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Lysine requirement of the spotted scat *Scatophagus argus* (Linaeus, 1766)

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Abstract

The study aimed to determine the lysine requirement of young spotted scat *Scatophagus argus* (Linaeus, 1776) (average body weight = 10.7 g). The feeding trial was conducted for 8 weeks with 5 experimental diets which were isonitrogenous, isocaloric and isolipidic (35% CP, 18.3 MJ·kg-1, containing lysine levels as treatments containing equal dietary protein (35%) and lipid levels (5%) in 3 replicates. The lysine content was from 1.22 g to 1.94 g lysine·kg^{-1} dry diet (35 to 55 g·kg^{-1} CP) with an average content of 0.18 g lysine·kg^{-1} dry diet per treatment. Results showed that the growth rate, feed conversion and protein efficiency ratios were significantly highest at lysine level of 1.58 g·kg dry diet^{-1}(45 g·kg^{-1} CP). Fish survival was not influenced by dietary lysine. Lysine requirement level for juvenile scat was estimated using quadratic regression of the percent weight gain (PWG) and specific growth rate (SGR) against dietary lysine level to have a mean value of 1.64 g·kg dry diet^{-1} (4.75 g·kg^{-1} CP).

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Introduction

The spotted scat is a tropical euryhaline species that is a candidate for commercial coastal aquaculture in Asia (Gupta, 2016) because of several interesting characteristics such as good adaptation to highly fluctuating environments (Barry and Fast, 1992). It is found in freshwater, brackishwater and marine habitats. It is a popular aquarium fish (Morgan 1983) and an important food fish in the Philippines and Vietnam where it is considered a delicacy. It belongs to the genus Scatophagus*,* family Scatophagidae*,* and order Perciformes*.* Successful breeding of this fish has been done in many countries (Chang and Hsieh, 1997; Ruensirikul et al., 2008; Cai et al., 2010; Khanh et al., 2012; Gandhi et al., 2014). Despite this, mass seed production of spotted scat is still limited partly because of its low survival rate from hatching, slow growing and long period of weaning. One of the main causes could be inappropriate food formulation given to fish fry or unattractive artificial diet (Yangthong & Ruensirikul, 2020). Up until now research has been scarce on the optimum formulation of feed for spotted scat.

In order to support the development of scat aquaculture, proper feeds should be formulated. Fish nutrition researchers should be able to search for alternative fish feed ingredients with high nutritive value especially the essential amino acid for growth and maintenance of scat fingerlings. Fish do not have a requirement for protein, but rather for an adequate balance of essential and nonessential amino acids. Thus, diets formulated only on the basis of the crude protein content may not necessarily meet the needs for all amino acids especially the essential ones. Amino acid imbalance could result in compromised growth performance and increased discharge of metabolic nitrogen compounds into the environment (Bomfim et al., 2010; Cyrino et al., 2010). Thus, for various fish species and stages of production, the determination of the requirement of each amino acid is necessary, especially for essential amino acids which are normally limited in plant protein sources (Nunes et al., 2014).

Lysine is an essential amino acid in fish diet for better growth performance, feed utilization (Zhou et al., 2007), tissue protein deposition (Sveier et al., 2000) enhancement of immune responses and gastrointestinal condition (Li et al., 2009), considering that its almost exclusive use in protein synthesis. In addition, since it is the amino acid found in the greatest proportion in the body of animals, it is one of the main limiting amino acids in the practical diets of fish (Abimorald et al., 2009; Bicudo et al., 2009; Nunes et al., 2014). Rawles et al. (2013) have emphasized that the if the lysine requirement in fish diet is met, the other amino acids are present in amounts that meet or exceed their requirements. Fish fed diets deficient in lysine show reduced weight gain and feed conversion ratio (Cabanero et al., 2016; Bomfim et al., 2010), fin rot as well as mortality (Li et al., 2009).

