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Effects of a single-phase fasting period and subsequent re-feeding on compensatory growth, digestive enzyme activities, and antioxidant capacity of Sobaity (*Sparidentex hasta*) and Yellowfin seabream (*Acanthopagrus latus*)

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Running head: Compensatory growth in sparids

Abstract

An eight-week research was carried out to examine the influence of fasting (FA) and refeeding (RF) episodes on the compensatory growth responses (CGR) in sobaity (*Sparidentex hasta*, 10 g) and yellowfin seabreams (*Acanthopagrus latus*, 4.3 g) juveniles. Fish were fed with a commercial feed (contained 500 g kg^{-1} crude protein and 150 g kg^{-1} crude lipid) as following regimes: control (C, fish were fed three times every day), T_1 (two weeks of feeding, one week of FA, and five weeks of RF), T_2 (one week of feeding, two weeks of FA and five weeks of RF) and T_3 (three weeks of FA and five weeks of RF). Two hundred and forty *S. hasta* juveniles were stocked into twelve 300-L tanks (20 fish $tank^{-1}$), and 360 *A. latus* juveniles were allocated into other 12 tanks (30 fish $tank^{-1}$). Each treatment was carried out in triplicates for each species, and each tank held only one of the species. The experiment was carried out for both species simultaneously. The weight and length of fish from the four groups were measured individually after the third week (after FA episode) and after eight weeks (after RF episode). After finishing the RF episode (eighth week), six fish of each tank were sacrificed with an overdose of 2-phenoxyethanol (1000 mg L^{-1}), and the liver and the whole gut of the sacrificed fish were sampled, dissected, and then kept in a freezer (−80 °C) until further analyses. Survival rate was decreased in *S. hasta* juveniles with increasing the FA period mainly due to their cannibalistic behavior, which was triggered by starvation, but it was not affected in *A. latus*. The fasted groups in both species were significantly lost their weight after FA episodes. After five weeks of RF, *S. hasta* showed full compensatory growth response; meanwhile *A. latus* had a partial compensatory response ($P<0.05$). Hepatosomatic index value decreased after the FA period in both species, but it was restored to the normal level after RF phase. The activities of liver catalase, superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase were increased in T_2 group in *S. hasta*, but liver antioxidant enzymes were

not affected in *A. latus*. In both species, the amount of the lipid peroxidation was significantly increased in the liver of fish groups subjected to T_2 or T_3 compared to T_1 and control groups ($P < 0.05$). Liver alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in *S. hasta* fasted for two weeks were higher than the other groups. The activities of trypsin, chymotrypsin, α-amylase, and lipase in *S. hasta* fasted for a week (T_1) were higher than control. In addition, the activities of trypsin and chymotrypsin in *A. latus* fasted for two or three weeks were lower than C and T_1 groups. The activity of ALP was increased with increasing FA period in both species. The findings of the present study showed that single-phase FA episodes reduce survival and induce oxidative stress in *S. hasta* juveniles; meanwhile *A. latus* juveniles did not show complete compensatory growth after RF episode.

Key words: Compensatory growth response, feeding regime, growth metrics, hepatic antioxidant system, Sparidae

Feed cost is the main expenditure in aquaculture (*Ca*. 40%–70% of the total expenses) and profoundly affect the profitability margins of this industry (Henry et al., 2015). Thus, improving feed efficiency through feeding management can maximize economic gain. It has been proven that the application of feeding strategies based on fasting (FA) and re-feeding (RF) by activating compensatory growth response (CGR) can promote the feed efficiency in different farmed aquatic species and enhance the profit margins (Ali et al., 2003; Jobling 2010). In addition, fasting can be applied for different processes in aquaculture such as control of overproduction, reduction of feed and labor expenditures, correction of errors in the feeding programs (Krogdahl and Bakke-McKellep, 2005), final production quality (Favero et al., 2020), recuperation of stress (Davis and

Gaylord, 2011; Waagbø et al., 2017), overwintering of fish (Triebenbach et al., 2009), and prevention of infectious diseases (Mohapatra et al., 2017).

CGR is a period of fast growth that manifested after the food deprivation period and mainly depends on species, age (size), health condition, pre-and post-nutritional history, and severity of the feed deprivation and/or extension of refeeding period (Ali et al., 2003; Pérez-Jiménez et al., 2007). Depends on the above-mentioned factors, the degree of CGR in fish might differ as no compensation, partial, full and over CGR (Ali et al., 2003; Jobling 2010). CGR in fish after RF period mainly associated with hyperphagia, abatement of metabolism and energy costs, regulation of liver key enzymes (*e.g.* alanine aminotransferase, ALT), better feed conversion ratio due to the increment of digestive enzyme activities, promotion of protein synthesis and enhancing synthesis and release of appetite and growth regulatory hormones (*e.g.* insulin-like growth factors, ghrelin, and leptin) (Gaylord and Gatlin, 2001; Ali et al., 2003; Won and Borski, 2013; Viegas et al., 2014; Bertucci et al., 2019). On the other hand, it has been demonstrated that feed deprivation may induce oxidative stress in fish by overproduction of reactive oxygen species (ROS) and decreasing the antioxidant reserves in tissues, especially in the liver that will, in turn, lead to disturbing the effective neutralization of ROS (Morales et al., 2004; Pascual et al., 2003; Pérez-Jiménez et al., 2007). In this context, it has been reported that FA can provoke the antioxidative enzyme activities and upregulate their related gene expressions in fish (Zheng et al., 2016). For example, Pascual et al. (2003) found that partial (25 and 50% of normal feeding rate) or total feed deprivation for 46 days enhanced lipid peroxidation, antioxidant enzyme activities and content of oxidized glutathione in the liver of gilthead seabream (*Sparus aurata*). It should be emphasized that the liver has a crucial role in metabolic adjustments of energy during the feed deprivation period in fish by regulating different metabolic pathways (Pérez-Jiménez et al., 2007, 2012). In this regard,

Pérez-Jiménez et al. (2012) reported that ALT activity was decreased in the liver of starved common dentex (*Dentex dentex*) to reduce the excessive gluconeogenesis, but aspartate aminotransferase (AST) activity was increased to fuel ß-oxidation by oxaloacetate. Interestingly, these authors found that the activities of the above-mentioned enzymes in the liver of common dentex were returned to the normal basal levels after RF episode. In addition, a plethora of research has reported that digestive enzyme activities could be remarkably affected by FA and RF in fish, but there are discrepancies in these findings mainly due to species-specific responses and differences in FA and RF strategies (Harpaz et al., 2005; Krogdahl and Bakke-McKellep, 2005; Abolfathi et al., 2012; Zeng et al., 2012). For example, digestive enzyme activities were pronouncedly decreased during the FA period in European glass eels (*Anguilla anguilla*, Gisbert et al., 2011) and juvenile roach (*Rutilus rutilus caspicus*, Abolfathi et al., 2012). The reduction of digestive enzyme activities mainly occurred as a consequence of the lack of mechano-chemical stimulators of the gut (Li et al., 2007), drastic structural alternation of the gut (*e.g.* reduction of goblet cells counts, mucosal thickness, and villus fold or brush border height) (Rodríguez et al., 2005) and resorption of the digestive enzymes in the gut (Gisbert et al., 2011). On the contrary, Krogdahl and Bakke-McKellep (2005) reported that the activities of leucine-alanine peptidase and maltase in brush border membranes of enterocytes in the pyloric caeca were significantly decreased after two days of FA, then their specific activities gradually increased over 40 days of FA period that may be attributed to the reduction of total protein content in the tissue.

