Growth performance of tinfoil barb (*Barbonymus schwanenfeldii*) fed with different protein levels and energy/protein ratios on diet

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Abstract. Tinfoil barb (*Barbonymus schwanenfeldii*) is a potential species which is developed as aquaculture commodity but it has slow growth. This study aimed to determine the best protein level and energy/protein ratio for the growth of tinfoil barb fingerlings. This study used factorial design of 4 x 2, i.e. protein levels (25, 30, 35 and 40%) and energy/protein ratios (8 and 10 kcal g^{-1} protein) with 3 replications. The rearing was done in 24 aquarium units sizing 60 x 40 x 40 cm filled with 60 L water. Then, tinfoil barb fingerlings with an initial weight of 6.89±0.62 g were reared for 60 days at a stocking density of 20 individuals per aquarium. The results showed that protein level and energy/protein ratio had a significant effect on protein retention (PR), fat retention (FR), energy retention (ER), final weight, specific growth rate (SGR), conditional factor (CF) and feed conversion ratio (FCR) ($p < 0.05$). While the interaction between protein level and energy/protein ratio only significantly influenced to FR and SGR ($p < 0.05$). The protein level at a value of 35% with an energy/protein ratio of 10 kcal g^{-1} protein could produce the highest PR and FR ($p < 0.05$). In addition, the feed containing 35% protein and an energy/protein ratio of 10 kcal g^{-1} protein also produced SGR (2.15±0.07%) which was higher than other treatments. There was no mortality of the fish during the experiment (survival rate, SR 100%). Hence, it was concluded that the feed containing 35% protein and an energy/protein ratio of 10 kcal g^{-1} which used as a treatment in this study resulted in the best growth performance of tinfoil barb fingerlings.

Key Words: nutrient, retention, feed, fingerling.

Introduction. Tinfoil barb (*Barbonymus schwanenfeldii*) is a fish species that has high economic value and potential to be developed as an aquaculture commodity. It is because the fish has many advantages especially biological aspects of this fish which makes it to be selected as a prospective aquaculture commodity. Tinfoil barb is a herbivore and placed in the second level of food pyramid (Sumiarsih 2014). In addition, the fish size when it reaches initial gonad maturation size is not too large which is about 150-200 g $fish^{-1}$. However, the weight of the fish can reach 500-1000 g $fish^{-1}$ if it grows in its natural habitat (Danau Sentarum, West Kalimantan). The advantages of tinfoil barb has not been followed by the development of this species cultivation. The demand for fish consumption continually increases along with the increasing of fish consumption by people and the increasing of population. It causes the decline of the fish's natural stock because of intensive fish exploitation. To prevent the decline of fish populations, it is needed to culture some species (Dewantoro et al 2011).

Aquaculture is a controlled fish-rearing activity in order to obtain high production. The production is largely determined by survival and the size of the fish when the fish are harvested. The size of the fish that can be reached in particular cultivation period depends on the fish growth rate. Tinfoil barb is one of the slow-growing-fish species. The fish at a size of 3-5 g which has been farmed for 10 months in the pond has absolute growth rate about 66.9 g $fish^{-1}$ (the specific growth rate is only 0.94% day^{-1}) (Huwoyon & Kusmini 2010). The approach that can be done to overcome that problem is to improve the feed quality, especially in improving macro nutrients. Macro nutrients are needed in large quantities as a source of energy and materials for the growth of the fish (Abowei & Ekubo 2011; Hasan & Khan 2013).

