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Selection for stress responsiveness and slaughter stress affect flesh quality in pan-size rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

The control of slaughter stress is of importance with regard to both fish welfare and flesh quality. Muscle characteristics and instrumentally measured quality parameters were determined in rainbow trout lines selected for high-responsiveness (HR) or low-responsiveness (LR) of plasma cortisol to an acute confinement stressor. Measurements were made in both unstressed and stressed fish (a 15 min period of confinement before slaughter) from both lines. Compared to LR fish, HR fish were smaller, had a slightly higher condition factor, lower fat-meter-values, and higher carcass yield. No difference between the lines was observed for muscle pH, either at slaughter or at 72 h *post-mortem* (pm). Fillets from HR fish had a lower muscle dry matter content and had higher lightness (L*) value for raw fillet. Fillet redness (a*) was lower for fish from the HR line for both raw fillet at slaughter and 72 h pm, and for cooked fillets. Fillet firmness was higher for fish from the HR line for raw fillet, but lower after cooking. Both white and red muscle fibers of HR fish were smaller than those in LR fish and HR fish had a thicker red muscle than LR fish. Imposition of an acute confinement stressor before slaughter induced a differential plasma cortisol response in the HR and LR fish. Pre-slaughter stress also lowered muscle initial pH, lowered red muscle mean diameter, and reduced raw fillet mechanical resistance, but increased cooked fillet firmness and had no effect on fillet color. Almost no interaction between selection line and pre-slaughter stress effects was observed showing that slaughter stress had similar consequences in both lines. Overall, the HR/LR trout model gave new insights in the comprehension of trout flesh quality and showed that the level of plasma cortisol response did not affect the impact of slaughter stress on fillet quality.

Statement of relevance: Stress in fish is a permanent concern in aquaculture, and the stress associated with slaughter needs to be minimized in order to preserve flesh quality. The present work shows that similar adverse effects of slaughter stress on flesh quality are seen in rainbow trout from both low stress-responding and high responding lines. Genetic selection for low stress responsiveness does not appear to offer benefits to manage slaughter-stress consequences on flesh quality.

1. Introduction

The stress response is an adaptive mechanism in animals that are disturbed or threatened by biological, physical or chemical stressors (Ashley, 2007). In fish farming, fish are subjected both to acute stressors, such as handling, and to chronic stressors including changes in their environment (temperature, water quality, salinity), interactions with other fish, and prolonged physical stressors (e.g. transport, crowding) (Pickering, 1992; Bonga, 1997). In fish, cortisol is the principal corticosteroid released following activation of hypothalamic-pituitary-interrenal axis (Pickering, 1992) and cortisol is widely used as an indicator of stress. A maladaptive stress response impairs feeding and growth, adversely affects immunocompetence, and interferes with reproductive processes (Pickering, 1992; Bonga, 1997; Portz et al., 2006). Selective breeding, to reduce the responsiveness of fish to stressors, has been investigated as a possible strategy to overcome some of the undesirable outcomes associated with aquacultural stressors (Pottinger and Carrick, 1999).

In rainbow trout (*Oncorhynchus mykiss*), the magnitude of cortisol response was shown to be a trait with a moderately high heritability ($h^2 \approx 0.41$) and families with divergent high (HR) or low (LR) responsiveness to a standardised stressor were constituted (Pottinger and Carrick, 1999).

Selection for stress responsiveness was found to affect growth (Fevolden et al., 2002; Pottinger, 2006; Trenzado et al., 2006), feed efficiency (Øverli et al., 2006) and behaviour (Pottinger and Carrick, 2001; Øverli et al., 2002; Schjolden et al., 2005). However, the impact of

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selection for stress response on muscle characteristics and fillet quality has not yet been investigated.

As previously mentioned, chronic stress can reduce growth performance of rainbow trout (Ashley, 2007) and may consequently therefore affect muscle characteristics. It is still unclear whether chronic stress affects muscle growth (Galt et al., 2014) and no consequences of chronic stress on flesh quality have been reported. Conversely, the adverse effects on flesh quality of acute stress before slaughter are well documented in most species. Pre-slaughter stress, caused by crowding and handling, often leads to an intense muscle activity associated with an accelerated entry into rigor mortis, nucleotide breakdown, and muscle pH fall (Sigholt et al., 1997; Skjervold et al., 1999; Thomas et al., 1999; Robb et al., 2000; Roth et al., 2002; Wills and Proctor, 2004). Some consequences of pre-slaughter stress on organoleptic properties of the fillet have been reported in various species (Poli et al., 2005). A negative impact on fillet color and a softer texture (Faergemand et al., 1995; Sigholt et al., 1997; Gomez-Guillén et al., 2000; Robb et al., 2000; Roth et al., 2002; Kiessling et al., 2004; Wills and Proctor, 2004; Lefevre et al., 2008) are the main effects that have been observed. More recently these effects have been linked with muscle proteome changes in Atlantic salmon, sea bream and sea bass (Veiseth-Kent et al., 2010; Addis et al., 2012; Silva et al., 2012) showing that both contractile proteins and sarcoplasmic proteins are impacted by slaughter stress. Moreover, slaughter stress was also shown to promote cathepsins expression and activity with consequences for muscle structural integrity (Bahuaud et al., 2010).

Although a genetic influence on the consequences of slaughter stress for flesh quality in fish has not yet been demonstrated, it can be assumed to be present by analogy with findings in terrestrial animals (Terlouw et al., 2008).

