EFFECT OF SHORT STARVATION AND REFEEDING ON GROWTH, BODY COMPOSITION, AND DIGESTIVE ENZYMES ACTIVITIES IN YELLOW RASBORA (*Rasbora lateristriata* Blkr.)

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ABSTRACT

Digestive enzyme activities of Yellow Rasbora (*Rasbora lateristriata*) had been reported, but only focused on a daily feeding strategy. The effect of starvation and refeeding on the growth, body composition, and digestive enzyme activities in Yellow Rasbora has never been reported. This study aimed to know the most efficient feeding strategy for growth, body composition, and digestive enzyme activities of Yellow Rasbora. The experiment was conducted in 20 aquariums using two months old fingerling. Four different feeding strategies were tested; twice feeding a day, one-day starvation and six days refeeding, two days starvation and five days refeeding, and four days starvation and ten days refeeding. The result showed that different feeding strategies had no significant effect on all growth parameters. The result proved that Yellow Rasbora could compensate for starvation through absolute growth, with the highest value achieved in two days of starvation and five refeeding. No significant value was also observed on crude protein, and lipid contents indicate that starvation does not affect the utilization of protein and lipid reserves. This result was consistent with fish condition factors which did not differ among treatments. The feeding strategy did not significantly affect total protease, trypsin, and amylase activities, but significantly affected chymotrypsin, lipase, and alkaline phosphatase activities. All parameter indicated that two days starvation and five days refed duration showed similar values to daily feeding. Therefore, it is concluded that two days starvation and five days refeeding method is the most efficient feeding strategy for Yellow Rasbora cultivation.

Key words: fasting, compensatory, proximate, protease.

مجلة العلوم الزراعية العراقية -2023 :54(4):914-927 سوسيلو وآخرون

تأثير التجويع قصير المدى واعادة التغذية في نمو وتركيب الجسم وفعالية انزيمات الهضم في سمك الراسبورا الاصفر (*Rasbora lateristriata* Blkr.)

سوسيلو سيستينا ويبو نوريانتو

المستخلص

تهدف الدراسة إلى معرفة استراتيجية التغذية الأكثر فاعلية للنمو وتركيب الجسم وفعالية إنزيمات الجهاز الهضمي لسمك الراسبورا الاصفر، أجريت التجربة في 20 حوض سمك باستخدام إصبعيات عمرها شهرين .تم اختبار خمس استراتيجيات تغذية مختلفة ؛ مرتين في اليوم من التغذية، والمجاعة ليوم واحد وستة أيام من إعادة التغذية، ويومان من الجوع وخمسة أيام من إعادة التغذية ، وأربعة أيام من الجوع وعشرة أيام من إعادة التغذية أظهرت النتائج أن استراتيجيات التغذية المختلفة ليس لها تأثير معنوي على جميع مؤشرات النمو .أثبتت النتيجة أن سمك الراسبورا الاصفر يمكن أن يعوض الجوع من خلال النمو المطلق، مع تحقيق أعلى قيمة في يومين من الجوع وأربعة إعادة تغذية .لم يلاحظ أي قيمة معنوية في البروتين الخام، وتشير محتويات الدهون إلى أن الجوع لا يؤثر على الاستفادة من احتياطيات البروتين والدهون .كانت هذه النتيجة متوافقة مع عوامل حالة الأسماك التي لم تختلف بين العلاجات .لم تؤثر إستراتيجية التغذية بشكل كبير على فعالية انزيمات البروتياز الكلي والتربسين والأميلاز، ولكنها أثرت بشكل كبير على فعالية الكيموتريبسين والليباز والفوسفاتيز القلوية . أشارت جميع المعلمات إلى أن الجوع لمدة يومين والمدة المرجعية لمدة خمسة أيام أظهرت قيمًا مماثلة للتغذية اليومية .لذلك ، استنتج أن التجويع لمدة يومين وطريقة إعادة التغذية لمدة خمسة أيام هي استراتيجية التغذية الأكثر فعالية لتنمية اسماك الراسبورا الصفراء.

الكلمات المفتاحية: الصوم، التعويض، التقريبي، البروتياز.

