WHOLE BLOOD GLUCOSE: BIOMARKER OF COMMON ON-FARM PROCEDURES IN JUVENILE *Clarias gariepinus*

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ABSTRACT
Juveniles of Clarias gariepinus (mean weight, 180.56 ± 15.76 g; mean total length, 13.57 ± 3.78 cm SD) were exposed to five on-farm procedures (exposure outside water, sorting, starvation, serial transfer and overcrowding) and whole blood was collected before each treatment and at 20, 40 and 60 mins. After fish were exposed to each procedure, blood glucose levels were determined using a glucose meter. In all the procedures the glucose level increased with duration ($p < 0.05$) with a peak at 60 minutes. Whole blood glucose was highest in fish exposed outside water in comparison to the other treatments ($p < 0.05$). This study revealed that common on-farm procedures such as the ones studied can cause stress in C. gariepinus as evident in a raised glucose level. Hence this should be minimized as much as possible to reduce the effect of these procedures on the fish.
Keywords: stress, aquaculture, fish, haematology.

INTRODUCTION

The African catfish, *Clarias gariepinus* belongs to the family Clariidae, and is the most popular fish for culture in Nigeria, next to the tilapine fishes (FAO, 1997; Adeogun *et al*., 2007). The choice of the clariids for culture is dependent on a number of desirable characteristics detailed in the studies by a number of workers (Legendre *et al.*, 1992; De-Graaf and Janssen, 1996; Ayinla and Nwadukwe, 2003). The commercial catfish industry in Nigeria has undergone rapid expansion in recent years. With increasing demand for catfish farmers have therefore intensified of production which has resulted in high stocking and feeding rates (Akinrotimi *et al*., 2007). Along with the increase in production, cultured fish in aquaculture has experienced an increase in stress resulting in fish health problems. Stress is any condition that causes physical or psychological discomfort, which results in the release of stress-related hormones, or specific physiological responses (Foster and Smith, 2007), "such that it causes an extension of a physiological state beyond the normal resting state" (Barton, 2002). Various stressors, such as grading, transportation and vaccination, are necessary components of modern procedures in intensive fish culture. The response of the fish to such stressors involves all levels of organization from the cell (Hur *et al*., 2007), the individual organism (Barton and Iwama, 1991) and the population (Barton, 2002).

In aquaculture on-farm procedures such as sorting, overcrowding, exposure outside water, starvation and serial transfer, transport, anaesthetics and water quality are commonplace and can cause temporal or permanent stress in fish depending on duration of exposure (Smart, 1981; Thomas and Robertson, 1991, Weaving *et al.*, 1996; Hemre *et al*., 2002). These may singly or jointly significantly affect survival rate, disease resistance, growth rate and production of farmed fish (Cheng *et al*., 2006). Altered homeostasis may pre-dispose the fish under such conditions to pathogenic and parasitic infections that can result in economic losses due to an interaction between the environment and immunological system of the fish (Martins *et al.*, 2002). Disease resistance is directly related to hypothalamic, pituitary and interrenal axis. As a result of environmental disturbances or stress of handling, changes in cortisol and catecholamines are responsible for immunosupression (Correa *et al.*, 2003). Corticosteroid levels may depend on severity and type of stressor stimulus varying from a period of one to 24 hours (Schreck, 1981).

This initial response to stress represents the perception of an altered state by the fish and initiates a neuroendocrine response that forms part of the generalized stress response in fish (Gamperl *et al.*, 1994). This response includes the rapid release of stress hormones catecholamines and cortisol into circulation, which in turn elicit some secondary response, which include elevation in plasma glucose levels (Mommsen *et al.*, 1999; Foster and Smith, 2007). Channel catfish under fasting conditions experienced hyperglycemia after 30 days; 22.8 $ngml^{-1}$ versus 4.7 $ngdml^{-1}$ in the control group (Peterson and Small, 2004). Glucose also varies between species and stage of development (Iwama *et al.*, 2004; Hemre *et al.*, 2002). Woodward and Strange (1987) observed that wild rainbow trout experienced a critical increase three times greater than hatchery fish when exposed to net confinement and electroshock.

The measurement of plasma glucose concentration has been proposed and used as an indicator of stressed states and probably the easiest and most commonly measured secondary (metabolic) response to stressors in fish (Ortuno *et al*., 2001; Barton, 2002). The plasma glucose concentration in circulation is dependent upon glucose production and its clearance from the circulation. The production of glucose with stress assists the animal by providing energy substrates to tissues such as brain, gills and muscles, in order to cope with the increased energy demand. Liver is the main source of glucose production and is achieved by glycogenolysis and gluconeogenesis. Adrenaline and cortisol levels have been shown to increase glucose production in fish and play important role in plasma glucose concentration (Pottinger, 1998; Soengas and Aldegunde, 2002).