The two main objectives of this study were 1) to examine (using HR and LR rainbow trout families) whether genetic selection on stress response had any impact on muscle characteristics and flesh quality and 2) to determine whether the effects of slaughter stress had similar effects on the quality of fillet in fish selected as HR or LR.

2. Material and methods

2.1. Fish and rearing conditions

The selection procedure and the effect of the breeding program on the magnitude of the cortisol response to a stressor has been described elsewhere (Pottinger and Carrick, 1999, 2001; Øverli et al., 2005). In brief, the HR and LR rainbow trout lines were initiated in 1996 at the Windermere facility of the Centre for Ecology & Hydrology (Cumbria, U.K.) by repeated measurement of the cortisol response to a standardised stressor (3 h confinement in 50 L water in groups of 6–7 individuals once monthly) in individually tagged (passive integrated transponder; PIT) 2-year-old rainbow trout (pedigree unknown) kept in groups of 25 in 1500 L holding tanks. Individuals were ranked according to their mean stress-induced plasma cortisol concentration across five episodes of confinement. The four fish in each tank exhibiting the highest response (HR) and the four exhibiting the lowest response (LR) were used to produce the F1 generation which consisted of 15 HR and 14 LR families each resulting from a unique male-female cross. These progeny were tested, using the standardised confinement stressor, on ten occasions between 1997 and 1999 and the six LR families with the lowest mean cortisol response during this period and the six HR families with the highest mean cortisol response were identified and used for further work. The F2 generation (2000) was created from unique crosses conducted within the two most divergent families of F1 fish following the testing of individuals within those families during 1999. The two most divergent families within the F2 generation were used to create F3 lines (2003) and the F4 lines were generated from pooled gamete crosses of randomly selected F3 parents (2006). The F4 fish were tested for stress responsiveness in 2006 and 2007 using the standardised confinement stressor and found to exhibit a divergence in their cortisol stress response that was consistent with that of earlier generations.

Fifty, 18 months old, F4 HR and LR rainbow trout were transferred to each of eight (4 × HR and 4 × LR) experimental tanks (circular, 1.8 m diameter, 1500 l, glassfibre tanks) in September, one month before the sampling date. The fish were taken randomly from holding tanks, of identical specification to the experimental tanks, within which the F4 HR and LR lines were separately maintained. Each tank was supplied with untreated lake water at a flow of 25 l/min and was exposed to a natural photoperiod and ambient temperature. Fish were fed five times per week at 1.5% body weight with a commercial diet (Skretting Standard Expanded 40, without added pigments).

The experimental work was carried out under Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Slaughter procedure

Fish were fasted for 48 h before slaughtering. Fish from the HR and LR lines (n = 40 per line; 10 fish in each of four tanks) were subjected (stress: S) or not (no stress: NS) to an acute stressor comprising 15 min confinement immediately before slaughter. In each tank, unstressed (NS) fish (n = 5 per tank) were netted rapidly and anesthetized in 2-phenoxyethanol (1:2000). Then the water level in the tank was lowered rapidly until the dorsal fins of the fish were exposed to air and after 15 min the stressed (S) fish were netted (n = 5 per tank) and anesthetized as for the NS fish. The experimental design comprised four groups, HR-NS, HR-S, LR-NS, and LR-S, each of 20 trout (n = 5 per treatment × 4 replicates).

However, sexually mature fish (gonad weight > 1% of body weight), which were not detected visually, were finally excluded from the analysis, so the actual number of fish employed in the study was ≥16 per treatment.

2.3. Measurements and sampling at slaughter

The fish traits measurements were indexed according to the Animal Trait Ontology for Livestock (ATOL: http://www.atol-ontology.com/index.php/en/les-ontologies-en/visualisation-en (Golik et al., 2012)).

A blood sample was taken immediately after sedation for plasma cortisol (ATOL:0002287) determination, and then fish were bled by gill arch section. Blood samples were centrifuged (3000 g, 1 min) and separated plasma was stored frozen at 20 °C until assay (Pottinger and Carrick, 2001) which was conducted within two weeks.

On whole fish, muscle lipid content (ATOL:0001663) was measured using the Torry Fish Fat Meter® (Distell Industries Ltd., Scotland). Fish were wiped with paper tissue to remove excess water and mucus. The instrument was firmly applied on dorsal musculature, parallel to the lateral line, between the head and the dorsal fin of both sides of the fish (Douirin et al., 1998). The fat-meter value is the mean of these two measurements.

Individual body weight (BW, ATOL:0000351) and standard length (L, ATOL:0,001,658) were measured and the condition factor (ATOL:0001653) was calculated as $K = BW/L^3$.

Fish were eviscerated and carcass, viscera, liver, and gonad were weighed. Yields were calculated as: carcass yield = carcass weight/BW (ATOL:0000548), Viscera-Somatic Index VSI = viscera weight/BW (ATOL:0002259), Hepato-Somatic Index HSI = liver weight/BW (ATOL:0001121), and gonado-somatic index GSI = gonad weight/BW (ATOL:0001799).

After filleting, fillet color (ATOL:0001017) was assessed using a portable Minolta Chromameter CR-200 (Minolta, France) equipped with light source C and a 2° observer angle, calibrated to a white standard. For each fillet, two measurements were conducted on the interior part of fillet, one anterior to the dorsal fin and the other anterior to the anal fin. The mean of these two measurements values was considered.