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Effects of Dietary Lysine for River Catfish Juveniles on Protein Digestibility and Body Composition

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Abstract

The success of intensive aquaculture for river catfish juveniles (*Pangasius hypophthalmus* Sauvage, 1878) is determined by the availability of good quality feed. Good quality feed does not only contain protein required by the fish; it should also contain essential amino acids to support fish growth. Lysine is a constraint of amino acids in the formulation diet due to the characteristics of the plant-based protein with lysine deficiency. This study was conducted to determine the dietary effects of lysine river catfish juveniles on protein digestibility, body composition, and relative growth rate. The river catfish juveniles used in the study had a mean weight of 8.21 g ± 0.32 g fish^{-1}. The test feed contained 37.1 % and 19.76 MJ kg^{-1}. Doses of lysine in the feed were added in an increase of 0.25 g 100 g^{-1} [1.48 g; 1.73 g; 1.98 g; 2.23 g; 2.48 g, and 2.67 g] 100 g^{-1} dry diet)]. The results showed that a lysine-added diet with the dose of 2.23 % 100 g^{-1} dry diet generated the highest values of protein digestibility (ADCp), relative growth rate (RGR), and fish body composition compared to other treatments. The optimal dose of lysine in feed for protein digestibility and relative growth rate of river catfish juveniles were 2.22 % to 2.24 % dry diet.

Keywords: Amino acid essential, Diet formulation, Fish growth, Fish nutrition, Intensive aquaculture, Lysine deficiency, *Pangasius hypophthalmus* (Sauvage, 1878), Quality of feed

1. Introduction

The availability of high-quality feed determines the success of intensive aquaculture. The excellent quality of diet formulation of feed does not only contain protein required by the fish; it should also contain essential amino acids to support fish growth. Ten types of amino acids are necessary for the feed to help maximum growth (Setyobudi *et al*., 2021; Yun *et al*., 2016). Lysine is a constraint of amino acids in the diet formulation of feed due to the characteristics of the plant-based protein that has lysine deficiency (Xie *et al.*, 2012a). Lysine is one of the amino acids the fish highly require since it is discovered in high concentration in the carcasses of most species (NRC, 2011). For example, adding 1.93 % lysine from the total feed could increase the growth of orange-spotted rabbitfish (*Siganus guttatus* Bloch, 1787) (Nhu *et al*., 2022). Furuya *et al*. (2012) also indicated that adding 1 % lysine from the total feed can increase the efficiency of protein utilization by Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), which is followed by an increase in growth rate. Lysine is needed to support growth, normal physiological function, and protein synthesis. Lysine is a limiting amino acid for river catfish (*Pangasius hypophthalmus* Sauvage, 1878) species, and if feed diets are formulated to meet the minimum lysine requirement, all other amino acids should be in excess (Yun *et al*., 2016). Moreover, metionin lysine has a role in carnitine synthesis, transporting fat acid to create energy through oxidation reactions (Furuya *et al.* (2012).

Lysine deficiency in the fish causes slow growth, not maximal protein utilization, and losing appetite (Nguyen and Davis, 2016). Excess lysine in the feed also causes low growth (Yang *et al*., 2011). The lysine deficiency due to using a plant-based diet can be compensated for by adding lysine to the feed (NRC, 2011). The addition of lysine to the diet can increase fish weight (Khan and Abidi, 2011). The additional lysine in the diet also reduces lipid content in the fish (Nguyen and Davis, 2016), increases protein retention, and metabolizes mass growth (Cao *et. al*., 2012). Some studies have been done on the lysine needs of some fish species, such as *Oncorhynchus mykiss* Walbaum, 1792 (Yun *et al*., 2016), *Heteropneustes fossilis* Bloch, 1794 (Farhat and Khan, 2013), *Ictalurus punctatus* Rafinesque, 1818 and *Oreochromis niloticus* Linnaeus, 1758 (Nguyen and Davis, 2016), *Oreochromis niloticus* Linnaeus, 1758 (Ovie and Eze, 2013), *Siganus guttatus* Bloch, 1787 (Nhu *et al*., 2022), *Trachinotus blochii* Lacépède, 1801 (Ebeneezara *et*

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al., 2019), *Pseudosciaena crocea* Richardson, 1846 (Xie *et al*., 2012a).

