



Effect of Various Lengths of Single Phase Starvation on Compensatory Growth in Rainbow Trout under Summer Conditions (*Oncorhynchus mykiss*)

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Abstract

This study was conducted to determine the effects of various lengths of starvation periods on following compensatory growth (CG) in rainbow trout under summer conditions (18.1°C and day length of 12.5-14.5 hours). Five treatments with triplicate tanks were as follows: control (C) fed to satiation over 84 days; one (S1), two (S2), three (S3), and four (S4) weeks of starvation; and then refeeding for the remaining eight weeks of the experiment. Starvation periods induced hyperphagia during refeeding but only S1 and S2 were able to catch up with C. Repeated measures of analysis of variance suggested a convergence in body mass but not in body length (structure). Organo-somatic indices of the starvation groups were significantly reduced at the end of starvation periods and restored to levels of the control fish within the first two weeks of the refeeding period. Broadly speaking, starvation longer than one week significantly reduced apparent digestibility of dry matter, lipid, and energy compared with the control group but did not affect protein and ash, and a complete recovery in the digestibility coefficients occurred within two weeks of satiation feeding. There was a linear increase in body moisture and a decrease in lipid and lipid/lean body mass ratio with the severity of starvation periods, but these divergences largely disappeared at the end of refeeding. During the starvation period, the protein synthesis rate (estimated using RNA/DNA ratio in the muscle and liver) reduced but in subsequent refeeding period, it increased in starved fish. The findings of the present experiment suggest that an application of single starvation episodes to elicit CG as a management tool in summer conditions should not be longer than two weeks.

Keywords: Rainbow trout, starvation, compensatory growth, body composition, organ indices, nutrient digestibility

Yaz Koşullarında Tek Fazlı Farklı Uzunlukta Açlık Sürelerinin Gökkuşluğu Alabalığında (*Oncorhynchus mykiss*) Telafi Büyümesine Etkisi

Özet

Bu araştırma, farklı uzunlukta açlık sürelerinin yaz koşullarında (18.1°C su sıcaklığı ve 12.5-14.5 saat gün uzunluğu) gökkuşluğu alabalığında telafi büyümesi (TB) üzerine etkilerinin belirlenmesi amacıyla yürütülmüştür. Üç tekerrürlü 5 grup, kontrol (K, 84 gün boyunca doyana kadar yemleme), bir (A1), iki (A2), üç (A3) ve dört (A4) hafta açlık ve ardından sekiz hafta doyana kadar yemlenen gruplardan oluşmuştur. Açlık süreleri yeniden besleme aşmasında yüksek iştaha neden olmuş, ancak sadece A1 ve A2 grupları K'ı yakalayabilmişlerdir. Tekrarlı ANOVA yapısal değil, vücut kitlesi bakımından gruplar arasında bir birleşmeyi işaret etmiştir. Aç bırakılan grupların organ-vücut indeksleri kontrole göre önemli derecede düşmüş, fakat yeniden beslemeye başladıktan iki hafta sonra kontrol grubu seviyesine kavuşmuştur. Genel olarak bir haftadan uzun açlık süreleri kuru madde, lipit ve enerji sindirilebilirliğini kontrole göre önemli derecede düşürmüştür, protein ve kül sindirimini etkilememiş; düşen sindirim değerleri yemleme başladıktan sonra iki hafta içinde kontrol düzeyine erişmiştir. Açlık şiddeti ile vücut nem düzeyinde doğrusal bir artış, lipit ve lipit/yağsız vücut kitlesi oranında ise doğrusal bir düşme olmuş, fakat bu farklılıklar deneme sonunda büyük çapta kaybolmuştur. Açlık protein sentez oranını (kas ve karaciğer RNA/DNA oranı) düşürmüştür, yemleme aşamasında ise önceden açlığa maruz kalanlarda (özellikle uzun sürelielerde) arttırmıştır. Bu araştırmanın bulguları, yaz koşullarında açlık ve ardından TB bir yetiştiricilik yönetim aracı kullanılacak ise, açlığın iki haftayı geçmemesi gerektiğini göstermektedir.

Anahtar Kelimeler: Gökkuşluğu alabalığı, açlık, telafi büyümesi, vücut kompozisyonu, organ indeksleri, sindirilebilirlik

Introduction

Compensatory growth (CG), a phenomenon of growth spurt following a growth retardation period, has been suggested as a rearing tactic in numerous aquatic organisms (Ali *et al.*, 2003). Dietary restriction and starvation with single phase and subsequent satiation feeding or cycled restriction and full feeding are the strategies that have been mostly resorted (Hayward *et al.*, 1997; Qian *et al.*, 2000; Nikki *et al.*, 2004; Eroldoğan *et al.*, 2006).

However, switching the rearing water temperature from either sub or supra optimums to the ideal levels can also be a tool to invoke CG in various fish species (McMillan and Houlihan, 1989; Mortensen and Damsgård, 1993; Mylonas *et al.*, 2005; Person-Le Ruyet *et al.*, 2006). Water temperature may have a remarkable effect on eliciting CG response in previously growth depressed fish (Wang *et al.*, 2000). Indeed, it has been reported that weight loss caused by fasting linearly elevated with the temperature (Brett *et al.*, 1969; Mäkinen, 1994; Wang *et al.*, 2000; van Dijk *et al.*, 2002), and during the summer with high water temperatures and longer photoperiods, an attempt of food deprivation or restriction to invoke CG in fish may create permanent depressions in fish body because of higher metabolic rate (Morgan and Metcalfe, 2001; Rodríguez *et al.*, 2009). Moreover, there are evidences indicating that fish undergoing a period of low plain of nutrition or starvation show a tendency of lower temperature optima than those abundantly fed (Brett *et al.*, 1969; van Dijk *et al.*, 2002; van Dijk *et al.*, 2005) and during realimentation, the restricted fish select lower temperature for some time (van Dijk *et al.*, 2002; van Dijk *et al.*, 2005). Despite an apparent interaction between water temperature and CG following a restriction phase, this issue has been little addressed in aquatic species and available data are rather controversial. For instance, Cho (2005) and Cho *et al.* (2006) reported a similar CG response in starved olive flounder (*Paralichthys olivaceus* L.) for up to four weeks during the winter (15°C) and summer (23.6°C) periods. Morgan and Metcalfe (2001) noted that food deprivation of juvenile Atlantic salmon during September and October permanently depressed body lipid reserves and structure, and CG in the following period lasted for a short time.