Although blood glucose level has been proposed as one of the easiest and quickest way of measuring stress in fish species yet the effect of common on-farm procedures on stress in the fish assessed by changes in the glucose level has not been reported in a number of cultured species in the tropics including *C. gariepinus*. This necessitates the need to carry out this experiment to assess the effect of selected on farm procedures on the whole blood glucose of this important aquaculture species. The objectives of the study is to assess the changes in blood glucose levels as a result of the difference in farm procedures in *C. gariepinus* juveniles determines which of the on farm procedures exerts more stress on the fish.

MATERIALS AND METHODS

Location of the Experiment Site/Source of Experimental Fish

The study was carried out at the Hatchery Unit of the African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt, Nigeria. Juvenile of *Clarias gariepinus* (mean weight, 180.56 ± 15.76 g; mean total length 13.57 ± 3.78 cmSD) of the same stock were harvested from ponds with drag net at the African Regional Aquaculture Centre, Aluu. They were held in circular plastic aquaria in the hatchery before being subjected to the various on farm procedures (treatments). All the experimental procedures were carried out in the hatchery same day.

Experimental Design and Experimental Procedure

The design of the experiment was a one way completely Randomized Design (CRD) with five treatment levels. **F**ish were subjected to handling stress common in aquaculture namely: 1. Sorting, 2. Overcrowding, 3. Exposure (without water), 4.Starvation (without feed) and 5. Serial transfer. Blood samples were collected from the fish by kidney puncture. Blood was collected from ten fish samples (initial value: time, 0min.) before they were subjected to each of the various on farm procedures. The blood samples collected was immediately put on the strip and the glucose level was read on a meter (ACCU "CHECK" Roche, Germany) and the value recorded. Subsequent blood samples were collected from ten fish each at 10, 20, 40 and 60 mins intervals after administering each of the treatment. Fish was capture and held with a towel. It was restrained physically by covering the head with a towel, placed on a table and blood collected as indicated above. Forty fish was sampled in each of the procedures.

Sorting

Sixty fish were placed on a sorting table and sorted into six rectangular plastic aquaria, ten fish per aquaria (dimension: 0.6 x 0.3 x 0.3 m^3).

Overcrowding

Twenty fish each was crowded in each of four small aquaria (dimension: 0.3 x 0.3 x 0.2 m^3).

Exposure outside water

Ten fish each was put in four rectangular aquaria without water (dimension: 0.6 x 0.3 x 0.3 m^3). Blood samples were collected from ten fish from one of the aquaria at each of the time intervals and the glucose level determined.

Starvation

Ten fish each was left inside the aquaria (dimension: 0.6 x 0.3 x 0.3 m^3) where it was held after harvesting without food for twenty four hours.

Serial transfer

Twenty fish was held in the four aquaria (dimension: 0.6 x 0.3 x 0.3 m^3) after harvest were transferred from one aquarium to another up to the fourth aquaria. The transfer was done by pouring the content (fish and water) from one aquarium to another.

Data Analysis

Data obtained from the experiment was tested for normality before it was subjected to ANOVA to determine variation in whole glucose at the various durations from each of the on-farm procedures and among the procedures Tukey HSD was used to separate the means where they existed (Wahua, 1999).

RESULTS

The data was normally distributed. The basal whole glucose level in *C. gariepinus* ranged between 2.5 to 4.5 mg/l. Exposure of the fish to the various on farm procedures in the hatchery caused changes in the blood glucose of the fish but the degree of alteration differed with the specific procedures and exposure duration. Fish in exposed outside water had the highest glucose level (10 mg/dl) was observed at 60 minutes against the lowest (1.98 ± 0.02 mg/dl) at the initial reading (Fig. 1). Sorting caused an increase in the glucose ($P \leq 0.05$) from 1.99 ± 0.46 mg/dl (initial) to 4.01 ± 1.12 mg/dl at 60 min (Fig. 2). The blood glucose exposed to serial transfer rose progressively ($P \leq 0.05$) with time to a peak, 4.33 ± 1.11 mg/dl at 60 min (Fig. 3).