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INTRODUCTION

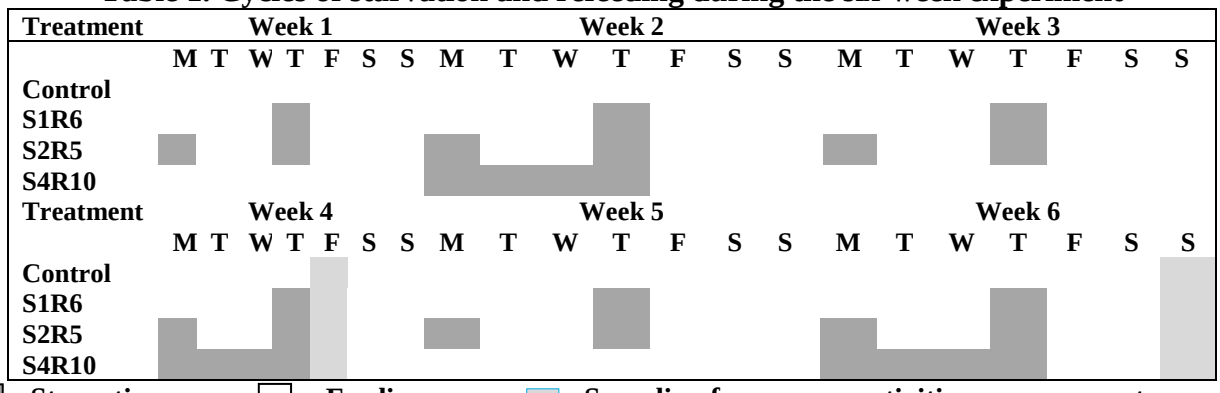
Fish nutritional status can be different between starvation and feeding conditions (27, 28, 30) . Natural starving conditions occur due to limited food availability, migration, and reproductive status (2, 9). In culture, starvation occurs due to stress, pathogen, and other environmental disturbances, such as temperature and water quality alteration (10). Starvation causes several physiological changes, i.e., increased protein level, reduced muscle lipid level, decreased body weight, or fish growth (19, 25). Differences in growth rate in fish experiencing food deprivation varied considerably among fish. In previous studies, short starvation can produce compensatory growth (3, 49), but long fasting are often challenging to produce compensatory growth in fish (12). Differences in the feeding strategy often make differences in growth rate. If compensatory growth is not stimulated, it will be difficult for fish receiving starvation and refeeding conditions to achieve the same weight gain as the control at the end of rearing. Body composition, especially fish's protein and lipid content, is also affected by nutritional status. Lack of feed can reduce body composition because fish need the energy to maintain the body without food. Previous studies have shown a variety of body composition responses in starved fish. Studies on *Acanthopagrus latus* with fasting for two to eight days followed by refeeding did not cause a decrease in body composition (45), but in *Oncorhynchus mykiss* fasting for one to two days followed by refeeding caused changes in body composition (46). Different species respond differently to the absence of feed. Changes in fish growth are related to changes in nutritional status, also correlated with changes in the activity of their digestive enzymes. Several studies of digestive enzyme activity related to fasting conditions and refeeding have also been conducted. In *Acipenser naccarii* and *Oncorhynchus myskiss*, the protease activity decreased slowly after fasting, and the fish recovered capacity for protein digestion after 60 days of refeeding (14). Experiments on Tilapia, which received a short period of fasting treatment, also showed that trypsin activity was lower than in fish that were fed daily, while chemotrypsin activity increased. However, refeeding after fasting increased trypsin and chemotrypsin activity (7). An increase in protease activity in fish that received feed after being fasted also occurred in *Labeo rohita* (54). A decrease in enzyme activity, especially trypsin and chymotrypsin, has also been found in *Salmo caspius* and *Paramisgurnus dabryanus*, and refeeding causes an increase in activity (35, 55). However, a previous study found different conditions in *Pagrus pagrus*, which did not experience a significant change in trypsin and chymotrypsin activity in the absence of feed (6). Alkaline phosphatase activity, as well as proteinase activity, is also affected by nutritional status changes. In *Rutilus rutilus* caspicus, alkaline phosphatase activity increases due to food consumption and decreases when there is no feed (1). The same phenomenon is also found in *Anguilla anguilla*; a lack of food for an extended period causes a decrease in alkaline phosphatase activity, but refeeding can improve the activity (17). Studies on *Acipenser persicus* and *Seriola quinqueradiata* also showed that alkaline phosphatase activity is affected by changes in the presence of feed (20, 53). Increasing the feed amount consumed also causes an increase in alkaline phosphatase activity, as shown in *Megalobrama amblycephala* (52). There appears to be a variety of digestive enzyme responses related to the different methods of fasting and refeeding used. Yellow Rasbora, *Rasbora lateristriata* Blkr., is a wild fish species with small body size and a maximum standard length of 11 cm. This species can be found in Indonesia on great Sunda Island, Jawa, Sumatera, and Kalimantan (22). It is an economically important freshwater fish species as a protein source. This condition makes the species highly exploited, which causes a population decrease. Therefore, it is necessary to protect the species through ex-situ conservation. A study on the biology of Yellow Rasbora is a preliminary need. Previous studies reported some biological aspects of Yellow Rasbora *Rasbora lateristriata* Blkr., such as spawning habitat (39), longitudinal distribution, population structure, genetic diversity (41, 42), molecular phylogeny (23), and cultivation and

conservation (36). A study on digestive enzyme activities in Yellow Rasbora had also been carried out, but only focused on samples that were fed daily (43, 44). Currently, there is no study about the effect of starvation and refeeding on the growth, body composition, and digestive enzyme activities of Yellow Rasbora. Therefore, a survey of the growth, body composition, and digestive enzymes activities in response to starvation and refeeding is still necessary for the Yellow Rasbora. This study was aimed to know the growth, body composition, and digestive enzyme activities of Yellow Rasbora (*Rasbora lateristriata*) after short starvation and refeeding. This study is essential to obtain an effective feeding strategy for Yellow Rasbora cultivation.