So far, CG in rainbow trout has been mostly studied between suboptimum and optimum temperatures (3°C-16°C) (Dobson and Holmes, 1984; Kindschi, 1988; Quinton and Blake, 1990; Farbridge *et al.*, 1992; Nikki *et al.*, 2004; Blake *et al.*, 2006; Bhat *et al.*, 2011; Guzel and Arvas, 2011). In one direct study of CG response of individually held rainbow trout at optimum and supraoptimum temperatures (17°C and 20.5°C), cyclic starvation and refeeding regimes have been employed (Nykänen, 2006), but the temperature effects appear to be masked by high interindividual variations and the

arbitrary termination of the study prior to ending of refeeding periods. Therefore, CG response of starved rainbow trout during high summer temperatures is yet to be established. This is particularly important for countries like Turkey, where supra or maximum critical temperatures can pose significant problems, which can even lead to suspension of the operations (Atasoy and Şeneş, 2004; Mefut *et al.*, 2007; Alpaslan and Pulatsü, 2008).

Therefore the present experiment was designed to determine the effects of various lengths of single-phase-starvation episodes on subsequent CG response, feed intake, organ indices, whole body composition, muscle and liver RNA/DNA ratio, and nutrient apparent digestibility coefficients (ADC) of rainbow trout under summer conditions.

Materials and Methods

Fish and Rearing Conditions

This study was conducted at the Kepez Unit of Mediterranean Fisheries Research and Training Institute, Antalya, Turkey using rainbow trout produced at the institute's trout hatchery. A total of 1,275 fish were selected from a large population, size graded and randomly distributed to fifteen 500 L experimental tanks with 400 L water holding capacity. Fish were adapted to the experimental conditions for two weeks. During this period, they were fed a commercial trout diet (450 and 200 g kg⁻¹ protein and lipid respectively, Çamlı Yem, İzmir, Turkey) at 2% of body weight. At the commencement of the trial, the number of fish in each tank was reduced from 85 to 75, and the average initial weight was 54.21±0.34 g. There were 5 treatments with three replications: satiation feeding throughout the experiment (C); one-week starvation and then eight-week satiation (S1), two-week starvation and then eight-week satiation (S2), three-week starvation and then eight-week satiation (S3) and four-week starvation and then eight-week satiation (S4). Each starvation treatment was started such that it was terminated at the end of the fourth week of the experiment. Prior to the starvation period, fish on S1, S2 and S3 were fed until apparent satiation. Feeding was done twice daily at 08:30 and 15:00 until the feeding activity of fish ceased. Water flow rate to each tank was set to about 20 L min⁻¹. The fish were weighed at the beginning, at the fourth week, and then two-week intervals until the end of the study after slight anesthetization with ethylene glycol monophenyl ether (0.3 ml L⁻¹). Experimental fish were subjected to a natural photoperiod between June 6 and September 15 (day length between 12.5 and 14.5 hours). Over the experimental period, water temperature, oxygen and pH were monitored every three days using YSI 58 dissolved oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA) and Lovibond SensoDirect pH200 (Tintometer

GmbH, Dortmund, Germany). The average water temperature, dissolved oxygen and pH were 18.1 ± 0.6 °C, (range 17.4-18.8), 7.9 ± 0.6 mg L⁻¹ and 7.6 ± 0.2 , respectively.

Diet

The ingredients were ground with a hammer mill, weighed at predetermined levels, and mixed through an experimental-type horizontal mixer (Şahin Torna, Antalya, Turkey) for five minutes (Table 1). Chromic oxide was included into the diet to serve as digestible indicator at 5.0 g kg⁻¹. Then the diets were pelleted using a pelleting machine (4 mm pellet diameter) without steam, packed in plastic bags, and kept at an ambient temperature in a dark room over the study.

Sample Collection and Analysis

Twenty fish were taken for determination of initial body composition and another set of twenty individuals for initial organo-somatic indices. At the termination of the starvation and refeeding phases, ten fish from each tank were separated: five for organo-somatic indices from which muscle and liver samples were taken for determination of tissue DNA and RNA concentrations, and the remaining five for whole body composition. At the second week of refeeding, five fish were also killed for determination of body indices, three fish for muscle and liver nucleic acids, and two fish for whole body nutrient concentrations. Fish separated for the further analysis were killed by over anesthetization with 2-phenoxyethanol, and the sampled tissues were stored at -20°C until analysis (Mohanta *et al.*, 2009).

During the first three weeks of the refeeding period, feces samples were collected by siphoning from the tank bottom. The tanks were cleaned after the evening meal, and then the following morning, the collection was done before the morning meal. Intact feces pellets were carefully collected into a small

bowl, and after a 10-minute decantation, excess water was discarded, and the remaining samples were dried overnight at 100°C. Three feces collections performed in a week were combined as representative of that week. The samples were kept in vacuumed bags in the refrigerator until analysis.

Proximate analysis of diet, feces, and fish were performed according to the methods of AOAC (1990): dry matter after drying in an oven at 104°C until constant weight, ash content by incineration in a muffle furnace at 600 °C for two hours; crude protein ($N \times 6.25$) by the Kjeldhal method after acid digestion, and lipid by petroleum ether extraction in a Soxhlet extractor. All analyses were conducted in duplicate. Carbohydrate levels of the diets and faeces were estimated by subtracting protein, lipid and ash from dry matter. Chromic oxide was determined after (Furukawa and Tsukahara, 1966). The DNA and RNA contents of liver and muscle were determined by a fluorometric procedure (Fluoroskan Ascent, Thermo LabSystems, Helsinki, Finland) following the method of Caldarone *et al.* (2001). Gross energy was calculated using conversion factors of 39.5, 23.7, and 17.2 MJ kg⁻¹ for fat, protein, and carbohydrate, respectively.