The river catfish (*P. hypophthalmus*) is a freshwater cultivated fish that is highly preferred and consumed in Indonesia. The river catfish is one of the original fish in Indonesian waters that have been successfully domesticated, as superior fish and economical; besides, the cultivation development is quite prospective. Support for developing this fish is quite easy to access – farming areas, farmers, cultivation technology, even markets. For catfish cultivation, the environment needed is not complicated because catfish is included in the class of catfish that can survive in bad aquatic environments such as circumstances with lack of oxygen (Rachmawati and Prihantoro, 2019). The taste of river catfish meat is unique, tasty, and delicious. These characteristics make people like the Catfish (Rachmawati and Prihantoro, 2019). The need for protein for the growth of Catfish juveniles is 37.12 %, as reported by Jayant *et al*. (2018); however, there are still few studies on the optimal requirement of lysine for Catfish juveniles that justify studying the matter.

2. Materials and Methods

2.1. Research design and test fish

The study method used an experimental design conducted at the Laboratory of Freshwater Hatchery and Aquaculture of Muntilan Subdistrict, Magelang District, Central Java, Indonesia, from April to June 2021. Test fish was obtained from the laboratory. Adaptation for the fish on cultivation media and feed was made 1 wk before the study was conducted. Fish were acclimatized and reared in fiber containers with the dimension of 1.50 m × 1 m × 1 m filled with 600 L water. During adaptation, amino acid lysine-free feed was administered. One day prior to the experiment, the fish fasted to remove any metabolic waste. Test fish were healthy with homogenous size, normal organs, and pathogen-free (Rachmawati *et al*., 2017).

The containers used in this study were 18 aquariums. The study consisted of six treatments, and each treatment was replicated three times. The fish (weight of 8.21 g ± 0.32 g fish^{-1}) was reared in the aquariums with the dimension of 100 cm × 80 cm × 80 cm equipped 20 fish stocked recirculation systems each. The test diet was given at the fixed rate of 5 % of the mass weight three times a day (07.00, 12.00, and 17.00). The study was conducted for 49 d. Each fish was weighed on weekly basis to determine the growth.

2.2. Test feed

The lysine used was L-lysine HCI which is produced by PT. Cheiljedang, Indonesia. Test feed consisted of isonitrogenous feed (37.1 %) and isoenergetic feed (19.76 MJ kg^{-1}) based on the study of Jayant *et al*. (2018). Doses of lysine in the feed were added an increase of 0.25 g 100 g^{-1} (1.48 g; 1.73 g; 1.98 g; 2.23 g; 2.48 g and 2.67 g) 100 g^{-1} dry diet, equals 3.99 %, 4.66 %, 5.34 %, 6.01 %, 6.68 % and 7.19 % protein diet. The ratio of casein-gelatin (4:1) accounted for the minimum of amino acids, and other maximum amino acids were maintained to increase the isonitrogenous diet as lysine increased but by sacrificing non-essential amino acid, glycine, to decrease. Crystalized lysine amino acid was weighed according to the treatments and stirred in hot water (Hotplate Stirrer Ika Hs-7, Germany) at 80 °C. In a separate container, the gelatine was diluted in the water and constantly stirred, then mixed with the amino acid solution (Xie *et al*., 2012b). Diet ingredients were mixed with amino acid and gelatine solution and added with NaOH 6 N to reach pH 7.0 (Ebeneezar *et al*., 2019). After the diet mixture had been ready, the mixture was formed as pellets with a size of 4.2 mm (MKS-PLT10, Indonesia) and dried in the oven (Memmert, UF30Plus Universal, Italy) at 70 ºC for 3 h. After the pellets had been dried, the pellets were saved in cold storage (Gea AB2226R, China) at 4 ºC until the pellets were ready to be used. Formulation of test feed used for determining the dietary lysine content of juvenile Catfish was shown in Table 1.

