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Fasting combined with long catch duration modifies the physio-metabolic response and flesh quality of rainbow trout

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Abstract

Pre-slaughter handling involves fasting fish and catching them, which can affect fish welfare and flesh quality, but few studies have considered their combined effects. In this study, adult rainbow trout (320 ± 10 g average weight) were fasted for 7 days (135.6 degree days) and subjected to a long catch duration (20 min), compared with controls (no fasting or short catch duration). Condition factor, organ weight indexes and carcass yield decreased with fasting but not catch duration. Plasma concentrations of cortisol, glucose and lactate increased after a long catch, while plasma triglycerides decreased with fasting. Liver glycogen concentration was lower in fasted fish, and liver luminosity and chroma were higher after fasting with a long catch. Regarding flesh quality, *rigor mortis* resolved more slowly and final muscle pH at 48 hr *post-mortem* was higher for fasted fish with a long catch time. Muscle glycogen concentration was higher in fasted fish, where chroma was also lower. Fasted fish had lower lipid oxidation, but there were no differences in fat content in muscle. Fasted fish with a long catch duration also had less monounsaturated and more saturated fatty acids. In conclusion, a long catch triggered a stress response that had negative effects on flesh quality, independently of fasting.

KEYWORDS

fast, fillet composition, flesh quality, harvesting method, rainbow trout, stress response

1 | INTRODUCTION

More attention is being paid to systematize and automate pre-slaughter handling processes in aquaculture since improved handling has beneficial effects for both fish welfare and flesh quality (Lines & Spence, 2012). Compared to terrestrial livestock production, fish culture usually involves handling thousands of small individuals (<500 g) in a confined space (Mood & Brooke, 2012). Thus, individual animal welfare is difficult to measure commercially, especially before slaughter when time constraints make sampling difficult and multiple stressors interact, including fasting and catching stress.

Pre-slaughter fasting empties the gut and decreases oxygen demand and waste production (Robb, 2008). Fish normally adjust to fasting by reducing their basal metabolic rate and the activity of organs related to exogenous nutrition (Waagbø, Jørgensen, Timmerhaus, Breck, & Olsvik, 2017). Although they may present motivational mechanisms for feeding when nutritional reserves are low (Metcalfe & Thorpe, 1992), farmed rainbow trout (*Oncorhynchus mykiss*) can fast for weeks with no apparent negative effects on stress physiology, behaviour or flesh quality (Jentoft, Aastveit, Torjesen, & Andersen, 2005; Pottinger, Rand-Weaver, & Sumpter, 2003). Rainbow trout can also adjust well to short-term fasting (1–3 days), with a similar level of cortisol, lactate or glucose in fasted and

unfasted trout (López-Luna, Vásquez, Torrent, & Villarroel, 2013) and with no effect on flesh quality (López-Luna, Torrent, & Villarroel, 2014), but less is known about the effect of catching duration, especially in relation to fasting.

Harvesting fish commercially usually involves grouping several hundred individuals in a net, often called crowding. Crowding depletes tissue energy stores, increases lactate and causes osmoregulatory imbalance in fish (Cooke & Suski, 2005) and increases cortisol and haematocrit levels (Merkin, Roth, Gjerstad, Dahl-Paulsen, & Nortvedt, 2010). In wild fish, the degree of physiological disturbance and recovery time correlate positively with the duration of capture (Cooke & Suski, 2005). Similarly, in aquaculture, fasting carp for 14 days makes them more susceptible to crowding stress, although short fasting times (i.e. 3 days) have significant effects on fish health (Hoseini, Abtahi, & Yousefi, 2019). However, little is known about the combined effects of fasting and prolonged catch duration on the welfare or product quality of farmed fish.

Flesh quality can be influenced by pre-slaughter handling (Hardy & Lee, 2010; Suárez et al., 2014) since poor handling will increase the stress response and subsequent physio-metabolic changes can decrease muscle pH, resulting in early onset of *rigor mortis* and a decreased shelf-life (Matos et al., 2011; Wilkinson, Paton, & Porter, 2008). Previous studies in trout have shown that a prolonged pre-slaughter fasting can slightly improve muscle colour, but at the expense of weight and carcass yield loss (Regost, Arzel, Cardinal, Laroche, & Kaushik, 2001). However, short-term fasting or alternating fasting and refeeding periods can improve flesh quality (Bermejo-Poza et al., 2015). In addition, an increase in physical activity prior to slaughter, such as during crowding, has a negative effect on flesh quality (Erikson, Digre, & Misimi, 2011), and also can affect muscle proteins and therefore modify muscle colour (Hultmann, Phu, Tobiassen, Aas-Hansen, & Rustad, 2012).

In this study, we analysed the impact of pre-slaughter fasting (up to 135.6 degree days) and catch duration in rainbow trout on morphometric measurements, physio-metabolic response and flesh quality, including variations in the fatty acid composition of muscle tissue.

2 | MATERIALS AND METHODS

2.1 | Experimental design and fish

The study was performed according to the Spanish Policy for Animal Protection RD53/2013. The experiment was specifically assessed and approved by the Polytechnic University of Madrid Committee of Ethics in Animal Research and by the Community of Madrid, which is the authority in charge for animal research (Ref. PROEX 302/15). The trial was carried out at the aquaculture facilities of the School of Forestry Engineering (Madrid, Spain), located on a slope where the water is extracted from an underground well and the downward water flow is distributed among different raceways and finally comes back to the well after passing by a biofilter. For the experiment, four parallel raceways (volume 5.16 m^3) were filled with freshwater from an underground well, supplying a constant water flow (recirculation). Regarding environmental conditions, during the whole experiment, animals were subjected to natural photoperiod (15L:9D) and average dissolved oxygen and water temperatures were 10.5 ± 0.5 mg O_2/L and 22.6 ± 0.9°C respectively. To calculate degree days (°C d), water temperature was recorded once every 30 min during the whole trial using temperature sensors (Hobo-U11, ONSET). Fish were fed twice a day at 1% body weight using commercial feed (42% crude protein, 23% fat, 4.1% ash and 2.0% crude fibre, 30 ppm astaxanthin), in compliance with recommendations for rainbow trout.

