Aquaculture 324-325 (2012) 135–144

Contents lists available at SciVerse ScienceDirect

Aquaculture

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

Conditions for instant electrical stunning of farmed Atlantic cod after de-watering, maintenance of unconsciousness, effects of stress, and fillet quality — A comparison with AQUI-S™

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ARTICLE INFO

Article history:
Received 7 January 2011
Received in revised form 20 July 2011
Accepted 9 October 2011
Available online 15 October 2011

Keywords:
Animal welfare
Unconsciousness
Electrical stunning
AQUI-S™
Atlantic cod
Quality

ABSTRACT

Electrical stunning of farmed Atlantic cod is a method used to render the fish unconscious before further processing. However, evaluations of the stunning method at plants have shown that the electrical parameters need to be optimized to achieve instant stunning and prolonged duration of unconsciousness. One aim of the present study was to establish suitable stunning conditions for cod to comply with future fish welfare regulations. AQUI-S™ is an anaesthetic capable of producing rested fish at slaughter. In the current study, we wanted to assess some welfare aspects of using this agent. In addition, the two stunning methods were compared in terms of the magnitude of the stress response, and the resulting effect on product quality. The data show it was possible to stun cod instantly (0.5 s) at 107 V_{rms}, 0.5 + 0.2 A_{rms}. However, it was necessary to expose the fish for a longer period (e.g. 15 s) to the same voltage to prolong the period of unconsciousness to facilitate killing without recovery. AQUI-S™ (68 mg L^{-1}) rendered the fish unconscious without recovery. No noticeable avoidance behaviour or distress was observed during stunning. Blood pH, lactate levels, and blood drainage, as determined after recovery, were similar for both stunning methods. The ability of the white muscle to twitch was not affected by treatment although electrical stunning caused a drop in initial white muscle pH. Hence, a tendency for a more rapid onset of rigor mortis was observed. No detrimental effects on product quality (Quality Index scores, tendency for gaping, ultimate pH, and fillet texture) were observed for either stunning method. Moreover, no blood spots and discolourations of fillets, or spinal fractures were observed. To comply with both good fish welfare protocols, and at the same time ensuring good product quality, we have fundamentally shown that this is indeed possible when cod are stunned with either of the methods described here.

1. Introduction

Recent research suggests that fish can experience fear (Yue et al., 2004) and they have anatomical features indicating they might also experience pain (Chandroo et al., 2004). As a precautionary approach, fish welfare issues should therefore be addressed in the aquaculture industry. In the harvesting step, crowding, pumping, air exposure, stunning, killing and exsanguination have all been identified as critical steps in which fish welfare can be severely compromised (EFSA, 2009). In Norway, carbon dioxide has traditionally been used to stun salmonids before exsanguination. Atlantic cod (*Gadus morhua*) has been introduced as a farmed species, where a basically similar slaughter line is also used for the slaughter of cod. Encouraged by the food authority and consumer demand, the use of carbon dioxide as a stunning agent in the salmon industry is currently being phased out. The major concern is that carbon dioxide merely immobilizes the salmon, meaning that the fish are actually conscious (Robb and Kestin, 2002) when they are subsequently bled. Indeed, Roth et al. (2006) showed that although the salmon were calm after 40 min of commercial live chilling at high CO_2 concentrations, they might not have been unconscious since eye rolling was observed in all fish. In addition, commercial use of carbon dioxide to stun Atlantic salmon (*Salmo salar*) generally causes stress and early rigor mortis (Erikson, 2008). Machines for percussion or electrical stunning are gradually replacing carbon dioxide as stunning (killing) methods. However, through experience and scientific testing, it is clear that both methods must be improved to impose less stress and promote better fish welfare. For instance, it has been shown that with the right conditions, using either percussion or electrical stunning, it is possible to stun salmon practically instantly, in less that 1 s (Lambooij et al., 2010a). When carried out correctly, electrical stunning can be a humane procedure for stunning or killing large numbers of fish (Lines et al., 2003; Robb et al., 2002).

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doi:10.1016/j.aquaculture.2011.10.011

In terms of fish welfare, the food authority in Norway requires that the fish are instantly rendered unconscious or killed. After stunning, the fish must not experience undue pain or distress when the gill arches are cut or during subsequent exsanguination. Should the fish be accidentally exsanguinated without proper stunning, it has been showed that exsanguination alone is a slow killing method as it takes about 3 to 7 min for Atlantic salmon to lose visual evoked reflexes (VERs) after gill cutting (Robb et al., 2000). Therefore, it is important to include assessment of recovery when the efficiency of various stunning methods is evaluated. When a stunning method is devised, it is therefore important that unconsciousness is maintained until death occurs. For salmonids and cod where the present commercial killing method is mostly exsanguination, with a certain safety margin, it seems reasonable to require that the fish should not recover for at least 10 min post stunning. There are three major factors that determine stun duration of fish in an electric field: (1) current magnitude, (2) duration, and (3) frequency (Robb et al., 2002). The use of eye roll (VOR) has been suggested as a simple field method to assess whether fish are unconscious or not (Kestin et al., 2002). However, a study of Lambooij et al. (2010a) revealed that caution is needed regarding interpretation on the use of VOR.

