

The Effects of starvation and refeeding on growth and digestive enzymes activity in Caspian brown trout (*Salmo caspius* Kessler, 1877) fingerlings

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Abstract

240 Caspian brown trout (*Salmo caspius*) fingerlings with initial weight of 13.74 ± 0.63 g were stored in 300 L tanks to investigate the effect of starvation and refeeding on compensatory growth and digestive enzymes activity. The fish were introduced to four different periods of starvation during 10 weeks including control (with no starvation: C), 2 weeks (S2), 4 weeks (S4) and 6 weeks (S6) and then fed to satiation during the refeeding period. Sampling for growth and enzymes activity measurements was conducted three times: day 1 (T1), after starvation (T2), after 2 weeks (R2) and 4 weeks (R4) of refeeding. Results have indicated a significant decrease in growth performance after starvation ($p < 0.05$). Final weight (W_2) was similar to the control group in treatment of S2 after 2 weeks of refeeding. Body weight increasing (BWI) and specific growth rate (SGR) were the highest in treatment of S6 at the end of trial ($p < 0.05$). Trypsin, chymotrypsin, lipase and amino peptidase declined after starvation while pepsin activity significantly increased in deprived fish ($p < 0.05$). Trypsin and chymotrypsin values were lower in S6 than of control whereas the lowest activity of pepsin was observed in the control fish. After 4 weeks of refeeding, trypsin and chymotrypsin values were similar to that of the control ($p > 0.05$). Generally, results declared that Caspian brown trout fingerlings could recover digestive capacity after 2 weeks of starvation by appropriate refeeding with no negative impact on growth performance.

Keywords: *Salmo caspius*, Digestive enzyme, Starvation, Refeeding, Growth, Fingerling.

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Introduction

Feeding is one of the major ongoing costs in fish farming which allocated 40 to 60 percent of current expenditures (Mahmoudi *et al.*, 2009). Using feed deprivation period and refeeding are efficient ways to decrease manpower and food costs by inducing compensatory growth. This strategy can also decrease the environmental damage by reducing fish feed. When abundant food is available and followed by a period of food deprivation, compensatory growth occurs in which the growth rate is faster than usual (Ali *et al.*, 2003; Wang *et al.*, 2005; Jobling, 2010; Adaklı and Taşbozan, 2015) due to overeating, increased nutrient absorption and improved food conversion ratio (Boujard *et al.*, 2000). Food and digestive capacity effect on growth rate have been proved (Bélanger *et al.*, 2002; Ditlecadet *et al.*, 2009). Analysis of digestive enzyme activities could be used as an indicator of digestive capacity and nutritional condition of fish (Abolfathi *et al.*, 2012).

Wild fish may challenge with starvation because of food limitation in their environment (Fang *et al.*, 2017; Skrzynska *et al.*, 2017). Fish species showed different tolerate ability (Eslamloo *et al.*, 2017), different respond to feed deprivation and had variety of digestive enzymes changes depending on food availability and environmental factors (Furné *et al.*, 2008). For example, trypsin and chymotrypsin activity decreased 2 days after feed deprivation in pancreas of Atlantic salmon (*Salmo salar*)

(Krogdahl and Bakke-McKellep, 2005). Moreover, lipase activity was affected by food deprivation in rainbow trout (*Oncorhynchus mykiss*) and decreased during experimental period (Imani and Iranparast, 2010). Fish digestive system is directly related to species and food regime. Nutrient digestion and absorption, which determine level of access to the nutrient needed for biological activity, are important processes in animal's metabolism. Study of digestive enzymes is important considering their roles in nutrient absorption and growth performance. Information about development of digestive enzymes could be useful to recognize growth-limiting factors, decrease mortality and formulate appropriate diet and could be as an indicator of digestive activity and nutritional condition in fish (Bolasina *et al.*, 2005; Gisbert *et al.*, 2009).

Caspian brown trout (*Salmo caspius*) from Salmonid family is one of the most valuable species in Iran, because of good flavor and texture. In the recent years, *S. caspius* was listed as an endangered species due to overfishing and destruction of its settlement and spawning sites (Naderi and Abdoli, 2005). Knowledge of nutritional requirements is necessary for farming and, as a result, protection of this species. Study of digestive enzymes activity can lead to predict requirements of this species (Furné *et al.*, 2005). Trypsin is an important protease enzyme (Einarsson *et al.*, 1996) that activates some enzymes like chymotrypsin (Jobling, 1995). Pancreatic enzymes including

