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Effect of Lysine and Poultry Slaughterhouse by Product Meal on Growth Performance, Feed Efficiency, and Blood Profile of Sangkuriang Catfish (*Clarias gariepinus* var. Sangkuriang)

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ABSTRACT

The increasing demand for livestock and poultry feeds results in the lack of fish meals (FM). Poultry slaughterhouse by-product (PSB) is one promising strategy due to its high protein content despite the limited content of lysine. Thus, supplementing lysine in dietary fish feed is necessary. The present study aimed to investigate how different lysine doses in feed with PSB and FM as animal protein sources affected protein digestibility, feed utilization, growth, hematology, and body composition of Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang). Sangkuriang catfish at the grow-out stage (15.54±0.17 g/fish) were used. The fish were fed six experimental diets with similar protein and energy content but different lysine levels at 1.25%, 1.75%, 2.25%, 2.75%, 3.25%, and 3.75%/kg (treatments 1 to 6). The addition of lysine to feed had a significant (P<0.05) effect on protein digestibility (ADCp), efficiency of feed utilization (EFU), and relative growth rate (RGR) of Sangkuriang catfish at a grow-out stage but had no significant (P>0.05) effect on survival rate, hematology, and nutrient content. The optimal doses of dietary lysine with PSB and FM to improve ADCp, EFU, and RGR of Sangkuriang catfish were 2.59%, 2.63%, and 2.62%/kg diet, respectively. However, the supplementation of PSB in experimental diets had no significant effect on glucose, triglyceride, total protein, urea, calcium, magnesium, albumin, globulin, hemoglobin, hematocrit, phosphorous, and mean corpuscular hemoglobin concentration (MCHC). The lysine addition in feed

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formulated with PSB and FM could improve the growth performance and increase the feed digestibility of Sangkuriang catfish at the grow-out stage.

Keywords: Feed, food efficiency, growth, lysine, nutrient

INTRODUCTION

Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) is a popular freshwater fish species widely cultured in Indonesia. The fish has distinct characteristics, including fast growth, adapting quickly to the environment, delicacy, and high nutrient content (Rachmawati et al., 2019). Feed cost is the most important variable in intensive culture, accounting for 80% of the total production costs of each culture cycle (Rawles et al., 2011). It is because protein is the costliest fish feed component compared to other nutritional components. It is a source of essential amino acids (EAA) for body tissue repair and fish growth (Khan & Abidi, 2011).

Fish meal is widely regarded as the finest animal protein source for feed components due to its stable amino acid profile (National Research Council, 2011). However, due to the rising demand for livestock and poultry feed, fish meals are scarce for fish feed (Suloma et al., 2014). Therefore, alternative protein sources must be found to substitute fish meals in the fish diet. Srour et al. (2016) identified poultry slaughterhouse by-products (PSB) as an alternative protein source due to their high protein content (60%–65%) and lower cost when contrasted to fish meal (Hernández et al., 2014). Poultry slaughterhouse by-product (PSB) differs from poultry by-product meal (PBM) since it still contains ether extract (EE), ash, and non-digestible part (feathers), which reduces degradability (Yones & Metwalli, 2015).

According to Khan & Abidi (2011), most poultry slaughterhouse by-products (PSB) have low lysine content. Thus, adding lysine to dietary feed based on fish requirements becomes one solution for improving fish growth performance (Khan & Abidi, 2011). Lysine is a vital nutrient for fish growth and normal physiological function. Lysine and methionine are involved in synthesizing carnitine, which is used in fatty acid transport activities to produce energy via oxidation (Nguyen & Davis, 2016). Lysine is frequently the first amino acid limiter in fish feed ingredients among the ten essential amino acids (Farhat & Khan, 2013). In fish, an insufficiency of lysine causes slow growth and poor protein utilization. In addition, several fish species have reported appetite loss and increased lipid accumulation (Mai et al., 2006). It has also been reported that excessive lysine and a lack of dietary feed caused lower growth in several fish species (Bicudo et al., 2009).

Although biochemical evaluation of blood and plasma could help assess fish health, there is limited evidence on the effect of dietary lysine on fish blood (Zhou et al., 2010). Therefore, the present study aimed to investigate how different lysine doses in feed with poultry slaughterhouse by-product (PSB) meal and fish meal (FM) as an animal protein

source affected protein digestibility, feed utilization, growth, hematology, and body composition of Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) at the grow-out stage.