One of the main components of macro nutrients is protein. Proteins are bio-macromolecules needed by the fish. The role of protein in feed is as a source of energy and material for the growth. Fish requires a high protein content in the feed which varies depending on food habits and fish species (Goddard 1996). The studies that determined protein requirements have been done in mahseer (*Tor putitora*) (Hossain et al 2002), silver barb (*Puntius gonionotus*) (Mohanta et al 2008), tilapia (*Oreochromis niloticus*) (Bahnasawy 2009), catfish (*Clarias nieuhofii*) (Kiriratnikom & Kiriratnikom 2012), cachara catfish (*Pseudoplatystoma reticulatum*) (Cornelio et al 2014), and Patagonian blennie (*Eleginops maclovinus*) (Sa et al 2014). The protein requirement of tinfoil barb fry sized from 1 to 2 grams has also been reported by Mansour et al (2017). However, the protein requirement for fingerlings sized from 6 to 7 grams has not been studied.

Protein is an expensive nutrient, therefore feed proteins should be utilized as much as possible just for the fish growth. To maximize the utilization of feed proteins for growth, fish feed should contain energy derived from non-protein ingredients. Thus the feed given to the fish should contain enough protein with a balanced ratio of energy to protein. The studies about feeding the fish with the required and balanced protein have been widely reported (Meyer & Fracalossi 2004; Mohan & Basade 2005; Pantazis 2005; Ali et al 2008; Bicudo et al 2009; Pirozzi et al 2010; Gao et al 2011; Li et al 2012; Shapawi et al 2014), but there was no study about protein required by tinfoil barb. Thus the present study aims to determine the best protein level and energy/protein ratio on diet for the growth of tinfoil barb fingerlings.

Material and Method

***Period and place of study**.* The study was conducted for 60 days from March to April 2017 at Fish Hatchery of Food Affairs Agency of Pontianak District, West Kalimantan, Indonesia.

Research procedure. The experimental design used in this study was the factorial complete randomized design consisting of two factors named protein level and energy/protein ratio on diet. The first factor (protein level) consisted of 4 levels, i.e. 25, 30, 35 and 40% of protein. While second factor (energy/protein ratio) consisted of 2 levels, ie. 8 and 10 kcal/g protein, so there were 8 treatments with 3 replications. The treatments and compositions of the diet used are listed in Table 1.

This study used 24 aquariums sized 60 x 40 x 40 cm. The medium used to rear the fish was the water from Kapuas River that had been deposited. Before it was used, the water quality was checked and installed the temperature control device (electric heater), so the temperature would not be too much different to the natural water temperature. To fulfill oxygen need, the water was aerated continuously. During the experiment, the water was replaced every 2 days as much as 40% of the total media volume. The water quality used were eligible for the cultivation of fish, i.e. the water temperature ranged between 29.0 and 32.0^{o}C, dissolved oxygen was 5.0-5.5 mg L^{-1}, pH was 6.0-6.9, total alkalinity was 24-36 mg L^{-1} $CaCO_3$, and ammonia was < 0.02 mg L^{-1}.

The experimental fish used was tinfoil barb in average and standard deviation length of 5.94±0.18 cm (weight was 6.89±0.62 g). The fish were obtained from the Fishery Cultivation Center in Anjongan, West Kalimantan. These fish were reared in an aquarium containing 60 L water with a density of 20 fish per aquarium (2.3 kg m^{-3}). During rearing, the fish were fed by pellets with appropriate protein level and energy/protein ratio according to the treatment. The feed was given in *ad satiation* model for twice a day, in the morning and afternoon, for 60 days of rearing.

Table 1
Experimental diet formulations for tinfoil barb fingerlings with different protein level and energy/protein ratio