chymotrypsin and trypsin along with intestinal and stomach enzymes including amino peptidase and pepsin act in protein digestion while lipase secreted from pancreas which hydrolyzes fats. In response to starvation and feed deprivation, some digestive enzymes activities can change which help fish digestive system (Gisbert *et al.*, 2011). The effects of food deprivation, starvation and refeeding on growth and some physiological factors have been studied in many species including Atlantic salmon (*Salmo salar*) (Krogdahl and Bakke-McKellep, 2005), Nile tilapia (*Oreochromis niloticus*) (Nebo *et al.*, 2013), Caspian trout (*S. caspius*) (Khodabandeh *et al.*, 2013), Persian sturgeon (*Acipenser persicus*) (Yarmohammadi *et al.*, 2015), Gilthead sea bream (*Sparus aurata*) (Skrzynska *et al.*, 2017), tongue sole (*Cynoglossus semilaevis*) (Fang *et al.*, 2017), Tinfoil barb (*Barbonymus schwanenfeldii*) (Eslamloo *et al.*, 2017) and Nile tilapia (*Oreochromis niloticus*) (Moustafa and Abd El-Kader, 2017), but the relation between compensatory growth and digestive enzymes activity in Caspian brown trout (*S. caspius*) has not been studied yet, so this study aimed to find the effects of starvation on growth and digestive enzymes in Caspian brown trout (*S. caspius*). As a result of this study, we could evaluate the response capacity of this fish to starvation and their ability to recover these alterations during refeeding and recommend the appropriate periods of starvation and refeeding without negative impact on

growth and digestive enzyme activity as well as probable compensatory growth.

Material and methods

Fish and trial conditions

Two hundred and forty Caspian brown trout fingerlings with an initial average weight of 13.74 ± 0.63 g were obtained from Kelardasht Institute of Rearing and Breeding Salmons and transferred to rearing center of Sari Agricultural Sciences and Natural Resources University. Fish were adapted to a new environment for 2 weeks and fed with the commercial diet of rainbow trout (Biomar, Denmark) up to satiation two times daily. Then, fish were randomly distributed in twelve fiberglass tanks (20 fish in each 300 L tank). The experimental period continued for 10 weeks and was divided into two periods including a starvation period (week 1 to 6) and a refeeding period (week 6 to 10). The control group (C) was fed to satiation two times daily throughout the experimental period. Starvation was timed so that the end of starvation period occurred at the end of week 6. General schematic of the experimental design is shown in Table 1. The experimental design was a factorial completely randomized design with two factors (starvation period and sampling occasion). Starvation was examined in four levels: zero (C), 2 weeks (S2), 4 weeks (S4) and 6 weeks (S6) and then fed during 4 weeks of refeeding and sampling occurred at 4 levels: day1 (T1), after starvation (T6), 2 weeks (R2) and 4 weeks (R4) after refeeding. All treatments in this study were defined and abbreviated in Table 2. For

growth parameters, all the fish in each tank were sampled at all sampling occasions but for digestive enzymes activity analysis, randomly 4 fish in each tank were sampled at all sampling date except 2 weeks after refeeding. Fish were fed up to satiation 2 times daily with a commercial diet of rainbow trout (Biomar, Denmark) containing 45.97 % protein, 18 % lipid, 8.22 % moisture and 5.87 % ash based on the experimental design. Feed proximal analysis was done by the method of AOAC, 2005. Analysis of Uneaten feed

has collected and used for growth indices measurement (Adel *et al.*, 2016; Adel *et al.*, 2017). The whole water was daily exchanged with fresh water. Some water physicochemical parameters such as temperature, DO and pH were measured daily and nitrite was measured weekly. The average water temperature, DO, pH and nitrite concentration during the study were 14.5 ± 0.27 °C, 6.34 ± 0.34 mg L⁻¹, 8.44 ± 0.17 and 0.15 mg L⁻¹, respectively.

Table 1: General schematic of the experimental design.

Treatments	Feeding (F), starvation (S) and Refeeding (Re)									
	*					*		*		*
Control	F	F	F	F	F	F	F	F	F	F
2wS	F	F	F	F	S	S	Re	Re	Re	Re
4wS	F	F	S	S	S	S	Re	Re	Re	Re
6wS	S	S	S	S	S	S	Re	Re	Re	Re

wS: weeks of starvation, *: sampling time (day 1, after starvation, after 2 weeks of refeeding and After 4 weeks of refeeding)

Table 2: Experimental treatments and their abbreviations

Abbreviation	Experimental treatments
0wS:be	Control with zero week of starvation which has analyzed at the beginning of the experiment or day 1
0wS:aS	zero week of starvation which has analyzed after starvation
0wS: 2wRe	zero week of starvation which has analyzed after 2 weeks of refeeding
0wS: 4wRe	zero week of starvation which has analyzed after 4 weeks of refeeding
2wS:be	2 weeks of starvation which has analyzed at the beginning of the experiment or day 1
2wS:aS	2 weeks of starvation which has analyzed after starvation
2wS: 2wRe	2 weeks of starvation which has analyzed after 2 weeks of refeeding
2wS: 4wRe	2 weeks of starvation which has analyzed after 4 weeks of refeeding
4wS:be	4 weeks of starvation which has analyzed at the beginning of the experiment or day 1
4wS:aS	4 weeks of starvation which has analyzed after starvation
4wS: 2wRe	4 weeks of starvation which has analyzed after 2 weeks of refeeding
4wS: 4wRe	4 weeks of starvation which has analyzed after 4 weeks of refeeding
6wS:be	6 weeks of starvation which has analyzed at the beginning of the experiment or day 1
6wS:aS	6 weeks of starvation which has analyzed after starvation
6wS: 2wRe	6 weeks of starvation which has analyzed after 2 weeks of refeeding
6wS: 4wRe	6 weeks of starvation which has analyzed after 4 weeks of refeeding