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

Methodology

Experimental design

The compositions of the experimental diets are shown in **Table 1**. Five isonitrogenous (35% crude protein) and isocaloric (18.3 $MJ \cdot kg^{-1}$) diets were formulated and supplemented with L-lysine HCL (Sigma Aldrich, St. Louis MO, USA) to add lysine in increments of 5 $g \cdot kg^{-1}$ crude protein (35, 40, 45, 50, 55 $g \cdot kg^{-1}$ crude protein, CP); each treatment was replicated three times. The mixture of nonessential amino acids (NEAA) was used to balance the nitrogen contents at the expense of lysine in each experimental diet.

The experiment was conducted in a completely randomized fashion (CRD) which lasted for 8 weeks.

Normally, to find the range of dietary levels of the essential amino acid of interest to be included in the experimental diets, the initial basis is the content of that amino acid analyzed from body of the fish (Yan Q et al., 2007). In the present study, in order to establish a reasonable level of lysine in the diet for the scat, we collected samples of the scat for analysis and were sent to the National Institute of Livestock, Vietnam for amino acid profiling. Briefly, 40 individuals of *Scatophagus argus* fingerlings with an average weight range of 7-11 g·fish^{-1} were collected, washed, internal organs removed, dried at 600^{o}C, pulverized and packed for analysis. Results showed that the average lysine content in the scat was 43.6 g·kg^{-1} CP. Based on this result, we prepared five dietary levels of lysine in which the expected optimum lysine level was placed somewhere in the middle of the following level range: 35, 40, 45, 50, 55 g·kg^{-1} crude protein (CP).

Experimental feed preparation

First, we determined the proximate composition and amino acid content of the ingredients. Based on the proximate composition, we balanced the diets in terms of protein source and 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 scat for 8 weeks.

Ingredients	Dietary Lysine level (g·kg^{-1} crude protein)				
	1.22	1.40	1.58	1.76	1.94
Casein[1]	10.0	10.0	10.0	10,0	10.0
Fish meal[2]	6.5	6.5	6.5	6.5	6.5
Dextrin	38.0	38.0	38.0	38.0	38.0
Gelatin	4.0	4.0	4.0	4.0	4.0
α-cellulose	6.5	6.5	6.5	6.5	6.5
EAA mix[3]	10.0	10.0	10.0	10,0	10,0
NEAA mix[4]	10.0	9.8	9.6	9.5	9.3
Crystalline lysine	0.0	0.2	0.4	0.5	0.7
Vitamins/mineral mix[5]	4.0	4.0	4.0	4,0	4.0
Binder	5.0	5.0	5.0	5.0	5.0
Soybean oil	5.0	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0	100.0
Analyzed protein content	35.2	34.9	35.1	34.8	35.2
Analyzed lysine content	1.21	1.39	1.57	1.77	1.92
Estimated energy content	18.3	18.3	18.3	18.3	18.3

Notes:

[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.

Substances that were high in the diet such as fishmeal, casein, cellulose, and dextrin were mixed together, and those that were added in small quantity such as vitamins, minerals, and oil were mixed together. Crystalline lysine was added to the two previous mixes. The binder was gelatinized, 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 600^{o}C and refrigerated to 4^{o}C until use.

Experimental fish and facility

Scat fingerlings (average body weight of 7-11 g·fish^{-1}, 5-7 cm·fish^{-1} total length) were purchased from two locations: Tan An village, Thuan An town, Phu Vang district and Vinh Hien commune, Phu Vang district, Thua Thien Hu province. Fish were transported to the laboratory and acclimatized in composite tanks for 3 weeks. During acclimation, scats 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 20 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 NH_4^+ were monitored twice in one week.

The experiment was conducted in a recirculating water system consisting of 15 composite tanks with a total volume capacity of 4 m^3 and contained 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.

Sample collection? State the process of collecting samples for this experiment.

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 (Tram et al., 2014):

$$\text{PWG (\%): PWG (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

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

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 homoscedascity with the 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, PWG, SGR, FCR and FBW was modeled aligned with lysine concentration in the diet using a quadratic model analysis.

Results

Water quality

The mean water quality parameters during the duration of the experiment are presented in **Table 2**. 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 scat.