Data Calculation and Statistical Analysis

The growth and feed utilization parameters were calculated as follows: weight gain (WG g) = $W_t - W_0$, weight specific growth rate (SGR_w % day⁻¹) = $100 (\ln W_t - \ln W_0) t^{-1}$, length specific growth rate (SGR_L % day⁻¹) = $100 (\ln L_t - \ln L_0) t^{-1}$, feed conversion ratio (FCR) = (dry feed intake (g)) (wet weight gain (g))⁻¹, protein retention efficiency (PER) = (weight gain (g)) (protein fed (g))⁻¹, condition factor (CF) = $W_t L^{-3}$, viscerosomatic index (VSI) = $100 [(\text{visceral weight (g)}) (\text{body weight (g)})^{-1}]$, hepato-somatic index (HSI) = $100 [(\text{liver weight (g)}) (\text{body weight (g)})^{-1}]$, nitrogen (N) retention (%) = $100 [(\text{N gain (g)}) (\text{N intake (g)})^{-1}]$ and N loss (g kg WG⁻¹) = (N fed (g)) - (N deposited (g)) WG (kg)⁻¹, Lipid/Lean body mass (L/LBM) =

Table 1. Formulation and nutrient composition of the experimental diet (g kg⁻¹)

Ingredients		Nutrient composition (g kg ⁻¹ dry matter)	
Fish meal	455.0	Dry matter	941
Soybean meal	200.0	Protein	450
Wheat middling	184.9	Lipid	218
Fish oil	143.6	Ash	105
Pellet binder ¹	3.0	Chromic oxide	4.9
Choline chloride ²	1.5	Gross energy (MJ kg ⁻¹)	23.2
Vitamin ³	5.0		
Mineral ⁴	2.0		
Chromic oxide ⁵	5.0		

¹ Calcium lignosulfonate, Korkutelim Feed and Food Company, Antalya, Turkey

² Ufuk Kimya İlaç San. ve Tic. Ltd. Şti., Istanbul, Turkey.

³ Vitamin mixture contains kg diet⁻¹: 4 000 000 IU vitamin A, 480 000 IU vitamin D₃, 40 000 mg vitamin E, 2400 mg vitamin K₃, 4 000 mg vitamin B₁, 6 000 mg vitamin B₂, 40 000 mg niacine, 10 000 mg calcium D- pantothenate, 4 000 mg vitamin B₆, 10 mg vitamin B₁₂, 100 mg D-biotin, 1 200 mg folic acid, 40 000 mg vitamin C and 60 000 mg inositol.

⁴ Mineral mixture contains kg diet⁻¹: 23 750 mg Mn, 75 000 mg Zn, 5 000 mg Cu, 2 000 mg Co, 2 750 mg I, 100 mg Se, 200 000 mg Mg.

⁵ Avocado Research Chemicals Ltd, UK.

(whole body lipid (g)) [(whole body protein (g)) + (whole body ash (g))]⁻¹, where W_t (g) is fish body weight at day t and W₀ at day 0, t (days) is the duration of experiment, L is total fish length. Apparent digestibility coefficients (ADC) of experimental diets were estimated as follows:

$$\text{ADC (dry matter \%)} = 100 - 100 \left[\frac{(\text{Cr}_2\text{O}_3)_{\text{Faeces}}}{(\text{Cr}_2\text{O}_3)_{\text{Food}}} \right]$$

$$\text{ADC (nutrient \%)} = 100 - \left[100 \frac{(\text{Cr}_2\text{O}_3)_{\text{Food}}}{(\text{Cr}_2\text{O}_3)_{\text{Faeces}}} \right] \left[\frac{(\text{Nutrient})_{\text{Faeces}}}{(\text{Nutrient})_{\text{Food}}} \right]$$

A statistical package JMP v.8.0 (SAS Institute Inc, Cary, NC, USA) for Windows was used for all statistical analyses. Normality and homogeneity were checked by Shapiro-Wilk W Test and Bartlett's test, respectively. All percentage values were arcsine transformed before analysis of variance (ANOVA). During the study, all fish in one tank of the control group died because of water supply failure; thus, the control was assessed as two replicates in statistical analysis. One-way ANOVA was employed to reveal the effects of treatments on the criteria selected. Tukey's *post hoc* test was used to discriminate differences between the treatments. Feed intake, FCR,

SGR_W and SGR_L during the realimentation period were analyzed with analysis of covariance (ANCOVA) using average body weight (or length in the case of SGR_L) at the fourth week as covariate. Biweekly feed intake and SGR_W were also analyzed with ANCOVA using the previous average weight as covariate. Prior to proceeding with the ANCOVA, the homogeneity of treatment slopes were verified by including an interaction term between the treatments and covariates at a significance level of P = 0.1 (Engqvist, 2005). Growth trajectories of control and starved fish were compared by repeated measures of ANOVA. Starvation period was a between-subjects factor and repeated measures of *ln* body weight and length at different times was a within-subject factor (Álvarez and Nicieza, 2005).

Results

At the end of the depletion phase, there was a progressive decline in body weight and WG with starvation lengths (Table 2). In terms of body length, there was also a significant depressive effect in fish starved for longer than two weeks. During the refeeding period, all the restricted groups except S1 showed significantly higher feed intake and SGR_W

Table 2. Growth and feed utilization performance of rainbow trout starved for up to 4 weeks and refed for 8 weeks

Variables	C	S1	S2	S3	S4
Weight (g)					
Initial	53.85±1.89	53.92±0.43	55.11±0.51	53.64±0.23	54.40±1.05
Week 4	81.35±2.59 ^a	68.54±0.15 ^b	60.24±0.29 ^c	52.44±0.73 ^d	46.64±1.68 ^e
Week 12	193.76±4.65 ^a	171.47±3.75 ^{ab}	170.29±4.93 ^{ab}	151.42±1.98 ^b	147.22±9.22 ^b
Weight gain (g)					
Initial	27.50±0.70 ^a	14.62±0.27 ^b	5.13±0.75 ^c	-0.74±0.08 ^d	-7.20±1.71 ^e
Week 4-12	112.40±2.06	102.94±3.68	110.05±5.20	98.98±1.27	100.58±7.62
Week 0-12	139.91±2.76 ^a	117.56±3.57 ^{ab}	115.18±4.45 ^{abc}	98.24±1.29 ^{bc}	93.37±8.80 ^c
Length (cm)					
Initial	15.52±0.18	15.53±0.04	15.64±0.05	15.46±0.07	15.52±0.08
Week 4	17.27±0.04 ^a	16.65±0.05 ^{ab}	16.52±0.11 ^b	15.56±0.16 ^c	15.48±0.20 ^c
Week 12	23.12±0.28 ^a	22.30±0.17 ^{ab}	21.89±0.41 ^{ab}	21.05±0.19 ^b	21.13±0.35 ^b
Feed intake (% body weight day ⁻¹)					
Week 0-4	1.54±0.04 ^a	1.23±0.03 ^b	0.82±0.03 ^c	0.44±0.01 ^d	0.00±0.00 ^e
Week 4-12*	1.42±0.02 ^c	1.50±0.03 ^c	1.64±0.02 ^b	1.77±0.02 ^a	1.80±0.03 ^a
Week 0-12	1.33±0.011 ^a	1.28±0.01 ^{ab}	1.26±0.02 ^{ab}	1.25±0.02 ^b	1.15±0.01 ^c
FCR					
Week 4-12*	0.97±0.02	0.97±0.02	0.96±0.03	1.02±0.02	0.97±0.03
Week 0-12	0.99±0.01	1.03±0.02	1.03±0.03	1.10±0.02	1.06±0.05
SGR _W (% day ⁻¹)					
Week 4-12*	1.55±0.01 ^c	1.64±0.04 ^c	1.85±0.06 ^b	1.89±0.01 ^b	2.05±0.06 ^a
SBO 0-12	1.52±0.01 ^a	1.38±0.02 ^{ab}	1.34±0.02 ^b	1.24±0.01 ^b	1.18±0.05 ^c
SGR _L (% day ⁻¹)					
4-12*	0.52±0.02	0.52±0.01	0.50±0.02	0.54±0.03	0.56±0.01
0-12	0.47±0.00 ^a	0.43±0.01 ^{ab}	0.40±0.02 ^{ab}	0.37±0.01 ^b	0.37±0.02 ^b
PER					
4-12	2.29±0.05	2.28±0.04	2.31±0.06	2.19±0.04	2.28±0.07
0-12	2.24±0.04	2.14±0.03	2.15±0.06	2.02±0.03	2.11±0.11
N utilization					
N retention (%)	35.32±2.96	32.83±1.75	32.61±2.18	29.58±0.93	29.37±2.52
N loss (g kg WG ⁻¹)	46.19±2.84	50.18±1.93	50.30±2.84	55.76±1.01	54.05±4.61

