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Determination of optimal degree days of fasting before slaughter in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Pre-slaughter fasting is a common practice in aquaculture to ensure emptying of the digestive tract, but little is known about how long fish should be fasted, in terms of degree days ($^{\circ}\text{C d}$), to clear the gut without affecting their welfare or flesh quality. In this study, 180 rainbow trout (*Oncorhynchus mykiss*) were fasted for 3, 4, 5, 6, 7 and 9 days (17.2, 22.3, 28.6, 35.3, 41.8 and 55.3 $^{\circ}\text{C d}$, respectively) and different morphometric, hematological and flesh quality parameters were measured. The slaughter weight, relative growth and hepato-somatic index did not decrease until after 7 days of fasting (41.8 $^{\circ}\text{C d}$) and stomach content was similar and near zero after 4 days of fasting (22.3 $^{\circ}\text{C d}$). Conversely, fasting time increased carcass yield. Plasma concentrations of cortisol, glucose and creatine phosphokinase enzyme were lower with increasing days of fasting. However, trout slaughtered after 5 days of fasting (28.6 $^{\circ}\text{C d}$) had a higher flesh pH at 0 hours *post-mortem* than 3 and 4 days fasted trout and, consequently, earlier establishment of *rigor mortis*. Furthermore, liver color could be useful as a stress indicator since its hue decreases after 5 days of fasting (28.6 $^{\circ}\text{C d}$). In conclusion, a pre-slaughter fasting period from 17.2 $^{\circ}\text{C d}$ to 22.3 $^{\circ}\text{C d}$ makes possible to obtain a complete emptying of the digestive system in rainbow trout and simultaneously it can minimize the stress response with consequent better flesh quality.

Keywords: Food deprivation; Water temperature; Rainbow trout; Cortisol; pH

1. Introduction

Fish are subjected to periods of fasting before certain management procedures, such as transport, treatment of diseases, transfer of smolts from fresh water to seawater and slaughter. That is done to reduce physiological stress (Ashley, 2007), as it evacuates the gut and reduces metabolism, oxygen demand and waste production (Lines and Spence, 2012). However, according to the Farm Animal Welfare Council (FAWC, 1996), depriving a farmed fish which has been fed regularly will normally have adverse effects on welfare. The critical question is whether the duration of the applied fasting fits with the time needed to empty the gut. Wall (2001) recommends that pre-slaughter fasting should last long enough to empty the stomach, but they do not provide information on the optimum period or rates of stomach emptying. The European Food Safety Authority (EFSA, 2008) indicates that it is not possible 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, Lines and Spence (2012) conclude that a fasting period of 1-5 days prior to slaughter is unlikely to cause significant

welfare problems and Nikki et al. (2004) report no significant effects of short term (14 days) fasting in rainbow trout. For fish farming, degree days are used to estimate the length of time required to develop to the next growing stage, such as the egg incubation, hatching or fattening (From and Rasmussen, 1991). However, very little research has been carried out on the effects of pre-slaughter fasting. Caggiano (2000) pointed out that the length of fasting prior to slaughter is influenced by feeding rate and water temperature, so recommendations should be given in degree days instead of only days. In addition, EFSA (2009) has also recommended avoiding fasting beyond 50 degree days.

There have been relatively few studies on the effects of fasting (typically less than two weeks) on stress physiology or behavior (Barton et al., 1988; Jentoft et al., 2005; Pottinger et al., 2003) and the majority concerns the effect of starvation (more than two weeks of food deprivation) on growth, muscle protein and fat composition (Einen et al., 1998; Pirhonen et al., 2003; Sumpster et al., 1991; Tripathi and Verma, 2003), not on its potential effects on stress indicators. More recently some authors have analyzed the effect of short term fasting (1-9 days) on plasma stress indicators in rainbow trout (Hoseini et al., 2013), including the effect of water temperature (López-Luna et al., 2013) and its effect on flesh quality (López-Luna et al., 2014), all suggesting that trout can adjust well to short term fasting, with a similar levels of cortisol, lactate or glucose in fasted and unfasted trout.