MATERIAIS AND METHODS

The study used an experimental method with a completely randomized design (CRD) using four treatments and five replications. The treatment includes fish fed daily (control); fish fasted for one day in one week of rearing (S1R6:); fish fasted two days in one week of rearing (S2R5); fish fasted four days in two weeks of rearing (S4R10). The treatment was different in fasting and refeeding periods. The experimental units were 20 glass aquariums with dimensions of 30x30x50 cm (35 L of water volume) arranged in a water recirculation model. Fish densities were 35 individuals in each aquarium. The fish were 3-4 cm in body length and weighed 0.44±0.05 g per fish; they were ± 2 months old. Fish rearing was carried out for six weeks and fed as much as 3% of the biomass weight per day (42), as shown in Figure 1. During rearing, we fed unfasted fish with commercial pellets (34.53% protein and 12.95% lipid), and the feeding frequency was twice a day. In the fourth and sixth weeks, we sampled fish to measure enzyme activity under fasting conditions (fourth week) and refeeding (sixth week) (Table 1).

Table 1. Cycles of starvation and refeeding during the six-week experiment



: Starvation **: Feeding** **: Sampling for enzyme activities measurement**

Growth and body composition measurement: Growth parameters included weight gain, relative growth rates, and condition factors. Body weights were calculated using the average final weight (g) minus the initial weight (g). The relative growth rate (RGR) is calculated from the final fish weight (g) minus the initial fish weight (g) divided by the initial fish weight (g) multiplied by 100%. The condition factor (CF) was calculated for the final fish of the experiment with the formula CF = 100 x fish weight (W) / fish length $(L)^3$. Body composition was estimated using proximate analysis at the end of the experiment. Body moisture was measured using oven drying (100°C for 3 hours) and 550°C for ash content. At the same time, the gravimetry method was for crude fiber measurement. Furthermore, this study applied the Kjeldahl method for crude protein and Soxhlet for crude lipids measurements (23).

Preparation of digestive organ homogenate

Ten fish samples were taken from each for digestive enzyme activity measurement in the fourth and sixth weeks. The fish was anesthetized using ice water, dissected in the abdomen, and its digestive organs were removed from the abdominal cavity. The isolated digestive organs were then crushed using an electric homogenizer in a cold buffer solution of 50 mM Tris-HCl pH 7.5 containing ten mM NaCl with a ratio of 1 : 8 w/v. The homogenate was centrifuged in a refrigerator

centrifuge (temperature 4 °C) at 12,000 rpm for 15 minutes. The supernatant was stored in a refrigerator at -80 °C (25). Protein levels were determined from the supernatant using the folin-phenol method and albumin as a standard (44).

Digestive enzyme activities measurement

The proteinase activity was estimated using total protease, chymotrypsin, and trypsin activities. This study used a modified azocasein hydrolysis method to measure total protease activity (43) using 0.1M phosphate (pH 7.6) and 0.1 M Tris-HCl (pH 8.1) as the buffer solutions. Absorbance was measured at 366 nm. Total protease-specific activity is expressed as (U) = $Abs_{366}.minute^{-1}.mg^{-1}$ protein. The chymotrypsin-specific activity is expressed as U = $Abs_{256}.min^{-1}.mg^{-1}$ protein. Trypsin-specific activity is defined as U = $Abs_{247}.minute^{-1}.mg\ protein^{-1}$. Chymotrypsin activity was measured using the N-Benzoyl-L-tyrosine ethyl ester (BTEE) method (32), which was calculated as the change in absorbance at a wavelength of 256 nm for 3 minutes. Trypsin activity was measured using Nα-*p*-Tosyl-L-arginine methyl ester hydrochloride (TAME) (32). TAME hydrolysis was calculated as the change in absorbance at a wavelength of 247 nm for 3 minutes.Lipase activity was measured using the *p*-Nitrophenylpalmitate (*p*-NPP) hydrolysis method (48). This study measured the hydrolysis product of *p*-NPP in p-nitrophenol with a spectrophotometer at 410 nm. Lipase concentration was calculated using the standard p-nitrophenol curve. Lipase-specific activity is expressed as U (µmol p-nitrophenol.h^{-1}).mg^{-1} protein. A spectrophotometer measured the absorbance of maltose as a product of starch hydrolysis at 540 nm. The hydrolysis of the starch substrate with 3,5-Dinitrosalicylic acid (15) was used to measure amylase activity. The amount of released maltose from this assay was determined from a standard maltose curve. The specific activity of amylase is expressed as U (µmol.h^{-1}) .mg^{-1} protein supernatant.Alkaline phosphatase activity was measured following the hydrolysis of *p*-nitrophenyl phosphate (*p*NPP) as a substrate (16). The absorbance was measured with a spectrophotometer at 405 nm. The standard p-nitrophenol curve determined alkaline phosphatase activity. Alkaline phosphatase activity is expressed in U (µmol p-nitrophenol.$minute^{-1}$).mg^{-1} protein.