Table 1. Formulation of tests feed used for Catfish juveniles

Ingredients (g 100 g^{-1}, dry diet)	**Diets**					
	P_1	**P_2**	**P_3**	**P_4**	**P_5**	**P_6**
Casein[a]	20	20	20	20	20	20
Gelatin[b]	5	5	5	5	5	5
Amino acid mix[c]	29.95	29.70	29.45	29.20	28.95	28.70
Dextrin	21.15	19.24	19.24	19.24	19.24	19.24
Corn oil	4	4	4	4	4	4
Cod liver oil	3	3	3	3	3	3
Mineral mix[d]	3	3	3	3	3	3
Vitamin mix[e]	4	4	4	4	4	4
Carboxymethyl cellulose	10	10	10	10	10	10
α- cellulose	0.06	0.06	0.06	0.06	0.06	0.06
Lysine	1.25	1.5	1.75	2.0	2.25	2.5
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Analyzed lysine	1.48	1.73	1.98	2.23	2.48	2.67
Proximate composition [(*)]						
Crude protein (%)	36.85	37.13	36.76	37.12	37.05	37.08
Crude Lipid (%)	7.43	7.46	7.45	7.51	7.25	7.45
Ash (%)	8.33	8.30	8.32	8.28	8.36	8.27
Gross energy (kcal 100 g^{-1}, dry diet)	471.87	471.89	471.88	471.87	471.87	471.88

[a]Crude protein (76 %)

[b]Crude protein (96 %)

[c]Amino acid mixture (g 100 g^{-1}) tryptophan 0.45; alanine 1.23; methionine 1.03; valine 1.67; proline 0.952; tyrosine 0.94; aspartie acid 0.09; cystine 0.85; arginine 1.20; glulamic acid 0.08; histidine 0.37; serine 0.06; isoleucine 2.15; leucine 1.89; threoinne 1.02; phenylalanine 1.55; lysine variable; glycine variable.

[d]Mineral mixture (g 100 g^{-1}) magnesium sulphate 13.20; potassium iodide 0.015; zinc sulphated $7H_2O$ 0.40; sodium chloride 4.35; magnus sulphated H_2O 0.080; calcium biphosphate 13.57; calcium lactate 32.69; potassium phosphate 23.98; alumunium chloride $6H_2O$ 0.0154; cuprous chloride 0.010; cobalt chloride $6H_2O$ 0.10; sodium biphosphate 8.72; ferric citrate 2.97 (Xie *et al*., 2012b).

[e]Vitamin mixture diluted in choline chloride 0.5 g 100 g^{-1}; pyridoxine hydrochloride 0.005; vitamin B12 0.00001; alpha cellulose 3 g 100 g^{-1}; biotin 0.0005; vitamin E as α-tocopherol 0.04; thiamin hydrochloride 0.005; ascorbic

acid 0.10; niacin 0.075; calcium pantothenate 0.05; riboflavin 0.02; menadione 0.004; folic acid 0.0015; inositol 0.20 (Loba Chemic India)

*) Animal Nutrient Laboratory, Faculty of Husbandry and Agriculture, Diponegoro University (2021)

2.3. Proximate analysis

Based on the method of Latimer (2019 and Setyobudi *et al*. (2018), AOAC proximate analysis was used to analyze diet and carcasses of the fish. Ash content was attained by incinerating the sample fish in the furnace (HPE, Indonesia) at a temperature of 550 °C for 24 h. The cooled and stable ash results were then weighed so that the formula can calculate the total ash content:

$$\text{Ash (\%)} = \frac{W_1 - W_2}{W} \times 100\ \% \qquad (1)$$

W = Sample weight before turn to ashes (g)

W_1 = Sample weight + porcelain dish after turn to ashes(g)

W_2 = Weight of an empty porcelain dish (g)

The fat level was measured with the eter extraction method based on the Soxhlet (FOSS Soxtec 2043, Denmark) method. Samples with known constant weight were put into Soxhlet extracted using hexane or petroleum ether. After extraction, the sample was removed from the Soxhlet and dried.

$$\text{Crude lipid (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Final Sample weight (g)}} \times 100\ \% \qquad (2)$$

