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Influence of Dietary Lysine Level on Growth Performance, Feed Efficiency, and Body Composition of Sangkuriang Catfish (*Clarias gariepinus* var. Sangkuriang) Fingerlings

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ABSTRACT

The high consumer demand in Indonesia encourages catfish farmers to conduct an intensive culture. A low feed efficiency mainly occurs in cultivating Sangkuriang catfish resulting in poor growth. This condition might be caused by low lysine content, as lysine is an essential amino acid that the fish cannot synthesize. The present study aimed to investigate the effect of lysine supplementation in feed on protein digestibility, feed efficiency, and growth of Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) fingerlings. The study used 270 Sangkuriang catfish with an average wet weight of 7.54 ± 0.13 g/fish. The experimental feed contained protein, energy, and amino acid, and then various doses of lysine were added to the experimental feed: (1) 0.0%, (2) 0.5%, (3) 1.0%, (4) 1.5%, (5) 2.0%, and (6) 2.5%. Weight gain (WG), protein digestibility (ADCp), the efficiency of feed utilization (EFU), relative growth rate (RGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein retention (PR) of catfish were evaluated for 8 weeks. The results found that the supplementation of lysine in feed significantly ($P < 0.05$) influenced WG, ADCp, EFU, RGR, FCR, PER, and PR of Sangkuriang catfish fingerling. However, there was no significant effect ($P > 0.05$) on the SR of Sangkuriang catfish fingerling. The supplementation of 1% lysine/kg feed was the optimal dose to improve the feed efficiency and growth of Sangkuriang catfish fingerlings by 83.79% and 3.94%/day,

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respectively. Therefore, the supplementation of lysine could increase Sangkuriang catfish production.

Keywords: Catfish, efficiency, feed, growth, lysine

INTRODUCTION

Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) is a common cultivated freshwater fish in Indonesia with rapid growth, a high nutritional value, and the ability to adapt to the environment (Rachmawati et al., 2019). Feed is the most expensive component of intensive Sangkuriang catfish cultivation, accounting for up to 70% of total production costs per cycle (Rawles et al., 2011). The success of intensive Sangkuriang catfish cultivation depends on the feed quality. A high-quality feed contains an essential amino acid (EAA) profile related to the fish's needs. If the fish feed contains the appropriate amount of EAA, then the required protein for the fish will be fulfilled without a shortage or excess of amino acids (Miles & Chapman, 2008). Each fish species has a different requirement for protein and essential amino acids; thus, the protein content and number of essential amino acids in the feed will only provide effective results when they are within the range required by the fish (National Research Council [NRC], 2011). Amino acids could be used to maintain health and synthesize a new protein structure to improve fish feed efficiency and growth.

Conversely, lacking amino acids decreases feed efficiency and fish growth (L. Nguyen & Davis, 2016). The new protein structure is a protein that has an essential amino acid profile according to the needs of fish. Feeds with protein that has an essential amino acid profile according to fish requirements can increase protein digestibility, thereby increasing fish feed efficiency and growth. Conversely, if essential amino acids are deficient, protein digestibility, feed efficiency, and fish growth will be decreased (Ebeneezara et al., 2019).

The low effectiveness of feed consumption and the high feed cost are issues confronting Sangkuriang catfish cultivation. This problem is mainly caused by the lack of lysine content in the feed. The price of fish meals is increasing due to higher aquaculture production and the limited availability of fish meals (K. A. T. Nguyen et al., 2021). Plant-based protein sources are increasingly being used as an alternative source of protein in fish feed (Elesho et al., 2021). If the amount of lysine in the feed does not meet the needs of the fish, it causes feed inefficiency (Ebeneezara et al., 2019). The lysine requirement in fish differs depending on the fish species, and in the same species, the lysine requirement also varies depending on the growth stage of the fish (NRC, 2011). For example, el-Husseiny et al. (2017) reported that the lysine requirement of African catfish (*Clarias gariepinus*) was 1% of a dry diet. If the amount of lysine in the feed according to the needs of the fish can increase the digestibility of the ileum (intestines) so that nutrients can be absorbed quickly, the growth rate is high, and feed efficiency increases (NRC, 2011). As the main feed component, the vegetable protein lacked amino acid lysine (Ebeneezara et al., 2019). Among all essential amino acids, lysine is a limited essential amino acid in most plant-based ingredients used for commercial fish feed (F. Zhou et al., 2010). NRC (2011) stated that lysine is an essential amino generally found in low amounts in the fish feed containing vegetable protein ingredients. One of the strategies to overcome the problems is the supplementation of lysine amino acid in the artificial feed of Sangkuriang catfish.

