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ALKALINE PROTEASE, AMYLASE AND CELLULASE ACTIVITIES OF YELLOW RASBORA, *Rasbora lateristriata* Blkr., AT DIFFERENT FEEDING LEVELS

AKTIVITAS PROTEASE ALKALIN, AMILASE DAN SELULASE IKAN LUNJAR, *Rasbora Lateristriata* Blkr., PADA LEVEL PEMBERIAN PAKAN BERBEDA

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ABSTRACT

Alkaline protease, amylase and cellulase activities of the digestive organ of yellow rasbora, *Rasbora lateristriata* Blkr., was evaluated with four different feeding levels of 0.34 g protein+0.03 g fiber, 1.01 g protein+0.10 g fiber, 1.69 g protein+0.16 g fiber and 2.36 g protein+0.23 g fiber/day/100 g fish biomass. A total of 280 fish with average body weight of 0.71±0.06 g were used in this study. The results showed that the difference in the feeding levels caused in a significant difference in trypsin and chymotrypsin activities ($P < 0.05$), but not for amylase and cellulase activities ($P > 0.05$). In conclusion, protein digestion capacity increased, but not to the digestion of starch and fiber in response to different feeding levels and the optimal feeding level for yellow rasbora was 1,01 g protein+0,10 g fiber/day/100 g fish biomass.

Keywords :Amylase, cellulase, chymotrypsin, rasbora, trypsin

ABSTRAK

Aktivitas protease alkalin, amilase dan selulase organ pencernaan ikan lunjar, *Rasbora lateristriata* Blkr., telah dievaluasi dengan empat tingkat pemberian pakan yang berbeda (0,34 g protein + 0,03 g serat, protein 1,01 g + 0,10 g serat, 1, 69 g protein + 0,16 g serat dan 2,36 g protein + 0,23 g serat / hari / 100 g biomassa ikan). Sebanyak 280 individu ikan dengan berat badan rata-rata 0.71 ± 0.06 g telah digunakan dalam penelitian ini. Hasil penelitian menunjukkan bahwa perbedaan level pemberian pakan menghasilkan perbedaan yang signifikan dalam aktivitas tripsin dan kimotripsin ($P < 0,05$), tetapi tidak untuk aktivitas amilase dan selulase ($P > 0,05$). Kesimpulan, kapasitas pencernaan protein meningkat, tetapi tidak untuk pencernaan pati dan serat dalam menanggapi level pemberian pakan berbeda dan level pakan optimum untuk ikan lunjar adalah 1,01 g protein + 0,10 g serat / hari / 100 g biomassa.

Kata kunci : Amilase, kimotripsin, rasbora, sellulase, tripsin

INTRODUCTION

Yellow rasbora in Indonesia known as lunjar, has a moderate economic value (Dina Boer, and Butet, 2011; Rosadi, Endang, Setyohadi, & Bintoro, 2014). This economic value leads to high exploitation which may decrease its production. It has been reported that yellow rasbora production in Agam, Sumatera Island has been dropped from 307.05 tons in 2006 to 142.21 tons in 2007 (Dina, *et al*., 2011). This happen also in Kalimantan Island (Sulistiyarto,

2012) and West Java (Astuti, 2012). Therefore, more attention should be paid on this yellow rasbora *Rasbora lateristriata* Blkr. to avoid extinction. In first study domestication was successfully performed in the wet laboratory of Faculty of Biology Unsoed, Purwokerto. However, this effort need to be optimized by providing scientific data, especially on nutritional physiology. Therefore, it is important to study a nutritional physiology of yellow rasbora, especially the ability of yellow rasbora on nutrients utilization.

The ability of fish to utilize the nutrients depends on the ability of digestion which is reflected by digestive enzyme activity. Therefore, the study on digestive capacity which is reflected in the activity of the digestive enzyme is required to harmonize the digestive capability and the consumed feed to improve feeding efficiency.

