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Effect of dietary lysine on the growth performance of *Pangasius hypophthalmus*

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ABSTRACT

The success of intensive striped catfish (*Pangasius hypophthalmus*) cultivation requires quality feed that contains not only protein according to fish needs but also a complete essential amino acid profile to support the fish growth. One solution to overcome this problem is done through the addition of lysine in the feed commercial. This study aimed to examine the effect of lysine addition on the efficiency of feed utilization, growth and body composition of *P. hypophthalmus*. The study consisted of 4 (four) treatments those were with dosages of 0% / kg of feed (A); 0.6% / kg of feed (B); 1.2% / kg of feed (C); and 2.4% / kg of feed (D) of lysine for every kg of feed. The experimental diet in this study was a commercial feed in the form of pellets which was added with the lysine. Fixed feeding rate of 5%/weight of biomass/day was applied at frequency 3 times a day. The experimental fish used were *P. hypophthalmus* fingerling has an average weight of 4.13 ± 0.26 g/individuals. The results showed that the addition 1,2% / kg of lysine to the diet increased SGR, EFU, FCR, PER and protein content of body composition of *P. hypophthalmus*.

Introduction

Striped Catfish (*Pangasius hypophthalmus*) is one of freshwater species that is very popular and widely consumed by the people of Indonesia (Rachmawati and Prihanto, 2019). *P. hypophthalmus* production in 2019 was 384,310 tons, fourth after catfish, tilapia, and goldfish (KKP, 2022). In the intensive cultivation of *P. hypophthalmus*, quality feed is required. Basically, quality feed does not only contain protein according to fish requirements but also must complete essential amino acids to support growth performance of fish. Fish require ten essential amino acids, which are indispensable in feed for maximum growth (Ahmed and Khan, 2004). However, it expected that commercial artificial diets are lack of lysine due to insufficient amount of lysine in plant-based protein source ingredients (Mai *et al*., 2006; Gatlin *et al.,* 2007).

One solution to overcome this problem is by supplementing lysine in dietary feed. Farhat and Khan (2013) found that lysine is an essential amino acid that the body cannot produced. Therefore, the addition of amino acids in fish feed supplements is required as an effort to increase growth. According to NRC (2011), lysine is an amino acid required by fish because lysine is found in the highest concentration in most fish species. Lysine is indispensable for growth, normal physiological function and protein synthesis (Robinson *et al.,* 2001). Walton *et al.* (1984) suggested that lysine and methionine play a role in the synthesis of carnitine which functions in the transportation of fatty acids to produce energy through oxidation. Nguyen *et al.* (2013) mentioned that the addition of lysine in feed can reduce the body fat content of fish, and increase protein retention (Cao *et al*., 2012) besides playing a role in metabolism for muscle growth (Khan and Abidi, 2011).

The addition of lysine in fish diet allows faster metabolic processes compared to the addition of other amino acids (Farhat and Khan, 2013). Many researches were done related to lysine in the diet of several fish species, namely *Penaeus monodon* (Biswas *et al*., 2006), *Macrobrachium rosenbergii* (Pramana *et al*.,

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2017), *Plectropomus leopardus* (Giri *et al.*, 2009), *Trachinotus blochii* (Ebeneezar *et al.*, 2019), *Litopenaeus vannamei* (Xie *et al.*, 2012), *Polydactylus sexphilis* (Deng *et al.*, 2010), *Cirrhinus mrigala* (Ahmed and Khan, 2004), and *Heteropneustes fossilis* (Farhat and Khan, 2013). Information of supplementary lysine in feed according to the needs of *P. hypophthalmus* is not yet known. Based on the description above, this study was conducted to determine the dietary lysine requirement for *P. hypophthalmus*. This study was carried out to examine the effect of addition on the amino acid lysine on the efficiency of feed utilization, growth performance and body composition of *P. hypophthalmus*.

Materials and Methods

Location and time of research

This research was conducted at the Muntilan Freshwater Seed and Cultivation Center, Central Java from May to August 2020.

