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Growth, Food Efficiency, and Enzyme Activities in Yellow Rasbora (*Rasbora lateristriata*) at Different Feeding Frequencies

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Abstract. *Many studies have been conducted on the feeding frequency in fish, but there is no information on R. lateristriata. Therefore, this study aimed to determine yellow rasbora growth performance, feed efficiency, and digestive enzyme activity at different feeding frequencies. This research was conducted experimentally with four treatments and three replications. The treatments include: P1 fish were fed two times/day; P2 fish were fed three times/day; P3 fish were fed four times/day; and P4 fish were fed five times/day. In this experiment, 420 two-month-old yellow rasboras were used. The experimental results showed that body weight gain, RGR, SGR, CF, FCR, PER, body lipid & protein levels, protein and lipid retention, protease, and amylase activity were not significantly different ($p>0.05$) between different feeding frequencies. However, lipase and alkaline phosphatase activities differed significantly among the different feeding frequencies. Growth performance, feed efficiency, protease activity, and yellow rasbora amylase are not affected by feeding frequency between two to five times a day. Still, the highest lipase and alkaline phosphatase activity were achieved at the feeding frequency four times a day, but no effect on growth. So, feeding twice a day is considered quite efficient for the culture of yellow rasbora. The results of this study can contribute to the development of fish farming, especially the yellow rasbora, in the future.*

Keywords: *digestive enzyme, feeding frequency, food efficiency, growth, yellow rasbora*

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INTRODUCTION

Many factors, including differences in species, size or age of fish, temperature, amount of feed given, and feeding frequency, influence growth and feeding efficiency in fish (Xie et al., 2011). Therefore, a low feeding frequency will result in low growth and feed efficiency. Conversely, a feeding frequency that exceeds the optimal needs of fish will not result in higher growth but will increase the cost of feed and waste discharged into the aquatic environment (Tian et al., 2015; Amadou et al., 2019).

The effect of feeding frequency on growth has been studied intensively in various fish species. Previous research has shown on tilapia, *Oreochromis niloticus* (Jegede &

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Olorunfemi, 2013), *Oreochromis mosambicus* (Luthada & Jerling, 2013), *Clarias gariepinus* (Haruna et al., 2014; Jamabo et al., 2015), *Carassius auratus* (Habib et al., 2014), that the feeding frequency three to four times a day led to an increase in growth. Still, *Acipenser stellatus* (Dicu et al., 2013), *O. niloticus* (Bin et al., 2014; Kaya & Bilguven, 2015; Alal, 2018), *Sebates inermis* (Oh et al., 2018), *C. auratus* (Hafeez-ur-Rahman et al., 2015) and *Epinephelus coiodes* (Amadou et al., 2019) achieved optimal growth on a feeding frequency twice a day. Different fish species produce different growth responses to the application of feeding frequency.

The relationship between feed efficiency and feeding frequency has also been studied in various fish species, such as *O. niloticus* and *C. auratus gibelio*, which experienced increased feeding efficiency with increasing feeding frequency. (Zhao et al., 2016; Daudpota et al., 2016). However, previous studies found no positive correlation between feeding efficiency and frequency on *Clarias gariepinus*, *O. niloticus* (GIFT), and *Trachinotus ovatus* fry (Ajani et al., 2011; Huang et al., 2015; Wu et al., 2015). In contrast, other studies reported a negative correlation between feeding frequency and efficiency in *O. niloticus*, *C. auratus*, and *Channa striatus* fry (Hafeez-ur-Rahman et al., 2015; Muntaziana et al., 2016; Alemayehu & Getahun, 2017; Alal, 2018).