During the past decade, several research studies have been done to examine the influence of FA and RE strategies in marine fish species (Pérez-Jiménez et al., 2007; Zheng et al., 2016; Ziheng et al., 2017), especially in various sparids such as gilthead seabream (Peres et al., 2011; Yilmaz and Eroldoğan, 2011), common dentex (Pérez-Jiménez et al., 2012), black seabream

(*Acanthopagrus schlegelii*, Oh et al., 2013; Xiao et al., 2013) and red seabream (*Pagrus major*, Oh et al., 2007; Mohapatra et al., 2017).

Among the different sparid species in the Persian Gulf and Oman sea regions, yellowfin (*Acanthopagrus latus*) and sobaity seabream (*Sparidentex hasta*) are considered potential candidates for developing mariculture activities in Iran. There was a well knowledge regarding the requirements of macronutrients in these fish species (Mozanzadeh et al., 2017a); however, more studies should be conducted regarding their feeding management is scarce (Mozanzadeh et al., 2017b; Mozanzadeh et al., 2020). Thus, the current research was aimed to evaluate the effects of a single-phase FA and subsequent RE on CGR, antioxidative capacity, and digestive enzymes in *A. latus* and *S. hasta* juveniles.

Material and Methods

Experimental design

The current research was carried out in a private marine fish hatchery center (Sarbandar, Khuzestan, Iran; 30°32′N, 49°20′E). The research design was conducted as previously described for Asian seabass (*Lates calcarifer*, Tian and Qin (2004) and red porgy (Oh et al., 2007). In this regard, four different feeding programs were designed, including the control (C) in which fish were fed to apparent satiation three times a day (09:00; 12:00 and 16:00 h) for eight weeks. The other three feeding schedules were T_1 (two weeks of feeding, one week of FA, and five weeks of RF), T_2 (one week of feeding, two weeks of FA, and five weeks of RF), and T_3 (three weeks of FA, and five weeks of RF) (Fig. 1). During RF period, the feeding frequency applied in the treatments was similar to that of the control (three times a day; 09:00; 12:00 and 16:00 h). Two fish species were stocked separately into two 5000-L circular fiberglass tanks acclimated to the culture condition for two weeks. The husbandry system consisted of twenty-four 300-L cylindrical polyethylene tanks

supplied with sand-filtered and disinfected running seawater (*Ca*. 200% water exchange daily). The mean (mean ± standard deviation) values of water salinity, temperature, pH, and dissolved oxygen were 48.0 ± 0.5‰, 29.2 ± 0.5 °C, 7.7 ± 0.3 and 6.5 ± 0.8 mg L^{-1}, respectively. At the beginning of the experiment, fish were individually weighed to determine their initial body weight (BW_i). Two hundred and forty *S. hasta* juveniles (BW_i = 10 ± 0.1 g, mean ± standard error) were stocked into 12 tanks (20 fish $tank^{-1}$), and 360 *A. latus* juveniles (4.3 ± 0.0 g) were distributed into other 12 tanks (30 fish $tank^{-1}$). Each treatment had three replicates for each species, and each tank held only for one of the species. The experiment was carried out for both species simultaneously. Fish were fed on a commercial feed (particle size: 2-3 mm, 500 g kg^{-1} crude protein, 150 g kg^{-1} crude fat, 107 g kg^{-1} ash, and 40 g kg^{-1} fiber, Beyza Feed Mill 21) and ensure that no feed was left uneaten (*i.e.* feeding to visual satiation).

Sampling

The weight and length of fish from the four groups were measured individually after the third week (after the fasting episode) and after eight weeks (after the re-feeding episode). For determining the effects of FA and RF strategies on somatic indices, antioxidant, and digestive enzymes, after finishing the RF episode (eighth week), six fish of each tank were sacrificed with an overdose of 2-phenoxyethanol (0.5 ml L^{-1}) (Merck, Schuchardt, Germany; Dehghani et al., 2020) to measure their liver and viscera weights. In addition, the liver and the whole gut of the sacrificed fish were dissected on ice and snap-frozen with liquid nitrogen, then were kept in a freezer (−80 °C).

Growth and feed utilization

Standard formulae were used to determine growth performance, feed utilization, and somatic indices:

SGR: specific growth rate (%) = $((\ln BW_f - \ln BW_i) / t) \times 100$

WGR: weight gain rate (%) = $((BW_f - BW_i)/BW_i) \times 100$

Survival (%) = (number of fish in each group remaining on day 56/initial number of fish) × 100

Feed intake (FI) = total feed intake per tank (g) / number of fish

Relative feed intake (RFI) = total feed intake per tank (g) / feeding days

FCR: feed conversion ratio = (total feed intake per tank (g) /weight gain in each tank (g))

HSI: hepatosomatic index (%) = (liver weight (g) / BW_f (g)) × 100

VSI: viscerosomatic index (%) = (visceral weight (g) / BW_f (g)) × 100

K: Fulton's condition factor = (BW_f (g)/ standard length $(cm)^3$) × 100

BW_i and BW_f are initial body weight and final body weight, respectively and t is the experimental period = 56 days.

Growth compensation in fish experienced FA was calculated by the compensation coefficient (CC) as follows: $CC = \Delta T/\Delta C$. In this formula, ΔT is the mean weight gain (g) in the FA groups divided by the number of feeding days, meanwhile, ΔC was the mean weight gain (g) in the control group (c) divided by the number of feeding days. Therefore, values of CC >1.0 would indicate CGR for WG (Mattila et al., 2009). The feeding days for the C, T_1, T_2, and T_3 groups were 56, 49, 42, and 35 days, respectively.

Enzyme activities

The frozen livers were homogenized in ice-cold physiological buffer saline (1:10, w/v; NaCl 0.9%, pH 7.0) then centrifuged (2900 g, 15 min, 4 °C) and the supernatants were separated, aliquoted, and kept in – 80 °C (Jaroli and Sharma, 2005). The activity of the antioxidant enzymes including glutathione peroxidase (GPX) (Noguchi et al., 1973), superoxide dismutase (SOD) (McCord and Fridovich, 1969), and catalase (CAT) (Aebi, 1984) as well as thiobarbituric acid reactive substances (TBARs) concentrations (Buege and Aust, 1978) in the liver were measured according to standard methods. Liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured utilizing an auto-analyzer (Technicon RA-1000, Technicon Instruments) using commercial kits (Pars Azmoon Kit, Iran). Bradford method (Bradford, 1976) was used for determining the soluble protein content of the homogenates.