Data analysis

Quantitative data were analyzed by one-way ANOVA using the SPSS 18.0 version of the Windows software package. We first transformed percentage data into Archsin before the analysis.

RESUITS AND DISCUSSION

Growth performance: The current study calculated fish growth based on weight gain, relative growth, and condition factors. The initial body weights were 0.42-0.46 g, and the final weight ranged from 0.73-0.78 g resulting in weight gain ranging from 0.26-0.37 g (Figure 1.). Calculation of condition factors produces values ranging from 0.83-0.91 (Figure 2a.). The relative growth rate (RGR) was 57.47-88.25 % (Figure 2b.). The results showed that fasting and refeeding duration did not significantly affect Yellow rasbora growth, as seen in all growth parameters (P>0.05). The phenomenon might indicate that fasting and refeeding duration promoted a complete compensatory growth in the Yellow Rasbora.

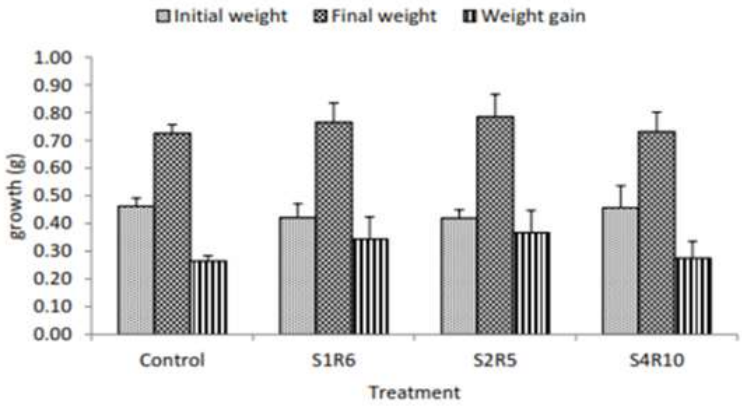


Figure 1. Weight gain of yellow Rasbora at the end experiment

This study resulted in similar phenomena to those previous studies in a wide range of fish species, such as *Cyoglosussus semilaevis* (18), tambacu hybrid (*Piaractus mesopotamicus* ♂x *Colossoma macropamum*♀) (21), *Lophiosilurus alexandri* (40), and *Oreochromis niloticus* (38). The similarity of the current result with previous studies could be due to the fasting duration applied in all the studies, including the present study. However, the present results were different from those observed in *Mylopharyngodon piceus* (33), *Acipencer stelatus* (13), and *Acipencer dabryanus* (51) with more prolonged fasting. According to the current data and those previous data, it is suggested that fasting duration might cause a difference in the ability of the fish to compensate for the growth rate that stopped during fasting.

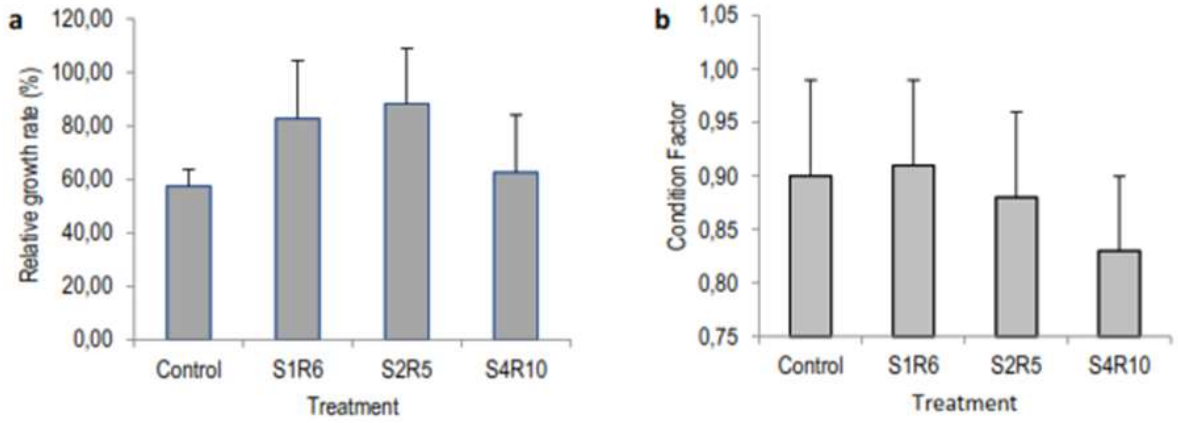


Figure 2. Relative growth rate (a) and condition factor (b) of yellow Rasbora at the end experiment