Lysine plays a role in protein deposition in the fish body, maintaining healthy blood vessels, producing antibodies, absorbing calcium, and repairing tissue damage (Robinson et al., 2007). In addition, lysine activates digestive enzymes, such as intestinal trypsin, to increase protein digestibility for growth (Zhang et al., 2021). Digested protein will then be stored in fish muscles through muscle hyperplasia or muscle size increase. Therefore, lysine is one of the factors that may regulate the hyperplastic process in fish skeletal muscle and thus become very important for optimizing fish muscle growth (Zhao et al., 2020). The addition of the lysine amino acid in the feed will quickly be used for metabolic processes compared to the addition of other amino acids (Farhat & Khan, 2013). Several studies have reported the requirement for the lysine amino acid for some species of fish, for example, in *Cirrhinus mrigala* (Ahmed & Khan, 2004), *Plectropomus leopardus* (Giri et al., 2009), *Pseudosciaena crocea* (Xie et al., 2012), *Heteropneustes fossilis* (Farhat & Khan, 2013), and *Trachinotus blochii* (Ebeneezara et al., 2019). However, until now, information on the requirement of lysine amino acids in Sangkuriang catfish is limited. The optimum lysine amino acid content in feed needs to be determined to develop an efficient fish feed for the growth of Sangkuriang catfish. Therefore, the present study aimed to investigate the effect of lysine supplementation in feed on protein digestibility, the efficiency of feed, and the growth performances of Sangkuriang catfish to improve the quality and increase Sangkuriang catfish production.

MATERIALS AND METHODS

Fish Preparation and Experimental Condition

Sangkuriang catfish fingerlings with an average weight of 7.54 ± 0.13 g/individual were obtained from a local commercial hatchery, Tambaksari Village, Rowosari District, Kendal District, Central Java, Indonesia. Then, the fish were acclimatized for 1 week before the experiment. During the acclimatization process, the fish were fed with feed that did not contain lysine by using the *ad satiation* method (until fish are satiety) three times a day, approximately at 8 a.m., 1 p.m., and 6 p.m. Before the experiment, fish were fasted to remove the remaining metabolism waste. Sangkuriang catfish used in the study were physically healthy, seen from the active swim, and had complete and normal body organs (Rachmawati et al., 2017). This research was conducted for 56 days.

Research Design and Treatment

A total of 270 Sangkuriang catfish fingerlings were divided randomly into six groups with three replications. Sangkuriang catfish fingerlings are maintained with a stocking density of 1 fish/L in a fiber tank (water capacity of 50 L) containing 15 liters of water, which is equipped with an aeration system where the temperature ranges between 26 °C - 29 °C, pH 7.0 - 8.5 and dissolved oxygen above 4 mg/L. A siphon was done to dispose of leftover fish feed and collect fish feces for digestibility of protein analysis to maintain the quality of the water. Siphoning was conducted before feeding the test at 8 a.m. and after feeding at 6 p.m. The fish per fiber tank biomass was weighed and calculated weekly for 8 weeks to determine weight gain and survival rate. The experiment was designed using a complete randomized design (CRD) with 6 different lysine content in a feed with three replications. The weight gain (WG), apparent digestibility of crude protein (ADCp), relative growth rate (RGR), efficiency of feed utilization (EFU), food conversion ratio (FCR), protein efficiency ratio (PER), retention of protein (PR), and survival rate (SR) were calculated based on following formulas:

$$WG\ (g) = Final\ body\ weight\ (g) - Initial\ body\ weight\ (g) \tag{1}$$

$$ADCp\ (\%) = 100 - \left\{\frac{100 \times Cr_2O_3\ in\ the\ fish\ feed}{\%\ Cr_2O_3\ in\ the\ feces} \times \frac{\%\ protein\ in\ the\ feces}{\%\ protein\ in\ diet}\right\}$$

$$\left\{\frac{100 \times Cr_2O_3\ in\ the\ fish\ feed}{\%\ Cr_2O_3\ in\ the\ feces} \times \frac{\%\ protein\ in\ the\ feces}{\%\ protein\ in\ the\ diet}\right\} \tag{2}$$