Electrically stunned grass carp (*Ctenopharyngodon idella*) resulted in faster ATP depletion and earlier onset of rigor mortis compared with fish that were not subjected to electrical stunning. Electrical stunning did not induce blood spots in the flesh or damages to the skin. Shelf-life was not affected by this stunning method (Scherer et al., 2005). Similarly, Roth et al. (2007) found that electrical stimulation in air of turbot (*Scopthalmus maximus*) resulted in a more rapid drop in muscle pH and early onset of rigor, although potentially excessive fillet gaping, inferior flesh texture, or injuries were not observed. In another study, the efficiency of electrical stunning of turbot depended on whether the electrical discharge was applied to the head only or through the whole body. For the latter group, flesh quality was also studied. Low initial pH and rapid onset of rigor mortis occurred, and the flesh was softer, redder and darker than fish killed by percussion or bleeding (Morzel et al., 2002). On the other hand, electrical stunning of Atlantic salmon in water did not accelerate rigor development and at no injuries were observed in the fish (Roth et al., 2002). To avoid carcass damage (haemorrhages and vertebrae fracture), the current duration when stunning Atlantic salmon in water should not exceed 1.5 s (Roth et al., 2003). For electrical stunning of rainbow trout in water, a high frequency electric field (1000 Hz) is necessary to ensure that no carcass damages occur (Lines et al., 2003).

Eugenol and isoeugenol (the active ingredient of AQUI-S™) have been demonstrated to be a safe and effective anaesthetic or euthanizing agent for several fish species (Holloway et al., 2004; Iversen et al., 2003). Moreover, when fish are anaesthetized in connection with slaughter, the use of these agents makes it possible to harvest truly rested fish, assuring optimal flesh quality is maintained and preventing premature onset of rigor mortis (Jerrett et al., 1996; Misimi et al., 2008). When adult Atlantic salmon are exposed to AQUI-S™ (17 mg L^{-1}) for at least 30 min, no fish showed VORs (Erikson, **2011**). To further study the welfare aspect of using AQUI-S™ in connection with slaughter, it is of interest to compare VORs with electrocardiograms (ECGs) and electroencephalograms (EEGs) on the same individuals (present research).

We have previously studied electrical stunning of cod in the laboratory using a portable version of the commercial dry-stunner (STANSAS #1). In the industry, electrical stunning takes place after dewatering as the fish are pumped to the stunner from the waiting cage or directly from the well-boat. The fish are transported through the stunner on a conveyor belt. In our previous laboratory study, single cod were quickly netted from a holding tank and transferred to the stunner where the average stunning voltage was 41 V DC (100 Hz, 0.2 A). The fish passed 10 rows of electrodes in 18–27 s while lying on the conveyor belt, that is, at least **one** row of electrodes was **in** contact with the fish at any one time. Since the fish were immediately bled, recovery was not studied. The fish may have been properly stunned, but when rested fish was used as input, it was shown that the electrical stunning partly depleted the high-energy phosphates and glycogen levels of the muscle. However, product quality assessed after ice storage was not affected by electrical stunning. Furthermore, the method was compared with stunning of cod in seawater where a bipolar square wave current (170 Hz, 33% duty cycle) was applied for 5 s. No differences between stunning methods were observed in terms of product quality (Digre et al., 2010). In a later study at a cod farm, the commercial version of the electrical stunner (STANSAS #1) was used. A complex pDC was applied where the voltage of first row of electrodes (initial stun) was 16 V, whereas the subsequent rows 2 to 10 had a voltage of 45 V. Post-stunning recovery assessments revealed that 20% of the fish were not properly stunned (unpublished results). This showed that the electrical parameters had to be optimized to ensure that no fish recovered before they had died due to loss of blood. Another factor that also necessitated another study on present stunner was to demonstrate that Atlantic cod could be instantly rendered unconscious to comply with expected future fish welfare legislation. Thus, the aims of the current research were to: (1) establish the electrical parameters necessary for instant stunning, and at the same time, assuring that the period of unconsciousness is maintained long enough (10 min), and that the treatment do not inflict carcass damage, (2) assess whether AQUI-S™ anaesthesia actually rendered the fish in an unconscious state for at least 10 min, and (3) compare the two stunning methods in terms of fish welfare and ability to produce high quality fillets.

2. Materials and methods

2.1. Fish

Fiftyfive farmed Atlantic cod (*Gadus morhua* L) were obtained in January 2010 from Frengen Havbruk AS at Fjøsvika, Ytterøy in the Trondheimsfjord in Central Norway. The seawater temperature at a depth of 6 m was 6 °C. Until 2 d before transport, the fish had been offered BioMar Classic Marine 800 feed containing 18% fat and 50% protein. The fish (mean ± SD), weighing 1660 ± 314 g with fork length 49 ± 4 cm were netted from the seacage into two 500 L tanks equipped with pumps for water renewal. After a 5 min boat transport, the fish were netted into 3 tanks filled with fresh seawater on a truck designed for transport of salmon smolts. The fish were then transported with oxygenation at a fish density of 15 kg m^{-3} for 3 h to our laboratory. The levels of dissolved oxygen, acidity, and temperature in the stagnant transport water in the tanks were upon arrival 67–112% saturation, pH 7.5–7.6, and 0.4–1.6 °C, respectively. To recover from possible transport stress, the fish were kept at a density of 14 kg m^{-3} in two 4000-L holding tanks in our laboratory. Fresh, sand-filtered seawater was constantly circulated through the tanks at a rate of 5 m^3 h^{-1}. The fish exhibited normal behaviour shortly after transfer to the holding tanks. Seawater temperature, acidity, and levels of dissolved oxygen ranged between 6.7–7.9 °C, pH 7.97–8.02, and 81–91% saturation, respectively. After 6 d without feed, the experiment started.

2.2. Experimental setup

2.2.1. Electrical stunning after dewatering

A small portable unit resembling the larger STANSAS #1 commercial dry stunner from SeaSide AS (Stranda, Norway) was used. Whereas the commercial version has 10–12 rows of 4 parallel electrodes per slot, the experimental unit used here had 3 rows of 4 electrodes (one slot). Since our main goal was to obtain immediate stunning (contact point between fish head and electrode), a larger