Digestive enzyme activities

The gut samples were homogenized according to Gisbert et al. (2016). The samples were homogenized in ice-cold Mannitol-Tris buffer (1:30, w/v) contained 50 mM Mannitol and 2 mM Tris-HCl (pH 7.0) for 30 s. After that, 100 µl of $CaCl_2$ (0.1 M) was added to the homogenate and centrifuged (10,000g, 10 min, 4 °C). Ultimately the supernatants were separated, aliquoted and kept at − 80 °C. Trypsin activity was measured with N-α-benzoyl-dlarginine-pnitroanilide (BAPNA) as substrate at 25 °C, and absorbance was recorded at 410 nm (Erlanger et al., 1961). Chymotrypsin activity was quantified at 25 °C using BTEE as substrate in 80 mM Tris-HCl, 100 mM $CaCl_2$ buffer (pH 7.2). Chymotrypsin activity (U) corresponded to the µmol BTEE hydrolyzed min^{-1} ml^{-1} of extract at 256 nm (Worthington, 1991). The alkaline phosphatase activity was measured according to the method described by Gisbert et al. (2018). The activity of α-amylase

was measured by starch (1% in a buffer contain 0.02 M Na_2HPO_4 and 0.006 M NaCl, pH 6.9) as substrate using the 3,5-dinitrosalicylic acid according to Worthington (1991). Bile-salt dependent lipase activity was measured using 0.4 mM 4-nitrophenylmyristate as substrate (at room temperature), according to Gawlicka et al. (2000).

Statistics

SPSS ver. 20 (Chicago, Illinois, USA) was used for data analyses. Data are presented as means ± standard error of the mean. Kolmogorov-Smirnov test was used to evaluate the normality of data, and Levene's test was applied to determine the homogeneity of variances. Significant differences among groups were evaluated by one-way ANOVA ($P < 0.05$), and the Tukey's post hoc test was used for multiple comparisons.

Results

Compensatory growth response

The survival rate and growth performance indices of *S. hasta* subjected to a single FA and subsequent RF were reported in Table 1. Survival rate decreased in *S. hasta* juveniles with increasing the FA mainly due to cannibalistic behavior. Thus, fish in T_3 group that experienced three weeks of FA had the lowest survival rate after FA (69.4 ± 8.0 %) and RF (52.8 ± 1.6 %) episodes ($P<0.05$). Fish in T_2 group which subjected to two weeks of FA, had a lower survival rate (86.1 ± 4.8%) compared to the C and T_1 groups after the FA episode, but after RF phase the survival rate in T_2 group did not differ from the C and T_1 groups ($P>0.05$). After the FA episode, *S. hasta* subjected to FA remarkably had lower WG and SGR than the C (Table 1). HSI in T_2 and

T_3 groups were lower than the other ones after the FA ($P<0.05$). VSI gradually increased with the increasing starvation period. Thus fish in the C and T_3 groups had the highest and the lowest VSI values, respectively ($P<0.05$). Condition factor did not affect by different FA and RF protocols. After RF episode, *S. hasta* juveniles showed complete CGR, and there were not significant differences in BW_f of fish ($P>0.05$). After the RF episode, SGR pronouncedly provoked, especially in T_3 that experienced long term FA period. Thus, fish in T_3 group demonstrated the highest SGR value (3.2 ± 0.2 %); meanwhile, fish in the C had the least value (1.5 ± 0.0 %). Fish in T_2 and T_3 remarkably got more WG than the other groups after the RF episode ($P<0.05$). Total FI in the C group was more than fish subjected to FA. However, RFI in T_3 was lower than in the other treatments ($P<0.05$). The feed conversion ratio in T_3 group was worse than the other experimental groups. After RF, somatic indexes (HSI, VSI, and K) were restored in fish that experienced FA same as the C ($P>0.05$).

Regarding *A. latus* juveniles, the survival rate did not affect by various FA ($P>0.05$, Table 2). After FA, fish that were subjected to FA had lower BW_f than the C. In addition, after the FA episode, the highest and the lowest SGR (2.58 *vs.* –3.15 %) and WG (72.09 *vs.* –10.8 %) were in the C and T_3 groups ($P<0.05$). HSI in T_3 group was lower than the C and T_1 ones and fish in T_2 showed intermediate value. Fulton's condition factor in T_3 and T_2 groups was lower than the C. VSI did not affect by different FA episodes ($P>0.05$). After RF, *A. latus* juveniles subjected to FA had lower BW_f compared to the C. After RF, fish in T_3 group had the highest SGR value (2.5 ± 0.07 %), meanwhile fish in the C had the least value (1.7 ± 0.4 %). After RF episode, WG in T_3 and T_2 groups was higher than the other ones ($P<0.05$). Fish in the C and T_3 groups had the highest and the lowest FI (14.31 vs. 11.27 g $fish^{-1}$). However, RFI in T_3 group was higher than C and T_1 groups. FCR did not affect by different FA and RF strategies. HSI and K were restored after RF

in *A. latus* juveniles. Fish in the T_3 and C groups had the highest and the lowest VSI (22.94 *vs.* 15.15 %), respectively ($P<0.05$).

Regarding the compensatory coefficient index in *S. hasta*, T_3 and T_1 groups had the greatest and the least values for WG, respectively, whereas T_2 had an intermediate value (Figure 2 A). However, *A. latus* juveniles subjected to different FA and RF protocols did not show a significant difference in CC values (Figure 2 B).

Antioxidant capacity and enzymes activities in the liver

The levels of GST, GPX, and SOD activities in the liver of fish subjected to a two-week FA (T_2) were higher than the C and T_1 groups, and the T_3 group had intermediate values ($P<0.05$, Table 3). CAT activity in the liver of fish in the T_2 group was higher than the other treatments. The amount of liver TBARs of the T_2 and T_3 groups was higher than the other groups ($P<0.05$).

In *A. latus* juveniles, there were no significant differences in values of antioxidant enzymes activities in the liver; however, fish that experienced longer FA including T2 and T3 groups had greater lipid peroxidation compared to the C and T_1 groups ($P<0.05$).

The level of ALT activity in the liver of *S. hasta* experienced a two-week FA was higher than the other groups ($P<0.05$; Table 4). There were no significant differences in the levels of AST and LDH among experimental groups. The ALP level in the liver of *S. hasta* juveniles in the T_2 group was higher than the C and T_1, meanwhile, those in the T_3 group had intermediate values ($P<0.05$; Table 4).