Flesh quality of fish is influenced by various factors such as species, age, feeding management, fat content, muscle fat distribution and by the stress response produced during the pre-slaughter period (Poli, 2009; Suárez et al., 2014). Evidence for quality benefits from prolonged fasting is weak and somewhat contradictory. In the past, long periods of fasting were used in the belief that this could improve the flesh quality by reducing oil levels (Wall, 2001), but other research has demonstrated that there is no significant loss of fat even over extended periods of food withdrawal (Einen et al., 1998). Pre-slaughter fasting is used in aquaculture to empty the digestive tract, but it might also be used to improve flesh quality since fasting can slightly enhance flesh color (Regost et al., 2001), increase muscle firmness (Álvarez et al., 2008) and even reduce bad taste (Palmeri et al., 2009).

In this study, we aimed to analyze the impact of pre-slaughter fasting on rainbow trout stress response and flesh quality using a wide range of degree days.

2. Material and methods

2.1. Fish and experimental design

The experiment was carried out at the aquaculture facilities of the College of Forestry Engineering, Polytechnic University of Madrid (Madrid, Spain). The fish farm is located on a small slope divided into terraces with raceways. The terrace arrangement allows the downward water flow to be distributed among the different raceways by means of channels. For the experiment, two parallel raceways (volume 5.16 m³) were filled with freshwater from an underground well, supplying a constant water flow. For the purposes of the study, we used 180 rainbow trout obtained from Fuente Campillo farm (Zaorejas, Guadalajara, Spain), with an initial average weight of 332 ± 34 g ($\pm$ SEM). Upon arrival they were randomly divided into six groups of 30 individuals (three separate sections in each of the two raceways, each separation measuring approximately 1.25 m x 2.3 m).

Each trout was individually identified using Pit-Tags (Pit Tag i-Tag 162, 2 x 12 mm) injected under the dorsal fin a month before the experiment. Fish were fed twice a day at 2 % body weight of commercial feed (same as source farm) for one month to adapt to their new environment (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. During the whole experiment animals were subjected to natural photoperiod (10 hours of light and 14 of darkness). Water temperature was measured every half hour and averaged 6.15 ± 0.6 °C. To calculate degree days, water temperature was recorded once every 30 min during the whole trial using underwater temperature sensors (Hobo-U11, ONSET, Bourne, Massachusetts, USA).

All fish were weighed (initial weight) and then we stopped feeding in all tanks and fish were sampled after 3 days (17.2 °C d, 3D), 4 days (22.3 °C d, 4D), 5 days (28.6 °C d, 5D), 6 days (35.3 °C d, 6D), 7 days (41.8 °C d, 7D) and 9 days (55.3 °C d, 9D). Care was taken to sample starting from the tank closest to the water outlet in the raceway so as not to stress other fish downstream.

2.2. Slaughtering and analyses

Trout were captured with dip nets, electrically stunned and killed immediately (< 15 seconds) by severing spinal cord.

Immediately after slaughter, blood samples were taken from the caudal vein. Each blood sample was divided into two Eppendorf tubes, one with sodium fluoride (NaF) for the determination of glucose and lactate and another with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for cortisol, triglycerides and creatine phosphokinase (CPK). Both tubes were centrifuged at 6000 rpm for 10 min to remove the plasma, and immediately stored at 4 °C until analysis. Cortisol was measured by enzyme immunoassay using a commercial Cortisol EIA well kit (Radim Ibérica S.A., Barcelona, Spain). Glucose and lactate ion concentrations were measured using enzymatic–spectrophotometric methods (Spinreact S.A., Sant Esteve de Bas, Spain). Triglycerides were measured using a fully enzymatic method from a commercial kit (Boehringer Mannheim, Barcelona, Spain). The CPK levels were measured using a Roche/Hitachi 717 Chemistry Analyzer (Roche Diagnostics, S.L., Sant Cugat del Valles, Spain) with Boehringer Mannheim reagents.