Protein content was measured with a semi-automatic Kjeldahl system (FOSS Kjeltec 2300, Denmark). Samples (2 g) were digested in the digestion unit for 45 minutes. The digester was then distilled in a distillation unit (Kjeldahl system, Indonesia). It was titrated with 0.2 N Hydrochloric acid HCL, and crude protein was obtained by multiplying the total nitrogen by a conversion factor of 6.25 (Setyobudi *et al*. 2021, Tonda *et al*., 2022)

$$\text{Crude protein (\%)} = \frac{\text{mL titration} \times N \times 14{,}007 \times 6.25 \times 100\ \%}{\text{Weight the sample (g)} \times 1\ 000} \qquad (3)$$

2.4. Protein digestibility analysis

The indirect method of adding Cr_2O_3 0.5 % in the diet to measure protein digestibility was employed (Pérez-Jiménez *et al*., 2014). Before the feces of the fish were collected, the fish was acclimated to the diet containing chromium for 1 wk. After the 8th day, the feces were collected for 49 d every morning, noon, and afternoon after the fish was fed. The collection process was performed 2 h after feeding – a small plastic hose of which tip was attached to a wooden stick for easy moving was used for the purpose, and the feces were put in a bucket. Then the feces were filtered with a plankton cloth net; the filtered feces were placed in small plastic bottles and stored in cold storage. Before the feces were analyzed, they were dried in the oven (Memmert, UF30Plus Universal, Italy) at 6 °C for 24 h. After that, protein and Cr_2O_3 content in the feces were analyzed using a spectrophotometer (SSA 320N, Denmark) with a wavelength of 350 nm (Pérez-Jiménez *et al*., 2014).

2.5. Water quality

Observation of water quality, including temperature, pH, and dissolved oxygen, was conducted every day during the study. Meanwhile, the observations of ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), and nitrate-nitrogen (NO_3-N) were done on the first and final day of the study. The quality of water was still in accordance condition of Catfish aquaculture. Those values for the temperature ranged from 26.4 °C to 30.5 °C; pH ranged 7.3 to 8.5; dissolved oxygen (DO) ranged from (4.6 to 6.8) mg L^{-1}; ammonia-nitrogen (NH_3-N) ranged between (0.002 to 0.002) mg L^{-1}; nitrite-nitrogen (NO_2-N) ranged (0.001 to 0.002) mg L^{-1}; nitrate-nitrogen (NO_3-N) ranged from (0.02 to 0.04) mg L^{-1} (Ut *et al*., 2016).

2.6. Observed variables

Variables that were observed included average growth weight (AWG), total feed consumption (TFC), relative growth rate (RGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR). The measurement of those variables was based on the method of Rachmawati *et al*. (2021), while protein digestibility (ADCp) and Efficiency Feed Utilization (EFU) measurement followed the method of Rachmawati and Prihantoro (2019). The Equation used to calculate the variables were in (4) to (11)

$$\text{AWG (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100\ \% \qquad (4)$$

TFC (g) = Amount of feed the first day + Amount of feed the second day + Amount of feed day n (5)

$$\text{SGR (\%)} = \frac{\text{Log final weight} - \text{Log initial weight}}{\text{Number of days}} \times 100\ \% \qquad (6)$$

$$\text{ADCp (\%)} = 100 - \left\{ \frac{100 \times Cr_2O_3\ \text{feed (\%)} \times \text{protein feces (\%)}}{Cr_2O_3\ \text{feed (\%)} \times \text{protein feed (\%)}} \right\} \qquad (7)$$

$$\text{AWG (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{The weight of feed consumed (g)}} \times 100\ \% \qquad (8)$$

FCR = feed consumtion (g) / body weight gain (g) (9)

PER = body weight gain (g) / protein intake (g) (10)

SR (%) = 100 (final count/initial count) (11)

2.7. Amino acid analysis

Analyze lysine of 1.48 %, 1.73 %, 1.98 %, 2.23 %, 2.48 %, and 2.67 % dry diet (Table 2) was determined by the HPLC (Column Chiller EW-42650-80, Japan) method followed by Ebeneezar *et al*. (2019), Setyobudi *et al.* (2021).