Various digestive enzymes can be found throughout the gastrointestinal tract with different activities, but in general they can be classified as digestive enzymes of protease, lipase and carbohydrase. Alkaline protease activity such as trypsin and chymotrypsin are found in intestine and pancreas of herbivores, omnivores and carnivores. Previous studies had demonstrated the activity of trypsin and chymotrypsin in the pancreas and intestine in a variety of fish such as *Parachanna obscura* (Odedeyi, 2007), *Mormyrus rume* (Odedeyi & Fagbenro, 2010), herbivores and carnivores stomachless fish (Day *et al*., 2011), *Solea senegalensis* (Rodiles, Santigosa, Herrera, Hachero-Cruzado, & Cordero, 2012), *Diplodus sargus* (Yufera, Moyono, Astola, Pousau-Ferreira, & Martinez-Rodriguez, 2012), *Horabagrus brachysoma* (Prasad & Suneesha, 2013), *Lates niloticus* (Namulawa et *al*., 2013), *Etroplus suratensis* and *Oreochromis mossambicus* (Sankar et *al*., 2014).

Trypsin has an optimum pH of 8.0 in *Theragra chleogramma* (Kishimura, Klomklao, Benjakul, & Chun, 2008), but in *Glyptosternum maculatum* (carnivores) the optimal pH for proteases found along the intestine are pH 9.0 to 10.0, (Xiong, Xie, Zhang, & Liu, 2009), and the same phenomenon is also observed in *Zosterisessor ophiocephalus*, *Raja clavata* and *Scorpaena scrofa* which has alkaline protease activity at the optimum pH 8-10 (Nasri *et al*., 2011). The end product of trypsin and chymotrypsin digestion in the form of peptide fragments will be the substrate for the aminopeptidase and carboxypeptidase.

Activity of carbohydrase, such as amylase, is also found in a variety of fish such as *Perca fluvialis, Salvelinus alpinus, Mormyrus rume,* and New Zealand herbivorous fish (Skea, Mounfort, and Clements, 2007; Odedeyi & Fagbenro, 2010 ; Langeland, Lindberg, and Lundh, 2013), but amylase activity is not found in three species of tuna, *Thunnus albacares, Katsuwonus pelamis* and *Thunnus tonggol* (Prasertsan & Prachumratama, 2008). In three Cyprinidae species, it has also shown that α-amylase activity is influenced by feeding habits and α-amylase in herbivorous fish is more superior than in omnivores and carnivores (Al-Tameemi, Aldubaikul, and Salman, 2010).

Amylase activity is also associated with the fish food category. Herbivorous fish such as prickleback fishes (*Cebidichthys violaceus*) has α-amylase activity higher than carnivorous fish (German, Horn, and Gawlicka, 2004). The same phenomenon is also found in halfbeaks fish (Hemiramphidae), a herbivorous fish, in which maltase activity is not different from carnivorous fish, but has a higher α-amylase activity (Day *et al*., 2011). In *Lates niloticus*, amylase activity is also observed in the digestion system (Namulawa *et al*., 2013). Amylase is active in neutral to alkaline, such as those found in four species of herbivorous minnows fish (German, 2009), *Betta splendens* (Thongprajukae, Kovitvadhi,

Engkagul, and Rungruangsak-Torrissen, 2010) and *Rhamdia quelen* (Lazzari *et al*., 2010), three species of cyprinid (Al - Tameemi *et al*., 2010) and *Pangasianodon gigas* (Tongsiri, Mang-Amphan, and Peerapornpisal, 2010).

Fish generally do not secrete cellulase, so the ability to hydrolyze the fiber in the diet is very low. The fish ability to digest fiber is associated with symbiotic microbes that have the ability to produce cellulase in the digestive tract, such as those found in *Labeo rohita* and *Channa punctatus* (Kar & Ghosh, 2008). The activities of cellulase is also observed in *Pangasianodon gigas* (Tongsiri *et al*., 2010) and *Diplodus puntazzo* (Savona, Tramati, and Mazzola, 2011).

Evaluation of the relationship between food quality and quantity on digestion capacity have also been carried out on several fish species, such as on *Labeo rohita* (Debnath *et al*., 2007), *Salmo salar* (Kraugerud *et al*., 2007), tilapia, *Oreochromis niloticus* (Chan, Lee, Cheng, Hsieh, and Weng, 2007), *Betta splendens* (Thongprajukaew, Kovitvadhi, Kovitvadhi, Somsueb, and Rungruangsak-Torrissen, 2011). and *Sparus sole senegalensis* (Rodiles *et al*. 2012).