Similar growth rates among individuals treated with short fasting and refeeding duration could be due to the fish's metabolisms not being significantly altered. We assumed this because temporary starvation does not affect protein and lipids in *Poecilia latipinna* fish (29) and *Huso huso* (31). In contrast, different phenomena were observed from prolonged fasting duration. The difference is suggested due to different metabolisms response in extended fasting, as indicated by reduced triglycerides, glycogen, and protein reserves in the liver under fasting conditions, such as in *Hoplosterum littorale* (37) and *Oncorhynchus mykiss* after ten days of fasting (5). A similar phenomenon has also been observed in the hepatosomatic index, visceral fat, and fat reserves in *Lutjanus guttatus* (8), which shows the utilization of food reserves to act as an energy source when there are no feed or fasting conditions. Therefore, it is easier for fish to adapt to short fasting duration to achieve compensatory growth than fish with prolonged fasting duration. Starvation also stimulates an increase in appetite when refed. Previous studies have proved that phenomena, such as those observed in hybrid grouper (*Epinephelus fuscoguttatus*♀ x *E. lanceolatus*♂) (26), *Oncorhynchus mykiss* (3), *Cynoglossus semilaevis* (11) and hybrid tambacu (*Piaractus mesopotamicus* ♂ x *Colossoma macropamum* ♀) (34). Increased food consumption is a determining factor for compensatory growth in fasted fish. If there is no increased food consumption during the refeeding phase, it will be difficult for fish to achieve absolute compensatory growth.

Body composition

The current phenomenon and phenomena reported in previous studies proved that species will respond differently to fasting and refeeding duration. Short fasting and feeding duration were not significantly affected the body composition of Yellow Rasbora after 42 days of rearing, as shown in moisture level, crude protein, and crude lipids ($p>0.05$). The phenomena are assumed because short fasting and feeding duration did not promote the fish to metabolize lipid and protein reserves as energy sources during fasting. The phenomena were also observed in varieties of fish species (18, 21, 38, 40). The results of this study were no different from previous studies on *Cynoglossus semilaevis* and *Acanthopagrus latus* for moisture and crude protein content (45, 11) but different for crude lipids, which showed a decrease with increasing duration of satiation on *Cynoglossus semilaevis* (11). A

different result was also observed compared to previous studies on *Oncorhynchus mykiss*. The current study showed similar protein and lipid levels among fasting and refeeding duration, while on *O. mykiss*, similar fasting and refeeding duration resulted in increased protein and lipid levels (46). Inversely, *Acipenser baerii* decreased body protein levels after fasting for eight days, followed by refeeding for 32 days (29). The absence of differences in the crude protein and body lipid levels of the Yellow Rasbora proved that short fasting followed by refeeding does not cause a decrease in body crude protein and lipid levels due to use for body maintenance during fasting conditions. Therefore, achieving absolute compensatory growth in Yellow Rasbora with a short starvation cycle followed by refeeding was easy. This condition is different from that which occurs in *Cynoglossus semilaevis*. Decreased body lipid levels in a 4-day fasting and 12-day refeeding duration cannot produce absolute compensatory growth (11). So two days of fasting and five days of refeeding is still a feasible feeding strategy for the Yellow Rasbora growing or cultivation (Table 2).

Table 2. Body composition of yellow Rasbora at different cyclic starvation and refeeding

Composition	Control	S1R6	S2R5	S4R10
Moisture (%)	70.37±1.06	70.14±1.15	70.35±2.34	70.51±1.76
Crude protein (%)	16.27±0.31	15.91±0.57	15.95±1.12	15.89±0.91
Crude lipid (%)	8.28±0.39	8.95±0.43	8.18±0.74	8.03±0.44
Crude fiber (%)	3.56±0.09[a]	3.18±0.16[a]	3.17±0.39[a]	2.74±0.21[b]
Ash (%)	1.92±0.08[a]	1.69±0.16[a]	1.40±0.22[b]	1.75±0.13[a]

Note: The different superscripts are significantly different (p<0.05)

