PORTABLE POINT-OF-CARE DEVICE AS ALTERNATIVE TOOL FOR MONITORING BLOOD GLUCOSE IN LAMBARI Astyanax altiparanae: STRESS AND SEX-SPECIFIC EFFECTS

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ABSTRACT

The efficacy of a point-of-care (POC) blood glucometer (Accu-Chek Active®) was evaluated in adult lambaris (Astyanax altiparanae), comparing the values obtained by this equipment to those obtained by the laboratory enzymatic method. The glycemia of adult males and females and the variations resulting from transporting in juveniles were also evaluated 0, 1, 24, 48 and 72 hours after procedure. Data were submitted to ANOVA, comparing the means by the Tukey’s Test (P> 0.05) and a regression analysis was performed to establish the correlations between values obtained by the two methods. The blood glucose values obtained by POC meter were 11.2% lower, in absolute terms, but did not differ significantly from those recorded by the laboratory enzymatic method, with linear correlation (R = 67.7%) between the methods. Higher glucose levels were recorded for males; glycemia increased 334.3% immediately after transportation, remained elevated (113.3%) up to 48 hours and returned to basal levels (fasting) after 72 hours. It was concluded that POC Accu-Chek Active® is an effective tool for glucose determination in lambari; there are sex-dependent differences in relation to blood glucose levels; the species is sensitive to stress transporting and glycemia is an immediate and appropriate physiological indicator for this evaluation.

Key words: glucometer; handheld; portable clinical analyser; transporting.

GLICOSÍMETRO PORTÁTIL COMO FERRAMENTA ALTERNATIVA PARA MONITORAMENTO DA GLICOSE SANGUÍNEA EM LAMBARIS Astyanax altiparanae: EFEITOS DO ESTRESSE E SEXO

RESUMO

A eficácia do medidor portátil de glicemia (Accu-Chek Active®) foi avaliada em lambaris adultos (Astyanax altiparanae), comparando-se os valores registrados com os obtidos por meio do método enzimático laboratorial. A glicemia de machos e fêmeas adultos e as variações decorrentes do transporte em juvenis também foram avaliadas 0, 1, 24, 48 e 72 horas após o procedimento. Os dados foram submetidos à ANOVA, comparando-se as médias pelo Teste de Tukey (P> 0,05), realizando-se posterior análise de regressão para estabelecimento das correlações entre os valores obtidos pelos dois métodos. Os valores de glicemia obtidos pelo glicosímetro foram 11,2% menores, em termos absolutos, mas não diferiram daqueles registrados pelo método laboratorial, observando-se correlação linear (R = 67,7%) entre ambos. Maiores concentrações de glicose foram registradas para os machos; a glicemia aumentou 334,3% imediatamente após o transporte, permanecendo elevada (113,3%) até 48 horas e retornou aos níveis basais (jejum) após 72 horas. Concluiu-se que o glicosímetro portátil Accu-Chek Active® é uma ferramenta eficaz para a determinação da glicose em lambari; existem diferenças dependentes do sexo em relação aos níveis de glicose no sangue; a espécie é sensível ao estresse de transporte, sendo a glicose um indicador fisiológico imediato e apropriado para esta avaliação.

Palavras-chave: glicosímetro; medidor clínico portátil; transporte.
INTRODUCTION

Hematological and biochemical characteristics reflect the internal state of the organism and can be used to evaluate the nutritional, reproductive and health status of the fish. Therefore, monitoring of blood parameters is important for study of metabolic imbalances, handling trauma, cellular damage caused by exposure to chemical agents, infections and physiological changes due to stress, which can be detected even before clinical signs are evident (BAHMANI et al., 2001; TAVARES-DIAS and MORAES, 2004; DE PEDRO et al., 2005; TAVARES-DIAS et al., 2007; SVOBODOVÁ et al., 2009; SATHEESHKUMAR et al., 2011; HARter et al., 2014; STOOT et al., 2014; BOSISIO et al., 2017). Blood glucose is a commonly parameter used to assess the physiological status and sanity of the fish and its concentration expresses the level of mobilization of hepatic and muscular glycogen reserves in stress situations (SVOBODA et al., 2001; ENDO et al., 2009; YONEMORI et al., 2009).

Glucose concentration varies according to the species and individuals, biotic factors such as sex, stage of development and feeding or abiotics, such as water temperature and seasonal variations, which may affect hepatic glycogen stores (BARTON et al., 1988; WEDEMEYER et al., 1990; HEMRE et al., 2002; SOENGAS and ALDEGUNDE, 2002; IWAMA et al., 2004; COZ-RAKOVAC et al., 2005; PRASAD and CHARLES, 2010; BARBIERI and BONDIOLI, 2015).