After slaughter, each fish was measured, weighed and placed into a cooler with ice. Half of the fish were eviscerated quickly (approximately 10 min after slaughter) and the other half were left whole to measure *rigor mortis*. Slaughter weight and fork length were used to calculate relative growth and condition factor:

$$\text{Relative growth (\%)} = [(\text{Slaughter weight (g)} - \text{Initial weight (g)}) / \text{Initial weight (g)}] \times 100$$

$$\text{Condition factor} = (\text{Slaughter weight (g)} / \text{Fork length}^3 \text{ (cm)}) \times 100$$

Stomach content was weighed to calculate the empty body weight (slaughter weight - stomach content) and is expressed as a percentage relative to the stomach weight (only stomach, without visceral fat). The weights of digestive tract (from stomach to anus, including visceral fat) and liver were also weighed and we calculated digestive somatic index [DSI = (digestive tract weight/empty body weight) * 100] and hepato-somatic index [HSI = (liver weight/empty body weight) * 100], both expressed as percentages. The carcass yield (%) was calculated as the ratio between slaughter weight and carcass weight (slaughter weight – digestive tract, liver and spleen weight).

Muscle pH was measured at 0 hours *post-mortem* at the front end of the dorsal muscle (after cutting it away from the head) using a pH-meter (HANNA HI9125, Rhode Island, USA) that adjusts for temperature. The progression of *rigor mortis* (4 and 24 hours *post-mortem*) was measured following Cuttlinger's method (Korhonen et al., 1990), placing each trout on a solid flat surface so that the body part behind the posterior end of the dorsal fin was hanging over

an edge, unsupported. The *rigor* angle was calculated as $\alpha = \tan^{-1} (X/Y)$, where X is the length (cm) of the horizontal leg of the right-angled triangle and Y is the length (cm) of the vertical leg of the right-angled triangle. Finally, color measurements were taken at 0 hours *post-mortem* for liver and skin using a Minolta Spectrophotometer CM-2500c (Minolta, Osaka, Japan). The CIEL*a*b* system recommended by the International Commission on Illumination (CIE, 1978) was chosen as the color scale. Using a* and b* parameters we calculated chroma (C*) and hue (h*):

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}}$$

$$\text{Hue (}^{\circ}\text{)} = \arctan (b^*/a^*) \times 57.29$$

Three colour measurements were taken of the liver and skin (dorsal portion on the right-hand side just behind the dorsal fin).

2.3. Statistical analysis

The data were analyzed using SAS software ver. 9.0 (Statistical Analysis System Institute Inc., Cary, NC, USA). A prior analysis of the normality and homogeneity of variance of all variables was performed using the Shapiro-Wilks test with the UNIVARIATE procedure and Bartlett's test with the ANOVA procedure for residues. We used the GLM procedure of SAS, with fasting time (17.2 °C d, 22.3 °C d, 28.6 °C d, 35.3 °C d, 41.8 °C d or 55.3 °C d) as fixed effect. For weight related parameters (slaughter weight, condition factor, stomach content, DSI, HSI and carcass yield), initial weight was included in the model as covariate. The Bonferroni test was used for mean comparison ($p < 0.05$).

3. Results

Table 1 shows the mean slaughter weight, relative growth, condition factor, stomach content, DSI, HSI and carcass yield of the trout after different fasting times. Slaughter weight and relative growth were affected significantly by fasting time, with a higher weight in 3D trout than 7D and 9D. Condition factor was higher in 3D, 4D and 5D than 9D. The stomach content of 3D trout was higher than the rest of the groups. The DSI tended to decrease with fasting time. HSI was higher in 3D trout than 7D and 9D. Carcass yield was higher in 7D trout than 3D.

Mean hematological parameters (cortisol, glucose, lactate, triglycerides and CPK) are shown in Figure 1 for the different fasting times. All hematological parameters varied significantly

with fasting. Plasma cortisol was higher in 3D and 4D trout than the rest of the groups. Glucose levels decreased with degree days of fasting, being highest after 3 days of fasting and lowest after 9 days. The 3D trout has the lowest plasmatic levels of lactate compared to the other groups. The triglyceride levels in 9D trout were higher than 5D, with the rest of groups showing similar levels. The CPK followed a similar pattern as glucose, decreasing as increased days of fasting with the highest value in 3D and 4D trout and the lowest in 9D.

The flesh pH was affected significantly by fasting time, with lower values in 3D and 4D trout than the rest of the groups. At 4 hours *post-mortem*, *rigor* angle was higher in 6D, 7D and 9D trout than 3D, while at 24 hours *post-mortem* it was similar between all groups (Figure 2).