Digestive enzyme activities

Fish that were fasted one day a week had a total protease activity of 14.25 ± 3.90 mU.mg^{-1} protein, and when refed, it became 22.11 ± 2.30 mU.mg^{-1} protein (Figure 3a.). The AMOVA showed that the feeding strategy did not significantly affect protease activity (P>0.05). The phenomena might indicate no significant protein metabolisms have occurred among different fasting and feeding duration because the fish lowered growth during short fasting periods directly compensated in the refeeding period. A previous study reported similar results in *Synechogobius hasta* (57). The current results differed from those on *Acipencer naccarii, Oncorhynchus myskiss, Oreochromis niloticus,* and *Paramisgunus dabryanus*, which showed a reduction in protease activity when the fish fasted and increased activity when the fish were refed (14, 35, 38). The difference in the results of this study with previous studies is related to differences in fasting time. Fasting duration is thought to be related to decreased protein substrates in the digestive system, thereby reducing secretion and protease activity (56). In this study, short fasting did not cause a decrease in protease activity, whereas previous studies that conditioned fish with longer satiation caused a reduction in protease activity. Trypsin activity ranged from 10.93 ± 1.59 mU.mg^{-1} protein in one day of fasted fish a week and 13.57 ± 14.23 mU.mg^{-1} protein in two days of fasted fish a week. When the fish were refed, the trypsin activity ranged from 9.31±7.72 mU.mg^{-1} protein to 13.21±6.06 mU.mg^{-1} protein (Figure 3b). The AMOVA result showed that the feeding strategy did not significantly affect trypsin activity (p>0.05). Likewise, refeeding does not increase trypsin activity. The results of this study did not differ from *Rutilis rutilis* and *Pagrus pagrus*, which did not experience a change in trypsin activity in the absence of feed (1, 6). Nevertheless, the current results differed from those studied in Tilapia, *Silurus meridionalis*, and *Salmo caspius*, which experienced decreased trypsin activity when fish were fasted (7, 55, 56). As reported in the current and previous studies, these similarities and different responses of fish could be due to similarities and differences in fasting and refeeding duration.

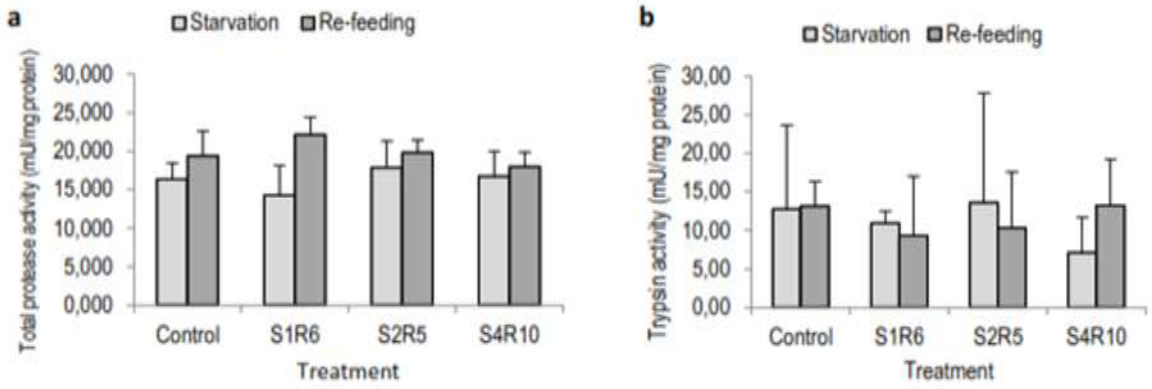


Figure 3. Total protease (a) and trypsin (b) activities of yellow Rasbora at starvation and refeeding.

Chymotrypsin activity in Yellow Rasbora ranged from 57.51-96.70 mU.mg^{-1} protein in the fasted fish and 8.19 – 32.40 mU.mg^{-1} protein in the refed fish (Figure 4a.). Statistical analysis showed that fasting duration resulted in a significant difference (P<.05) in chymotrypsin activity. Chymotrypsin activity under fasting conditions was also higher than trypsin (Figures 3b and 4a). Extended food lack increases chymotrypsin activity but refed decreases chymotrypsin activity in the Yellow Rasbora. We assumed that metabolisms occurred on aromatic protein reserves during the fasting period. Since aromatic protein is less abundant than non-aromatic protein, metabolisms of aromatic protein during the fasting period did not significantly affect total protein content in body fish. The phenomenon was indicated by not significant protein body content among treatments. However, the argument needs scientific proof through further study. A previous study reported increased chymotrypsin activity in fasted Tilapia (7). However, our results differed from those found in *Rutilus rutilus* (1), and in Salmo caspius, fasting caused a decrease in chymotrypsin activity (55). In the refeeding condition, chymotrypsin activity in this study was lower than in the fasting condition (Figure 5a.). This study's results differ from previous studies on *Rutilus rutilus* and *Pagrus pagrus*, which experienced increased chymotrypsin activity under refeeding conditions (1, 6). An increase in chymotrypsin activity in fasted fish, not accompanied by an increase in trypsin activity, will cause a decrease in the trypsin-chymotrypsin ratio (T/C ratio). Changes in the T/C ratio generally correlate with changes in the growth rate of fish. Reduced T/C ratio or increased chymotrypsin activity occurs typically in fish that experience growth inhibition (7). Fish with four days of fasting and ten days of refeeding (S4R10) showed higher chymotrypsin activity than controls. So when the fish are not given food, they are stunted, but their growth increases when fed again. This phenomenon is why fasted fish can achieve compensatory growth when the fish get fed again.