We used 144 sterile female triploid rainbow trout, average weight 320 ± 10 g, originally obtained from a local farm (Cifuentes, Guadalajara, Spain). Each trout was individually identified using Pit-Tags (Pit Tag i-Tag 162, 2 × 12 mm) injected under the dorsal fin and were randomly distributed into four groups with 36 individuals in four different raceways (average density of 0.06 kg/m^3). After 2 weeks of acclimatization period next to Pit-Tag identification, two groups were not fasted and two groups were fasted for 7 days (or 135.6°C d; Figure 1). We decided to use 7 days since it is within normal commercial practice, and we wanted to assure a clear effect of fasting. Previous findings by our group using similar fish suggest that fasting for less than 5 days does not always produce important effects on metabolism or an important stress response in rainbow trout

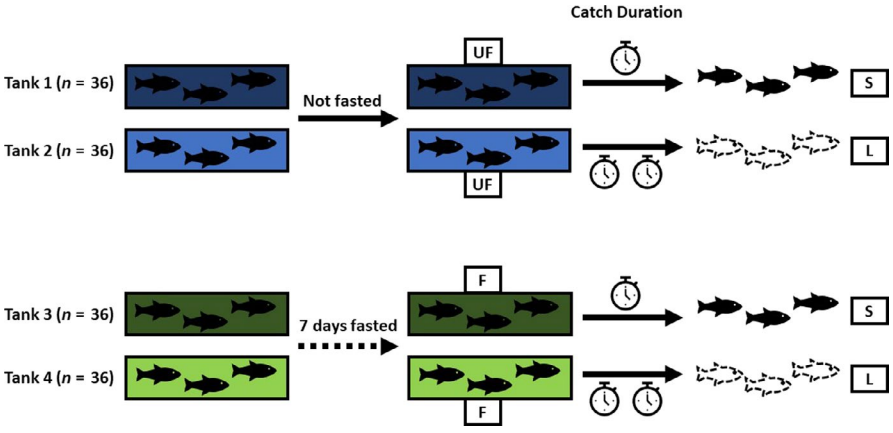

FIGURE 1 General scheme of experimental design. UF: unfasted; *F*: fasted; *S*: short catch duration; *L*: long catch duration

(see, e.g. Bermejo-Poza et al., 2017, 2018; López-Luna et al., 2013). Within the two unfasted (UF) and fasted groups (F), two groups were also either captured quickly, stunned and slaughtered (short catch duration, *S*) or crowded and released repeatedly for 20 min before stunning (long catch duration, *L*). We decided to use the term 'catch duration' instead of crowding duration since the latter fish were not kept at a high density throughout the harvest until slaughter. All fish with a short catch duration (n = 36 UF and 36 F trout) were only crowded for less than 30 s, just enough time to get them all into the net and then stunned. The fish with a long catch duration (n = 36 UF and 36 F trout) were crowded and released a total of six times over 20 min, before stunning. We chose a catch duration of 20 min since previous studies have shown that is enough to provoke a stress response in fish (Olsen, Sundell, Mayhew, Myklebust, & Ringø, 2005; Olsen et al., 2008).

After harvest, all fish were electrically stunned individually (head only, 90 W, 2 s), after which they were blood-sampled, weighed and measured to calculate the condition factor (CF = slaughter weight (g)/fork length $(cm)^3$).

2.2 | Blood and tissue sampling

Blood samples of 2 ml were taken from the caudal vein from 18 trout per treatment and were divided into two tubes, one with sodium fluoride (NaF) for the determination of glucose and lactate and another with ethylenediaminetetraacetate acid as an anticoagulant for cortisol, triglycerides and creatine phosphokinase enzyme (CPK). Both tubes were centrifuged at 1,200 *g* for 10 min to obtain plasma and immediately stored at 4°C until analysis on the same day.

After blood sampling, fish were killed immediately (<15 s) by sectioning the spinal cord at the base of the head. Fish were eviscerated, and gastrointestinal content was weighed to calculate the empty body weight (fish weight − gastrointestinal content) and % relative weight of the gastrointestinal contents (gastrointestinal content (g)/digestive tract weight (g) × 100). The digestive tract (from stomach to anus, including visceral fat) and liver were weighed to calculate digestive somatic index (DSI = digestive tract weight (g)/empty body weight (g) × 100) and the hepato-somatic index (HSI = liver weight (g)/empty body weight (g) × 100). The carcass yield was calculated as the ratio between slaughter weight and carcass weight (slaughter weight − viscera weight) and expressed as a percentage too. In addition, a sample of the liver and dorsal musculature (at the level of the dorsal fin) was taken from each of the 18 fish blood samples per treatment. Each sample was frozen immediately in liquid nitrogen to determine liver and muscle glycogen levels and flesh quality parameters, except pH and colour, which were determined in fresh liver and muscle.

2.3 | Assay procedures

In plasma, we determined cortisol, glucose, lactate, triglycerides and CPK concentration. Cortisol was measured using enzyme immunoassay using a commercial Cortisol EIA well kit (Radim Ibérica S.A.). Plasma concentrations of glucose and triglycerides were determined using reflectance spectrophotometry (Reflotron® System, Roche Diagnostics) based on the methods described by Trinder (1969) and Bucolo and David (1973) respectively. Lactate concentration was measured using enzymatic–spectrophotometric methods (Spinreact, S.A.). The CPK levels were measured using a Roche/Hitachi 717 Chemistry Analyzer (Roche Diagnostics S.L.) with Boehringer Mannheim reagents.

Liver and muscle samples were homogenized with perchloric acid to determine glycogen concentration as described by Dreiling, Brown, Casale, and Kelly (1987). Liver colour measurements were taken in fresh liver in triplicate, using a Minolta Spectrophotometer CM-2500c (Minolta). Three colour measurements were taken of the dorsal muscle on the right-hand fillet just behind the dorsal fin. The International Commission on Illumination (CIE) $L^*a^*b^*$ system (CIE, 1978) was chosen as the colour scale. Using a^* and b^* parameters, we calculated chroma ($C^* = \sqrt{(a^{*2} + b^{*2})}$) and hue ($h^* = \arctan(b^*/a^*) \times 57.29$).

The progression of *rigor mortis* (0, 5, 24 and 48 hr *post-mortem*) was measured following Cuttinger's method (Korhonen, Lanier, & Giesbrecht, 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 mortis* 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. Muscle pH was measured at the front end of the dorsal muscle after cutting it away from the head at 0 and 48 hr *post-mortem* using a pH meter that adjusts for temperature (HANNA, mod. HI9125). Lipid oxidation in muscle was measured at 0 hr *post-mortem*, quantifying the reactive substances with thiobarbituric acid (TBARS), and performed in duplicate in frozen muscle samples following the method described by Maraschiello, Sárraga, and García Regueiro (1999).