Procedures as the capture, biometry, vaccination and transportation induce increase of blood glucose levels, which characterizes the “secondary response to acute stress” that provides energy support for homeostasis (BARTON and IWAMA, 1991; MORGAN and IWAMA, 1997; WENDELLAR BONGA, 1997; BARTON, 2002). This increase arises initially from the activity of catecholamines (VIJAYAN and MOON, 1992), is maintained by the later release of cortisol and depends on the intensity and exposure time to stressor agent, species, size and nutritional status of the fish (BARTON, 2000).

Therefore, the determination of blood glucose levels is a simple and low cost tool that when analyzed in association with the cortisol provides the evaluation of physiological stress responses of fish, either in intensive production or in laboratory research conditions (BARTON, 1997; CARNEIRO et al., 2009; SVOBODOVÁ et al., 2009).

Regardless the variable investigated, blood collection is a necessary and ordinary procedure in studies related to fish physiology (SHI et al., 2010; STOOT et al., 2014). However, blood sampling is difficult when research is performed small specimens or individuals, considering the small size of their vessels and the small blood volume (EAMES et al., 2010). The significant distance between the collection site (field situations) and the laboratory can affect the results, once physical and chemical changes occur during the storage and transportation of the samples (BEECHAM et al., 2006).

The storage of whole blood can also affect the concentrations of glucose and lactate, because erythrocytes are nucleated and show intense metabolic activity while refrigeration of blood samples may be inappropriate considering fish ectothermia (WELLS and PANKHURST, 1999). Therefore, the use of portable equipment for in situ determination of glucose is an important diagnostic alternative which provides immediate evaluations in laboratory or field conditions (HARRESTIEN et al., 2005; BEECHAM et al., 2006; COOKE et al., 2008; HARter et al., 2014; STOOT et al., 2014).

The use of point-of-care (POC) device to measure physiological blood parameters related to veterinary diagnostics and laboratory research has become popular due to its speed of measurement (seconds) and in loco portability over traditional laboratory methods but it should be considered that these equipments were developed based on human blood samples and that there are differences between species and taxonomic classes; therefore, species-specific validation studies to confirm the accuracy and reliability of these tools are still necessary (STOOT et al., 2014).

Validation studies of portable glucometers have already been performed in coho salmon Oncorhynchus kisutch (IWAMA et al., 1995), rainbow trout Oncorhynchus mykiss (WELLS and PANKHURST, 1999; HARter et al., 2014), Nile tilapia Oreochromis niloticus (EVANS et al., 2003), black e blue rockfish Sebastes melanops e S. mystinus (HARRESTIEN et al., 2005), channel catfish Ictalurus punctatus (BEECHAM et al., 2006), bonefish Albula vulpes (COOKE et al., 2008), zebrafish (EAMESet al., 2010) and ballan wrasse Labrus bergylta (LECLERCQ and MIGAUD, 2014).

The yellow-tailed lambari, Astyanax altiparanae, is a small fish of the family Characidae, native of
Brazil and previously classified as *A. bimaculatus*, of omnivorous habit with insectivorous tendency (ANDRIAN et al., 2001). Although initially considered an invader of fish ponds, represents today an interesting species for fish farming due to its high prolificacy, adaptation to environmental conditions and early growth (GARUTTI, 2003; ABIMORADI and CASTELLANI, 2011).

The species is considered a significant part of the diet of larger carnivorous fish (GARUTTI and BRITSKI, 2000), is appreciated in the human diet, used as live bait for sport fishing and can be industrialized in canned form, which characterizes it as promising for the market (PORTO-FORESTI et al., 2005). The objectives of this work were to evaluate the potential and validate the efficacy of a portable commercial glucometer (Accu-Chek Active - Roche®), originally developed for monitoring human blood glucose, to investigate sex-dependent and stress effects on glycemic profile in lambaris, *Astyanax altiparanae*.

**METHODS**

**Fish**

Lambaris of the species *Astyanax altiparanae* were obtained from induced spawning performed at São Paulo Agribusiness Technology Agency (APTA) Pirassununga, São Paulo State, Brazil, and the experiment was conducted at APTA and Laboratory of Fish Behavior, Department of Basic Sciences, College of Animal Science and Food Engineering, University of São Paulo, Brazil.