$$EFU\ (\%) = \frac{Final\ weight - Initial\ weight}{Weight\ of\ diet\ consumed} \times 100 \tag{3}$$

$$RGR\ (\%) = 100 \times \frac{(Final\ weight - Initial\ weight)}{(Times\ of\ experiment \times Initial\ weight)} \tag{4}$$

$$FCR = \frac{Feed\ intake\ (g)}{Body\ weight\ gain\ (g)} \tag{5}$$

$$PER = 100 \times \frac{(Final\ weight - Initial\ weight)}{The\ amount\ of\ diet\ consumed \times Protein\ content\ of\ diet} \tag{6}$$

$$PR = 100 \times \left(\frac{The\ total\ protein\ in\ fish\ body\ gain\ (g)}{The\ total\ protein\ consumed\ (g)}\right) \tag{7}$$

$$SR\ (\%) = 100 \times \left(\frac{Final\ count}{Initial\ count}\right) \tag{8}$$

Diet Preparation

The experimental diets with the same protein, energy, and amino acid content and different amounts of lysine amino acid were used. There were six lysine levels supplemented in the experimental diet: 0%, 0.5%, 1%, 1.5%, 2%, and 2.5% dry diet. Meanwhile, based on the percentage of lysine in the protein content of the feed experiment, the dose of lysine in the feed as treatment was equivalent to 3.72%, 5.13%, 6.52%, 7.94%, 9.39%, and 10.78% of feed protein (Table 1). The lysine used in this study was in the form of a brown powder with the brand name L-lysine hydrochloric acid (HCl) produced by Limited Liability Company Cheil Jedang (Indonesia). This experiment's range of the lysine level covers the lowest until the highest lysine content in Sangkuriang catfish fingerlings protein. Lysine is an essential amino acid for fish growth and physiological function (Deng et al., 2010). Among the ten essential amino acids, lysine is the first limiting amino acid in ingredients used in fish feed (Forster & Ogata, 1998). Feed was formed into pellets with a diameter of 4.2 mm, dried using an oven at 70 ºC for 3 h, and then stored in a refrigerator at 4 °C before being used. The experiment diet contained 34.1% – 34.8% protein and 4.1 - 4.3 kcal/g of energy. The formulation of the experimental diet (g/100 g feed) is shown in Table 1. The amino acid composition of the feed experiment and the amino acid composition of the Sangkuriang catfish fingerling are provided in Table 2.

Table 1
Formulation of the feed experiment (g/100 g feed)

Ingredients	Feed experiment					
	1	2	3	4	5	6
Fish meal	24.50	24.50	24.50	24.50	24.50	24.50
Casein	10.00	10.00	10.00	10.00	10.00	10.00
Dextrin	25.27	25.27	25.27	25.27	25.27	25.27
Squid oil	7.50	7.50	7.50	7.50	7.50	7.50
Vitamin mix[1]	1.31	1.31	1.31	1.31	1.31	1.31
Mineral mix[2]	3.00	3.00	3.00	3.00	3.00	3.00
Lecithin	1.50	1.50	1.50	1.50	1.50	1.50
Astaxanthin	0.10	0.10	0.10	0.10	0.10	0.10
Carboxymethyl cellulose (CMC)	2.50	2.50	2.50	2.50	2.50	2.50
Glutamic acid*	2.50	2.00	1.50	1.00	0.50	0.00
L-lysine	0.00	0.50	1.00	1.50	2.00	2.50
Amino acid mix[3]	21.32	21.32	21.32	21.32	21.32	21.32
Chromium oxide (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5	0.5
Total	100.00	100.00	100.00	100.00	100.00	100.00
Total lysine: % of diet**	1.25	1.75	2.25	2.75	3.25	3.75
% of protein***	3.59	5.06	6.52	8.06	9.39	10.84

