Aquaculture 462 (2016) 109–114

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effect of degree-days of fasting stress on rainbow trout, *Oncorhynchus mykiss*

Javier López-Luna [a,*], Ruben Bermejo-Poza [c], Fernando Torrent Bravo [d], Morris Villarroel [b]

[a] Department of Evolution, Ecology and Behaviour, Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool L69 7ZB, UK
[b] Department of Animal Production, College of Agronomics Engineering, Polytechnic University of Madrid. Ciudad Universitaria s/n, Madrid, Spain
[c] Department of Animal Production, Complutense University of Madrid, Avenida Puerta de Hierro s/n, Madrid, Spain
[d] Department of Forestry Engineering, College of Forestry Engineering, Polytechnic University of Madrid, Ciudad Universitaria s/n, Madrid, Spain

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form 5 May 2016
Accepted 10 May 2016
Available online 11 May 2016

Keywords:
Fasting
Degree days
Trout
Cortisol
Glucose
Lactate

ABSTRACT

Most farm animals are fasted before slaughter to empty the digestive system but in fish, the appropriate fasting time also depends on the water temperature. To analyze how the physiological and haematological response vary with degree-days (°C days), 180 rainbow trout (*Oncorhynchus mykiss*) were fasted for 1, 2 or 3 days in two different trials with water at 22.7 or 11.1 °C. In general, water temperature had a significant influence on most variables measured, although the number of degree days had a less important effect on trout physiological response, being only important in the depletion of reserves rate. The condition factor decreased at 2 and 3 days of fasting. At warmer temperatures (22.7 °C), relative weight of the gut content, hepatosomatic index and plasma levels of glucose were lower while cortisol, lactate and haematocrit were higher than at colder ones (11.1 °C). Fasting up to 68 °C days did not seem to have a negative effect on stress but a high water temperature above 20 °C was stressful for trout.

Statement of relevance: This paper present data on the effect of the number of degree days of fasting on body measurements and the haematological response of rainbow trout prior to slaughter. Fasting up to 68 degree days did not have a major effect on their welfare and that their haematological response was solely affected by the water temperature and not by food deprivation

1. Introduction

Pre-slaughter handling involves several processes which can potentially have negative effects on fish welfare (Lines and Spence, 2012), including fasting, transport, crowding, and stunning, all of which must be performed in water, where the temperature cannot always be controlled easily. Short term fasting (i.e., the early phase of food deprivation during which animals mobilize readily available metabolic reserves), normally lasts 7–10 days (Soengas et al., 1996) as is different from starvation (i.e., chronic fasting associated with weight loss and pronounced protein and lipid catabolism). Very little is known about the effect of short term fasting (measured in degree days) on fish that are normally fed regularly. Several interest groups have proposed maximum limits to fasting, but measured in hours as 48 h (FAWC, 1996) and 72 h (HSA, 2005; CIWF, 2009). More prudently, EFSA (2008) indicates that it is difficult to specify a maximum acceptable duration of fasting, since its impact on welfare is related to size, lipid reserves, life stage and water temperature. However, EFSA (2009) does point out that fasting for 50 °C days (degree days) can stimulate the use of body fat reserves and then functional tissue, which is associated with poor welfare. Robb (2008) considers that in salmon there is no evidence that fasting for 72 h is needed to void the digestive tract. Lines and Spence (2012) conclude that fasting for 1–5 days prior to slaughter is unlikely to cause significant welfare problems, and Nikki et al. (2004) report no significant effects of 14 day fasting on live weight in rainbow trout.

Little is also known about the effect of fasting at relatively high water temperatures, although previous studies have found that the accumulated amount of degree days may be more important than fasting duration itself (Lopez-Luna et al., 2013). The optimum water temperature range to grow rainbow trout is suggested to range from 13 to 19 °C, when oxygen is not a limiting factor (Schurmann et al., 1991), and up to 20–22 °C (FAO, 2011) without loss of appetite. Water temperatures in raceways used for trout production the Mediterranean region are often in the upper part of that range, particularly around summer, but few studies have considered fasting effects at the high end. The aim of the present study was to compare the stress response of trout to fasting at two different water temperatures that represent harvesting in winter and summer months.