Experimental fish

The *P. hypophthalmus* fingerlings of 5-7 cm length with an average weight of 4.13±0.26 g were used in this study. The experimental fish were obtained from the Muntilan Breeding and Freshwater Cultivation Center, Central Java. Fish were selected based on the completeness of the organs, physical health, uniformity of size, and no potential for disease (Rachmawati *et al.*, 2019). An adaptation of the *P. hypophthalmus* to feed and the environment was carried out for a week prior to treatment, fish were further fasted for 1 day to remove metabolic waste from the diets previously given.

Experimental design and diet preparation

The method of study was experimental, using completely randomized design. The containers used in this study were hapa cages made of waring net at dimension: 1 m x 1m x 1m (15 units). Hapa cages were placed in a soil pond. The range of water quality parameters during the study were maintened within range of 7.6-8.4 for pH, 27-30ºC for temperature, 4-6 mg/l for DO, alkalinity 120-150 ppm for alkalinity, and 0.01-0.1 ppm for total ammonia (SNI, 2014).

The experimental diets were commercial feed in the form of pellets. Diets were added with lysine (L-lysine HCl) according to dose 0%/kg feed (A); 0.6%/kg feed (B); 1.2%/kg feed (C); and 2.4%/kg feed (D). The determination of amino acid level dose in this study was based on the modified results of Khalida *et al.* (2017). The amino acid lysine used is the brand L-lysine HCl in a brown powder produced by PT. Cheiljedang Indonesia. Lysine was dissolved in 100 ml of distilled water for each dose until homogeneous to be further mixed with the pulverized artificial feed. Later, the mix was added with 1% CMC as a binder, reprinted using a pellet molding machine and dried in an oven at temperature of 40°C to dry (Khalida *et al.*, 2017). The results of the proximate analysis of the experimental diet after adding lysine can be seen in Table 1. The diets were given with a fixed feeding rate 5%/biomass weight/day. The frequency of feed given was 3 times a day, at 08.00, 14.00, and 18.00 WIB for 56 days. Data sampling was taken one time/week.

Proximate analysis of the experimental diet and fish body referred to the AOAC (2005) method. Ash content was determined by burning the sample in a furnace at 550°C for 24 hours. Fat content was determined by the ether extraction method using the Soxhlet System (FOSS Soxtec2043). Protein content was determined by the Kjeldahl method using a semi-automated Kjeldahl System (FOSS Kjeltec 2300).

Table 1. Composition and proximate analysis results of experimental diets (%).

Diet Composition	**Diets**			
	1	**2**	**3**	**4**
Commercial Feed	100	98.40	97.80	96.60
Lisin	0	0.6	1.2	2.4
CMC	0	1	1	1
proximate analysis results				
Moisture*	4.89	7.43	11.44	5.50
Ash*	8.00	9.41	6.00	7.50
Protein*	29.89	30.67	30.93	32.31
Lipid*	8.90	10.84	10.23	5.77
Fiber*	0.12	0.22	0.12	0.32

*Analysis results obtained at the Laboratory of Animal Feed, Faculty of Animal Sciences and Agriculture, Diponegoro University.

Test parameters

Growth performance parameters included specific growth rate (SGR) and food conversion ratio (FCR) according to Jin *et al.* (2018), efficiency feed utilization (EFU), and protein efficiency ratio (PER) according to Tacon (1987), and Survival Rate (SR) according to Wang *et al.* (2018). The equations are as follows:

$$\mathrm{SGR} = \frac{\ln Wt - \ln Wo}{t}$$

Notes: Wt= final weight (g); Wo= initial weight (g); t = number of days in the feeding period (day).

$$\mathrm{EFU}\ (\%) = \frac{Wt - Wo}{F} x\ 100\%$$

Notes : Wt= final weight (g); W0= initial weight (g); F= the amount of feed consumed (g).

$$FCR = \frac{F}{Wt - Wo}$$

Notes : Wt = final weight (g); Wo= initial weight (g); F= the amount of feed consumed (g).

$$PER = \frac{Wt - Wo}{Pi} x\ 100\%$$

Notes: Wt= final weight (g); W0= initial weight (g); Pi= Protein intake (g).

$$SR = \frac{Nt}{No} x\ 100\%$$

Notes: No= initial count; Nt= final count.

Data analysis

Research data were analyzed by the one-way Analysis of Variance (ANOVA). Significant results of the ANOVA were followed up by the Duncan's multiple range tests (P<0.05) (Stell *et al*., 1996).

