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Short-term fasting and welfare prior to slaughter in rainbow trout, *Oncorhynchus mykiss*

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ABSTRACT

Fasting fish before slaughter is a common practice in aquaculture but it is not clear how long rainbow trout can be starved before suffering unnecessary stress, nor at what moment of the day slaughter is least stressful. We fasted 90 rainbow trout (*Oncorhynchus mykiss*; initial average weight 215.0 ± 22.6 g) for 24, 48 and 72 h (19.5, 38.8 and 58.0 °C days) and slaughtered them in the morning (08 h00), afternoon (14 h00) and night (20 h00) to observe the effect of fasting duration and slaughter time on welfare indicators, including plasma cortisol, glucose and lactate concentrations as well as hematocrit and leucocyte count. The values of the fasted fish were compared with 90 control fish kept under similar conditions but not fasted. Body weight was not significantly different between fasted fish and controls during the trial but the relative weight of the gut was higher in control trout. Cortisol levels were similar between fasted and control fish and among the treatment groups. Similar results were found for glucose and lactate concentrations in plasma. Hematocrit values were also normal and similar between fasted fish and controls throughout the experiment, but leucocyte count was slightly lower in fasted fish by day three. There were no clear differences in any of the stress parameters in the morning, afternoon and night in either treatment. These results suggest that rainbow trout can cope with fasting up to three days (58.0 °C days) prior to slaughter and that their welfare is therefore not seriously compromised.

1. Introduction

Fasting fish before slaughter is a common practice in aquaculture but concerns have been raised regarding the maximum duration of fasting in different species commonly produced in continental (Barcellos et al., 2010) and marine aquaculture (Morkore et al., 2008), including trout (FAWC, 1996).

Fasting prior to transport or slaughter serves to evacuate the gut and reduce oxygen demand and waste production (Lines and Spence, 2012). Some authors suggest that fasting in exotherms may be less stressful than in endotherms (FSBI, 2002), however fish have motivational mechanisms for feeding when nutritional reserves are low (e.g. Metcalfe and Thorpe, 1992). On the other hand, rainbow trout (*Oncorhynchus mykiss*) in the wild commonly survive long periods of food deprivation (e.g. several months over winter, Ashley, 2007), suggesting that fasting may be a common occurrence in fish and not as stressful as for mammals.

There have been a few studies on the effects of starvation on fish physiology (Jentoft et al., 2005; Pottinger et al., 2003) and most analyze the effect of long-term fasting on growth, muscle protein and fat composition (Einen et al., 1998), not the effect on stress indicators. Less is known about the effect of short term fasting on fish that are normally fed regularly. If prolonged, fasting will decrease live weight (Sumpter et al., 1991) but it is not clear how much pre-slaughter fasting affects trout, since it normally only lasts a few days. Wall (2001) recommends that pre-slaughter fasting should last enough to empty the stomach, but they do not provide information on the optimum period or rates of stomach emptying, nor do they relate time to degree days. Despite the lack of data, both the Farm Animal Welfare Council and the Humane Slaughter Association recommend that the maximum limit for fasting should be 48 h before slaughter (FAWC, 1996; HSA, 2005).

Regarding stress indicators, plasma levels of cortisol are consistently reported to be elevated in response to fasting in mammals (Ortiz et al., 2001) but the evidence in fish is contradictory. In rainbow trout (*O. mykiss*), Pottinger et al. (2003) found that cortisol was higher in fed and re-fed fish than in fasted fish. However, no consistent treatment-related patterns could be discerned. Those authors concluded that fasting appeared to have no direct effect on plasma cortisol levels, but to obtain a better idea of the metabolic challenge, more welfare indicators could be used, including hematocrit or glucose and lactate levels. Less is known about the best time of day to slaughter trout. Rainbow trout (*O. mykiss*) have a daily peak of cortisol after ingestion and lower concentrations at night (Polakof et al., 2007; Small,

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2005), which may imply that slaughtering at night may be less stressful, but no study has been specifically performed to analyze that. In this study we aimed to analyze the impact of short term fasting (up to three days) and time of day of slaughter on several hematic stress indicators, including cortisol in rainbow trout.

2. Material and methods

2.1. Fish material and experimental design

The study was carried out at the aquaculture facilities of the School of Forestry Engineering (Madrid, Spain). The fish farm is located on a small slope divided into terraces where raceways are set. The terrace arrangement allows the downward water flow to be distributed among the different raceways by means of channels. For the experiments, two parallel raceways (volume 5.16 m^3) were filled with freshwater from an underground well, supplying a constant water flow. We used 180 rainbow trout (*O. mykiss*) obtained from Truchas de la Alcarria S.L. farm (Valderrebollo, Guadalajara, Spain), with an initial average weight of 215.0 ± 22.6 g and initial length of 29.3 ± 1.0 cm (±s.d.). When the fish arrived from the farm, they were randomly divided into two groups of 90 individuals (fasted and controls) and then stocked in the two independent raceways for two weeks (adaptation period). After these two weeks, each raceway was divided into three different sections using net separators. Each section had the same capacity (1.72 m^3) and housed 30 animals. Fish were kept under these conditions two additional weeks until the trial started. During the whole experiment animals were subjected to natural photoperiod (sunrise at 08 h15 and sunset at 19 h48) at a constant water temperature (averaging 19.2 ± 0.6 °C; range: 17.9–20.4 °C). They were fed the same commercial feed given on the source farm twice a day (45% crude protein, 24% fat, 11.7% ash and 1.1% crude fiber; 1% feeding rate) and in compliance with recommendations for rainbow trout.

