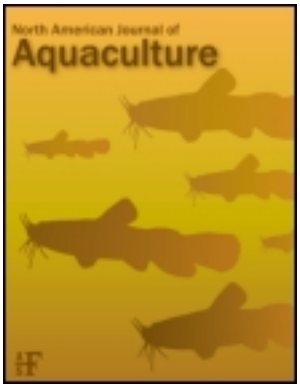

North American Journal of Aquaculture
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/unaj20

Using Portable Lactate and Glucose Meters for Catfish Research: Acceptable Alternatives to Established Laboratory Methods?

Rachel Venn Beecham [a] , Brian C. Small [b] & C. Douglas Minchew [c]

[a] Department of Natural Sciences, Mississippi Valley State University, Post Office Box 7254, 14000 Highway 82 West, Itta Bena, Mississippi, 38941, USA

[b] U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi, 38776, USA

[c] Delta Research and Extension Center and Department of Wildlife and Fisheries, Mississippi State University, Mail Stop 9753, Mississippi, Mississippi State, 39762, USA

Version of record first published: 09 Jan 2011.

To cite this article: Rachel Venn Beecham, Brian C. Small & C. Douglas Minchew (2006): Using Portable Lactate and Glucose Meters for Catfish Research: Acceptable Alternatives to Established Laboratory Methods?, North American Journal of Aquaculture, 68:4, 291-295

To link to this article: http://dx.doi.org/10.1577/A05-074.1

North American Journal of Aquaculture 68:291–295, 2006

DOI: 10.1577/A05-074.1

[Technical Note]

Using Portable Lactate and Glucose Meters for Catfish Research: Acceptable Alternatives to Established Laboratory Methods?

RACHEL VENN BEECHAM*

Department of Natural Sciences, Mississippi Valley State University, Post Office Box 7254, 14000 Highway 82 West, Itta Bena, Mississippi 38941, USA

BRIAN C. SMALL

U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi 38776, USA

C. DOUGLAS MINCHEW

Delta Research and Extension Center and Department of Wildlife and Fisheries, Mississippi State University, Mail Stop 9753, Mississippi State, Mississippi 39762, USA

Abstract.—Simple and portable methods for assessing the physiological state of channel catfish *Ictalurus punctatus* would be valuable tools in field situations where problems with blood storage and transportation occur. This study compared the use of handheld lactate and glucose meters with established laboratory methods in stressed (fatigued) and unstressed (control) channel catfish fingerlings. The results obtained from the Accutrend (Roche Diagnostics Corp.) lactate meter and the Accu-Chek Advantage (Roche Diagnostics) glucose meter were consistently lower ($P < 0.05$) than those obtained with the laboratory reference method. However, significant differences ($P < 0.0001$) were found between the control and fatigued fish for both lactate and glucose, regardless of the method of analysis. Both handheld meters were found to be reliable and suitable for use in field or laboratory situations where relative measurements are acceptable. The costs associated with using the handheld meters were higher than those associated with accepted laboratory methods; however, the initial capital investment was lower for the handheld meters. Ease of use, portability, and rapidity of sample analysis make the handheld meters attractive alternatives to traditional laboratory methods.

The development of inexpensive and accurate methods for monitoring metabolic indices, such as blood lactate and glucose levels, has great potential for improving husbandry and research protocols for both aquaculture producers and researchers. Use of a handheld meter to determine lactate and glucose levels in field situations would greatly assist fishery personnel because it could potentially eliminate problems of blood storage and transportation. Storage of whole blood can lead to problems because the erythrocytes possess a high metabolic capacity, which can cause large fluctuations in lactate and glucose concentrations (Korcock et al. 1988; Nikinmaa 1990; Wells and Pankhurst 1999). Transportation further complicates the situation, since whole blood cannot be frozen without cells rupturing and refrigeration may be inappropriate for blood from exothermic animals (Wells and Pankhurst 1999).

Portable, handheld lactate and glucose meters have been evaluated for use in numerous mammalian species, including dogs *Canis* spp. (Osi et al. 2003), horses *Equus* spp. (Schulman et al. 2001), and humans (Dillon et al. 1997; Pyne et al. 2000; Newman et al. 2002; Solnica et al. 2003) as well as rhinoceros auklets *Cerorhinca monocerata* (Lieske et al. 2002). Evaluations of handheld meters for use with fish are sparse and have been primarily limited to salmonids (Iwama et al. 1995; Wells and Pankhurst 1999). Results of those studies suggest that the meters tested provided reliable measures of metabolic changes but that the values obtained with the handheld meters were consistently lower than those obtained by established laboratory techniques. Both Iwama et al. (1995) and Wells and Pankhurst (1999) concluded that the meters had strong potential for use in aquaculture and field monitoring situations where relative, rather than absolute, values could be used to evaluate metabolic responses.

