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Research Article
Influence of Different Protein Levels and Protein to Energy Ratios on Growth, Feed Efficiency and Survival of Bonylip Barb (*Osteochilus vittatus* Cyprinidae) Fingerlings

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Abstract

Background and Objective: Bonylip barb *Osteochilus vittatus* (*O. vittatus*) is a freshwater fish with economic value. This study was conducted to evaluate the effect of protein levels and energy levels on the growth, feed efficiency and survival of *O. vittatus* fingerlings. **Materials and Methods:** A 3×2 factorial feeding trial was conducted to evaluate the production response of bonylip barb fingerling fish (*O. vittatus*). Six diets containing 3 protein levels (28, 30 and 32%) and 2 protein-energy ratios (12 and 14) were formulated and fed to triplicate groups of *O. vittatus* fingerlings (3.77±0.02 g/fish) for 60 days. The formulated diets were P28E12, P28E14, P30E12, P30E14, P32E12 and P32E14 (P-protein and E-energy). **Results:** Fish fed diets with the lowest protein and highest energy combination (P28E14) had the lowest growth performance. The percent weight gain, specific rate growth, feed conversion ratio, feed efficiency and protein efficiency ratio of *O. vittatus* fingerlings was highest with the 32% crude protein feed and a protein-energy ratio of 12 (P32E12). Meanwhile, the best fat retention was found in the P28E14 treatment. The protein retention and survival rate showed no significant effect ($p>0.05$) between treatments. **Conclusion:** Feed with 32% crude protein and a protein-energy ratio of 12 is good for growth, feed efficiency and survival of *O. vittatus* fingerlings.

Key words: Aquaculture, diet, growth rate, protein, energy

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The success of aquaculture operations not only depends on the fish species, feeding rate, stocking density, integrated multi-trophic aquaculture, water quality management, feed type and feed conversion ratio[1-10], but also on the protein level and protein to energy ratio[11-13]. The protein level and protein to energy ratio is one of the most important variables in aquaculture because it directly affects the specific growth rate, condition factor, viscerosomatic index and gonadosomatic index of female Nile tilapia[13], the growth and feed conversion ratio of juvenile spotted Babylon[11] and the whole body composition of gilthead sea bream[14].

Protein and energy intake have a great influence on growth speed[14-16], which is determined by the dietary nutrient level and feed intake. Diet composition and feed intake should be designed to cover the needs and maintenance of the growth of the fish[17]. Meanwhile, protein, which is the most expensive ingredient in fish feed, is also deemed to be the most important because its regular intake is required for the fish to utilize amino acids to build new proteins during growth[18]. Reduced feeding might result in less growth and economic loss, whereas, over-feeding may lead to feed waste, economic loss and a large amount of waste output[10,19].

To increase the growth of fish, the dietary composition and feed intake should be efficiently designed during maintenance[2,20,21]. However, the amount of digestible energy or protein that fish need to retain a determined level of growth depends not only on the initial body weight and temperature but also on the efficiency of the retention of the digestible energy intake or digestible protein intake[14,22]. Therefore, the optimum protein levels and energy for each species, as well as the cultivation phase needs, must be determined to allow for maximum profitability.

Osteochilus vittatus Cyprinidae, is a herbivorous freshwater fin-fish and a native specie in Indonesia[23,24]. These species live in lake Singkarak, the Antokan river, the Koto Panjang reservoir[25] and the Kampar Kanan river[26] in Indonesia. The over-exploitation of these natural fish stocks has occurred due to increased market demand and due to the increase in retail fish sales[26]. Studies performed by Syandri *et al.*[25], Azrita *et al.*[27], Syandri *et al.*[28,29] and Aryani *et al.*[30] describe the information relevant to aquaculture activity. *Osteochilus vittatus* has been identified as one the best prospect species for middle-scale commercial aquaculture in Indonesia.

The aim of this study was to evaluate the effects of different protein levels and the protein to energy ratio on the growth performance, feed efficiency and survival of *O. vittatus*. The results of this study increase the scientific understanding of *O. vittatus*.