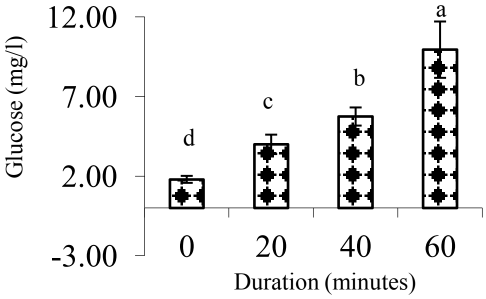

Fig. 1: Effect of exposure outside water on whole blood glucose in *C. gariepinus* juveniles at various time intervals (Bars with different alphabets are significant, P≤ 0.05)

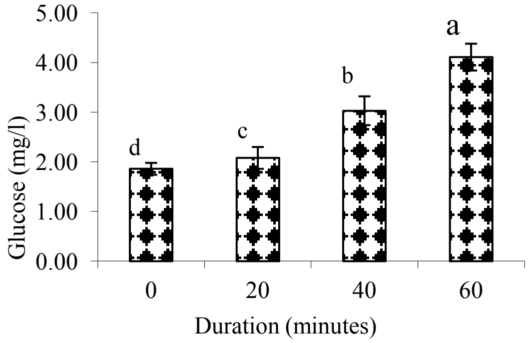

Fig.2: Effect of sorting on whole blood glucose in *C. gariepinus* juveniles at various time intervals (Bars with different alphabets are not significantly different at P ≤ 0.05)

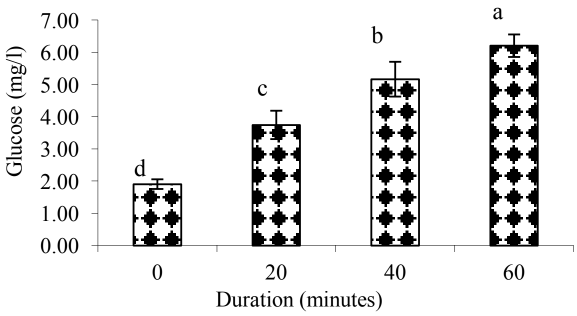

Fig. 3: Effect of serial transfer on whole blood glucose in *C. gariepinus* juveniles at various time intervals (Bars with different alphabets are significantly different at P ≤ 0.05)

Starving the fish for 24hrs caused elevation ($P \leq 0.05$) in the blood glucose of *C. gariepinus* which increased with the duration of exposure with a maximum, 4.46 mg/dl at 60 min (Fig. 4). Overcrowding stressed the fish leading to the highest glucose level 5.48 ± 1.12 mg/dl was observed at 60 min compared to the lowest 1.98 ± 0.11 mg/dl at initial reading (Fig. 5). The comparative whole blood glucose levels resulting from the various stressful procedures in *C. gariepinus* juveniles indicated the highest (5.88 ± 1.94 mg/dl) glucose level was recorded in the fish kept outside water, while the lowest (2.84 ± 1.61 mg/dl) was observed in fish exposed to sorting (Fig. 6).

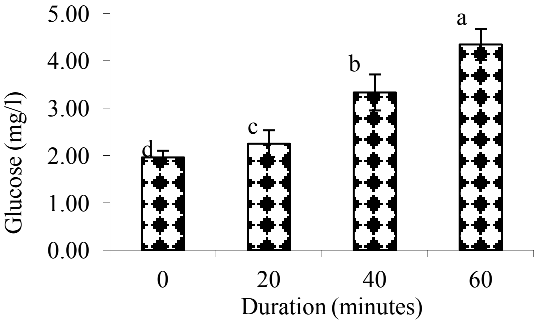

Fig 4: Effect of starvation on whole blood glucose in *C. gariepinus* juveniles at various time intervals (Bars with different alphabets are not significantly different at P≤0.05)

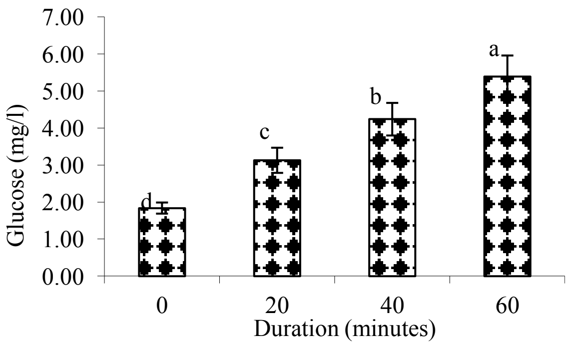

Fig.5: Effect of overcrowding on whole blood glucose in *C. gariepinus* juveniles at various time intervals (Bars with different alphabets are not significantly different at P≤0.05).

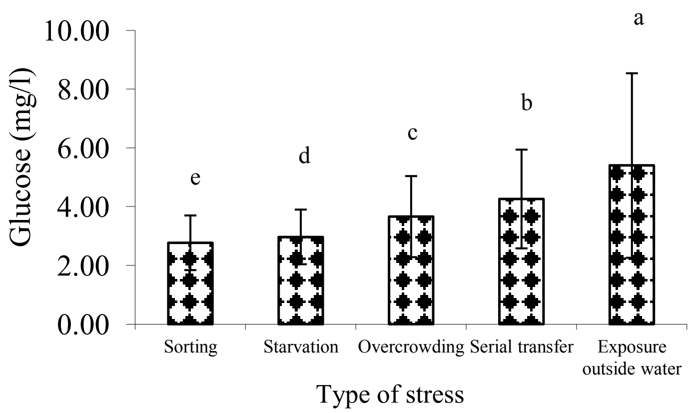

Fig. 6: Comparative whole blood glucose levels in *C. gariepinus* subjected to various on farm procedures (Bars with different alphabets are not significantly different at P ≤ 0.05)