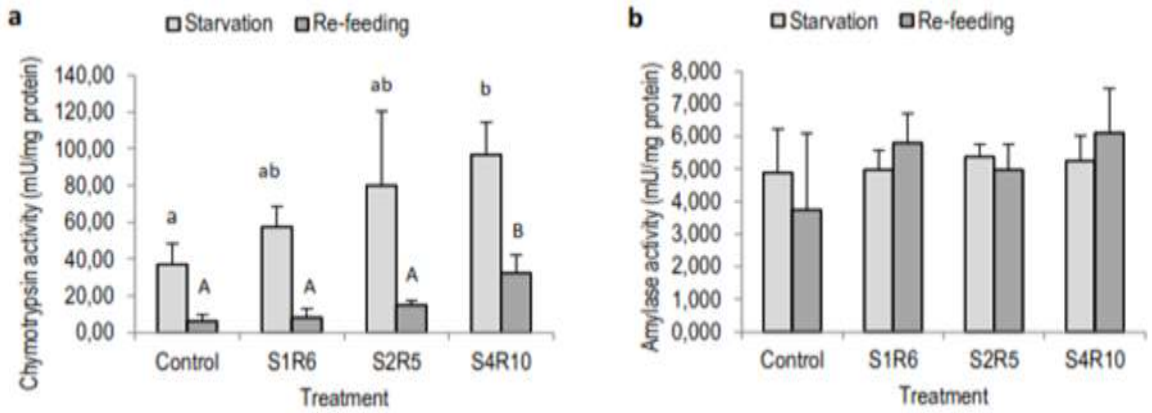


Figure 4. Chymotrypsin (a) and amylase (b) activities of yellow Rasbora at starvation and refeeding. The different superscripts in a bar are significantly different (p<0.05)

The amylase activity of fasted Yellow Rasbora ranged from 4.976-5.250 $mU.mg^{-1}$ protein. The refed fish had amylase activities ranging from 4.971-6.099 $mU.mg^{-1}$ protein (Figure 4b.). Analysis of variance showed that the fasting and refeeding duration was not significantly affected amylase activity ($P>0.05$). So changes in the presence of feed in the live medium of Yellow Rasbora do not result in significant changes in amylase activity. This study's results were similar to previous studies on *Pagrus pagrus* and *Synecogobius hasta* (6, 57). The similarity could be due to the equal fasting duration in both studies. However, this study's results differ from those in *Labeo rohita*, *Acanthopagrus latus*, and *Acipenser baerii*, which decreased amylase activity when fish fasted and increased activity when refeeding (4, 45, 54). The lipase activity of the fasted Yellow Rasbora ranged from 5.139-14.593 $mU.mg^{-1}$ protein, while in refed fish ranged from 12.478-16.207 $mU.mg^{-1}$ protein (Figure 5b.). The results showed that the fasting period and refeeding had a significant effect on lipase activity ($P<.05$). So, fasting or no feed on the longitude fish caused a decrease in lipase activity only in the S1R6 treatment and caused an increase in lipase activity when the fish were refed at S1R6. Still, it was not different from the other treatments. This study's results differ from the previous results on *Paramisgurnus dabryanus* and *Salmo capsius*, which experienced a decrease in lipase activity under conditions of longer satiation (35,55). However, no lipase activity alteration was found in *Synechogobius hasta* when fasting for over three days (57). So, Yellow Rasbora responds differently related to their lipase activity in the absence or limitation of feed. The lack of differences in lipase activity between S2R5 and control fish indicated that fasting for up to 2 days and refeeding for five days did not change the digestive capacity of the Yellow Rasbora for lipids. Therefore, from the lipase activity, fasting up to two days per week can be applied to the maintenance of yellow rasbora fish. Alkaline phosphatase activity of the Yellow Rasbora ranged from 9.932-12.604 $mU.mg^{-1}$ protein under fasting conditions, whereas in refeeding, it ranged from 12.550-13.724 $mU.mg^{-1}$ protein (Figure 6b.). This data proved that fasting duration significantly affects alkaline phosphatase activity ($P<.05$). So it seems that the Yellow Rasbora response to fasting duration by alkaline phosphatase activity alteration. This phenomenon differs from that found in *Rutilus rutilus* and *Anguilla anguilla*, which experience a decrease in alkaline phosphatase activity when faced with a lack of feed (1, 17). However, the results of this study were similar to the previous research on *Acanthoparus latus*, which experienced an increase in activity on four days of fasting followed by 16 days of refeeding (45). Alkaline phosphatase is one of the digestive enzymes whose activity is greatly influenced by the presence of feed in the digestive tract. Alkaline phosphatase activity usually decreases when fish are in deprived conditions but will recover when conditions are refeeding. This phenomenon is related to the role of alkaline phosphatase in the digestive system, which works to help lipid absorption (24). In this study, the activity of alkaline phosphatase with fasting one to two days per week was not different from the control, except in fish that were fasted four days per two weeks, the activity was higher (S4R10). So short fasts of up to two days do not cause changes in alkaline phosphatase activity, reflecting that there has been no change in the presence of substrates in the digestive tract that triggers a decrease in enzyme activity. In line with the findings of other digestive enzyme activities, based on the measurements of lipase and alkaline phosphatase activity in the S2R5 treatment, they still support the achievement of compensatory growth and the body composition of the Yellow Rasbora.

