TISSUE GLUCOSE AND HAEMOGLOBIN LEVELS IN THE CATFISH *Clarias albopunctatus* DURING ANAESTHESIA WITH KETAMINE HYDROCHLORIDE

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ABSTRACT

Adult Clarias albopunctatus (mean weight 64.8 ± 1.09 g) were subjected to anaesthesia for 72h with ketamine hydrochloride. The fish demonstrated uncoordinated and gyratory movements before reaching anaesthesia. The time taken to reach anaesthesia at the tested concentrations of ketamine differed in the treatment groups (P < 0.05). The haemoglobin, liver and plasma glucose concentrations were determined at 2, 4, 12, 48 and 72h interval. The mean liver and glucose concentrations were significantly elevated by ketamine hydrochloride (p < 0.05).The mean haemoglobin concentration was also significantly increased in the anaesthetized fish (p < 0.05). Both the liver and plasma glucose concentrations were significantly increased in the anaesthetized fish. The result showed that ketamine hydrochloride appeared to have long-lasting anaesthetic effect on the fish.

Keywords: *Clarias*, Anaesthesia, Ketamine hydrochloride, Haemoglobin, Glucose

INTRODUCTION

Aquaculture is a legitimate agricultural enterprise and the increased volume in world trade in ornamental fish coupled with increased contribution of aquaculture to food security entails among others, the transport of fish over long distances, which can be stressful to the fish. During management protocols such as fish transport, stripping for gametes, sorting and even weighing, chemical anaesthetics are used to reduce stress and /or shock during such management protocols (Barton *et al.,* 1980; Barton and Peter, 1982; Robertson *et al.*, 1988; Rose *et al.* 1993). The commonly used anaesthetics in aquaculture include tricaine methane sulphonate (MS222), benzocaine, quinaldine and 2-phenoxyethanol (Blasiola, 1977; Hattingh, 1977; Soivio *et al.*, 1977; Molinero and Gonzalez, 1995) and metomidate (Guo *et al.*, 1995). These conventional anaesthetics are expensive and most times difficult to get in Nigeria. Another problem of these anaesthetics is their high dosage. For example, Eze (1991) observed that the dosage of MS 222 for juvenile *Clarias gariepinus* was 150g/L.

Ketamine hydrochloride is an injectable anaesthetic (William *et al.*, 1988) widely used in human and veterinary medicine. However, Graham and Iwama (1990) had applied it to trout and salmonids, and reported that it affected the ventilation and the partial pressures of oxygen and carbon dioxide in the blood.

This study was conducted with a view to evaluating the efficacy of ketamine hydrochloride as a water-borne anaesthetic and also to investigate its effect on the tissue glucose and the haemoglobin levels of *Clarias albopunctatus.*

MATERIALS AND METHODS

Fish Collection and Experimental Design: The catfish juveniles used in this study were collected from Anambra River at Ogurugu using local traps in the flood plain pond. The fish were transported to the laboratory in a plastic container. The fish were disinfected with 0.1 % formalin for 2 minutes and thereafter the fish were rinsed in tap water for 10 minutes. The fish were acclimatized for two weeks before the commencement of the study. During this period the fish were fed 32 % crude protein diet at 3 % body weight daily. A total of 135 fish (mean weight 64.8 ± 1.09g) were used for the study. The fish were randomly distributed into three groups of 45 fish per group. Each group was further divided into three replicates of 15 fish per replicate using hand net to minimize stress. Each aquarium consisted of 50 L container containing 30 L water. The water in each aquarium was drained with the aid of siphon.

The fish in treatments 1 & 2 were exposed to 0.025 and 0.05 mg/l ketamine hydrochloride, respectively. The fish in treatment 3 were exposed to tap water only (control). Instead of administering the ketamine hydrochloride by injection, it was added to the water in each aquarium. The commercial pharmaceutical preparation of ketamine (Park-Davis, USA) containing 10mg/ml ketamine was used as the stock solution for the study and the static bioassay system was use throughout the study. The water temperature remained at 27-28^{o}C throughout the duration of the study.

