Aquaculture 285 (2008) 135–140

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Evaluation and use of the Lactate Pro, a portable lactate meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*)

J. Anne Brown [a,b,*], Justin Watson [c,1], Alan Bourhill [c,2], Tony Wall [d]

[a] *Aquatonics Ltd, Glenthorne, Searle Street, Crediton EX17 2DB, UK*
[b] *School of Bioscience, Hatherly Laboratories, University of Exeter, Exeter EX4 4PS, UK*
[c] *NoCatch Ltd, Marine Park, Vidlin, ZE2 9QB Shetland Isles, UK*
[d] *Fish Vet Group, 22 Caresgate Road, Inverness IV3 8EX, UK*

ARTICLE INFO

Article history:
Received 31 January 2008
Received in revised form 28 August 2008
Accepted 29 August 2008

Keywords:
Atlantic cod
Gadus morhua
Welfare
Crowding
Stress
Blood lactate
Plasma lactate

ABSTRACT

Measurement of blood lactate in teleost fish is a common procedure in assessing the impacts of aquacultural procedures such as crowding and pre-harvest handling, or the metabolic consequences of swimming and fatigue, but processing of blood and laboratory assays is not easily applicable at aquaculture sites or in the field. Lactate meters offer an attractive alternative, but few studies have assessed their viability for analysis of fish blood. We have carried out the first evaluation of the Lactate Pro™ meter for on-farm determination of the blood lactate of teleost fishes, specifically the cod, *Gadus morhua*. Blood lactate of farmed cod, caught by rod and line, was below detection limits of the meter (<0.8 mM), and confirmed by laboratory assay as 0.459 ± 0.037 mM (mean ± SEM, $n = 34$). After 20 min crowding in a sweep net, as used in grading or harvesting, blood lactate was measurable with the meter. Mean blood lactate during crowding for 120 min was 2.723 mM ± 0.122 ($n = 102$). In the two crowded cages, blood lactate concentrations was 4 mM or above in 9% and 24% of cod, indicating possible exhaustion. Meter readings were stable for at least 8 h in blood samples held on ice in tubes containing sodium fluoride and oxalate as an anticoagulant and antiglycolytic mix. The coefficient of variation of lactate readings was 4.35% at 1.26 mM, 4.53% at 2.96 mM, 2.89% at 3.94 mM. Lactate Pro™ meter readings were compared to blood or plasma lactate concentrations measured by laboratory enzymatic assay. Regression analysis gave an R^2 value of 0.9315 for meter readings and laboratory-derived values for whole blood, (mean ± SE slope 1.138 ± 0.082), and an R^2 value of 0.943 for meter readings against laboratory-derived plasma lactate concentrations (slope 0.942 ± 0.024). Laboratory-derived plasma lactate concentrations of crowded cod were ~5% above meter readings of whole blood lactate concentrations for the same samples ($P < 0.003$, $n = 95$), but haematocrit did not significantly affect meter readings. Our data lead to the conclusion that the Lactate Pro™ meter is a valuable tool for easy on-farm measurement of blood lactate in aquaculture and that the meter can be employed in assessing the welfare of farmed cod.

1. Introduction

Measurement of blood lactate in teleost fish is a common procedure in research laboratories investigating the metabolic (secondary) responses to stressors (Barton, 1997), the physiological consequences of swimming and fatigue (Gustaveson et al., 1991; Young and Cech, 1994; Milligan, 1996; Johansen et al., 2006; Peake and Farrell, 2006), and the effects of aquacultural manipulations (Barton, 1997) and fisheries techniques (Hopkins and Cech, 1992; Pottinger, 1998; Kojima et al., 2004). Lactic acid (lactate) is a by-product of carbohydrate metabolism in white muscle cells. When oxygen availability for tissue function is compromised, glycolysis gives rise to increased lactate production. For example, blood lactate concentrations rise after exhaustive exercise (Gustaveson et al., 1991; Nelson et al., 1996), in severe hypoxia (Herbert and Steffensen, 2005) and in fatigue (Kojima et al., 2004). Therefore, the measurement of blood or plasma lactate can provide valuable insights into the aerobic status of fish in aquaculture, where both oxygen availability and use may vary.