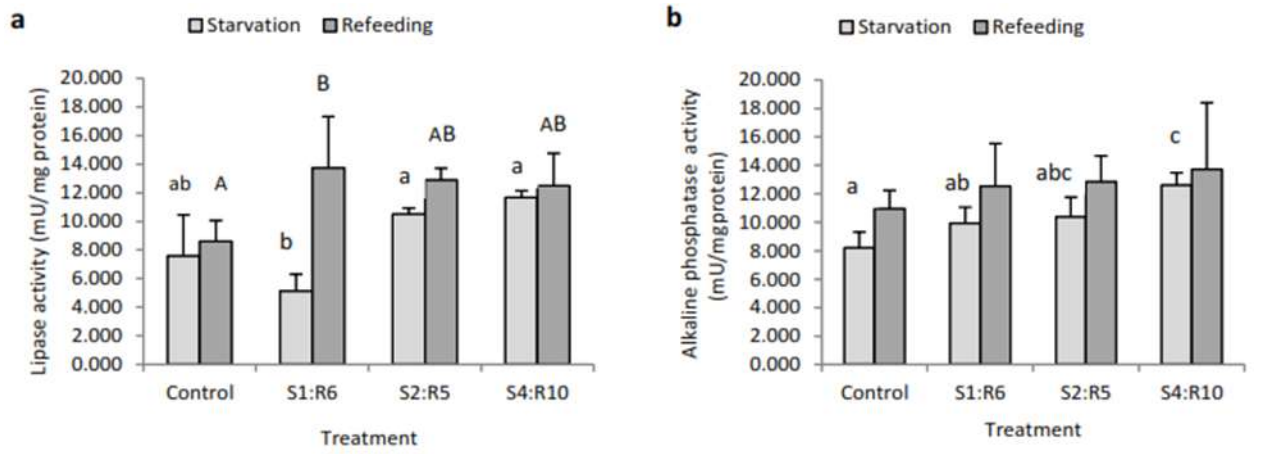


Figure 5. Lipase (a) and alkaline phosphatase (b) activities of yellow Rasbora at starvation and refeeding. The different superscripts in the bar are significantly different (p<0.05)

In the case of Yellow Rasbora, insignificant protease and trypsin activities among treatment indicated that short fasting duration did not reduce the digestive capacity of feed protein. This condition leads to no difference in body protein levels and absolute compensatory growth in fasted fish than those given daily feeding. Moreover, this study also showed that the digestive capacity of the Yellow Rasbora for protein and starch was still good up to 2 days of fasting and five days of refeeding (S2R5). The phenomena indicated that chymotrypsin and amylase activity might support compensatory growth in S2R5 treatments. The S4R10 treatment showed higher chymotrypsin activity than the control, but it was not significantly different from the S2R5 treatment. Therefore, since our study focused on a short fasting period, we suggested that S2R5 was a more efficient feeding strategy than that S4R10. At the same time, chymotrypsin activity was low in the control and S2R5 treatments, which indicated no growth inhibition during the fasting period. In this study, the activity of alkaline phosphatase with fasting one to two days per week was not different from the control, except in fish that were fasted four days per two weeks, the activity was higher (S4R10). So short fasts of up to two days do not cause changes in alkaline phosphatase activity, reflecting that there has been no change in the presence of substrates in the digestive tract that triggers a decrease in enzyme activity. In line with the findings of other digestive enzyme activities, based on the measurements of lipase and alkaline phosphatase activity in the S2R5 treatment, they still support the achievement of compensatory growth and the body composition of the Yellow Rasbora. Therefore, we could apply the S2R5 treatment for the yellow Rasbora to produce compensatory growth as shown in its body composition, which was no different from the control treatment (daily feeding). In conclusion, the two-day fasting and the five-day refeeding cycles did not cause a decrease in growth; instead, they resulted in an absolute compensatory growth in the yellow Rasbora. Body composition, especially crude protein and lipids, did not change during the S2R5 cycle application. The activity of digestive enzymes did not change significantly, and the yellow Rasbora fish still had good digestive capacity in the S2R5 cycle. Two days fasting and five days feeding (S2R5) is the most efficient and appropriate feeding method for growing yellow Rasbora.

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