*Values were tested by ANCOVA using previous body length as covariate and the observed values are given to prevent confusion. Values with different superscripts in the same row are significantly different (P<0.05). Data are mean ± SE.

than C (Table 2). As far as the whole refeeding period is concerned, feed intake of all treatment groups except S1 was significantly higher than the control (Figure 1). A similar trend but with a little bit obscurity was observed in SGR_w values during refeeding periods, generally being higher for S2, S3, and S4 than for S1 and C (Figure 2). In terms of structural growth, there was only numerical increment in SGR_L in the starved fish compared with C. There were also no significant effects of starvation periods on the following FCR, PER, and N utilization values. Briefly, fish subjected to starvation up to two weeks caught up with the control fish in terms of body mass and length even though CG response of S1 was invisible by ANCOVA. The repeated measures of ANOVA revealed that time \times treatment interaction was significant only for body mass, but not for length (Table 3).

Whole body moisture increased with the starvation lengths and became significantly higher in S4 than in C (Table 4). However, there was a continuing oscillation in the moisture concentrations during the realimentation phase, being significantly higher in S2, S3 and S4 than in C and S1 at the second week of refeeding, and a replenishment in S2 and S3, but not in S4, at the end of the experiment. While whole body protein levels of fish were significantly altered by starvation lengths at the end of starvation and the second week of refeeding, they were comparable among the treatments at the end of the experiment. Starvation periods linearly decreased whole body lipid level, but only S4 was significantly lower than C at the end of the starvation phase. Body lipid of S3 and S4 were significantly less than C at the second week of the realimentation period, but this difference disappeared at the end of the experiment

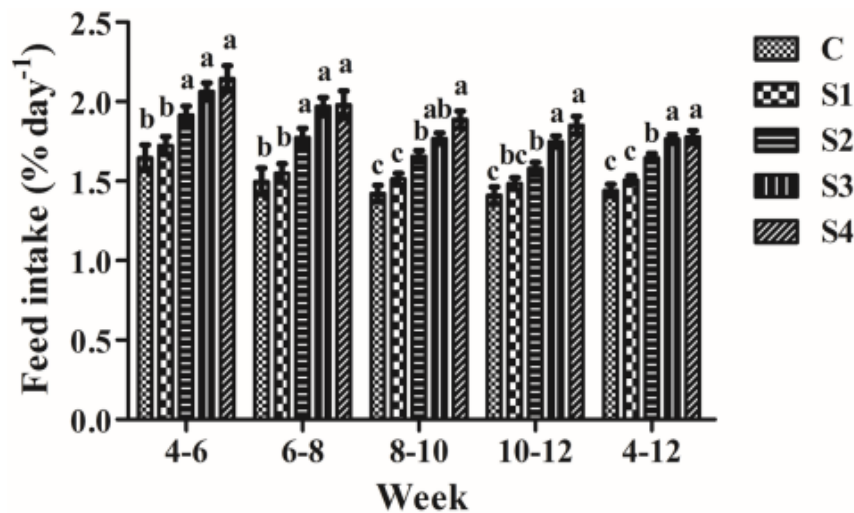


Figure 1. Changes of feed intake of rainbow trout previously starved for 1 to 4 weeks during refeeding period. Different letters in the same duration indicates significant differences ($P < 0.05$). Data are mean \pm SE.

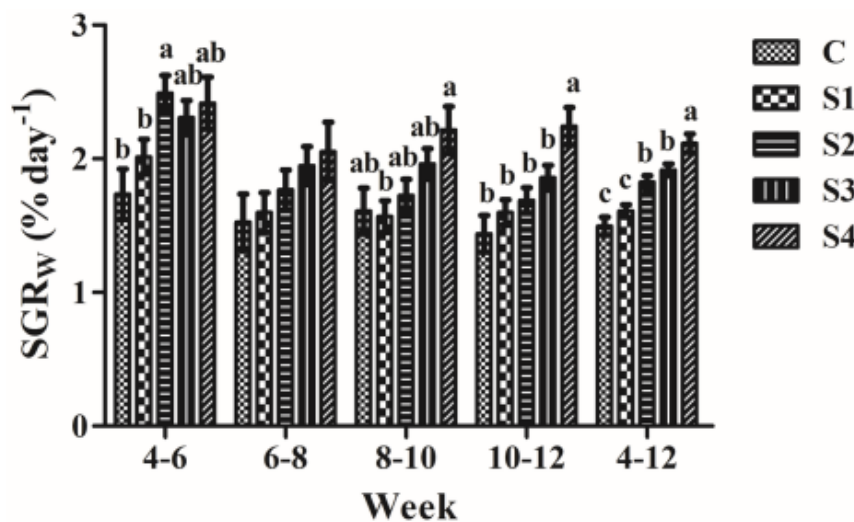


Figure 2. Changes of SGR_w of rainbow trout previously starved for 1 to 4 weeks during refeeding period. Different letters in the same duration indicates significant differences ($P < 0.05$). Data are mean \pm SE.

