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Effect of dietary lysine level on the growth performance of orange-spotted rabbitfish (*Siganus guttatus)* fingerlings

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Abstract

The study aimed to determine the optimum dietary lysine level of the orange-spotted rabbitfish *Siganus guttatus* Bloch, 1787 (average body weight range of 5.2 to 7.6 g·fish^{-1}). The feeding trial was conducted for 8 weeks employing six experimental diets which were isonitrogenous, isocaloric, and isolipidic (35% crude protein (CP), 18.3 MJ·kg^{-1} of gross energy, and 5% lipid). The lysine content ranged from 1.22 g to 2.12 g lysine ·kg^{-1} dry diet (35 to 70 g·kg^{-1} CP). Results showed that percent weight gain (PWG), and specific growth rate (SGR) peaked at a lysine level of about 55 g·kg^{-1} CP equivalent to 1.93 g·kg dry diet^{-1}). Feed efficiencies i.e., protein efficiency ratio and food conversion ratio values as well as survival were not affected by the dietary treatments. Lysine requirement level for juvenile rabbitfish was estimated using both broken-line and quadratic regression analyses of PWG and SGR against dietary lysine levels to be between 15.9 and 18.3 g·kg^{-1} diet (=45.3 and 52.3 g·kg^{-1} CP).

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Introduction

Orange-spotted rabbitfish *(Siganus guttatus)* is one of the marine fishes with high economic value, a good market, and is a euryhaline species i.e. has wide adaptability to salt concentrations from 1 ppt to seawater. The fish is relatively fast-growing and has a wide food spectrum (Carumbana and Luchavez, 1979; Tacon et al., 1990). In Vietnam, rabbitfish is a fish of particular interest because of its many advantages such as its delicious meat and is popular with consumers (Phu, 2021). In the Philippines, it is a commodity very well suited for aquaculture since it has a high consumer preference and market value (Tabugo et al., 2012).

Several species of rabbitfish species are candidates for culture, namely, *S. argenteus, S. canialiculatus, S. guttatus, S. javus* and *S. rivalutus*. These fish display high tolerance to environmental factors, rough handling and crowding (Carumbana and Luchavez, 1979). The highest growth rate was reported by Horstmann (1975) at 5.0-6.5 g per week for all the rabbitfish species. They are opportunistic omnivores, capable of feeding on amphipods, copepods, sponges, Foraminifera, crustaceans, and brittle stars (Bwathondi, 1982). Under controlled conditions, they can be trained to readily accept artificial feeds, making them feasible to be grown on a commercial scale (Parazo, 1990). While rabbitfish larvae are zooplankton feeders, the fry and adults are primarily herbivorous (Suyehiro, 1942). They nibble on the marine vegetation (Munro, 1967). In captivity, rabbitfish become omnivorous, ingesting algae and a variety of feedstuffs, e.g. fish scraps, mussel and shrimp meat, rice bran, chicken and rabbit pellets (Von Westernhagen, 1974). This makes it easy to feed rabbitfish with artificial dry diets.

For the *S. guttatus* fry, feeds containing 35% protein and 3832 $kcal \cdot kg^{-1}$ were considered economical (Parazo, 1990). However, protein requirements may vary for rabbitfish species e.g., *S. javus*, diets containing 35-46% protein grow best (Basyari and Tanaka, 1989) while in *S. canaliculatus* fry, rapid growth was observed when fed diets containing 58% protein (Basyari and Tanaka, 1989). For the juvenile *S. canaliculatus*, Tacon et al. (1990) have found that the best growth and feed efficiency was observed for rabbitfish fed a diet containing 31% crude protein 8% lipid, and 38% carbohydrate. In supplemental feeding in the grow-out phase, Emata (1991) reported that a supplemental diet containing 26% protein promoted higher growth of rabbitfish in ponds than fish-fed diets containing 21% protein. The variable observations in fry and juvenile rabbitfish could be due to the fact that there are no real protein requirements but rather, there are amino acid requirements.

The protein utilization efficiency of fish is highly dependent on the proportion and ratio of the essential amino acids (EAAs) and non-essential amino acids (NEAAs) content of the feed (Akiyama et al., 1997). However, all the EAAs must be included in the fish feed at an optimum requirement level (NRC, 2011) and deficiency of these EAAs in the diets can result in suboptimal growth because of low nutrient utilization (Andersen et al., 2016).

Lysine is the first limiting essential amino acid in most plant-based feedstuffs incorporated in aquafeed (Mai et al., 2006). Lysine is found in high amount in fish muscle tissue, has an important role in the growth, nitrogen balance, collagen synthesis and carnitine synthesis (Sandell and Daniel, 1988; Michelato et al., 2016; Dias et al., 2001). Lysine deficiency in feed leads to decreased growth, appetite and protein utilization with fin erosion and high mortality in many fish (Khan and Abidi, 2011; NRC, 2011). Garg et al. (2022) note that there is a large difference in dietary lysine requirement for fish in literature and suggested that dietary lysine levels should be optimized.

The present study was conducted to determine the requirement for lysine of the rabbitfish by assessing the effect of various levels of dietary lysine on growth, survival rate, feed conversion ratio and protein use efficiency of *Siganus guttatus* at the fingerling stage.

Lysine requirements have been quantified and reported for other aquaculture species. The need to quantify a dietary lysine requirement for the orange-spotted rabbitfish is essential. Therefore, the objective of the present study was to determine the dietary lysine requirement of this fish species by assessing the effect of various levels of dietary lysine on growth, survival rate, feed conversion ratio and protein use efficiency of *Siganus guttatus* fingerlings.

Materials and Methods

Experimental design

The composition of the experimental diets is shown in **Table 1**. Five isonitrogenous (35% crude protein) and isocaloric (18.3 MJ·kg^{-1}) diets were formulated and supplemented with L-lysine HCL (Sigma Aldrich, St. Louis MO, USA) at an expected increment of 5 g·kg^{-1} crude protein. However, analyzed lysine level were reflected on the label of the dietary treatments (35, 40, 44, 53, 61, and 70 g·kg^{-1} crude protein, CP); each treatment was replicated three times. The added crystal lysine replaced an equal mixture of nonessential amino acids (NEAA) in each experimental diet. The experiment was conducted in a completely randomized design (CRD) which lasted for 8 weeks.

The range of dietary levels of the essential amino acid of interest to be included in the experimental diets must be based on the content of that amino acid in the body of the fish (Yan Q et al., 2007). In the present study, we collected samples of the rabbitfish for analysis and were sent to the National Institute of Livestock, Vietnam for amino acid profiling. Briefly, 40 individuals of *Siganus guttatus* fingerlings with an average weight range of 5.2 to 7.6 g·fish^{-1} were collected, washed, internal organs removed, dried at 100°C, pulverized, and packed for analysis. Results showed that the average lysine content in the rabbitfish was 46.8 g·kg^{-1} CP. Based on this result, we prepared six dietary levels of lysine in which the expected optimum dietary lysine level was situated somewhere in the middle of the range.