Growth performance and feed efficiency

For calculating of growth performance, some indices including length, weight, hepatic weight and viscera weight were determined at all sampling time (day 1,

after starvation, after 2 weeks of refeeding and After 4 weeks of refeeding). Growth parameters including specific growth rate (SGR), body weight increasing (BWI), feed conversion ratio (FCR), condition

factor (CF), hepatosomatic index (HSI), visceral somatic index (VSI) and feed conversion ratio were measured according to formula that are given below:

SGR=100 (ln final weight–ln initial weight)/time, BWI=100 (final body weight–initial body weight)/initial body weight, FCR=100 (Feed intake/weight gain), CF= (100 total weight)/L³, HSI= (100 hepatic weight)/total weight, VSI= (100 visceral weight)/total weight (Kestemont *et al.*, 2007).

Determination of enzymes activity

For digestive enzymes activity measurement, brown trout were overdosed with 250 mg L⁻¹ of clove oil solution. Afterward, digestive tract has been completely separated from the body and moved to liquid nitrogen container immediately. Then, the samples were transferred to a -80 °C freezer (GFL) and stored until utilization.

Pancreatic (trypsin, chymotrypsin and lipase) and gastric enzymes extract were obtained via method of Furné *et al.* (2008). 1 g of tissue was homogenized at 0–4 °C using an electric homogenizer (T18, Germany) with 4 ml Tris–HCl buffer and 50 mM CaCl₂, 20 mM KCl (pH=8.5). The homogenates were centrifuged at 30000 g at 4 °C for 30 min (D78532, Germany). The supernatant was extracted after centrifugation and stored at –80 °C.

Intestinal enzyme extract (amino peptidase) was collected using 50 mM Manitol and 2 mM Tris–HCl buffer in a proportion of 1:20 and centrifuged at

22000 g at pH 7 for 30 min (Cahu and Zambonino Infante, 1995). After homogenizing by the methods of Crane *et al.* (1979), 0.1 M CaCl₂ was added and centrifuged at 11000 rpm for 10 min and the supernatant was collected for enzyme assessment.

Trypsin activity has evaluated by *N*-α-benzoyl dl-arginine-*p*-nitroanilide (BAPNA) as substrate. 25 µl enzyme extract was mixed with 1.25 ml substrate solution and incubated at 37 °C for 1 min. Then, 0.5 ml of acetic acid 30% was added in order to stop the reaction. Finally, the absorbance of supernatant was measured using a spectrophotometer at 410 nm (Erlanger *et al.*, 1961).

Chymotrypsin activity was measured by adding 590 µl Succinyl-(Ala) 2-Pro-Phe-*p*-nitroanalidine (SAPNA) solution to 10 µl pancreas extract at 25 °C (Erlanger *et al.*, 1961).

Assessment of lipase activity was conducted according to Iijima and Tanaka (1998) method. Five µl of digestive extract was added to 0.5 ml substrate solution at 30 °C for 15 min. Then 0.7 ml of acetone-*n* heptane solutions (with a ratio of 5:2) was added in order to stop the reaction and centrifuged at 6080 g and 4 °C for 2 min. At final step, the absorbance of the supernatant was measured at 405 nm.

Activity of amino peptidase was determined by adding 100 µl intestinal enzyme extract to 900 µl L-leucine P-Nitroanilide substrate. Then the absorbance then was measured every minute for 5 min at 405 nm (Prescott and Wilkes, 1976; Spungin and Blumberg, 1989). For determination of

pepsin, hemoglobin powder used as substrate and the absorbance was read at 280 nm according to Anson (1938).

Statistical analysis

Normality distribution of data was firstly checked by Kolmogorov-Smirnov test, and then a two-way ANOVA was used to specify the effect of starvation period and sampling occasion with their interaction on growth and enzyme activity. Duncan's test was utilized to compare any differences among means when $\alpha=0.05$. All data were reported as mean \pm SD and analyzed using a statistical package of SPSS ver. 22.

Results

Growth performance and feed utilization

Results of growth performance and feed efficiency demonstrated significant changes after starvation ($p<0.05$; Table 3). The most values of final weight (W_2), SGR, BWI, HSI and VSI were observed after starvation in the control (C: T2) whereas it was lower after 4 weeks of refeeding (C: R4). After 2 weeks of refeeding (R2), FCR value was higher in the control (C: R2, $p<0.05$), whereas the lowest value was observed in treatment of 2 weeks of starvation (S2: R2). Survival rate was similar among all groups during the trial ($p>0.05$). After 2 weeks of refeeding (R2), BWI and SGR were significantly lower in the control (C: R2) than those of other treatments (S2: R2, S4: R2 and S6: R2). FCR showed no significant differences between the fish exposed to starvation for 2(S2) and

4(S4) weeks in all sampling times ($p>0.05$) but the S2 fish showed higher FCR after 4 weeks of refeeding (R4) compared to the other times (T2 and R4, $p<0.05$). 6 weeks of starvation (S6: R2) led to a significant increase in HSI and VSI. After 4 weeks of refeeding, 6 weeks of starvation (S6: R4) showed higher BWI and SGR than those of the other groups (S0: R4, S2: R4 and S4: R4). Moreover, HSI in the control group (C: R4) was significantly lower than that of S4 (S4: R4) and S6 fish (S6: R4, $p<0.05$).