Muscle total fat content was determined using the ANKOM method (AOCS, 2005) and expressed as percentage in fresh muscle. Freeze-dried muscle was assessed in duplicate, using a solvent (heated diethyl ether) to extract lipids and using gravimetric loss to determine total fat content. Muscle fatty acid methyl esters (FAMEs) at 48 hr *post-mortem* were formed in duplicate according to Lee, Tweed, Kim, and Scollan (2012). The final organic layers with the FAMEs were transferred to a 2-ml vial, then capped and stored at −20°C until analysis. Chromatographic analysis of FAMEs was performed using a gas chromatograph (PerkinElmer, Autosystem-1:A) equipped with a split–splitless injector and a flame ionization detector, using a fused silica capillary column (0.32 mm internal diameter and 30 m long). The mobile phase consisted of helium C-50 at a flow of 9 psig. Fatty acids were identified using the standard FAME mixture (Sigma-Aldrich Co.). Chromatographic procedures are described in detail in Díaz et al. (2011). Results were expressed as a percentage of the total FAMEs.

2.4 | Statistical analyses

The data were analysed using SAS software ver. 9.0 (Statistical Analysis System Institute Inc.). A prior analysis of the normality

and homogeneity of variance of all variables was performed using the Shapiro–Wilk test with the UNIVARIATE procedure and Bartlett's test with the ANOVA procedure for residues. Data were analysed using a two-way ANOVA, with fasting and catch duration as fixed effects, including the interaction between both effects in the model. For biometric data, initial weight was introduced in the model as covariate. The Bonferroni test was used as a post hoc test ($p < .05$).

3 | RESULTS

3.1 | Morphometric measurements

As expected, most biometric parameters varied significantly with fasting, which was longer, but not with catch duration, which was quite short in comparison (Table 1). Slaughter weight did not differ statistically among treatments. Condition factor (1.11 ± 0.02 vs. 1.06 ± 0.01), DSI (10.1 ± 0.43 vs. 7.1 ± 0.18%), gastrointestinal content (46.1 ± 4.97 vs. 5.3 ± 0.63%) and HSI (1.54 ± 0.03 vs. 1.46 ± 0.04%) decreased with fasting, being higher in UF trout. Carcass yield was lower in UF trout (88.0 ± 0.46 vs. 91.1 ± 0.19%).

3.2 | Blood and plasma parameters

Blood and plasma parameters are presented in Figure 2. Plasma cortisol showed a significant interaction between fasting and catch duration ($p < .05$). *L* fish presented higher levels of cortisol regardless of fasting, but in *S* trout, cortisol was higher in UF compared with F trout. Glucose levels increased significantly with catch duration, with lower levels in *S* compared with *L* trout (75.1 ± 2.05 vs. 114 ± 5.13 mg/dl, $p < .001$). Fasting produced a significant decrease in lactate (6.8 ± 0.32 vs. 5.3 ± 0.32 mmol/L, $p < .001$), and lactate levels were lower in *S* trout than *L* trout (5.1 ± 0.25 vs. 7.0 ± 0.39 mmol/L, $p < .001$). Triglyceride levels in plasma were higher in UF than F trout (362 ± 39 vs. 177 ± 14.1 mg/dl, $p < .01$). Regarding CPK plasma levels, there was a significant interaction between fasting and catch duration ($p < .05$), with no differences with regard to fasting in *L* trout, but in *S* trout, UF presented a higher CPK plasma levels than F trout.

3.3 | Liver glycogen and colour

Liver-related parameters are shown in Table 2. Liver glycogen was significantly higher in UF than F trout (106.6 ± 12.3 vs. 39.2 ± 4.97 mg/g). Liver L^* and C^* showed a significant interaction between fasting and catch duration. In F fish, the L^* was higher in *L* than *S*, but not in UF fish. F trout presented a lower C^* in *S* than *L* fish, but there were no differences with regard to catch duration in UF trout, although they had a lower C^* than F fish. Liver h^* was significantly affected by fasting and catch duration, being higher in UF than F trout (48.3 ± 1.37 vs. 44.0 ± 1.62°) and lower in *S* than *L* fish (43.6 ± 1.77 vs. 48.7 ± 1.22°).

3.4 | Flesh quality parameters

Data on *rigor mortis* are presented in Figure 3. At 0 hr *post-mortem*, there was a significant interaction between fasting and catch duration in *rigor mortis* angle ($p < .01$). In F trout, *S* presented a higher angle than *L*, but no differences with regard to catch duration were found in UF fish. No significant differences were found at 5 hr *post-mortem*, with all groups presenting the maximum angle (90°). However, at 24 hr *post-mortem*, there was a significant interaction between both factors ($p < .05$), with no differences with regard to catch duration in F fish, but in UF trout, *rigor mortis* angle was lower in *S* than *L* fish. Finally, at 48 hr *post-mortem*, *rigor mortis* angle presented significant differences due to fasting, with lower angles in UF than F fish (69.7 ± 2.75 vs. 77.6 ± 1.36°, $p < .05$), and catch duration, being lower in *S* than *L* fish (70.9 ± 2.30 vs.

TABLE 1 Biometric parameters of adult rainbow trout subjected to 7 days of fasting (135.6 degree days) and a short or long catch duration

	UF		F		*p* value		
	S	*L*	*S*	*L*	*F*	*C*	*F* × *C*
Slaughter weight (g)	322 ± 6.95	356 ± 9.06	319 ± 7.27	319 ± 7.66	.21	.41	.37
Condition factor	1.11 ± 0.02	1.11 ± 0.02	1.06 ± 0.01	1.06 ± 0.01	.002	.86	.87
DSI (%)	9.81 ± 0.44	10.5 ± 0.42	7.35 ± 0.17	6.94 ± 0.20	<.001	.70	.11
GI (%)	44.1 ± 4.91	48.1 ± 5.03	6.56 ± 0.74	4.05 ± 0.53	<.001	.87	.41
HSI (%)	1.56 ± 0.04	1.52 ± 0.03	1.42 ± 0.05	1.50 ± 0.04	.04	.58	.11
Carcass yield (%)	88.3 ± 0.46	87.6 ± 0.45	90.9 ± 0.18	91.2 ± 0.21	<.001	.55	.17

Note: Values are mean ± *SEM*, $n = 36$ per treatment. Condition factor = fish weight (g)/fork length $(cm)^3$; DSI (digestive somatic index) = digestive tract weight (g)/empty body weight (g) × 100; GI (gastrointestinal content) = gastrointestinal content (g)/digestive tract weight (g) × 100; HSI (hepatosomatic index) = liver weight (g)/empty body weight (g) × 100. Carcass yield = carcass weight (g)/slaughter weight (g) × 100.

Abbreviations: *C*: catch duration; *F*: fasted; *L*: long catch duration; *S*: short catch duration; UF: unfasted.