MATERIALS AND METHODS

Experimental animals: Three hundred *O. vittatus* fingerlings (3.77±0.02 g/fish) were produced from the Hatchery Laboratory, Faculty of Fisheries and Marine Sciences, Bung Hatta University. This study was performed from April-June, 2017. A total of 3,000 individual fingerlings were used in the study. The samples were acclimated for 45 days prior to the experiment. The samples were placed in a concrete tank with a capacity of 5,600 L. The concrete tank had supplemental aeration with a continuous flow of underground water at a velocity of 5 L min^{-1}. During acclimatization, the fingerlings were fed with commercial feed, which had a proximate composition (dry weight %) of moisture content (10%), crude protein (41%), lipid (5%), crude fiber (6%) and crude ash (16%). The feeding was conducted twice daily and the fish were fed at predetermined ratio of 5% b.wt./day.

Eighteen 60×60×50 cm framed nets of PVC pipe (75 L capacity) were placed inside a 400×200 cm concrete tank with a water volume of 5,600 L. Each net contained 30 fish. The fish were fed twice a day at 09:00 and 16:00. Aeration was provided for 24 h. The water quality parameters were measured for temperature (26-28°C), dissolved oxygen (6.5-7.0 mg L^{-1}) and pH (6.5-7.0) twice daily.

Experimental diets: This study involved a 3×2 factorial design with 3 levels of dietary crude protein (CP) (28, 30 and 32%) and 2 levels of dietary protein-energy ratio (12 and 14). The formulated diets were named P28E12, P28E14, P30E12, P30E14, P32E12 and P32E14 (P-protein and E-protein to energy ratio) (Table 1). The major dietary protein sources were soybean meal and fish meal and the dietary protein level increased in proportion to its percentage. Pollar and DDGS were used as carbohydrate sources. Lemuru oil and palm oil were used as the lipid sources. Mineral and vitamin mixes were added to the diets. The PMC was used as a binder. The diets were prepared by weighing the dry ingredients and mixing thoroughly in a mixer. The lipid sources were added dropwise while the mixture was further blended to ensure homogeneity. Approximately 200 mL of hot water was then added for each kg of this mixture. The diets were extruded and dried at room temperature for 48 h. Upon feeding, the feeds were made into small pieces (round shape of 2 mm diameter) to facilitate consumption by the fish. All of the experimental

Table 1: Percentage (dry weight basis) and proximate analysis (%) of the formulated diets and natural food

	Diets					
Items	P28E12	P28E14	P30E12	P30E14	P32E12	P32E14
Ingredients (%)						
Soybean meal	29.0	29.0	30.0	30.0	32.0	32.0
Fish meal	11.0	11.0	13.0	13.0	14.0	14.0
Bone meal	7.0	7.0	8.5	8.5	10.0	9.0
Pollar	20.0	17.0	19.0	14.0	19.0	7.5
DDGS	9.0	10.5	9.0	11.0	9.0	15.0
Rice bran	15.5	11.0	10.0	7.0	4.5	4.0
Lemuru oil	2.0	5.0	3.0	6.0	3.5	7.0
Palm oil	2.0	5.0	3.0	6.0	3.5	7.0
Premix	4.0	4.0	4.0	4.0	4.0	4.0
Polymethylolcarbamide	0.5	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition						
Crude protein (%)	28.16	27.99	30.11	29.84	32.09	31.57
Crude lipid (%)	6.86	12.19	9.1	15.22	10.28	17.64
BETN	29.19	26.54	27.48	24.11	26.69	21.08
GE (kkal kg^{-1})	3428.93	3872.79	3668.19	4089.86	3857.26	4290.68
C/P ratio (kkal g^{-1})	12.14	13.84	12.18	13.71	12.02	13.59

DDGS: Distiller's dried grain with soluble

diets were then stored in a refrigerator at 4°C until use. All of the diets were analyzed in triplicate for the proximate compositions according to the standard methods[31]. The samples were analyzed in the Animal Science Lab, Department of Aquaculture, Faculty of Fisheries and Marine Science, Institute Pertanian Bogor, Indonesia.