The objective of this study was to determine the reliability and cost-effectiveness of using portable, handheld lactate and glucose meters in the field for research on channel catfish *Ictalurus punctatus* and to provide a prediction model for comparing meter values with values determined via established spectrophotometric analyses in the laboratory. The ability to use these instruments has the potential to make field sampling more accessible and less time-consuming, which may ultimately lead to more field-based research and a better understanding of fish metabolism outside of the laboratory environment.

* Corresponding author: rvbeecham@yahoo.com

Received August 19, 2005; accepted January 5, 2006
Published online October 2, 2006

Methods

Fish care and sampling protocol.—Channel catfish fingerlings (mean weight ~ 80 g) used for this study were obtained from a mixed-family population reared at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, Mississippi. One week before the start of the experiment, all fish were acclimated to a 750-L circular tank continuously supplied with well water and maintained at room temperature (19–20°C). The fish were fed a commercial catfish feed containing 32% protein (Indi-Bel, Inc., Indianola, Mississippi) once every other day to apparent satiation.

At the start of the experiment, fish were caught at random and assigned to one of two groups: control ($N = 40$) or fatigued ($N = 40$). The control fish were removed from the tank and immediately placed in water containing MS-222 (tricaine methanesulfonate) anesthetic at a concentration of 0.2 g/L of water. The stressed (fatigued) fish were chased around the tank with a dip net for 10 min, quickly removed, and placed in the anesthetic solution. As soon as the fish were anesthetized, blood was collected from the caudal vasculature into a 1-mL heparinized syringe. Blood collection of all fish was completed within 5 min of anesthetization. Immediately after blood was collected, blood lactate and glucose were analyzed with the handheld meters as described below. The remaining blood was placed on ice for approximately 2 h until it was returned to the laboratory. Plasma was separated from whole blood by centrifugation and then analyzed for lactate and glucose as described below. Whole-blood and plasma samples were analyzed in duplicate.

Plasma lactate.—The Accutrend lactate meter (Roche Diagnostics Corp., Indianapolis, Indiana), which was designed for clinical use, was evaluated against an established laboratory spectrophotometric method using the lactate oxidase procedure (Pointe Scientific, Inc., Lincoln Park, Missouri; No. L7596). In the laboratory method, lactic acid is converted to pyruvate and hydrogen peroxide by lactate oxidase (enzyme number 1.13.12.4; IUBMB 1992). Peroxidase (1.11.1.7) then catalyzes the reaction of hydrogen peroxide with a hydrogen donor in the presence of 4-aminophenazone to form a color. Color intensity, measured at 550 nm, is then proportional to the lactate concentration in the sample relative to the standard curve.

The Accutrend lactate meter uses enzymatic determination and reflectance photometry (wavelength, 660 nm) to determine the concentration of plasma lactate in a sample of whole blood. The plasma portion of the whole blood is automatically separated in the test strip before analysis. At ambient temperatures between 5°C and 35°C, the meter is capable of displaying values in plasma concentrations (0.7–27.0 mmol/L) or in whole-blood concentrations using an internal correction factor (0.8–22.0 mmol/L). The meter was calibrated with the provided calibration strip, the calibration strip was then removed, a test strip was inserted into the meter, and a small drop of blood (~25 μL) was placed on the strip. For ease of comparison, the meter was set to display values as plasma concentrations. Values were presented on the meter approximately 60 s after blood was applied to the test strip.

Plasma glucose.—The Accu-Chek Advantage glucose meter (Roche Diagnostics), also designed for clinical use, was evaluated against a standard laboratory spectrophotometric method using the glucose oxidase procedure (Pointe Scientific; No. G7519). In the laboratory method, glucose is oxidized by glucose oxidase (1.1.3.4) to gluconate and hydrogen peroxide. Peroxidase then catalyzes the reaction of hydrogen peroxide, phenol, and 4-aminophenazone to form a color. Color intensity, measured at 500 nm, is then proportional to the glucose concentration in the sample relative to the standard curve.