However, the study on the relationships between different feeding levels and digestive enzyme activity, especially in yellow rasbora, *Rasbora lateristriata* Blkr., has never been done. Information related to a response of digestive enzyme to feeding levels of yellow rasbora is needed to understand the capacity of fish digestion. Therefore, this study aimed to determine the activity of alkaline protease (trypsin and chymotrypsin), amylase and cellulase in response to different feeding levels on yellow rasbora.

EXPERIMENTAL SECTION

Materials and Instruments

Tris (hydroxymethyl) amino-methane (Tris) (Sigma-Aldrich, ACS Reagent, >99.8%), hydrochloric acid (Merck, 36.5-38.0%), 3,5-dinitrosalicylic acid (DNS) (Sigma-Aldrich, >98%), Nα-*p*-tosyl-L-arginine hydrochloride methyl ester (Sigma-Aldrich, AG), N-benzoyl-L-tyrosine ethyl ester (Sigma-Aldrich, AG), Sodium acetate (Merck, AG), Folin & Ciocalteu's phenol reagent (Sigma-Aldrich), Starch (Bio Basic Canada, High Purity), carboxymethylcellulose (Merck, Technical Grade), centrifuge (Eppendorf, 5415 R), spectrophotometry (Hitachi, U-2900), single channel pipette (Serana), water bath (JEIO-TECH, WB-20E).

Experimental Design

The study was carried out by experimental methods using completely randomized design (CRD) with four treatments and three replications. The treatment was as follow:
A. feed containing 0.34 g protein+0.03 g fiber/day/100 g biomass; B. (1.01 g protein+0.10 g fiber/day/100 g biomass); C. (1.69 g protein+0.16 g fiber/day/100 g biomass); D. (2.36 g protein+0.23 g fiber/day/100 g biomass).

A total of 280 fish with an average weight of 0.71 ± 0.06 g were used in this study. Fish samples were obtained from the wet laboratory of Faculty of Biology, Unsoed. Fish were reared for one month in four concrete tanks(1.0 x 1.2 m^2) filled with 0.24 L of water and were fed artificial feed. During the experiment, fish were fed two times a day in accordance with the treatment applied. Proximate analysis based on dry matter of artificial feed showed in **Table 1**.

Table 1. Mean nutrient content of the artificial feed

Water (%)	Dry Matter (%)	Dry weight				
		Crude protein	Crude fat	Crude fiber	Ash	NFE
9.85		33.71	5.67	3.27	8.55	38.96

Preparation of Tissue Homogenate

In the final experiment, fish in each concrete tank was divided into six pool samples. The digestive organs were taken out from fish in pool sample (10-12 fish) and were homogenized in ice-cold buffer solutions (50 mM Tris-HCl, pH 7.5) at a ratio of 1: 9, and than were centrifuged at 12,000 rpm for 15 minutes at 4 ^{o}C. All supernatant were collected in 1500 µL eppendorf tubes, and then stored at -80 °C for future enzymatic assay. Protein contents in the supernatant were measured using Lowry method (Thongprajukaew, Kovitvadhi, Engkagul, & Rungruangsak-Torrissen, 2010) with albumin as a standard.

Enzyme Activity Essay

Trypsin activity essay

Modification method of Kishimura, (2008) using Nα-p-tosyl-L-argnine hydrochloride methyl ester (TAME) was used to measure trypsin activity. 10 mM TAME substrate was prepared by dissolving 37.9 mg TAME into 10 mL of double-distilled water. Buffer 46.0 mM Tris-HCl at pH 8.1 containing 11.5 mM $CaCl_2$ prepared by dissolving 1.39 g Tris into double-distilled water to a volume of 200 mL, then added 6.4 mL of 5% (w / v) $CaCl_2$. The solution was then adjusted its pH by adding a solution of 1 N HCl until the pH reached 8.1. The solution was then diluted to a volume of 250 mL reached. Mixture reagent consisted of 100 µL enzymes extract, 300 µL substrates and 1500 µL buffer. Reactions were started by addition of enzyme extract into TAME and buffer solution. The reaction mixture was then shaken and poured into a quartz cuvette. After the cuvette was placed in a spectrophotometry, absorbance was setup at zero conditions; absorbance measurement performed every 1 minute for 3 minutes. Hydrolysis of TAME was measured as the change in absorbance at a wavelength of 247 nm for 3 minutes at temperatures of 30 °C. The activity of trypsin expressed as U = change Abs247 /min/mg protein.