Each pen within a raceway was assigned a slaughter time: 08 h00, 14 h00 and 20 h00 (the former group located upstream and the latter downstream to avoid feed pellets passing from one group to another; see Fig. 1). Within each pen, fish were fasted for 1, 2 or 3 days depending on the day they were slaughtered (first, second or third day of the trial, three consecutive days). Fish from the control raceway were divided similarly and slaughtered at the same times as the fasted fish but were fed every day during the experiment. To calculate degree days, water temperature was recorded once every 5 min during the whole trial using underwater temperature sensors (Hobo®-U11). We then calculated the average temperature per day, which was 19.5 ± 0.6 °C on day 1, 19.3 ± 0.5 °C on day 2 and 19.2 ± 0.6 °C on day 3, obtaining 19.5, 38.8 and 58.0 °C days for days 1, 2 and 3 of fasting respectively. At the same time, the other 90 fish from the control group were kept in a neighboring raceway (in parallel), fed twice a day (at 10 h00 and 18 h00) and slaughtered at the same times as the fasted fish.

2.2. Slaughtering and analyses

After 1, 2 or 3 days of fasting, 10 treatment fish and 10 control fish from each pen (08 h00, 14 h00 and 20 h00) were alternatively captured and immediately anesthetized in clove oil for 2 min (60 mg l^{-1}). Care was taken to quickly capture fish, avoiding unnecessary stress. They were then weighed and measured individually and sampled to evaluate hematological variables. Blood samples were withdrawn from the caudal vein using 2 ml syringes (BD Plastipak), 1 ml was centrifuged at 1500 ×*g* for 10 min and the plasma was extracted to measure cortisol, glucose and lactate (using potassium fluoride as anticoagulant). Another 1 ml of blood was used to measure leucocytes and the hematocrit (with ethylenediaminetetraacetic acid, EDTA, as anticoagulant) and immediately stored at 4 °C until analysis. Immediately after blood collection, fish were killed by sectioning the spinal cord at the base of the head. Finally, all the fish were gutted and the whole gut was weighed. All the work described has been carried out in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the Animal Ethics Committee of the Polytechnic University of Madrid (Spain), in compliance with the Spanish guidelines for the care and use of animals in research (BOE, 2005).

2.3. Statistical analyses

With the data on body weight, gut weight and body length, we calculated the relative weight of the gut (GRW = 100 ∗ gut weight/body weight) and the coefficient of condition (100 ∗ body weight/length^3). Mean ± standard error of the mean (S.E.M.) of the body measurements and blood parameters per treatment were calculated and analyzed using SAS software ver. 9.0 (Statistical Analysis System Institute Inc., Cary, NC, USA). Data from fasted and control groups and within groups were compared by two-way analysis of variance (ANOVA) using the General Linear Method procedure with starvation (1, 2 and 3 days) and slaughter time (morning, afternoon and night) as fixed variables. Means were compared using the LSD test with 5% ($P < 0.05$) as the level of significance.

3. Results

3.1. Body measurements

The mean body weight of fasted trout at slaughter was 210.7 ± 20.5 g (±S.E.M.) and 219.3 ± 23.9 g for control fish, with no differences between groups. Gut weight averaged 18.5 ± 5.0 g and 25.5 ± 6.2 g in fasted and control groups respectively, and was significantly higher in controls after 14 h00 on day 2 of fasting (except at 20 h00 on day 2). The GRW ranged between 8.0% and 11.7% in fasted fish (averaging 8.9 ± 0.9%), and between 11.0% and 12.0% in controls (with an average ratio of 11.6 ± 1.1%), being significantly higher for controls at every slaughter time except at 14 h00 on day 1 (see Fig. 2). Body length was similar among groups averaging 29.3 ± 1.0 and 29.3 ± 1.1 cm in fasted and control trout respectively. Finally, the average coefficient of condition in fasted trout was 0.84 ± 0.08 and in fed trout was 0.88 ± 0.09, with no significant differences between groups (Table 1). No differences were found within groups (fasted and control fish) at any slaughter time.

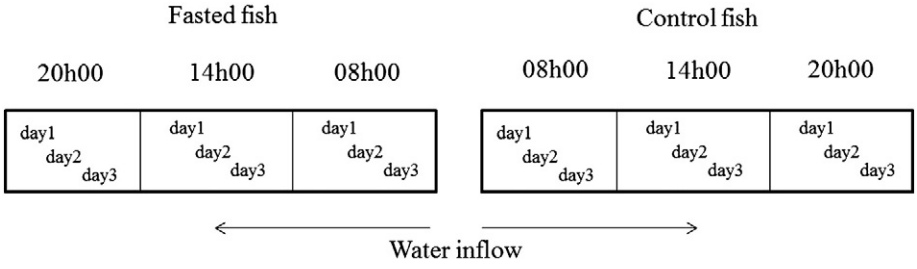

Fig. 1. Raceway setup. Each day (1, 2, and 3) within each pen represents a batch of 10 fish.