According to Table 3, study of growth performance at different times of sampling occasions showed higher BWI and SGR in the control group after starvation period (C: T2, $p<0.05$). Values of BWI and SGR in treatments of S2: R2, S4: R2 and S6: R2 were higher than S2: T2, S4: T2 and S6: T2 ($p<0.05$). BWI and SGR have increased significantly in S6: R4 than treatments of C: R4, S2: R4 and S4: R4. FCR has showed the lowest value in S6: T2 and S6: R4, whereas, after 2 weeks of refeeding, lower level of FCR was observed in 2 weeks starvation (S2: R2) which showed no significant differences with the time of after starvation (S2: T2). There were no significant differences in S4 fish at different times of sampling (S4; T2, S4: R2 and S4: R4, $p>0.05$). Lower value of HSI was observed in the brown trout treated by S6 after starvation (S6: T2) and it was significantly different from the other sampling times ($p<0.05$). VSI was higher in S2: R4, which was significantly different from S2: T2 ($p<0.05$). In treatments of 4 and 6

weeks of starvation, higher value of VSI has been found after 2 weeks of refeeding (S4: R2 and S6: R2). Additionally, CF decreased significantly in S2: T2 and the lower value has been observed in S4: T2 and S6: T2 ($p<0.05$).

Results of two-way ANOVA analysis showed significant differences in times

of sampling and starvation periods on growth parameters except CF ($p<0.05$; Table 3). Interaction effect between times of sampling and starvation periods was significant in all the studied parameters except CF and final weight ($p<0.05$).

Table 3: Growth performance and feed efficiency of brown trout after starvation and refeeding.

Parameters	Treatment	sampling times				p value		
		Day 1	After starvation (aS)	After 2 weeks of refeeding (2wRe)	After 4 weeks of refeeding (4wRe)	starvation periods	Sampling time	starvation periods × Sampling time
W ₁ (g)	Control	14.55±0.82 ^{Aa}	32.28±3.37 ^{Bb}	42.66±4.63 ^{Cc}	-	0.00	0.00	0.00
	2wS	13.39±0.87 ^{Aa}	22.07±1.68 ^{Cb}	37.43±3.37 ^{Cc}	-			
	4wS	14.04±0.30 ^{Aa}	15.95±0.94 ^{Ba}	26.89±3.84 ^{Bb}	-			
	6wS	12.97±0.53 ^{Aa}	9.87±1.29 ^{Aa}	15.24±1.29 ^{Aa}	-			
W ₂ (g)	Control	-	32.28±3.37 ^{de}	42.66±4.63 ^f	21.35±5.72 ^g	0.00	0.00	0.214
	2wS	-	22.07±1.68 ^C	37.43±3.37 ^{ef}	48.41±5.07 ^g			
	4wS	-	15.95±0.94 ^B	26.89±3.84 ^{cd}	33.77±2.43 ^{ef}			
	6wS	-	9.87±1.29 ^a	15.24±1.29 ^{cd}	27.67±1.00 ^{cd}			
BWI (%)	Control	-	121.47±13.79 ^D	32.13±3.11 ^A	20.40±4.21 ^A	0.001	0.012	0.00
	2wS	-	64.80±1.90 ^C	69.24±2.50 ^B	29.22±2.48 ^A			
	4wS	-	13.61±5.22 ^B	67.95±15.14 ^B	42.35±21.22 ^A			
	6wS	-	-3.97±8.43 ^A	55.55±17.55 ^B	82.11±10.15 ^B			
CF (g cm ⁻³)	Control	-	1.05±0.11 ^{bc}	1.05±1.03 ^{bc}	0.93±0.16 ^{abc}	0.317	0.00	0.173
	2wS	-	0.82±0.15 ^{ab}	1.10±0.08 ^c	0.95±0.07 ^{abc}			
	4wS	-	0.74±0.08 ^a	1.06±0.07 ^{bc}	0.98±0.15 ^{bc}			
	6wS	-	0.71±0.00 ^a	1.05±0.26 ^{bc}	0.92±0.13 ^{abc}			
SGR (% day ⁻¹)	Control	-	1.89±0.15 ^D	1.87±0.26 ^A	1.32±0.25 ^A	0.087	0.00	0.00
	2wS	-	1.18±0.02 ^C	3.37±0.10 ^B	1.83±0.13 ^{AB}			
	4wS	-	0.30±0.11 ^B	3.68±0.66 ^B	2.74±0.05 ^B			
	6wS	-	-0.66±0.25 ^A	3.12±0.81 ^B	4.27±0.39 ^C			
FCR	Control	-	1.26±0.17 ^B	1.96±3.03 ^B	1.79±0.28 ^A	0.002	0.034	0.001
	2wS	-	1.48±0.30 ^B	1.32±0.12 ^A	1.78±0.15 ^A			
	4wS	-	2.34±1.26 ^B	1.61±0.39 ^{AB}	1.78±0.15 ^A			
	6wS	-	0 ^A	1.60±0.15 ^{AB}	1.53±0.11 ^A			
HSI (%)	Control	-	1.62±0.28 ^B	1.42±0.45 ^A	1.55±0.28 ^A	0.001	0.00	0.00
	2wS	-	1.01±0.07 ^A	1.16±0.14 ^A	1.77±0.05 ^{AB}			
	4wS	-	0.83±0.10 ^A	2.05±0.67 ^A	2.12±0.36 ^B			
	6wS	-	0.68±0.15 ^A	3.47±0.82 ^B	2.23±0.14 ^B			
VSI (%)	Control	-	8.92±1.09 ^C	8.54±1.38 ^A	8.76±0.84 ^A	0.026	0.00	0.00
	2wS	-	7.25±0.60 ^B	9.0±1.54 ^{AB}	11.66±3.07 ^A			
	4wS	-	6.01±0.24 ^B	10.51±2.99 ^A	9.8±1.43 ^A			
	6wS	-	4.68±1.19 ^A	16.71±0.29 ^B	11.17±0.58 ^A			