Experimental feed preparation

Proximate composition and amino acid content of the ingredients were analyze and formed the basis for the formulation of the diets in terms of gross energy, crude protein, lipid as well as lysine level. A mixture of crystalline amino acids was added to ensure balance with the body's amino acids, except for the lysine levels. The experimental diet was so formulated to contain CP of 35% and gross energy (GE) of 18.3 MJ·kg^{-1}. The diets were a blend of fish meal, casein, gelatin, dextrin, cellulose, carboxymethyl cellulose, fish oil, vegetable oil, vitamin and mineral mix, crystalline lysine.

Table 1 Composition of the five experimental diets containing different levels of lysine fed to the rabbitfish fingerlings for 8 weeks.

Ingredients	Additional lysine levels					
	lysine 35	lysine	lysine	lysine	lysine	lysine
Casein[1]	10.0	10.0	10.0	10.0	10.0	10.0
Fish meal[2]	6.5	6.5	6.5	6.5	6.5	6.5
Dextrin	38.0	38.0	38.0	38.0	38.0	38.0
Gelatin	4.0	4.0	4.0	4.0	4.0	4.0
α-cellulose	6.5	6.5	6.5	6.5	6.5	6.5
EAA mix[3]	10.0	10.0	10.0	10.0	10.0	10.0
NEAA mix[4]	11.0	10.8	10.6	10.5	10.3	10.1
Crystalline lysine	0.0	0.2	0.4	0.5	0.7	0.9
Vitamins/mineral mix[5]	4.0	4.0	4.0	4,0	4.0	4.0
Carboxymethyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Soybean oil	5.0	5.0	5.0	5.0	5.0	5.0

Total	100.0	100.0	100.0	100.0	100.0	100.0
Analyzed CP (%)	35.2	34.9	35.1	34.8	35.2	35.1
Analyzed lysine (%)	1.22	1.40	1.54	1.83	2.14	2.45
Est. GE (MJ kg^{-1})	18.3	18.3	18.3	18.3	18.3	18.3

[1]Casein (g·100g^{-1}): Leucine 9.2, lysine 8.9, valine 6.8, histidine 3.8, isoleucine 5.6, methionine 1.8, threonine 4.4, phenylalanine 5.3, arginine 3.3
[2]Fish meal (g·100g^{-1}): Methionine 1.63, Cystine 0.56, Lysine 5.31, Threonine 2.48, Arginine 3.50 Isoleucine 2.20, Leucine 4.20, Valine 2.29, Histidine 1.71, Tryptophan 0.82
[3]EAA mix (g·100g^{-1}): arginine 1.658, histidine 0.392, isoleucine 2.262, leucine 1.072, lysine variable, methionine 1.086, phenylalanine 1.648, threonine 1.092, tryptophan 0.472, valine 1.770.
[4]NEAA mix (g·100g^{-1}): cystine 0.896, tyrosine 0.980, alanine 1.420, aspartic acid 0.144, proline variable, glycine variable
[5]Vitamin mix: vitamin A, 4.000.000UI; vitamin D3, 800.000UI; vitamin E, 8.500UI; vitamin K3, 750UI; vitamin B1, 375UI; vitamin C, 8.750UI; vitamin B2, 1.600mg; vitamin B6, 750mg; folic acid, 200mg; vitamin B12, 3.000mcg; biotin, 20.000mcg; methionine, 2.500mg; Mn, Zn, Mg, K and Na, 10mg.

Ingredients in relatively larger amounts in the diet such as fishmeal, casein, cellulose, and dextrin were mixed together, and those that were in relatively smaller quantity such as vitamins, minerals, and oil were mixed together. The binder was gelatinized, and mixed with the above mixture to create a bond for the feed when extruding the pellets. The feed was then extruded into a pelletizing machine with a mesh size of 3mm, the resulting pellets were dried at 100°C and refrigerated to 4°C until use.

Table 2 Analysis of 10 essential amino acids composition in the experimental diet (lysine = Lys) and in the body of the rabbitfish (g·100g^{-1} dry diet).

Treatments/	Lys 35	Lys 40	Lys 44	Lys 53	Lys 61	Lys 70	Rabbitfish
Arginine	2.74	2.75	2.78	2.73	2.79	2.80	2.78
Histidine	0.44	0.46	0.45	0.46	0.45	0.48	0.45
Leucine	1.61	1.64	1.61	1.65	1.69	1.65	1.66
Isoleucine	1.41	1.40	1.47	1.46	1.44	1.45	1.44
Lysine	1.22	1.40	1.54	1.83	2.14	2.45	1.56
Methionine	0.61	0.64	0.62	0.61	0.63	0.62	0.62
Phenylalanine	1.49	1.51	1.53	1.51	1.53	1.54	1.53
Threonine	1.22	1.25	1.25	1.27	1.27	1.24	1.26
Valine	1.11	1.14	1.16	1.12	1.12	1.18	1.14
Tryptophan	1.26	1.26	1.29	1.23	1.26	1.28	1.23

* Number of replicates for analyzes n = 3; Rabbitfish has an average crude protein content of 35.6% (% in dry matter).

Experimental fish and facility

Rabbitfish fingerlings (initial average body weight of 5.28g $fish^{-1}$) were procured from the Center for Aquaculture Research, Application and Technology Transfer, University of Agriculture and Forestry, Hue University. Fish were transported to the laboratory and acclimatized in composite tanks for 3 weeks. During acclimation, rabbitfish were fed with commercial feed for 2 weeks. Fish then were weaned into the experimental diet by feeding initially mixed commercial and corresponding experimental feed for 1 week with the test feed being gradually increased in proportion until it became 100% pure formulated test diet and this point was considered the start of the experiment. Following random distribution of fingerling scats to their respective experimental units at 30 fish·$tank^{-1}$, fed with the experimental diet twice daily at 0800 and 1600 h at the rate of 5% body weight. Feeding was closely monitored to ensure that the fish consumed the daily ration completely. Water quality parameters such as temperature, salinity, pH, DO, NH_3 were monitored twice a week. Fifteen to 20 fish in each tank were randomly caught, batch-weighed, and individual length measured.

The experiment was conducted in a recirculating water system consisting of 18 composite tanks with a total volume capacity of 4 m^3 and containing 3.5 m^3 of water.

Additionally, there were also storage tanks, settling tanks, chlorine reduction tanks, aerators, water pumps, circulating water purifiers, electrical systems, fresh water systems.