Liver and skin color parameters (L^* , C^* and h^*) for the different fasting times are presented in Table 2. Liver L^* and C^* parameters showed no significant differences due to fasting time but liver h^* was higher in 3D and 4D trout than the rest of the groups. Skin L^* was higher in 3D and 4D trout than 9D while skin C^* and h^* presented similar values between all groups.

4. Discussion

4.1. Morphometric measurements

According to Pottinger et al. (2003) and Sumpter et al. (1991), slaughter weight begins to decrease in rainbow trout with starvation periods of 2 to 6 weeks. However, in our study, a pre-slaughter fast of 7 days ($41.8\text{ }^{\circ}\text{C d}$) was enough to decrease slaughter weight and relative growth (compared to 3D trout). These differences with the aforementioned studies could be since fasting effects depend on various factors, such as fish size, lipid reserves, life cycle phase or water temperature (EFSA, 2008). In addition, studies in other species such as salmon found significant differences in body weight after only 3 days of food deprivation, equivalent to $13.5\text{ }^{\circ}\text{C d}$ (Einen et al., 1998). The lack of changes in slaughter weight until 7 days of fasting may be due to an adjustment of fish metabolic rate in response to food availability (Lines and Spence, 2012), reducing body mass loss. Some authors conclude that a 24-48 hours fast before any stressful procedure (handling, transport or slaughter) appears to increase fish stress tolerance (Davis and Gaylord, 2011), implying that short-term fasting may be beneficial before slaughter, requiring less time at higher temperatures (Robb, 2008). Furthermore, the condition factor reflects the nutritional status of fishes (Bavcevic et al., 2010; Chatzifotis et al., 2011) and allows a more accurate measure of fish size, including fork length in its calculation. It has been reported to decrease with fasting periods between $31.5\text{ }^{\circ}\text{C}$

d (Pottinger et al., 2003) and 98 °C d (Sumpter et al., 1991) in rainbow trout, so the 55.3 °C d maximum duration of fasting in our trial was enough to decrease it.

Stomach content decreased to values near zero from 4 days of fasting (22.3 °C d) and DSI also tended to decrease with increasing degree days of fasting. Consequently, carcass yield was lower in 3D trout compared to the other groups. Our results are agreed with López-Luna et al. (2013), who found that trout stomachs emptied after 24 hours at 19 °C (19 °C d), emphasizing that digestive tract clearance is a time and temperature dependent process, which requires a longer time at low temperatures (Usher et al., 1991). Our results agree with Lines and Spence (2012) and McMillan and Houlihan (1992), who estimated an average of 1-5 days of fasting to ensure a completely empty stomach. Therefore, according to our results, we can consider that a pre-slaughter fasting of 22.3 °C d is enough to ensure an empty stomach in rainbow trout, avoiding potential problems of flesh contamination during evisceration.

As with slaughter weight and relative growth, HSI decreased from 7 days of fasting (41.8 °C d), with respect to 3D trout. A similar decrease was found by Farbridge and Leatherland (1992) in rainbow trout, but their average values in fasted fish were lower than our trial (1.3 vs. 1.8), probably since they used smaller fish. Liver weight normally decreases with food deprivation in fish (Barcellos et al., 2010; Costas et al., 2011) but the time that it takes to start decreasing may vary with species and experimental conditions. In rainbow trout, the minimum period varies from 2 days to 6 days of pre-slaughter fasting (Farbridge and Leatherland, 1992; McMillan and Houlihan, 1992). In other species such as sea bass (*Dicentrarchus labrax*) (Pérez-Jiménez et al., 2007) or sea bream (Metón et al., 2003) HSI decreases with increasing degree days of fasting. This reduction in liver weight is a consequence of the utilization of nutrients stored (Davis and Gaylord, 2011), mainly glycogen (Soengas et al., 1996), but other liver metabolites, such as lipids or proteins, have been found to decrease (Blasco et al., 1992; McMillan and Houlihan, 1992).