MATERIALS AND METHODS

Preparation of Experimental Fish

Sangkuriang catfish at the grow-out stage were obtained from the Teaching Factory, Faculty of Fisheries and Marine Sciences, University of Diponegoro, Indonesia. Experimental fish were previously acclimatized for one week. During acclimatization, the fish were adapted to an environment with the same water quality as the research environment. The commercial feed has a protein content of 30% and 10% fat. Fish were fed 3 times daily at 7 am, 12 pm, and 4 pm. Fish were fasted one day before the experiment to remove metabolic waste from the fish's body. Experimental fish were chosen based on uniform size, physically healthy, active swimming, and no organ deformation (Rachmawati et al., 2017).

Experimental Design

The current study was experimental research with a completely randomized design (CRD), 6 treatments, and 3 replicates. This study was conducted in the Wet Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Diponegoro, Indonesia. A total of 360 Sangkuriang catfish with an average weight of 15.54±0.17 g/fish at the grow-out stage were used. The fish were then randomly divided into six groups (n=60) with a stocking density of 1 fish/liter (20 fish/fiber tank). The experiment was carried out in a plastic fiber tank (capacity of 70 L) filled with 20 L of water at 25–28°C, pH 7.0–7.5, and dissolved oxygen above 5 mg L^{-1} (Boyd, 2003). Fish were sampled weekly by calculating the total fish biomass in each plastic fiber tank. At satiation, feeding was given 3 times a day for 42 days at 8 am, 1 pm, and 5 pm. During the experiment, siphoning was done to maintain water quality by removing uneaten feed and collecting fish feces for protein digestibility analysis.

Feed Preparation

Sangkuriang catfish at the grow-out stage were treated with 6 experimental diets with similar protein (30%) and energy content (8.30 Kcal $g^{-}1$) and different lysine levels at 1.25%, 1.75%, 2.25%, 2.75%, 3.25%, and 3.75%/kg (treatment 1 to 6). The proximate and amino acid analyses of animal and plant protein sources are demonstrated in Table 1. The experimental diets containing 30% of protein (Rachmawati et al., 2022) were then added with 0.5% chromium (III) oxide (Cr_2O_3) as the indicator of protein digestibility. Experimental diets consisted of fish meal, poultry slaughterhouse by-product (PSB) meal as the animal protein source, and soybean meal as a plant protein source. According

Table 1
Protein and amino acid content in experimental diet (g/100 g)

Composition	Fish meal	Soybean meal	Poultry slaughterhouse by-product
Dry matter	89.18	89.18	89.18
Lipid	7.56	3.90	13.14
Crude protein	68.35	39.82	52.31
Alanine	4.96	1.87	2.89
Glutamic acid	5.93	3.99	5.89
Proline	4.30	3.10	5.29
Tyrosine	2.28	1.70	1.87
Serine	2.76	2.20	3.95
Aspartic acid	3.96	2.75	3.87
Glycine	5.87	2.28	3.87
Cystine	0.7	0.69	3.2
Arginine	4.90	3.2	3.06
Histidine	1.51	1.13	0.64
Isoleucine	3.30	1.97	2.70
Leucine	5.18	3.36	4.29
Lysine	5.30	2.76	1.98
Methionine	1.96	8.58	0.50
Phenylalanine	2.80	2.16	2.79
Threonine	4.06	1.80	2.70
Tryptophan	0.69	0.58	0.47
Valine	3.76	2.10	3.47

to El-Husseiny et al. (2018), PSB is high in protein, approximately 61.5%; hence, it is suitable to replace a fish meal with lysine partially. The composition of PSB that replaced fish meal in experimental diets up to 50% and the doses of lysine used in this study based on El-Husseiny et al. (2018) with slight modification, including 1.25%, 1.75%, 2.25%, 2.75%, 3.25%, and 3.75%/kg diet (treatment 1 to 6). PSB meal is a poultry meal derived from the broiler and layer industry sectors, particularly from dead animals and giblets that were heat-peeled at 112°C for 2 h. PSB was obtained from the Animal Slaughterhouse Semarang, Central Java, Indonesia.

Experimental diets were made by mixing feed ingredients until homogenous. The acidity of the mixed experimental diets was neutralized with 6 N sodium hydroxide (NaOH) (Nose et al., 1974) to eliminate the effect of pH on growth performance and feed efficiency (Wilson et al., 1977). Furthermore, the mixed experimental diets were produced using a 4 mm-sized pellet molding machine to be further dried at room temperature and stored at -4°C until use. Formulation and proximate analysis of experimental diets (g/100 g diet) of Sangkuriang catfish is presented in Table 2. Amino acid profiles and AA requirements of experimental diets according to National Research Council (2011) are shown in Table 3.

