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Effects of weekend starvation and the duration of daily feeding on production parameters of rainbow trout *Oncorhynchus mykiss*

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ABSTRACT

It would often be practical to starve the cultivated fishes over the weekends, e.g. to save in labour costs. We evaluated the possibility that domesticated juvenile rainbow trout (*Oncorhynchus mykiss*) could compensate for the lost growth of the 2-day weekend starvation by either hyperphagic response and/or by lower feed conversion ratio, compared to the fish fed every day in an 8-week experiment. Rainbow trout (initial weight c. 30 g, temperature 16 °C) starved during the weekends were able to increase feed intake during the weekdays clearly above the intake of the control fish after the first two weekends, also seen as an increase of the compensation coefficients over the last four weeks of the experiment. However, the control fish had significantly greater total absolute feed intake than the fish in the treatment group inducing significantly higher specific growth rate and final weight (176.7 ± 4.7 g) compared to the treatment group (153.1 ± 8.4 g) at the end of the experiment. The coefficient of variation of weight did not differ between the two groups, but the feed conversion ratio was significantly higher in the 2-day starving group (0.93 ± 0.03) than in the control group (0.88 ± 0.01). Growth compensation was only partial, and our data indicated clear differences in the capacity for compensation between the tanks. The tank-wise compensation capacity appears to be rather persistent, seen as a significant positive correlation in the compensation coefficients between the first and last four weeks of the experiment. Body moisture and hepatosomatic index were significantly higher in the 2-day starving groups than in the control group, but there were no treatment differences in stomach capacity (weight or volume), liver weight or in the relative amount of visceral fat. The lack of the increase in stomach capacity in the treatment group fish was hypothesized to be a consequence of selective breeding for several decades and being an unnecessary trait in farmed fishes supplied feed constantly. We conducted also a 3-week follow-up experiment to test the effects of feeding frequency (continuous feeding vs. feeding twice per day) on growth responses in rainbow trout experiencing the weekend starvation, and these results confirmed that the lack of full growth compensation in the first experiment was not due to feeding frequency. Our results suggest that weekend starvation cannot be recommended for domesticated rainbow trout without negatively affecting their growth.

1. Introduction

Compensatory growth (CG) is a phenomenon known as unusually rapid growth supported by hyperphagia and in some cases also by improved feed utilization, following a period of restricted food availability or adverse environmental conditions (Ali et al., 2003). CG studies have been done on a variety of fish species and other animals, and the growth responses to feed deprivation can be classified into non-existing, partial, full or overcompensation in respect to the growth of the control group animals (Jobling, 1993). Despite ample research, this phenomenon is not commonly utilized in commercial fish farming.

From the fish farmer's and researcher's point of view alike it would often be practical if feed withdrawal during the weekends was an option without affecting fish growth negatively. Starvation of fish during the weekends would bring savings in labour costs, and a 2-day starvation would also allow researchers to monitor the animals from different treatments in a similar condition in respect to their stomach fullness if the sampling day would be Monday (Salgado-Ismodes et al., 2020).

Abbreviations: CC, compensation coefficient; CG, compensatory growth; CV, coefficient of variation; CF, condition factor; FCR, feed conversion ratio; SGR, specific growth rate; HSI, hepatosomatic index; VFSI, visceral fat somatic index; TED, twice every day; TEWD, twice every weekday; CEWD, continuously every weekday.

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Despite the fact that feed withdrawal decreases biomass gain, the growing animals can be capable of compensating for the missed growth by increasing intake on the days following starvation.

Surprisingly few CG studies have investigated the effects of 2-day (i.e. weekend) starvation followed by 5-day feeding. Nikki et al. (2004) found in individually grown juvenile rainbow trout (*Oncorhynchus mykiss*) that when the fish were starved for two days, the consequent hyperphagia continued typically for five days, and the starved trout were even slightly bigger than the control fish at the end of the 80-day trial, but there was no indication of improved feed utilization. More recently, Taşbozan et al. (2016) found in a 10-week experiment that juvenile rainbow trout starved for two days and fed for five days grew significantly bigger than the control fish fed every day. In that experiment, daily feed intake in the 2-day starving group was slightly lower than in the controls, suggesting improved feed utilization (was not reported). The results with non-salmonid fish are contradictory, e.g. the growth of fingerlings of *Piaractus brachypomus* (initial weight 2.4 g) was negatively affected by weekend starvation as compared to the fish starved 0 or 1 days per week, but feed conversion ratio and protein efficiency ratio were unaffected (Favero et al., 2021). Yengkokpam et al. (2013) did an experiment with *Labeo rohita* fingerlings, and one of their treatments was starvation for two consecutive days per week; however they evaluated only the effects of starvation on metabolic, digestive and anti-oxidative enzyme activities and they did not report possible effects on production parameters. Their conclusion was that longer periods of starvation than two days per week had a negative effect on L. *rohita* fingerlings (Yengkokpam et al., 2013).