* Corresponding author.
E-mail addresses: jlopez@liverpool.ac.uk (J. López-Luna), rbermejo@ucm.es (R. Bermejo-Poza), fernando.torrent@upm.es (F. Torrent Bravo), morris.villarroel@upm.es (M. Villarroel).

http://dx.doi.org/10.1016/j.aquaculture.2016.05.017
0044-8486/

2. Materials and methods

2.1. Study animals and experimental design

We carried out two trials, one in winter and one in summer, at the aquaculture facilities of the School of Forestry Engineering, at the Polytechnic University of Madrid (Madrid, Spain). The fish farm is located on a small slope divided into terraces with raceways filled with freshwater from an underground well and re-circulated. In each trial we used 180 rainbow trout (*Oncorhynchus mykiss*) obtained from Fuente Campillo farm (Zaorejas, Guadalajara, Spain) with an initial average weight (± standard deviation) of 417.7 ± 61.3 g in trial 1 (T1) and 368.8 ± 63.7 g in trial 2 (T2). Upon arrival, fish were randomly divided into two groups of 90 individuals in two parallel raceways (volume 5.16 m^3), for control and treatment fish. They were kept in the raceway for two weeks, after which each raceway was divided into three sections of 1.72 m^3 (housing 30 fish) using net separators, with 30 trout in each section. Fish were kept under these conditions for two additional weeks until the trials started, to make sure they had adjusted to the new arrangement. During the whole experiment, animals were subjected to natural photoperiod (T1: sunrise at 07 h40 and sunset at 19 h12; T2: sunrise at 06 h52 and sunset at 21 h31). Water temperature averaged 11.1 ± 0.6 °C in T1 and 22.7 ± 0.9 °C in T2, whereas the water pH varied between 7.7 and 8.0 in the two trials and the average oxygen concentration was 9.9 mg l^{-1} in T1 and 7.8 mg l^{-1} in T2. Before fasting, fish were fed the same commercial feed given on the source farm, twice a day (42% crude protein, 23% fat, 4.1% ash and 2.0% crude fibre, 30 ppm astaxanthin; 1% feeding rate) and in compliance with recommendations for rainbow trout.

Fish in the treatment raceway were fasted for 1, 2 or 3 days before slaughter (n = 30 trout for each day), while fish in the control raceway were fed twice a day (10 h00 and 18 h00) and also slaughtered on the same days. To calculate degree-days, water temperature was recorded once every 5 min during the whole trial using underwater temperature sensors (Hobo-U11, ONSET, Bourne, Massachusetts, USA). The average temperatures per day for T1 were 11.1 ± 0.3 °C on day 1, 11.1 ± 0.3 °C on day 2 and 11.2 ± 0.2 °C on day 3, which meant 11.1, 22.2 and 33.4 °C days for days 1, 2 and 3 of fasting respectively. In T2, the average temperature was 22.7 ± 0.9 °C on day 1, 22.6 ± 0.9 °C on day 2 and 22.7 ± 0.9 °C on day 3 (averaging 22.7 °C), obtaining 22.7, 45.3 and 68.0 °C days for days 1, 2 and 3 of fasting, respectively.

2.2. Slaughter and analyses

All fish were slaughtered and all samples taken at 14 h00. After 1, 2 or 3 days of fasting, 30 treatment fish and 30 control fish were quickly captured (alternating between treatment and control fish) and immediately anesthetized in clove oil for 2 min (60 mg l^{-1}), to avoid unnecessary stress. They were then weighed and measured individually and sampled to evaluate haematological variables. Blood samples were withdrawn from the caudal vein using 2 ml syringes (BD, Franklin Lakes, New Jersey, USA), 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 the anticoagulant). Another 1 ml of blood was used to measure leucocytes and haematocrit (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. Then, half of the fish were gutted and the gut content and liver were extracted and weighed separately. Using the body weight, body length, gut content and liver weight, we calculated the condition factor (CF) = body weight (g) / body length $(cm)^3$, the relative weight of the gut content (RWGC) = 100 * gut content (g) / body weight (g) and the hepatosomatic index (HSI) = 100 * liver weight (g) / body weight (g).