Table 2
Formulation, chemical composition, and proximate analysis of experimental diet (g/100 g diet)

Ingredients Composition	1	2	3	4	5	6
Soybean meal	20.0	20.0	20.0	20.0	20.0	20.0
Fish meal	20.0	20.0	20.0	20.0	20.0	20.0
PSB	22.76	22.76	22.76	22.76	22.76	22.76
Corn	20.0	20.0	20.0	20.0	20.0	20.0
Starch	1.14	1.14	1.14	1.14	1.14	1.14
Wheat	8.7	8.2	7.7	7.2	6.7	6.2
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin and Mineral Premix[1)]	1.5	1.5	1.5	1.5	1.5	1.5
Chemical composition (g kg^{-1})						
Carboxymethylcellulose	0.6	0.6	0.6	0.6	0.6	0.6
Vitamin C	0.15	0.15	0.15	0.15	0.15	0.15
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0
Lysine	1.25	1.75	2.25	2.75	3.25	3.75
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Results of Proximate Analysis						
Dry matter	9.26	9.26	9.26	9.26	9.26	9.26
Crude protein	30.40	30.60	30.60	30.56	30.55	30.60
Crude lipid	12.22	12.32	12.43	12.29	12.37	12.28
Crude Ash	8.23	8.27	8.23	8.25	8.27	8.29
Gross energy (Kcal g^{-1})[3)]	8.15	8.23	8.30	8.28	8.27	8.25

[1)]Vitamin mix (mg/100 g diet): riboflavin 5.0; cyanocobalamin 0.01; inositol 200; menadione 4.0; folic acid 1.5; b-carotene 15.0; a-tocopherol 2.0; vitamin C-sty 120.0; Ca-pantothenate 10.0; choline chloride 900.0; thiamin-HCl 5.0; niacin 2.0;pyridoxin-HCl 4.0; calciferol 1.9; biotin 0.6; p-aminobenzoic acid 5.0
[2)]Mineral mix (mg/100 g diet): Calcium carbonate ($CaCO_3$) 282; Iron (II) chloride tetrahydrate ($FeCl_3.4H_2O$) 166; Magnesium sulfate ($MgSO_4$) 240; Manganese sulfate ($MnSO_4$) 6.3; Cobalt (II) Sulfate Heptahydrate ($CoSO_4.7H_2O$) 0.05; Potassium dihydrogen phosphate (KH_2PO_4) 412; Calcium biphosphate [$Ca(H_2PO_4)$] 618; Zinc sulfate ($ZnSO_4$) 9.99; Copper sulfate ($CuSO_4$) 2; Potassium iodide (PI) 0.15
[3)]Total energy based on: protein = 4 kcal/g, lipid = 9 kcal/g, and carbohydrate = 4 kcal/g (NRC, 2011)

Table 3
Amino acid profile of the experimental diet (g/kg experimental diet)

Amino Acids	1	2	3	4	5	6	Catfish *
Essential amino acids							
Arginine	24.62	24.50	24.72	25.94	24.76	24.56	10.3
Tryptophan	3.80	3.82	3.83	3.89	3.83	3.62	5.3
Threonine	18.23	18.24	18.45	19.20	18.12	18.43	12.0
Methionine	18.10	18.76	18.63	18.87	18.25	18.49	15.0
Isoleucine	18.20	18.27	18.30	18.20	18.13	18.26	6.2
Histidine	7.63	7.65	7.49	7.25	7.48	7.39	3.7

Table 3 *(continue)*

Amino Acids	1	2	3	4	5	6	Catfish *
Phenylalanine	18.71	18.46	18.53	18.23	18.79	18.84	4.6
Lysine	19.50	22.31	22.50	28.42	32.53	38.56	12.3
Leucine	28.42	28.57	28.10	28.36	28.61	28.47	8.4
Valine	19.15	19.23	19.67	19.39	19.73	19.59	7.1
Non-essential amino acid							
Alanine	22.54	22.19	21.53	21.26	21.38	21.17	–
Serine	20.36	20.53	21.64	20.23	22.62	20.76	–
Glycine	27.63	26.67	24.73	26.13	24.26	24.74	–
Glutamic	35.78	35.39	35.27	35.72	35.87	35.35	–
Aspartic	24.52	24.20	24.72	24.12	24.89	24.75	–
Proline	27.19	29.53	31.36	27.79	29.58	28.47	–
Tyrosine	12.57	12.89	12.78	12.67	12.77	12.57	–

Note. *NRC (2011)

Chemical Analysis

Feed ingredients, experimental diets, and the whole body of fish and fish fillet were proximately analyzed through the standard model (Horwitz, 1975). Protein content was measured using the Kjeldahl method (BÜCHI, Auto-KjeldahlK-370, Swiss). Kjeldahl factor 6.25 (100/16) was used to convert total nitrogen into total protein content as a dry mass percentage. The total lipid content of fish was extracted with petroleum benzene using the Soxhlet method (Barnstead/Electrothermal, UK). Fiber content was analyzed with a fiber analyzer (VELP® Scientifica, Italia), while ash content analysis was applied to each dried sample in a porcelain crucible using a muffle furnace (Finetech, Shin Saeng Scientific, South Korea) at 600°C for 8 h. Moisture content was determined using moisture analyzer (AM B5 0, AD AM, UK). Protein digestibility was analyzed using a spectrophotometer (Millipore, Merck KGaA, Germany) at 350 nm.