The standard feeding protocol of rainbow trout farming is to feed the fish every day, except during the times of unfavourable environmental conditions (e.g. extreme temperature or low oxygen level). The present study hypothesizes that if domesticated juvenile rainbow trout are fasted during the weekends, they would compensate for the lost growth by hyperphagia and lower feed conversion ratio during the weekdays when compared to the fish fed every day of the week. We also made a follow-up experiment to test the effects of daily feeding duration on CG response in fish starved for two days.

2. Materials and methods

2.1. Fish and experimental conditions

Two experiments were carried out in a laboratory scale Recirculating Aquaculture System (RAS) at the Department of Biological and Environmental Science, University of Jyväskylä, Finland. The first experiment was done between 21 January and 18 March 2019, 57 days in total, and the second one between 08 April and 28 April 2019, 21 days in total. In growth experiments in general, the very minimum requirement is that weight of the control fish should double, but preferably quadruple, during the experiment. The second experiment, which was a follow-up experiment based on the result of the first experiment, slightly exceeded the minimum requirement while in the first experiment control fish weight increase was almost six-fold. The RAS consisted of twelve, 185 L circular fish tanks, swirl separator, drum filter, moving bed biofilter, aerator tower and a sump tank. The total volume of the system was *c.* 4.5 m^3, and the amount of daily incoming water was *c.* 0.6 m^3. The water originated from a bore-whole, and it was passed through a water softener and a limestone column before use. Average water temperature in the tanks was 16.0 °C (min 15.5 °C, max 16.5 °C, controlled by Titan Professional 6000, Bissendorf, Germany), pH varied between 6 and 7, and oxygen between 8 and10 mg L^{-1}. KOH was used for buffering, and each tank was aerated through an airstone. The concentration of ammonium-nitrogen (NH_4-N; varied between 0.061 and 0.287 mg L^{-1}), nitrite-nitrogen (NO_2-N; 0.015–0.271 mg L^{-1}) and nitrate-nitrogen (NO_3-N; 5.9–17.2 mg L^{-1}) were determined weekly with a mobile laboratory spectrophotometer (LASA 100, Dr. Lange, Germany) using testing kits LCK304, LCK341 and LCK339 (Hach, Colorado, USA) for ammonium, nitrite and nitrate, respectively. Photoperiod was set at 16 h light and 8 h dark.

Fish were fed in excess with Circuit RED 2.5 dry feed (composition 48% protein, 17% fat, 3.2% fiber and 5.2% ash according to the manufacturer Raisioaqua Ltd., Finland) with belt feeders. A sieve under the outlet of each tank collected uneaten pellets and these were removed and counted in the morning and in the afternoon. The number of uneaten pellets was converted to weight of dry feed knowing that 31 dry pellets equals to 1.00 g. The amount of daily feed intake was calculated as the difference between the fed and uneaten feed. The feeding was adjusted daily for each tank to match the growth and appetite (based on the amount of uneaten feed) of the fish.

In the first experiment 200 all-female rainbow trout (mean initial weight *c.* 30 g) were divided into each of the 8 experimental tanks, 25 fish in each tank. Four of the tanks were fed every day and four tanks on weekdays only, feeding time was 9:00–24:00.

In the second experiment of 108 all-female rainbow trout (mean initial weight *c.* 49 g) were divided into each of the 9 experimental tanks, 12 fish in each tank. These fish originated from a different population than the fish in the first experiment. Three of the tanks were fed twice every day (TED; 09:00–10:00 and 15:00–16:00), three tanks were fed twice every weekday (TEWD) and three tanks were fed continuously (9:00–24:00) every weekday (CEWD).