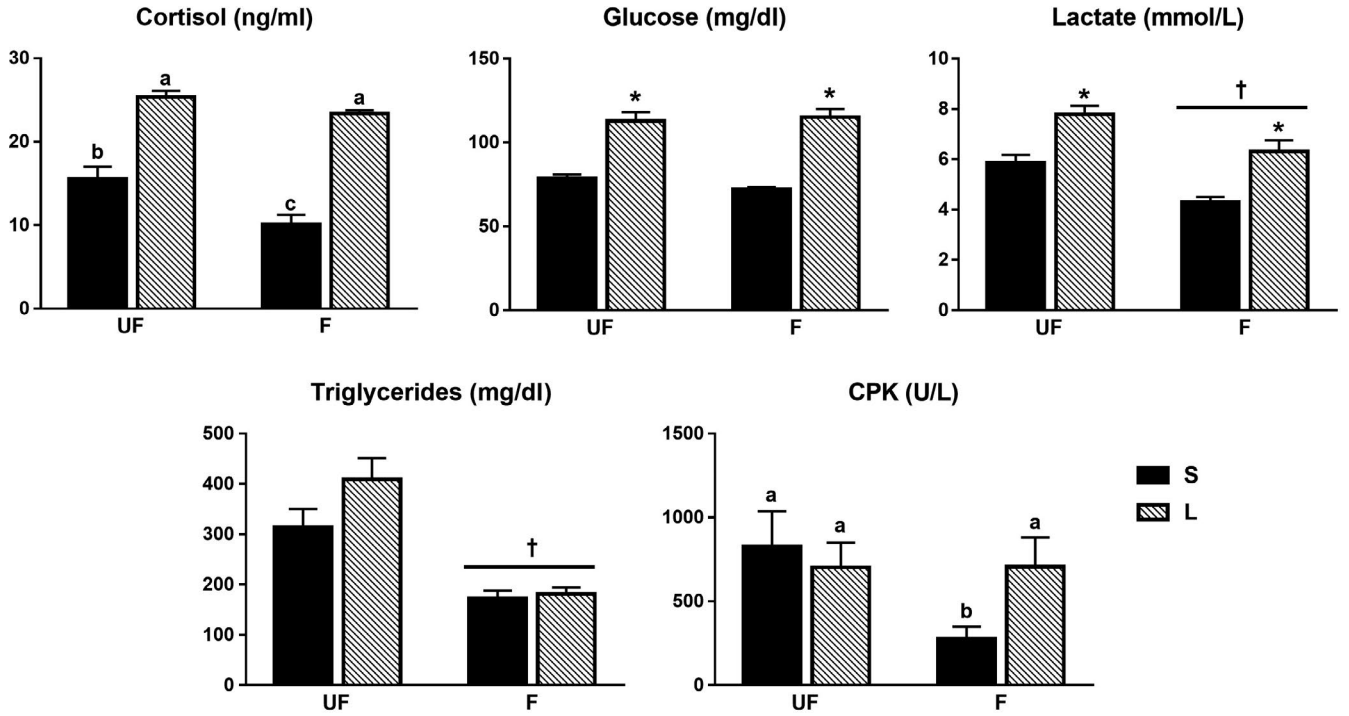

FIGURE 2 Biochemical parameters in blood and plasma of adult rainbow trout at different fasting times and catch duration (mean ± *SEM*, *n* = 18 per treatment). [a,b,c]Different letters indicate significant differences among groups ($p < .05$). †Significant differences due to fasting ($p < .05$). *Significant differences due to catch duration ($p < .05$). UF: unfasted; *F*: fasted; *S*: short catch duration; *L*: long catch duration

76.4 ± 1.81°, $p < .05$). Muscle pH at 0 hr *post-mortem* was significantly affected by catch duration ($p < .05$; Figure 4), being lower in *S* than *L* trout (6.62 ± 0.03 vs. 6.76 ± 0.02, $p < .05$). At 48 hr *post-mortem*, muscle pH showed a significant interaction between fasting and catch duration ($p < .05$), with no differences with regard to fasting in *S* fish, but in *L* trout, muscle pH was lower in UF than F trout.

Flesh quality parameters are presented in Table 3. Muscle glycogen concentration increased with fasting (1.6 ± 0.37 vs. 3.6 ± 0.83 mg/g). Regarding muscle colour parameters, L^* showed significant differences with catch duration, with higher values in *S* than *L* fish (47.4 ± 1.46 vs. 43.9 ± 0.93), while C^* was affected significantly by fasting, with higher values in UF than F trout (14.8 ± 0.88 vs. 11.7 ± 0.75). However, muscle h^* presented no significant differences by fasting or catch duration. TBARS were higher in UF than F fish (0.86 ± 0.21 vs. 0.52 ± 0.09 mg MDA/mg muscle).

3.5 | Muscle total fat content and fatty acid profiles

Muscle total fat content did not show significant differences by neither of the effects, with similar levels between all groups (Table 4).

The mean muscle fatty acid profiles at 48 hr *post-mortem* are presented in Table 4. The total percentage of saturated fatty acids was significantly affected by fasting, being lower in UF than F fish (18.5 ± 0.10 vs. 18.8 ± 0.12%). C16:0 and C20:0 presented significant differences due to fasting, with a lower percentage of C16:0 in UF than F fish (12.5 ± 0.08 vs. 12.9 ± 0.10%) and a

TABLE 2 Liver glycogen concentration and colour parameters of adult rainbow trout subjected to 7 days of fasting (135.6 degree days) and a short or long catch duration

	UF		*F*		*p* value		
	S	*L*	*S*	*L*	*F*	*C*	*F* × *C*
Liver glycogen (mg/g)	104 ± 13.5	111 ± 11.1	44.7 ± 5.42	32.4 ± 4.53	<.001	.78	.31
Liver L^*	38.9 ± 0.85[a]	37.8 ± 1.27[a]	33.4 ± 1.52[b]	39.5 ± 0.79[a]	.10	.04	.01
Liver C^*	16.2 ± 0.66[c]	15.0 ± 0.67[c]	17.4 ± 1.27[b]	21.1 ± 0.64[a]	<.001	.15	.01
Liver h^* (°)	45.4 ± 1.77	49.4 ± 1.84	35.7 ± 2.90	47.3 ± 1.48	.01	.001	.07

Note: Values are mean ± *SEM*, *n* = 12 per treatment.

Different superscripts letters within a row indicate significant differences among groups (fasting × catch duration, $p < .05$).

Abbreviations: *C*: catch duration; C^*: chroma; *F*: fasted; h^*: hue; *L*: long catch duration; L^*: lightness; *S*: short catch duration; UF: unfasted.