The liver and plasma glucose concentration were assayed at 2,4,12, 48 and 72 h intervals following anaesthesia.

The haemoglobin concentration was determined at these intervals. After 72 h in anaesthesia, the time taken for these parameters to return to normal (recovery phase) was also studied. This was done by draining completely the water containing ketamine with a siphon to avoid disturbance. Each plastic aquarium was rinsed with tap water and thereafter, 30 L of tap water was added to each aquarium containing the anaesthetized fish and aerated continually. The water quality of the tap water (total hardness 21mg/L as $CaCo_3$;conductivity 3.8 mS/m alkalinity 66mg/L;pH 7.8; dissolved oxygen 7.6 mg/L; temperature 28^o C) were determined using the standard methods (APHA, 1981).

Sample Collection and Assay Methods: Two fish from each replicate experiment were removed at 2, 4, 12, 48 and 72 h intervals and dried with an adsorbent paper. The fish were immediately killed and the liver dissected out. The liver was rinsed with physiological saline and kept in a deep freezer. The liver and plasma glucose concentrations were determined within 30 minutes of collection using the method of Wedermeyer and Yasutake (1977).

Blood was collected by cardiac puncture and by the severance of the caudal peduncle using heparinized capillary tubes. The haemoglobin concentration was determined by the cyanmethemoglobin method (Blaxhall and Daisley, 1973).

Statistical Analysis: One-way analysis of variance was used to analyze the data at 5 % significant levels followed by the Fisher's least significant difference (F-LSD) post-hoc test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The time taken to reach anaesthesia in the fish exposed to 0.025 and 0.05 mg/l ketamine was significantly different (P >0.05) being 47 and 26 minutes, respectively. This time taken to reach anaesthesia in the group treated with 0.025 mg/l ketamine was comparable to 40 minutes reported for MS 222 to induce anaesthesia in adult *Clarias gariepinus* (Eze, 1991). Compared with the control, the movement of the fish treated with ketamine was uncoordinated and some turned their belly up. Some demonstrated gyrated movement and attempted to jump out of the plastic aquarium before reaching anaesthesia. Similar uncoordinated movements were observed during the period of recovery when the anaesthetized fish were put in tap water only. It took 1h 10 minutes and 1h 52 minutes for the fish treated with 0.025 and 0.05 mg/L ketamine to recover from anaesthesia. Such abnormal behaviour was reported in the clariids treated with extracts of the leaves of *Erythrophleum suaveolens* (Mgbenka and Ejiofor, 1998) and in shad exposed to metomidate (Ross *et al.*, 1993).

The changes in the liver glucose concentrations in *C. albopunctatus* during anaesthesia with ketamine are showed that the liver glucose concentrations were significantly elevated in the anaesthetized fish when compared with the control value of 0.09mg/g (p<0.05) which did not differ significantly with the pre-treatment value of 0.08 mg/g liver weight (Figure 1). The liver glucose increased maximally (0.64mg/g) after 4 h in the fish exposed to 0.05mg/l ketamine. This represented 700 % increase over the control. In the fish treated with 0.025mg/l ketamine, the liver glucose was maximally stimulated (0.58 mg/g) after 12 h, representing 544 %.After the anaesthetized fish was put in tap water to recover, the liver glucose decreased to 0.1mg/g and 0.14 mg/g liver in the fish treated with 0.025 and 0.05 mg/l ketamine, respectively after 1h.

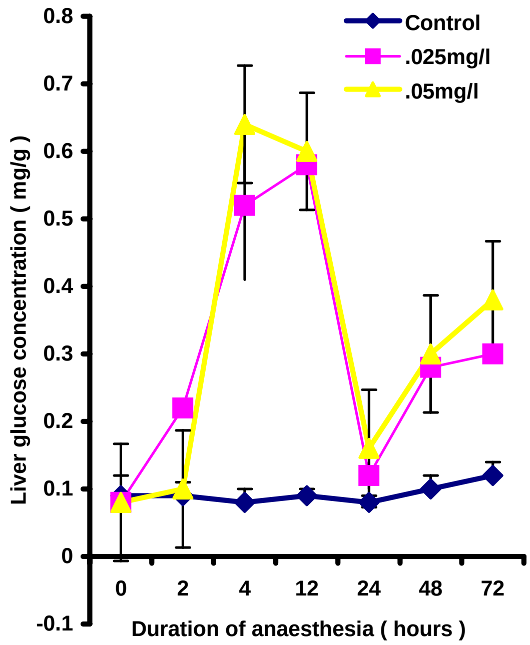

Figure 1: The effect of ketamine on the liver glucose concentration in C. albopunctatus