Table 3. Results of repeated measures of ANOVA test for starvation length (week 0-3) and time (week 3-12) on body weight and length of rainbow trout

Variables	Source of variation	Degree of freedom	F value	P
Weight	Starvation length	4	33.21	0.0001
	Time	4	3817	0.0001
	Time × Starvation length	16	9.523	0.0001
	Error	36		
Length	Starvation length	4	25.57	0.0001
	Time	2	1758	0.0001
	Time × Starvation length	8	1.007	0.4646
	Error	18		

Table 4. Whole body composition of rainbow trout starved up to 4 weeks and then refed for 8 weeks (%)

	C	S1	S2	S3	S4
Moisture	Initial (68.4)				
Week 4	68.0±0.2 ^b	67.9±0.3 ^b	69.6±0.8 ^{ab}	70.0±0.9 ^{ab}	72.1±0.3 ^a
Week 6	67.2±0.0 ^b	67.9±0.3 ^b	69.8±0.2 ^a	70.0±0.5 ^a	71.4±0.5 ^a
Week 12	66.2±0.3 ^b	66.1±0.2 ^b	65.8±0.6 ^b	67.1±0.3 ^{ab}	68.1±0.3 ^a
Protein	Initial (16.4)				
Week 4	14.1±0.3 ^b	15.3±0.2 ^a	14.8±0.4 ^{ab}	14.7±0.1 ^{ab}	14.5±0.1 ^{ab}
Week 6	15.0±0.0 ^a	14.5±0.1 ^{ab}	13.8±0.0 ^{bc}	14.1±0.3 ^{bc}	13.7±0.2 ^c
Week 12	15.9±0.8	15.7±0.5	15.6±0.5	15.3±0.3	14.8±0.2
Lipid	Initial (11.8)				
Week 4	13.9±0.2 ^a	12.8±0.4 ^a	11.6±0.5 ^{ab}	11.1±0.9 ^{ab}	9.2±0.4 ^b
Week 6	14.1±0.4 ^a	14.1±0.3 ^a	12.4±0.2 ^{ab}	11.8±0.7 ^b	10.8±0.4 ^b
Week 12	15.0±0.1 ^{ab}	15.1±0.2 ^{ab}	15.5±0.4 ^a	14.4±0.4 ^{ab}	13.8±0.1 ^b
Ash	Initial (2.4)				
Week 4	2.3±0.0 ^b	2.6±0.0 ^a	2.6±0.0 ^a	2.7±0.1 ^a	2.7±0.0 ^a
Week 6	2.2±0.1	2.2±0.0	2.2±0.0	22.3±0.0	2.3±0.1
Week 12	2.3±0.2	2.2±0.0	2.2±0.0	2.3±0.1	2.1±0.1
L/LBM	Initial (0.62)				
Week 4	0.84±0.02 ^a	0.71±0.02 ^{ab}	0.66±0.03 ^b	0.64±0.05 ^b	0.53±0.02 ^c
Week 6	0.82±0.02 ^{ab}	0.85±0.02 ^a	0.78±0.01 ^{ab}	0.72±0.05 ^{ab}	0.68±0.03 ^b
Week 12	0.82±0.02	0.85±0.03	0.87±0.02	0.82±0.03	0.81±0.01

Values with different superscripts in the same row are significantly different ($P < 0.05$).

Data are mean±SE.

when the only significant difference was recorded between S2 and S4. Whole body ash contents were significantly increased with starvation but they were recovered at the termination of the experiment (Table 4). There was a progressive drop in L/LBM ratio with increasing starvation, and all starvation groups except S1 were significantly different from C. At the second week of refeeding the only significant difference was detectable between S1 and S4. However, a complete restoration in L/LBM ratio occurred at the end of the experiment.

Muscle RNA/DNA ratio was significantly reduced in S3 and S4 compared with C (Table 5). After two weeks of refeeding, previously starved fish exhibited numerically higher RNA/DNA ratio than C, but only S4 was significantly higher than C. However, at the end of the experiment, there was no significant difference among the treatments. There were strong relationships between muscle RNA/DNA ratio and SGR_w during the respective periods, especially when growth was highly manipulated (eg. starvation and early period of refeeding). However, the relationship faded with the fall of CG response toward the end of

the study. The liver nucleic acid ratio was significantly lower in S2, S3, and S4 relative to C after starvation, but an opposite trend was observed during the early period of refeeding. During the refeeding period, no change in liver RNA/DNA ratio was recorded among the treatments. The correlation of SGR_w with liver nucleic acids ratio was strong in depletion phase, while it was much weaker during the refeeding period, and there was no relation at the end of the study.

Dry matter digestibility showed significantly lower values for S2, S3, and S4 than for C and S1 at the first week of refeeding, an increase at the second week despite still being lower for S4 than for C, and eventually a complete restoration at the third week (Table 6). A similar trend was seen in ADCs for lipid and energy among the treatments. However, neither protein nor ash digestibility was significantly affected by the treatments.

Organ indices of fish were highly affected by the starvation (Figure 3). Starvation groups except S1 significantly reduced CF values. Likewise, there was a strong reducing effect of starvation including S1 on

Table 5. Effect of various starvation periods on muscle and liver RAN/DNA ratios and their relationship with the corresponding SGR_w

	C	S1	S2	S3	S4	Linear relationship with the corresponding SGR_w		
						Equation	R ²	P value
Muscle								
Week 4	4.11±0.35 ^a	3.68±0.12 ^a	3.11±0.34 ^{ab}	2.50±0.14 ^{bc}	1.56±0.06 ^c	$SGR_{w(0-4)} = -1.55 + 0.65 R/D$	0.81	<0.0001
Week 6	4.24±0.10 ^b	4.55±0.23 ^b	4.68±0.03 ^{ab}	4.84±0.07 ^{ab}	5.16±0.04 ^a	$SGR_{w(4-6)} = -2.08 + 0.90 R/D$	0.83	<0.0001
Week 12	3.84±0.30	4.12±0.28	4.14±0.18	4.30±0.11	4.82±0.18	$SGR_{w(4-12)} = 0.71 + 0.22 R/D$	0.37	<0.05
Liver								
Week 4	3.99±0.10 ^a	3.80±0.06 ^a	3.02±0.05 ^b	2.92±0.07 ^b	2.05±0.01 ^c	$SGR_{w(0-4)} = -2.49 + 0.92 R/D$	0.89	<0.0001
Week 6	4.07±0.05 ^c	3.96±0.03 ^c	4.48±0.02 ^b	4.53±0.06 ^{ab}	4.78±0.10 ^a	$SGR_{w(4-6)} = -1.47 + 0.84 R/D$	0.57	<0.05
Week 12	4.05±0.09	4.09±0.11	4.18±0.13	4.07±0.03	4.14±0.11	$SGR_{w(4-12)} = 1.81 + 0.002 R/D$	0.01	>0.05

Values with different superscripts in the same row are significantly different ($P < 0.05$). Data are mean ± SE. R/D is RNA/DNA ratio.