Table 1 *(continue)*

Ingredients	Feed experiment					
	1	2	3	4	5	6
Proteins (%)	34.8 ± 0.12	34.6 ± 0.15	34.5 ± 0.13	34.1 ± 0.10	34.6 ± 0.11	34.6 ± 0.13
Lipid (%)	9.4 ± 0.23	9.8 ± 0.22	10.5 ± 0.26	10.7 ± 0.20	10.7 ± 0.24	10.5 ± 0.21
Ash (%)	7.8 ± 0.30	7.8 ± 0.35	7.9 ± 0.32	7.8 ± 0.31	7.7 ± 0.33	7.8 ± 0.31
Crude fiber (%)	4.7 ± 0.09	2.5 ± 0.10	4.1 ± 0.13	2.5 ± 0.12	3.9 ± 0.11	2.0 ± 0.09
Nitrogen-free extractives (NFE)	33.0 ± 0.10	34.7 ± 0.14	32.3 ± 0.12	33.8 ± 0.13	32.7 ± 0.12	34.4 ± 0.12
Energy (kcal/g fish meal)[4]	4.1 ± 0.11	4.3 ± 0.12	4.2 ± 0.10	4.3 ± 0.11	4.3 ± 0.14	4.3 ± 0.11

[1]Vitamin mix (mg/100 g diet): choline chloride 900.0 mg/100 g diet; riboflavin 5.0 mg/100 g diet; biotin 0.6 mg/100 g diet; cyanocobalamin 0.01 mg/100 g diet; folic acid 1.5 mg/100 g diet; inositol 200 mg/100 g diet; niacin 2.0 mg/100 g diet; p-aminobenzoic acid 5.0 mg/100 g diet; menadion 4.0 mg/100 g diet; pyridoxin-HCl 4.0 mg/100 g diet; Ca-panthothenate 10.0 mg/100 g diet; calciferol 1.9 mg/100 g diet; α-tocopherol 2.0 mg/100 g diet; vitamin C- sty 120.0 mg/100 g diet; thiamin-HCl 5.0 mg/100 g diet; β-carotene 15.0 mg/100 g diet;
[2]Mineral mix (mg/100 g diet): potassium iodide (PI) 0.15 mg/100 g diet; iron(II) chloride tetrahydrate ($FeCl_{3,}$ $4H_2O$) 166 mg/100 g diet; calcium carbonate ($CaCO_3$) 282 mg/100 g diet; zinc sulfate ($ZnSO_4$) 9.99 mg/100 g diet; manganese sulfate ($MnSO_4$) 6.3 mg/100 g diet; copper sulfate ($CuSO_4$) 2 mg/100 g diet; cobalt sulfate heptahydrate ($COSO_4$ $.7H_2O$) 0.05 mg/100 g diet; calcium dihydrogen phosphate [$Ca(H_2PO_4)$] 618 mg/100 g diet; magnesium sulfate ($MgSO_4$) 240 mg/100 g diet; potassium dihydrogen phosphate (KH_2PO_4) 412 mg/100 g diet;
[3]Refer to Table 2
[4] Total energy based on: protein = 4 kcal/g, lipid = 9 kcal/g, and carbohydrates = 4 kcal/g (NRC, 2011)
*The difference in the percentage of lysine was the treatment used in this study. While the difference in the presentation of glutamic acid is used in the feed formulation to obtain the protein content of the test feed, which is relatively the same
**% of diet: treatment in feed experiment
***% of protein: the total of lysine compared to the protein content of the feed experiment

Table 2
The composition of amino acid in the feed experiment (g/100 g feed)

Amino acids	Feed experiment						Sangkuriang catfish
	1	2	3	4	5	6	
Non-Essential amino acids							
Alanine	8 .4	14.3	10.2	16.1	17.8	6.6	10.0
Aspartic	11 .2	16 .6	8.1	18 .6	21.4	6.3	8.0
Glutamate	11 .7	16.5	11.3	14.2	15.2	7.2	11.0
Glycine	14 .8	15.3	8.5	17.4	17.2	16 .9	8.3
Proline	18.8	16.3	14.6	17.9	18.7	17.5	14.1
serine	19.3	19.1	15.2	18.1	14.5	13.5	15.0
Tyrosine	12.9	14.7	11.4	13.2	13.8	8.7	11.0
Essential amino acids							
Arginine	16.9	15.4	10.8	15.6	12.4	8.4	10.3
histidine	7.6	6.4	4.0	6 .6	6.8	5 .3	3.7
Isoleucine	10.9	9.3	6.9	8.9	9.7	9.1	6.2