2.8. Statistic analysis

Data on growth performance and feed utilization variables before ANOVA analysis were tested for homogeneity, additivity, and normality. Analysis of

Variance (ANOVA) was used to determine growth performance and feed utilization variables. Duncan's Multiple Range Test was used to identify specific differences between means (Adinurani 2016, 2022). Finally, the Polynomial Orthogonal test was used to determine the optimum dose of lysine in the test feed (Xie *et al*., 2012a). The ethical clearance of this research was issued by the research ethics committee of Brawijaya University, Malang, East Java, Indonesia (No.114-KEP-UB-2021).

3. Results and Discussion

3.1. Result

3.1.1. Amino acids of test feed

The results were as shown in Table 2. It can be incurred that the essential amino acids in the test feed contains the lysine dose of 2.23 % dry diet, which was close to Catfish juveniles' essential amino acid profile

3.2. Composition of fish carcasses

The results of the proximate test of the fish carcasses, which were fed with various doses of lysine, are depicted in Table 3. The protein content in fish carcasses given additional lysine was higher than in the initial fish carcasses. The treatment with 2.23 % lysine in a dry diet showed the highest range of protein in the carcasses. The level of water, lipid, and ash contents decreased as the lysine in the fish increased.

Table 2. Content of amino acid on test feed (% dry matter)

Amino acid essential	Analyzed dietary lysine levels (% dry diet)						*P.hypophthalmus**
	1.48	1.73	1.98	2.23	2.48	2.67	
Threonine	1.69	1.58	1.55	1.40	1.29	1.28	1.358
Lysine	1.48	1.73	1.98	2.23	2.48	2.67	2.268
Phenylalanine	1.13	1.22	1.59	1.47	1.50	1.58	1.398
Arginine	1.18	1.26	1.39	1.45	1.47	1.49	1.447
Methionine	0.86	0.84	0.83	0.76	0.80	0.84	0.755
Leucine	3.59	3.64	3.68	4.09	4.20	4.25	4.128
Isoleucine	1.38	1.59	1.80	2.10	2.18	2.21	2.019
Histidine	0.81	0.83	0.82	0.84	0.82	0.81	0.841
Valine	1.52	1.63	1.74	1.81	1.90	1.98	1.805

Note : * Jayant *et al*. (2018)

Table 3. Body composition of Catfish juveniles containing different doses of lysine

$g\ 100\ g^{-1}$ wet weight	Analyzed dietary lysine doses (% dry diet)						
	Initial	1.48	1.73	1.98	2.23	2.48	2.67
Moisture	70.4 ± 0.1	70.2 ± 0.2	70.1 ± 0.2	70.0 ± 0.1	69.6 ± 0.3	68.6 ± 0.3	67.9 ± 0.1
Protein	11.6 ± 0.3	14.4 ± 0.2	15.7 ± 0.3	17.8 ± 0.2	19.5 ± 0.3	18.3 ± 0.1	17.0 ± 0.5
Lipid	9.6 ± 0.1	8.3 ± 0.3	7.5 ± 0.4	6.5 ± 0.3	6.2 ± 0.1	5.2 ± 0.2	5.0 ± 0.2
Ash	5.4 ± 0.1	4.4 ± 0.2	4.0 ± 0.2	3.5 ± 0.3	3.4 ± 0.2	3.2 ± 0.3	3.0 ± 0.2

Responses of Catfish juveniles fed with various dosages of lysine were displayed in Table 4. During the research, diseases that caused fish mortality were not found. The research study showed that the responses of AWG, TFC, ADCp, EFU, RGR, FCR, and PER were significant ($P < 0.01$) due to the availability of lysine in the feed; however, the existence of lysine in the feed did not affect SR. The highest values of AWG, TFC, ADCp, EFU, RGR, FCR, and PER were obtained in the fish which was given the feed containing lysine of a 2.23 % dry diet. The polynomial orthogonal test resulted in the optimum lysine dosages on ADCp and RGR as much as 2.24 % and 2.22 % dry feed, respectively (Figure 1 and Figure 2).