wS: weeks of starvation

*Different superscript and subscript letters show significant differences in starvation period and sampling time, respectively ($p<0.05$).

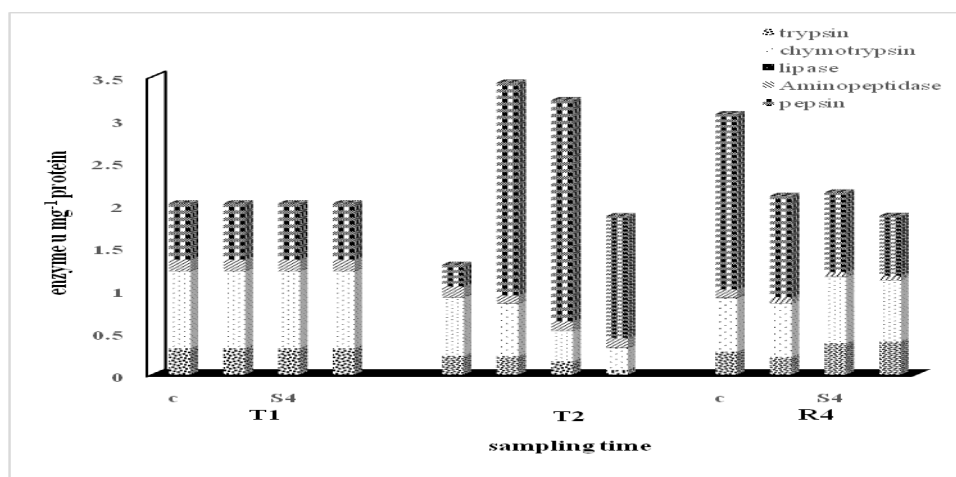


Figure 1: Comparison of digestive enzyme activity of samples (4 treatments including: C: control, S2, S4 and S6: 2 weeks, 4 weeks and 6 weeks of starvation, respectively) during the experimental period (sampling time including: T1: day1, T2: after starvation and R4: after 4 weeks of refeeding).

Digestive enzymes

Study of digestive enzymes activity including trypsin, chymotrypsin, lipase, amino peptidase and pepsin has shown in Table 4 and Fig. 1. After starvation, trypsin activity was higher in the control group whereas lower value was observed in S6 (S6: T2, $p < 0.05$). There were no significant differences among C: T2, S2: T2 and S4: T2 ($p > 0.05$). Specific activity of chymotrypsin in S2 and S4 fish showed higher value on first day (S2: T1 and S4: T1, $p < 0.05$). After starvation, chymotrypsin was lower in 6 weeks starvation (S6: T2) while the higher level has been detected in S4: R4 ($p < 0.05$). Moreover, in S4 and S6 treatments, it was higher after 4 weeks of refeeding (S4: R4 and S6: R4) than the time after starvation (S4: T2 and S6: T2) and similarly to the value of enzyme on first day (S4: T1 and S6: T1, $p > 0.05$). Determination of lipase showed lower value in treatment of S6: R4 ($p < 0.05$). There were no significant difference among other groups and also among experimental treatments at

different sampling times ($p > 0.05$). In addition, the value of aminopeptidase decreased significantly after 4 weeks of refeeding compared to first day and after starvation. The lowest value of this enzyme was observed in S6: R4 S4: R4, S2:R4 and the highest value has determined in C: R4, respectively. Specific activity of pepsin increased in those fish that experienced starvation (S2: T2, S4: T2 and S6: T2) than the control group after starvation period (C: T2). No significant change in pepsin activity occurred after 4 weeks of refeeding among treatments (C: R4, S2: R4, S4: R4 and S6: R4, $p > 0.05$).

As shown in Table 4, two-way ANOVA analysis showed significant effect of time on trypsin, chymotrypsin, amino peptidase and pepsin ($p < 0.05$) while, the effect of starvation period was not significant ($p > 0.05$). Furthermore, the interaction of sampling time and starvation periods was significant in trypsin and pepsin ($p < 0.05$) whereas, there was no significant effect in chymotrypsin and

amino peptidase ($p>0.05$). Similarly, the effects of time, starvation period and their interaction were not significant in lipase activity.