Table 2 *(continue)*

Amino acids	Feed experiment						Sangkuriang catfish
	1	2	3	4	5	6	
Leucine	16.7	13.4	9.7	11.8	10.9	11.2	8.4
Lysine	16.3	14.7	12.9	14.6	15.5	16.7	12.3
Methionine	18.2	17.9	16.8	18.2	19.9	19.7	15.0
Phenylalanine	8.4	7.8	4.9	7.3	6.7	3.2	4.6
Threonine	18.9	15.2	13.5	17.4	17.3	16.6	12.0
Valine	10.8	9.6	8.0	8.9	10.4	10.5	7.1
Tryptophan	6.9	6.6	5.8	4.6	4.7	3.9	5.3

Chemical Analysis

The composition of amino acids in the experimental diet was analyzed using a High-Speed Amino Acid Analyzer LA8080 AminoSAAYA (Hitachi High Technologies, Japan). A total of ± 1 mg of the sample was weighed, put in a closed tube, and hydrolyzed with 6 N HCl for 22 h at 110 ºC. After being filtered through 0.2 mm, the sample was ready to be injected into a High-Speed Amino Acid Analyzer LA8080 AminoSAAYA (Hitachi High Technologies, Japan) with an ion exchange resin column measuring 4.6 x 150 mm, temperature 53 ºC. The separation of amino acids was done using a gradient system with sodium citrate buffer solution (Merck KGaA, Germany) at pH 3.3, pH 4.3, and pH 4.9 with a flow rate of 0.225 mL/min. A reagent post column with a ninhydrin solution (Merck KGaA, Germany) at the speed of 0.3 mL/min was used to identify each acidic amino in length wave 570 nm and 440 nm (Ju et al., 2008). The Kjeldahl and Soxhlet methods determined the protein and fat content in the experimental diet and fish (El-Husseiny et al., 2017). The ash and water content of fish feed and body was defined based on Steffens's method (1989). The protein digestibility was analyzed using a spectrophotometer (Millipore, Merck KGaA, Germany) at 350 nm (Steffens, 1989).

Statistical Analysis

Data on growth, feed efficiency, and survival rate of fish were analyzed using the analysis of variance (ANOVA) test and Duncan's multiple range test (DMRT) (Steel et al., 1997). Lysine requirement was determined by the regression method (Ziethoun et al., 1976). All statistical analyses were performed using the SAS 9.4 (SAS Institute Inc.) software for Windows (SAS, 2004).

RESULTS

The initial average weight of fish was 7.54 ± 0.13 g/individual in all treatment groups. Table 3 shows that the lysine content in the feed significantly influenced ($P < 0.05$) WG, FI, ADCP, EFU, RGR, FCR, PER, and PR of Sangkuriang catfish fingerlings. However, the effect of lysine content in the fish feed was not significantly influenced ($P > 0.05$) SR of Sangkuriang catfish, indicated by an SR value of 100% during the study.

Table 3
Data of WG, FI, ADC_P, EFU, RGR, FCR, PER, PR, and SR of Sangkuriang catfish fingerlings feed with different lysine content

Parameters	Feed Experiment					
	1	2	3	4	5	6
Initial body weight (g)	7.67 ± 0.25^{a}	7.54 ± 0.28^{a}	7.54 ± 0.24^{a}	7.54 ± 0.25^{a}	7.54 ± 0.26^{a}	7.41 ± 0.25^{a}
Final body weight (g)	19.64 ± 0.12^{ed}	21.42 ± 0.10^{d}	24.18 ± 0.16^{a}	21.86 ± 0.13^{b}	20.49 ± 0.17^{c}	18.58 ± 0.12^{fed}
WG (g/fish)	11.97 ± 0.34^{e}	13.88 ± 0.32^{c}	16.64 ± 0.38^{a}	14.32 ± 0.39^{b}	12.95 ± 0.37^{d}	11.17 ± 0.35^{fe}
FI (g)	23.89 ± 0.10^{b}	21.75 ± 0.17^{c}	19.86 ± 0.19^{f}	20.29 ± 0.14^{e}	21.63 ± 0.18^{dc}	24.32 ± 0.16^{a}
ADCp (%)	60.26 ± 0.43^{e}	75.64 ± 0.46^{b}	83.47 ± 0.44^{a}	70.22 ± 0.42^{c}	65.49 ± 0.49^{d}	55.34 ± 0.41^{f}
EFU (%)	50.10 ± 0.37^{e}	70.50 ± 0.30^{b}	83.79 ± 0.35^{a}	64.17 ± 0.31^{c}	59.54 ± 0.35^{d}	45.93 ± 0.39^{f}
RGR (%/day)	2.79 ± 0.29^{e}	3.39 ± 0.25^{b}	3.94 ± 0.23^{a}	3.29 ± 0.27^{cb}	3.07 ± 0.22^{d}	2.69 ± 0.23^{fe}
FCR	1.97 ± 0.06^{e}	1.42 ± 0.03^{c}	1.19 ± 0.07^{a}	1.32 ± 0.04^{b}	1.56 ± 0.09^{d}	2.18 ± 0.03^{f}
PER	1.44 ± 0.52^{e}	1.72 ± 0.57^{d}	2.43 ± 0.51^{a}	2.08 ± 0.56^{b}	1.85 ± 0.51^{c}	1.33 ± 0.54^{f}
PR	47.87 ± 0.63^{d}	52.11 ± 0.68^{cb}	57.56 ± 0.62^{a}	52.67 ± 0.67^{b}	45.84 ± 0.61^{e}	41.88 ± 0.65^{f}
SR (%)	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}