Previous studies showed that increasing the feeding frequency to three times a day only caused an increase in α-amylase activity and not for proteases and lipases. However, a further increase in the feeding frequency caused a decrease in α-amylase activity. (Tian et al., 2015). Another study in Atlantic salmon, *Salmo salar*, that was fed continuously also showed lower trypsin, lipase, and amylase activity compared to fish fed three times a day (Shi et al., 2017). However, studies on *O. niloticus* showed no differences in digestive protease, lipase, and amylase activity with increasing feeding frequency (Thongprajukaew et al., 2017). There appears to be a different response to digestive enzyme activity associated with different feeding strategies. Therefore, research related to differences in feeding frequency is deemed necessary.

Yellow rasbora, *R. lateristriata* is one of 42 species owned by Indonesia (Eschmeyer et al., 2016) and known as *seluang* in Kalimantan and Sumatera, and as wader in Java (Asrial et al., 2017). Previous studies on the biology of *R. lateristriata* have been carried out, including determining changes in the activity of digestive enzymes at different feeding levels (Susilo et al., 2016) and digestive enzyme activity of yellow rasbora at different ages (Susilo et al., 2018). However, further information is still needed to improve the success of the domestication of the yellow rasbora. Among the scientific studies that still need to be carried out are examining growth, feed efficiency, and digestive enzyme activity in fish receiving different feeding frequencies. Therefore, this study aimed to determine yellow rasbora growth, feed efficiency, and digestive enzyme activity at different feeding frequencies. The results of this study will contribute to the bio-enrichment of yellow rasbora cultivation, so it is expected to increase the success of yellow rasbora domestication in the future.

MATERIALS AND METHODS

Time and Research Location

The study was conducted for culture and growth measurement in June-September 2020 at the PSDP D3 Experimental Station, Faculty of Biology. Enzyme activity was prepared dan measured at the Laboratory of Animal

Physiology and Molecular Biology, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto.

Experimental Design

This research was conducted experimentally using a basic design in the form of a completely randomized design (CRD) consisting of four treatments and three replications. The treatments included: P1: Feeding two times/day (07.00 and 15.00); P2: Feeding three times/day (07.00, 11.00, and 15.00); P3: Feeding four times/day (07.00, 09.40, 12.20 and 15.00); P4: feeding five times/day (07.00, 09.00, 11.00, 13.00 and 15.00). The treatment applied was in the form of differences in the feeding frequency. During rearing, fish were fed as much as 3% of the daily biomass weight (Susilo et al., 2016). The feed was commercial, with a protein content of 34.53% and fat of 12.95. The aquariums were cleaned daily to remove waste and maintain water quality.

Experimental Animals

The study used the Yellow Rasbora (F1) aged two months produced by the Biology D3 PSDP Experimental Laboratory Universitas Jenderal Soedirman Purwokerto. The weight of the fish used in this study ranged from 0.3-0.5 g/head. In this study, 420 fish were used and kept in 12 glass aquariums, each measuring 30x30x50 cm (about 35 L of water volume) with a density of 35 fish per aquarium. Experimental aquariums were arranged into four groups with a recirculation system. Acclimation of the test fish was carried out for one week. Initially, the experimental fish fasted for 24 hours, and their length and body weight were measured. Fish body length and weight were measured during rearing in the third and sixth weeks. At the end of the experiment, the fish were fasted for 24 hours, and then some of the fish were sampled to isolate their digestive organs for measuring digestive enzyme activity.

Calculation of Growth Parameters, Efficiency, and Feed Retention

Growth and efficiency parameters are calculated as follows: Relative growth rate (RGR) = [(Wt (g) – Wo (g)/Wo (g)] x 100 % Specific Growth Rate (SGR) = [(ln Wt (g)– ln Wo (g)))/ t (days)] x 100. Condition Factor (FK) = 100 x fish weight (W) / fish length $(L)^3$. Feed Conversion Ratio (FCR) = amount of feed given (g)/weight gain of fish (g). Protein Efficiency Ratio (PER) = [Wt(g)–Wo(g)]/ protein given (g). Protein Retention (RP) = 100 x (increase in body protein (g)/protein given (g). Lipid retention (RL) = 100 x (body lipid gain (g)/feed lipid provided (g) Total protein content was measured by the Kjeldahl method, and levels of total fat were measured by the Soxhlet method (Nielsen, 2017).