2.2. Experimental procedures and measurements

Fish were weighed (to 0.1 g) individually at the beginning of the experiment and then biweekly (not in the second experiment) and total length was measured (to 0.1 cm) at the start and the end of the experiment. Measurements were done under anesthesia (clove oil:ethanol mixture 1:10; clove oil concentration 40 mg L^{-1}). In the first experiment, 10 extra fish (these were not assigned into any tank) were sampled in the beginning, and at the end of the experiment five fish were sampled from each tank. These sampled fish were dissected and eviscerated, their liver and visceral fat were separated and weighed (to 0.01 g). Visceral fat was separated by pulling it out with fingers from around the gastrointestinal tract, including the pyloric caeca, but due to visceral fat being relatively loose and sticky, 100% removal was likely not achieved. Stomach volume was measured by tying a string around the pyloric sphincter and the esophagus was tied to a 50 cm burette. Stomach volume was estimated as the volume of water required to dilate the stomach under a pressure head of 50 cm (Jobling et al., 1977). After the volume measurement, the stomach was separated from the intestine, tapped dry and weighed (to 0.01 g). The whole carcass was dried in an oven at 85 °C for three days to determine dry weight.

2.3. Calculations and statistical analyses

Specific growth rate (SGR) was calculated as $100(\ln W_2 - \ln W_1) * t^{-1}$, where W_1 and W_2 were fish weights (g) in the beginning and end of the experiment, respectively, and t was the length (d) of the experiment. Feed conversion ratio (FCR) was calculated as feed intake (g) * biomass $gain^{-1}$, and condition factor (CF) as $100\ W * L^{-3}$, where W was fish weight (g) and L total length (cm). Relative feed intake was calculated as total feed intake (g) * number of feeding $days^{-1}$ * W^{-1} *100 where W was the average weight ((initial weight + final weight)/2) of the fish. Body moisture (%) was calculated as $100(W - dryW) * W^{-1}$, hepatosomatic index (HSI) as 100(liver weight * W^{-1}), visceral fat somatic index (VFSI) as 100(visceral fat weight * W^{-1}). Compensation coefficient (CC) was calculated as $\Delta T * \Delta C^{-1}$, where ΔT is the weight gain or feed intake (g) in each treatment group tank divided by the number of feeding days and ΔC is the average weight gain or intake (g) in the control group tanks divided by the number of feeding days (Mattila et al., 2009). CC > 1 indicates compensation. CC was calculated for the first and last 4 weeks of the first experiment separately. Coefficient of variation (CV) of weight was calculated for each tank as 100(S.D. *

avarage weight^{-1}).

The data are presented as mean values ± S.D., using tank average values as an observational unit, i.e. n was 4 and 3 in the first and second experiment, respectively. SPSS 21 for Windows (IBM SPSS Software, USA) was used for analyzing the data. Possible differences between means were analysed using Student's *t*-test in the first experiment and ANOVA in the second experiment, and when needed, *post-hoc* comparisons were done by Tukey's test. One-sample *t*-test was used to test the possible difference of CC from 1.0. Linear regression analysis was used for analyzing tankwise relationship of CC-values between growth and feed intake, and CC and CV of weight. In the analyses $P < 0.05$ was used as the level of significance, and when $0.10 > P > 0.05$, the slope of the regression was regarded as indicatively significant (Robinson et al., 2006).