Sample collection and analysis: The wet weight of the fingerlings from each of the framed nets was measured individually at the beginning of the experiment and every 2 weeks in the feeding trials for growth estimation. The initial length (cm) and weight (g) of the fish were determined using a measuring scale and a digital electronic balance (OHAUS, Model CT 1200-S, USA) and these values were recorded. To determine the growth performance of the fish, the following parameters were calculated: Weight gain (WG), weight gain percentage (WG%), specific growth rate (SGR %/day), coefficient of variation (CV%) for SGR, feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER) and survival rate (SR). The parameters were analyzed according to the methods described by Aryani *et al.*[1], Iqbal *et al.*[32] and Wang *et al.*[33] with the following equations:

$$\text{WG} = \text{Final fish weight (g)-Initial fish weight (g)}$$

$$\text{WG (\%)} = \left[\frac{\text{Final weight-Initial weight}}{\text{Initial weight}}\right]\times 100$$

$$\text{SGR \%/day} = \left[\frac{(\ln \text{Wt-ln} \text{Wi})}{\text{T}}\right]\times 100$$

Where:
Wt = Mean final weight
Wi = mean initial weight
T = Total experimental days

$$\text{CV (\%) for SGR} = \frac{\text{SD}}{\text{Mean}}\times 100$$

Where:
Mean = Mean of the sample
SD = Standard deviation

$$\text{FCR} = \frac{\text{Total feed fed (g)}}{\text{Total wet weight gain (g)}}$$

$$\text{FE} = \frac{\text{Fish weight gain}}{\text{Feed intake}}$$

$$\text{PER} = \frac{\text{Protein efficiency ratio:weight gain}}{\text{Crude protein intake}}$$

$$\text{PR (\%)} = \frac{\text{Total protein weight of fish at the end of the experiment (g)-Total protein weight of fish before the experiment (g)}}{\text{Total weight of protein in diet fed to fish (g)}}\times 100$$

$$\text{FR (\%)} = \frac{\text{Raw fat weight of fish at the end of the experiment (g)-Raw fat weight of fish before the experiment (g)}}{\text{Raw fat weight in diet fed to fish (g)}}\times 100$$

$$\text{SR (\%)} = \frac{\text{Number of fish survived}}{\text{Number of fish stocked}}\times 100$$

Water quality: The water samples were collected at a depth of 10 cm from each synthetic sheet pond for the determination of the dissolved oxygen (DO) contents. An oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA) was used *in situ* and the pH values were determined with a pH meter (Digital Mini-pH Meter, 0-14PH, IQ Scientific, Chemo-science [Thailand]) Co., Ltd, Thailand). The levels of phosphorus, ammonia-nitrogen and alkalinity and the hardness of the water in each replicate was measured according to the standard procedures[34].

Statistical analysis: The data were presented as the Mean±SD of the three replicates. The growth data from each treatment were analyzed by one-way and two-way analysis of variance (ANOVA) to test the effects of the dietary protein and protein-energy ratio[35]. All statistical analyses were performed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL). If significant ($p<0.05$) differences were found in the one-way ANOVA test, a least significant difference test was used to compare the means. The treatment effects were considered significant at $p<0.05$.

RESULTS

During the experimental period, the growth performance of *O. vittatus* at 15, 30, 45 and 60 days showed significant differences ($p<0.05$). The lowest final weight was observed in P28E12 followed by P30E14, P28E14, P30E12, P32E14 and P32E12 (Fig. 1).

Significant interactions were observed between dietary protein and protein-energy ratio levels ($p<0.05$) regarding the average weight at 15 and 60 days and the average total length at 60 days and the percent weight gain (Table 2). The percent body weight gain (BWG) per day, feed conversion ratio and coefficient of variation for SGR of *O. vittatus* was significantly influenced by dietary protein and protein-energy ratio levels ($p<0.05$) (Table 3).

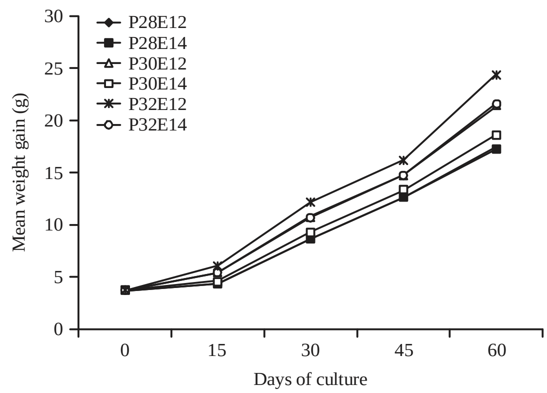

Fig. 1: Growth in body weight of *O. vittatus* fingerlings fed experimental diets containing different dietary protein and protein-energy ratio for 60 days