The water quality indicators (temperature, pH and dissolved ammonia, nitrite and oxygen concentrations) were continuously monitored using, respectively, digital thermometer (Checktemp - Hanna Instruments Brazil), portable pH meter (ExStik II - Extech Instruments), portable ammonia and nitrite meters (HI 93700 and HI 93707 - Hanna Instruments Brasil) and dissolved oxygen meter (DO-5519 - Lutron Electronic Enterprise).

Fish were fed *ad libitum* twice daily (8:00 and 17:00 hours) with a commercial extruded ration (Ambar Amaral Group®, Santa Fé do Sul, São Paulo, Brazil) with 2 mm grain diameter and 32% crude protein, until 18 h prior to assays. Procedures were approved by the Animal Research Ethics Committee at Faculty of Animal Science and Food Engineering, University of São Paulo Protocolo - 20062011).

**Anesthesia and blood sampling**

The assays were performed after induction to the stage of deep anesthesia with clove oil in the proportion of 50 mg.L⁻¹ of facility water, according to the methodology described by PEREIRA-DA-SILVA et al. (2009) and whole blood samples were collected in up to 1 min by puncturing the caudal vein (100 μL) using syringes containing sodium heparin (Heptar® - Lot 184728A).

**Blood Glucose in captive conditions**

Adult fish (n = 30; 15.0 ± 4.6 g) from a tank (30 m³) were caught using dip net and anesthetized to proceed the blood samplings. An aliquot of each sample (10 μL) was immediately transferred to a test strip used for determination of whole blood glucose (mg.dL⁻¹) in loco on a point-of-care (POC) blood analyzer (Accu-Chek Active - Roche®) and the remainder of the samples was immediately stored in Eppendorf tube and centrifuged 3000 rpm to separate the plasma, which was maintained in freezer (-20°C) until determination of glucose by enzymatic method - glucose oxidase (Laborlab kit, Guarulhos, SP, Brazil - Lot 0610210), with absorbance evaluated in a spectrophotometer at 505nm.

**Glycemic profile of males and females**

Adult fish (males n = 40; 10.5 ± 1.5 g; females n = 40; 11.4 ± 1.5 g) were captured and anesthetized for sexing and subsequent blood sampling to determine the glucose levels by the POC device, as described in the previous item.

**Glycemic profile after transporting**

To evaluate the stressor effect of transporting 95 juveniles (6.0 ± 0.4 g) were packed in a polyethylene bag (50L) containing 25L of facility water, inflated with oxygen and tied with rubber strips for transportation (density 5 fish.L⁻¹ of water), through a paved road for 45 minutes. Then, blood sampling were performed at 0 (arrival to laboratory), 1, 24, 48 and 72 hours after the procedure (n = 19 fish per treatment) and during this period, the fish were fasted and grouped in a tank (250 L).

Glucose levels were measured using the POC device and the values were compared to those...
obtained for fish from a control group (n = 19), not submitted to transportation and that were acclimatized to laboratory conditions.

Statistical Analysis

The data showed as the mean ± standard deviation presented a normal distribution (Shapiro-Wilk normality test) and were submitted to ANOVA one-way, comparing the means by Tukey’s Test (p < 0.05). Subsequently a regression study was performed to verify the correlation between the values of blood glucose recorded using the conventional laboratory enzymatic method or the POC device.

RESULTS

The temperature remained at 26.2 ± 0.6, pH 7.4 ± 0.1, the concentration of dissolved oxygen 5.6 ± 0.5 mg.L⁻¹ and the concentrations of ammonia and nitrite respectively 0.32 ± 0.06 mg.L⁻¹ and 0.06 ± 0.01 mg.L⁻¹.

The mean glucose levels of fish from captive conditions and recorded by means of the POC device or the enzymatic laboratorial method are presented in Figure 1.

The values of blood glucose recorded using the POC were 11.2% lower, but there was no significant difference between the methods. After the regression study, a significant correlation (R = 67.7%) was found between the values obtained by means of the POC or the laboratory method (Figure 2). The blood glucose levels recorded for males were higher than females (Figure 3). The blood glucose levels increased 334.3% immediately after transporting and although a significant reduction of glucose was observed one hour after the procedure, the values remained at high levels up to 48 hours (113.3%), returning to baseline levels after 72 hours (Figure 4).

![Figure 1](image_url). Mean ± SE comparison of glucose levels for lambaris (Astyanax altiparanae) (n=30), as determined by POC device (Accu Chek Active - Roche®) and laboratorial enzymatic method (Laborlab®). ANOVA and Tukey’s test were used to determined statistical differences (p<0.05) and means followed by (*) indicates statistical differences between the treatments.
Figure 2. Regression analysis of blood glucose levels for samples obtained from lambaris, *Astyanax altiparanae* (n=30) using POC device (Accu Chek Active - Roche®) and laboratorial enzymatic assay (Laborlab®).