The analytical techniques to measure blood lactate in research laboratories involves rapid processing of blood to precipitate proteins, and centrifugation, prior to enzyme-based spectrophotometric assays (Wedemeyer and Yasutake, 1977; Nelson et al., 1996), but these procedures are not easily employed at aquacultural sites, or in the field. In recent years, several inexpensive, battery-driven hand-held lactate meters have been marketed, but largely targeted at the sports

* Corresponding author. Aquatonics Ltd, Glenthorne, Searle Street, Crediton, Devon EX17 2DB, UK. Tel.: +44 1363 776456; fax: +44 1392 263700.
E-mail addresses: anne@aquatonics.com, J.A.Brown@exeter.ac.uk (J.A. Brown), justin@qufish.com (J. Watson), Alan.Bourhill@nafc.uhi.ac.uk (A. Bourhill).
[1] Current address: Kergord Hatchery, Weisdale, Shetland Isles, ZE2 9LW, UK.
[2] Current address: NAFC Marine Centre, Port Arthur, Scalloway, Shetland Isles, ZE1 0UN, UK.

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.08.027

industry. These meters offer the potential for rapid, direct and easy on-farm measurement of blood lactate alongside sea cages or besides ponds of on-growing fish. The simplest meters work with single-use strips that are inserted into the meter. For example, the Accusport™ meter (Boehringer Mannheim) involves placing a drop of blood on a test strip impregnated with dried reagents; the resulting reaction, based on lactate oxidase, is measured by reflectance photometry. The Lactate Pro™ meter (Akray Inc, Japan) is again operated with one-use test strips, but in this case performs an electrochemical (oxidation-reduction) based on a method initially developed by Shimojo et al. (1993). Reaction of lactate in the sample with lactate oxidase, in the presence of potassium ferricyanide, forms pyruvate and potassium ferrocyanide that is then oxidised to potassium ferricyanide and produces an anodic current proportional to the lactate concentration of the sample.

Several studies working with mammalian blood samples (the majority using human blood) have assessed the comparability of lactate measurements obtained using simple hand-held lactate meters with those derived from research laboratory assays (Nordstrom et al., 1998; Medbo et al., 2000; Pyne et al., 2000; Schulman et al., 2001; Mc Naughton et al., 2002; Saunders et al., 2005). In some cases, these studies indicated concentration-dependent errors with overestimation of low concentrations and underestimation of high concentrations (Nordstrom et al., 1998; Mc Naughton et al., 2002), or consistent errors in meter readings (Saunders et al., 2005), but other studies have concluded that some low cost portable meters can provide reliable measurements of human blood lactate (Medbo et al., 2000).

Despite their wide use in analysing mammalian blood, only two studies have so far investigated the possibility of employing these inexpensive portable lactate meters for aquacultural or field monitoring of fish. The first study of rainbow trout concluded that the Accusport™ lactate meter was a potentially valuable tool for use in the field (Wells and Pankhurst, 1999), even though the blood lactate concentrations were consistently only about one third of laboratory-derived lactate concentrations. This indicates a significant, but unresolved technical problem. More recently, analysis of catfish blood (Beecham et al., 2006) using the Accutrend™ meter (Roche Diagnostics Corp) again reported lower lactate meter readings that those measured in laboratory assays, but readings were within 10% of each other and a significant elevation in blood lactate was shown by fatigued catfish (chased around the tank in the laboratory) compared to control catfish, whichever technique was employed. As yet there has been no evaluation of the potentially more reliable Lactate Pro™ meter in any fish species.

