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bream is the limited supply of
This type of diet is di cult to sto
quality, poor feed conversion rate and easily to result in
water pollution. So there is an urgent need to develop a cost-
e ective practical diet for grow-out production of black sea
bream. It is well known that lysine plays a significant role in
fish, therefore, the objective of the present investigation was
undertaken to study the influence of varying dietary lysine
levels in isoenergetic diets on growth performance, protein
utilization, body compositions and biochemical parameters
so as to determine the optimum dietary lysine requirement
for black sea bream juvenile.

Materials and methods

Experiment diets

Ingredients and proximate composition of the experimental diets are presented in Table 1, amino acid compositions (g kg⁻¹ dry diet) of dietary ingredients in Table 2 and the analysed EAA contents for each diet in Table 3. Six isonitrogenous and isoenergetic diets were formulated with graded levels of crystalline lysine and dietary lysine was quantitatively increased at the expense of glutamic acid. Experimental diets contained 380 g kg⁻¹ crude protein, which was slightly lower than the optimum protein requirement suggested in our preliminary experiment (410 g kg⁻¹, unpublished data) to

		Diet					
		Diet 5		Diet 6		Diet 7	
		280	280	280	280	280	280
		120	120	120	120	120	120
		20	20	20	20	20	20
		90	90	90	90	90	90
		8	12	16	20	24	28
		12	8	4	0	0	0
		50	50	50	50	50	50
		80	80	80	80	80	80
		15	15	15	15	15	15
		15	15	15	15	15	15
		310	310	310	310	310	310
		1000	1000	1000	1000	1000	1000
		Analysis (g kg ⁻¹ dry matter)					
		385.0	383.7	386.4	384.4	387.3	387.8
		148.4	150.6	149.9	152.0	148.5	149.1
		120.4	120.6	120.3	120.1	120.0	120.1
		68.9	70.9	70.3	69.1	69.6	68.4
		20.8	25.2	28.8	32.5	36.8	40.5

¹ Crystalline amino acid premix: showed in Table 2.

² Mineral premix (g kg⁻¹ of premix): Na₂SiO₃, 0.4; CaCO₃, 350; NaH₂PO₄·H₂O, 200; KH₂PO₄, 200; MgSO₄·7H₂O, 10; MnSO₄·H₂O, 2; CuCl₂·2H₂O, 1; ZnSO₄·7H₂O, 2; FeSO₄·7H₂O, 2; NaCl, 12; KI, 0.1; CoCl₂·6H₂O, 0.1; Na₂MoO₄·2H₂O, 0.5; AlCl₃·6H₂O, 1 and KF, 1.

³ Vitamin premix (mg kg⁻¹ diet): retinyl acetate, 40; cholecalciferol, 0.1; α-tocopheryl acetate, 80; menadione, 15; niacin, 168; riboflavin, 22; pyridoxine HCl, 40; thiamin mononitrate, 45; D-Ca pantothenate, 102; biotin, 0.4; folic acid, 10; vitamin B₁₂, 0.04; and inositol, 450.

⁴ Others: zoelite, 30; α-starch, 200; carboxymethylcellulose, 50; sodium dihydrogen phosphate, 25; betaine, 5.

assure maximum utilization of the limiting amino acid (Wilson 1989). The 270 g kg⁻¹ diets protein was supplied by fish meal, soybean protein concentrate and gelatine, and the remaining by a mixture of crystalline amino acids (CAAs) without lysine to simulate an amino acid profile found in 380 g kg⁻¹ whole body protein of black sea bream. The basal diet (diet 1) contained the minimum level of lysine from fish meal, soybean protein concentrate and gelatin, and the proximate ratios of synthetic/natural bound lysine to be zero to about 50% of total lysine in the diet. The final levels of lysine were confirmed by amino acid analysis and the values were 20.8, 25.2, 28.8, 32.5, 36.8 and 40.5 g kg⁻¹, respectively, by adding incremental levels of crystalline L-lysine ranging from zero to 20 lysine g kg⁻¹ diet (Table 3).

All diets were individually blended in a mixer and then homogenized after fish oil and corn oil were added.