Note. WG = Weight gain; FI = Feed intake; ADCp = Apparent digestibility of crude protein; EFU = Efficiency of feed utilization; RGR = Relative growth rate; FCR = Food conversion ratio; PER = Protein efficiency ratio; PR = Retention of protein; SR = Survival rate. Mean ± Standard deviation values with different *superscripts* showed significant difference ($P < 0.05$)

The WG, FI, EFU, RGR, FCR, PER, and PR of Sangkuriang catfish fingerlings which were fed with 2.25% of lysine (feed experiment 3), was significantly higher ($P < 0.05$) than in other treatments. On the other hand, fish-fed with 3.75% of lysine (feed experiment 6) had the lowest values ($P < 0.05$) on ADCP, EFU, FCR, and PR.

Proximate analysis of the fish body after receiving feed containing different lysine levels is shown in Table 4. There were significant differences ($P < 0.05$) in the crude protein of the fish body. However, between treatment groups, the fish body's dry matter, crude lipid, and ash levels were not significantly different ($P > 0.05$). Supplementation of 2.75%, 3.25%, and 3.75% of lysine in Sangkuriang catfish fingerlings feed significantly decreased crude protein content.

A regression polynomial analysis was conducted to determine the optimum amount of lysine in the Sangkuriang catfish fingerling feed. Based on the efficiency of feed utilization percentage, the optimum lysine in the feed was 2.37% of the dry diet (Figure 1) or 6.89% of feed protein. Sangkuriang catfish fingerlings fed with

Table 4
Body chemical composition (g/kg) of Sangkuriang catfish

Composition	Feed experiment					
	1	2	3	4	5	6
Dry matter	28.36[a]	28.61[a]	29.13[a]	28.55[a]	28.78[a]	29.43[a]
Crude protein	16.66[d]	18.03[b]	19.86[a]	17.96[c]	15.86[ed]	14.49[fde]
Crude lipids	12.32[a]	13.08[a]	12.87[a]	12.98[a]	13.42[a]	12.35[a]
Ash	19.10[a]	18.86[a]	19.27[a]	19.09[a]	18.79[a]	18.72[a]

Note. Values within columns with the same letter are not significantly different ($P > 0.05$)