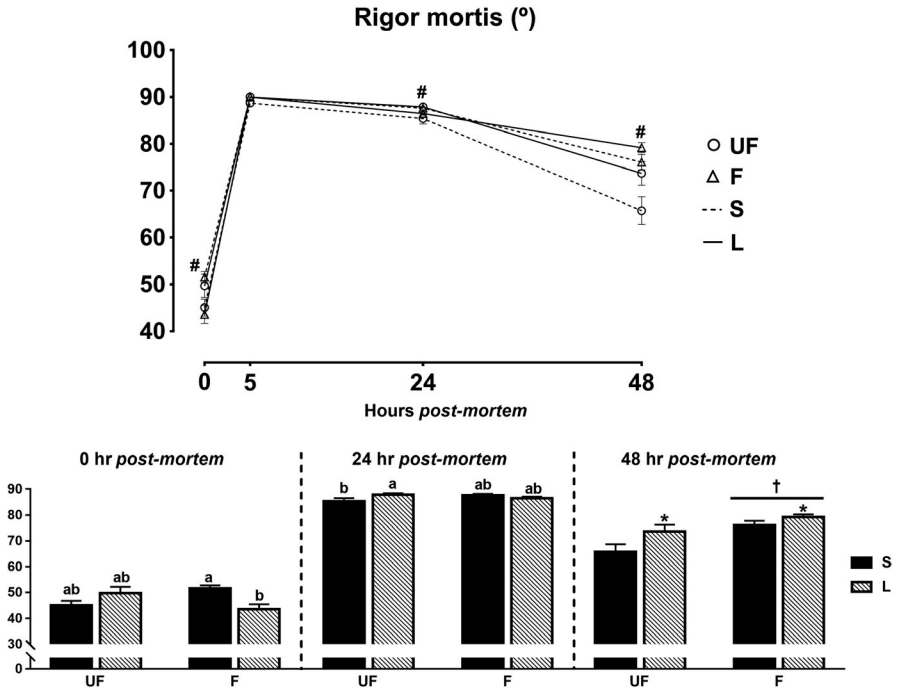

FIGURE 3 *Rigor mortis* angle evolution, from slaughter to 48 hr *post-mortem*, of adult rainbow trout subjected to 7 days of fasting (135.6 degree days) and a short or long catch duration. #Significant differences represented in the graphics below. [a,b]Different letters indicate significant differences among groups ($p < .05$) †Significant differences due to fasting ($p < .05$). *Significant differences due to catch duration ($p < .05$). UF: unfasted; *F*: fasted; *S*: short catch duration; *L*: long catch duration

higher percentage of C20:0 in UF than F fish (0.27 ± 0.003 vs. 0.25 ± 0.004%). Levels of C20:0 were also significantly affected by catch duration, being higher in *S* than *L* fish (0.27 ± 0.003 vs. 0.26 ± 0.004%). The percentage of C17:0 and C18:0 presented a significant interaction between fasting and catch duration, so, in F fish, *S* showed higher percentages than *L* fish, but not differences with regard to catch duration were presented in UF fish. Total percentage of monounsaturated fatty acids (MUFA) was higher in UF than F fish (46.0 ± 0.31 vs. 45.3 ± 0.33%). C18:1 n-9 showed significant differences in terms of fasting, being higher in UF than F fish (38.2 ± 0.26 vs. 36.7 ± 0.35%), and by catch duration, being higher in *S* than *L* fish (37.9 ± 0.29 vs. 37.0 ± 0.32%). Percentages of C16:1 n-9, C17:1, C18:1 n-7 and C22:1 n-9 presented a significant interaction between fasting and catch duration. In UF fish, C16:1 n-9 and C18:1 n-7 presented no differences with regard to catch duration, but in F trout, these fatty acids were lower in *S* than *L* fish. In F fish, percentages of C17:1 and C22:1 n-9 were higher in *S* than *L* fish, but UF fish presented no differences with regard to catch duration. With respect to PUFA, fasting significantly affected C18:2 n-6, with higher percentages in UF than F fish (14.0 ± 0.08 vs. 13.7 ± 0.11%), and C20:4 n-6, with lower levels in UF than F trout (0.76 ± 0.013 vs. 0.80 ± 0.016%). The percentage of C18:4 n-3 showed a significant interaction between both effects, with no differences with regard to catch duration in UF trout, but in F fish, a higher percentage was observed in *S* than *L* fish.

4 | DISCUSSION

4.1 | Morphometric measurements

Catch duration prior to slaughter had no effect on the biometric parameters, but pre-slaughter fasting affected them all, except for slaughter weight. Slaughter weight has been reported to decrease with starvation periods from 1 to 6 weeks (Bermejo-Poza et al., 2017; Pottinger et al., 2003; Sumpter, Le Bail, Pickering, Pottinger, & Carragher, 1991). However, in our study, a pre-slaughter fast of 7 days (135.6°C d) was not enough to decrease slaughter weight, possibly due

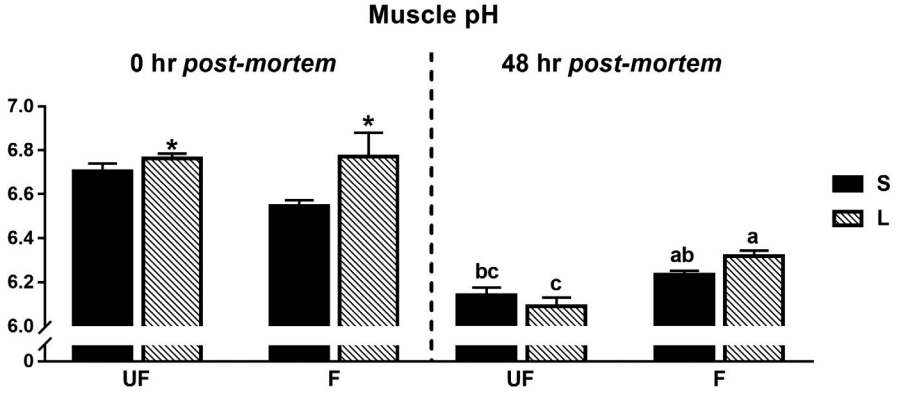

FIGURE 4 Muscle pH at 0 and 48 hr *post-mortem* of adult rainbow trout subjected to seven days of fasting (135.6 degree days) and a short or long catch duration. [a,b,c]Different letters indicate significant differences among groups ($p < .05$) *Significant differences due to catch duration ($p < .05$). UF: unfasted; *F*: fasted; *S*: short catch duration; *L*: long catch duration

TABLE 3 Flesh quality parameters of adult rainbow trout subjected to 7 days of fasting (135.6 degree days) and a short or long catch duration

	UF		F		p value		
	S	L	S	L	F	C	F × C
Muscle glycogen (mg/g)	1.76 ± 0.44	1.45 ± 0.31	4.43 ± 0.98	2.80 ± 0.69	.01	.15	.32
Muscle L^*	46.0 ± 1.24	43.6 ± 1.01	48.7 ± 1.68	44.1 ± 0.85	.20	.01	.37
Muscle C^*	15.3 ± 0.90	14.3 ± 0.86	11.0 ± 0.92	12.3 ± 0.59	.001	.86	.15
Muscle h^* (°)	67.8 ± 2.04	69.0 ± 1.96	73.1 ± 3.22	65.0 ± 2.29	.80	.16	.06
TBARS (mg MDA/mg muscle)	0.79 ± 0.22	0.93 ± 0.20	0.45 ± 0.08	0.59 ± 0.11	.04	.38	.99

Note: Values are mean ± *SEM*, n = 36 per treatment (TBARS), 24 per treatment (muscle total fat content) or 12 per treatment (muscle glycogen, muscle colour parameters).