Amino acid content of experimental diets was analyzed using High-Speed Amino Acid Analyzer LA8080 AminoSAAYA (Hitachi High Technologies, Japan). Approximately ± 1 mg sample was weighed, put into a closed tube, and hydrolyzed with 6N hydrogen chloride (HCl) for 22 h at 110ºC. The sample was filtered through a 0.2 mm filter and further injected into High-Speed Amino Acid Analyzer LA8080 AminoSAAYA (Hitachi High Technologies, Japan) with ion-exchange resin columns at size 4.6 × 150 mm at 53ºC. Amino acids were separated by a gradient system using sodium citrate buffer solution of pH 3.3, 4.3, and 4.9 at a flow rate of 0.225 mL minute^{-1}. Post-column ninhydrin reagent at a flow rate of 0.3 mL min^{-1} was used to identify each amino acid at 570 nm and 440 nm, respectively.

Protein Digestibility Analysis

The indirect method of adding Cr_2O_3 0.5% to the diet was employed to measure protein digestibility (Pérez-Jiménez et al., 2014). Before the feces of the fish were collected, the fish was acclimated to the diet containing chromium for 1 week. After the eighth day, the feces were collected for 49 d every morning, noon, and afternoon after the fish was fed. The feces were collected two hours after feeding that its collection used a small plastic hose with the tip attached to the wooden stick to move around easier and put the collected feces in the 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, it was 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 was analyzed using a spectrophotometer (SSA 320N, Denmark) with a wavelength of 350 nm (Pérez-Jiménez et al., 2014).

Parameter Observed

All parameters related to fish growth were measured, including weight gain (WG), relative growth rate (RGR) (National Research Council, 2011), feed efficiency expressed by protein digestibility (ADCp) (Fennuci, 1981), the efficiency of feed utilization (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), and survival rate (SR) (National Research Council, 2011). These parameters were calculated based on Equations 1-8:

$$WG\ (g) = Final\ body\ weight\ (g) - Initial\ body\ weight\ (g) \quad (1)$$

$$ADCp\ (\%) = 100 - \left\{\frac{100 \times Cr_2O_3\ in\ the\ fish\ feed}{\%\ Cr_2O_3\ in\ the\ feces} \times \frac{\%\ protein\ \ in\ the\ feces}{\%\ protein\ \ in\ diet}\right\} \quad (2)$$

$$EFU\ (\%) = \frac{Final\ \ weig\,ht - Initial\ \ weig\,ht}{Weig\,ht\ of\ \ diet\ \ consumed} \times 100 \quad (3)$$

$$RGR\ (\%) = 100 \times \frac{(Final\ \ weig\,ht - Initial\ \ weig\,ht)}{(Times\ \ of\ \ experiment\ \ \times Initial\ \ weig\,ht)} \quad (4)$$

$$FCR = \frac{Feed\ \ intake\ \ (g)}{Body\ \ weig\,ht\ gain\ \ (g)} \quad (5)$$

$$PER = 100 \times \frac{(Final\ \ weig\,ht - Initial\ \ weig\,ht)}{The\ amount\ \ of\ \ diet\ \ consum\ ed \times Protein\ \ content\ \ of\ \ diet} \quad (6)$$

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

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

Hematology and Biochemical Analysis

Hematocrit analysis (Hct%) was measured using micro-centrifugation (Barros et al., 2002). The packed cell volume percentage was determined after blood centrifugation in a standard heparinized micro-hematocrit capillary tube of 3500 g for 10 min at room temperature. The concentration of hemoglobin (Hb; g dl^{-1}) was measured using a spectrophotometer through the cyanmethemoglobin method (Blaxhall & Daisley, 1973). Blood indices, including (MCHC) were calculated using the formula (g dl^{-1})=Hb(g dl^{-1})/ Hct(%) (Dacie & Lewis, 1991). Bioc8hemical parameters were analyzed with automatic analysis tools (Mindray BK-3.00, China) using the commercial clinical kit (Pars Azmoon Kit, USA). Biochemical measurement was conducted for glucose, total protein, albumin, total cholesterol, triglyceride, calcium, magnesium, and inorganic phosphorus, as Kumar et al. (2005) referred to.