Ingredients (%)	*Composition of experimental diet (%) on each treatment*							
	D1 (25;8)	*D2 (25;10)*	*D3 (30;8)*	*D4 (30;10)*	*D5 (35;8)*	*D6 (35;10)*	*D7 (40;8)*	*D8 (40;10)*
Fish meal	36.0	26.5	42.0	28.0	50.0	35.0	50.0	50.0
Soybean meal	7.0	12.5	9.0	30.0	10.5	38.0	30.0	32.0
Pollard	4.0	15.5	19.0	13.0	20.5	4.5	8.0	0.5
Rice bran	20.0	20.0	7.0	8.0	6.0	3.0	2.5	0.5
Corn meal	0.5	8.5	1.0	7.5	2.0	5.0	2.5	0.5
Tapioca meal	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fish oil	0.5	1.0	1.0	3.5	1.5	8.0	2.5	13.5
Carboxy methyl cellulose	29.0	13.0	18.0	7.0	6.5	3.5	1.5	0.0
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Protein level (%)	24.11	24.75	29.96	31.17	34.97	35.01	39.85	39.88
Digestible energy (kcal 100 g^{-1})	196.01	248.93	241.98	291.70	282.34	334.30	320.63	385.43
E/P ratio (kcal g^{-1})	8.13	10.06	8.08	9.36	8.07	9.55	8.05	9.66

Note: D1 (25; 8): protein level 25%, energy/protein ratio 8 kcal/g protein; D2 (25; 10): protein level 25%, energy/protein ratio 10 kcal/g protein; D3 (30; 8): protein level 30%, energy/protein ratio 8 kcal/g protein; D4 (30; 10): protein level 30%, energy/protein ratio 10 kcal/g protein; D5 (35; 8): protein level 35%, energy/protein ratio 8 kcal/g protein; D6 (35; 10): protein level 35%, energy/protein ratio 10 kcal/g protein; D7 (40; 8): protein level 40%, energy/protein ratio 8 kcal/g protein; D8 (40; 10): protein level 40%, energy/protein ratio 10 kcal/g protein.

Observation and calculation of variables. The weight of fish was measured every 15 days in all experimental units. The measurement was also done to know the remaining feed and the feed provided for the next observation periods. These observational data were used to calculate fish survival, specific growth rate, conditional factor and feed conversion ratio. The proximate analysis of the fish body at the beginning and at the end of the treatment was done to calculate the nutrient and energy retention.

The formulas to calculate variables such as nutrient retention (protein and fat) and energy retention can be seen in the following formulas by Pirozzi et al (2010):

$$PR = \text{body protein gain (g)/protein consumed (g)} \times 100$$

$$FR = \text{body fat gain (g)/fat consumed (g)} \times 100$$

$$ER = \text{body energy gain (kcal)/energy consumed (kcal)} \times 100$$

where: PR = protein retention (%);
FR = fat retention (%);
ER = energy retention (%).

Specific growth rate was calculated using the formula of Liu et al (2015):

$$SGR = (\ln Wt - \ln Wo)/T \times 100$$

where: SGR = specific growth rate (% day^{-1});
Wo = the average weight of fish at the beginning of the study (g);
Wt = the average weight of fish at the end of the study (g);
T = duration of the experiment (day).

The conditional factor was calculated by the equation recommended by Froese (2006):

$$CF = W/L^3 \times 100$$

where: CF = conditional factor;
W = body weight (g);
L = standard length (cm).

Feed conversion ratio was calculated by using the formula of Abowei & Ekubo (2011):

$$FCR = FI/(Wt–Wo) \times 100$$

where: FCR = feed conversion ratio (%);
Wo = the weight of fish biomass at the beginning of the study (g);
Wt = the weight of fish biomass at the end of the study (g);
FI = the weight of feed consumed (g dry weight).

The survival was calculated using the formula of Rivas-Vega et al (2013):

$$SR = Nt/No \times 100$$

where: SR = survival (%);
Nt = the number of fish that lived at the end of the study (individual);
No = the number of fish at the beginning of the study (individual).

Statistical analysis. The parametric variables observed in this study were nutrient retention (protein and fat), energy retention, specific growth rate, conditional factor, feed conversion ratio and survival. The data obtained is presented in tabulation and statistic analysis was done with the IBM SPSS Statistics 20 program.