Homogenate of Digestive Organs Preparation

Ten fish were taken from each aquarium as samples in the sixth week to measure their digestive enzyme activity. The fish was anesthetized by immersing it in ice water until it passes out, then the fish's stomach was dissected, and its digestive organs were taken.

The isolated digestive organs were then crushed using an electric homogenizer in a cold solution of 50 mM Tris-HCl buffer pH 7.5 containing 10 mM NaCl at 1: 8 w/v. The homogenate obtained was centrifuged in a refrigerator centrifuge (temperature 4 ºC) at 12,000 rpm for 15 minutes, and the supernatant obtained was stored in a refrigerator at -80 ºC (Thongprajukaew et al., 2010). The supernatant's protein content was determined with Folin-phenol reagent and using albumin as a standard (Gangadhar et al., 2017).

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Enzyme Activity Measurement

This study used the azo-casein hydrolysis method (Thongprajukaew et al., 2017) with modification to measure total protease activity. The buffer used was Tris-HCl 0.1 M (pH 8.1). Absorbance was measured at 366 nm. The specific activity of the total protease is expressed as U (Abs366.h-1).mg protein-1.

The *p*-Nitrophenylpalmitate (p-NPP) hydrolysis method has been used to measure lipase activity (Klahan et al., 2009). The p-nitrophenol concentration resulting from the hydrolysis of *p*-NPP was measured at 410 nm and calculated from the standard p-nitrophenol curve. Lipase-specific activity is expressed as U (µmol p-nitrophenol.h-^{1}).mg-^{1} protein.

Measured alkaline phosphatase activity following the hydrolysis of p-nitrophenyl phosphate (*p*NPP) as a substrate (Susilo et al., 2018). The absorbance was measured with a spectrophotometer at 405 nm. Standard p-nitrophenol curves determined alkaline phosphatase activity. Alkaline phosphatase activity is expressed in U (µmol p-nitrophenol.h-^{1}). mg-^{1} protein supernatant.

The present study used the 3,5-Dinitrosalicylic acid method with starch as a substrate to measure amylase activity (Thongprajukaew et al., 2017). The buffer used was Tris-HCl 0.1 M (pH 8.1). The absorbance of maltose from starch hydrolysis was measured with a spectrophotometer at 540 nm. The amount of maltose released 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.

Data Analysis

Quantitative data obtained at this stage were analyzed by one-way ANOVA using the Windows software package SPSS version 18.0. Data in the form of percentages are transformed first into the Archsin form.

RESULTS AND DISCUSSION

The results of the final weight measurement for yellow rasbora showed an increase in weight after rearing for six weeks or 42 days, as shown in Table 1.

Table 1. Growth of yellow rasbora at different feeding frequencies

Parameter	Feeding Frequency			
	2 times/day	3 times/day	4 times/day	5 times/day
Initial weight (g)	0.29±0.01	0.27±0.01	0.28±0.01	0.29±0.01
Final weight (g)	0.43±0.01	0.41±0.01	0.44±0.03	0.44±0.03
Weight gain (g)	0.14±0.02	0.14±0.01	0.16±0.02	0.15±0.01
Relative Growth Rate (%)	47.46±7.52	54.03±5.07	56.36±4.50	50.52±3.23
Specific Growth Rate (%)	1.16±0.23	1.16±0.27	1.24±0.06	1.38±0.25
Condition Factor (CF)	0.90±0.07	0.85±0.07	0.90±0.04	0.90±0.13

The average weight gain for yellow rasboras ranged from 0.14 ± 0.2 g in fish fed twice a day, while the who received a feeding frequency four times a day of 0.16 ± 0.2 g (Table 1). The relative growth rate (RGR) was 47.46 ± 7.52% at the feeding frequency twice a day, while the fish given the feeding frequency four times a day had an RGR of 56.36 ± 4.5%. The specific growth rate (SGR) was 1.16 ± 0.23% in fish fed twice a day, while

in fish fed five times daily, the SGR was 1.38 ± 0.25%. The fish condition factor obtained was less than 1 (Table 1). The analysis of variance showed that body weight gain, relative growth, specific growth rate, and condition factors did not show any difference between the feeding frequency ($p>0.05$). The results of this study indicated that changes in the feeding frequency more than twice a day did not result in changes in the growth performance of yellow rasbora.