4.2. Hematological parameters

In the scientific literature, data about the effect of fasting on cortisol levels in rainbow trout are inconsistent. There are results reporting an increase in cortisol in fasted rainbow trout depending on the degree of food deprivation (Sumpter et al., 1991). Other studies report no effect (Holloway et al., 1994; Reddy et al., 1995) or even decreased levels in fasted fish (Farbridge and Leatherland, 1992), as we observed. In other species, the evidence is also

contradictory, with cortisol being higher (Barcellos et al., 2010), lower (Barton et al., 1988) or not varying (Weber and Bosworth, 2005) in fasted jundia (*Rhamdia quelen*), salmon and channel catfish (*Ictalurus punctatus*), respectively, compared to fed fishes. In our case, 3D and 4D trout showed higher cortisol levels than groups with longer fasting times, which agrees with other authors (Hultmann et al., 2012; Olsen et al., 2008) who report that feed-deprived cod (*Gadus morhua*) display increased and prolonged responsiveness to stress as compared to fed cod. The low cortisol levels at 5, 6, 7 and 9 days of fasting could be explained by an adaptation mechanism of trout to fasting reducing their metabolism after five days of food deprivation (28.6 °C d) due to a chronic stress. Another reason for the decrease of plasma cortisol could be a depletion of the hypothalamic-pituitary-interrenal axis after 5 days of fasting.

Cortisol stimulates the hydrolysis of glycogen in the liver, which increases blood glucose levels (Menezes et al., 2015). Glucose levels can reach 150 mg/100 ml in rainbow trout during a stress response (Pottinger and Carrick, 1999), with basal levels being 70-90 mg/100 ml (Jentoft et al., 2005). Pottinger et al. (2003) found that glucose levels decrease after 38 °C d of fasting and it has been demonstrated that a fast of 70 °C d is enough to induce hypoglycemia in this species (Furné et al., 2012). In our case, a fast of 4 days (22.3 °C d) began to decrease glucose levels and with 7 days of fasting (41.8 °C d), glucose levels suggested hypoglycemia (55 mg/100 ml). Generally, fish are fasted 24 h before sampling to reach the postprandial stage and begin the post absorptive stage. During the post absorptive stage, circulating metabolites return to basal levels and remained stable. In our trial, further decrease in glucose may indicate an adaptive mechanism depressing basal metabolism to adapt energy restriction.

Lactate is considered an acute stress indicator in fishes because its increases under adverse situations (Grutter and Pankhurst, 2000; Thomas et al., 1999). Although lactate is more associated with acute stress, it can be affected by fasting. Lactate levels should decrease in fishes with longer fasting times (Blasco et al., 1992) to enhance the formation of glucose in the liver (Liew et al., 2012). This may explain the tendency to decrease lactate levels from 4D to 9D trout. However, the lowest lactate levels were observed in 3D trout, which can be considered a result of a lower stress response than in the other groups.

Triglycerides should decrease with increased fasting since fish will mobilize reserves to cope with fasting but those reserves will decrease with time (Costas et al., 2011; Takahashi et al.,

2011). However, in our study plasma triglycerides remained quite stable, with a slight rise after 9 days, which may indicate that fish were beginning to mobilize lipid reserves from muscle and adipose tissue (Li et al., 2011).

The CPK was highest in 3D, 4D and 5D trout than 9D trout. The decrease in CPK levels during starvation has also been reported in other fish species like sea bream (Peres et al., 2013). The decline of CPK activity is attributed to a decrease of enzyme synthesis and turnover rates due to lower metabolic demands in unfed fishes (Echevarria et al., 1997; Evans and Watterson, 2009).

4.3. Flesh quality

In fishes, an initial pH reduction right after death is attributed to the concentration of lactic acid produced by the anaerobic metabolism of glycogen in the muscle (Grigorakis et al., 2003), and this pH reduction is accompanied by the onset of *rigor mortis* (Huss, 1995). The establishment of *rigor mortis* was reached earliest in 6D, 7D and 9D fish compared to 3D, which means a lower flesh quality in these groups (Borderías and Sánchez-Alonso, 2011). The flesh pH was lower at 0 hours *post-mortem* in 3D and 4D trout compared to the other groups, which may be associated with a high muscle activity (Lowe et al., 1993; Thomas et al., 1999), which in turn can be related to higher CPK levels in these groups, who reduced their activity to meet the longer periods of fasting. We did not measure flesh pH at 24 hours *post-mortem* but a negative correlation between pH at 0 hours *post-mortem* and *rigor* angle at 24 hours *post-mortem* has been observed in previous studies in fasted fish (Bermejo-Poza et al., 2015), so observing the lower *rigor* angle at 24 hours *post-mortem* in 6D, 7D and 9D trout than 3D trout, we could expect a higher pH at 24 hours *post-mortem* in 6D, 7D and 9D trout.