Results and Discussion

Nutrient retention (protein and fat) and energy retention. Protein level and energy/protein ratio on diet had a significant effect on protein retention, but their interactions did not show any significant effect (Table 2). Fish diet with 35% protein level produced the highest protein retention, while the other protein levels showed no significant difference. The data illustrates that the highest protein level on diet may not necessarily produce the highest protein retention, and vice versa. It was also found in Indian catfish (*Heteropneustes fossilis*) (Ahmed 2010) and in *Parachanna obscura* (Kpogue et al 2013). In Patagonian blanchie fish (*Eleginops maclovinus*), the increase of protein retention occurred because the change in feed protein level from 9% to 15% and the increase of protein level from 15% to 44% had no significant effect on protein retention (Sa et al 2014). In contrast, the higher feed protein level, the protein retention of lower silver barb (*Puntius gonionotus*) (Mohanta et al 2008). The energy/protein ratio of 10 kcal g^{-1} protein produced higher protein retention in tinfoil barb than that of 8 kcal g^{-1} protein. The increase of protein retention also occurred in increasing feed energy which was also found in silver barb (Mohanta et al 2009).

Protein retention described the effective use of energy contained in the feed to fulfil the energy requirement of the body, thus the feed protein could be optimally utilized and converted into body proteins. The more protein feed consumed with a balanced energy, the more protein that could be retained. The proper protein level and energy requirement to produce the highest protein retention was 35% protein with energy/protein ratio at a value of 10 kcal g^{-1} protein (treatment D6) resulting average protein retention at a value of 36.70%. In the brown-marbled grouper (*Epinephelus fuscoguttatus*), the highest protein retention was produced from feed with 45% protein level and a lipid level of 16 g kg^{-1} of feed (energy/protein ratio was 11.5 kcal g^{-1} protein) (Shapawi et al 2014). The highest protein retention of grass carp (*Ctenopharyngodon* *idella*) and hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) were found from feed with low protein level and energy/protein ratio, i.e. 25% protein and a lipid level of 40 g kg^{-1} of feed (energy/protein ratio was 10.13 kcal g^{-1} protein) and highest protein retention of Nile tilapia (*Oreochromis niloticus*) was produced from 20% protein level with energy content of 2800 kcal kg^{-1} of feed (energy/protein ratio was 14 kcal g^{-1} protein) (Li et al 2012).

Table 2

Retention of protein (PR), fat (FR), and energy (ER) of tinfoil barb fingerling after rearing for 60 days at various treatments

Diet (protein; energy/protein)	*PR*	*FR*	*ER*
D1 (25; 8)	29.45 ± 3.06^{a}	87.11 ± 8.75^{a}	20.82 ± 2.39^{a}
D2 (25; 10)	32.83 ± 1.81^{b}	74.62 ± 5.16^{b}	21.75 ± 1.33^{a}
D3 (30; 8)	29.47 ± 1.41^{acd}	82.82 ± 4.38^{a}	22.69 ± 1.11^{a}
D4 (30; 10)	31.30 ± 0.86^{ab}	61.74 ± 1.82^{c}	26.47 ± 0.75^{b}
D5 (35; 8)	32.77 ± 0.76^{bd}	68.93 ± 1.47^{bc}	29.78 ± 0.65^{cd}
D6 (35; 10)	36.70 ± 3.10^{e}	48.32 ± 4.26^{d}	30.91 ± 2.67^{d}
D7 (40; 8)	30.52 ± 0.64^{abd}	46.23 ± 0.85^{d}	27.91 ± 0.55^{bc}
D8 (40; 10)	29.14 ± 1.83^{ac}	32.37 ± 2.11^{e}	26.43 ± 1.69^{b}
Two-way Anova			
Protein	0.002	0.00	0.000
Energy/protein	0.025	0.000	0.111
Interaction	0.113	0.232	0.075
Pairwise comparisons			
25% protein	31.14 ± 2.91^{a}	80.86 ± 9.38^{a}	21.29 ± 1.81^{a}
30% protein	30.39 ± 1.45^{a}	72.28 ± 11.93^{b}	24.58 ± 2.24^{b}
35% protein	34.74 ± 2.95^{b}	58.63 ± 11.64^{c}	30.35 ± 1.84^{c}
40% protein	29.83 ± 1.44^{a}	39.30 ± 7.73^{d}	27.17 ± 1.39^{d}
8 kcal/g protein	30.56 ± 2.06^{a}	71.27 ± 17.18^{a}	25.30 ± 4.00^{a}
10 kcal/g protein	32.49 ± 3.38^{b}	54.26 ± 16.68^{b}	26.39 ± 3.70^{a}