Table 2: Average weight and total length* and percent weight gain of fish fed various dietary treatments

Diets	Average initial weight (g)	Average weight (g) 0-15 days	Average weight (g) 0-60 days	Average total length (cm) at 60 days	Percentage weight gain 0-15 days	Percentage weight gain 0-60 days
P28E12	3.76±0.00	4.38±0.02^a	17.50±0.32^a	9.39±0.09^a	16.48±0.53^a	365.03±9.96^a
P28E14	3.76±0.00	4.33±0.01^b	17.30±0.30^b	9.23±0.20^b	15,16±0.40^b	360.10±10.54^b
P30E12	3.76±0.00	5.32±0.02^c	21.36±0.10^c	11.41±0.01^c	41.66±0.61^c	535.06±6.70^c
P30E14	3.37±0.05	4.66±0.01^d	18.63±0.17^d	10.05±0.03^d	23.81±0.43^d	452.77±4.24^d
P32E12	3.77±0.00	6.11±0.02^e	24.42±0.26^e	13.00±0.05^e	62.58±0.40^e	547.69±5.47^e
P32E14	3.75±0.05	5.39±0.02^f	21.59±0.34^f	11.61±0.01^f	43.39±0.27^f	475.36±9.16^f

P28E12: Crude protein 28% and C/P 12, P28E14: Crude protein 28% and C/P 14, P30E12: Crude protein 30% and C/P 12, P30E14: Crude protein 30% and C/P 14, P32E12: Crude protein 32% and C/P 12 and P32E14: Crude protein 32% and C/P 14. Values (Means±SD of three replicates) in the same row with different superscripts are significantly different ($p<0.05$). *Initial lengths of *O. vittatus* fingerlings 5.5 cm. CP: Crude protein (%), GE: Gross energy (kcal kg^{-1}). WG: Weight gain: (final weight- initial weight). Percent weight gain: [Final weight-initial weight/initial weight]×100

Table 3: Percent body weight gain (BWG) per day*, feed conversion ratio and coefficient of variation for SGR of fish fed various dietary treatments

Diets	BWG/day (%) 0-15 days	BWG/day (%) 0-60 days	CV of SGR (%) at 60 days	FCR 0-15 days	FCR 0-60 days
P28E12	1.01±0.03^a	2.56±0.03^a	1.39±0.01^a	1.28±0.005^a	1.29±0.02^a
P28E14	2.31±0.02^b	2.89±0.30^b	1.07±0.10^b	1.05±0.005^b	1.26±0.02^b
P30E12	2.31±0.02^c	3.08±0.20^d	0.47±0.06^c	1.06±0.005^c	1.02±0.01^c
P30E14	1.42±0.03^d	2.85±0.01^d	0.35±0.00^d	1.21±0.005^d	1.09±0.00^d
P32E12	3.23±0.01^e	3.11±0.01^e	0.45±0.00^e	0.92±0.002^e	1.00±0.01^e
P32E14	2.40±0.01^f	2.92±0.02^f	0.91±0.00^f	1.04±0.003^f	1.04±0.01^f

Values are Mean±SD (n = 3), column values with different superscripts are significantly different ($p<0.05$), *BWG per day = $e^{GW^{-1}}$·100, where, GW is the instantaneous growth rate (ln final weight-ln initial weight)/time in days

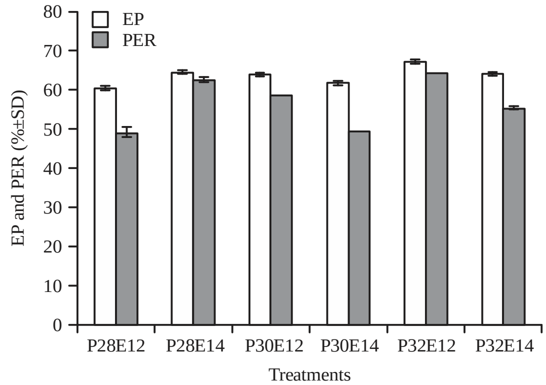

Fig. 2: Feed efficiency (FE) and protein efficiency ratio (PER) of *O. vittatus* fingerlings fed experimental diets containing different dietary protein and protein-energy ratio levels for 60 days