The Accu-Chek Advantage glucose meter uses the enzyme glucose dehydrogenase (1.1.1.22) to create a reaction that generates an amperometric reaction between two silver bars. This generates a small current that is read by the monitor. The Accu-Chek Advantage test strips, which use a whole-blood referenced system, has been calibrated to deliver "plasma-like" values from 10 to 600 mg/dL at ambient temperatures between 14°C and 40°C. Briefly, the provided calibration chip was inserted into the back of the meter, a test strip was then inserted into the meter, and a small drop of blood (~25 μL) was placed on the strip. Values appeared on the meter approximately 45 s after the blood was applied to the test strip.

Hematocrit.—A hematocrit tube was immediately filled for each sample taken, and hematocrit was determined by centrifuging the tubes and measuring the percentage of packed cells relative to the whole-blood volume.

Statistical analysis.—A two-way analysis of variance was used to detect differences in results obtained from the meter and laboratory methods and differences between control and fatigued fish. Linear regressions between values obtained with the laboratory methods and values obtained with the handheld meters were plotted for both lactate and glucose, and a simple linear correlation was conducted. Coefficients of variation (CVs) were calculated on the replicates of 40 samples, and the CVs were used to calculate the variation between the samples run in duplicate. Data were analyzed with the Statistical Analysis System (SAS

2003), and significance testing was at the 0.05 probability level.

Results and Discussion

The control and fatigued values for blood lactate and glucose were within the normal range for pre- and poststress channel catfish as previously reported (Beecham 2004; Small 2004). Significant differences were found between the control and fatigued fish for both lactate ($P < 0.0001$; Figure 1) and glucose ($P < 0.0001$; Figure 2), regardless of the method of analysis.

Significant differences were found between the laboratory and handheld meters for both lactate ($P = 0.023$) and glucose ($P < 0.0001$). There were no interactions ($P > 0.05$) between analysis type and fish stress for either lactate or glucose. On average, lactate and glucose values determined by the handheld meters were 10% and 30% lower, respectively, than values determined by the laboratory reference methods. These results might have been further confounded by the temporary storage of the heparinized whole blood for up to 2 h before centrifugation, an inherent problem with field sampling. Plasma glucose and lactate concentrations can decrease when left in contact with red blood cells (Tietz 1976, 1996). This might result in a greater difference between the two methods. Even so, the handheld meters were able to effectively detect metabolic changes in response to stress and fatigue in the present study. Lieske et al. (2002) found the Accu-Chek Advantage glucose meter to be reliable for use with seabirds but observed that the values determined by the meter averaged 33% lower than those determined by an established laboratory method. A low degree of variability was also found in the present evaluation of the Accutrend lactate meter (CV = 4.93) and the Accu-Chek Advantage glucose meter (CV = 3.32) when assaying channel catfish blood.

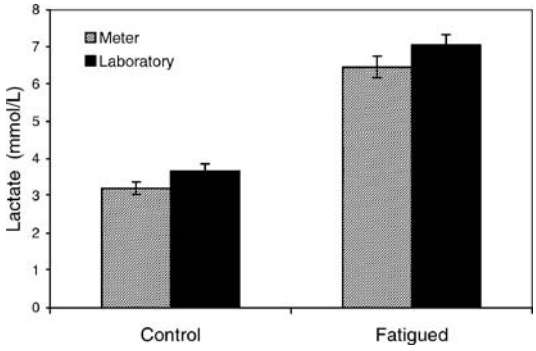

FIGURE 1.—Mean ± SE comparison of plasma lactate levels for control and fatigued channel catfish as determined by handheld meters and a laboratory reference method. Means are significantly different ($P < 0.0001$) for control and fatigued fish, and meter and laboratory means within each group are significantly different ($P = 0.0231$). There was no significant interaction ($P = 0.7768$).

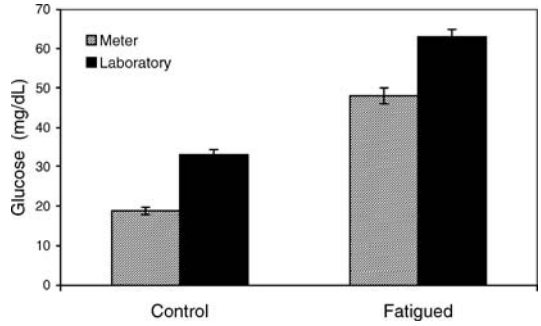

FIGURE 2.—Mean ± SE comparison of plasma glucose levels for control and fatigued channel catfish as determined by handheld meters and a laboratory reference method. Means are significantly different ($P < 0.0001$) for control and fatigued fish, and meter and laboratory means within each group are significantly different ($P = 0.0001$). There was no significant interaction ($P = 0.8420$).