Different superscript letters in the same column show a significant difference ($p < 0.05$).

The protein level and energy/protein ratio of feed had a significant effect on fat retention ($p < 0.05$), but there was no significant effect in the interaction between protein and energy/protein ratio (Table 2). There was a tendency for higher protein content resulting in significant decreasing of fat retention. This phenomenon was also found in the silver barb fed with low protein level feed (20%), resulting higher fat retention than the feed with the high protein level (25%-40%) (Mohanta et al 2008). In contrast, the common sole fish (*Solea solea*) fed with 39 to 57% protein in the feed increased its body fat retention that was in line with the increasing of protein level in the feed (Gatta et al 2011).

The treatment with energy at a value of 8 kcal g^{-1} protein produced higher body fat retention of tinfoil barb fingerling than that of 10 kcal g^{-1} protein. Grass carp and hybrid tilapia fed with the feed with a lipid level of 40 g kg^{-1} lipid (low energy) had increased their body fat retention than that of 100 g kg^{-1} (high energy) (Gao et al 2011). In silver pompano (*Pampus argenteus*), the increasing of fat level in the diet up to 16% or the energy/protein ratio at a value of 10.57 kcal g^{-1} protein caused the increasing of body fat retention, but if fat level in the diet was increased, it did not significantly affect the increasing of body fat retention (Hosain et al 2011).

In this study, the energy/protein ratio at a value of 8 kcal g^{-1} protein resulted in higher body fat retention than that of 10 kcal g^{-1} protein. The feed energy required by tinfoil barb fingerlings to produce the highest fat retention was lower than the energy required by silver barb and silver pompano. The silver barb required 12.5 MJ kg^{-1} energy with 30% protein level in the feed (energy/protein was about 9.96 kcal g^{-1} protein) to produce the highest fat retention (Mohanta et al 2009), while silver pompano required fat level at a value of 16% in feed (energy/protein was about 10.57 kcal g^{-1} protein) (Hossain et al 2011).

Treatments D1 and D3 produced the highest fat retention (87.11 and 82.82%), while the lowest fat retention was in treatment D8. In hybrid tilapia, the highest fat retention was produced by giving the feed with protein level at a value of 25% and lipid at a value of 40 g kg^{-1} of feed (energy/protein ratio was 10.13 kcal g^{-1} protein) (Gao et al 2011). In grass carp, a treatment with protein level at a value of 38% and lipid at a value of 40 g kg^{-1} of feed (energy/protein ratio was 8.74 kcal g^{-1} protein) could produce the highest fat retention. It means that the protein level required to produce the highest fat retention in this study was not much different from the protein requirement of hybrid tilapia, but it required lower energy/protein ratio. Comparing to grass carp, the protein level and energy/protein ratio required by tinfoil barb fingerling to produce the highest fat retention was much lower.