Results

Results of growth performance included: the Specific Growth Rate (SGR), Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) of striped catfish in this study were shown in Table 2.

The results of the proximate analysis of striped catfish fed with different level of lysine were presented in Table 3. The protein content of fish fed with lysine-containing diet was higher than that the initial fish. The highest protein content was observed in fish fed a lysine at dose of 1.2%/kg of feed. The moisture, lipid, and ash content of fish decreased with the dose of lysine supplemented in fish diet.

Discussion

The results of the analysis showed that the addition of lysine to the fish diets led to had a very significant effect on the SGR of *P. hypophthalmus*. The results indicated that *P. hypophthalmus* fed diets contained the lysine at a level dose of 0.6-2.4%/kg feed (B, C and D) had a higher SGR value than those fed diets contained lysine of 0%/kg feed (A). However, fish fed diets that were lack of lysine resulted in low SGR values. Mai *et al.* (2006) stated that dietary lysine deficiency causes loss of appetite which contributes to slow fish growth. The SGR value was found the highest in fish fed diets added with lysine at of 1.2%/kg feed (C) of 5.38%/day, followed by 0.6%/kg feed (B) of 4.15%/day, 2,4%/kg feed (D) of 3.40%/day and 0%/kg feed (A) of 3.18%/day. The high value of SGR in treatment C was influenced by the low FCR in the treatment, indicated that the feed could be digested and converted for fish growth due to the role of lysine in protein synthesis. Abimorad *et al.* (2014) stated that lysine is the most limiting amino acid in feed that can affect protein synthesis and catabolism in muscle tissue, thereby affecting fish growth. Lysine is also required in carnitine formation which is associated in the formation of energy. According to Biswas *et al.* (2006), lysine plays a role in the formation of carnitine which is responsible for the process of fat oxidation in the mitochondria. Moreover, Nguyen *et al.* (2013) mentioned that carnitine is an essential metabolic intermediary needed to oxidize long-chain fatty acids in the mitochondria, producing metabolic energy.

The presence of lysine in diets had a very significant effect on the EFU of *P. hypophthalmus*. The presence of amino acids in fish diets increased the EFU value of *P. hypophthalmus*, presumably due to an increase in feed protein digestibility. Xie *et al.* (2012) reported that the presence of lysine in feed can increase protein digestibility and increase feed efficiency. The results of the study (Table 2) showed that *P. hypophthalmus* fed diets contained lysine (B, C and D) had higher EFU values than that without the dietary amino acid (A). The treatment C (1.2%/kg feed) was found to obtain the highest EFU value of 77.05% besides the other excellent results for SGR, PER, FCR, and protein retention compared to other treatments.

The EFU value obtained in this study was higher than 50%, hence it is classified as high. Wu (2013) reported that the efficiency of feed utilization in good fish farming activities should have a value of more than 50% or even close to 100%. Khalida *et al.* (2017) stated that lysine has a role for feed quality which functions as an amino acid limiter in fish feed. Palavesam *et al.* (2008) suggested that lysine is one of the ten indispensable amino acids needed in feed protein. It was also reported in the study of Deng *et al.* (2010), that among the ten required amino acids, lysine is often used as the first amino acid limiter in ingredients used in fish feed. The high efficiency value of feed utilization reflected the feed quality. Hence, feed was considered of good quality and possibly converted into body energy. Ebeneezar *et al.* (2019) stated that high feed efficiency indicates efficient use of feed, therefore only a small amount of protein is metabolized to energy required. Protein is mostly used for growth.

Table 2. Mean of growth performance of *P. hypophthalmus* fed experimental diets contained different level of lysine.

Diets	Parameters				
	SGR (%/day)	**EFU (%)**	**FCR**	**PER**	**SR (%)**
A	3.18±0.28^{cb}	55.32±5.22^{c}	1.69±0.17^{b}	1.79±0.17^{c}	87.50±1.50^{a}
B	4.15±0.90^{b}	67.46±4.76^{b}	1.46±0.15^{ab}	2.26±0.16^{b}	95.83±3.22^{a}
C	5.38±0.38^{a}	77.05±2.08^{a}	1.27±0.03^{a}	2.57±0.07^{a}	95.83±2.22^{a}
D	3.40±0.77^{bc}	60.53±5.32^{bc}	1.57±0.10^{b}	2.01±0.18^{bc}	87.50±2.50^{a}

Note: Mean values ± SD. with different superscripts indicated a significant difference (P<0.05)

Table 3. Composition nutrition of *P. hypophthalmus* fed experimental diets containing different level of lysine.