	Supplied by 280 g fish meal kg ⁻¹ diet	Supplied by 120 g soybean protein concentrate kg ⁻¹ diet	Supplied by 20 g gelatine kg ⁻¹ diet	Supplied by crystalline amino acids	Total	380 g kg ⁻¹ whole body protein	Ta 2 Amino acid composition (g kg ⁻¹ diet) of dietary ingredients (excluding tryptophan)
Amino acids							
EAAs							
Val	10.6	4.8	0.4	9.1	24.9	24.9	
Leu	15.1	6.9	0.6	10.0	32.5	32.5	
Ile	9.2	4.2	0.3	8.4	22.1	22.1	
Met	4.4	1.3	0.1	8.7	14.5	14.5	
Phe	8.7	4.7	0.3	2.3	16.0	16.0	
Thr	7.1	2.8	0.4	8.1	18.4	18.4	
His	4.4	2.0	0.1	0.9	7.4	7.4	
Arg	13.0	6.0	1.3	7.4	27.6	27.6	
Lys	14.7	5.2	0.7	Variable	Variable	32.9	
NEAAs							
Glu	24.5	14.5	2.3	Variable	Variable	59.5	
Gly	14.7	3.4	5.0	1.4	24.5	24.5	
Ala	13.0	3.5	2.0	0.5	19.0	19.0	
Tyr	4.9	2.2	0.2	9.4	16.7	16.7	
Asp	16.7	9.1	0.2	15.3	41.3	41.3	
Ser	5.6	2.8	0.8	5.9	15.1	15.1	
Pro	10.4	3.5	0.1	2.6	16.6	16.8	

EAAs, essential amino acids; NEAAs, non-essential amino acids.

Ta 3 Analysed essential amino acid contents (excluding tryptophan) in the experimental diets

EAAs (g kg ⁻¹ diet)	Diets					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Val	21.9	21.5	21.6	22.2	22.1	21.9
Leu	33.6	32.9	33.2	33.1	33.9	33.3
Ile	22.6	22.3	21.9	22.2	22.1	22.1
Met	14.1	14.2	14.3	14.2	14.2	13.9
Phe	16.1	16.1	16.3	16.9	16.7	17.4
Thr	18.6	18.5	18.1	18.6	18.3	18.2
His	6.8	7.1	7.2	6.9	6.7	7.1
Arg	26.7	27.4	26.9	27.5	27.2	26.9
Lys	20.8	25.2	28.8	32.5	36.8	40.5

EAAs, essential amino acids.

During the mixing 6 N NaOH was added to establish a pH level of 7–8 according to the method described by Nose (1979). Gelatin was dissolved separately in a volume of water with constant heating and stirring and then transferred to the above mixture. Distilled water was then added to achieve a proper pellet consistency, and the mixture was further homogenized and extruded through a 3-mm die. The noodle-like diets were dried at 23 °C for 72 h with air condition and electrical fan. Dried noodles were broken into particles by a food processor, sieved to remove particles above 3 mm and then stored in a refrigerator at –20 °C. A representative sample was taken for proximate analysis.

Experiment procedure

Black sea bream were obtained from Marine Fisheries Research Institute of Zhejiang Province in Zhoushan, China. Prior to initiation of the feeding trial, all fish were kept in 300-L circular fiberglass tanks and fed with diet 1 for 2 weeks. At the beginning of the experiment, 20 uniform-sized and healthy fish (initial mean weight: 9.13 ± 0.09 g, mean \pm SD) were stocked in each fibreglass tank (300-L water volume). Each experimental diet was randomly assigned to triplicate tanks in a completely randomized design. Each tank was supplied with sand-filtered aerated seawater at a flow rate of 2 L min⁻¹. Fish were maintained under a natural photoperiod, the temperature and salinity of the seawater in tanks were 28 ± 1 °C and 29 g L⁻¹ respectively. Dissolved oxygen concentrations were above 5.0 mg L⁻¹ at any point during the experiment by using air stones with continuous aeration. Experimental fish were fed by hand twice daily (08:00 and 16:00 hours) which were fed slowly little by little to prevent waste of dietary pellets. When the experimental feeds were supplied, the fish would swim to the water surface to ingest the feeds. As long as fish were fed to satiation, they would never come up to water surface again. Hence, their apparent satiation could be judged by feeding behaviour observation, the feed losses could also be avoided almost completely. The experiment lasted for 8 weeks and feed consumption was recorded daily. Tanks were thoroughly cleaned as needed and mortality was checked daily.

and the modified procedure of (1976) for methionine and cystine, with performic acid at -10°C for 2 h. The residue was hydrolysed with methanesulfonyl chloride to form methanesulfone and cysteic acid, then extracted with distilled and deionized water. The freeze-dried residue was analysed as the process of other amino acids in a laboratory using an automatic amino acid analyser (model 105-50) equipped with a column (Hitachi 105-50) and a resin (Hitachi 105-50). Tryptophan could not be analysed by acid hydrolysis and it was excluded from the present experiment.