The results of this study were no different from studies on *Rachycentron canadum*, *C. gariepinus*, and tilapia GIF, which showed no differences in body weight gain and specific growth rate of fish when the feeding frequency was between two to five times a day. (Ajani et al., 2011; Huang et al., 2015; Moreira et al., 2015). However, this study's results differed from previous studies on *O. mossambicus*, *C. auratus*, *O. niloticus*, and *Scortum barcoo*, which showed significant differences in weight gain and specific growth rates when fish fed with different feeding frequencies. between one and five times a day (Jegede & Olorunfemi, 2013; Luthada & Jerling, 2013; Habib et al., 2014; Al-Khafaji et al., 2017). The difference in the results of this study with previous studies was not only caused by differences in species' response to different treatments but also thought to be due to differences in the feeding frequency. Previous studies showed that *O. niloticus*, *C. gariepinus* x *Heterobranchus longifilis* hybrids, *Sebastes inermis*, and *Sadinella brasiliensis* showed the lowest wet weight gain and specific growth rate at once-daily feeding frequency. There was an increase in fish body weight gain and specific growth rate at the feeding frequency twice a day, but feeding frequency more than twice a day did not increase wet weight gain and specific growth rate of fish (Ndome et al., 2011; Baloi et al., 2014; Thongprajukaew et al., 2017; Oh et al., 2018). This study found that the condition factor of yellow rasbora did not differ from previous studies on *Trachinotus ovatus*, *Platichthys flesus luseus*, and *Megalorama amblycephala*. Previous studies showed that differences in feeding frequency did not cause changes in condition factors (Küçük et al., 2014; Tian et al., 2015), but this is different from the results of previous studies on *S. brasiliensis* which produced various condition factors at different feeding frequencies (Baloi et al., 2014). In this study, the amount of feed consumption did not differ between the differences in the frequency of feeding (Table 2), this resulted in no differences in fish growth, which was reflected in weight gain, SGR, RGR, and CF between differences in the frequency of feeding (Table 1). Increased fish growth due to increased feed consumption has been shown in *O. niloticus* (Kaya & Bilguven, 2015) and juvenile *Pseudobagrus ussuriensis* (Bu et al., 2017).

The calculation of individual feed consumption at 42 days of rearing results showed about 9.46 ± 0.56 g in fish fed four times a day and 11.76 ± 1.24 g in fish fed five times a day. Feed conversion (FCR) was 2.19±0.3 in fish fed five times daily and 1.73±0.16 in fish fed four times daily. Protein efficiency (PER) ranged from 1.32 ± 0.02 in fish fed five times a day, while fish fed four times a day produced a PER of 1.68 ± 0.16. Body protein levels ranged from 49.52 ± 0.07% to fish fed three times a day, whereas fish fed twice a day had a protein content of 52.58 ± 0.06%. The body protein content of the tested fish was no different from the experimental fish, which had a protein content of 49.58%. Body lipid levels ranged from 33.56 ± 1.23% in fish fed three times a day, while fish fed twice daily had 34.76 ± 1.76%. The lipid content of the tested fish was higher than the initial lipid level of the experiment, namely 26.63%. Protein retention (PR) was 12.68 ± 1.11% in fish fed four times a day, while fish fed twice a day

had a PR of 17.77 ± 4.64%. Lipid retention (LR) was 36.00 ± 8.51% in fish fed four times a day, while fish fed five times a day had an LR of 44.05 ± 7.78% (Table 2). Analysis of the variety of feed consumption, FCR, PER, protein content, lipid content, PR, and LR showed no significant difference (P>0.5) between different feeding frequencies. These results indicate that the difference in the feeding frequency between two to five times a day in yellow rasboras resulted in the same feed consumption performance, FCR, PER, protein, and body fat content, as well as PR and LR.