Chymotrypsin activity essay

Chymotrypsin activity was measured using N-benzoyl-L-tyrosine ethyl ester (BTEE) as a substrate (Lazzari *et al*., 2010). 2 mM BTEE substrate was prepared by dissolving 31.4 mg BTEE up to 50 mL to 50% methanol. 80.0 mM Tris-HCl buffer at pH 7.8 containing 0.1 M $CaCl_2$ was prepared by dissolving 2.42 g Tris into double distilled water to a volume of 150 mL, then added 55 mL of 5% (w / v) $CaCl_2$. The solution is then adjusted pH by adding a solution of 1 N HCl until the pH reached 7.8. The solution was then diluted to a volume of 250 mL. The mixture reaction consisting of 100 µL of enzyme extract, 1400 µL substrate and 1500 µL buffer. Reactions were started by addition of enzyme extract to the mixture of BTEE and buffer solution. The reaction mixture was then shaken and poured into a quartz cuvette. After the cuvette was placed in a spectrophotometry, absorbance was setup in zero condition; absorbance measurements performed every 1 minute for 3 minutes. BTEE hydrolysis was measured as a change in absorbance at a wavelength of 256 nm for 3 minutes at temperatures of 30 °C. Chymotrypsin activity expressed as U = change Abs256 /min/mg protein.

Amylase activity essay

Modification method of Klahan, Areechon, Yoonpundh, & Engkagul, (2009) using 3,5-Dinitrosalicylic acid with the starch as substrate was used to measure amylase activity. The reagent of 0.1 M Tris-HCl (pH 8.1) was used as a buffer. Buffer 0.1 M Tris-HCl (pH 8.1) was prepared by dissolving 1.212 g Tris into double distilled water to a volume of 80 mL. The solution was then adjusted pH with 1 N HCl solution drop by drop until the pH reached 8.1. Buffer solution was then diluted until they reached a volume of 100 mL. 1% starch substrate was

prepared by dissolving 1 g of starch to 100 mL of 0.1 M Tris-HCl buffer solution containing 0.01 M NaCl. The starch solution was then heated and boil for 5 minutes. Reagent 1% 3,5-dinitrosalicylic acid (DNS) was prepared by dissolving 1.0 g DNS into 30 mL of double-distilled water, then add 40 mL of 1 N NaOH and 30 g Na-K tartrate $4H_2O$, then added double-distilled water up to a volume of 100 mL. Mixture Reaction consisting of substrate (750µL), buffer (70 µL) and the enzyme extract (50µL) were incubated for 20 min at 37 °C, and the reaction was stopped by addition of 1500 µL of 1 % DNS reagent. The mixture reaction was put into boiling water for 5 minutes. The same procedure was done on the blank tube except that enzyme extract was added after the 1 % DNS reagent. The maltose as the product of hydrolysis of the substrate was determined by the standard curve of maltose at 540 nm. Maltose standard curve made with maltose concentrations between 0.21 to 3.36 µmol/mL. The activity of amylase was calculated as the amount of maltose released (µmol) /min/mg protein extract.

Cellulase activity essay

Modification method of Savona *et al*. (2011) using 3,5-Dinitrosalicylic acid and substrate carboxymethylcellulose (CMC) were used to measure the cellulase activity. Reagent 0.1 M sodium acetate (pH 5.0) was used as a buffer. 0.1 M sodium acetate buffer (pH 5.0) was prepared by dissolving 578.3 mg $C_2H_3O_2Na$ into double-distilled water to a volume of 80 mL. Then set a pH buffer solution with added 178.5 mg of glacial acetic acid until the pH reached 5.0 and the buffer was diluted with double-distilled water until they reached a volume of 100 mL. Substrate carboxymethyl cellulose 1% was prepared by dissolving 0.5 g of CMC in 50 mL acetate buffer solution. Reagent, 1% DNS was prepared by dissolving 1 g DNS, 0.2 g of phenol, 0.05 g of sodium sulfite and 20 g of Na-K tartrate in 50 mL of 2% NaOH. DNS reagent was then diluted with double-distilled water until they reached a volume of 100 mL. Mixture Reaction consisting of substrate (300 µL), buffer (300 µL) and the enzyme extract (60 µL) were incubated for 20 min at 40 °C, and the reaction was stopped by addition of 900 µL of 1% DNS reagent. The mixture reaction was put into boiling water for 5 minutes. The same procedure was done on the blank tube except enzyme extract was added after the 1% DNS reagent. The releasing amount of maltose from substrate were determined from a standard curve of maltose at 540 nm. Maltose standard curve was prepared in the same manner as that used for the measurement of amylase activity. The activity of cellulase was calculated as the amount of maltose released (µmol)/min/mg protein extract.