Regarding *A. latus* juveniles, ALT level in the fish liver subjected to a two-week FA (T2) was higher than the T_1 group. The levels of AST and ALP in the liver of fish in different treatments did not significantly change. The amount of LDH in the C and T_1 groups was higher than in the other groups ($P<0.05$).

Digestive enzyme activities

The digestive enzyme activities were pronouncedly affected by different procedures of FA and RF in *S. hasta* (Table 5). The level of trypsin in T_1 was higher than in the C and T_3 groups ($P<0.05$). The highest and the lowest chymotrypsin, ALP, and lipase activities were in T_1 and C groups, respectively ($P<0.05$). Fish in T_1 had higher α-amylase activity than the other groups.

Regarding *A. latus* juveniles, the amounts of trypsin and chymotrypsin activities in the C were higher than T_2 and T_3 groups ($P<0.05$). However, ALP and α-amylase in the T_2 and T_3 groups were higher than the C and T_1. Lipase activity was not affected by different fasting and re-feeding procedures ($P<0.05$).

Discussion

Previous studies in *S. hasta* juveniles demonstrated that feed restriction or cyclical FA and RF did not compromise its survival rate (Mozanzadeh et al., 2017 b, 2020). However, the results of the current study revealed that the survival rate in *S. hasta* juveniles was significantly reduced with increasing the severity of the FA period, mainly due to provoking of the cannibalistic behavior in this species. But, the survival rate of *A. latus* did not affect by the severity of feed restriction, which suggesting species-specific behavior of these sparids relative to feed deprivation. It has been well known that any quantitatively, spatially, temporally, or even qualitatively feeding restriction

may profoundly trigger intracohort cannibalistic behavior in fish, especially in fast-growing tropical fish species as a consequence of food deprivation and inducing size heterogeneity in a cohort (Baras et al., 2002). In this regard, it has been reported that feed deprivation remarkably triggered cannibalism in two fast-growing tropical species, including juvenile yellowtail (*Seriola quinquevadiata*, Sakakura and Tsukamoto, 1998) and Asian seabass (*Lates calcarifer*, Liu et al., 2017). According to the findings of Liu et al. (2017), feed deprivation adversely affects the recognition capability of fish to identify siblings that eventually result in intracohort cannibalism. In the present study, one, two, or three weeks of FA period remarkably abated weight in both sparids mainly related to the mobilization of the body energy storages (e.g. glycogen and fat in the liver and viscera cavity, respectively) for vital physiological processes (Ali et al., 2003; Navarro and Gutierrez, 1995). Regarding *S. hasta* juveniles, after the RF episode, fish subjected to three weeks of FA (T_3) had higher BW_f, SGR, and WG than fish that experienced two weeks of FA (T_2) that mainly because of higher cannibalism in the T_3 group which adversely reduced survival rate (*Ca.* –16.4%) compared to T_2 group.

After the RF period, fish subjected to feed deprivation demonstrated an accelerated growth rate, and fish that experienced more severe feed deprivation (T_2 and T_3) had higher SGR and WG compared to the control and T_1 groups in both species. Furthermore, in this study, increasing FA duration resulted in increasing compensatory coefficient (CC) of WG in *S. hasta* that eventually led to complete CGR indicating adaptability of this species to feed restriction, as previously proved by Mozanzadeh et al. (2017). In this regard, it has been reported that *S. hasta* juveniles subjected to a cyclic FA and RE strategy, including one-day FA followed by two days of RE for 60 days, demonstrated full CGR (Mozanzadeh et al., 2017 b). But it should be mentioned that in the present research, complete CGR in T_3 group in *S. hasta* associated with more cannibalism ultimately

reduced the survival rate in this species. Moreover, it has been reported that *S. hasta* fingerlings subjected to 30 days feeding restriction (feeding rates were at 2, 4, 6, and 8% of BW_i during feed restriction phase) demonstrated partial CGR following 30 days of RF at satiation level. Thus, long term FA periods (more than one week) is not recommended in *S. hasta* as it may provoke cannibalism in this species juveniles in the same stocking density. Full CGR is also demonstrated in other sparids. For example, full CGR reported in gilthead seabream that experienced a restricted feeding (feeding rate at 1.4% of BW_i) for 30 days then re-fed up to satiation for another for 30 days (Bavcevic et al., 2010). On the other hand, 7, 14, and 28 days of FA that followed by 84, 77, and 63 days of RF (long term RF periods), respectively, resulted in full CGR in red porgy (*Pagrus pagrus*, Reuda et al., 1998) that accompanied by hyperphagy in this species. Furthermore, cyclical FA (1 or 2 days) followed by 5 to 6 days of RF also resulted in full CGR in black seabream (*Acanthopagrus schlegelii schlegelii*, Oh et al., 2013; Xiao et al., 2013).

In contrast, in this study BW_f in *A. latus* that subjected to feed deprivation did not reach to BW_f in the control group, and this species did not show CC. It seems that partial CGR more obvious in T_2 and T_3 compared to T_1 because they showed elevated SGR and elevated WG but they didn't reach the control mass, meanwhile fish of T_1 only showed higher SGR. In contrast, *A. latus* juveniles subjected to a short-term FA and RE periods two days of FA that followed by 8 days of RF for 80 days showed full CGR. In addition, Mozanzadeh et al. (2020) reported that *A. latus* fingerlings subjected to 30 days of moderate feeding restriction (feeding rate at 6-8% of BW_i) demonstrated full CGR following 30 days of RF at satiation level. But *A. latus* subjected to a severe feed restriction (feeding rate at 2-4% of BW_i) for 30 days showed partial CGR (Mozanzadeh et al., 2020). These findings suggested that *A. latus* juveniles can adapt to short-term cyclical FA and RE strategies and moderate feed restriction rather than severe feed deprivation periods. Previous

studies in gilthead seabream subjected to different cyclical FA and RF strategies also demonstrated partial CGR in this species (Eroldoğan et al., 2006, 2008; Yilmaz and Eroldoğan, 2011).

In the current research, total feed intake in fish that experienced feed deprivation was lower than the control group; however, RFI (feed intake during RF phase) increased in these groups suggesting hyperphagia during the RF period in both fish species. It should be mentioned that RFI in *S. hasta* in the T_3 group was lower than the other groups, mainly due to cannibalism. In agreement with the results of the present study hyperphagia demonstrated in *S. hasta* and *A. latus* fingerlings that experienced feed restriction for 30 days then refed at satiation level for another 30 days (Mozanzadeh et al., 2020). The hyperphagic behavior in response to FA and RF strategies also demonstrated in other Sparids such as *S. aurata* (Yilmaz and Eroldoğan, 2011), *Dentex dentex* (Pérez-Jiménez et al., 2012), *A. schlegelii* (Oh et al., 2013). Thus, in the present study, the CGR in both species is mainly related to hyperphagia during the RF phase.