Statistical Analysis

Data on growth, feed intake, feed efficiency, nutrient content, and survival rate of fish were analyzed using the homogeneity test and analysis of variance (ANOVA). Duncan's multiple range test was further applied if ANOVA revealed a very significant difference ($P<0.01$) or a significant difference ($P<0.05$). The optimal dose of lysine was determined with polynomial orthogonal (Steel et al., 1996). All statistical analysis used SPSS ver. 19.0 (Chicago, Illinois, USA).

RESULTS

The Efficiency of Feed Utilization and Growth

The initial weight of fish observed for growth and feed efficiency parameter was 15.54±0.17 g/fish. There was no statistically significant difference ($P>0.05$) between experimental diet treatments. Protein digestibility (ADCp), efficiency of feed utilization (EFU), relative growth rate (RGR), food conversion ratio (FCR), protein efficiency ratio (PER), and protein retention of fish given experimental diets increased until a certain dose of lysine (2.75%) (Table 4). However, it later decreased along with the increasing lysine dose of higher than 2.75%. The survival rate of Sangkuriang catfish for all treatments was 100% during the study. Moreover, feed intake during the research did not indicate a significant difference between treatments of experimental diets.

The optimum lysine levels in feed with PSB and FM for Sangkuriang catfish at the grow-out stage were measured using the orthogonal polynomial test. Based on EFU, PER, and RGR data, the optimum lysine levels in the diet of Sangkuriang catfish at the grow-out stage were 2.63% (Figure 1), 2.47% (Figure 2), and 2.62% (Figure 3), respectively.

Table 4
Protein digestibility (ADCp), the efficiency of feed utilization (EFU), relative growth rate (RGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), and survival rate (SR) of Sangkuriang catfish at grow-out stage fed experimental diets contained different lysine level

Parameters	Experimental diets					
	1	2	3	4	5	6
Initial body weight (g)	15.54±0.17	15.58±0.19	15.54±0.15	15.60±0.16	15.50±0.17	15.50±0.18
Final body weight (g)	63.32±0.27^{f}	75.78±0.28^{c}	79.26±0.25^{b}	88.14±0.22^{a}	70.56±0.21^{d}	68.64±0.23^{e}
Weight gain (g/fish)[1]	47.82±0.36^{f}	60.20±0.36	63.72±0.30	72.54±0.34^{a}	55.06±0.37^{d}	53.10±0.31^{e}
Feed intake (g)	276.48±0.23	278.42±0.35	279.75±0.26	274.43±0.27^{a}	276.88±0.32	275.88±0.31
ADCp (%)	58.43±0.21^{f}	65.34±0.27^{d}	69.21±0.20^{b}	76.52±0.25^{a}	67.29±0.20^{c}	61.35±0.23^{e}
EFU (%)	55.34±0.36^{f}	63.29±0.33^{d}	67.46±0.36^{b}	73.82±0.38^{a}	65.31±0.36^{c}	60.37±0.30^{e}
RGR (%/day)	2.03±0.18^{f}	2.36±0.14^{d}	2.94±0.10^{b}	3.48±0.16^{a}	2.73±0.17^{c}	2.25±0.15^{e}
FCR	2.08±0.22^{f}	1.65±0.24^{c}	1.48±0.23^{b}	1.36±0.20^{a}	1.74±0.21^{d}	1.89±0.27^{e}
PER	1.34±0.12^{f}	1.73±0.17^{d}	2.09±0.13^{b}	2.44±0.16^{a}	1.85±0.17^{c}	1.45±0.12^{e}
PR	42.67±0.30^{f}	54.23±0.31^{c}	58.62±0.35^{b}	59.43±0.34^{a}	48.64±0.30^{d}	46.35±0.33^{e}
SR (%)	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Note. Mean values with different superscripts showed a significant difference ($P < 0.05$)