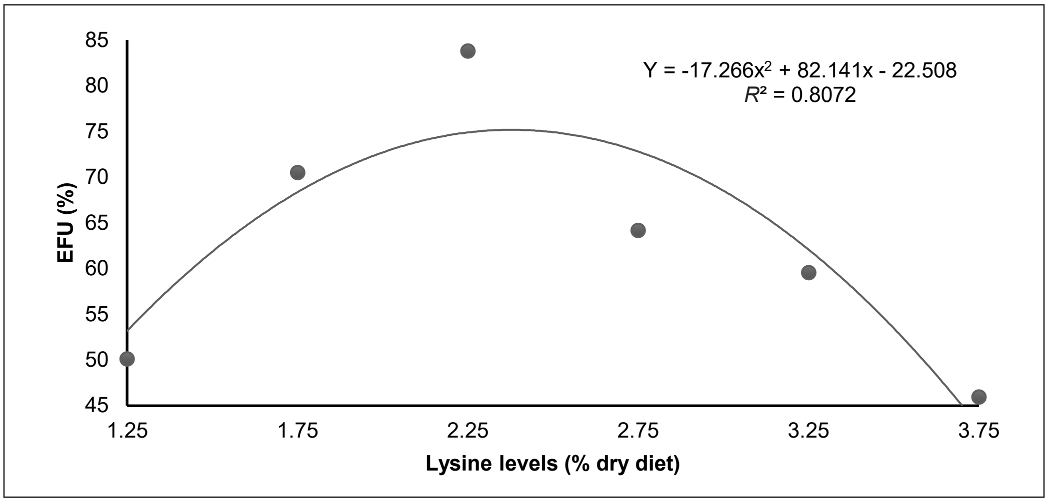

Figure 1. The correlation between lysine content in feed with the percentage of Sangkuriang catfish fingerling efficiency of feed utilization

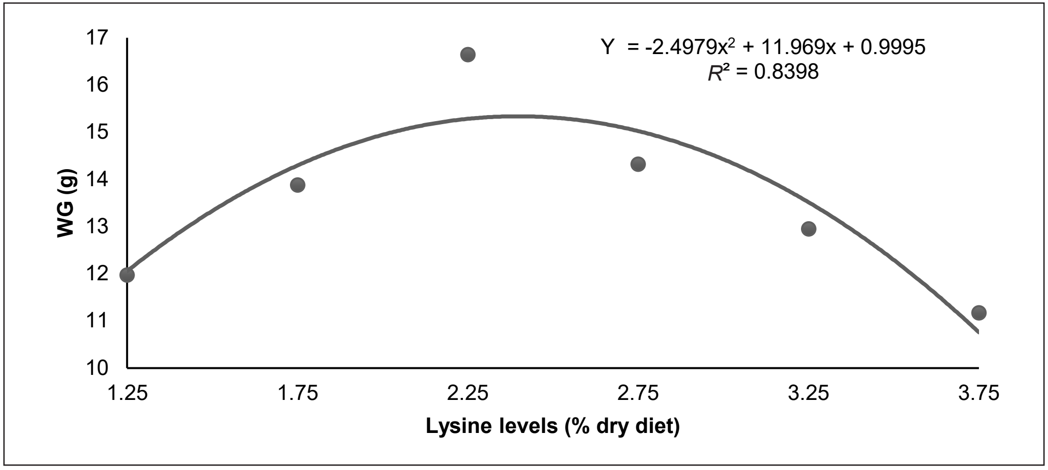

Figure 2. The correlation between the lysine content in feed with the percentage of Sangkuriang catfish fingerling weight increase

lysine addition of 2.75%, 3.25%, and 3.75% dry feed showed a decrease in crude protein content. Based on the weight of Sangkuriang catfish fingerling, the optimum lysine in the feed was 2.39% of the dry diet (Figure 2) or 6.93% of feed protein.

DISCUSSION

The success in intensive fish cultivation highly depends on the quality of feed. A good quality fish feed contains protein according to the need of fish and contains a complete essential amino acid (EAA) profile (El-Husseiny et al., 2017). On the other hand, incomplete EAA profiles in fish diets have been linked to protein synthesis inhibition, leading to reduced growth (Hansen et al., 2007). Therefore, one of the efforts to complete EAA is to formulate fish feed with a balanced essential amino acid that meets the requirements of the fish (Khan & Abidi, 2011).

The response of fish growth to the number of amino acids in fish feed is the most widely used and accurate method for measuring the need for essential amino acids in fish (Bureau & Encarnacao, 2006). The need for amino acids can be statistically measured using polynomial regression, "broken-line" regression analysis, or the math-specific model depending on the fish growth response pattern (Dairiki et al., 2007). However, due to many factors, such as differences in the formulation of fish feed, size and age of the fish, genetics, feed management, and cultivation condition, the lysine need varies among fish species, even within the same species (Bureau & Encarnacao, 2006). According to Santiago and Lovell (1988), the percentage of feed protein determines the amino acid requirements of fish.