DISCUSSION

The biological concept of stress applied to fish has attracted considerable attention in recent years (Barreto and Volpato, 2006). Stress is a generalized response attributed to the fact, that fish has complete adaptive measures to cope with stressors (Barton, 2002). Although stress can be considered to be a generalized response, it can be modulated by specific stressful conditions (Brown *et al*., 2005). The present study indicated that stressors such as normal on farm procedures like those assessed in this study caused distinct stress response in juvenile of *C. gariepinus* thus reinforcing the specificity of stress in fish (Correa *et al*., 2003).

Basal plasma glucose levels vary substantially among teleost fish (Hur *et al*., 2007). The basal levels observed in this study ranged between 2.5 and 4.6 mg/dl, which falls within the values of that reported for carp(West *et al*., 1994), and brown trout (Blasco *et al*., 2001) and various salmonids (Barton *et al.,* 1998, Fevolden *et al*., 1993). Fish respond to stress with characteristic increase in blood glucose levels and the levels of glucose in *C. gariepinus* exposed to sorting was comparable to that recorded by Mack *et al*. (2008) in *Piaractus mesopotamicus* subjected to repeated sorting in the hatchery.

Changes in blood glucose levels in *C. gariepinus* exposed to stress of exposure outside water elicited high glucose levels, which increased with duration similar to the results obtained in rainbow trout, that was left out of water for an hour (Holloway *et al*., 1994). The authors observed that the blood glucose levels increased five folds after exposure. On the contrary Fevolden *et al*. (1993), recorded a decline in whole blood glucose in Atlantic salmon, *Salmon salar* exposed to the same condition. The differences observed may be as a result of influence of species, diet and nutritional status of the fishes (Vijayan and Moon, 1992). These have been reported to significantly modulate the blood levels under stress (Hemre *et al*. 2002; Cheng *et al.* 2006).

The effects of serial transfer and overcrowding on the plasma glucose dynamics in some fish species have been reported (Rotllant *et al*., 2001; Skjervold, *et al*., 2001). The result obtained in this work confirms that of Davis *et al*., (2002) in *Ictalurus punctatus* following a serial transfer and one hour. The increase in glucose level may be as a result of increase in energy demand, necessitating the release of glucose from liver into the blood stream through glycolysis to meet the elevated metabolic activities. (Jentoft *et al*., 2005). Elevation of blood glucose from starvation induced stress was equally observed by Pereira *et al*. (1995) in rainbow trout starved for 24 hours. The increase may be as a result of breakdown and release of glycogen from liver cells into the blood stream. According to Soengas *et al*. (1994) the presence of glycogen in the blood stream guarantees a fast supply of glucose in adverse conditions like starvation.

Exposure outside water induced a more rapid and higher glucose response in *C. gariepinus* than the other on-farm procedures suggesting that exposure outside water is a most stressful procedure than all others judged by the changes in whole blood glucose. A possible reason for this may be the very facts that outside water, gaseous exchange and excretion of waste products are greatly impaired leading to a much more increased internal metabolic/physiological distress. Such an internal condition will lead to increased energy demand than usual and hence a greater and faster rate of release of glucose from the store (liver) into the blood stream for sustained activities/homeostasis.

Methodologically, it is difficult to provide stressful conditions of equivalent intensity (Barton, 1996). However the magnitude of glucose response represents a suitable parameter for the comparative goal of the present study. Thus, the differences found for the glucose response in this species depends more probably on stressor modalities than on the intensity. A similar conclusion has been reported for some species subjected to different stressors (Banya, 1997). Vijavan and Moon (1994) suggests that "the rearing history including nutritional status may affect the stress response and glucose clearance rates". That affirmation is supported by other authors who concluded that blood glucose results have to be interpreted with care, taking into account extrinsic factors such as diet, life stage, time since last feeding and season of the year that they may affect liver glycogen stores (Nakano and Tomlison 1967; Barton *et al*., 1988; McLeay 1977; Wedemeyer *et al*., 1990). The liver glucose level may the release into the blood stream under the environmental stress such as those examined in this study

The various handling stress studied raised the blood glucose level of the treated fish which increases with duration. Exposure outside water caused the highest glucose level in comparison to others. The fish culturist/researcher should bear in mind the implications of on-farm procedures while subjecting to them. Therefore fish should be handled with care as many of the procedures induce a degree of stress in the fish which will ultimately leads to poor performance in aquaculture.

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