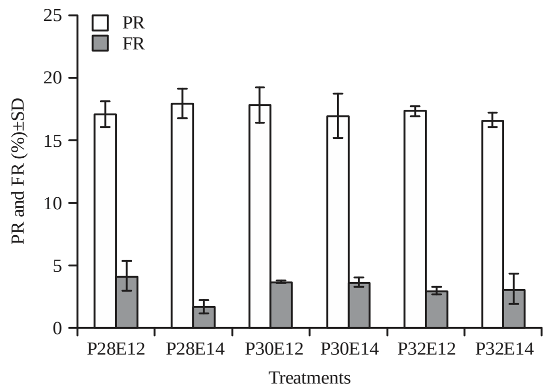

Fig. 3: Protein retention (PR) and fat retention (FR) of *O. vittatus* fingerlings fed experimental diets containing different dietary protein and protein-energy ratio levels for 60 days

After 60 days of rearing, the difference between the feed efficiency and protein efficiency ratio showed a significantly increase ($p<0.05$) among the different dietary protein and protein-energy ratio levels. At the end of the rearing period (60 days), *O. vittatus* fingerlings that were observed between P32E12 showed better feed efficiency (FE) and protein efficiency ratio (PER) compared to the other treatments (Fig. 2). Furthermore, the protein retention (PR, %) and fat retention (FR, %) of each treatment are presented in Fig. 3.

Over the 15 days of the experiment, the mortality rate of *O. vittatus* fingerlings ranged from 12 ± 1.73 to 14 ± 2.12% and after 45 and 60 days, the mortality rate tended to increase and ranged from 14.34 ± 1.60 to 18.34 ± 2.50%. The mortality may have been caused by the handling activity and water quality. The water quality parameters in all of the experiments in the framed nets were within the normal range (temperature 26-28°C, dissolved oxygen 6.5-7.0 mg L^{-1} and pH 6.5-7.0).

DISCUSSION

In the present study, the diet with a low protein content and high energy ratio significantly decreased the average weight, average total length, percentage weight gain, CV of SGR and feed conversion ratio of *O. vittatus* fingerlings. This may be mainly caused by a deficiency in the dietary amino acids because of inadequate dietary protein. Interactions observed between dietary protein and the protein-energy ratio have been successful in increasing the growth performance of some fish species[10,11,15,36]. In general, as the protein content increased the growth was increased, but at any given protein content, the growth was reduced by a high protein-energy ratio. Similarly, Setiawati *et al*.[36] evaluated the effect of protein and the protein-energy ratio in common carp (*Cyprinus carpio*) fingerlings concluded that growth was positively affected by the increased protein level and decreased protein-energy ratio. Meanwhile, Jiang *et al*.[12] evaluated the effect of protein-energy levels in hybrid grouper (*Epinephelus fuscoguttatus* ♀×*Epinephelus lanceolatus* ♂) juveniles and concluded that growth was positively affected by the increase in protein content in the diets and that within a protein level, growth was reduced by the increasing energy content of the diet. These authors also observed that the weight gain (WG%) of fish fed the P/E191 diet was significantly lower than that of fish fed the P/E173 or P/E157 diet.

After 60 days of the experiment, the interactions observed between dietary protein and protein-energy ratio levels showed a significant difference ($p<0.05$) on BWG, the CV of SGR and the feed conversion ratio. Meanwhile, *O. vittatus* fingerlings fed with P32E12 and P30E12 obtained a higher percent body weight gain per day. In general, an increase of CP from 28-32% affected any significant improvement in fish growth and protein retention. This could be an indication that a minimal amino acid requirement was met at 32% protein with a protein-energy ratio of 12 (P32E12). Meanwhile, P28E12, P28E14, P30E14 and P32E14 diets might not have met the requirements and hence produced the lowest significant growth. Sun *et al*.[17] stated that the growth of fish requires nutrients and energy and the protein levels of feed and energy are negatively correlated with the feed intake for most aquatic animals. In terms of FCR, fish fed the P32E12 and P30E12 diets had better values than those fed P28E12 and P28E14, which indicated that the feed was best utilized in the former two diets. This result showed that *O. vittatus* fingerlings (herbivorous) may have the lowest protein requirement compared to carnivorous species. According to Thoman *et al.*[37], protein is one of the most important nutrients for growth. Therefore, it is important to determine the exact protein requirement for each species to obtain the highest efficiency of nutrient utilization.