The aim of the present studies was to assess the accuracy of the Lactate Pro™ meter in measuring blood lactate in farmed cod. We have assessed the reproducibility of Lactate Pro™ readings for blood lactate and their stability over time. We have compared lactate concentrations measured on-farm using the meter with laboratory-derived values obtained for the same samples using an enzymatic assay. We also compared meter readings with laboratory-derived plasma lactate concentrations in the same samples. Variations in blood haematocrit will deliver variable proportions of plasma and erythrocytes in the set volume (5 µl) aspirated by the lactate meter, and could influence meter readings because of unequal distribution of lactate between plasma and erythrocytes. For example, in human venous blood, plasma lactate concentration has been reported to be 50% more than the lactate concentration of erythrocytes (Foxdal et al., 1999). Similarly, the plasma lactate concentrations of rainbow trout blood were reported to be about 25% above blood lactate concentration of the same samples (Wells and Pankhurst, 1999). These differences may reflect different water content of plasma and erythrocytes (Foxdal et al., 1999) and/or the effects of metabolic processes in the erythrocytes (Tiihonen and Nikimaa, 1991), and may be further affected by sample handling procedures. We therefore examined the effect of haematocrit on Lactate Pro™ meter readings and whether haematocrit influenced the relationship between meter readings and laboratory assay of the same samples. As a further means of biologically validating the application of the Lactate Pro™ meter for use in aquaculture, we collected blood samples from both un-manipulated cages of farmed cod and cod subjected to the normal commercial practise of crowding in a sweep net for grading and harvesting.

2. Materials and methods

2.1. Cod

The cod, *Gadus morhua* used in these studies were held at NoCatch Ltd (formerly Johnson Seafarms) in Vidlin Voe (Outer), Shetlands (Lat: 60°23' 17'' N, Long: 01°07' 21'' W) in four 100 m circumference suspended sea cages, at a mean density of 10.8 kg m^{-3}. We collected blood samples from cod held in each of the four cages that were being actively harvested.

Cod reared in sea cages are only rarely visible at the surface, but after a short period (4 days) of food deprivation, as is common practise prior to harvesting of fish (Ashley, 2007) cod were readily captured by rod and line. In two cages, routinely active cod weighing (mean ± SE) 3.22 ± 0.16 kg and 2.9 ± 0.16 kg respectively were caught by rod and line fishing and rapidly killed by percussion stunning before blood removal. The time from initial hooking to stunning was approximately 30 s. Immediately after blood sampling the gills were severed, according to humane killing protocols. The remaining two cages were used to collect blood samples from a group of cod before crowding (by rod and line fishing) and then at 20 min intervals during crowding for grading and harvesting.

Crowding involved using a crane to lower a sweep net with a grading panel (Flexipanel™, Grading Systems) into the cage and moving the net towards the edge of the cage, allowing small cod to escape through the panel. Cod in the crowd were randomly captured, individually, in a hand net, killed by percussive stunning and blood sampled. These cod weighed 4.60 ± 0.11 kg (n = 54) and 4.98 ± 0.16 kg (n = 59) respectively.

2.2. Blood sampling

Blood samples were collected from the caudal vein, within 1.5 min of capture. First, blood for measurement of haematocrit was rapidly collected through a double-sided 18 G needle held in a one-use holder, into a vacutainer tube (5 ml, containing 85 IU lithium heparate; Beckton Dickinson Vacutainer System). This was immediately followed by collection of a second sample for measurement of lactate, into a vacutainer tube (4 ml) containing 10 mg sodium fluoride and 8 mg oxalate (NaF/Ox) to inhibit coagulation and any glycolysis. Tubes of blood were collected on ice.

2.3. Haematocrit

Blood from the vacutainer tubes containing heparin was used to fill duplicate haematocrit capillary tubes, and centrifuged (Hawksley haematocrit centrifuge). Packed cell volume (PCV) expressed as haematocrit (%) was measured with a Hawksley haematocrit reader.

2.4. Lactate Pro™ measurement of blood lactate

Blood from NaF/Ox tubes was transferred to a microfuge tube and used to measure whole blood lactate using the Lactate Pro™ meter (Akray Inc, Japan) before freezing the remainder of the blood on dry-ice for later laboratory assays of blood lactate.

Initially the meter was calibrated by insertion of a “check strip”, following manufacturers guidelines. Then, a test strip (single-use per