3. Results

3.1. First experiment

There was no mortality during the experiment. After the first two weeks daily feed intake of the weekend starving group was at a similar level than in the control group but increased thereafter clearly (Fig. 1). However, when calculated for the whole experiment, the control group fish ate significantly more than fish in the treatment group but the relative feed intake was significantly higher in the treatment group than in the controls (Table 1). The higher absolute feed intake in the control group induced significantly higher SGR and final weight and length than in the treatment group, and also CF in the control group was significantly higher (Table 1). Starvation during the weekends did not affect significantly size variability (CV of weight) but it increased feed conversion ratio by 0.05 units, causing a significant difference between the treatments (Table 1). Compensation coefficients (CC) for growth of the first and last four weeks of the experiment in the starving group were on average (SD) 0.84 (0.06) and 1.11 (0.09), respectively, and for feed intake the respective values were 0.98 (0.05) and 1.09 (0.07). The CC-values did not differ significantly from 1, except for CC for growth of the first four weeks ($P = 0.015$). When looking at the tank level, there was a positive correlation between the CC-values of the first and last four weeks for growth ($P = 0.027$), and the correlation was indicatively significant also for feed intake ($P = 0.057$) (Fig. 2a). The positive correlation between CC of growth and feed intake during the first four weeks was indicatively significant ($P = 0.081$), and statistically significant ($P = 0.031$) during the second four-week period (Fig. 2b). There was a significant negative correlation between CC of growth ($P = 0.040$) and CV of weight but for feed intake this correlation was not statistically significant ($P = 0.10$) (Fig. 2c).

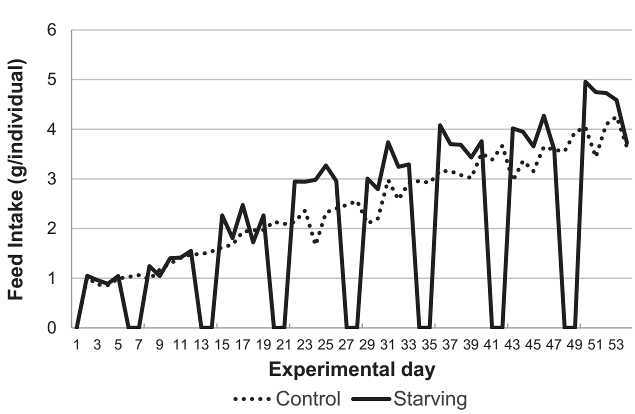

Fig. 1. Daily average individual feed intake of rainbow trout during the 56-day experiment in the control group (fed in excess every day) and in the treatment group (fed in excess but starved during the weekends). Data presented as tank average values, $n = 4$, error bars omitted for clarity.

Table 1
Initial and final wet weight, total length, condition factor, coefficient of variation (CV) of weight, specific growth rate (SGR), absolute and relative feed intake and feed conversion ratio (FCR) of rainbow trout, *Oncorhynchus mykiss*, fed every day (control) and starved over the weekends for 56 days.

	Initial		Final or Whole period		sig.
	Control	2-d starving	Control	2-d starving	
Weight (g)	29.2 ± 0.54	30.7 ± 0.82	176.7 ± 4.7	153.1 ± 8.4	*
Length (cm)	14.1 ± 0.14	14.0 ± 0.27	23.2 ± 0.17	22.5 ± 0.40	*
Condition factor	1.05 ± 0.02	1.07 ± 0.02	1.41 ± 0.02	1.34 ± 0.03	*
CV of weight	15.8 ± 1.02	16.8 ± 2.22	16.5 ± 1.20	16.6 ± 2.73	ns
SGR	–	–	3.34 ± 0.03	2.97 ± 0.10	*
Feed Intake (g/fish)	–	–	129.8 ± 2.92	113.2 ± 6.74	*
Relative Intake (% eaten/fed day)	–	–	2.30 ± 0.03	2.58 ± 0.15	*
FCR	–	–	0.88 ± 0.01	0.93 ± 0.03	*

Values are means ± SD, $n = 4$. Possible statistical difference (sig.) in the final values between control and starving groups is indicated by an asterisk, ns = not significant (Student's *t*-test, $P < 0.05$).

Weekend starvation induced no significant differences in any stomach parameter (Table 2). At the end of the experiment body moisture and hepatosomatic index were significantly higher ($P = 0.044$ and $P = 0.028$, respectively) in the treatment group than in the control group. Liver weight and visceral fat somatic index did not differ between the treatments (Table 2).

3.2. Second experiment

There was no mortality during the experiment. At the end of the experiment the average weight of the fish fed twice every day (TED) was significantly higher than in the groups fed twice every weekday (TEWD) and continuously every weekday (CEWD), while there was no significant difference between TEWD and CEWD, and the result was similar regarding SGR (Table 3). Fish in the TEWD group were significantly shorter than those in the TED group while the length of the fish in the CEWD group was intermediate and did not differ statistically from the other groups (Table 3). Despite the condition factor being slightly lower in TEWD and CEWD groups than in the TED group, there were no statistically significant differences between the treatments ($P = 0.081$). Absolute feed intake was highest and relative intake lowest in the TED group ($P < 0.05$) (Table 3).