The response of energy retention was slightly different than fat retention. The protein level had a significant effect on energy retention ($p < 0.05$), but the energy/protein ratio and the interaction between protein level and energy/protein ratio did not give a significant effect. Feeding with a diet containing 35% protein resulted in the highest energy retention (mean was 30.35%), followed by the feed containing 40%, 30% and 25% protein. Zehra & Khan (2012) reported that the increasing of protein level in the feed of snakehead fish (*Channa punctata*) could significantly increase energy retention, if the protein level increased more than 40%, it would affect the decreasing of energy retention. Zebra sea bream (*Diplodus cervinus*) juvenile increased its energy retention that was in line with the increasing of protein level in the feed up to 25%, but the increasing of protein level from 25 to 55% has no significant effect on the increasing of the energy retention (Coutinho et al 2016). The same pattern of increasing the energy retention was also found in Indian catfish which achieved maximum energy retention from 16 to 20% protein (Ahmed 2010) and silver barb at 30% protein (Mohanta et al 2008).

Treatment D6 resulted in the highest average of energy retention, i.e. 30.91%, but it was not significantly different from treatment D5. The energy retention of treatment D5 was not significantly different to the energy retention produced by treatment D7. The lowest energy retention was found in treatments D1, D2 and D3 (Table 2). In the rearing of Nile tilapia fingerlings, the highest energy retention was achieved in feeding with the feed contained 20% protein and digestible energy at a value of 2800 kcal/kg of feed (energy/protein ratio was 14 kcal g^{-1} protein) (Li et al 2012). It showed that tinfoil barb fingerlings required a higher protein level contained in a diet to achieve the highest energy retention, but the energy required for each gram of protein was smaller than tilapia required.

Nutrient retention (protein and fat) and energy in the fish body highly depends on fish species, feeding habits, fish size, feed consumption and nutrient content of feed (Pirozzi et al 2010; Gao et al 2011; Li et al 2012; Shapawi et al 2014). Tinfoil barb are omnivorous fish that tend to be herbivorous (Sumiarsih 2014). Fish with these feeding habits require low protein level (30-35%) with a balanced amount of energy (Mohanta et al 2008; Bahnasawy 2009; Gao et al 2011; Li et al 2012). Thus, the feed can be utilized properly if it was given by the feed with low protein and energy levels, and the body also stored low protein and energy. On the other side, if the protein level and energy contained in the feed was too high, the feed could not be digested and absorbed perfectly, thus nutrients stored were relatively in a little amount.

Growth, condition factor, feed conversion ratio and survival. In 60 days, the fish could grow with an initial average weight of 6.86-6.92 g $fish^{-1}$ became 17.18-25.07 g $fish^{-1}$ with specific growth rate (SGR) ranging from 1.51 to 2.15 % day^{-1}. Protein level and energy/protein ratio had a significant influence on final weight and SGR. However, the interaction between feed protein level and energy/protein ratio only had a significant effect on SGR ($p < 0.05$) (Table 3).

The increase of protein level from 25 to 35% caused the increasing of the growth, but it did not cause the increasing of the SGR if the protein level was increased up to 40%. The protein level resulted the best results in each parameter observed in this study was higher than previous study. Previous study showed that silver barb at a size of 0.88 g required 31.77% protein (Mohanta et al 2008) and tinfoil barb at a size of 1.2 g required 32% protein (Mansour et al 2017). This difference was due to different fish species, fish size and maximum protein level used as a treatment.

The influence of energy/protein ratio on growth could be seen from the high SGR of tinfoil barb fingerling. Feeding the fish with a diet containing an energy/protein ratio of 10 kcal g^{-1} protein produced higher SGR than that of 8 kcal g^{-1} protein. Himalayan golden mahseer *Tor putitora* (Hamilton) required energy/protein ratio in diet at a value of 10.39 kcal g^{-1} protein to achieve the best growth performance (Mohan & Basade 2005). It means the higher energy contained in the feed, the proportion of energy to fulfil the energy required for basal metabolism, specific dynamics action and voluntary activity will be higher, thus the energy produced by protein could be used for the growth (Glencross et al 2011).