Protein analysis and statistical analysis

The following variables were calculated:

Weight gain rate (WGR %) = $100 \times (\text{final mean weight} - \text{initial mean weight}) / \text{initial mean weight}$ in g.

Specific growth rate (SGR) (% day^{-1}) = $100 \times (\ln \text{ final mean weight} - \ln \text{ initial mean weight}) / \text{day}$.

Feed conversion rate (FCR) = feed intake in g / (final body weight - initial body weight).

Protein efficiency ratio (PER) = weight gain in g / protein intake in dry basis in g.

Protein productive value (PPV) = protein gain in g / protein fed in dry basis in g.

Condition factor (CF) (g cm^{-3}) = $100 \times (\text{live weight, g}) / (\text{body length, cm})^3$.

Hepatopancreas index (HSI) = $100 \times (\text{liver weight, g}) / (\text{body weight, g})$.

Intraperitoneal fat ratio (IPR) = $100 \times (\text{intraperitoneal fat weight}) / (\text{body weight})$.

All data were subjected to analysis of variance and regression analysis where appropriate using SPSS for windows (version 16.0, USA). Differences between the means were tested by Tukey's multiple range test. Differences were considered significant at $P < 0.05$. The optimum dietary lysine requirement based on SGR, which was estimated by second-order polynomial regression analysis ($y = a + bx + cx^2$) (Zeitoun *et al.* 1976).

Results

Crude protein and L-lysine in juvenile bream were 16.5% and 0.15%, respectively. L-lysine for 56 days showed no outward pathological changes. The effect of dietary lysine in juvenile bream on growth, survival, and body composition with lysine from 0 to 1.5% showed no significant differences.

Ta 4 Growth performance, body-organ indices and feed utilization of black sea bream juvenile fed the diets with graded levels of lysine for 8 weeks

Parameters	Diets (lysine)					
	Diet 1 (20.8)	Diet 2 (25.2)	Diet 3 (28.8)	Diet 4 (32.5)	Diet 5 (36.8)	Diet 6 (40.5)
IBW	9.13 ± 0.13	9.13 ± 0.15	9.12 ± 0.10	9.13 ± 0.08	9.13 ± 0.08	9.10 ± 0.00
FBW	32.20 ± 0.13	34.32 ± 0.30	36.35 ± 0.44	37.77 ± 0.67	37.21 ± 0.43	34.35 ± 0.68
Survival	96.7 ± 5.8	96.7 ± 5.8	98.3 ± 2.9	95.0 ± 5.0	100.0 ± 0.0	91.7 ± 2.9
WG	252.6 ± 3.8 d	275.8 ± 4.5 c	298.7 ± 6.6 b	313.5 ± 4.6 a	307.4 ± 3.8 ab	277.4 ± 7.4 c
SGR	2.25 ± 0.02 c	2.39 ± 0.03 b	2.50 ± 0.03 a	2.53 ± 0.02 a	2.51 ± 0.02 a	2.40 ± 0.02 a
CF	1.93 ± 0.04 b	2.01 ± 0.02 a	2.02 ± 0.03 a	2.02 ± 0.03 a	2.04 ± 0.02 a	1.99 ± 0.01 a
HSI	1.99 ± 0.04	1.94 ± 0.15	1.94 ± 0.12	1.93 ± 0.13	1.86 ± 0.03	1.85 ± 0.04
IPR	2.55 ± 0.07 a	2.38 ± 0.23 b	2.37 ± 0.05 b	2.36 ± 0.07 b	2.34 ± 0.21 b	2.34 ± 0.17 b
FCR	1.36 ± 0.02 a	1.31 ± 0.02 a	1.24 ± 0.02 b	1.22 ± 0.02 b	1.24 ± 0.02 b	1.25 ± 0.01 b
PER	1.76 ± 0.03 c	1.84 ± 0.03 ab	1.92 ± 0.03 ab	1.96 ± 0.03 a	1.94 ± 0.04 a	1.94 ± 0.01 a
PPV	0.28 ± 0.02 b	0.29 ± 0.02 ab	0.32 ± 0.00 ab	0.34 ± 0.00 a	0.33 ± 0.00 a	0.32 ± 0.01 a