Table 4: Digestive enzyme activity of brown trout after starvation and refeeding.

Parameters	Treatments	sampling times			starvation periods	p value	
		Day 1	After starvation (aS)	After 4 weeks of refeeding (4wRe)		Sampling time	starvation periods × Sampling time
Trypsin U mg ⁻¹ protein	Control	0.308±0.05 ^{Aa} *	0.219±0.07 ^{Ba}	0.266±0.11 ^{Aa}	0.748	0.00	0.007
	2wS	0.308±0.05 ^{Aa}	0.215±0.04 ^{Bb}	0.206±0.07 ^{Ab}			
	4wS	0.308±0.05 ^{Ab}	0.154±0.05 ^{Ba}	0.367±0.09 ^{Ab}			
	6wS	0.308±0.05 ^{Ab}	0.053±0.02 ^{Aa}	0.387±0.08 ^{Ab}			
Chymotrypsin U mg ⁻¹ protein	Control	0.906±0.14 ^c	0.687±0.15 ^{bc}	0.634±0.22 ^{abc}	0.945	0.00	0.100
	2wS	0.906±0.14 ^c	0.619±0.21 ^{abc}	0.629±0.25 ^{abc}			
	4wS	0.906±0.14 ^c	0.361±0.08 ^{ab}	0.781±0.42 ^c			
	6wS	0.906±0.14 ^c	0.258±0.20 ^a	0.721±0.2 ^c			
Lipase U mg ⁻¹ protein	Control	0.003±0.001 ^{abc}	0.0042±0.001 ^{bc}	0.005±0.001 ^c	0.068	0.912	0.233
	2wS	0.003±0.001 ^{abc}	0.003±0.001 ^{abc}	0.002±0.0 ^{abc}			
	4wS	0.003±0.001 ^{abc}	0.0022±0.008 ^{abc}	0.0033±0.001 ^{abc}			
	6wS	0.003±0.001 ^{abc}	0.0031±0.001 ^{abc}	0.0017±0.0 ^a			
Aminopeptidase U mg ⁻¹ protein	Control	0.134±0.01 ^d	0.126±0.02 ^{cd}	0.096±0.03 ^{bc}	0.080	0.00	0.245
	2wS	0.134±0.01 ^d	0.094±0.02 ^{bc}	0.073±0.01 ^{ab}			
	4wS	0.134±0.01 ^d	0.108±0.02 ^{cd}	0.053±0.09 ^a			
	6wS	0.134±0.01 ^d	0.116±0.009 ^{cd}	0.052±0.01 ^a			
Pepsin U mg ⁻¹ protein	Control	0.660±0.18 ^{Ab}	0.254±0.09 ^{Aa}	2.06±1.37 ^{Ab}	0.408	0.006	0.003
	2wS	0.660±0.18 ^{Aa}	2.5±0.54 ^{Bb}	1.19±0.77 ^{Ab}			
	4wS	0.660±0.18 ^{Aa}	2.6±0.67 ^{Bb}	0.935±0.32 ^{Aa}			
	6wS	0.660±0.18 ^{Aa}	1.43±0.47 ^{Ba}	0.703±0.40 ^{Aa}			

wS: weeks of starvation

*Different superscript and subscript letters show significant differences in starvation period and sampling time, respectively ($p<0.05$).

Discussion

Results of the present study showed that significant changes in growth performance of Caspian brown trout (*S. caspius*) fingerlings could be attributed to different feeding strategies. After starvation period, all of growth factors declined significantly ($p<0.05$). After 2 weeks of refeeding, final weight (FW) in S2 (S2: R2) reached to the control (C: R2) while, in other treatments (S4: R2 and S6: R2) it was lower than the control group (C: R2). BWI and SGR increased in those treatments that passed starvation as compared to the control with compensatory growth that lasted 4 weeks of refeeding in treatment of S6 (S6: R4). In the study done by Taheri and Aliasghari (2012) on *Rutilus rutilus caspicus* with four sporadic

times of food deprivation and refeeding (24, 48, 72 and 96h fasting and refeeding consecutively) had lower growth performance like BWI and SGR than the control group. This disagreement might be due to differences in their natural environment as Caspian brown trout may occasionally challenge with starvation which make it adopted to starvation periods.