Table 3

Initial body weight (IBW), final body weight (FBW), specific growth rate (SGR), condition factor (CF), feed conversion ratio (FCR) and survival rate (SR) of tinfoil barb fish fingerling reared for 60 days in various treatments

Diet (protein; energy/protein)	*IBW*	*FBW*	*SGR*	*CF*	*FCR*	*SR*
D1 (25; 8)	6.92±0.38	17.18±1.46[a]	1.51±0.07[a]	3.09±0.04[a]	2.20±0.23[a]	100
D2 (25; 10)	6.86±0.31	18.68±0.55[ab]	1.67±0.09[b]	3.20±0.02[b]	1.91±0.11[b]	100
D3 (30; 8)	6.89±0.35	19.53±0.75[b]	1.74±0.04[bc]	3.19±0.04[bc]	1.78±0.08[bc]	100
D4 (30; 10)	6.92±0.38	20.43±0.28[b]	1.81±0.07[c]	3.22±0.06[bc]	1.62±0.05[cd]	100
D5 (35; 8)	6.86±0.31	22.60±0.25[c]	1.99±0.09[d]	3.26±0.06[cd]	1.43±0.03[de]	100
D6 (35; 10)	6.88±0.33	25.07±1.83[d]	2.15±0.07[e]	3.34±0.04[d]	1.29±0.11[e]	100
D7 (40; 8)	6.91±0.31	23.78±0.83c[d]	2.06±0.04[de]	3.33±0.07[d]	1.37±0.03[e]	100
D8 (40; 10)	6.91±0.27	22.87±1.41[c]	1.99±0.08[d]	3.33±0.10[d]	1.46±0.10[de]	100
Two-way Anova						
Protein	0.996	0.000	0.000	0.000	0.000	1.000
Energy/Protein	0.996	0.038	0.014	0.035	0.014	1.000
Interaction	0.996	0.084	0.041	0.296	0.054	1.000
Pairwise comparisons						
25% protein	6.89±0.31	17.93±1.28[a]	1.59±0.11[a]	3.12±0.07[a]	2.05±0.22[a]	100
30% protein	6.91±0.32	19.98±0.71[b]	1.77±0.06[b]	3.20±0.05[a]	1.70±0.11[b]	100
35% protein	6.87±0.29	23.83±1.79[c]	2.07±0.12[c]	3.30±0.06[b]	1.36±0.10[c]	100
40% protein	6.91±0.26	23.32±1.15[c]	2.02±0.07[c]	3.33±0.07[b]	1.41±0.08c	100
8 kcal/g protein	6.89±0.29	20.78±0.28[a]	1.83±0.23[a]	3.22±0.10[a]	1.69±0.36[a]	100
10 kcal/g protein	6.89±0.28	21.76±2.72[b]	1.91±0.20[b]	3.27±0.08[b]	1.57±0.25[b]	100

Different superscript letters in the same column show a significant difference ($p < 0.05$).

Based on statistical analysis, treatment D6 was the best treatment resulting in the best growth (SGR) of tinfoil barb (mean was 2.15 % day^{-1}), but it was not significantly different from treatment D7. Treatment D1 had the lowest effect to the fish growth (Table 3). The nutritional requirement of fish were determined by various factors, such as fish species, age, size, physiological activity and environment. Black catfish (*Rhamdia quelen*) required 37% protein and energy/protein ratio at a value of 10.13 kcal g^{-1} protein to achieve maximum growth (Salhi et al 2004). Pacu (*Piaractus mesopotamicus*) required the feed with 27.1% protein with energy/protein ratio at a value of 10.78 kcal g^{-1} protein (Bicudo et al 2009).

The improvement of SGR had a similar pattern with the increasing of protein and energy retention. This phenomenon was found in several treatments, especially treatment D6. The more nutrients from the feed that can be converted into nutrients stored in the body, the faster fish growth (Gao et al 2011). Ali et al (2008) stated that the same protein level and higher energy/protein ratio caused protein contained in the feed to be maximally utilized for the growth and the maintenance of protein in the body. This supported the conclusion that there was the nutrient and energy accumulation in body that triggered the growth of tinfoil barb fingerlings on treatment D6.