The effect of ketamine on the plasma glucose concentrations is shown in Figure 2. Compared with the control, the plasma glucose concentrations were significantly increased in the anaesthetized fish. The plasma glucose concentration in the anaesthetized fish differed throughout the study except at 2 and 72 h intervals. After 72 h in anaesthesia, the plasma glucose concentration increased from a pre-treatment value of 1.18mg/dl to 4.88 mg/dl and 6.7mg/dl in the fish treated with 0.025 and 0.05 mg/l ketamine, respectively. When the anaesthetized fish were put in tap water after 72 h in anaesthesia, the plasma glucose concentration decreased to 0.3 and 0.41 mg/dl after 1 h and 2 h in the group exposed to 0.025 and 0.05 mg/l, respectively.

The increase in liver and plasma glucose concentrations in this study were similar to the trend reported in sea bream anaesthetized with tricaine methanesulphonate (MS 222) and 2-phenoxyethanol for 6 h (Molinero and Gonzalez, 1995) even though

Morales *et al.* (1990) did not observe a significant increase in the blood glucose of trout anaesthetized with MS222. However, Soivio *et al.* (1977) reported hypoglycaemia in *Salmo gairdneri* anaesthetized with MS 222. Also, Ladu and Ross (1997) reported significant increase blood glucose in *Oncorhynchus mykiss* anaesthetized with either benzocaine or electroanaesthetized. Although the mechanism by which ketamine induced the hyperglycaemia in *C. albopunctatus* is not known with certainty. Earlier reports showed that anaesthetics activate the hypothalamic-pituitary-interenal axis (Strange and Schreck, 1978; Robertson *et al*. 1988; Molinero and Gonzalez 1995). The stimulation of this pathway would cause physiological processes that would promote glycogenolytic and glucogenetic functions that may induce hyperglycaemia during anaesthesia (REF?).

The changes in the mean haemoglobin concentration of the fish treated with ketamine are shown in Figure 3. At the end of the study, the haemoglobin concentrations in the anaesthetized fish were significantly higher than the control ($p<0.05$) and the baseline group. The mean haemoglbin concentration of the anaesthetized fish did not vary after 2 and 48 h ($p<0.05$). The highest in the haemoglobin concentration occurred in the treatment group within the first two hours of exposure. The haemoglobin concentration in the anaesthetized groups decreased during the period of recovery to approximate the control value after 2h in the tap water.

The observed increase in the haemoglobin in *C. albopunctatus* following anaesthesia with ketamine agreed with the report of Ladu and Ross (1997) on *Oncorhynchus mykiss*. Also increased haemoglobin concentration was reported by Molinero and Gonzalez (1995) in sea bream *Spratus aurata* anaesthetized with 2-phenoxyethanol. The increase in haemoglobin level during anaesthesia may represent an adaptive response to enhance oxygen carrying capacity during anaesthesia which according to Wells *et al* (1984) is to increase arterial oxygen concentration.

The result of this study demonstrated that instead of being injected, ketamine could conveniently be used as water borne anaesthetic and still has a long-lasting effect. This mode of application would reduce stress during injection. Similar long lasting effect of ketamine was reported in *Oncorhynchus kisutch* and *O. mykiss* by Graham and Iwama (1990) and in the shad (Ross *et al*., 1993). The result of the study has shown that ketamine, though an intramuscular anaesthetic could conveniently be applied as water borne anaesthetic. Its advantage in Nigeria over the other anaesthetics like benzocaine, 2-phenoxyethanol or quinaldine, lies in its long-lasting effect, low cost and in being readily available.

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