In this study, except for the T_3 group in *S. hasta*, which had a lower survival rate, FCR did not affect by FA and RF strategies suggesting other factors such as hyperphagia, reduction of basal metabolic rate or maintenance energy costs, and adaptation of the endocrine system during FA phase could be induced CGR in both species (Ali et al., 2003; Davis and Gaylord, 2011). Similar to this result, Mozanzadeh et al. (2017b) reported that *S. hasta* juveniles subjected to six days of FA followed by six days of RF for 60 days had worse FCR than the control group fish was not adapted to this feeding schedule. In addition, Tamadoni et al. (2020) demonstrated that FCR did not affect by cyclical FA and RF in *A. latus* juveniles.

At the end of the FA period, somatic indices, including HSI and VSI in *S. hasta* and HSI and K in *A. latus* decreased especially in T_2 and T_3 groups, and after the RF period these parameters restored to the normal values. Reduction of K value after FA period also reported in other Sparids

such as *P. pagrus* (Caruso et al., 2012) and *D. dentex* (Perez-Jimenez et al., 2012). It seems that mobilization and depletion of nutrients and energy reserves (*e.g.* lipid and glycogen) in the liver and visceral cavity for sustaining gluconeogenesis and restriction of protein synthesis during the FA period resulted in lower somatic indices in fish (McCue, 2010). Furthermore, recuperation of somatic indices after the RF phase may be associated with hyperphagia and modulation of metabolic pathways for recovering nutrients and energy storages (Furné et al., 2012). In addition, in the current research, VSI increased during the RF period by increasing FA duration that may require a metabolic response of *A. latus* for excess lipid storage for future feed restriction periods. Similar to our results, it has been reported that FA and RF protocols induced lipid deposition in the visceral cavity of *S. hasta* (one-day FA and two days RF for 60 days, Mozanzadeh et al., 2017b) as well as provoked overcompensation of HSI in *A. latus* (8 days FA and 32 days FR for 80 days, Tamadoni et al., 2020). In this context, there is some evidence for lipostatic involvement (i.e. the body fat reserves) in the regulation of the hyperphagic response seen in fish that are undergoing catch-up growth after deprivation fasting period (Jobling and Johansen 1999).

It has been proved that the most adverse influence of food deprivation on fish health could be associated with the generation of ROS due to the depletion of antioxidant reserves in the body (Morales et al., 2004). In this context, Dar et al. (2019) reported that SOD and CAT activities pronouncedly increased in the liver and gills of rohu (*Labeo rohita*) subjected to one week of FA that was associated with overexpression of antioxidant related genes, but antioxidant activities and their related genes remarkably reduced in the tissues after one week RF period. In the present study, the values of TBARs were pronouncedly increased in the liver of T_2 and T_3 groups in both species suggesting a longer RF period is required for recuperation of oxidative stress in both groups. Increasing the level of lipid peroxidation also reported in large yellow croaker

(*Pseudosciaena crocea*, Zhang et al., 2008) and Yangtze sturgeon (*Acipenser dabryanus*, Yang et al., 2019) subjected to different FA and RF strategies. In this regard, Tamadoni et al. (2020) reported that the activities of antioxidant enzymes (*i.e.* ACT, GR, and GST) in *A. latus* subjected to two and four days of FA followed by 8 and 16 days of RF, respectively restored to the normal range after 80 days of the feeding trial, but extending the FA period to 8 days followed by 32 days of RF triggered oxidative stress in this species. It should be mentioned that antioxidant enzymes values in the T_3 group were generally lower than those in the T_2 group in *S. hasta* may as a result of cannibalism that could be reduced feed deprivation stress in the T_3 group.

During FA and RF periods results in an increment of liver enzymes activities, especially transaminases (*e.g.* ALT, and AST) for gluconeogenesis that eventually rise the leakage of these enzymes into the blood circulation (Congleton and Wagner, 2006). In the present study, the levels of liver ALT and ALP were increased in *S. hasta* that experienced two weeks of FA (T_2), indicating these enzymes have a vital role in providing energy and metabolic processes to support CGR in this species. On the other hand, high levels of ALT and ALP in *S. hasta* in the T_2 group associate with an elevation of antioxidant enzymes in the liver, suggesting liver damage due to oxidative stress. In this regard, it has been reported that plasma AST and ALP in Persian sturgeon (*Acipenser persicus*) that subjected to four weeks of FA remarkably increased, and their values recuperated to the normal range after the RF period (Yarmohammadi et al., 2015). Furthermore, starvation of *L. rohita* for a week pronouncedly elevated serum ALT and AST in the gills and the liver, but their levels significantly decreased after a week of RF. Ashouri et al. (2020) demonstrated that levels of AST, ALP, and LDH in plasma juvenile Siberian sturgeon (*Acipenser baerii*) were subjected to one, two, and three weeks of FA were increased, but their values restored to normal ranges after four weeks of RF. The activity of ALT in the liver of *A. latus* in T_2 increased, suggesting elevation

amino acids mobilization for providing energy. Similarly, AST and ALT levels increased after four weeks of starvation in olive flounder (*Paralichthys olivaceus,* Park et al., 2012). In contrast, LDH decreased with increasing FA period in *A. latus,* indicating anaerobic glycolysis reduced during RF period, and amino acids rather than lactate might be utilized as substrate for gluconeogenesis for supporting CGR. Similar to these results, it has been reported that ALT and LDH activities decreased, but AST activity increased during three days of in white muscle of Adriatic sturgeon (*Acipenser naccarii,* Furné et al., 2012).

In the present study, after five weeks of RF period, digestive enzyme activities of *S. hasta* in the T_1 group were remarkably higher than the C. Other groups (T_2 and T_3) also recovered the activity of the digestive enzymes after the RF period suggesting these enzymes could compensate for deficiencies of the FA period. This result indicating that total CGR in S. hasta connected with the fast recovery of digestive enzymes during RF period, which was also reported in Atlantic cod (*Gadus morhua*, Bélanger et al., 2002) and European sea bass (*Dicentrarchus labrax*, Cara et al., 2007). Similarly, it has been reported that different FA periods suppress digestive enzymes activities in *Megalobrama pellegrini* (Zheng et al., 2015) and *L. rohita* (Dar et al., 2018), but their activities were rebound after the RF period. Furthermore, in the current research, the activity of ALP in both Sparids subjected to FA was over-compensated compared to the control indicating nutrient absorption efficiency promoted during the RF period as previously described in European eel (*Anguilla anguilla*, Gisbert et al., 2011). On the other hand, the activities of trypsin and chymotrypsin after five weeks of RF were not completely recovered in A. *latus* that eventually resulted in lower growth and partial CGR in fish subjected to different FA periods. In contrast, Tamadoni et al. (2020) demonstrated that the activity of total alkaline proteases in *A. latus* juveniles subjected to 4 or 8 days FA followed by 16 and 32 days of RF, respectively, was higher

than the control that consequently resulted in complete CGR in this species. In the present study, α-amylase activity increased in T_2 and T_3 groups in *A. latus*, suggesting glycolysis of glycogen storages in the liver and mobilization of glucose as also demonstrated in gilthead seabream (Eroldoğan et al., 2008) and *A. latus* (Tamadoni et al., 2020) subjected to different FA and RF strategies. These findings suggested that CGR in both Sparid species profoundly related to the activity of digestive enzymes during the RF period.