Despite the high values of BWI and SGR in the starved fish, final weight (W_2) was not as high as the control group (C: R4) except for S2: R4. It could be stated that long terms of starvation are needed to be compensated by longer duration of refeeding. As compensatory growth in Atlantic cod (*Gadus morhua*) led to

increased final weight in the groups of 1-3 weeks starvation after 22-33 days of refeeding (Jobling, 1995). Study on Atlantic halibut (*Hippoglossus hippoglossus*: 11D (days of deprivation): 20R (days of refeeding), 14D: 22R, 16 D: 28 R and 32 D: 67 R) and hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*: 1, 2 and 4 weeks of starvation and 4 weeks of refeeding) showed low compensation in growth and final weight which was lower than that of the control, in agreement with our results (Wang *et al.*, 2005; Heide *et al.*, 2006). In the present study, final weight in 2wS: 4wRe against other groups (S4: R4 and S6: R4) reached the control (C: R4), indicating more capability of this species for compensatory growth. In addition, periods of starvation, age and developmental stage of fish can affect the performance of compensatory growth. In most experimental species with long duration of refeeding, fish could compensate the growth (Aliasghari *et al.*, 2013). Using 1 (D1), 2 (D2) and 4 (D4) days of starvation followed by refeeding of 3 (R3, R6 and R12), 7 (R7, R14 and R28) and 11 (R11, R22 and R44) folds of starvation days in tongue sole *Cynoglossus semilaevis* showed complete compensatory growth in D1R11, D2R14 and D2R22 (Fang *et al.*, 2017). Adaklı and Taşbozan (2015) observed partial compensatory growth of sea bass (*Dicentrarchus labrax*) starved for 2 days and refed for 8 days (5 cycles of 2 days' starvation/8 days of refeeding). *Barbonymus schwanenfeldii* could not get back to its normal growth after 5

weeks of refeeding in long terms of food deprivation except for those with 1-week deprivation. Species demonstrates different feeding behavior in time of starvation that affects feeding intake and growth performance (Ali *et al.*, 2003). Asian sea bass (*Lates calcarifer*) showed no significant differences in growth and feeding performance exposed to different regimes of starvation and refeeding including control, 4 days of starvation followed by 16 days of refeeding (2 cycles) and 8 days of starvation followed by 32 days of refeeding (one cycle) (Azodi *et al.*, 2016). Moustafa and Abd El-Kader (2017) reported no significant differences in growth of Nile tilapia (*Oreochromis niloticus*) exposed to 4, 7 and 10 days of starvation followed by 30 days of refeeding and longer period of starvation caused negative effects on growth. Results of feed efficiency ratio in the present research showed an improvement in Caspian brown trout growth after starvation. Starvation led to FCR= 0, in S6: T2 which caused negative growth after 6 weeks of starvation. Falahatkar *et al.* (2009) obtained different results in great sturgeon (*Huso huso*) and observed growth. These contradictory results are due to different responses of species to starvation.

Condition factor is a factor represented fish physiological state (Řehulka, 2000). In the present research, condition factor declined after starvation and then increased to the same as the control group after refeeding in all treatments. Falahatkar *et al.* (2009) has reported similar results

in *H. huso*. Furthermore, visceral index and hepatosomatic index decreased significantly after starvation that may be due to the utilization of glycogen and fat resources in liver during starvation resulting in weight loss. HSI values increased 2 weeks after refeeding in all fish experienced starvation and reached to the maximum in 6wS (6wS: 2wRe) and after 4 weeks of refeeding, in S4 (S4: R4) and S6 (S6: R4) showed higher HSI than the control (C: R4). The reason seems to be polyphagia in long terms of feed deprivation. This result is similar to the findings reported by Wang *et al.* (2005). Moreover, lack of food leading to use protein resources (Krogdahl and Bakke-McKellep, 2005) in the intestine, could explain VSI value after feed deprivation. VSI value showed no significant difference among treatments after 4 weeks of refeeding. Zaefarian *et al.* (2016) reported that the whole body protein content of Caspian brown trout (*S. caspius*) in treatments of S2: R2 and S4: R2 showed no significant difference compared to the C: R2. This can be inferred that Caspian brown trout can tolerate starvation for 4 weeks without any negative impact on whole body protein contents.

Digestive enzymes and their responses to environmental changes such as starvation are good indicators of feeding conditions (Imani *et al.*, 2010). Trypsin, as a protease enzyme, plays a role in activating and stimulating the reactions related to the growth. In the present study, activity of trypsin declined in starved fish compared to the control group. This difference between

the control and other experimental fish was not detected in Atlantic salmon (*S. salar*) which might be because of feeding along starvation period (Rungruangsak-Torrissen *et al.*, 2006). Similarly, Zeng *et al.* (2012) found that trypsin decreased thorough food deprivation. After 4 weeks of refeeding, no significant differences have been observed in trypsin activity between experimental fish and control fish which was in agreement with the study performed on rainbow trout (*O. mykiss*) and Japanese flounder larvae (*Paralichthys olivaceus*) (Bolasina *et al.*, 2005; Imani *et al.*, 2010). In addition, Gisbert *et al.* (2011) observed that trypsin reduced after 40 days of starvation and then compensated after 30 days of refeeding as we found here. Trypsin activity was not affected by fasting and refeeding in *Rutilus rutilus caspicus*. Fang *et al.* (2017) reported higher trypsin activity in tongue sole *Cynoglossus semilaevis* starved for 2 days followed by 22 days of refeeding as compared to the control group and this treatment introduced as the optimum starvation and refeeding strategy.