Table 2. Food efficiency and retention of yellow rasbora at different feeding frequencies

Parameter	Feeding Frequency			
	2 times/day	3 times/day	4 times/day	5 times/day
Feed consumption (g)	10.35±0.21	9.78±0.41	9.46±0.56	11.76±1.24
Feed conversion Ratio (FCR)	2.14±0.34	2.00±0.16	1.73±0.16	2.19±0.03
Protein efficiency ratio (PER)	1.37±0.22	1.45±0.11	1.68±0.16	1.32±0.02
Fish protein content (%)	52.58±0.61	49.52±0.07	50.37±1.76	50.63±2.28
Fish lipid content (%)	34.76±1.72	33.56±1.23	33.85±2.47	34.70±0.40
Protein retention (PR) (%)	17.77±4.64	14.34±1.45	12.68±1.11	17.64±5.91
Lipid retention (LR) (%)	43.07±12.16	38.68±4.97	36.00±8.51	44.05±7.78

Differences in species are one of the factors causing differences in fish response to changes in the feeding frequency. The results of this study on *R. lateristriata* were no different from those of studies on *O. niloticus*, which did not result in changes in feed efficiency and protein content by increasing the feeding frequency from one to three times a day (Thongprajukaew et al., 2017). A previous study reported a similar pattern in *Sebates inermes* which showed no differences in FE, PR, PER, PR, protein, and body fat levels with increasing feeding frequency from twice to three times a day (Oh et al., 2018). However, the results of this study on *R. lateristriata* differed from previous studies on C. *auratus gibelio*, which resulted in feed efficiency when the feeding frequency increased from two to four times a day (Zhao et al., 2016). Increased efficiency of feed and protein in fish fed three times a day compared to those only fed twice a day also occurred in Scortum barcoo (Al-Khafaji et al., 2017). In *Epinephelus coioides*, there was an increase in body lipid levels as the feeding frequency increased, but this is not the case with body protein levels (Amadou et al., 2019).

Feed efficiency is often correlated with the quality and quantity of feed consumed by fish. In this study, differences in the feeding frequency did not result in differences in feed consumption and growth. The absence of differences in feed consumption causes no difference in nutrient intake, so fish growth becomes relatively the same. The lack of differences in the amount of feed consumed and growth also impacts the absence of differences in FCR, PER, PR, and LR in yellow rasbora (Table 2.). Previous studies have been conducted on *O. mosambicus* and *C. auratus gibelio*, which showed an increase in feed efficiency when the amount of feed consumed

increased (Luthada & Jerling, 2013; Zhao et al., 2016).

Measurements of digestive enzyme activity showed protease activity ranging from 0.292 ± 0.047 Abs366.h-¹.mg-¹ in fish fed three times a day and protein 0.489 ± 0.147 Abs366.h-¹.mg-¹ in fish fed twice a day. In contrast, amylase activity ranged from 0.366 ± 0.088 mol.h-¹.mg-¹ protein in fish fed thrice daily to 0.610 ± 0.212 mol.h-¹.mg-¹ protein (Table 3). Analysis of the variance of protease and amylase activity did not show significant differences ($p>0.05$) between the different feeding frequencies. Lipase activity measurements ranged from 7.339 ± 3.643 mol p-nitrophenol.h-¹.mg-¹ protein in fish fed twice daily and 17.267 ± 1.24 mol p-nitrophenol.h-¹.mg-¹ protein in fish fed four times. While the activity of alkaline phosphatase was 28.04±4.771 mol p-nitrophenol.h-¹.mg-¹protein in fish fed three times a day, and 59.881±2.394 mol p-nitrophenol.h-¹.mg-¹protein on fish were fed four times a day (Table 3). The analysis of variance showed that the lipase and alkaline phosphatase activities were significantly different ($p<0.05$) between the different feeding frequencies. These results indicated that the increased feeding frequency did not change yellow rasbora's protease and amylase activities. In contrast, lipase activity showed that the feeding frequency of four times a day was higher than those only being fed twice daily. This study also showed a higher alkaline phosphatase activity in fish fed four to five times a day than those feed twice to three times daily.