Abbreviations: *C*: catch duration; C^*: chroma; *F*: fasted; h^*: hue; *L*: long catch duration; L^*: lightness; *S*: short catch duration; UF: unfasted.

to an adjustment of fish metabolic rate in response to food availability (Lines & Spence, 2012), which maintained body mass. Condition factor is a more reliable indicator than slaughter weight when it comes to assessing fish growth, since it also takes into account the body length of the fish (Chatzifotis, Papadaki, Despoti, Roufidou, & Antonopoulou, 2011), which could explain significant differences in this coefficient, as a 7-day fast (135.6°C d) is sufficient to reduce this parameter in rainbow trout (Pottinger et al., 2003; Sumpter et al., 1991).

Both DSI and gastrointestinal content decreased with fasting, and consequently, carcass yield increased. In our study, a fasting time of 135.6°C d was enough to reduce the gastrointestinal content considerably, supporting the theory that gastrointestinal emptying is a highly temperature-dependent process in fish and a fasting period of 1–5 days is required to produce a nearly complete emptying (Bermejo-Poza et al., 2017; Lines & Spence, 2012; López-Luna et al., 2013). HSI also decreased with fasting, possibly due to the use of glycogen reserves, mainly, and other metabolites such as lipids or proteins to maintain homeostasis (Davis & Gaylord, 2011; Torfi Mozanzadeh et al., 2017). Together, these findings support our original idea that 7 day of fasting was enough to bring about metabolic changes associated with feed deprivation, and thus, the fish were under fasting stress.

4.2 | Blood and plasma parameters

In general, plasma cortisol values in this study were in the range of basal values described in rainbow trout by other authors (5–20 ng/ml; Pottinger et al., 2003), except for the long catch duration, where they increased. An increase in cortisol after physical activity has been described by other authors, since it triggers a stress response (Merkin et al., 2010; Poli, Parisi, Scappini, & Zampacavallo, 2005). There were no significant differences in cortisol response after a long catch duration between fasted and unfasted fish, which suggests that the catch duration is more of an acute stressor than fasting. On the other hand, fasting fish had a lower increase in cortisol than unfasted fish after the short catch duration, which implies that their basal levels were lower, in accordance with their metabolic state. Data about the effect of fasting on cortisol plasma levels in rainbow trout are diverse (Bermejo-Poza et al., 2018), with studies reporting no effect (Holloway, Reddy, Sheridan, & Leatherland, 1994; Reddy, Vijayan, Leatherland, & Moon, 1995), increased levels (Sumpter et al., 1991) or, as in our study, decreased plasma levels (Farbridge & Leatherland, 1992). That may be because the fasted fish had already adapted to the lack of food and reduced their metabolic rate (Hultmann et al., 2012; Lines & Spence, 2012; Olsen et al., 2008). Another reason for the decrease in plasma cortisol could be a depletion of the hypothalamic–pituitary–interrenal axis after 7 days of fasting (Bermejo-Poza et al., 2017). In any case, plasma cortisol was quite variable among individuals and values should be considered together with other indicators (Ellis et al., 2012).

A long catch duration increased plasma glucose levels above basal levels described in rainbow trout (70–90 mg/dl; Jentoft et al., 2005). López-Patiño et al. (2014) observed a similar increase after an acute stressful stimulus related to management operations, which they suggest is due to an increase in gluconeogenesis to supply energy to the organism and maintain homeostasis. In our study, higher levels of plasma cortisol in long catch duration fish could trigger an increase in gluconeogenesis and glycogenolysis, thereby increasing blood glucose (Milligan, 2003). Fasting had no effect on glucose levels, possibly since trout were able to keep glucose concentrations stable via the use of glycogen reserves (Caruso et al., 2011) and the lipid reserves stored in the liver. Plasma lactate levels were increased by a long catch duration, which coincides with Merkin et al. (2010), who observed that intense exercise increases anaerobic metabolism in the white muscle at the level of fast muscle glycolytic fibres. Other authors have also observed that an increase in physical activity prior to slaughter of about 15 min increases plasma glucose and lactate levels (Olsen et al., 2005, 2008). Although lactate is more associated with an acute stressful stimulus such as catching, it can also be affected by fasting. Lactate levels were lower in fasted trout than unfasted ones, possibly due to its utilization as a substrate for hepatic gluconeogenesis (Liew et al., 2012).

Plasma triglycerides decreased with fasting, which indicates that the fat reserves were used by fish to cope with the lack of food, as described in other studies (Costas et al., 2011; Millán-Cubillo,

TABLE 4 Total fat content and fatty acid profiles of muscle at 48 hr *post-mortem* expressed as percentage of total fatty acids of adult rainbow trout subjected to 7 days of fasting (135.6 degree days) and a short or long catch duration