Calculations

At the end of the experiment, the following performance and feed efficiency indices were evaluated: percent weight gain (PWG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), and protein efficiency ratio (PER) according to the equation below:

PWG (%): $\text{PWG (\%)} = \frac{W_{-2} - W_1}{W_1} \times 100$

$$\text{SGR (\%/d)} = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \times 100$$

Where

W_1: Average weight at start of the experiment, t_1

W_2: Average weight at termination of the experiment, t_2

$t_2 - t_1$: Feeding experiment period

$$\text{Survival rate (SR): SR (\%)} = \frac{\text{Total number of fish at the end of the experiment}}{\text{Total number of fish initially stocked in the experiment}} \times 100$$

$$\text{Feed conversion ratio (FCR): FCR} = \frac{\text{The amount of feed the fish has consumed (kg)}}{\text{The weight of the fish increases (kg)}}$$

$$\text{Protein efficiency ratio (PER): PER} = \frac{\text{Weight gain of fish (g)}}{\text{Amount of protein consumed (g)}}$$

Statistical analysis

Data were analyzed as a completely randomized design and presented as means ± standard deviation (SD, n=3). All data were tested for normality using the Kolmogorov-Smirnov test and homoscedasticity with Levene's test. Subsequently, a one-way analysis of variance (ANOVA) was applied to determine significant differences among dietary treatments, and Tukey's ranking test when significant differences were found ($p<0.05$). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 20). Percent data were transformed into arcsine values prior to analysis. To determine the optimal dietary lysine requirement. The value of PWG and SGR was fitted in the broken-line linear and second-order polynomial regression analysis model to assess the optimum dietary lysine requirement of *S. guttatus* fingerlings.

Results

Water quality

The mean water quality parameters during the duration of the experiment are presented in **Table 3**. No major fluctuations occurred in water temperature (°C), pH, DO ($mg \cdot l^{-1}$), salinity (S‰) and NH_3 ($mg \cdot l^{-1}$) and were within the recommended range for the rabbitfish.

Table 3 Fluctuation of environmental factors during the feeding experiment (lysine = Lys).

Treatments (%) *Factors*	Lys 35	Lys 40	Lys 44	Lys 53	Lys 61	Lys 70
			min : max *Mean ± SEM*			
Temperature (°C),	24.4-28.5 27.3±0.9^{a}	25.22-28.4 27.1± 0.44^{a}	25.2-28.40 27.34±0.62^{a}	25.55-28.4 27.33±0.82^{a}	25.32-28.45 27.24±0.41^{a}	25.24-28.5 27.32±0.63^{a}
DO (mg·l^{-1})	3.55-5.67 4.62±0.78^{a}	3.54-5.65 4.60±0.84^{a}	3.54-5.62 4.67±0.72^{a}	3.56-5.58 4.63±0.83^{a}	3.52-5.65 4.58±0.86^{a}	5.10-5.65 4.65±0.84^{a}
pH	7.31-8.20 7.6±0.33^{a}	7.22-8.40 7.62±0.41^{a}	7.24-8.45 7.6±0.24^{a}	7.22-8.51 7.67±0.22^{a}	7.18-8.42 7.65±0.22^{a}	7.33-8.45 7.67±0.23^{a}
NH_3/NH_4 (mg·l^{-1})	0.01-0.03 0.018±0.004^{a}	0.01-0.03 0.02±0.001^{a}	0.01-0.03 0.018±0.004^{a}	0.01-0.03 0.02±0.003^{a}	0.01-0.03 0.02±0.003^{a}	0.01-0.03 0.02±0.004^{a}
Salinity (‰)	20.0-25.0 21.4±0.64^{a}	20.0-25.0 21.47±0.51^{a}	20.0-25.0 21.50±0.49^{a}	20.0-25.0 21.20±0.47^{a}	20.0-25.0 21.42±0.43^{a}	20.0-25.0 21.30±0.37^{a}

Growth performance and feed utilization efficiency

Table 4 Effect of lysine (Lys) levels on the growth performance of rabbitfish fingerlings (mean ± SEM)

Parameter	Experimental diets					
	Lys 35	Lys 40	Lys 44	Lys 53	Lys 61	Lys 70
IBW (g)	5.35± 0.40$^{\mathbf{a}}$	5.23± 0.51$^{\mathbf{a}}$	5.32± 0.36$^{\mathbf{a}}$	5.25± 0.40$^{\mathbf{a}}$	5.30± 0.44$^{\mathbf{a}}$	5.32± 0.41$^{\mathbf{a}}$
FBW (g)	20.42±0.23$^{\mathbf{c}}$	22.31±0.35$^{\mathbf{b}}$	23.41±0.13$^{\mathbf{a}}$	23.33±0.42$^{\mathbf{a}}$	22.83±0.12$^{\mathbf{ab}}$	22.55±0.35$^{\mathbf{b}}$
PWG (%)	281.3±1.45$^{\mathbf{d}}$	326.3±3.53$^{\mathbf{c}}$	340.0±3.46$^{\mathbf{ab}}$	344.0±6.66$^{\mathbf{a}}$	331.3±3.76$^{\mathbf{bc}}$	324.0±1.16$^{\mathbf{c}}$
SGR (% d^{-1})	2.39±0.01$^{\mathbf{c}}$	2.59±0.02$^{\mathbf{b}}$	2.66±0.00$^{\mathbf{a}}$	2.66±0.03$^{\mathbf{a}}$	2.61±0.02$^{\mathbf{b}}$	2.58±0.01$^{\mathbf{b}}$

^{a,b,c} means with different letter superscript in the same row are significantly different (P< 0.05); SEM: Sample mean standard error. IBW=mean initial body weight; FBW=mean final body weight; PWG=percent weight gain; SGR= specific growth rate.

For the growth trial, mean FBW ranged from 20.42 to 22.83 g, PWG was between 281.3 and 344.0% (**Table 4**), FCR ranged from 2.62 to 2.93 (**Table 5**), and survival ranged from 90.47% to 97.62% (**Table 6**). Results indicated significant differences in mean FBW, PWG, SGR, while there were no significant differences in either PER or FCR. Fish fed the Lys 35 diet performed poorly and significantly differed in the growth parameters from the fish reared on the other experimental diets.

FBW of the fish increased significantly from Lys 35 to Lys 45, peaked at Lys 55, and decreased significantly at Lys 60. There were no significant differences in the PWG values of Lys 40, Lys 45, and Lys 55 groups (P > 0.05), and only the extreme lysine levels of Lys 35 and Lys 60 groups showed significant differences from the rest of the dietary lysine treatments. For the feed utilization efficiency, both PER and FCR did not differ significantly between dietary groups (P>0.05, **Table 5**). Similarly, survival rates were very high (greater than 90%) and were not significantly different among dietary treatments. No signs of stress or other health problems were observed.

Table 5 Effect of dietary lysine levels on feed utilization efficiency (lysine = Lys).

	Experimental diets					
	Lys 35	Lys 40	Lys 44	Lys 53	Lys 61	Lys 70
PER	1.10±0.03[a]	1.21±0.07[a]	1.16±0.12[a]	1.28±0.05[a]	1.26±0.05[a]	1.14±0.03[a]
FCR	3.05±0.09[a]	2.77±0.15[a]	2.93±0.30[a]	2.62±0.10[a]	2.65±0.09[a]	2.93±0.07[a]

[a,b,c] means with different letter superscript in the same row are significantly different (P< 0.05); SEM: standard error of the mean; PER=protein efficiency ratio; FCR=food conversion ratio.

Dietary estimate requirements

Mean FBW, PWG, and SGR were fitted against analyzed dietary lysine levels (% DM) using a quadratic model (QM) to estimate the quantitative lysine requirement. The QM fits a second-degree polynomial for values of x below and above the requirement; 95% of the abscissa of the breaking point (*Ymax*) defines the requirement.