Statistical analysis

Data of enzyme activity was analysis with One Way ANOVA, followed with post test using Least Significant Difference (LSD) at 0.05 significance level. Statistical analysis was performed using SPSS 18.0 package version of Windows software.

RESULT AND DISCUSSION

Trypsin and chymotrypsin activities

A significant difference of trypsin activity was observed among feeding levels ($P <. 0.05$) with the lowest activity in fish fed by A (0.34 g protein+0.03 g fiber/day/100 g biomass). However, no statistical difference among trypsin activities on fish fed by B (1.01 g protein+0.10 g fiber/day/100 g biomass), C (1.69g protein+0.16 g fiber/day/100 g biomass) and D (2.36 g protein+0.23 g fiber/day/100 g biomass) (**Figure 1**). The results indicated that optimal trypsin activity on fish was fed by B (1.01 g protein+0.10 g fiber/day/100 g biomass).

Chymotrypsin activity among feeding levels showed a similar pattern

with trypsin activity. It was significantly different among feeding levels ($P < 0.05$) with the lowest chymotrypsin activity obtained from fish fed by A (0.34 g protein+0.03 g fiber/day/100 g biomass) and the highest activity was observed in fish fed by B (1.01 g protein+0.10 g fiber/day/100 g biomass). However, there was no significant difference between fish fed by C (1.69 g protein+0.16 g fiber/day/100 g biomass) and by D (2.36 g protein+0.23 g fiber/day/100 g biomass) (**Figure 1**). The changes in nutrient intake in this study resulted in the alteration of trypsin and chymotrypsin activities, which was indicated by the increase of trypsin and chymotrypsin activities in yellow fish rasbora fed by B (1.01 g protein+0.10g fiber/day/100 g biomass) compared to the activity of trypsin and chymotrypsin in fish fed by A (0.34 g protein+0.03 g fiber/day/100 g biomass). The feed quantity of 1.01g of protein +0.10 g fiber/day/100 g biomass was optimum for yellow rasbora to live because the further addition of protein and fiber content did not give specific activities of protease significantly.

In this study, we used TAME as substrate and change of absorbance as unit activities, but previous studies used N-benzoyl-DL-arginine *p*-nitroanilide (BAPNA) as substrate and *p*-nitroaniline as the standard unit of activities. Therefore, our result could not be compared to previous studies, such as the study from *Betta splendens* (Thongprajukaew *et al*., 2010), *Silurus meridionalis* (Zeng, *Li*, Fu, Can, & Zhang, 2012), *Oreochromis niloticus* (Abdel-Warith, Younis, Al-Asgah, & Abdualla, 2013) and *Poecilia reticulata* (Thongprajukaew & Kovitvadhi, 2013).

Differences among protease activities were due to differences in nutrient intake as observed in this study that was also found in *Labeo bata* (Mondal, Kaviraj, & Mukhopadhyay, 2012), *Megalobrama amblycephala* (Habte-Tsion *et al*., 2013) and *Labeo rohita* (Kumar, Jain, Munilkumar, Sahu, & Pal, 2013).

So it seems that yellow rasbora only responded to increased level of up to 1.01 g protein+0.10 g fiber/day/100 g biomass because it did not increase trypsin and chymotrypsin activities at upper levels. The capacity of digestion and absorption of proteins was due to the increase intake of protein in a feed (Karasov, Mart´ınez del Rio, & Caviedes-Vidal, 2011).

Chymotrypsin activity was higher than trypsin activity resulted in low trypsin and chymotrypsin ratio (the ratio of T/C). The T/C ratio in this study ranged from 0.56 to 1.69 (**Figure 2**).