In summary, the findings of the current study clearly revealed that sparids demonstrate species-specific CGR mainly due to different physiological responses to FA and RF episodes. According to the data, the FA period over two weeks detrimentally affects the survival rate in *S. hasta* juveniles as it provokes cannibalism in this species. Fasting triggered hyperphagia in both species that eventually resulted in boosted SGR during the RF period and led to complete and partial CGR in *S. hasta* and *A. latus* juveniles, respectively. Feed deprivation also induces lipid peroxidation in both species, and *S. hasta* experienced two weeks of FA could not down-regulate the activity of antioxidant enzymes suggesting oxidative stress in this group. Feed deprivation also modulates metabolic pathways in both fish species by affecting liver enzymes to provide energy to compensate for growth during RF episodes. Finally, CGR in both species mainly associated with digestive enzymes activities, especially proteases. By considering the findings of the present study, applying short-term cyclical FA and RF period strategies recommended for inducing CGR in these species because a single-phase FA reduced survival and induced oxidative stress in *S. hasta;* meanwhile, *A. latus* could not achieve complete compensatory growth after RF episode.

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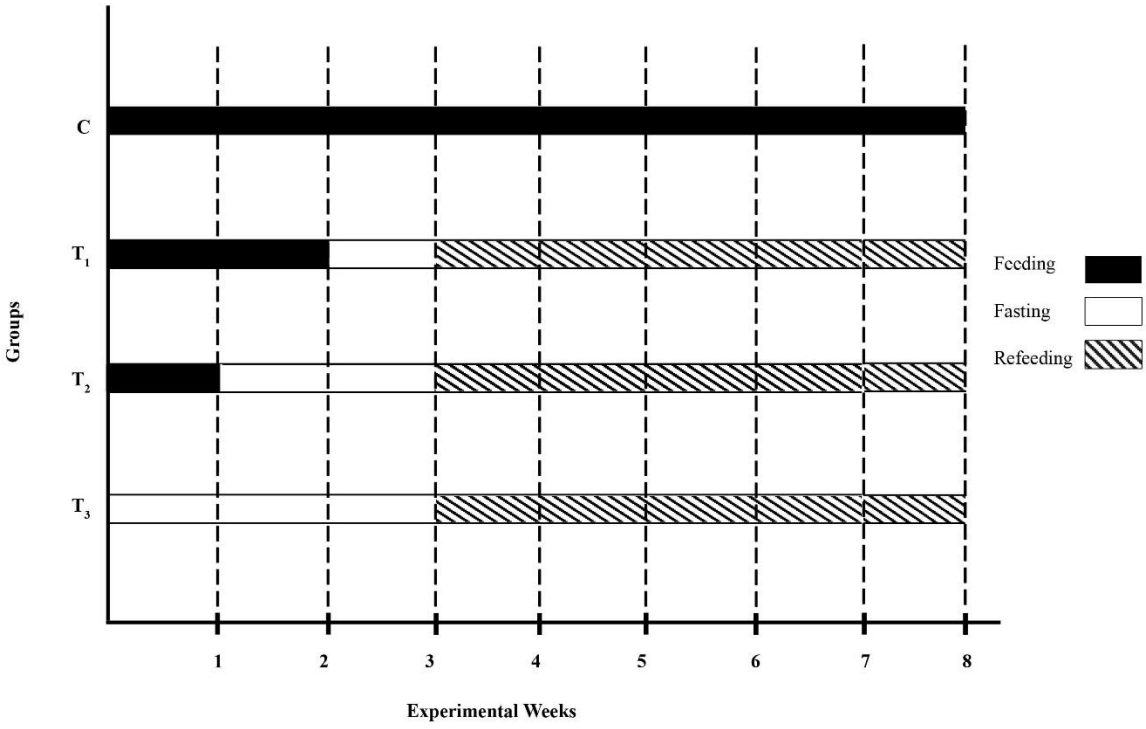

Figure 1. Flow chart delineating feeding schedule to test different scenarios of fasting and re-feeding. The control (c) was fed along the eight weeks, while T_1 received two weeks feeding, one week fasting and five weeks re-feeding; T_2: received one week feeding, two weeks fasting and five weeks re-feeding; T_3: received three weeks fasting and five weeks re-feeding