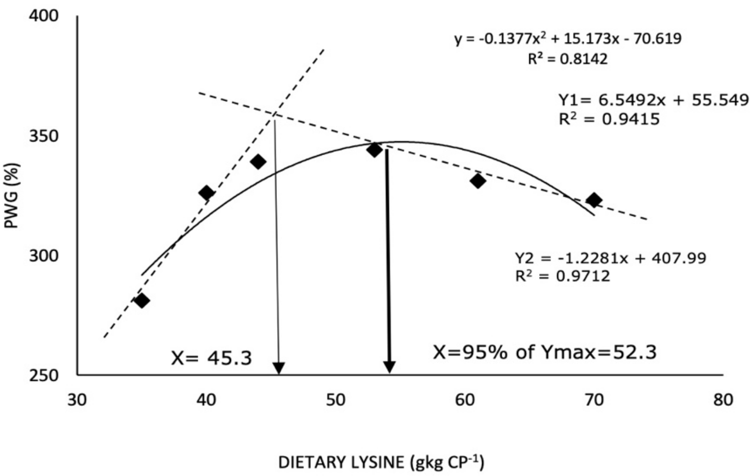

Figure 1 Broken-line linear (dash line) and second-order polynomial (solid line) regression analysis for optimization of dietary lysine requirement in relation to percent weight gain (PWG, %) of *Siganus guttatus* fed with graded dietary lysine levels for a period of 8 weeks.

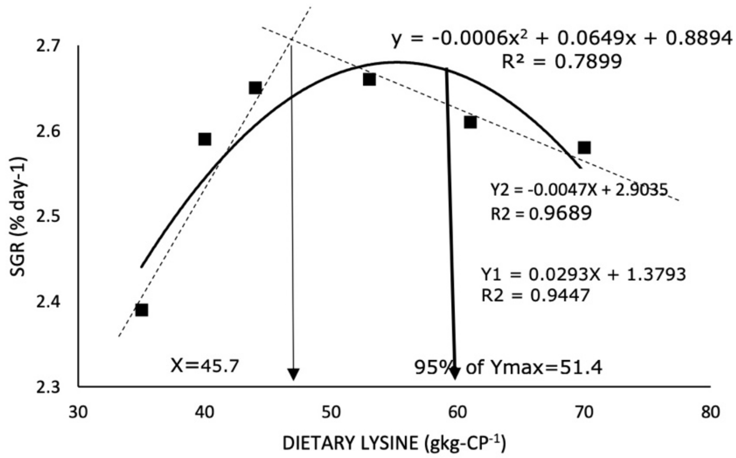

Figure 2 Broken-line linear (dash line) and second-order polynomial (solid line) regression analysis for optimization of dietary lysine requirement in relation to the specific growth rate (SGR, % day^{-1}) of *Siganus guttatus* fed with graded dietary lysine levels for a period of 8 weeks.

Table 6 Effect of lysine on the survival rate of fingerlings rabbitfish

Experimental rations	Survival rate (%)
Lys 35	97.62±2.38[a]
Lys 40	92.80±4.13[a]
Lys 44	85.71±8.25[a]
Lys 53	92.85±4.13[a]
Lys 61	95.23±2.38[a]
Lys 70	90.47±2.38[a]

[a,b,c] different characters in the same row have a statistically significant difference (P< 0,05); SEM: Sample mean standard error

Discussion

In formulating feeds for aquaculture species, information on individual EAA requirements is important in replacing or complementing animal protein sources with plant-based sources to decrease costs. Protein source and quality in amino acid profiles in diets are essential for maximum growth in fish (Ahmed and Khan, 2004).

As in other animals, fish require the same ten EAAs (Wilson & Cowey, 1985). EAA deficiency leads to suboptimal growth and diet utilization (Wilson and Halver, 1986) thus economic inefficiencies. Lysine is often one of the most limiting amino acids in commercial fish feeds (Harris, 1980). It serves an important role in the structure of collagen and regulates carnitine synthesis responsible for the transport of long-chain fatty acids into the mitochondria for the breakdown of lipids. Additionally, it is involved in maintaining acid-base concentrations and balances the osmotic pressure in the body (Chiu et al., 1988). Adding lysine to the diets of fish decreases mortality and inhibits fin rot (Li et al., 2009), and enhances protein deposition in the body.

Dietary lysine inclusion at an adequate level can enhance growth performance (Santiago and Lovell, 1988; Regmi et al., 2018). In the current study, growth indices (FBW, WG%, and SGR) were enhanced with the increasing LYS level up to 53 g·kg^{-1} CP (=1.86% CP) and growth retardation was observed with a subsequent increase in dietary LYS level. Suboptimal growth beyond the optimum level of dietary LYS might be due to the excess catabolism of LYS to generate energy with increased ammonia production causing stress, and leading to reduced growth of fish (Takishita et al., 2009; Liao et al., 2015; Kotzamanis et al., 2021). Dietary lysine at satisfactory levels could increase the growth of freshwater reared GIFT juveniles (Prabu et al., 2020). Similarly, growth performance was also improved in *Oreochromis niloticus* due to feeding optimal dietary LYS levels (Hua et al., 2019; de Souza Romaneli et al., 2021). In the current study, feed and nutrient utilization (FCR and PER) were not influenced by the dietary LYS contrary to the observation of Furuya et al. (2012) and Ovie and Eze (2010) in freshwater reared *O. niloticus*.

Lysine is often used as a reference amino acid because of its critical role in protein deposition, interaction with other amino acids, and critical physiological roles (Ball et al., 2007; Robinson and Li, 2007). The so-called ideal protein concept in which both EAA and NEAA are limited makes use of the utilization of lysine to estimate the requirements of the rest of the amino acids (van Milgen and Dourmad, 2015).

Fitting the PWG in broken-line linear and second-order polynomial regression model, the optimal LYS requirement in the diet of the orange-spotted siganid was determined as 45.3 and 54.1 gkg^{-1} CP, respectively (equivalent to 15.9 and 18.3 g·kg^{-1} diet, respectively) (**Figure 1**) and based on SGR, the optimal LYS requirement in the diet was found to be 45.7 and 51.4 gkg^{-1} CP, respectively, equivalent to 16.0 and 18.0 g·kg^{-1} diet, respectively) (**Figure 2**). Thus, the overall optimal LYS requirement in the diet of *Siganus guttatus* ranged between 15.9 and 18.3 g·kg^{-1} diets. The estimated range of dietary requirement level of lysine in the present study for the orange-spotted siganid were within a 2-unit of the value for the GIFT, Nile tilapia *Oreochromis niloticus*, milkfish *Chanos chanos* and Asian seabass *Lates calcalifer* (**Table 7**). Values found for the Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, Nile tilapia *Oreochromis niloticu*s, hybrid striped bass *Morone chrysops X Morone saxatilis*, the African catfish *Clarias gariepinus*, coho salmon *Oncorhyncus kisutch*, red drum *Sciaenops ocellatus*, scat *Scatophagus argus*, golden pompano *Trachinotus ovatus*, Japanese yellowtail *Seriola quinqueradiata* and common carp *Cyprinus carpio* were all lower than the requirement level estimated in the present study. However, estimates were higher in the Indian major carp *Cirrhinus mrigala,* silver perch *Bidyanus bidyanus*, cobia *Rachycentron canadum*, Atlantic cod *Gadus morhua*, Chinese sucker *Myxoprinus asiaticus*, silver pompano *Trachinotus blochii*, catla *Catla catla*, Japanes seabass *Lateolabrax japonicus*, striped bass Morone saxatilis, totoaba *Totoaba mcdonaldi*, golden pompano Trachinotus ovatus, Japanese flounder *Paralycthys olivaceous* and red seabream *Pagrus major* (**Table 7**). Furthermore, previous studies indicate that dietary LYS requirement may be influenced by species, body size, life stage, dietary composition, salinity and other water quality parameters etc. (De Silva and Perera, 1985; Dairiki et al., 2007; He et al., 2013; Nguyen and Davis, 2016; de Souza Romaneli et al., 2021).