IBW, initial mean body weight; FBW, final mean body weight; SGR, specific growth rate; WG, weight gain; CF, condition factor; HSI, hepatopancreas index; IPR, intraperitoneal fat ratio; FCR, feed conversion rate; PER, protein efficiency ratio; PPV, protein productive value. Values are presented as mean ± SD ($n = 3$); values with different superscripts in the same row differ significantly ($P < 0.05$).

fish fed graded levels of lysine differed significantly among treatments. WGR of fish increased with increasing levels of lysine up to 32.5 g kg⁻¹ of diet and peaked at 313.5% ($P < 0.05$), beyond which it showed a declining tendency. SGR increased with increasing dietary lysine level up to 28.8 g kg⁻¹ (diet 3) and remained nearly the same thereafter, except for diet 6 which was lower than fish fed diet 3 to diet 5 ($P < 0.05$). The highest SGR (2.53% day⁻¹) was observed when dietary lysine reached 32.5 g kg⁻¹ (diet 4). Second-order polynomial regression analysis on the basis of SGR indicated that the optimum dietary lysine requirement of juvenile black sea bream was 33.2 g kg⁻¹ dry diet (86.4 g kg⁻¹ of dietary protein) (Fig. 1). The most efficient FCR and the highest PPV were observed in groups fed diet 4. Fish fed the diets exceeding 32.5 g kg⁻¹ lysine level did not show any

improvement in PER whereas the efficiency of protein utilization was reduced when fish fed the diets with lower lysine level ($P < 0.05$). HSI was higher in fish fed lysine deficient diets than in fish fed adequate lysine levels, but differences among the treatments were not significant ($P > 0.05$). The lowest CF (1.93), highest IPR (2.75) were observed for fish fed the diet 1 ($P < 0.05$), but remained nearly the same for other treatments ($P > 0.05$).

Body compositions were significantly affected by dietary lysine level ($P < 0.05$). The whole body, muscle and liver protein were positively correlated with dietary lysine level, while lipid was negatively correlated with it. The ash and moisture contents were independent of dietary treatment (Table 5).

Lysine contents of fish muscle were significantly affected by dietary lysine levels ($P < 0.05$). Fish fed the diet with 20.8 g kg⁻¹ lysine showed the lowest lysine content (8.99 g kg⁻¹) in dorsal muscle, while fish fed the diet with 32.5 g kg⁻¹ lysine had the highest value (9.69 g kg⁻¹). The concentration of Val and His were more variable and was not related to dietary treatment. However, other EAAs showed an increasing trend with increasing dietary lysine levels except Thr content decreased ($P < 0.05$). Increasing dietary lysine level enhanced EAAs contents of muscle ($P < 0.05$) although there was a slight decline in fish fed the diet 5 and diet 6 (Table 6).

In serum profile, total protein content increased with increasing dietary lysine level ($P < 0.05$) (Table 7). Serum-free lysine remained at a relatively low and constant level up to fish fed diet 3. However, an increased level was observed in fish fed diet 4 and diet 5 thereafter and return

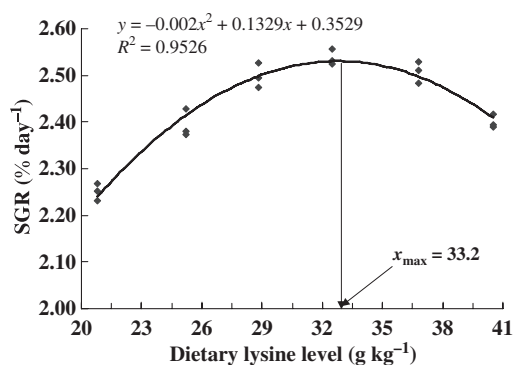


Fig 1 The relationship between specific growth rate (SGR, % day⁻¹, .) and dietary lysine levels (g kg⁻¹, .) in juvenile black sea bream.

Table 5 Effect of dietary lysine level on proximate compositions of whole body, dorsal muscle and liver of juvenile black sea bream (g kg⁻¹ live weight)