4. Discussion

Rainbow trout starved over the weekends in the first experiment had compensation coefficients for growth and feed intake between 0.84 and 1.11 meaning that there was no or only very small, statistically insignificant, compensation. The increase of compensation coefficients (CC) during the last four weeks of the experiment suggests that rainbow trout are able to adapt, at least to certain extent, to weekend starvation. The adaptation to weekend starvation is clearly seen in Fig. 1, where the treatment group fish did not exceed feed intake of the control fish after the first weekend, slightly exceeded those after the second weekend, and only after the third weekend they ate clearly more on the weekdays than the control fish. Relatively poor adaptation to 2-day starvation in juvenile rainbow trout concurs with the results of Salgado-Ismodes et al. (2020) but on the other hand contrasts the findings of Nikki et al. (2004) and Taşbozan et al. (2016). Känkänen and Pirhonen (2009) conducted a

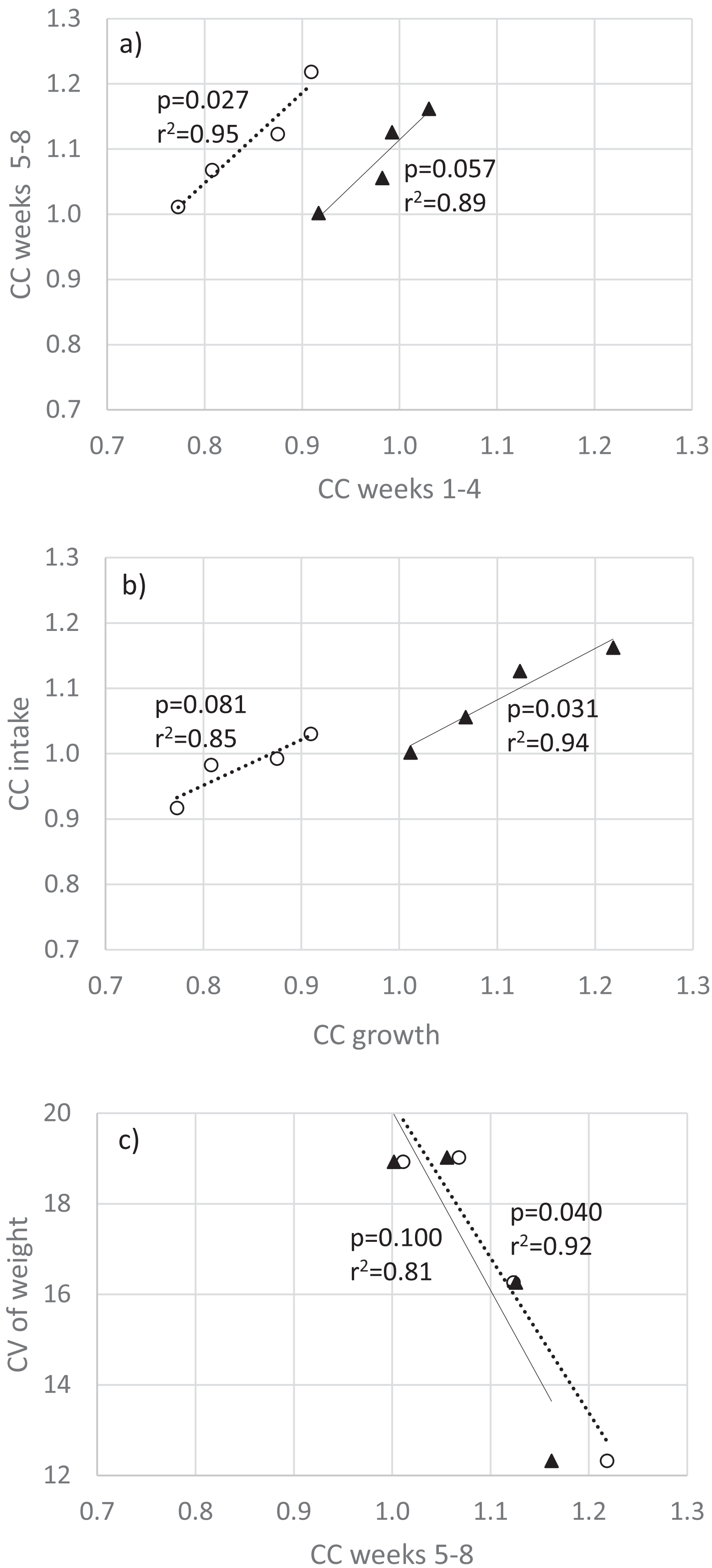

Fig. 2. a) The effect of feeding period (first and last four weeks of the 8-week experiment) on the relationship of compensation coefficients (CC) for growth (circles) and feed intake (triangles), b) the relationship of CCs of growth and feed intake during the experimental weeks 1–4 (circles) and weeks 5–8 (triangles) and c) relationship of CC of growth (circles) and feed intake (triangles) during the weeks 5–8 and coefficient of variation of final weight in juvenile rainbow trout. Data points represent the calculated CC for each of the four treatment group tanks, data analysed by linear regression analysis and dotted line is for circles, solid line for triangles.

Table 2
Measured and calculated stomach variables, body moisture, liver weight, hepatosomatic index (HSI), and visceral fat somatic index (VFSI) of rainbow trout, *Oncorhynchus mykiss*, fed every day (control) and starved over the weekends in the beginning and end of the 56-day experiment.