Table 7 List of lysine requirement (gkg-1 diet) of some aquaculture fish in literature.

$g{\cdot}kg^{-1}$ diet	Common name	Species	Authors
12.6	Atlantic salmon	*Salmo salar*	Espe et al., 2007
13.0	Rainbow trout	*Oncorhynchus mykiss*	Kim et al., 1992
13.1	Nile tilapia	*Oreochromis niloticus*	Furuya et al., 2013
14.0	Hybrid striped bass	*Morone chrysops X Morone saxatilis*	Griffin et al., 1992
14.2	Nile tilapia	*Oreochromis niloticus*	Furuya et al., 2004
14.3	Nile tilapia	*Oreochromis niloticus*	Santiago & Lovell 1988
14.3	African catfish	*Clarias gariepinus*	Fagbenro et al., 1998
14.4	Nile tilapia	*Oreochromis niloticus*	Furuya et al., 2006
14.4	Nile tilapia	*Oreochromis niloticus*	Nguyen and Davis, 2016
14.6	Nile tilapia	*Oreochromis niloticus*	Michelato et al., 2016
15.2	Nile tilapia	*Oreochromis niloticus*	Furuya et al., 2012
15.2	Coho salmon	*Oncorhynchus kisutch*	Arai & Ogata, 1991
15.5	Red drum	*Sciaenops ocellatus*	Craig & Gatlin, 1992
15.6	Nile tilapia	*Oreochromis niloticus*	Diogenes et al., 2016
15.9	Nile tilapia	*Oreochromis niloticus*	do Nascimento et al., 2020
16.4	Spotted scat	*Scatophagus argus*	Binh et al., 2021
16.7	Golden pompano	*Trachinotus ovatus*	Stites et al., 2022
17.0	Nile tilapia	*Oreochromis niloticus*	Liebert and Benkendorff, 2007
17.8	Japanese yellowtail	*Seriola quinqueradiata*	Ruchimat et al., 1997
18.0	Nile tilapia	*Oreochromis niloticus*	Bomfim et al., 2010
18.8	Tilapia	*GIFT*	Prabu et al., 2020
19.2	Orange-spotted siganid	*Siganus guttatus*	Present study
20.0	Milkfish	*Chanos chanos*	Borlongan & Benitez, 1990
20.0	GIFT Tilapia	*Oreochromis niloticus*	Garg et al.(2022)
20.6	Asian sea bass	*Lates calcalifer*	Murillo-Gurrea et al., 2001
22.0	Common carp	*Cyrprinus carpio*	Nose, 1979
23.0	Indian major carp	*Cirrhinus mrigala*	Murthy & Varghese 1997
23.2	Nile tilapia	*Oreochromis niloticus*	Takishita et al., 2009
23.2	Silver perch	*Bidyanus bidyanus*	Yang et al., 2011
23.3	Cobia	*Rachycentron canadum*	Zhou et al., 2007
23.8	Atlantic cod	*Gadus morhua*	Grisdale-Helland et al., (2011
24.2	Chinese sucker	*Myxocyprinus asiaticus*	Lin et al., 2013
24.3	silver pompano	*Trachinotus blochii*	Ebaneezar et al., 2019
24.9	Nile tilapia	*Oreochromis niloticus*	Ovie and Eze, 2010
24.9	Catla	*Catla catla*	Ravi & Devaraj, 1991
24.9	Japanese sea bass	*Lateolabrax japonicus*	Mai et al., 2006
27.0	Striped bass	*Morone saxatilis*	Small & Soares, 2008
27.8	Totoaba	*Totoaba macdonaldi*	Madrid et al., 2019
29.4	Golden pompano	*Trachinotus ovatus*	Du et al. (2011)
33.0	Japanese flounder	*Paralichthys olivaceous*	Forster & Ogata, 1998
36.0	Red sea bream	*Pagrus major*	Forster & Ogata, 1998

Normally, dietary amino acids at optimum levels promote protein synthesis in fish by inhibiting amino acid catabolism in the presence of sufficient non-protein energy sources (Benevenga et al., 1993). However, deficient or excessive LYS in feed can increase amino acid catabolism for energy production at the cost of body protein synthesis, accretion, and growth (Mai et al., 2006). Therefore, high dietary LYS did not promote protein synthesis since it would imbalance the normal protein metabolism (Wilson and Halver, 1986).

Many studies have shown that lysine deficiency reduces appetite leading to reduced feed intake, reduced feed efficiency, and slow growth in many fish species such as freshwater catfish (Tantikitti and Chimsung, 2001), and Indian carp (Ahmed and Khan, 2004). Reduced fish PWG at lysine levels higher than the estimated optimum in the present study may be due to an adverse effect (lysine-arginine interaction) and an excess of free lysine (Wilson, 1985). Studies on salmon by Kaushik and Fauconneau (1984) show that there is an opposing metabolic effect between lysine and arginine, the authors found that increasing dietary lysine levels affected urea and arginine content in plasma as well as ammonia excretion. Excess lysine reduces arginine breakdown (Kaushik et al., 1998).

In conclusion, the lysine requirement level for juvenile rabbitfish was estimated using the combined broken-line linear and second-order polynomial regression analyses of the percent weight gain (PWG) and specific growth rate (SGR) against dietary lysine level to be between 15.9 and 18.3 g·kg^{-1} diets (=45.3 and 52.3 g·kg^{-1} CP).

References

Ahmed, I., Khan, M.A., 2004. Dietary lysine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquaculture*, 235, 499–511. https://doi.org/10.1016/j.aquaculture.2003.12.009

Akiyama, T., Oohara, I., Yamamoto, T., 1997. Comparison of essential amino acid requirements with A/E ratio among fish species. *Fisheries Science* 63(6), 963–970. https:// doi.org/10.2331/fishsci.63.963.

Andersen, S.M., Waagbø, R., Espe, M., 2016. Functional amino acids in fish health and welfare. *Front. Biosci.* 8, 143–169. https://doi.org/10.2741/757.