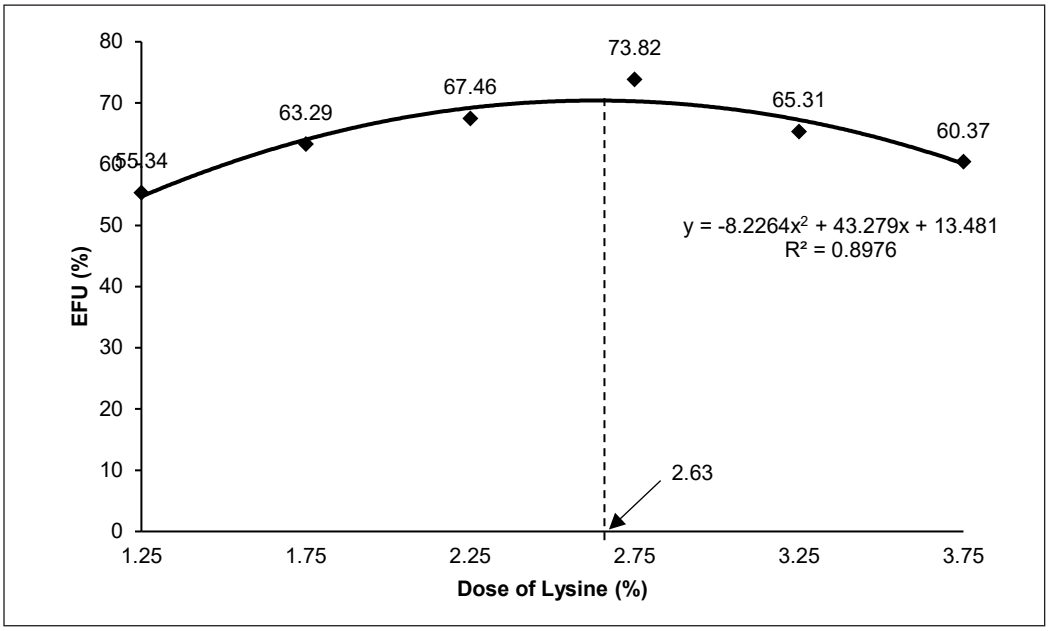

Figure 1. Correlation between dietary lysine level and efficiency of feed utilization (EFU) of Sangkuriang catfish at grow-out stage

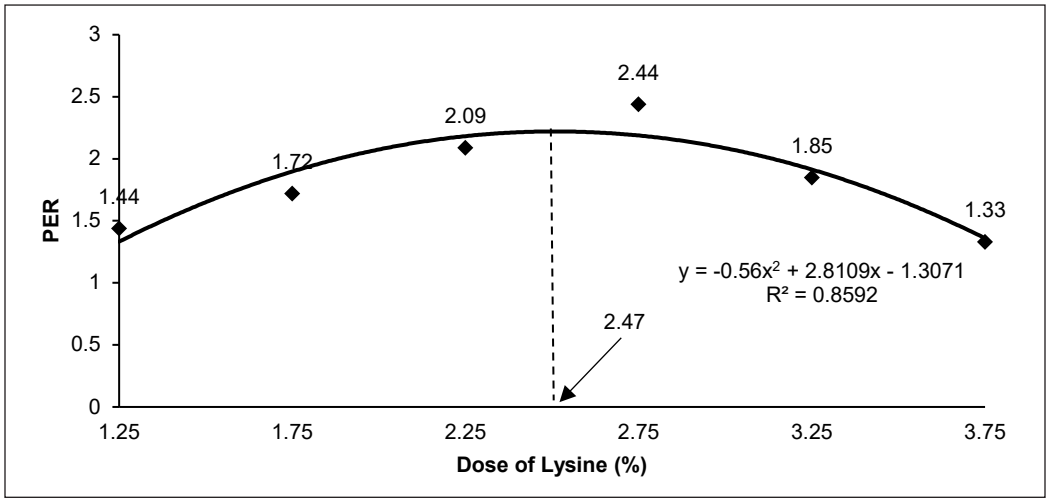

Figure 2. Correlation between dietary lysine level and protein efficiency ratio (PER) of Sangkuriang catfish at the grow-out stage

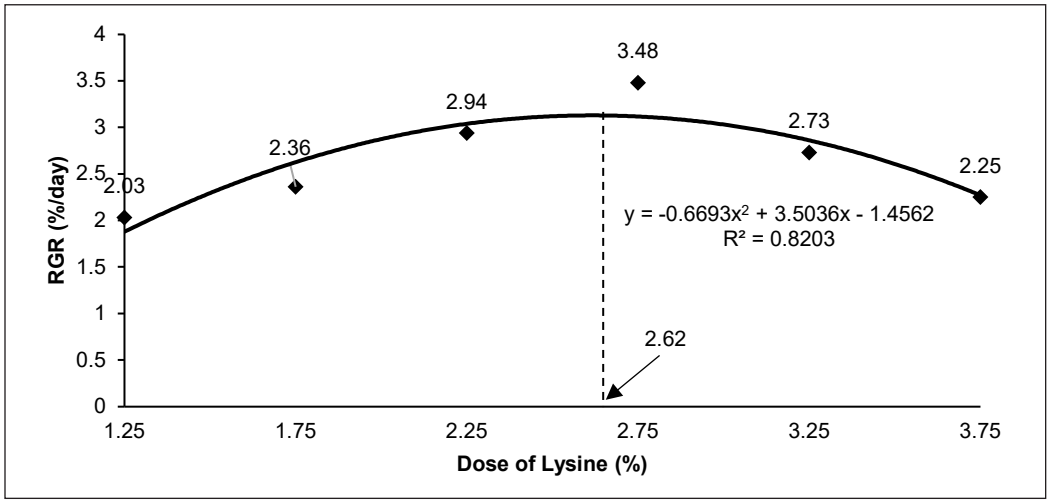

Figure 3. Correlation between dietary lysine level and relative growth rate (RGR) of Sangkuriang catfish at grow-out stage

Proximate Analysis of Experimental Diets

A proximate analysis of the whole body of fish-fed experimental diets is presented in Table 5. The proximate analysis showed an insignificant difference (P<0.05) in moisture, crude protein, crude lipid, and ash of carcass of fish-fed experimental diets.