	Initial	Final (n = 4)		sig.
	(n = 10)	Control	2-d starving	
Stomach				
volume (mL)	–	5.77 ± 0.55	6.12 ± 0.72	ns
volume (% of weight)	–	3.27 ± 0.37	4.01 ± 0.56	ns
weight (g)	0.39 ± 0.07	1.38 ± 0.04	1.31 ± 0.09	ns
weight (% of weight)	1.29 ± 0.14	0.78 ± 0.01	0.86 ± 0.09	ns
weight/volume	–	0.24 ± 0.02	0.22 ± 0.02	ns
Body moisture (%)	72.63 ± 1.61	68.78 ± 2.31	72.12 ± 0.78	*
Liver weight (g)	0.38 ± 0.06	1.64 ± 0.28	1.59 ± 0.29	ns
HSI (% of weight)	1.26 ± 0.18	0.90 ± 0.04	1.04 ± 0.05	*
VFSI (% of weight)	1.29 ± 0.14	3.80 ± 0.64	3.13 ± 0.36	ns

Values are means ± SD. Initial values are calculated from individuals (n = 10), final values from tank average values (n = 4). Possible statistical difference (sig.) in the final values between control and starving groups is indicated by an asterisk, ns = not significant (Student's *t*-test, $P < 0.05$).

Table 3
Average weight, length, condition factor, specific growth rate (SGR), feed intake, relative intake and feed conversion ratio (FCR) in rainbow trout (*O. mykiss*) fed twice every day (TED), twice every weekday (TEWD) or continuously every weekday (CEWD).

	Initial	Final (n = 3)		
	(n = 10)	TED	TEWD	CEWD
Weight (g)	49.2 ± 0.84	111.7 ± 2.75a	92.8 ± 0.77b	96.9 ± 4.21b
Length (cm)	16.7 ± 0.10	20.4 ± 0.21a	19.6 ± 0.11b	19.9 ± 0.18ab
Condition Factor	1.06 ± 0.02	1.33 ± 0.04	1.24 ± 0.02	1.23 ± 0.03
SGR	–	3.89 ± 0.14a	2.92 ± 0.24b	3.18 ± 0.15b
Feed Intake (g/fish)	–	45.3 ± 1.59a	32.1 ± 0.29c	36.3 ± 1.49b
Relative Intake (% eaten/ fed day)	–	2.68 ± 0.06b	3.08 ± 0.12a	3.30 ± 0.11a
FCR	–	0.73 ± 0.02	0.78 ± 0.09	0.77 ± 0.04

Values are means ± SD, $n = 10$ (initial) or $n = 3$ (final). Values on the same row with different letters denote significant differences ($P < 0.05$). TED; feeding twice every day (09:00–10:00 and 15:00–16:00), TEWD; feeding twice every weekday (09:00–10:00 and 15:00–16:00), CEWD; fed continuously (9:00–24:00) every weekday.