Table 6. Effect of starvation on nutrient digestibility coefficients (%) during the first three weeks of refeeding period

	C	S1	S2	S3	S4
Dry matter					
Week 1	77.93±0.95 ^a	76.59±0.35 ^a	74.45±0.37 ^b	73.45±0.26 ^b	72.84±0.18 ^b
Week 2	80.46±0.74 ^a	78.84±0.23 ^{ab}	78.43±1.00 ^{ab}	76.26±0.20 ^{ab}	75.70±1.29 ^b
Week 3	79.57±0.07	77.93±0.41	77.58±0.94	79.03±0.48	77.97±0.22
Protein					
Week 1	92.34±0.42	92.33±0.21	91.72±0.14	91.47±0.27	91.32±0.20
Week 2	92.83±0.38	92.57±0.24	92.68±0.40	91.90±0.04	91.53±0.62
Week 3	92.52±0.08	92.11±0.22	92.21±0.28	92.62±0.21	92.12±0.13
Lipid					
Week 1	96.47±0.56 ^a	95.72±0.65 ^a	95.12±0.35 ^{ab}	92.87±0.43 ^b	91.22±0.55 ^c
Week 2	96.50±0.12 ^a	94.38±0.46 ^b	94.38±0.03 ^b	92.41±0.23 ^b	91.98±0.88 ^c
Week 3	97.54±0.26	96.88±0.47	96.81±0.19	97.10±0.28	96.56±0.08
Ash					
Week 1	45.43±3.87	44.53±1.79	41.63±0.87	42.47±0.53	40.37±1.67
Week 2	52.77±1.64	51.42±1.99	52.12±1.88	50.55±0.33	48.48±3.05
Week 3	53.59±0.52	50.38±2.20	49.38±1.84	55.08±0.59	51.15±0.59
Energy					
Week 1	86.17±0.33 ^a	85.07±0.24 ^a	83.50±0.18 ^b	82.17±0.14 ^c	81.53±0.30 ^c
Week 2	87.55±0.49 ^a	85.96±0.29 ^{ab}	85.60±0.65 ^{ab}	83.59±0.13 ^b	83.21±0.97 ^b
Week 3	86.98±0.07	85.82±0.12	85.63±0.57	86.37±0.43	85.72±0.14

Values with different superscripts in the same row are significantly different ($P < 0.05$). Data are mean ± SE.

HSI, VSI and PSI. However, all organ indices of previously starved fish were rapidly increased to the level of the control group within two weeks of full feeding and remained unchanged until the termination of the study.

Discussion

In the present study, weights of S1, S2, S3, and S4 at the end of depletion phase were 84.3%, 74.1%, 64.5%, and 57.3% of the control, respectively. Extending starvation lengths induced an increasing feed intake and SGR_w during the refeeding period, but only fish on S1 and S2, despite being numerically lower, were able to catch up with the control at the end of satiation feeding. Starvation for longer than two weeks resulted in a partial compensation. These results are contradictory with some of the earlier studies in rainbow trout, reporting that much more severe starvation and refeeding cycles resulted in a

weight catch-up (Dobson and Holmes, 1984; Quinton and Blake, 1990; Blake *et al.*, 2006). The reason for this discrepancy between the reported literature and ours is highly likely because of high summer temperature, considering that a more pronounced CG response in starved-recovering trout during June and October compared with remaining of the year was attributed to a more favorable water temperature (11–16°C *versus* 3–11°C) (Dobson and Holmes, 1984). Moreover, under optimal temperature (15°C), Nikki *et al.* (2004) observed a catch-up in rainbow trout subjected to fixed starvation (2, 4, 8, and 14 days) and variable refeeding periods during an 80-day trial, being partly in disagreement with our observations. Although rainbow trout is one of the mostly studied species, the reports in the literature are inconsistent with each other, and there are also no fixed schedules of restriction or starvation and subsequent refeeding to elicit CG as a management tool in practice. For instance, Kindschi (1988) (who maintained rainbow

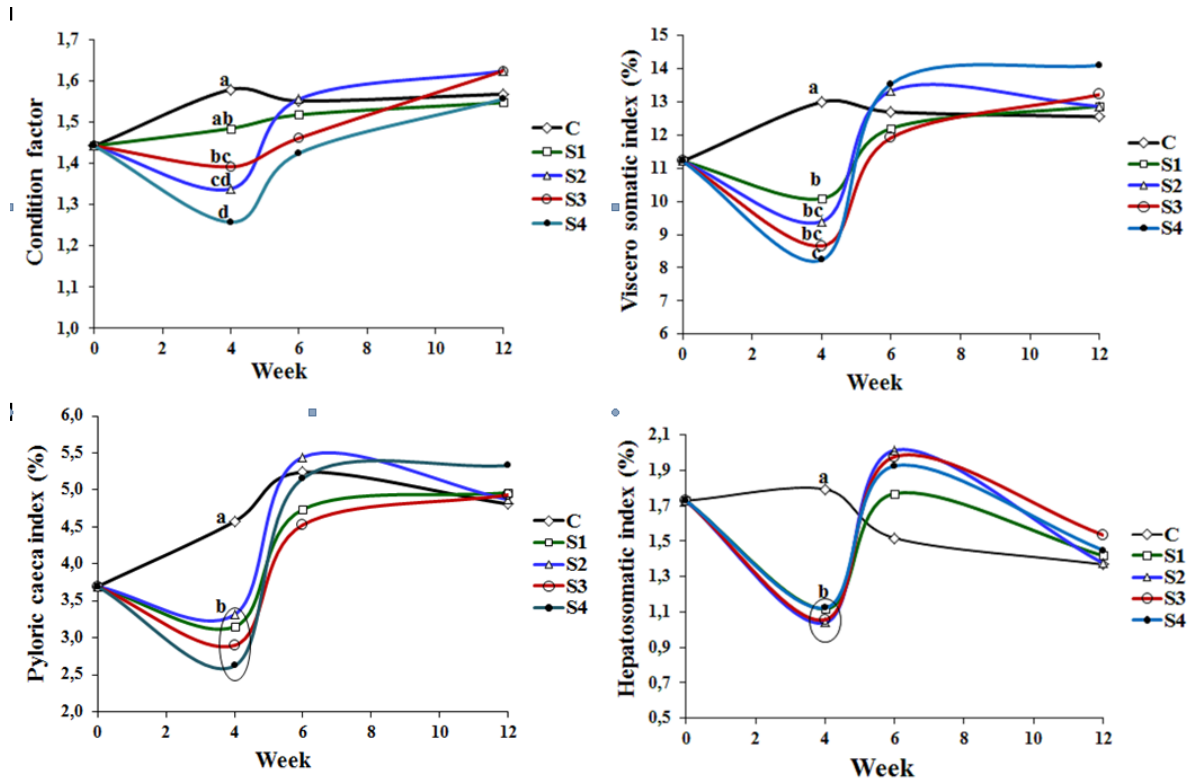


Figure 3. Effects of various starvation periods and subsequent refeeding on organ indices (%) of rainbow trout. Different letters in the same week denote that values are significantly different ($P < 0.05$).