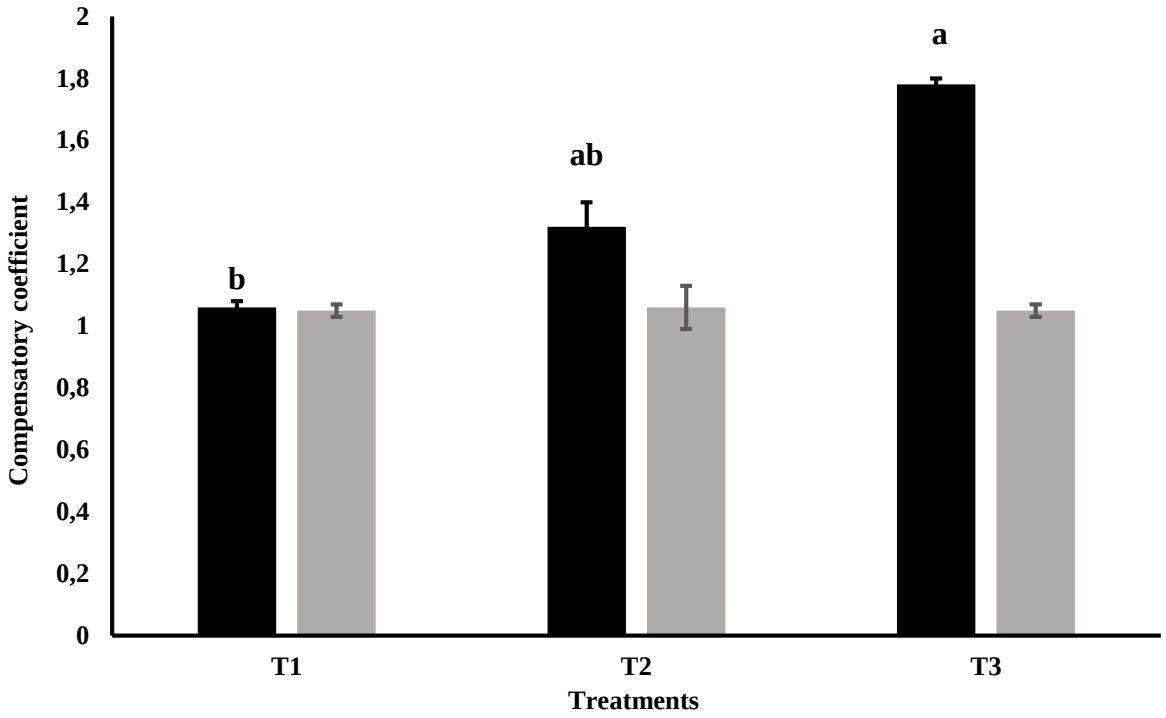

Figure 2. Compensation coefficients (CC) for weight gain in *S. hasta* (black bars) and *A. latus* (grey bars) juveniles fed according to different feeding regimes for eight weeks. CC >1 indicates compensation. Each bar represents the treatment average of three replicates (mean ± SEM). Fish fasted for one (T1), two (T2) or three (T3) weeks and then five weeks of re-feeding period. Different letters in each column represent significant differences among groups (Duncan's test, $P<0.05$)

Table 1. Growth performance of *S. hasta* subjected to the following feeding regimes: (C) fish were fed to satiation for eight weeks; (T_1) fish were fasted for one week then they were refed for seven weeks; (T_2) fish were fasted for two weeks then they were refed for six weeks; (T_3) fish were fasted for three weeks then they were refed for five weeks. A different superscript in the same row denotes statistically significant differences ($P<0.05$)

	Groups			
	C	T_1	T_2	T_3
	Fasting(at the end of three weeks)			
BW_f (g)	18.5 ± 0.1^a	9.9 ± 0.0^b	9.3 ± 0.0^c	9.7 ± 0.1^b
SGR (% BW_i day^{-1})	2.9 ± 0.03^a	-0.2 ± 0.0^b	-0.5 ± 0.1^c	-0.1 ± 0.0^b
WG (%)	85.3 ± 1.0^a	-1.2 ± 0.0^b	-6.5 ± 0.7^c	-3.0 ± 0.6^b
HSI (%)	1.5 ± 0.1^a	1.5 ± 0.2^a	1.2 ± 0.3^b	1.3 ± 0.1^b
VSI (%)	9.5 ± 1.2^a	9.0 ± 0.7^b	8.3 ± 0.3^c	7.8 ± 1.4^d
K (%)	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1
Survival (%)	100.0 ± 0.0^a	100 ± 0.0^a	86.1 ± 4.8^b	69.4 ± 8.0^c
	Re-feeding(at the end of eight weeks)			
BW_f (g)	31.2 ± 1.6	26.3 ± 0.2	27.4 ± 1.0	29.6 ± 2.2
SGR (% BW_i day^{-1})	1.5 ± 0.0^c	1.8 ± 0.0^{bc}	2.2 ± 0.0^b	3.2 ± 0.2^a
WG (%)	68.2 ± 0.8^b	86.1 ± 1.7^b	175.5 ± 13.1^a	205.5 ± 20.9^a
Total FI (g $fish^{-1}$)	34.9 ± 0.8^a	28.2 ± 0.6^b	24.1 ± 0.3^b	23.1 ± 2.1^b
RFI (g $tank^{-1}$ feeding day^{-1})	9.4 ± 0.2^a	8.9 ± 0.1^a	8.6 ± 0.1^a	6.3 ± 0.7^b
FCR	1.8 ± 0.1^a	1.9 ± 0.1^a	1.6 ± 0.1^a	2.4 ± 0.5^b
HSI (%)	1.4 ± 0.1	1.5 ± 0.2	1.5 ± 0.3	1.5 ± 0.1
VSI (%)	8.8 ± 0.8	11.6 ± 0.7	9.0 ± 0.3	8.6 ± 1.4
K (%)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	1.3 ± 0.1
Survival (%)	93.3 ± 5.6^a	86.1 ± 1.6^a	83.3 ± 4.5^a	52.8 ± 1.6^b

Abbreviations: BW_f: final body weight; SGR: specific growth rate; WG: weight gain; FI: feed intake; FCR: feed conversion ratio; HSI: hepatosomatic index; VSI: viscerosomatic index; K: Fulton's condition factor.

Table 2. Growth performance of *A. latus* subjected to the following feeding regimes: (C) fish were fed to satiation for eight weeks; (T_1) fish were fasted for one week then they were refed for five weeks; (T_2) fish were fasted for two weeks then they were refed for five weeks; (T_3) fish were fasted for three weeks then they were refed for five weeks. A different superscript in the same row denotes statistically significant differences (P<0.05)

	Groups			
	C	T_1	T_2	T_3
	Fasting (at the end of three weeks)			
BW (g)[a]	7.4 ± 0.07[a]	4.16 ± 0.08[b]	4.0 ± 0.09[b]	3.9 ± 0.0[b]
SGR (% BW_i day^{-1})[b]	2.58 ± 0.05[a]	-0.47 ± 0.03[b]	-0.51 ± 0.05[b]	-0.46 ± 0.03[b]
WG (%)[c]	72.1 ± 1.79[a]	-3.25 ± 2.02[b]	-7.0 ± 0.1[b]	-9.3 ± 0.30[c]
HSI (%)[f]	3.5 ± 0.21[a]	3.1 ± 0.60[a]	2.5 ± 0.50[ab]	1.9 ± 0.10[b]
VSI (%)[g]	16.4 ± 2.07	15.9 ± 3.26	14.5 ± 4.10	12.8 ± 2.65
K (%)[h]	1.3 ± 0.04[a]	1.21 ± 0.12[ab]	1.16 ± 0.01[b]	1.15 ± 0.05[b]
Survival (%)	100 ± 0.0	98.3 ± 0.93	96.7 ± 1.92	93.3 ± 3.84
	Re-feeding (at the end of eight weeks)			
BW (g)	13.41 ± 0.1[a]	12.27 ± 0.17[b]	11.23 ± 0.42[b]	10.01 ± 0.1[c]
SGR (% BW_i day^{-1})	1.7 ± 0.4[c]	1.94 ± 0.01[b]	2.04± 0.01[b]	2.5 ± 0.07[a]
WG (%)	81.33 ± 3.23[b]	97.86 ± 1.17[b]	158.3 ±10.8[a]	140.71 ± 5.72[a]
Total FI (g $fish^{-1}$)[d]	14.31 ± 0.5[a]	12.86 ± 0.23[b]	13.12 ± 0.70[b]	11.27 ± 0.70[c]
RFI (g $tank^{-1}$ feeding day^{-1})	7.4 ± 0.26[b]	7.6 ± 0.14[b]	8.7 ± 0.06[ab]	9.17 ± 0.47[a]
FCR[e]	1.6 ± 0.04	1.65 ± 0.07	1.5 ± 0.07	1.75 ± 0.34
HSI (%)[f]	3.72 ± 0.03	3.0 ± 0.31	2.63 ± 0.41	3.65 ± 0.20
VSI (%)[g]	15.15 ± 0.82[b]	18.42 ± 1.50[ab]	15.97 ± 0.76[ab]	22.94 ± 2.62[a]

K (%)[h]	1.25 ± 0.04	1.21 ± 0.12	1.11 ± 0.01	1.07 ± 0.05
Survival (%)	96.7 ± 0.0	96.7 ± 0.0	93.3 ± 5.8	90.0 ± 1.31

Abbreviations: BW: body weight; SGR: specific growth rate; WG: weight gain; FI: feed intake; RFI: relative feed intake; FCR: feed conversion ratio; HSI: hepatosomatic index; VSI: viscerosomatic index; K: Fulton's condition factor.