Figure 3. Mean ± SD comparison of glucose levels between males (n=40) and females (n=40) lambaris (*Astyanax altiparanae*) after capture, as determined by POC device (Accu Chek Active - Roche®). ANOVA and Tukey’s test were used to determined statistical differences (p<0.05) and means followed by (*) indicates statistical differences.
DISCUSSION

The recorded values for the water quality indicators remained in the range considered adequate for the maintenance of tropical fish species (VINATEA, 2004).

The glucose oxidase laboratory method, used for clinical evaluations in human blood, is also routinely used for the determination of plasma glucose in fish (EAMES et al., 2010). It is based on the glucose reaction of the sample with the enzyme glucose oxidase which, in the presence of oxygen, forms gluconic acid. This, in turn, combines with hydrogen peroxide ($H_2O_2$) and peroxidase and a chromogenic substance (usually toluidine and dianisidine) forming a colored product whose absorbance is measured in a spectrophotometer (TRINDER, 1969).

In general, portable glucose meters are based on the glucose reaction with the enzymes glucose oxidase or PQQ glucose dehydrogenase (pyrroloquinoline quinone-dependent glucose-mediated dehydrogenase reaction), the principles of which are detailed in the review by HONES et al. (2008). COOKE et al. (2008) recorded higher blood glucose values (approximately 9.3%) for fish using POCs but in general the values obtained using these devices are lower (WELLS and PANKHURST, 1999), which can be attributed to total blood utilized, unlike the conventional laboratory method that employs the plasma for such evaluations (COOKE et al., 2008). Furthermore, the measurements and calculations of many variables are based on constants and algorithms of human blood and the differences between species and taxonomic classes must be considered (HARTER et al., 2014).

BEECHAM et al. (2006) observed in $I. punctatus$ blood glucose levels 30% lower when the POC device was utilized and attributed the differences to storage period of heparinized blood until centrifugation, a commonly problem in field samplings. WELLS and PANKHURST (1999) and STOOT et al. (2014) suggest the use of portable glucometers for recording relative rather than absolute values, considering that these devices underestimate glucose values. In fact the values recorded in the present study were lower than those recorded for the same samples using the enzymatic laboratory method but this difference was only 11.2%.

EAMES et al. (2010) evaluated the efficacy of four portable devices for glucose determination in zebrafish Danio rerio, a biological model of extremely small size and widely used in research related to disorders of carbohydrate metabolism in humans. The authors considered as advantages the speed of reading (seconds) and in loco portability of POCs over traditional laboratory methods, once these

Figure 4. Mean ± SD comparison of glucose levels for lambari (Astyanax altiparanae) after transporting, as determined by POC device (Accu Chek Active - Roche®). ANOVA and Tukey’s test were used to determined statistical differences (p<0.05) and means followed by different lower case letters indicates statistical differences between the treatments.
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Accu-Chek®, a portable glucometer, cortisol variations were observed in this study, as verified in rainbow trout *O. mykiss* (WELLS and PANKHURST, 1999), Nile tilapia *O. niloticus* (EVANS et al., 2003), black rock fish *S. melanops* and adult blue rockfish *S. mystinus* (HARRENSTIEN et al., 2005), channel catfish *I. punctatus* (BEECHAM et al., 2006), and bonefish *A. vulpes* (COOKE et al., 2008). Considering this linear relationship and that application of correction factors was already proposed for zebrafish *D. rerio* (EAMES et al., 2010), black rock fish *S. melanops* and adult blue rockfish *S. mystinus* (HARRENSTIEN et al., 2005) and ballan wrasse *L. bergylta* (LECLERCQ and MIGAUD, 2014), it is suggested posterior investigations in order to determine specific correction factor for lambari, in order to make feasible the use of portable device Accu-Chek® for determination of absolute values in this species.