Protein level and energy/protein ratio of feed also significantly influenced the conditional factor (CF) in tinfoil barb fingerlings ($p < 0.05$), but the interaction did not show any significant effect (Table 3). The CF value was one of growth indicators that described the fattening of the fish (Froese 2006). The CF value was also used as an indicator of environmental conditions, feed, and fish health (Helland et al 2010; Okunsebor et al 2015; Hama et al 2015). The increasing of protein level up to 35% and energy/protein ratio at a value of 10 kcal g^{-1} protein caused the increasing of tinfoil barb CF. The increasing of CF values was also in line with the increasing of protein level on the feed that was found in brown-marbled grouper (*Epinephelus fuscoguttatus*) (Shapawi et al 2014). However, in shi drum (*Umbrina cirrosa*), the increasing of protein level on the feed did not cause the increasing of CF although the growth of the fish increased (Akpinar et al 2012).

Treatment D6 produced the highest CF (mean was 3.34), but it was not significantly different to treatments D5, D7 and D8 (Table 3). It showed that protein level at a value of 35% with an energy/protein ratio of 10 kcal g^{-1} protein was the best treatment for tinfoil barb fingerlings diet. Nutritional status was one of the factors that determined CF. This phenomenon was found in Nile tilapia fingerlings fed with different levels of maltose (Ighwela et al 2011) and brown-marbled grouper after feeding with different protein and lipid levels (Shapawi et al 2014).

The protein level and energy/protein ratio also had a significant effect on FCR ($p < 0.05$), but the interaction did not show any significant effect (Table 3). The protein levels at values of 35 and 40% were the factor levels that produced the lowest average FCR, but they were not significantly different. Furthermore, FCR increased along with low protein level on the diet. Energy/protein ratio had a similar phenomenon with protein level. Energy/feed ratio at a value of 10 kcal g^{-1} protein significantly resulted lower FCR than that of 8 kcal g^{-1} protein.

The results of statistical analysis on all treatments showed that treatments D6 and D7 were the treatments that produced the best FCR, which was 1.29 and 1.37 respectively. Then it was followed by treatments D5 and D8 that did not significantly differ with treatment D4. The treatment produced the highest FCR was treatment D1. In Nile tilapia, the best FCR occurred when it was fed by the feed with a protein level of 36% and 19 MJ kg^{-1} of feed (energy/protein ratio was 12.19 kcal g^{-1} protein) (Ali et al 2008), while black catfish (*Rhamdia quelen*) required higher protein (38%) and fat at a value of 14% (energy/protein ratio was 10.13 kcal g^{-1} protein) to produce the best FCR (Salhi et al 2004).

The FCR was determined by several factors such as genetics, nutrient content of feed, rearing management, and environment (Robinson & Li 2015; Hasan & Soto 2017). If all conditions were homogen, the suitability between the nutrient content and the fish nutritional requirement determined the amount of the feed that could be converted. Rohu (*Labeo rohita*) showed a high FCR value when it was fed with higher proportion of carbohydrates and fats than it was needed (Hasan & Khan 2013).

There was no dead fish in all treatments in this study (Table 3). The survival of the fish was determined by the water quality, especially the existence of substance dissolved in water that gave a lethal effect on the fish. The water quality in this study was not only in minimum tolerable level for the fish to live, but it was also set in optimum level to keep the fish alive.

Conclusions. The increasing of protein level up to 35% has a significant effect on growth performance, but it does not not give a significant effect if the protein level is increased more than that level. Energy/protein ratio at a value of 10 kcal g^{-1} protein gives a better effect comparing to the lower energy/protein ratio (8 kcal g^{-1} protein). Tinfoil barb fingerlings will show the best growth performance if it is fed a diet with a protein level of 35% and an energy/protein ratio of 10 kcal g^{-1} protein.

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