The association between the results from the handheld meter and the laboratory reference method for both blood lactate ($r = 0.96$; $P < 0.0001$) and blood glucose ($r = 0.99$; $P < 0.0001$) demonstrated a high correlation between the two methods. From these data, predictive linear regression equations were developed to describe the relationship between the laboratory blood lactate (Figure 3) and glucose (Figure 4) values and the values determined with the handheld meters.

The acute stress in fatigued fish caused a significant ($P = 0.04$) elevation in hematocrit (mean ± SE = 33.55 ± 0.72%) relative to that of control fish (31.38 ± 0.71%). There are three possible causes of elevated

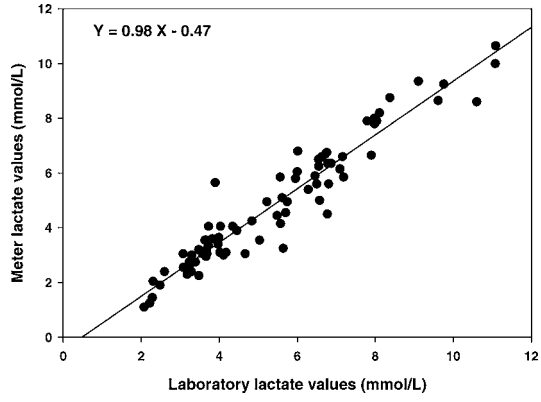

FIGURE 3.—Regression between plasma lactate values determined by means of the Accutrend lactate meter and those derived from the laboratory reference method.

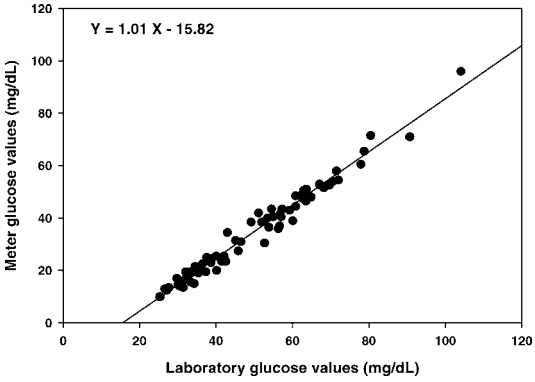

FIGURE 4.—Regression between plasma glucose values determined by means of the Accu-Chek Advantage glucose meter and those derived from the laboratory reference method.

hematocrit during stress: decreased plasma volume, swelling of erythrocytes, and release of additional erythrocytes into the blood (Witters et al. 1990; Pearson and Stevens 1991). In the absence of other hematological measurements, it is not clear what mechanisms were responsible. The differences in hematocrit between the two treatment groups did not result in a significant interaction between method of analysis and fish physiological state ($P > 0.05$). The acceptable range of hematocrit values for measurements using the Accu-Chek Advantage glucose meter is 20–65% according to the documentation supplied with the meter. Since the Accutrend lactate meter separates plasma from packed cells before measuring lactate concentration, hematocrit does not affect the resulting plasma lactate concentrations.

The expense of the disposable supplies associated with using the handheld meters is greater than that of standard laboratory methods. The cost of running both lactate and glucose analyses in the laboratory was approximately US$1.90 per sample run in duplicate, while the cost of using the handheld meters for both analyses was approximately $4.00 per sample run in duplicate. These estimates do not take into consideration associated labor costs or extra costs that may be incurred during sample storage and transportation. The meters have the advantages of (1) being lightweight, battery powered, portable, and simple to use; (2) eliminating transport and storage problems; and (3) providing rapid results in the field. Traditional laboratory methods require a centrifuge, spectrophotometer, cuvettes, pipettes, and reagents. In addition, the initial investment in laboratory equipment and supplies could easily be several thousand dollars. Portable, handheld lactate and glucose meters like those tested in this study can be purchased for approximately $100.00 per meter. The ease of use, portability, and rapidity of sample analysis make the handheld meters attractive alternatives to traditional laboratory methods and provide researchers and aquaculturists with the ability to quickly obtain results in field situations.

In conclusion, these data demonstrated that both the Accutrend lactate meter and the Accu-Chek Advantage glucose meter can be useful tools for measuring plasma lactate and glucose values in the field or laboratory. Results with both meters demonstrated low variability between replicates, but values were consistently lower than those obtained by established laboratory methods and should only be used in situations where relative measurements are suitable.

Acknowledgments

This research was supported through a faculty development grant through Title III at Mississippi Valley State University. Special thanks go to Jimmie Warren and Susan Bailey for their help with data collection and analysis. Reference to trade names does not imply endorsement by the U.S. Government.

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