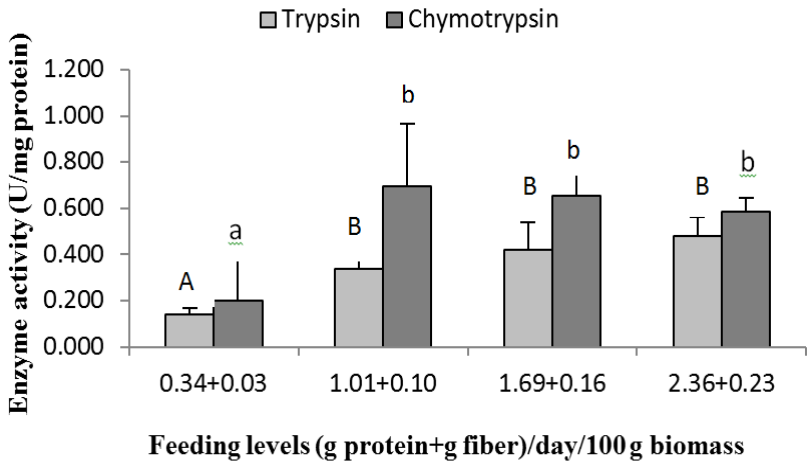

Figure 1. Mean (+S.D) trypsin and chymotrypsin activities of yellow rasbora at different feeding levels. Mean with different superscript was different significantly ($P<0.05$).

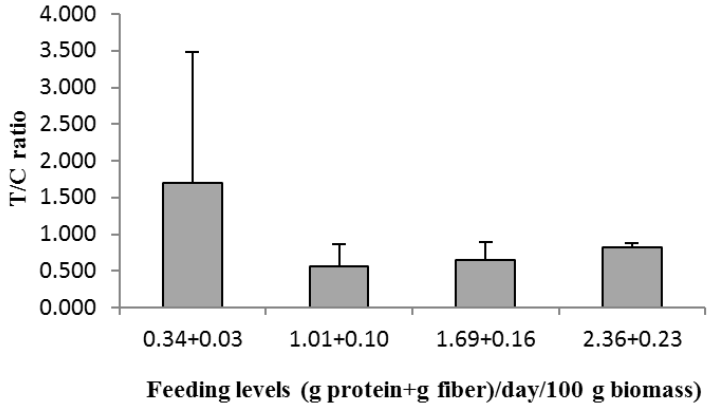

Figure 2. Mean (+S.D) trypsin/chymotrypsin ratio of yellow rasbora at different feeding levels.

The trypsin / chymotrypsin (T/C) ratio was not different significantly among feeding levels (P > 0.05). The T/C ratio in this study was lower than that obtained in *Salmo salar* (Rungruangsak-Torrissen, Moss, Andresen, Berg, &Waagbo 2006) and *Poecilia reticulate* (Thongprajukaew & Kovitvadhi, 2013), but higher than those obtained at *Oreochromis mossambicus* (Chan *et al*. 2007). The low T/C ratio at all feeding levels might reflect a low growth rate of yellow rasbora, because the T/C ratio is an indicator of growth rate. The T/C ratio often related to feeding efficiency and growth rate (Rungruangsak-Torrissen *et al*., 2009; Rodiles *et al*., 2012).

Amylase activity

In this study, amylase activity ranged from 0.83 to 0.86 U/mg protein (**Figure 3**) and showed no significant differences among feeding levels (P > 0.05). Amylase activity obtained in this study was lower than previous studies on Cyprinidae (Al-Tameemi *et al*., 2010), giant catfish, *Pangasianodon gigas* Chevey (Tongsiri *et al*., 2010), but was higher than on carnivorous fish *Glyptosternum maculatum* (Siluriformes) (Xiong *et al*., 2010).

No significant differences in amylase activity in increasing the quality and quantity of nutrients in this study also occurred in previous studies on *Seriola lalandi* (Bowyer, Qin, Adams, Thomson, & Stone, 2012), and *Rutilus rutilus* (Soleimani, Hoseinifar, Merrifield, Barati, & Abadi, 2012), but the different responses found at *Dentex dentex* (Perez-Jimenoz *et al*., 2009), *Rhamdia quelen* (Lazzari *et al*., 2010), *Labeo bata* (Mondal *et al*., 2012), *Megalobrama amblycephala* (Habte-Tsion *et al*., 2013) and *Labeo rohita* (Bhilave Nalawade, & Kulkarni, 2014).