4.4. Liver and skin color

Trout subjected to 3 (17.2 °C d) and 4 days of fasting (22.3 °C d) had a higher h^* than the other groups. This could be an indicator of increased mobilization of liver reserves after 5 days of fasting (28.6 °C d) due to increased stress response to fasting (Davis and Gaylord, 2011; Pérez-Jiménez et al., 2012), changing liver color. This color change could result from a decrease in liver lipid composition, as seen in other animals such as fasted broiler chickens (Trampel et al., 2005).

Regarding skin color, we found a decrease in brightness (L^*) for 9D trout (55.3 °C d) with respect to 3D (17.2 °C d) and 4D (22.3 °C d). Höglund et al. (2000) showed that social stress produced a darkening of the skin in Arctic char (*Salvelinus alpinus*) because of the dispersing effect of ACTH on chromatophores and a similar process has been reported in salmonid species like salmon (O'Connor et al., 1999) and rainbow trout (Bermejo-Poza et al., 2016). This may explain our results, suggesting that fish were under a considerable amount of stress after 9 days of fasting.

5. Conclusions

Based on the results obtained in this study, we can underline that one of the main objectives of pre-slaughter fasting, i.e., the complete emptying of the digestive system, is reached after 22.3 °C d (4 days of fasting) in rainbow trout. Rainbow trout are able to adjust their metabolic rate in response to food deprivation, depressing basal metabolism to adapt to the energy restriction produced by fasting. This adjustment of the metabolic rate can be observed in plasma cortisol, glucose or CPK levels, which decreased with increasing degree days of fasting. In the case of plasma cortisol, further research is necessary to confirm whether its decrease after 5 days of fasting (28.6 °C d) was due to a chronic stress response or by a depletion of hypothalamic-pituitary-interrenal axis. Through this mechanism of adaptation, trout are also able to reduce the loss of body mass until 7 days of fasting (41.8 °C d), which could suggest that, from a production point of view we can save on feed costs reaching these degrees day of fasting without a decrease in fish live weight. However, we have observed that periods of fasting above 5 days (28.6 °C d) have a negative effect on flesh quality because fish presented a higher muscle pH at 0 hours *post-mortem*, leading to an early onset of *rigor mortis* and poorer flesh quality, also presenting a darker skin color that could cause rejection by consumers. Therefore, we can conclude that a pre-slaughter fast from 17.2 to 22.3 °C d can minimize the stress response in rainbow trout and produce a better flesh quality.

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Figure captions

Figure 1. Means ($\pm$ SEM) of plasma cortisol, glucose, lactate, triglycerides and creatine phosphokinase enzyme of rainbow trout with different fasting time. Different superscripts indicate significant differences among fasting time ($p < 0.05$).

Figure 2. Means ($\pm$ SEM) of flesh pH (0 hours *post-mortem*) and *rigor* angle (4 and 24 hours *post-mortem*) of rainbow trout with different fasting time. Different superscripts indicate significant differences among fasting time ($p < 0.05$).

Table 1 Means ($\pm$ SEM) of slaughter weight, relative growth, condition factor, stomach content, digestive somatic index (DSI), hepato-somatic index (HSI) and carcass yield of rainbow trout with different fasting time (3D: 3 days or 17.2 degree days-°C d; 4D: 4 days or 22.3 °C d; 5D: 5 days or 28.6 °C d; 6D: 6 days or 35.3 °C d; 7D: 7 days or 41.8 °C d; 9D: 9 days or 55.3 °C d).