Arai, S., Ogata, H., 1991. Quantitative amino acid requirements of fingerling coho salmon. M.R. Collie, J.P. McVey (Eds.), Proceedings of the Twentieth US–Japan Symposium on Aquaculture Nutrition, UJNR Department of Commerce, Newport, OR, USA (1991), pp. 19-28.

Ball, R.O., Urschel, K.L., Pencharz, P.B., 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. *Journal of Nutrition* 137, 1626S–1641S.

Basyari, A., Tanaka, H., 1989. Studies on rearing of rabbitfish—1. Effect of different protein level on the growth of *Siganus jarus*. *Scientific report of Mariculture Research and Development Project (ATA-192) in Indonesia* 85:pp. 10-16.

Benevenga, N.J., Gahl, M.J., Blemings, K.P., 1993. Role of protein synthesis in amino acid catabolism. *Journal of Nutrition* 123 (2), 332–336. https://doi.org/10.1093/jn/123.suppl_ 2.332.

Binh M.N, Dan V. T, Thuy T.T N, Chat T.T, Tram N.D.Q., Serrano Jr., A.E., 2021. Lysine requirement of the spotted scat *Scatophagus argus* (Linaeus, 1766). *The Israeli Journal of Aquaculture-Bamidgeh*. ISSN 0792-156X. https://doi.org/10.46989/001c.30713

Bomfim, M.A.D., Lanna, E.A.T., Donzele, J.L., Quadros, M., Ribeiro, F.B., Sousa, M.P.D., 2010. Lysine levels, based on the ideal protein concept, in diets for Nile tilapia fingerlings. *Rev. Bras. Zootec.* 39, 1–8. https://doi.org/10.1590/S151635982010000100001

Borlongan, I.G., Benitez, L.V., 1990. Quantitative Lysine Requirement of Milkfish (*Chanos chanos*) Juvenile. *Aquaculture* 87,341-347. https://doi.org/10.1016/0044-8486(90)90071-T

Bwathondi, P.O.J., 1982. Preliminary investigations on rabbitfish, *Siganus canaliculatus* cultivations in Tanzania. *Aquaculture* 27, 205-210. https://doi.org/10.1016/0044-8486(82)90058-8

Carumbana, E.E. and Luchavez, J.A., 1979. A comparative study of the growth rates of *Siganus canaliculatus*, *Siganus spinus* and *Siganus guttatus* reared under laboratory and semi-natural conditions in Southern Negros Oriental. Philipp. *Silliman J.*, 26: 187-209.

Chiu, Y., Austic, R., Rumsey, G., 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (*Salmo gairdneri*). *Aquaculture* 69, 79–91. https://doi.org/10.1016/0044-8486(88)90188-3

Craig, S.R., Gatlin III, D.M., 1992. Dietary lysine requirement of juvenile red drum *Sciaenops ocellatus*. *J. World Aquacult. Soc*. 23, 133–137. https://doi.org/10.1111/j.1749-7345.1992.tb00761.x

Dairiki, J.K., Dias, C.T.D.S., Cyrino, J.E.P., 2007. Lysine requirements of largemouth bass, *Micropterus salmoides*: a comparison of methods of analysis of dose-response trials data. *Journal of Applied Aquac.* 19 (4), 1–27. https://doi.org/10.1300/J028v19n04_01.

De Silva, S.S., Perera, M.K., 1985. Effects of dietary protein level on growth, food conversion, and protein use in young *Tilapia nilotica* at four salinities. *Trans. Am. Fish. Soc.* 114 (4), 584–589. https://doi.org/10.1577/1548-8659(1985)114% 3C584:EODPLO%3E2.0.CO;2.

de Souza Romaneli, R., do Nascimento, T.M.T., Gous, R.M., de Paula Reis, M., Mansano, C.F.M., Khan, K.U., Fernandes, J.B.K., 2021. Response of Nile tilapia (*Oreochromis niloticus*) to lysine: performance, body composition, maintenance and efficiency of utilization. *Aquaculture* 538, 736522. https://doi.org/10.1016/j. aquaculture.2021.736522.

Dias, J., Arzel, J., Corraze, G., Kaushik, J., 2001. Effects of dietary L-carnitine supplementation on growth and lipid metabolism in European seabass (*Dicentrarchus labrax*). *Aquac. Res.* 32 (1), 206–215. https://doi.org/10.1046/j.1355557x.2001.00016.x.

Diogenes, A.F., Fernandes, J.B.K., Dorigam, J.C.P., Sakomura, N.K., Rodrigues, F.H.F., Lima, B.T.M., Gonçalves, F.H., 2016. Establishing the optimal essential amino acid ratios in juveniles of Nile tilapia (*Oreochromis niloticus*) by the deletion method. *Aquac. Nutr.* 22 (2), 435–443. https://doi.org/10.1111/anu.12262.

do Nascimento, T.M.T., Mansano, C.F., Peres, H., Rodrigues, F.H., Khan, K.U., Romaneli, R.S., Fernandes, J.B., 2020. Determination of the optimum dietary essential amino acid profile for growing phase of Nile tilapia by deletion method. *Aquaculture* 523, 735204. https://doi.org/10.1016/j.aquaculture.2020.735204.

Du, Q., Lin, H.Z., Niu, J., Ding, X., Huang, Z., Chen, X., Chen, Y., 2011. Dietary lysine requirements of juvenile pompano (*Trachinotus ovatus*). *Chin. J. Anim. Nutrit.* 23, 1725–1732. https://doi.org/10.5713/ajas.15.0627

Ebaneezar, S., Vijayagopal, P., Srivastava, P.P., Gupta, S., Varghese, T., Prabu, D.L., Wilson, L., 2019. Dietary lysine requirement of juvenile silver pompano, *Trachinotus blochii* (Lacepede, 1801). *Aquaculture* 511, 734234. https://doi.org/10.1016/j. aquaculture.2019.734234.

Emata, A.C., 1991. Milkfish, rabbitfish and mullet studies 1988-1991: a review. *A Lecture Note on Aquaculture*. Aquaculture Department, SEAFDEC, Tigbauan, Iloilo, Philippines, 15 pp.

Espe, M., Lemme, A., Petri, A., El-Mowafi, A., 2007. Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. *Aquaculture* 263, 168–178. **DOI:** 10.1016/j.aquaculture.2006.10.018

Fagbenro, O.A., Balogun, A.M. Fasakin, E.A., Bello-Olusoji, O.A., 1998. Dietary Lysine Requirement of the African Catfish *(Clarias gariepinus)*. *Journal of Applied Aquaculture* 8: 71-77. 10.3923/jfas.2013.94.100

Forster I. & Ogata H.Y. (1998) Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea bream *Pagrus major*. *Aquaculture* 161, 131–142. https://doi.org/10.1007/s10499-005-0614-6

Furuya, W.M., Botaro, D., Neves, P.R., Silva, L.C.R., Hayashi, C., 2004. Lysine requirement of Nile Tilapia (*Oreochromis niloticus*), for grow-out phase. *Cienc. Rural* 34, 1571–1577. https://doi.org/10.1590/S0103-84782004000500038.