similar experiment with another salmonid species, European whitefish (*Coregonus lavaretus*), and the fish exposed to 2-day starvation followed by 5-day feeding were not significantly smaller than the controls at the end of the 6-week trial, and also in their experiment CCs increased towards the end of experiment suggesting adaptation to intermittent feeding. Regarding non-salmonids, in matrinxã (*Brycon amazonicus*) a 2-day starvation followed by three or four days of feeding increased feed intake significantly during the feeding days and also FCR improved to the extent that the treatment fish did not differ in weight from the controls at the end of the 60-day experiment (Urbinati et al., 2014). In fingerling dusky grouper (*Epinephelus marginatus*; Spandri et al., 2021) 2-day starvation followed by five days of feeding for two months significantly decreased growth rate and also FCR.

Differences in CCs between the tanks were rather large, and our results suggest a persistent level of compensation within the tank (Fig. 2a): the higher is the compensation during the first 4-week period, the higher is the compensation during the following four weeks both in terms of intake and growth. The variation in CCs between the tanks can be a result of individual differences in the capacity for growth compensation due to two alternative reasons: first, all or most individuals within a tank are compensating more or less equally or second, some individuals are compensating really much while others are compensating much less, suggesting competition between individuals. An increase in the coefficient of variation of weight has been used as a proxy of interindividual competition for food within a tank (Jobling, 1995). Thus, our result (Fig. 2c) suggests that in the tanks with a higher CV of weight interindividual competition may limit or hinder feeding and consequent compensatory growth of many individuals.

Nikki et al. (2004) reported that individually reared rainbow trout of the similar size than in the present experiment were able to fully compensate the 2-day fasting period during the feeding days, typically five days (number of feeding days was adjusted according to their appetite). The difference between the present results and those of Nikki et al. (2004) could be explained by the possible negative effects of rearing in groups on CG, as found in hybrid sunfish, *Lepomis cyanellus* × *L. niacrochirus* (Hayward et al., 1997, 2000), and this inference is also supported by the negative correlation between CV of weight and CC (Fig. 2c). On the other hand, in the experiment of Taşbozan et al. (2016) group reared rainbow trout grew larger than the control fish when they were starved for two days and fed for five days. The most clear difference between our first experiment and that of Taşbozan et al. (2016) was the feeding frequency. Taşbozan et al. (2016) fed their fish only twice per day while we fed the fish continuously with belt feeders throughout the daylight hours. Therefore our second experiment was designed to test the possible effect of daily feeding duration on intake and growth performance. However, there was no significant difference in growth of rainbow trout when fed either continuously or only twice per day on weekdays, indicating that the feeding frequency was not the reason for the difference in our result and that of Taşbozan et al. (2016). Despite the short duration of the second experiment, rainbow trout fed every day more than doubled their initial weight, and they grew significantly larger than the groups fed on weekdays only, supporting the result of the negative effect of weekend starvation on growth found in the first experiment.

Growth compensation (partial, full or over-compensation) is in most cases induced only by hyperphagic response (e.g. Hayward et al., 1997, 2000; Nikki et al., 2004; Känkänen and Pirhonen, 2009; Yengkokpam et al., 2014; Favero et al., 2020) but in some cases improvement of FCR has been reported (Qian et al., 2000; Li et al., 2005; Urbinati et al., 2014; Savoie et al., 2017). It has been suggested that fishes do not maintain extra stomach capacity but the stomach size is adjusted to the size of expected ration (Ruohonen and Grove, 1996). Thus, during the periods of increased feed intake due to hyperphagia the growth of stomach has been reported (Jobling, 1982), and the hyperphagic whitefish were also capable of filling a bigger part of the stomach than normally eating fish (Känkänen and Pirhonen, 2009). However, in the present experiment we observed only a small, statistically insignificant, increase in stomach volume of the treatment group fish. This observation of the incapability of rainbow trout to enlarge their stomach capacity may be related to domestication and selective breeding, as suggested by Salgado-Ismodes et al. (2020). Tens of generations spent under captivity is possibly causing the inability of the cultivated fish to increase their stomach capacity due to the lack of variability in feeding as they obtain their ration without large daily variation what would typically occur in the wild.