trout on four days of starvation and 3 days of feeding or four weeks of starvation and four weeks of feeding at 12°C) and Farbridge *et al.* (1992) (who fed the fish with restricted schedules for 42 days and then fed them during the following 56 days at 13°C) observed a CG reaction resulting in an incomplete recovery. The intermittent feeding of rainbow trout at 8.5°C by employing starvation for one or two days and refeeding during remaining week days resulted in a lower growth performance than unrestricted control (Okumus and Bascinar, 2001), whereas the analogue treatments yielded a similar growth rate to the control fish at 17.3 and 11.2°C (Başçınar *et al.*, 2008) and (Guzel and Arvas, 2011), respectively.

Our observations are consistent with previous studies reporting hyperphagia as the main mechanism of CG in fish (Bull and Metcalfe, 1997; Hayward *et al.*, 1997; Ali *et al.*, 2003; Eroldoğan *et al.*, 2006; Bavčević *et al.*, 2010), but contradictory with those addressing better FCR with or without appetite increase is a contributing factor to CG (Dobson and Holmes, 1984; Miglavs and Jobling, 1989; Quinton and Blake, 1990; Boujard *et al.*, 2000; Qian *et al.*, 2000; Blake *et al.*, 2006; Xiao *et al.*, 2012). Considering that feed consumption was the main growth determinant in the present experiment, there should be a similarity between the recovered groups and the control. Indeed, overall feed intake rates of S1 and S2 were similar to C but S3 and S4 lagged behind the control. A somewhat short refeeding period could also be a factor in the incomplete catch-up growth in S3 and S4 in the present study since feed intake of

these groups were still higher than C during weeks 10-12. Starvation and following refeeding applications did significantly affect neither the PER or N utilization in this study, being inconsistent with findings of Qian *et al.* (2000), who observed better protein utilization in starved-refed gibel carp (*Carassius auratus gibelio*).

Álvarez and Nicieza (2005) suggested that after a period of depletion phase, CG occur selectively in body mass but not in structure (length), and once the body mass reaches the target, there would be no compensation at all. In other words, there will be no true catch-up growth as long as structural loss is not recovered (Nicieza and Álvarez, 2009) since muscle growth in fish must be matched with structural components including bone and cartilage (Mommsen, 2001). In the present experiment, we tested if weight and structural compensations were accompanied using the linear relationship between SGR_W and SGR_L during the refeeding period and did not find a linear relationship between SGR_L and SGR_W during weeks 4-12 ($SGR_{W(4-12)} = 0.695 + 2.117 \times SGR_{L(4-12)}$; $n = 14$, $R^2 = 0.153$, $P = 0.177$). The final weight and length of S1 and S2 were similar to C according ANOVA, whilst repeated measures of ANOVA did verify the mass convergence, but not length. This clearly suggests that compensatory response in SGR_W does not necessarily accompany SGR_L , and in agreement with the observations of Álvarez and Nicieza (2005) and Bavčević *et al.* (2010), there is a regulation of allocation between investments in body mass and structure.

Fish respond to food deprivation with a down-regulation of metabolic rate to save energy and minimize the body mass loss (Cook *et al.*, 2000; O'Connor *et al.*, 2000; van Dijk *et al.*, 2002; Ali *et al.*, 2003). The most notable difference is observed in visceral organs, which are the main body parts in terms of maintenance energy requirement (Gaylord and Gatlin, 2000; Bélanger *et al.*, 2002; Ali *et al.*, 2003; Cho, 2005; Cho *et al.*, 2006; German *et al.*, 2010). That's why, upon commencement of starvation, the required energy for maintenance is met first with glycogen deposited in the liver and partly in white muscle, then with sequential mobilization of lipid depots in and around the liver and viscera and finally, muscle proteins (Black and Love, 1986; Cook *et al.*, 2000; Rios *et al.*, 2006). Accordingly a remarkable shrinkage occurs in organ sizes through either reduction in size or number of tissue cells (Bélanger *et al.*, 2002; Rios *et al.*, 2004; German *et al.*, 2010). Depending on the severity of starvation, digestive systems can significantly be degenerated and atrophied in animals (Wang *et al.*, 2006). Behavioral changes also take place during starvation, beginning with an increased activity of food searching, then a reduction in locomotor activity and adaptation with low activity and metabolism (Méndez and Wieser, 1993; van Dijk *et al.*, 2002). The results of the present study concerning the whole body changes are in harmony with studies reporting that moderate starvation causes tissue hydration, lipolysis, and increase in ash (Farbridge *et al.*, 1992; Cook *et al.*, 2000; Ali *et al.*, 2003; Peres *et al.*, 2011). However, there were variations in whole body moisture, protein, and lipid levels at the end of the first two weeks of refeeding, which could be related to differences of priority to be compensated and concomitant imbalances in body proximate compositions. Since protein levels were not affected by the starvation lengths, it appears that body lipid had priority for compensation. In Atlantic salmon (*Salmo salar*), a somewhat different allocation of resource has been observed by Johansen *et al.* (2001), who starved or restricted fish for six weeks and then refed them for sixteen weeks. While the starved Atlantic salmon first restored protein leading to further reduction of body lipid (which was the protein in our case), the restricted fish restored body lipid. The discrepancy between these findings and ours could be due to the difference in starvation lengths (one to four weeks *versus* six weeks). At the end of the experiment, all body nutrient components of previously starved fish, except moisture which was still lower in S4 than C, were restored to the control fish. The reason for the lower moisture of S4 may be the lower body size rather than an influence of starvation.