Furuya, W.M., Graciano, T.S., Vidal, L.V.O., Xavier, T.O., Gongora, L.D., Righetti, J.S., Furuya, V.R.B., 2012. Digestible lysine requirement of Nile tilapia fingerlings fed arginine-tolysine-balanced diets. *Rev. Bras. Zootec.* 41 (3), 485–490. https://doi. org/10.1590/S1516-35982012000300003.

Furuya, W.M., Michelato, M., Graciano, T.S., Vidal, L.V.O., Xavier, T.O., Furuya, V.R.B., de Moura, L.B., 2013. Digestible lysine requirement of Nile tilapia from 86 to 227 g fed arginine to lysine balanced diets. *Semina* 34 (4), 1945–1954. https://doi.org/10.5433/1679-0359.2013v34n4p1945.

Furuya, W.M., Santos, V.G., Silva, L.C.R., Furuya, V.R.B., Sakaguti, E.S., 2006. Digestible lysine requirements of Nile tilapia juveniles (Bras). *Rev. Bras. Zootec.* 35 (3), 937–942. https://doi.org/10.1590/S1516-35982006000400001.

Furuya, W.M., Santos, V.G.D., Silva, L.C.R., Furuya, V.R.B., Sakaguti, E.S., 2006.

Garg, C.K., Sardar, P., Sahu, N.P., Maiti, M.K., Shamna, N., Varghese, T., Deo, A.D., Harikrishna, V., 2022. Dietary lysine requirement of genetically improved farmed tilapia (GIFT) juvenile reared in inland saline water of 10 ppt salinity. *Aquaculture* 555, 738223. https://doi.org/10.1016/j.aquaculture.2022.738223.

Griffin, M.E., Brown, P.B., Grant, A.L., 1992. The dietary lysine requirement of juvenile hybrid striped bass. *J. Nutr.* 122, 1332–1337. https://doi.org/10.1093/jn/122.6.1332

Grisdale-Helland, B., Gatlin, D.M., Corrent, E., Helland, S.J., 2011. The minimum dietary lysine requirement, maintenance requirement and efficiency of lysine utilization for growth of Atlantic salmon smolts. *Aquac. Res.,* 42 (2011), pp. 1509-1529, 10.1111/j.1365-2109.2010.02743.x

Harris, L.E., 1980. Feedstuffs. UNDP/FAO, Rome, Italy.

He, J.Y., Tian, L.X., Lemme, A., Gao, W., Yang, H.J., Niu, J., Liu, Y.J., 2013. Methionine and lysine requirements for maintenance and efficiency of utilization for growth of two sizes of tilapia (*Oreochromis niloticus*). *Aquac. Nutr.* 19 (4), 629–640. https://doi. org/10.1111/anu.12012.

Horstmann, U.. 1975. Some aspects of the mariculture of different siganid species in the Philippines. *Philipp. Sci.,* 12: 5-20.

Hua, K., Suwendi, E., Bureau, D.P., 2019. Effect of body weight on lysine utilization efficiency in Nile Tilapia (*Oreochromis niloticus*). *Aquaculture* 505, 47–53. https://doi.org/10.1016/j.aquaculture.2019.02.030. *Journal of Animal Science and Biotechnology* 6(15), 11 pages. DOI 10.1186/s40104-015-0016-1

Kaushik S.J, Fauconneau, B., Terrier L., Gras, J., 1988. Arginine requirement and status assessed by different biochemical indices in rainbow trout (*Salmo gairdneri* R.). *Aquaculture* 70:75-95. https://doi.org/10.1016/0044-8486(85)90154-1

Kaushik, S.J., Fauconneau, B., 1984. Effects of lysine administration on plasma arginine and on some nitrogenous catabolites in rainbow trout. *Comp. Biochem. Physiol.* 79A(3): 459-462.

Khan, M.A., Abidi, S.F., 2011. Effect of dietary L-lysine levels on growth, feed conversion, lysine retention efficiency and haematological indices of *Heteropneustes fossilis* (Bloch) fry. *Aquac. Nutr.* 17 (2), 657–667. https://doi.org/10.1111/j.13652095.2010.00815.x.

Kim, K.-I., Kayes, T.B., Amundson, C.H., 1992. Requirements for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 106, 333–344. https://doi.org/10.1016/0044-8486(92)90265-M

Kotzamanis, Y., Fawole, F.J., Brezas, A., Kumar, V., Fontanillas, R., Antonopoulou, E., Ilia, V., 2021. Dietary lysine requirement of greater amberjack juvenile (*Seriola dumerili*, Risso, 1810). *Aquac. Nutr.* 27 (6), 2107–2118. https://doi.org/10.1111/ anu.13344.

Li, P., Mai, K., Trushenski, J., Wu, G., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids* 37(1):p.43-53. https://doi.org/10.1007/s00726-009-0320-1

Liao, S.F., Wang, T., Regmi, N., 2015. Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. *Springerplus* 4 (1), 1–12. https://doi.org/10.1186/s40064-015-0927-5.

Liebert, F., Benkendorff, K., 2007. Modeling lysine requirements of *Oreochromis niloticus* due to principles of the diet dilution technique. *Aquaculture* 267 (1–4), 100–110. https://doi.org/10.1016/j.aquaculture.2007.02.022.

Lin, Y., Gong, Y., Yuan, Y., Gong, S., Yu, D., Li, Q., Luo, Z., 2013. Dietary L-lysine requirement of juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquac. Res*., 44, pp. 1539-1549. https://doi.org/10.1111/j.1365-2109.2012.03161.x

Madrid, J., Pohlenz, C., Viana, M.T., Lazo, J.P., 2019. Dietary lysine requirement for juvenile, *Totoaba macdonaldi*. *Aquaculture* 500, 92–98. https://doi.org/10.1016/j. aquaculture.2018.10.003.

Mai, K.,Zhang., L., Ai, Q., Duan, Q., Zhang, C., Li, H., Wan, J., Liufu, Z., 2006. *Dietary lysine requirement of juvenile Japanese seabass (Lateolabrax japonicus). Aquaculture* 258, 535–542. https://doi.org/10.1016/j.aquaculture.2006.04.043

Michelato, M., de Oliveira Vidal, L.V., Xavier, T.O., de Moura, L.B., de Almeida, F.L.A., Pedrosa, V.B., Furuya, W.M., 2016. Dietary lysine requirement to enhance muscle development and fillet yield of finishing Nile tilapia. *Aquaculture* 457, 124–130. https://doi.org/10.1016/j.aquaculture.2016.02.022.

Milgen, J.v., Dourmad, J.-Y., 2015. Concept and application of ideal protein for pigs

Munro, I.S.R., 1967. Spine-feet, rabbittish (suborder Siganoidei; family Siganidae). In: *The Fishes of New Guinea*. Port Moresby, New Guinea Department of Agriculture, Stock and Fisheries, pp. 472-479.