Table 3. Digestive enzyme activities of yellow rasbora at different feeding frequencies

Parameter	Feeding Frequency			
	2 times/day	3 times/day	4 times/day	5 times/day
Protease (U.mg-¹protein)	0.489±0.147	0.292±0.047	0.434±0.112	0.372±0.122
Lipase (U.mg-¹ protein)	7.339±3.643[a]	11.984±4.048[ab]	17.267±1.240[b]	8.789±7.813[ab]
Amylase (U .mg-¹ protein)	0.556±0.213	0.366±0.088	0.610±0.212	0.476±0.149
Alkalinephosphatase(U.mg-¹protein)	33.993±11.751[a]	28.04±4.771[a]	59.881±2.394[b]	58.524±24.349[b]

The increase in lipase and alkaline phosphatase activity did not increase the fish's growth rate and final weight. Still, body lipid levels rose at the end of the experiment compared to the beginning of the experiment (Table 2.). This study's results differed from studies on *C. auratus gibelio*, which increased protease activity as the feeding frequency increased (Zhao et al., 2016). The results of this study are also different from those studied in Atlantic salmon, *Salmo salar*, which showed a decrease in trypsin, lipase, and amylase activity in fish that were fed continuously compared to fish that were fed three times a day (Shi et al., 2017). However, the results of this study were not different from those in *O. niloticus*, which did not show differences in protease and digestive amylase activity with increasing feeding frequency (Thongprajukaew et al., 2017).

Increasing the feeding frequency causes lipids and digestive products to be more available in the digestive tract of fish, but not in this study. Feed without lipids can suppress alkaline phosphatase activity, while lipid intake stimulates intestinal alkaline phosphatase expression (Lalles 2020). Studies on juvenile *Rutilus rutilus caspisus* have also shown a

stimulatory effect of increasing alkaline phosphatase activity with increasing feed intake (Abolfathi et al., 2012). In this study, the increase in lipid and alkaline phosphatase activities was not caused by an increase in feed consumption but by other factors that still need to be investigated. It seems that different species produce different responses to the application of the feeding frequency. In the yellow rasbora increase in the feeding frequency does not result in changes in the activity of digestive enzymes, except for lipase and alkaline phosphatase, it produces a growth rate that is not different in yellow rasbora.

CONCLUSION

Growth performance, feed efficiency, protease, and amylase activities of yellow rasbora were not affected by the feeding frequency between two and five times a day. Still, the highest lipase and alkaline phosphatase activity were achieved at the feeding frequency of four times a day, although they did not affect growth. Therefore, feeding twice daily is considered quite efficient for the culture of yellow rasbora.

AUTHOR CONTRIBUTION

All authors contributed to the writing of this manuscript. U.S. designed and wrote a draft of the manuscript. Y.S. contributed to processing data on enzyme activity and writing draft manuscripts, while E.S.W. contributed to measuring growth, tabulating, and analyzing growth data. H.S. did language editing and manuscript review. H. contributed to measuring feed efficiency parameters, tabulation, and analysis of feed efficiency, and R. wrote and edited the language. All authors contributed to preparing the final draft of the manuscript through a discussion and conclusion.

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CONFLICT OF INTEREST

There is no conflict of interest between the authors regarding the order of authors and the manuscript contents. All authors agree to submit this manuscript to Jurnal Biodjati.

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