11^{a}
Nitrite (mg/L)	0.0039 ±0.01^{a}	0.0043 ±0.12^{a}
Sulfide (mg/L)	0.01 ±0.01^{a}	0.01 ±0.01^{a}

Means within the column with same superscript are not significant ($p < 0.05$)

The effects of anaesthetics on the plasma enzymes indicated that ALP activity in the plasma of the treated fish increased from the control 0.00ml/L (23.00±2.09IU/1) and peaked (59.23±1.37IU/L) at 12.00ml/L (Figure 1). Similar trends were recorded for AST and ALT (Table 2) AST activity in most of the concentrations were about twice that of ALT.

The highest values of AST, (145.66±11.01U/L), ALT (76.67±1.141U/L) LDH (82.66±1.531U/L) were recorded fish exposed to 12.00ml/I of metomidate, while the lowest values were in all the enzymes were recoded in the control (Table 2).

Table 2. Effects of anaesthetics (metomidate) on enzymes profiles gravid of *C. gariepinus* (mean ±SD)

Conc (ml/L)	Plasma Enzymes			
	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
0.00	23.00 ±2.09	18.54-27.46	5.75 ±2.48^{a}	16.25 ±5.98^{av}
0.25	31.75 ±2.00^{b}	22.25 ±1.01^{c}	4.50 ±2.48^{a}	34.00 ±2.91^{b}
0.50	36.25 ±2.14^{c}	46.75 ±8.14^{d}	14.25 ±2.48^{b}	59.25 ±1.91^{c}
0.75	38.75 ±1.52^{d}	79.75 ±4.50^{ab}	40.25 ±2.48^{c}	70.75 ±5.13^{c}
1.00	42.25 ±2.02^{e}	88.25 ±2.08^{b}	46.50 ±2.48^{c}	75.00 ±1.00^{c}
2.00	44.67 ±1.54^{f}	92.33 ±2.88^{e}	49.67 ±1.52^{bc}	75.33 ±2.08^{c}
4.00	46.68 ±1.52^{f}	99.99 ±1.00^{e}	54.00 ±2.01^{e}	76.33 ±2.51^{b}
6.00	49.33 ±1.02^{ab}	107.33 ±3.05^{ef}	60.06 ±2.3^{c}	78.33 ±1.15^{b}
8.00	51.66 ±1.8^{ef}	115.00 ±5.04^{f}	67.67 ±1.52^{f}	79.01 ±2.00^{b}
10.00	55.00 ±1.00^{gh}	130.00 ±5.11^{g}	71.33 ±2.08^{f}	82.66 ±1.53^{b}
12.00	59.23 ±1.37^{gh}	145.66 ±11.01^{h}	76.67 ±1.14^{f}	82.66 ±1.53^{b}

Key: ALP – Alkaline Phosphate, AST – Aspartate Transaminase, ALT – Alanine Transaminsase, LDH – Lactate dehydrogenase
Means within the column with same superscript are not significant ($p > 0.05$).

4. Discussion

Transaminases are both plasma and non-plasma specific enzymes that are found in the tissues of fish and normally give information about organ dysfunction. In most teleost fish, enzyme activities affect various chemical and biological reactions in the body of the fish [25]. According to Gabriel and George [26], transamination is one principal pathway for synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the organism under various adaptive conditions. Beyer *et al*., [27] opined that alanine and aspartate transaminase activities may be utilized as sensitive markers in experimental insecticide or herbicide intoxication in teleost fish.

Alkaline phosphatase (ALP) is an enzyme present practically in all tissues especially in the cell membranes, where active transport normally takes place and has hydrolase and transphosphorylase function. ALP activity in *C. gariepinus* brood fish increased significantly ($p<0.05$) and peaked at 12.00ml/l, similar result was recorded by Iverzen *et al*. [28], in atlantic salmon. Wagner et al. [29] reported an increase ($p<0.05$) in ALP activity in rainbow trout exposed to higher concentrations of clove oil (anaesthetics). Available report shows that increase or decrease in ALP activity can be used as index of hepatic parenchymal damage and hepatocytic necrosis [30]. Therefore an increase in the ALP enzyme activities reflects activities in inflammation and necrosis of hepatic cells.

The activities of ALT and AST increased correspondingly, as the concentrations of the metomidate increased, a pattern noted by Veliseck and Svobodova, [31], in common carp after exposure to higher concentrations of MS-222. Also, Iwama and Aerkerman, [32], observed the same result in rainbow trout exposed to increasing concentration of MS222 and clove oil. The disorder in plasma enzymes could be from of hepatic injury as a result of reactive metabolites from xenobiotic metabolism in the liver [33]. A concentration dependent activities of LDH noted in fish has previously been reported by Spotte *et al*., [34] in sea water adapted mummithoges exposed to 2-phenoxyethanol in the laboratory, also, Fereira et al. [35], in rainbow trout exposed to benzocainne hydrochloride and Guo et al. [36], in *Xiephophorus maculates* treated with MS 222 in the hatchery. The trend suggests an increase in the glycolytic process due to the lower metabolic rate as a result of the effect of anaesthetics. This is because the level of tissue lactate content acts as an indicator of anaerobic respiration as this enhances the animal tolerance to internal hypoxic condition [37].

5. Conclusion

The activities in the plasma enzymes of *C. gariepinus* was impaired by anaesthetics metomidate, which was more pronounced at higher concentration of (8.00 – 12.00m/L^{-1}). Therefore, caution should be exercise in the application of this anaesthetics in sedation of *C. gariepinus*.

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