2.3. Statistical analyses

Mean ± standard deviations (s.d.) of each sampling group were calculated using the SAS software ver. 9.0 (Statistical Analysis System Institute Inc., Cary, NC, USA) for each parameter analysed. The data were tested for normality (Shapiro-Wilks) and homogeneity (Levene). A nested analysis of variance using the MIXED procedure of SAS was run to compare biometric and haematological indicators both in fasted and fed trout. Prior to analysis, all the fish samples were classified as fasted or not fasted and this new nominal variable (treatment) was nested to the day of sampling. The nested ANOVA was performed with the day of sampling and treatment nested to day of sampling (hereafter, treatment (day of sampling)) as the main factors. To determine the effect of water temperature, the average water temperature of trials 1 and 2 was also included as a fixed effect and the fish sample as the random effect:

$$y_{ijkl} = \mu_0 + D_i + T(D)_{j(i)} + M_k + TE_l + DTE_{il} + TET(D)_{lj(i)} + \varepsilon_{l(ijk)}$$

where D_i is the day of sampling (1st, 2nd and 3rd; d.f. = 2), $T(D)_{j(i)}$ is the treatment (fasted or fed) nested to the day of sampling (fasted 1, 2 and 3 days and fed and slaughtered on day 1, 2 and 3; d.f. = 5), M_l represents the fish sample, TE_l is the water temperature (11.1 °C and 22.7 °C; d.f. = 1), DTE_{il} is the interaction between the day of sampling and the water temperature (d.f. = 5) and $TET(D)_{lj(i)}$ is the interaction between the treatment (day of sampling) and the water temperature (d.f. = 11).

3. Results

Overall, the water temperature had a significant influence on most variables measured (Table 1), but the effect of degree-days of fasting on trout physiology was not so apparent. There was a significant interaction between day of slaughter and treatment for the condition factor (CF). It only decreased significantly after 2 days of fasting (an average of 28% compared to one day of fasting), while was the same in all control trout (Fig. 1). The relative weight of the gut content (RWGC), was lower for fasted trout at higher temperatures (from 22.7 to 68 °C days; Fig. 2), and more similar between fasted and control fish at lower temperatures. The HSI was significantly lower in fasted trout at higher temperatures (Fig. 3) at all sampling points, i.e. from 22.7 °C d (around 24% less than fasted fish in winter or control fish in summer or winter). There were no statistical differences in HSI between fasted and controls at lower temperatures in winter, with the exception of a small reduction in fasted trout on day 3 (12.9% less than days 1 and 2).

The duration of fasting did not have a significant effect on plasma cortisol, which decreased in fish held at the higher temperatures (by 40% on average; Fig. 4), and remained stable at lower temperatures (averaging 137 ± 13 ng ml^{-1}). Plasma glucose levels were lower at higher temperatures after 45.3 and 68.0 °C days (2 and 3 days of fasting) compared to the other groups (Fig. 5). Plasma lactate levels were consistently higher on day 3 in both fasted and fed trout (27 ± 1 mmol l^{-1}), compared to day 1 or 2 (23 ± 1 mmol l^{-1} for both groups). With regard to the effect of water temperature, lactate was lower in trout held at 11.1 °C compared to 22.7 °C (22 ± 1 mmol l^{-1} vs. 27 ± 1 mmol l^{-1}, respectively). The haematocrit was affected by water temperature (but not fasting), with higher levels at 22.7 °C (35.9 ± 0.5%) compared to 11.1 °C (30.4 ± 0.5%). Finally, the leucocyte count did not vary significantly with day of slaughter, water temperature or fasting.

4. Discussion

Based on our results, it appeared that water temperature itself had the greatest effect on the haematological response in the fish and that the number of degree days of fasting had more of an effect on body measurements (CF, RWGC and HSI) than on plasma measurements associated with welfare. The CF (an index of fish volume) has no units (as