The L/LBM ratio has been suggested as an indicator of hyperphagia and concomitant growth acceleration in fish undergoing CG after starvation or dietary restriction (Jobling and Johansen, 1999;

Johansen *et al.*, 2001; Johansen *et al.*, 2002). According to this hypothesis (the lipostatic model), dietary restriction causes reduction in lipid reserves and thus L/LBM ratio (Jobling and Johansen, 1999; Johansen *et al.*, 2001) and after removal of restriction, the depleted fish show high food intake and accelerated growth as long as L/LBM ratio remains low compared with unrestricted individuals. In the present experiment, starvation periods longer than one week created a significant reduction in L/LBM ratio. Upon refeeding, a quick restoration of L/LBM ratio in the starved to the control level occurred, and at the end of the experiment, there were no longer differences among the experimental groups. Considering the change of L/LBM ratio at the end of the starvation phase and feed intake over the refeeding period, there first appears to be a harmony with the lipostatic hypothesis (Jobling and Johansen, 1999). However, a closer look at feeding rate, SGR_w, and L/LBM ratio during the refeeding period reveals a contradiction with the lipostatic model. For instance, despite similar L/LBM ratios of the treatments at the end of the experiment, feed consumptions of S2, S3 and S4 in last two weeks of refeeding period were still higher than C. Moreover, SGR_w of S4 was still superior to the others. All these suggest that fish starved for more than one week still have the tendency of CG response, even with a comparable L/LBM ratio. A partial support to the lipostatic model in barramundi (*Lates calcarifer*) undergoing compensatory growth has also been documented by Boujard *et al.* (2000), Tian and Qin, (2003, 2004) and Peres *et al.* (2011). One possible explanation of the contradiction could be that lipostatic regulation in rainbow trout may be activated at only extremely elevated lipid concentrations as suggested for sablefish (*Anoplopoma fimbria*) (Sogard and Spencer, 2004).

Effects of starvation on nutrient digestibility coefficients during refeeding periods have been rarely addressed in fish. Existing studies by Wang *et al.* (2000) and Wang *et al.* (2005) in hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) and by Tian and Qin (2004) in barramundi did not find a significant impact of starvation on the digestibility values during the refeeding period, being in disagreement with our results. Another finding consistent with ours to some degree was reported by Qian *et al.* (2000), who found that gibel carp starved for four weeks had significantly lower dry matter and energy digestibility than the control fish. On the other hand, cyclically fed black sea bream for one or two days and then refed in remaining week days showed an improved dry matter digestibility than those abundantly fed control (Xiao *et al.*, 2012). Since Qian *et al.* (2000), Tian and Qin (2004), Wang *et al.* (2000), Wang *et al.* (2005) and we collected the feces by siphoning, the collection method may not be considered as a contributing factor to the observed differences between studies. There seems to be

another factor involved, such as species, size, starvation length, etc. In the present study, the severity of the starvation period appears to have played the major role. Indeed, as stated above, digestive organs of fish undergoing starvation are diminished in size, functions of enzyme activities, and nutrient absorption capacity (Gaylord and Gatlin, 2000; Rios *et al.*, 2004; German *et al.*, 2010; Peres *et al.*, 2011). Therefore, there must be a restoration time for the effected organs upon refeeding (Ali *et al.*, 2001; Johansen *et al.*, 2001). Changes of body condition and organ indices in the present experiment are consistent with the literature cited above. It is noteworthy that the CF, VSI, HSI and PCI were drastically reduced with the starvation periods, but quickly recovered after two weeks of refeeding, interestingly coinciding with the amelioration of ADCs for dry matter, lipid, and energy. Our results suggest that regardless of starvation length, rainbow trout have the ability to restore organ sizes within as early as two weeks after full feeding, but the restoration of nutrient ADCs appears to be dependent on the severity of deprivation. However, an effect of possible change of gut microbiota during the deprivation phase on the following nutrient ADCs in the present experiment warrants further exploration because a substantial change in intestinal microbial flora in quantity and species diversity has been observed in starving Syrian hamsters by Sonoyama *et al.* (2009).

In the present experiment, muscle and liver RNA/DNA ratios were highly responsive to the severity of starvation lengths and there was a descending trend in both tissue samples with the magnitude of starvation at the end of the depletion phase. Also, we found a highly significant correlation between tissue RNA/DNA ratios and SGR_w during the starvation period, which is consistent with the published observations (McMillan and Houlihan, 1989; Miglavts and Jobling, 1989; Ali *et al.*, 2003; Tripathi and Verma, 2003). During the first two weeks of the refeeding period, muscle RNA/DNA ratio of starvation groups were higher than the control, but only significant in S4. The nucleic acid ratios of the treatments were highly correlated with SGR_w in the same period. In this phase, liver RNA/DNA ratio appeared to be more affected by previous starvation periods, being significantly higher in S2, S3 and S4 than S1 and C. However, liver nucleic acid ratios are more weakly associated with SGR_w in that period when compared with the muscle RNA/DNA ratio. This may be more related with, upon refeeding, a requirement of rapid tissue replenishments of organs that are seriously atrophied because of starvation (Farbridge *et al.*, 1992; Rueda *et al.*, 1998; Gaylord and Gatlin, 2000). This issue is particularly important for starved fish to provide increased capacity of digestion of the excessive amount of nutrient intake through the hyperphagic response. At the end of the experiment, the nucleic acid ratios in the starved fish muscle were

numerically higher than the control, and a significant but weak relationship between RNA/DNA ratio and SGR_w during refeeding was seen, suggesting a slowing of CG toward the end of the study. But it should be noted once again that there was still an increased appetite in S2, S3 and S4 and a superior SGR_w in S4 during the last two weeks of the refeeding period. Thus, RNA/DNA ratio should be carefully considered when associated with growth rate, since it represents a simplistic estimation of protein synthesis and growth (Miglavts and Jobling, 1989). Our results suggest that muscle liver RNA/DNA ratios may only be a reliable indicator of growth when a recent growth depression or acceleration period is experienced by fish.

In conclusion, rainbow trout under summer conditions respond to various lengths of starvation sessions by decreasing body weight, length, body lipid, protein, organ sizes, and CF as well as muscle and liver RNA/DNA ratio. Upon refeeding, organ sizes and ADCs of macro nutrients are recovered within the first two weeks of the refeeding period. During refeeding, the starvation induced hyperphagia and thereby CG, depending on the severity of deprivation; but S1 and S2 could catch up with the control fish, an opposite conclusion to most of the previous trout studies reporting a catch-up after more severe restriction periods under lower water temperatures. In addition, structural loss occurring at the end of more severe depletion periods cannot be compensated, which reflects to restoration of body mass. The correlation of muscle and liver RNA/DNA ratios with growth rate is high during either downward or upward growth manipulations, but poor when the effect of manipulation abates. Under summer conditions like in the present study, an application starvation period of longer than two weeks to evoke CG response as a management tool should be avoided. Future studies should deal with possible reasons of poor nutrient ADCs observed in starved fish during early weeks of realimentation session.

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