Table 2 Fluctuation of environmental factors during experimental fish culture

Treatments (%)	*Lys 1.22*	*Lys 1.40*	*Lys 1.58*	*Lys 1.76*	*Lys 1.94*
Factors			*min : max* / *Mean ± SEM*		
Temperature (°C),	25.55-28.47 / 27.1±0.59[a]	25.75-28.47 / 27.17± 0.64[a]	26.00-28.45 / 27.66±0.69[a]	25.65-28.58 / 27.03±0.62[a]	25.55-28.5 / 27.01±0.61[a]
DO ($mg \cdot l^{-1}$)	3.15-5.80 / 4.37±0.85[a]	3.05-5.85 / 4.4±0.86[a]	3.1-5.55 / 4.37±0.89[a]	3.15-5.50 / 4.43±0.83[a]	3.10-5.65 / 4.45±0.84[a]
pH	7.15-8.00 / 7.56±0.22[a]	7.12-8.00 / 7.55±0.21[a]	7.14-8.00 / 7.6±0.24[a]	7.12-8.00 / 7.57±0.21[a]	7.00-8.00 / 7.58±0.23[a]
NH_3/NH_4 ($mg \cdot l^{-1}$)	0.010-0.035 / 0.019±0.006[a]	0.009-0.104 / 0.02±0.01[a]	0.009-0.037 / 0.018±0.007[a]	0.010-0.036 / 0.018±0.007[a]	0.009-0.037 / 0.02±0.007[a]
Salinity (‰)	20.00-22.40 / 21.46±0.57[a]	20.25-22.40 / 21.47±0.51[a]	20.09-22.03 / 21.50±0.49[a]	20.89-22.50 / 21.89±0.37[a]	20.15-22.10 / 21.56±0.47[a]

Growth performance and feed efficiency

Survival rate did not differ significantly between experimental diets and were very high (86.7-90.0%) during the experimental period; there were not any external pathological signs observed, even in the group fed diets containing a low level of dietary lysine (**Table 3**). After 8 weeks of feeding, growth performance as well as efficiency performance exhibited an evident effect in scat juveniles with significant differences found between FBW of fish fed the diet Lys 35 and those fed diets Lys 40, 45, 50 and 55 (Table **3**). Quadratic regression of PWG and SGR yielded similar requirement levels of 1.64 g $lysine \cdot kg^{-1}$ dry diet (**Figure 1**). FCR and FBW followed a similar pattern as that of both PWG and SGR.

Table 3 Mean values of final body weight (FBW), percent weight gain (PWG), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and survival rate of juvenile scats fed with different lysine levels in the diet for 8 weeks.

Parameter	*Lysine level (% diet)*					*p-value*
	Lys 1.22	*Lys 1.40*	*Lys 1.58*	*Lys 1.76*	*Lys 1.94*	
IBW (g)	10.77±0.12^{a}	10.83±0.14^{a}	10.82±0.03^{a}	10.63±0.17^{a}	10.78±0.03^{a}	0.241
FBW (g)	29.55±0.57^{a}	31.35±0.09^{b}	33.48±0.73^{c}	31.80±0.48^{b}	31.08±0.18^{b}	<0.001
PWG (%)	174.4 ± 3.0^{a}	189.4±3.5^{b}	209.37±7.06^{c}	199.29±6.46^{bc}	189.26±1.86^{b}	<0.001
SGR (% d^{-1})	1.60±0.00^{a}	1.69±0.00^{b}	1.79±0.00^{c}	1.74±0.00^{bc}	1.68±0.00^{b}	<0.001
FCR	3.00±0.09^{b}	2.85±0.01^{ab}	2.73±0.05^{a}	2.87±0.10^{ab}	2.91±0.11^{ab}	0.032
PER	0.95±0.02^{a}	0.98±0.01^{ab}	1.03±0.03^{b}	1.03±0.03^{b}	0.99 ±0.01^{ab}	0.003
Survival (%)	86.7±2.9^{a}	86.7±7.6^{a}	88.3±2.88^{a}	90.0±5.0^{a}	88.3±5.8^{a}	0.574

Different characters $^{(a,b,c)}$ in the same row show statistically significant differences ($p < 0.05$).