The requirement of lysine in the percentage of feed protein has been reported for some economically important fish, such as *Seriola quinqueradiata* (4.13%) (Ruchimat et al., 1997), *Chanos chanos* (4%) (Borlongan & Coloso, 1993), *Micropterus* *salmoides* (4.9%) (Dairiki et al., 2007), *Cirrhinus mrigala* (5.75%) (Ahmed & Khan, 2004), and *Trachinotus blochii* (5.71–5.83%) (Ebeneezara et al., 2019). The levels of lysine required by the fingerling catfish Sangkuriang in our study are higher than those needed for the fish reported in the previous references. The value of necessary lysine in Sangkuriang catfish fingerlings obtained in this experiment was based on the percentage of feed protein which was relatively higher than the lysine requirement in the fish mentioned in the reference above. The best treatment was obtained by adding lysine to 2.25% of the dry diet, equivalent to 6.52% of protein. Variations in amino acid requirements can also be affected by the method of determination, environmental factors, and experimental design (Moon & Gatlin, 1991).

This study revealed that increasing lysine supplementation to 2.25% in the fish diet could enhance feed efficiency (EFU, FCR, PER, PR) and fish growth (WG and RGR). The Sangkuriang catfish fingerlings were shown to utilize the pure lysine added to the feed, indicating that the acid amino lysine is required for Sangkuriang catfish growth. The highest WG, ADCP, EFU, RGR, FCR, PER, and PR were found in Sangkuriang catfish fingerlings fed a diet containing 2.25% lysine. Fish will use a small amount of amino acid for energy and a larger amount for protein synthesis and metabolism, resulting in high fish feed efficiency and growth. Previous references were also similarly found on Japanese sea bass (Mai et al., 2006) and *Ictalurus punctatus* (L. Nguyen & Davis, 2016).

Our results showed that the addition of lysine above 2.25% stunted growth. Lower growth due to excess lysine in the diet has also been documented in several species (Bicudo et al., 2009; Mai et al., 2006; Q.-C. Zhou et al., 2007), but the mechanism is unclear. Furthermore, Ahmed and Khan (2004) suggested that decreased growth in fish fed high lysine was due to the antagonism effect of lysine to arginine, which disrupts the process of protein synthesis. Further research is needed to understand better the mechanism of interaction between lysine and other amino acids in fish. The addition of lysine in the feed above 2.25% showed a decrease in feed efficiency parameters (EFU, FCR, PER, PR). Lower fish growth due to excess lysine in feed has also been documented in several species (Bicudo et al., 2009; Mai et al., 2006; Q.-C. Zhou et al., 2007). The decrease in protein content fed with the addition of lysine above 2.25% can be caused by the excess amount of lysine used as an energy source. Suppose the amount of essential amino acids is excessive or there is a lack of non-protein energy sources (carbohydrates and protein). In that case, the body will use amino acids as an energy source (Lehninger & Nelson, 1993). Essential amino acids cannot be stored by the body and cannot be synthesized by fish (Ebeneezara et al., 2019). The effectiveness of pure amino acid use by fish was also reported to depend on the protein concentration of feed, with feed efficiency being low in high protein content and *vice versa* (Williams et al., 2001). It is also associated with the absorption of free amino acids much faster than protein amino acids (Ahmed & Khan, 2004; Bicudo et al., 2009; Ebeneezara et al., 2019; F. Zhou et al., 2010; Xie et al., 2012).

The influence of the lysine content in feed on the fish composition proximate is provided in Table 4. The addition of lysine in the feed significantly affected ($P < 0.05$) the protein body of Sangkuriang catfish fingerlings but was not significant ($P > 0.05$) with the substance of the dried fish fat and ash. The highest crude protein content of Sangkuriang catfish showed in the feed containing a 2.25% lysine compared to other feed treatments. It is possible because the 2.25% lysine level in the feed has an EAA profile similar to the EAA profile of Sangkuriang catfish fingerling. Therefore, feed containing 2.25% lysine is the most suitable feed for Sangkuriang catfish fingerling. Akiyama et al. (1991) stated that the most appropriate feed had an amino acid profile similar to fish. In this way, fish will use very few amino acids for energy and more for body protein synthesis and metabolic functions resulting in high efficiency and growth of fish (Ebeneezara et al., 2019).

CONCLUSION

The different lysine content in the fish feed significantly affects feed efficiency and growth of Sangkuriang catfish fingerling. Therefore, the supplementation of 1% lysine/kg feed was the optimal dose to improve the feed efficiency and growth of Sangkuriang catfish fingerlings by 83.79% and 3.94%/day, respectively. Therefore, the supplementation of lysine could increase Sangkuriang catfish production.

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