Table 4. Feed utilization and growth performance of juvenile catfish feed diets containing varying dietary lysine doses

Variables	Dietary lysine doses					
	1.48	1.73	1.98	2.23	2.48	2.67
Initial weight	8.21 ± 0.32	8.20 ± 0.30	8.25 ± 0.28	8.28 ± 0.18	8.20 ± 0.15	8.21 ± 0.30
Final weight	19.38 ± 0.12[f]	24.51 ± 0.16[e]	31.52 ± 0.10[b]	42.56 ± 0.11[a]	30.75 ± 0.12[c]	27.73 ± 0.14[d]
AWG (%)	11.17 ± 0.22[f]	16.31 ± 0.23[e]	23.27 ± 0.19[b]	34.28 ± 0.14[a]	22.55 ± 0.14[c]	19.52 ± 0.22[d]
TFC (g)	95.10 ± 0.26[f]	96.53 ± 0.78[dc]	97.43 ± 0.75[b]	99.76 ± 0.42[a]	96.83 ± 0.36[c]	96.02 ± 0.27[e]
ADCp (%)	58.27 ± 0.19[f]	63.53 ± 0.14[e]	75.15 ± 0.16[c]	85.27 ± 0.14[a]	77.63 ± 0.12[b]	70.31 ± 0.17[d]
EFU (%)	60.27 ± 0.12[f]	65.17 ± 0.15[e]	76.51 ± 0.18[c]	85.24 ± 0.17[a]	78.25 ± 0.15[b]	71.46 ± 0.12[d]
SGR ($\% \ d^{-1}$)	0.76 ± 0.08[f]	0.97 ± 0.09[e]	1.19 ± 0.12[c]	1.45 ± 0.15[a]	1.17 ± 0.12[b]	1.08 ± 0.11[d]
FCR	2.48 ± 0.10[f]	2.12 ± 0.14[e]	2.00 ± 0.10[d]	1.43 ± 0.12[a]	1.75 ± 0.13[b]	1.98 ± 0.03[c]
PER	1.78 ± 0.13[f]	2.56 ± 0.10[e]	3.05 ± 0.10[c]	4.18 ± 0.13[a]	3.78 ± 0.12[b]	2.89 ± 0.11[d]
SR (%)	93.85 ± 2.47[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]

Notes : Mean values with same superscript showed insignificant difference ($P > 0.05$)

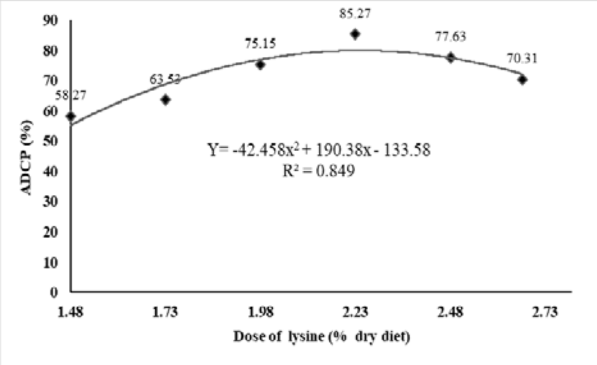

Figure 1. The correlation between the addition of lysine in the diet and ADCp

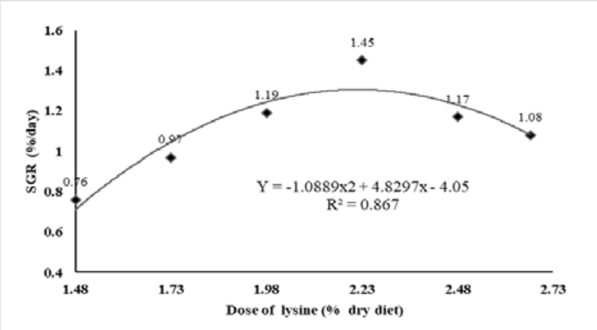

Figure 2. The correlation between the addition of lysine in the diet and SGR

4. Discussion

The river Catfish juveniles that were fed with a low dose of lysine caused low values of AWG, TFC, ADCp, EFU, SGR, FCR, and PER. Farhat and Khan (2013) disclosed that the lack of lysine caused low growth of the fish. The study results on *Litopenaeus vannamei* Boone, 1931 showed that lysine deficiency reduced appetite (Xie *et al*., 2012a). Otherwise, the results of this study proved that the river Catfish juveniles fed with various doses of lysine did not cause the fish appetite to decrease. The low values of ADCp, EFU, SGR, FCR, and PER were because of lysine deficiency. The lysine deficiency hindered protein synthesis and metabolism. The same results were discovered in several fish, such as in the *H. fossilis* (Farhat and Khan, 2013), *O. niloticus* (Ovie and Eze, 2013), *T. blochii* (Ebeneezara *et al*., 2019), *P. crocea* (Xie *et al*., 2012b), *O. mykiss* (Yun *et al*., 2016).