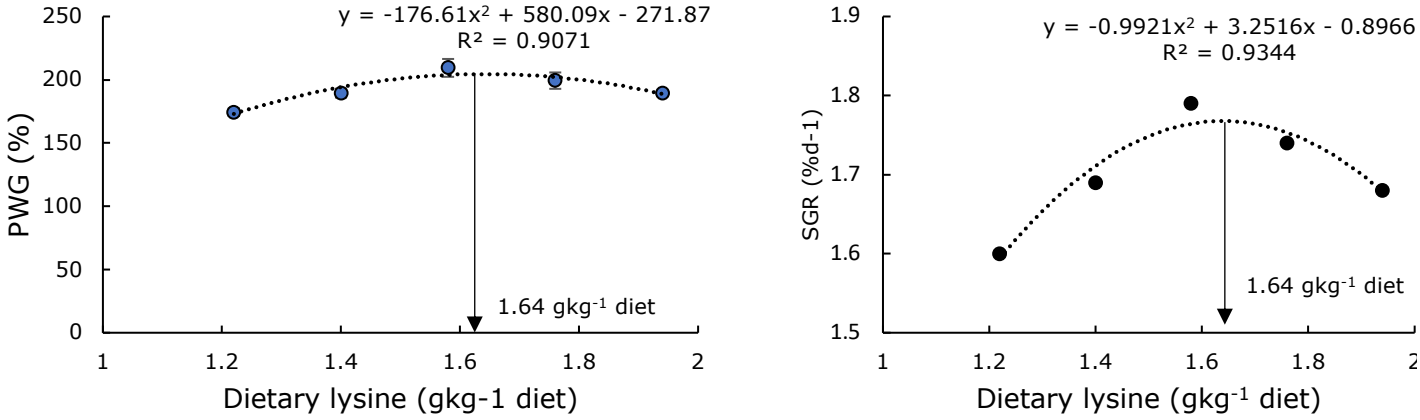

Figure 1 Graphical representation of PWG and SGR as a function of dietary lysine level in the diets of juvenile *Scatophagus argus*.

Discussion

At the end of the experimental trial, high PWG (174.4 – 209.37%) was observed in fish from all dietary treatments. Water quality parameters were under control in the laboratory set up as evidenced by the no-significant difference among the parameters. Thus, it was safe to assume that the water quality parameters did not affect the results of the present requirement study which allowed for the maximal growth expressed as either PWG or SGR.

The estimated requirement level of 16.4 g·kg^{-1} diet (i.e., 4.7 g·kg^{-1} CP) determined in the scat for the maximum PWG in the present study was lower or higher than the estimates of other researchers for other fishes. For example, 5.1% dietary protein was the estimated value of Santiago and Lovell (1988) for the maximum WG of the Nile tilapia fingerlings. For the Indian major carp *Labeo rohita* and African catfish *Clarias gariepinus* juveniles, the estimates were 5.6% and 5.7% dietary protein, respectively, for maximum WG (Murthy & Varghese, 1997; Fagbenro et al., 1988), 5.4% dietary protein for maximum WG in juvenile cobia *Rachycentron canadum* (Zhou et al., 2007), 6.0% dietary protein for the maximum WG in the finishing adult tilapia (ABW=274.9 g), 6.0% dietary protein for the maximum WG in silver perch *Bidyanus bidyanus* (Yang et al., 2011), and 5.5% for Chinese sucker *Myxocyprinus asiaticus* (Lin et al., 2013). Other values for other species in literature that were higher than the value in the present study are 20.7 g·kg^{-1} diet for striped bass *Morone*