	UF		*F*		*p* value		
	S	*L*	*S*	*L*	*F*	*C*	*F* × *C*
Total fat content (%)	1.27 ± 0.02	1.28 ± 0.04	1.26 ± 0.03	1.27 ± 0.03	.79	.84	.88
SFA	18.5 ± 0.12	18.4 ± 0.12	18.9 ± 0.12	18.7 ± 0.12	.009	.44	.65
C14:0	2.09 ± 0.02	2.07 ± 0.02	2.09 ± 0.02	2.13 ± 0.02	.18	.57	.14
C15:0	0.219 ± 0.01	0.219 ± 0.01	0.220 ± 0.01	0.227 ± 0.01	.07	.13	.12
C16:0	12.5 ± 0.10	12.5 ± 0.10	12.8 ± 0.10	13.1 ± 0.10	<.001	.12	.24
C17:0	0.219 ± 0.01^{a}	0.217 ± 0.01^{a}	0.223 ± 0.01^{a}	0.175 ± 0.01^{b}	.04	.009	.001
C18:0	3.23 ± 0.05^{a}	3.18 ± 0.05^{a}	3.28 ± 0.05^{a}	2.88 ± 0.05^{b}	.02	<.001	.001
C20:0	0.270 ± 0.01	0.265 ± 0.01	0.263 ± 0.01	0.247 ± 0.01	.003	.02	.16
MUFA	46.1 ± 0.32	45.9 ± 0.32	45.3 ± 0.32	45.3 ± 0.32	.03	.85	.79
C16:1 *n*-9	0.283 ± 0.01^{b}	0.290 ± 0.01^{b}	0.309 ± 0.01^{b}	0.438 ± 0.01^{a}	<.001	.001	.001
C16:1 *n*-7	2.97 ± 0.04	2.95 ± 0.04	2.91 ± 0.04	2.94 ± 0.04	.41	.77	.52
C17:1	0.227 ± 0.01^{a}	0.226 ± 0.01^{a}	0.228 ± 0.01^{a}	0.197 ± 0.01^{b}	.009	.004	.007
C18:1 *n*-9	38.3 ± 0.31	38.1 ± 0.31	37.4 ± 0.31	36.0 ± 0.31	<.001	.01	.09
C18:1 *n*-7	2.14 ± 0.14^{b}	2.33 ± 0.14^{b}	2.40 ± 0.14^{b}	3.73 ± 0.14^{a}	<.001	<.001	.002
C20:1	1.70 ± 0.02	1.66 ± 0.02	1.66 ± 0.02	1.63 ± 0.02	.20	.22	.95
C22:1 *n*-9	0.391 ± 0.01^{a}	0.384 ± 0.01^{a}	0.380 ± 0.01^{a}	0.322 ± 0.01^{b}	<.001	.003	.003
PUFA	35.4 ± 0.33	35.6 ± 0.33	35.9 ± 0.33	36.0 ± 0.33	.25	.65	.92
C18:2 *n*-6	14.0 ± 0.10	14.0 ± 0.10	13.7 ± 0.10	13.8 ± 0.10	.02	.64	.72
C18:3 *n*-6	0.184 ± 0.03	0.179 ± 0.03	0.146 ± 0.03	0.197 ± 0.03	.70	.37	.29
C18:3 *n*-3	3.73 ± 0.04	3.69 ± 0.04	3.68 ± 0.04	3.68 ± 0.04	.42	.52	.65
C18:4 *n*-3	0.922 ± 0.02^{a}	0.958 ± 0.02^{a}	0.919 ± 0.02^{a}	0.829 ± 0.02^{b}	.005	.24	.007
C20:2 *n*-6	0.573 ± 0.01	0.552 ± 0.01	0.561 ± 0.01	0.534 ± 0.01	.30	.09	.85
C20:3 *n*-6	0.349 ± 0.01	0.333 ± 0.01	0.329 ± 0.01	0.312 ± 0.01	.12	.21	.93
C20:4 *n*-6	0.755 ± 0.01	0.772 ± 0.01	0.806 ± 0.01	0.792 ± 0.01	.02	.93	.29
C20:3 *n*-3	0.294 ± 0.03	0.283 ± 0.03	0.310 ± 0.03	0.366 ± 0.01	.12	.47	.29
C20:5 *n*-3	3.66 ± 0.07	3.74 ± 0.07	3.78 ± 0.07	3.66 ± 0.07	.76	.74	.15
C22:4 *n*-6	0.144 ± 0.02	0.148 ± 0.02	0.171 ± 0.02	0.185 ± 0.02	.08	.63	.77
C22:5 *n*-3	1.20 ± 0.03	1.21 ± 0.03	1.25 ± 0.03	1.24 ± 0.03	.24	.93	.79
C22:6 *n*-3	9.66 ± 0.30	9.78 ± 0.30	10.2 ± 0.30	10.4 ± 0.30	.06	.59	.88

Note: Values are mean ± *SEM*, *n* = 12 per treatment.

Different superscript letters within a row indicate significant differences among groups (fasting × catch duration, $p < .05$).

Abbreviations: *C*: catch duration; *F*: fasted; *L*: long catch duration; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; *S*: short catch duration; SFA: total saturated fatty acids; UF: unfasted.

Martos-Sitcha, Ruiz-Jarabo, Cárdenas, & Mancera, 2016). However, the long catch duration did not decrease plasma triglycerides, which coincides with other species such as carp (*Cyprinus carpio*), where plasma triglycerides do not vary after an acute stress-type stimulus (Li, Fu, Wang, Zhu, & Hu, 2011). Regarding CPK enzyme levels, they were lower in fasted fish after a short catch duration, possibly due to their lower metabolic demands (Evans & Watterson, 2009). However, CPK levels were similar in all fish after a long catch duration, since physical exertion may have been intense enough to release it from cytoplasm of muscle cells to plasma (Peres, Santos, & Oliva-Teles, 2013).

4.3 | Liver glycogen and colour

Fasting produces a decrease in liver glycogen levels when used to replace the absence of dietary carbohydrate intake (Dias Junior et al., 2016). It has been reported to decrease in rainbow trout after 5 day of fasting (Furné et al., 2012), which agrees with our results. However, we did not find any effect of catch duration on liver glycogen, which agrees with other authors such as Vijayan, Pereira, Forsyth, Kennedy, and Iwama (1997), who submitted rainbow trout to netting and chasing before slaughter and did not find a decrease in liver glycogen concentration. In other species, liver glycogen decreases after a

few minutes of stressful stimulus, as in yellow perch (*Perca flavescens*; Schwalme & MacKay, 1991) or sea bass (*Dicentrarchus labrax*; Reubush & Heath, 1996), but rainbow trout is characterized by low utilization of carbohydrates (Panserat, Médale, Breque, Plagnes-Juan, & Kaushik, 2000), so we would expect little effect.

Rainbow trout, such as most ectotherms, follow a capital breeder reproductive strategy, with high lipid reserves in the liver (Stephens, Houston, Harding, Boyd, & McNamara, 2014), which plays an important role in maintaining homeostasis during fasting (Bermejo-Poza et al., 2018). That helps explain why we observed a darker liver colour in fasted trout (lower h^* and higher C^*), possibly due to a decrease in liver lipid concentration, as described in other species such as broiler chickens (Trampel, Sell, Ahn, & Sebranek, 2005). This hypothesis is supported by the lower levels of plasma triglycerides in fasted trout, which could indicate depletion of liver lipid reserves. However, the long catch duration produced an opposite effect, with a brighter liver (higher L^* and C^*) and with a higher hue. Merkin et al. (2010) observed that an increase in physical activity produces a stress response in fish that increases its metabolic activity (lower partial pressure of O_2 and higher partial pressure of CO_2), which reduces the blood supply to the liver, which could be related to this loss of tonality.