The relative size of liver, as indicated by HSI, may or may not change due to the change in the feeding level, depending on the length of feed deprivation, fish species and fish size. For example, in fingerling (start weight 3.2 g) hybrid striped bass (*Morone chrysops* × *M. saxatilis*) one-week starvation did not induce significant change in HSI but starvation for two or four weeks significantly decreased HSI, and during refeeding HSI increased above that of the control fish (Turano et al., 2007), concurring with the results of larger fish of the same species (Picha et al., 2006). However a shorter duration (two days) of feed withdrawal repeatedly did not induce significant drop in HSI in

European whitefish (start weight c. 50 g; Känkänen and Pirhonen, 2009), hybrid tilapia (*Oreochromis mossambicus* x *O. niloticus*, start weight 5.5 g; Gabriel et al., 2017) nor in dusky grouper (start weight c. 10 g; Spandri et al., 2021) and similar results about insignificant changes in HSI in response to repeated short feed deprivation has been observed in rainbow trout (Nikki et al., 2004; Taşbozan et al., 2016; Salgado-Ismodes et al., 2020). However, in the present experiment the treatment group fish starved over the weekends had significantly higher final HSI (1.04 $\pm$ 0.05%) than the control fish (0.90 $\pm$ 0.04%), and this result contrasts with the experiments listed above but is supported by results of Favero et al. (2020) with juvenile pacu (*Piaractus mesopotamicus*) where HSI increased above the controls during the refeeding period. On the other hand, Taşbozan et al. (2016) reported a bigger absolute difference in HSI than in our experiment between their control (1.94 $\pm$ 0.47%) and the treatment fish (2.12 $\pm$ 0.20%), but that difference was not statistically significant. Taşbozan et al. (2016) reported HSI values about double to what we measured here and in our earlier studies with about the same size of rainbow trout (Nikki et al., 2004; Salgado-Ismodes et al., 2020), suggesting differences in HSI between different strains of rainbow trout.

During starvation body lipids are used as the primary energy source thus causing a decrease in body fat e.g. in juvenile rainbow trout (Denton and Yousef, 1976). In the current experiment we did not measure body fat directly, but as body fat and moisture are known to be inversely correlated (Shearer, 1994), the higher moisture of the group starved during the weekends (72.12 $\pm$ 0.78%) than in the group fed every day (68.75 $\pm$ 2.31%) indicates that weekend starvation caused a decline in body fat. Our results are similar to those of Taşbozan et al. (2016) who reported that rainbow trout starved for two days a week had higher body moisture (70.80 $\pm$ 0.56%) than the control group fed every day (68.15 $\pm$ 0.26%), and in their experiment body fat content was significantly higher in the controls than in the treatment group fish.

The second experiment was designed to evaluate the effect of feeding frequency during the weekdays on growth and on compensatory growth. In general, there is much variation in the results with rainbow trout regarding the possible growth-promoting effects of feeding frequency. Grayton and Beamish (1977; feedings from once every second day up to six times per day) and Rasmussen et al. (2007; feedings 1 or 3 times per day) found no significant effects of feeding frequency on growth of rainbow trout while findings of Holm et al. (1990; feeding frequency from once per hour to continuous feeding) and Ruohonen et al. (1998; 1, 2 or 4 feedings per day) suggested improved growth along with the increase of feeding frequency. We could not show any significant effect of increasing daily feeding hours from two to continuous feeding during the three-week experiment. However, our results showed that the lack of full growth compensation in the first experiment was not due to the feeding frequency.

5. Conclusions

The results of the two experiments indicated that for achieving maximal growth rate in juvenile rainbow trout, the fish should be fed every day. The lack of full compensatory growth is suggested to be a consequence of selective breeding for decades. This has likely led to the reduced capacity for stomach enlargement and thus decreased the ability for hyperphagia, which in turn would facilitate full growth compensation. The original hypothesis of full growth compensation through hyperphagic response and improved feed conversion was not supported. We were unable to demonstrate significant difference in growth of rainbow trout fed either twice per day or continuously, and compensatory growth response was unaffected by feeding frequency.

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Ethical approval

The experiments were carried out according to the EU Directive 2010/63/EU for animal experiments, and under the national permit number ESAVI/10412/04.10.07/2016.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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