The optimal growth of *O. vittatus* occurred at the 32% crude protein level and a protein-energy ratio of 12 (P32E12) was nearly the same as *Cyprinus carpio*, which requires a diet containing 31.15% protein with a protein-energy ratio of 7.81[36]. This lower amount is comparable to carnivorous species such as *Babylonia areolata* (35% crude protein and 4.0 kcal g^{-1} energy)[11], *Nibea miichthioides* (36% crude protein and 14 kcal g^{-1} energy)[15], *Totoaba macdonaldi* (52% crude protein and 8.5% lipid)[22] and *Epinephelus fuscoguttatus* (49% crude protein and 11% lipid)[38]. Pirozzi *et al.*[39] stated that protein utilization is affected by several dietary factors including protein content and quality (i.e., digestibility and amino acid balance) and the amount of available energy and thus, the P:E ratio is typically adjusted to optimize the protein retention "protein sparing" depending on the energy requirements of the sh under the culture conditions.

Based on the PER data, in the present study, a significant effect of protein utilization ("protein sparing effect") by the high lipid inclusion levels did not be detected, where the highest growth was achieved at a low lipid level and a high protein content. Higher lipid levels decreased ingestion rates and limited growth. According to NRC[18], the addition of lipids into diets needs to consider the quality. A high lipid content will cause the storage of lipids in the fish body and can decrease the consumption of feed and fish growth.

Results of the current study showed that protein and protein-energy ratio levels show no significant difference (p>0.05) on protein retention values. In general, the higher protein retention rates are found in the treatment of low protein and energy ratios (C/P = 12). This is due to the feed with protein values of 28 and 30% and the energy-protein ratio of 12, which may be an appropriate protein balance. Thus, the energy sourced from fats and carbohydrates in feed protein 28-30% and C/P = 12, can be used as a protein sparing effect in tissue formation. Meanwhile, the protein levels and energy protein ratios were 31.22:9.48, 31.15:7.81, 28.08:9.12 and 28.27:8.38%. In addition, there were no significant effects on the protein retention values of *Cyprinus carpio*[36]. In addition, the protein retention values can be influenced by the main sources of feed proteins[21,40], feed formulations, feeding percentages, feeding times[14,17] and stocking densities[41].

The fat retention values of each treatment were P28E12 (4.14±1.18%), P28E14 (1.67±0.51%), P30E12 (3.68±0.14%), P30E14 (3.66±0.38), P32E12 (2.97±0.29%) and P32E14 (3.08±1.19%). The higher fat retention rates were found in the 28% protein treatment and at a protein-energy ratio of 12. This is because the P28E12 feed has a low fat content that may be suitable for the growth of *O. vittatus*. According to Setiawati *et al.*[36], feeds containing high fat will cause low fat retention because it exceeds the needs of fat for fish and takes more energy to hydrolyze the fat. For grass carp (*Ctenopharyngodon idella*) juveniles, the higher the protein level (35-45%) the lower the fat retention value[40].

CONCLUSION

Its is concluded that the results of the present study indicate that a diet containing 32% dietary protein with a protein-energy ratio of 12 is recommended for the growth and feed efficiency of *O. vittatus* fingerlings in a concrete tank. Despite the positive effects on growth, feed conversion ratio and feed efficiency, the effects on energy retention and nitrogen retention remain to be investigated.

SIGNIFICANT STATEMENT

This study analyzed the different protein levels and protein-energy ratios of the diets of bonylip barb (*Osteochilus vittatus*) on growth performance, feed efficiency and survival rate. The diet containing the 32% dietary protein and a protein-energy ratio of 12 (P32E12) showed the best results in terms of growth performance and feed efficiency compared with P30E12, P32E14, P28E14, P30E14 and P28E12. In the future, the diet containing 32% dietary protein and a protein-energy ratio fo 12 should be considered for the culture of *O. vittatus* and for the development of new species.

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