Besides the lack of lysine, the excess lysine also caused the values of TFC, ADCp, EFU, SGR, FCR, and PER to be low. However, the quantity of lysine did not indicate a toxic effect on the fish. So far, there is no information yet explaining the lysine toxicity in the fish. He *et al.* (2013) stated that the low growth of the fish fed with the excess lysine was due to the opposite function between lysine and arginine, although the mechanism has not been understood yet.

The protein content of fish carcasses fed with lysine supplementation was higher than that in the initial state. The increase in protein content in the fish carcass was due to the availability of lysine in the feed He *et al*. (2013). Hamid *et al*. (2016) expressed that lysine promotes protein formation in fish. Cao *et al*. (2012) noted that lysine could boost nitrogen retention. Lysine is essential to synthesize carnitine which transports fat acids and oxidizes fat to create energy. Wu (2013) also described that lysine is a critical factor for synthesizing keratin, which transports long-chain fatty acids from the cytoplasm to mitochondria for β-oxidation. Fat content in the fish carcass fed with a low dosage of lysine (1.48 % dry diet) was higher than those given with higher dosages of lysine (1.73 %, 1.98 %, 2.23 %, 2.48 %, and 2.67 % dry diet). The decrease in carnitine synthesis was thought to be due to lysine deficiency that hindered lipid metabolism and caused an excess of fat and energy. Similar studies were proclaimed by Xie *et al*. (2012b) in the white shrimp (*L vannamei*), Yang *et al*. (2011) in the silver perch (*Bidyanus bidyanus* Mitchell, 1838), Helland *et al*. (2011) in the Atlantic salmon (*S. salar*), Farhat and Khan (2013) in the black seabream (*Spondyliosoma cantharus* Linnaeus, 1758).

The highest values of AWG, TFC, ADCp, EFU, SGR, FCR, and PER were obtained from the fish fed with the lysine dosage of 2.23 % dry diet. It was thought that profile of amino acids in the feed containing lysine in a 2.23 % dry diet has a similar essential amino acids profile in the Catfish juveniles (Table 2). Therefore, He *et al*. (2013) declared that the appropriate feed for fish was the feed with a similar amino acid profile. Thus, the optimum lysine for protein digestibility and relative growth rate in the feed of Catfish juveniles ranged from 2.22 % to 2.24 % dry diet (5.96 % to 6.03 % protein feed).

NRC (2011) disclosed that the requirement of lysine among fish species and within the same species has various levels ranging from 3.32 % to 6.61 % protein feed. Therefore, the study's results were consistent with the need for lysine for fish, according to NRC (2011). The lack of lysine for Catfish juveniles (*P. hypophthalmus*) – 5.96 % to 6.03 % protein feed – was similar to the lysine requirement for stinging Catfish fingerlings (*H. fossilis*) at 5.3 % to 6.1 % (Farhat and Khan, 2013) and silver perch (*B. bidyanus*) at 6.0 % (Yang *et al.*, 2011), but lower than for *Trachinotus ovatus* Linnaeus, 1758 at 6.7 % (Du *et al*., 2011). However, it was higher than one for Indian major carp (*C. mrigala*) at 5.75 % (Ahmed and Khan, 2011), large yellow croaker (*P. crocea*) at 3.95 % (Xie *et al*., 2012), and juvenile Silver pompano (*T. blochii*) at 5.71 % to 5.83 % (Ebeneezara *et al*., 2019).

5. Conclusion

Supplementation of lysine in feed with the dose of 2.23 % dry diet generated the highest values of protein digestibility (ADCp), relative growth rate (SGR), and fish body composition compared to other treatments. The optimal dose of lysine in feed for protein digestibility and relative growth rate of juvenile Catfish were 2.22 % to 2.26 % dry diet.

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