Blood glucose levels may vary depending on the water temperature, diet and sex of the fish. In colder periods lower blood glucose concentration is observed due to reduced food intake and increased use of energy reserves (SHERIDAN and MOMMSEN, 1991; BANI and VAYGHAN, 2011). Significant variations resulting from energy displacement required for ovarian maturation or even fasting periods were observed in species that migrate to spawn (SVOBODA et al., 2001; SVOBODOVÁ et al., 2009; PRASAD and CHARLES, 2010). SVOBODA et al. (2001) recorded high concentrations of plasma glucose in females of the *Tinca tinca* species evaluated during the reproductive period and suggested an effect of gonadal development on this parameter. Otherwise lower levels of glucose were observed in common carp females *Cyprinus carpio*, response that was attributed to the accumulation of glycogen in the ovaries during maturation (SVOBODOVÁ et al., 2009) and in females *Rutilus frisii kutum*, an anadromous species of the Cyprinidae family that exhibits energy displacement for egg production and faces periods of fasting during migration to spawning (PRASAD and CHARLES, 2010). However no sex effect on mean values of blood glucose in *Clarias batrachus* (KUMARI and AHSAN, 2011) and zebrafish *D. rerio* (EAMES et al., 2010) were observed. In this study the fish weight were similar and the temperature and fed were controlled, therefore the lower blood glucose levels recorded for females suggest a sex-dependent effect on glycemia.

The increasing blood glucose is a classical secondary response of fish to stress (BARTON, 1997; MORGAN and IWAMA, 1997; BARTON, 2002) and results mainly from the release of catecholamines, mainly epinephrine (MAZEAUD et al., 1977; RANDALL and PERRY, 1992; REID et al., 1998) which promotes the phosphorylation of the enzyme glycogen phosphorylase, resulting in an increase in glycogenolysis and, consequently, in blood glucose levels, in order to meet the increase in the energy demand (VIJAYAN and MOON, 1992; WEDEMEYER, 1996; WENDELAAR BONGA, 1997). Transporting is a stressful procedure for intensive fish farming, since it includes handling, capture and confinement at high densities, with consequent changes in water quality, mainly due to the increase in temperature and reduction of oxygen, which causes a series of physiological changes in order to reestablish homeostasis (CARNEIRO and URBINATTI, 2001; URBINATI and CARNEIRO, 2004; URBINATI et al., 2004; ACERETE et al., 2004; BRANDÃO et al., 2006; TAKAHASHI et al., 2006; OLIVEIRA et al., 2009). Hyperglycemia is an immediate responses of fish after transportation and was observed in native species in Brazil as matrixxa *Brycon cephalus* (CARNEIRO and URBINATTI, 2001, 2002), tambaqui *Colossoma macropomum* (GOMES et al., 2003; CHAGAS et al., 2012), jundiá *Rhamdia quelen* (CARNEIRO et al., 2009 a) and pirarucu *Arapaima gigas* (GOMES et al., 2006; BRANDÃO et al., 2008).

In this study, the blood glucose levels increased significantly after transporting and this response should not be attributed to the activity of catecholamines that have a short half-life (clearance up to 10 minutes), due to tissue accumulation/binding and metabolic degradation (ROTHWELL et al., 2005) but rather to cortisol. Considering the aim of this research was to evaluate the efficacy of the portable glucometer, cortisol variations were...
not recorded, and it can only be inferred that the maintenance of hyperglycemia must have occurred due to the activity of this hormone, whose release occurs approximately after one hour with return to basal levels after six hour (BARTON et al., 2002; IWAMA et al., 2006).

The physiological stress responses due to transporting are intense and dependent on the species and intensity, duration and type of the management and the return to the basal blood glucose levels is an indicative of fish recovery. Blood glucose returned to basal levels 24 hours after transporting in tambaqui C. macropomum (GOMES et al., 2003; CHAGAS et al., 2012), pirarucu A. gigas (GOMES et al., 2006), jundiá Rhamdia quelen (CARNEIRO et al., 2009) and matrinxã Brycon amazonicus (CARNEIRO and URBINATTI, 2002; URBINATTI et al., 2004). BRANDÃO et al. (2008) observed the time 48 hours in pirarucu A. gigas and CARNEIRO and URBINATTI (2001) 96 hours in matrinxã B. amazonicus. TAKAHASHI et al. (2006) registered progressive reduction in blood glucose in pacu P. mesopotamicus maintained in ground tank (after 72 hours) and laboratory (after 120 hours). In this study the reestablished of glucose basal levels occurred only 72 hour after a short period transporting, indicating the stressor potential of transporting for the species and also interspecific variability of the responses.

CONCLUSIONS

The Accu-Chek Active® portable glucometer represents an efficient tool and can be used in laboratory or field to determine the glycemia of lambari, a species whose small size makes it difficult to collect significant volumes of blood samples. There are sex-dependent differences with respect to blood glucose levels which are lower in females; the species is sensitive to stress transporting and glycemia is an immediate and appropriate physiological indicator for this evaluation.

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