(g/ 100 g wet weight)	Diets				
	Initial	**A**	**B**	**C**	**D**
Moisture	72.2±0.2	70.8±0.2	68.4.1±0.1	68.0±0.2	66.2±0.2
Protein	10.7±0.3	12.6±0.1	14.2±0.2	18.3±0.2	16.9±0.1
Lipid	8.4±0.2	7.8±0.2	7.6±0.3	7.0±0.2	6.6±0.1
Ash	4.8±0.2	4.5±0.1	4.0±0.2	3.7±0.1	3.0±0.1

The results showed that the highest FCR value was found in treatment A (0%/kg feed) of 1.69, while the lowest was in treatment C (1.2%/kg feed) of 1.27. The low value of FCR in diet C (1.2%/kg feed) was expected due to the highest EFU value produced by diet C. Xie *et al.* (2012) mentioned that the lower feed conversion value will lead to high level of feed utilization efficiency, vice versa. The addition of lysine to diet for each treatment resulted in the decreased FCR value. Li *et al.* (2012) reported that lysine has a major role in maximizing food conversion, thus lowering feed conversion ratio. According to Salama *et al.* (2013), increasing levels of lysine in feed will increase the ratio of protein efficiency and decrease the ratio of feed conversion.

The results of the analysis of variance showed that the dietary lysine addition had a significant effect on the PER of *P. hypophthalmus*. As observed, the use of lysine in fish diets led to higher increase in protein efficiency ratio of *P. hypophthalmus* compared to those fed diets without the addition of lysine. The highest PER value was obtained in diet C (1.2%/kg feed) of 2.57 and the lowest value was obtained in diet A (0%/kg feed) of 1.79. Newsome *et al.* (2011) confirmed that the higher value of protein efficiency ratio indicated improvement of feed quality given. Li *et al.* (2012) stated that higher value of the protein efficiency ratio indicated optimal used of feed.

The addition of lysine to diet did not have a significant effect on the SR of *P. hypophthalmus*. The SR value of *P. hypophthalmus* during the study ranged from 87.50-95.83% which was still within the ideal range for fish culture. Referring to Fuady *et al.* (2013) the survival of fish is considered good if SR value is higher than 84%. Wang *et al.* (2018) mentioned that the high survival value of fish in aquaculture activities represented an excellent quality and quantity of feed provided, thus allowing no mortality in large numbers which would reduce the survival value of these cultivars.

The protein content of the body composition of *P. hypophthalmus* fed the diet contained lysine was higher than the body of the fish initial. An increase in the protein content of fish is a response to the presence of lysine in the feed required by fish (Luo *et al.*, 2006). Lysine plays a role in increasing protein deposition in the body and fillet content (Furuya and Furuya, 2010; Hamid *et al.*, 2016; Mukhtar *et al.*, 2017). Cao *et al.* (2012) reported that lysine could increase nitrogen retention. Lysine is very important for the synthesis of carnitine which is involved in the transport of fatty acids and fat oxidation to produce energy (Walton *et al.*, 1984). Observation of fat content in the body of *P. hypophthalmus* fed with a diet of 0%/kg feed (A) indicated a higher fat content than the fish fed a diet contained lysine 0.6-2.4%/kg feed (B, C, and D). To say, a decrease in carnitine synthesis possibly occurred due to lysine deficiency which interfered with lipid metabolism and resulted in excess body fat and energy storage as observed in this study. The results of similar studies were reported by Walton *et al.* (1984) in rainbow trout, Zhou *et al.* (2010) in black sea bream, Helland *et al.* (2011) in atlantic salmon, Yang *et al.* (2011) in silver perch, Xie *et al.* (2012) in yellow croakers, and Ovie and Eze (2013) in tilapia.

Conclusion

Based on the results of the study, it is concluded that the addition of the amino acid lysine to diet increased SGR, EFU, PER, and protein in the body of *P. hypophthalmus*. The optimal dose of lysine supplementation in *P. hypophthalmus* diet was 1.2%/kg lysine.

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