	Fasting time						Significance (p)
	3D	4D	5D	6D	7D	9D	
Slaughter weight (g)	403 $\pm$ 1.28 ^a	374 $\pm$ 2.34 ^{ab}	374 $\pm$ 4.25 ^{ab}	375 $\pm$ 2.11 ^{ab}	359 $\pm$ 3.30 ^b	365 $\pm$ 2.71 ^b	0.004
Relative growth (%)	15.30 $\pm$ 1.69 ^a	12.05 $\pm$ 1.07 ^{ab}	13.37 $\pm$ 1.33 ^{ab}	13.47 $\pm$ 0.91 ^{ab}	7.82 $\pm$ 1.35 ^b	9.21 $\pm$ 1.07 ^b	0.003
Condition factor	1.67 $\pm$ 0.04 ^a	1.67 $\pm$ 0.04 ^a	1.62 $\pm$ 0.03 ^a	1.55 $\pm$ 0.02 ^{ab}	1.56 $\pm$ 0.03 ^{ab}	1.50 $\pm$ 0.02 ^b	0.001
Stomach content (%)	22.09 $\pm$ 5.40 ^a	7.37 $\pm$ 3.11 ^b	8.17 $\pm$ 2.78 ^b	3.76 $\pm$ 3.02 ^b	2.17 $\pm$ 1.12 ^b	3.00 $\pm$ 0.97 ^b	0.002
DSI (%)	10.80 $\pm$ 0.65	9.35 $\pm$ 0.39	9.78 $\pm$ 0.53	9.31 $\pm$ 0.57	8.82 $\pm$ 0.28	8.70 $\pm$ 0.47	0.06
HSI (%)	2.31 $\pm$ 0.08 ^a	2.13 $\pm$ 0.09 ^{ab}	2.02 $\pm$ 0.11 ^{ab}	1.97 $\pm$ 0.10 ^{ab}	1.86 $\pm$ 0.08 ^b	1.85 $\pm$ 0.06 ^b	0.01
Carcass yield (%)	83.54 $\pm$ 0.60 ^b	84.67 $\pm$ 0.45 ^{ab}	85.27 $\pm$ 0.69 ^{ab}	85.48 $\pm$ 0.40 ^{ab}	86.57 $\pm$ 0.49 ^a	85.82 $\pm$ 0.47 ^{ab}	0.005

^{a, b} Different superscripts within a row indicate significant differences among fasting time ($p < 0.05$).

Table 2 Means ($\pm$ SEM) of liver and skin color parameters (L*: lightness; C*: chroma; h*: hue) of rainbow trout with different fasting time (3D: 3 days or 17.2 degree days-°C d; 4D: 4 days or 22.3 °C d; 5D: 5 days or 28.6 °C d; 6D: 6 days or 35.3 °C d; 7D: 7 days or 41.8 °C d; 9D: 9 days or 55.3 °C d).

	Fasting time						Significance (p)
	3D	4D	5D	6D	7D	9D	
Liver color							
L*	37.60 ± 1.51	35.01 ± 1.54	34.40 ± 1.35	33.36 ± 0.94	33.32 ± 0.74	35.71 ± 1.02	0.15
C*	13.02 ± 0.49	10.83 ± 0.60	12.30 ± 0.60	11.60 ± 0.43	11.16 ± 0.40	11.36 ± 0.55	0.06
h* (°)	35.49 ± 1.95 ^a	39.74 ± 1.96 ^a	25.96 ± 2.49 ^b	23.69 ± 1.81 ^b	27.85 ± 1.89 ^b	25.79 ± 3.49 ^b	<0.001
Skin color							
L*	28.54 ± 0.66 ^a	28.23 ± 0.82 ^a	26.46 ± 0.64 ^{ab}	25.63 ± 0.54 ^{ab}	26.21 ± 0.74 ^{ab}	25.12 ± 0.50 ^b	0.001
C*	4.50 ± 0.35	4.86 ± 0.32	4.29 ± 0.36	4.04 ± 0.23	3.63 ± 0.33	4.31 ± 0.27	0.11
h* (°)	70.84 ± 1.28	65.70 ± 2.34	63.09 ± 4.25	66.79 ± 2.11	62.73 ± 3.30	64.64 ± 2.71	0.34

^{a, b} Different superscripts within a row indicate significant differences among fasting time ($p < 0.05$).

Fig. 1

Fig. 2

Highlights

- We determined the optimal period of pre-slaughter fasting in rainbow trout.
- Fish were subjected to different degree days of fasting previous slaughter.
- Liver color could be useful as a stress indicator in fish.
- Pre-slaughter fasting from 17.2 to 22.3 degree days can minimize stress response.

ACCEPTED MANUSCRIPT