Hematology and Biochemical Parameter

The use of poultry slaughterhouse by-product (PSB) meal added with different doses of lysine in experimental diets had no significant effect on glucose, triglyceride, total protein,

Table 5
Body chemical composition (g kg^{-1}) of Sangkuriang catfish at grow-out stage fed experimental diets during the study

Composition	Experimental Diets					
	1	2	3	4	5	6
Moisture	751.79±0.2	751.74±0.27	751.78±0.23	751.79±0.28	751.74±0.25	751.78±0.27
Crude protein	153.38±0.20	153.39±0.22	153.39±0.25	153.39±0.24	153.38±0.22	153.39±0.21
Crude lipid	57.54±0.13	57.59±0.10	57.59±0.17	57.54±0.15	57.59±0.13	57.54±0.14
Ash	37.29±0.34	37.28±0.30	37.24±0.35	37.24±0.30	37.29±0.37	37.29±0.33

Note. Mean values with different superscripts showed a significant difference ($P < 0.05$)

Table 6
Results of hematological analysis and biochemical parameters of Sangkuriang catfish at grow-out stage fed experimental diets during the study

Parameters	Experimental Diets					
	1	2	3	4	5	6
Hemoglobin	8.88±0.19	8.47±0.14	8.67±0.71	8.62±0.84	8.34±1.33	8.21±0.63
Hematocrit (%)	43.00±2.16	42.67±2.26	44.67±2.52	43.33±2.31	43.00±2.45	43.33±2.27
MCHC (g dl^{-1})	21.77±1.04	21.87±1.28	21.51±1.23	21.98±1.35	21.01±1.52	21.71±1.14
Total protein	5.67±0.09	5.37±0.12	5.25±0.18	5.41±017	5.41±0.16	5.29±0.14
Albumin	0.53±0.12	0536±0.20	0.58±0.19	0.57±0.17	0.57±0.21	0.57±0.25
Globulin	4.86±0.26	4.61±0.23	4.77±0.24	4.74±0.20	4.34±0.31	4.82±0.30
Glucose	84.67±0.32	85.67±0.37	85.00±0.36	84.67±0.40	85.67±0.39	85.00±0.39
Cholesterol	412.00±3.54^{f}	464.00±3.68^{e}	519.33±3.37^{d}	575.00±3.49^{c}	607.33±3.38^{b}	630.67±3.57^{a}
Triglyceride	252.67±4.35	253.67±4.40	254.33±4.39	258.00±4.2	255.00±4.49	256.33±4.52
Urea	8.63±1.25	8.43±1.15^{a}	8.50±1.32^{a}	8.43±1.04^{a}	8.50±1.13^{a}	8.47±1.41^{a}
Calcium	15.60±0.87	15.50±0.75	15.13±0.54^{a}	15.23±0.65^{a}	15.57±0.73^{a}	15.83±0.69^{a}
Phosphorous	10.87±0.27	10.58±0.29	10.49±0.3	10.33±0.62	10.67±0.34	10.30±0.39
Magnesium	4.80±0.62	4.77±0.63	4.90±0.72	4.50±0.58	4.60±0.63	4.58±0.53

Note. Mean values with different superscripts showed a significant difference ($P < 0.05$)

urea, calcium, magnesium, albumin, globulin, hemoglobin, hematocrit, phosphorous, MCHC concentrations. The fish-fed experimental treatment diets 1 and 6 had the lowest and highest cholesterol levels, respectively (Table 6).

DISCUSSION

The study showed that lysine addition in feed ingredients formulated with PSB and FM as animal protein sources were suitable for Sangkuriang catfish at the grow-out stage. The highest efficiency of feed utilization (EFU) was shown by the fish-fed experimental diet 4 (Table 3). An experimental diet containing lysine levels according to the fish requirement

will support tissue formation, increasing feed efficiency that is further converted into body protein (Marchao et al., 2020). Fish fed with PSB and FM and supplemented with 2.75% lysine (experimental diet 4) possessed a higher EFU than other experimental diets. It occurred since fish fed the treatment diet exhibited the highest protein digestibility (ADCp) compared to other diets. Higher protein digestibility in the diet will result in higher efficiency of feed utilization by fish (National Research Council, 2011). Lysine that is added to dietary feed can increase feed digestibility through the activation of digestive enzymes. Aristasari et al. (2020) stated that lysine addition in the feed would increase ileal (intestine) digestibility. Thus, the nutrient is quickly absorbed, resulting in fish getting full faster, a high growth rate, and improved feed efficiency. Furthermore, Jiang et al. (2015) reported that lysine could increase feed intake, protein digestibility, and growth of grass carp.