4.4 | Flesh quality parameters

The onset of *rigor mortis* in rainbow trout flesh occurs between 2 and 9 hr *post-mortem* (Bermejo-Poza et al., 2015; López-Luna et al., 2014). The onset of *rigor mortis* was at 5 hr *post-mortem* in all treatments, but resolution at 48 hr *post-mortem* was slower in fasted and long catch duration fish compared with unfasted and short catch duration. That could be because fasted fish had a lower condition factor, which may delay the resolution of *rigor mortis* (Gokoglu & Yerlikaya, 2015).

Muscle pH at 0 hr *post-mortem* was higher after the long catch. The increase in physical activity during fish harvest promotes anaerobic glycogenolysis, which decreases muscle pH (Poli et al., 2005). However, the long catch duration did not cause the glycogen levels in muscle to decrease; therefore, there may have not been enough conversion of glycogen to lactic acid to lower the pH (Bermejo-Poza et al., 2015). Alternatively, lactic acid has already been released into plasma, as indicated by higher plasma lactate in *L* trout. It is widely known that there is a relationship between muscle pH and glycogen values (Warriss, 2010), but in our study, muscle glycogen content did not correlate with decreasing muscle pH. Van Laack, Kauffman, and Greaser (2001) analysed the role of glycogen levels in determining the pH of meat and showed that only 37% of the variation in ultimate pH can be accounted for by glycogen concentration, so other factors may be responsible of pH variation. Muscle glycogen was higher at 0 hr *post-mortem* in fasted fish than unfasted ones, so, to cope with fasting, fish used liver glycogen reserves as the first option instead of muscle glycogen, whose decrease is related more to muscle activity (Navarro & Gutierrez, 1995). The increase in these reserves with fasting could be due to the lower concentrations of plasma cortisol, which would not provoke the breakdown of muscle glycogen (Milligan, 2003).

Fasting produced a decrease in muscle colour saturation, as has been described in other studies in salmonids subjected to food deprivation periods (Álvarez, García, Garrido, & Hernández, 2008). These changes in saturation could be related to changes in the muscle pH that produces protein denaturation in the muscle fibres, changing their light refractive properties (Swatland, 2003). According to Kamireddy et al. (2011), fasted trout have less reactive substances to thiobarbituric acid (TBARS) as a product of the oxidation of lipids present in the muscle. Those authors observed a decrease in TBARS after 3 days of fasting, likely due to activation of energy reserves in muscle tissue, which may decrease the effects of lipid peroxidation.

4.5 | Muscle total fat content and fatty acid profile

The total muscle fat content was similar in all groups, indicating similar body condition in general, but we found significant differences in fatty acid profiles in relation to both pre-slaughter fasting and catch duration. The major muscle fatty acids were MUFA and PUFA, due to the capacity of freshwater fish, such as rainbow trout, to endogenously desaturate SFA to MUFA (Tocher, 2003). The most common were C16:0 and C18:1 n-9, which are the main sources of energy for rainbow trout (Suárez et al., 2014), followed by the polyunsaturated fatty acids C18:2 n-6 and C22:6 n-3, which coincides with muscle fatty acid profiles described by other authors in rainbow trout (Suárez et al., 2014; Taşbozan, Gökçe, & Erbaş, 2016).

At 48 hr *post-mortem*, an increase in total percentage of SFA by fasting was observed. Jezierska, Hazel, and Gerking (1982) observed an increase in SFA after 27 days of fasting in rainbow trout muscle, caused by a decrease in MUFA, while the content of PUFA remained constant, which is consistent with our results. Specifically, the percentage of C16:0 was higher in fasted trout, which agrees with Einen, Waagan, and Thomassen (1998) in fasted Atlantic salmon, which could indicate a reduction in Δ9-desaturation upon fasting. The C18:0 was lower in fasted and long catch duration fish than the rest of the groups. Sargent, McEvoy, and Bell (1997) observed that SFA are generally considered as substrates for energy production, in the same way as Penney and Moffitt (2015), who studied the fatty acid profiles in rainbow trout during migrations and observed that they selectively catabolize SFA and MUFA via mitochondrial β-oxidation. As for MUFA, C18:1 n-9 was lower in the muscle of fasted fish at 48 hr *post-mortem*. Similarly, Sidell, Crockett, and Driedzic (1995) observed, through substrate competition experiments, that tissues catabolize MUFA first and are the preferred source for energy metabolism. Tocher (2003) indicates that long-chain MUFA can easily be oxidized in stressful situations, especially C18:1 n-9, which decreased in the present trial after a long catch duration, as did C17:1 and C22:1 n-9, which were lower in fasted and long catch duration fish.

In salmonids subjected to fasting, the oxidation of SFA and MUFA is more common than PUFA. The β-oxidation of SFA and MUFA is carried out by mitochondria to obtain energy for cell metabolism,

while it is more complicated for PUFA. In general, in the present study PUFA were conserved when the animals were stressed, being less affected by fasting and catch duration than SFA or MUFA. PUFA concentrations could be preserved due to their important functions in biological membranes, namely providing fluidity to the membranes. Kiessling, Pickova, Eales, Dosanjh, and Higgs (2005), who compared muscle fatty acid profile between feed-restricted salmon and others fed the maximum ration and subjected to exercise, only obtained significant results in fish fed 75% of the daily ration, which suggests that an increase in energy demand is not enough to evoke changes in fatty acid profiles, but must be accompanied by a restriction in the diet. Zengin, Vural, and Çelik (2013) observed how C22:6 n-3 was conserved, since numerous physiological functions are attributed to it, being incorporated in the retina or the brain.

5 | CONCLUSIONS

Subjecting trout to a fasting time of 7 days or 135.6°C d produced a decrease in the majority of biometric parameters, plasma triglyceride concentration and liver glycogen, suggesting a clear mobilization of reserves to maintain fish homeostasis. In addition, *rigor mortis* was slower to resolve and final muscle pH was higher at 48 hr *post-mortem* in fasted trout with a long catch period. Feed withdrawal tended to decrease MUFA and increase SFA in muscle, with less effects on PUFA. The combined effect of fasting and a long catch duration increased plasma cortisol, glucose and lactate levels, suggesting a negative effect on animal welfare, and decreased muscle luminosity, suggesting decreased product quality. Those trout also had a brighter liver colour and with a higher saturation, which could be used a post-mortem indicator of animal welfare. Catch duration alone did not appear to evoke changes in the composition of fatty acids in muscle.

Regardless of the pre-slaughter fasting period, a long catch duration produces an important stress response in the fish during the pre-slaughter period, so it is essential to minimize the time in which this practice is carried out. So, in view of our results, since these effects are enlarged with pre-slaughter fasting, common practice in aquaculture, it seems indispensable to reduce the time devoted to fish harvest in the case of rainbow trout.

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