Effect of starvation on chymotrypsin activity showed a significant decrease in 6wS whereas it declined numerically in S2: T2 and S4: T2. Chymotrypsin activity enhanced in S4 and S6 after 4 weeks of refeeding (S4: R4 and S6: R4) and was similar to the control. This improvement declared that 4 weeks is adequate time to recovery chymotrypsin enzyme. Since specific growth rate was higher in treatments of S4 and S6 after two and four weeks of refeeding, this

result could also be explained by high enzyme performance and increased protein synthesis to use for growth. The important factor associated to growth rate is proteolytic capacity of fish digestive system (especially trypsin activity) (Ditlecadet *et al.*, 2009). These results were different to those reported by Imani *et al.* (2010) who showed that chymotrypsin increased in the control, 1 and 2 weeks starved rainbow trout after 4-8 weeks of refeeding. Since, longer refeeding may have induced time to secrete more enzymes; it is possible for Caspian brown trout to indicate a chymotrypsin increasing trend in long terms of refeeding. Moreover, Abolfathi *et al.* (2012) declared a chymotrypsin reduction in 3 weeks fasted juvenile roach (*R. rutilus caspicus*) after refeeding which might be due to destruction in parts of pancreatic tissue impressed by starvation (Ueberschär, 1993; Tanaka *et al.*, 1996; Gawlicka *et al.*, 2000). In the present study chymotrypsin increased after 4 weeks of refeeding which shows more ability of Caspian brown trout to recovery the pancreatic tissues. Fat is important in carnivorous diet to supply energy (Iijima *et al.*, 1998). During digestion process, triglycerides are hydrolyzed by the action of pancreatic lipase (Tanchaorenrat, 2012). Starvation can effect on lipase activity. In the study done by Zeng *et al.* (2012), lipase activity declined to 52% after starvation. In the present study, long term starvation led to a decrease in lipase value after refeeding while no changes were found in S2: R4 and S4: R4 compared to the control (C: R4).

These results were similar to that reported by Bolasina *et al.* (2005) in which the lipase activity was used as an indicator of nutritional condition in Japanese flounder larvae (*Paralichthys olivaceus*). Similarly, lipase activity was affected by food deprivation (for 72 days) in rainbow trout (*O. mykiss*) and Adriatic sturgeon (*Acipenser naccerr*) and have changed after 60 days of refeeding (Furné *et al.*, 2008). Contradictory, Rivera-Pérez *et al.* (2010) observed an increase in lipase activity after 5 days of starvation and 24 hours of refeeding in whiteleg shrimp postlarvae (*Penaeus vannamei*). Different life history and nourishment in shrimps could also affect lipase activity.

Amino peptidase assay showed significantly a reduction after starvation period and also continued in 4 weeks of refeeding (S4: R4 and S6: R4) and did not reach the control (C: R4). Decreasing in some enzymes activity can help regulating digestive system that is a response to food deprivation (Gisbert *et al.*, 2011). This result was in accordance with those achieved in the work of Zeng *et al.* (2012), Krogdahl and Bakke-McKellep (2005) and Gisbert *et al.* (2011). It has been illuminated that temporary responses to food deprivation are different depending on intestine parts (Zeng *et al.*, 2012). On the opposite, amino peptidase was compensated completely in Atlantic salmon (*S. salar*) after a week of refeeding (Krogdahl and Bakke-McKellep, 2005). In a research on European glass eels (*Anguilla anguilla*), amino peptidase value

decreased after 5 days of starvation and had not change until 10 days. A sharp decline in the intestine enzymes affected by starvation shows more sensitivity of intestine to food deprivation than pancreas (Gisbert *et al.*, 2011). As Gisbert *et al.* (2011) reported normal value of amino peptidase activity after 30 days of refeeding while the present study showed different results. It could be obtained from these results that intestine enzymes of Caspian brown trout fingerlings are sensitive to starvation and maybe needed more time to recover.

Pepsin increase in Caspian brown trout during present experiment showed that this species possibly stores pepsin deactivated substrate (pepsinogen) in stomach during starvation to release it in digestive time. Similarly, study of 72 h of starvation followed by a 3-day refeeding on juvenile South catfish (*Silurus meridionalis* Chen) indicated an increase up to 106 percent in pepsin activity (Zeng *et al.*, 2012). On the contrary, researches on *Lutjanus sebae* juveniles (Youjun and Zewei, 2007) and *Monopterus albus* (Yang *et al.*, 2007) showed that pepsin decreased after 11 and 12 days of starvation, respectively. Pepsin activity in experimental fish did not have significant differences with the control meaning that food digestion does not impressed by starvation and refeeding in this experiment. The same results were observed in white sturgeon (*Acipenser transmontanus*) (Buddington and Doroshov, 1986) and

Atlantic salmon (*S. salar*) (Einarsson *et al.*, 1996).

Considering the results, it can be generally declared that 2 weeks of starvation was compensated after 4 weeks of refeeding but recovery of growth performance and body weight in longer periods of starvation needs more refeeding times, which should be considered, to study. Enzymatic results suggest that Caspian brown trout fingerlings could recovery digestive capacity after 2 and sometimes 4 weeks of starvation, which depends on adequate refeeding; implying that destructive impact could be affected by long terms of starvation and inappropriate nourishment. The results of digestive enzyme activity showed to be approximately related to the growth performance. Overall, it seems applying 2 weeks of starvation followed by 4 weeks of refeeding can have economic profit with positive effects on growth performance.

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