saxatilis (Small, 2000), 20.6 g·kg^{-1} diet for Asian sea bass *Lates calcalifer* (Murillo-Gurrea et al., 2001), 23.8 g·kg^{-1} diet for Atlantic cod *Gadus morhua* (Grisdale-Helland et al., (2011), 33 g·kg^{-1} diet for dusky cob *Argyrosomus japonicus* (Adesola et al., 2017), 24.8 g·kg^{-1} diet for yellow croaker *Pseudosciaena crocea* (Zhang et al., 2006), 33 g·kg^{-1} diet for Japanese flounder *Paralichthys olivaceous* (Forster & Ogata, 1998), 36 g·kg^{-1} diet for red sea bream *Pagrus major* (Forster & Ogata, 1998), 17.8 g·kg^{-1} diet for Japanese yellowtail *Seriola quinqueradiata* (Ruchimat et al., 1997), 27.8 g·kg^{-1} diet for totoaba *Totoaba macdonaldi* (Madrid et al., 2019), 24-24.5 g·kg^{-1} diet for silver pompano *Trachinotus blochii* (Ebaneezar et al., 2019), 58 g·kg^{-1} diet for Japanese sea bass *Lateolabrax japonicus* (Mai et al., 2006), 5.7% dietary protein for common carp *Cyrprinus carpio* (Nose, 1979), 5.7% dietary protein for African catfish *Clarias gariepinus* (Fagbenro et al., 1998), 5.6% dietary protein for rohu *Labeo rohita* (Murthy & Varghese, 1997), 5.3% dietary protein for stinging catfish *Heteropneustes fossilis* (Bloch) (Farhat & Khan, 2013), 6.7% for golden pompano *Trachinotus ovatus* (Du et al., 2011), 6.2% dietary protein for catla *Catla catla* (Ravi & Devaraj, 1991), 6.0% dietary protein for silver perch *Bidyanus bidyanus* (Yang et al., 2011), 2.32% dry diet in another study for silver perch (Yang et al., 2011) and 20 g·kg^{-1} diet for milkfish *Chanos chanos* (Borlongan & Benitez, 1990). Conversely, the requirement level in the present study was lower in some reports for other fish species e.g., 15.5 g·kg^{-1} diet for red drum *Sciaenops ocellatus* (Craig & Gatlin, 1992), 14.0 g·kg^{-1} diet for hybrid striped bass *Morone chrysops X Morone saxatilis* (Griffin et al., 1992), 12.6 g·kg^{-1} diet for Atlantic salmon *Salmo salar* (Espe et al., 2007), 3.7% dietary protein for rainbow trout *Oncorhynchus mykiss* (Kim et al., 1992) and 3.8% dietary protein for coho salmon *Oncorhynchus kisutch* (Arai & Ogata, 1991).

The variation of the estimated values of dietary lysine requirement among diverse cultured finfish compared with the present study may be due to different protein sources, culture conditions and the methodological approaches used (Salze et al., 2017). Some of the factors reported to influence lysine requirement assessment are: fish life-stage, amount of food consumed, cultured conditions (Hauler and Carter, 2001), protein sources and their digestibility (Da Silva et al., 2000). In addition, the proximate composition of diets, feeding protocol and fish strains (Kim et al., 1992) can have a significant effect.

The mathematical model used to estimate the requirement value has been shown to over- or under-estimate the nutrient requirements (Rodehutscord et al., 2000; Nguyen Duy Quynh Tram, 2013. These models include broken-line model, quadratic model, and saturation-kinetic model and no one model is better than the other since each has its own pros and cons (Madrid et al., 2019). There is still no model that is the most appropriate (Shearer, 2000; Pesti et al., 2009). The quadratic regression analysis has been suggested as resulting in the lowest error to estimate requirements (Wilson, 2002) and the expression g·kg-1 of lysine in the diet provides the highest fit (Hua, 2013).

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), Indian carp (Ahmed and Khan, 2004). Reduced fish WG 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 at this feed (Wilson, 1985). Kaushik and Fauconneau (1984) studies on salmon 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). The research results on WG of Spotted scat fingerlings when feeding with different lysine content is quite consistent with the studies of Craig and Gatlin (1992) on Red Amur (*Sciaenops ocellatus*); Liou (1989) on blue tilapia (*Oreochromis aureus*); Forster and Ogata (1998) studied on Japanese flounder (*Paralichthys olivaceus*); Brown et al (1988) on red bream (*Pagrus major*) and Walton et al (1984) on rainbow trout (*Oncorhynchus mykiss*). But most of the effects of lysine on WG vary with fish species.

In conclusion, Lysine requirement level for juvenile scat was estimated using quadratic regression of the percent weight gain (PWG) and specific growth rate (SGR) against dietary lysine level to be1.64 g•kg dry diet^{-1} (4.75 g•kg^{-1} CP).

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