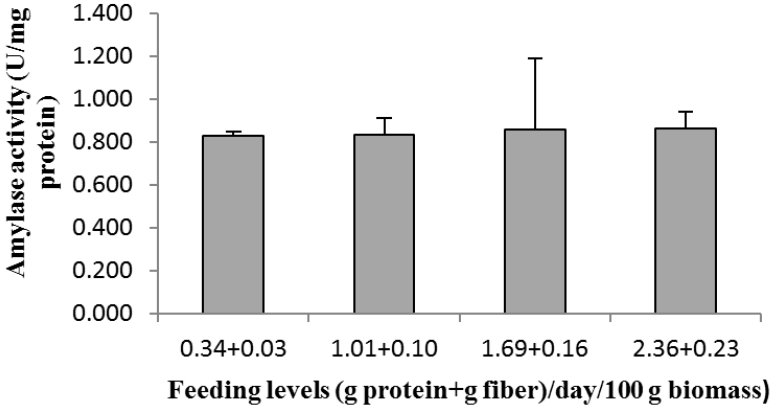

Figure 3. Mean (+S.D) amylase activity of yellow rasbora at different feeding levels.

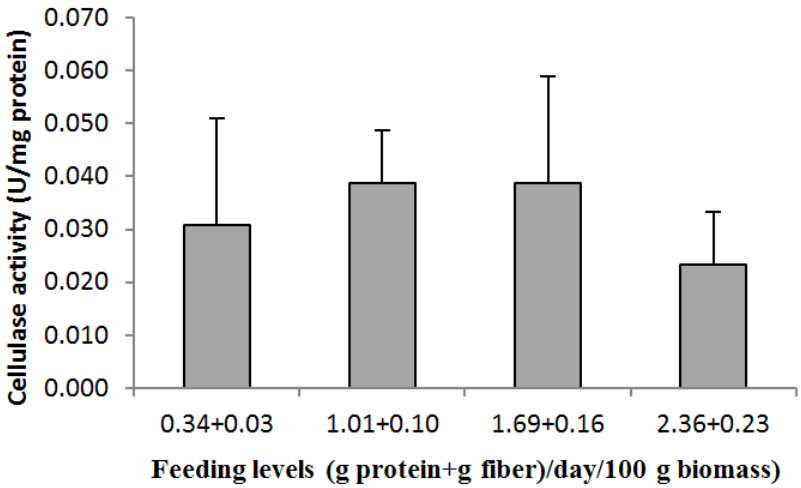

Figure 4. Mean (+S.D) cellulase activity of yellow rasbora at different feeding levels.

The low amylase activity in this study and the differences in the category of feed suspected to cause no significant difference of amylase activity. Yellow rasbora, *Rasbora lateristriata* Blkr., is a member of Cyprinidae, and species differences also seem to produce differences in the ability of fish to digest starch.

Some Cyprinidae were omnivorous fish, like yellow rasbora (Sulistiyarto, 2012), so it has a lower capacity to digest carbohydrates than herbivorous. It seems that changes in feed intake were not significantly altered amylase activity.

Cellulase activity

Cellulase activity in this study was measured with CMC substrates ranging from 0.02 to 0.04 U/mg protein (**Figure 4**) and showed no significant difference among feeding levels ($P > 0.05$). Cellulase activity obtained in this study was higher than in previous studies on carnivorous fish such as *Parachanna (Channa) obscura* and *Gymnarchus niloticus*, but was lower than tilapia, *Oreochromis niloticus* (Fagbenro *et al.* , 2005) and the giant catfish, *Pangasianodon gigas* (Tongsiri *et al.*, 2010). The low population of microbial symbioses in the lumen gut of yellow rasbora is believed to be the cause of low activity of cellulose, so it does not appear to have changed significantly with the increase of feed intake levels. The existence of cellulolytic bacteria on fish omnivores and carnivores have the ability to digest fiber because it generally consumes invertebrates that contain cellulose-digesting microbes (Kar & Ghosh, 2008). Therefore, yellow rasbora reared in the concrete tank do not have the opportunity to consume invertebrates, resulting in low activity of cellulase in its digestion tract. Fish generally do not secrete cellulase, and therefore the ability to digest cellulose in fish was highly dependent on the presence of microbial symbioses in lumen digestion of fish (Ganguly, 2013).

CONCLUSION

Capacity to digest protein increased, but the ability to digest starch and fiber does not change with the increase of feeding levels. Feed containing 1.01 g protein+0.10 g fiber/day/100 g fish biomass was the optimal feeding levels for yellow rasbora.

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