Murillo-Gurrea, D.P., Coloso, R.M., Borlongan, I.G., Serrano Jr., A.E., 2001. Lysine and arginine requirements of juvenile Asian sea bass (*Lates calcarifer*). *J. Appl. Ichthyol.*, 17 (2001), pp. 49-53. https://doi.org/10.1046/j.1439-0426.2001.00242.x

Murthy H.S. & Varghese T.J. (1997) Dietary requirements of juveniles of the Indian major carp, *Labeo rohita*, for the essential amino acid lysine. *Israeli Journal of Aquaculture (Bamidgeh)* 49, 19–24.

National Research Council (NRC), 2011. *Nutrient Requirements of Fish and Shrimp.* National Academies Press, Washington DC, pp. 70–76. https://doi.org/10.17226/ 13039.

Nguyen, L., Davis, D.A., 2016. Comparison of crystalline lysine and intact lysine used as a supplement in practical diets of channel catfish (*Ictalurus punctatus*) and Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 464, 331–339. https://doi.org/10.1016/j. aquaculture.2016.07.005.

Nose, T., Arai, S., Lee, D.L., Hashimoto, Y., 1974. A note on amino acids essential for growth of young carp. *Bull. Jpn. Soc. Sci. Fish.* 40 (9), 903–908. https://doi.org/10.2331/suisan.40.903.

Ovie, S.O., Eze, S.S., 2010. Lysine requirement and its effect on the body composition of *Oreochromis niloticus* fingerlings. In: *Proceedings of Fisheries Society of Nigeria* (FISON), Badagry, Nigeria 25th–29th OCTOBER 2010, pp. 573–579. http://hdl.ha ndle.net/1834/38235.

Parazo, M.M., 1990. Effect of dietary protein and energy level on growth, protein utilization and carcass composition of rabbitfish, *Siganus guttatus*. *Aquaculture* 86: 41-49. https://doi.org/10.1016/0044-8486(90)90220-H

Phu V.V., 2001. The composition of fish species in the lagoon system of Thua Thien Hue after the flood since 1999 and solutions for sustainable development of resources. *Journal of Biology* 20. 36 pp. (Vietnamese).
Prabu, E., Felix, N., Uma, A., Praveenraj, J., 2020. Effects of dietary *L*-lysine supplementation on growth, body composition and muscle-growth-related gene expression with an estimation of lysine requirement of GIFT tilapia. *Aquac. Nutr.* 26(2), 568–578. https://doi.org/10.1111/anu.13018.
Ravi, J., Devaraj, K.V., 1991. Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). *Aquaculture* 96, 281-291. https://doi.org/10.1016/0044-8486(91)90158-4
Regmi, N., Wang, T., Crenshaw, M.A., Rude, B.J., Liao, S.F., 2018. Effects of dietary lysine levels on the concentrations of selected nutrient metabolites in blood plasma of late-stage finishing pigs. *J. Anim. Physiol. Anim. Nutr.* 102 (2), 403–409. https:// doi.org/10.1111/jpn.12714.
Robinson, E.H., Li, M.H., 2007. *Catfish Protein Nutrition Revised,* Division of Agriculture, Forestry, and Veterinary Medicine at Mississippi State University. Office of Agricultural Communications.
Ruchimat, T., Masumoto, T., Hosokawa, H., Itoh, Y., Shimeno, S., 1997. Quantitative lysine requirement of yellowtail (*Seriola quinqueradiata*). *Aquaculture* 158, 331–339. https://doi.org/10.1016/S0044-8486(97)00215-9
Sandell, L.J., Daniel, J.C., 1988. Effects of ascorbic acid on collagen mRNA levels in short term chondrocyte cultures. *Connect. Tissue Res.* 17 (1), 11–22. https://doi.org/10.3109/03008208808992790.
Santiago, C.B., Lovell, R.T., 1988. Amino acid requirements for growth of Nile tilapia*. J. Nutr.* 118 (12), 1540–1546. https://doi.org/10.1093/jn/118.12.1540.
Small, B.C., Soares Jr., J.H. 2008. Quantitative dietary lysine requirement of juvenile striped bass *Morone saxatilis*. *Aquaculture Nutrition* 6, 207–212. https://doi.org/10.1046/j.1365-2095.2000.00140.x
Stites, W., Weldo, A., Reis, J., Walsh, S., Davis, R., Rhodes, R., Davis, D.A., 2022. Quantitative lysine requirement for juvenile Florida pompano, *Trachinotus carolinus* fed plant-based diets. *Aquaculture* 547, 737548. https://doi.org/10.1016/j.aquaculture.2021.737548
Suyehiro, Y., 1942. A study on the digestive system and feeding habits of fish. *Jpn J. Zool.*, 10: 301-303.
Tabugo, S.R., Rose, M., Sendaydiego, J.P., Requieron, E.A., 2012. Embryonic developmental stage in cultured rabbitfish (*Siganus guttatus*, Bloch 1787). *Int. Res. J. Biol. Sci.* 1, 65–70.
Tacon, A.G.J., Rausin, N., Kadari, M. and Cornelis, P., 1990. The food and feeding of marine finfish in floating net cages at the National Seafarming Development Centre, Lampung, Indonesia: rabbitfish, *Siganus canalicultus* (Park). *Aquac. Fish. Manage.,* 21: 375-390.
Takishita, S.S., Lanna, E.A.T., Donzele, J.L., Bomfim, M.A.D., Quadros, M., Sousa, M.P.D., 2009. Digestible lysine level in feed for Nile tilapia fingerlings. *Rev. Bras. Zootec.* 38(11), 2099–2105. https://doi.org/10.1590/S1516-35982009001100004.
Tantikitti,C., Chimsung,N., 2001. Dietary lysine requirement of fresh water catfish *(Mystus nemurus* Cuv.&Val.*). Aquacult.Res.*32:135–141.https://doi.org/10.1046/j.1355-557x.2001.00011.x
Von Westemhagen, H., 1974. Food preferences in cultured rabbitfish (Siganidae). *Aquaculture* 3: 109-117. https://doi.org/10.1016/0044-8486(74)90106-9
Wilson, R.P., Cowey, C.B., 1985. Amino acid composltion of whole body tissue of rainbowtrout and Atlantic salmon. *Aquaculture* 48:373-376. https://doi.org/10.1016/0044-8486(85)90140-1
Wilson, R.P., Halver, J.E., 1986. Protein and amino acid requirements of fishes. *Annual Review of Nutrition* 6:225-44.
Yan, Q., Xie, S., Zhu, X., Lei, W., Yang, Y., 2007. Dietary methionine requirement for juvenile rockfish, *Sebastes schlegeli. Aquaculture Nutrition* 13(3): 163-169. DOI:10.1111/J.1365-2095.2007.00461.X
Yang, S.D., Liu, F.G., Liou, C.H., 2011. Aquaculture Assessment of dietary lysine requirement for silver perch *(Bidyanus bidyanus)* juveniles*. Aquaculture* 312: 102–108. https://doi.org/10.1016/j.aquaculture.2010.12.011
Zhou, Q.C., Wu, Z.H., Chi, S.Z., Yang, Q.H., 2007. Dietary lysine requirement of juvenile cobia *(Ranchicentron canadum)*. *Aquaculture* 273(4): 634-640. https://doi.org/10.1016/j.aquaculture.2007.08.056