Table 3. Liver antioxidant enzymes (U mg protein^{-1}) and lipid peroxidation (TBARs, nmol g^{-1} tissue) in *S. hasta* and *A. latus* subjected to the following feeding regimes: (C) fish were fed to satiation for eight weeks; (T_1) fish were fasted for one week then they were refed for five weeks; (T_2) fish were fasted for two weeks then they were refed for five weeks; (T_3) fish were fasted for three weeks then they were refed for five weeks. A different superscript in the same row denotes statistically significant differences (P<0.05)

	Groups			
	C	T_1	T_2	**T_3**
		S. hasta		
GST	1.46 ± 0.0[b]	1.40 ± 0.0[b]	1.79 ± 0.06[a]	1.57 ± 0.14[ab]
GPx	1.55 ± 0.01[b]	1.26 ± 0.26[b]	1.87 ± 0.04[a]	1.63 ± 0.13[ab]
SOD	1.26 ± 0.0[b]	1.06 ± 0.31[b]	1.78 ± 0.09[a]	1.35 ± 0.21[b]
CAT	0.76 ± 0.0[b]	0.75 ± 0.1[b]	1.05 ± 0.13[a]	0.69 ± 0.15[b]
TBARs	2.33 ± 0.0[b]	2.01 ± 0.29[b]	2.69 ± 0.02[a]	2.50 ± 0.16[a]
		A. latus		

GST	1.70 ± 0.16	1.64 ± 0.05	1.60 ± 0.02	1.82 ± 0.15
GPx	1.30 ± 0.14	1.18 ± 0.03	1.28 ± 0.02	1.14 ± 0.05
SOD	1.16 ± 0.16	1.13 ± 0.04	1.14 ± 0.05	1.08 ± 0.13
CAT	1.14 ± 0.14	1.09 ± 0.03	0.91 ± 0.02	1.0 ± 0.1
TBARs	1.60 ± 0.0^{b}	1.65 ± 0.10^{b}	1.98 ± 0.02^{a}	2.0 ± 0.10^{a}

Abbreviations: GST: glutathione-S-transferase; GPx: glutathione peroxidase; SOD: superoxide dismutase; CAT: catalase; TBARs: Thiobarbituric acid reactive substances.

Table 4. Liver enzymes (U mg protein^{-1}) in *S. hasta* and *A. latus* subjected to the following feeding regimes: (C) fish were fed to satiation for eight weeks; (T_1) fish were fasted for one week then they were refed for five weeks; (T_2) fish were fasted for two weeks then they were refed for five weeks; (T_3) fish were fasted for three weeks then they were refed for five weeks. A different superscript in the same row denotes statistically significant differences (P<0.05)

	Groups			
	C	T_1	T_2	T_3

		S. hasta		
ALT	0.74 ± 0.0[b]	0.86 ± 0.14[b]	1.55 ± 0.19[a]	0.92 ± 0.17[b]
AST	0.94 ± 0.0	0.95 ± 0.12	1.37 ± 0.14	1.0 ± 0.15
LDH	1.03 ± 0.0	1.0 ± 0.15	1.44 ± 0.09	1.12 ± 0.13
ALP	1.5 ± 0.0[b]	1.64 ± 0.26[b]	2.75 ± 0.33[a]	1.73 ± 0.33[ab]
		A. latus		
ALT	1.45 ± 0.21[ab]	1.28 ± 0.03[b]	1.76 ± 0.04[a]	1.64 ± 0.10[ab]
AST	1.21 ± 0.11	1.08 ± 0.02	1.43 ± 0.10	1.46 ± 0.18
LDH	1.53 ± 0.15[a]	1.49 ± 0.02[a]	1.20 ± 0.01[b]	1.18 ± 0.07[b]
ALP	1.75 ± 0.18	1.66 ± 0.04	1.42 ± 0.14	1.53 ± 0.20

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; ALP: alkaline phosphatase.

Table 5. Digestive enzymes (U mg protein^{-1}) in *S. hasta* and *A. latus* subjected to the following feeding regimes: (C) fish were fed to satiation for eight weeks; (T_1) fish were fasted for one week then they were refed for five weeks; (T_2) fish were fasted for two weeks then they were refed for five weeks; (T_3) fish were fasted for three weeks then they were refed for five weeks. A different superscript in the same row denotes statistically significant differences ($P<0.05$)

	Groups			
	C	T_1	T_2	T_3
		S. hasta		
Trypsin	1.26 ± 0.0^{b}	1.60 ± 0.08^{a}	1.39 ± 0.03^{ab}	1.26 ± 0.12^{b}
Chymotrypsin	1.44 ± 0.01^{b}	1.79 ± 0.08^{a}	1.49 ± 0.03^{ab}	1.48 ± 0.14^{ab}
Alkaline phosphatase	1.23 ± 0.01^{c}	1.64 ± 0.1^{a}	1.54 ± 0.09^{ab}	1.32 ± 0.18^{b}
α-Amylase	0.17 ± 0.01^{b}	0.20 ± 0.01^{a}	0.17 ± 0.01^{b}	0.18 ± 0.01^{b}
Lipase	1.03 ± 0.0^{b}	1.36 ± 0.05^{a}	1.25 ± 0.04^{ab}	1.11 ± 0.11^{ab}
		A. latus		
Trypsin	1.64 ± 0.17^{a}	1.49 ± 0.03^{ab}	1.27 ± 0.02^{b}	1.32 ± 0.06^{b}
Chymotrypsin	1.84 ± 0.17^{a}	1.76 ± 0.04^{ab}	$1.5 \pm 0.0b^{b}$	1.59 ± 0.06^{b}
Alkaline phosphatase	1.1 ± 0.15^{b}	1.0 ± 0.02^{b}	1.5 ± 0.0^{a}	1.51 ± 0.01^{a}
α-Amylase	0.68 ± 0.10^{b}	0.52 ± 0.0^{b}	1.09 ± 0.03^{a}	1.09 ± 0.06^{a}
Lipase	1.49 ± 0.11	1.50 ± 0.05	1.39 ± 0.05	1.57 ± 0.13