Adding 2.75% lysine in feed with PSB and FM (experimental diet 4) was the best treatment, with the highest relative growth rate (RGR) of 3.48±0.16%/day. It was expected that treatment diet 4 exhibited the highest protein efficiency ratio (2.44%) compared to other treatments. Lysine supplementation with a dose according to fish diet requirements could increase feed conversion and fish growth while decreasing lipids in the fish body (Obado et al., 2018). Lysine and methionine are also involved in carnitine synthesis (Walton et al., 1984). L-carnitine increases energy production in mitochondria by oxidizing fatty acids and improves the efficiency of energy utilization from fatty acid oxidation of fish to increase growth rate and reduce tissue fat concentration (Suloma et al., 2014).

The supplementation of lysine at different doses in dietary feed with PSB and FM significantly affected (P<0.05) PER of Sangkuriang catfish. It indicated that lysine added to feed could increase feed quality. Thus, the protein of experimental diets is possibly utilized optimally by fish for growth. Table 3 showed that the experimental diets used were of good quality, as seen from the complete essential amino acid profile. The good quality feed contains protein based on fish requirements and a full essential amino acid profile (EAA) (El-Husseiny et al., 2017). An incomplete EAA profile in feed is associated with inhibited protein synthesis and causes slow growth (Hansen et al., 2007). Therefore, one effort to fulfill EAA requirements in fish feed is possibly made by formulating diets that contain balanced essential amino acids according to fish needs (Khan & Abidi 2011). Lu et al. (2014) mentioned that dietary amino acid levels of feed affected protein efficiency ratio (PER), feed efficiency ratio (FER), and crude protein significantly. Furthermore, Xie et al. (2012) reported that PER would increase along with the increasing lysine level in feed but later will remain constant.

Fish-fed experimental diets added with lysine below 2.75% (1.25, 1.75%, and 2.25%) were observed to have lower growth and were expectedly caused by lysine doses that

did not follow fish requirements to support growth. This result was confirmed by de Vareilles et al. (2012) that feed containing lysine that is not following fish needs will result in imbalanced amino acids and poor protein retention. Thus, dietary protein will be used more for energy formation, inhibiting fish growth. Besides, other study results also showed that experimental diets with lysine doses above 2.75% (3.25% and 3.75%) inhibited fish growth due to increased carnitine production in the body. This finding was confirmed by Putra et al. (2019) that high carnitine would lead to excessive oxidation of long-chain fatty acids, thus resulting in fatty acid deficiency that inhibits body protein synthesis. Fatty acid deficiency will induce inhibited fish growth. Akbary et al. (2011) reported that essential fatty acid deficiency resulted in slow growth of fish body weight gain, increased moisture content of muscle, high level of hepatic lipid, and low feed efficiency. Several long-chain fatty acids are required for the growth process. Katan et al. (2020) found that long-chain polyunsaturated fatty acids (LC-PUFA), namely eicosapentaenoic (EPA;20:5ω3), arachidonic acid (ARA; 20:4ω6), and docosahexaenoic acid (DHA;22:6ω3) play an important role in fish body weight gain and metabolism. Cholesterol significantly increased along with the increasing lysine dose in experimental diets, even though the value approached the normal range, as reported in *Sparidentex hasta*-fed PSB-based diets (Mozanzadeh et al., 2016). An increase in cholesterol was also reported in fish given feed with a higher animal protein source (Kjaer et al., 2008; Mozanzadeh et al., 2015) compared to fish-fed plant-protein-based diets (Yaghoubi et al., 2016). According to Gaylord et al. (2007), the protein source is important in controlling cholesterol levels by either increasing or decreasing metabolic rate, hence inducing bile salt synthesis in the liver. The addition of lysine with PSB and FM in the feed of another fish species is required to confirm the efficacy of the feed formulation.

CONCLUSION

The lysine addition in feed formulated with PSB and FM could improve the growth performance, increase feed digestibility, and have no significant effect on the hematology of Sangkuriang catfish at the grow-out stage. The optimal doses of dietary lysine with PSB and FM as an animal protein source for ADCp, EFU, and RGR parameters of Sangkuriang catfish at the grow-out stage were 2.59%, 2.63%, and 2.62%, respectively.

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