Parameters	Diets (lysine g kg ⁻¹)					
	Diet 1 (20.8)	Diet 2 (25.2)	Diet 3 (28.8)	Diet 4 (32.5)	Diet 5 (36.8)	Diet 6 (40.5)
Whole body						
Protein	169.7 ± 1.4 c	172.0 ± 1.8 b	173.7 ± 1.7 b	176.3 ± 1.7 ab	177.5 ± 1.4 ab	179.2 ± 2.0 a
Lipid	113.8 ± 2.6 a	111.4 ± 2.5 a	109.5 ± 5.5 b	103.7 ± 1.9 b	102.3 ± 3.2 b	101.2 ± 2.5 b
Ash	51.0 ± 0.5	51.8 ± 0.2	51.6 ± 2.1	52.7 ± 0.9	52.8 ± 2.3	53.9 ± 1.3
Moisture	658.0 ± 4.8	661.5 ± 3.7	658.5 ± 5.8	660.8 ± 4.8	659.8 ± 3.4	659.2 ± 3.5
Muscle						
Protein	188.9 ± 0.6 bc	197.6 ± 1.1 b	203.6 ± 0.5 ab	207.8 ± 1.6 a	205.6 ± 2.1 ab	209.0 ± 0.8 a
Lipid	75.7 ± 4.3 ab	77.9 ± 4.8 a	72.7 ± 8.2 b	67.7 ± 4.9 c	68.9 ± 6.3 c	67.1 ± 5.7 c
Ash	14.5 ± 0.8	13.4 ± 0.1	14.1 ± 0.2	14.0 ± 0.9	13.7 ± 0.1	13.8 ± 2.0
Moisture	728.9 ± 10.4	713.6 ± 20.8	716.1 ± 19.1	710.5 ± 9.9	718.4 ± 16.7	713.8 ± 26.7
Liver						
Protein	225.9 ± 4.2 c	226.4 ± 4.9 bc	230.8 ± 2.6 b	241.2 ± 1.6 a	248.5 ± 1.9 a	247.0 ± 2.5 a

to relatively low level in fish fed diet 6 similar to those observed in fish fed diet 1 to diet 3. TG and GLU contents were more variable and could not be related to dietary

treatments. From Table 8, it can be seen that dietary lysine concentrations had no significant effect on WBC and RBC, however, Hb was significantly different among treatments

Ta 8 Haematological characteristics of juvenile black sea bream fed graded levels of dietary lysine for 8 weeks

Parameters	Diets (lysine g kg ⁻¹)					
	Diet 1 (20.8)	Diet 2 (25.2)	Diet 3 (28.8)	Diet 4 (32.5)	Diet 5 (36.8)	Diet 6 (40.5)
Haemoglobin (g 100 mL ⁻¹)	11.10 ± 0.24 c	11.61 ± 0.21 b	11.89 ± 0.59 b	12.65 ± 0.15 a	12.05 ± 0.46 ab	11.26 ± 0.32 c
WBC (×10 ⁹ L ⁻¹)	4.37 ± 0.18	4.44 ± 0.40	4.88 ± 0.49	5.18 ± 0.32	4.84 ± 0.21	4.78 ± 0.21
RBC (×10 ¹² L ⁻¹)	5.32 ± 0.16	5.28 ± 0.16	5.20 ± 0.22	5.18 ± 0.19	5.08 ± 0.16	4.92 ± 0.17

WBC, white blood cell count; RBC, red blood cell count.

Values are presented as mean ± SD (*n* = 3); values with different superscripts in the same row differ significantly (*P* < 0.05).

and reached the top at 12.85 g 100 mL⁻¹ when fish fed diet 4 (*P* < 0.05).

Discussion

The present study results indicated that lysine is essential for growth of juvenile black sea bream, and this marine omnivorous fish is able to utilize crystalline lysine. Dose–response experiments with increasing supply of amino acid are accepted in principle as a method for determining dietary amino acid requirements (Cowey 1995). The optimal dietary crystalline lysine requirement for juvenile black sea bream was estimated to be 3.35% dry diet, corresponding to 8.71% dietary protein. This value is higher to values reported for certain other species, milkfish (4.0%, Borlongan & Benitez 1990); catla (6.20%, Ravi & Devaraj 1991); African catfish (5.7%, Fagbenro *et al.* 1998); striped bass (4.9%, Small & Soares 2000); Indian major carp (5.75%, Ahmed & Khan 2004); grass carp (5.44%, Wang *et al.* 2005); black catfish (4.5%, Montes-Girao & Fracalossi 2006); grouper (5.56%, Luo *et al.* 2006), Japanese seabass (5.80–6.07%, Mai *et al.* 2006); gilthead seabream (5.04%, Marcouli *et al.* 2006). These wide variations observed in lysine requirements among fish species may be the result of laboratory variances: differences in dietary protein sources, the reference protein which amino acid pattern is being imitated (Forster & Ogata 1998), different diet formulation and digestible energy (Encarnação *et al.* 2004), different feeding regime and allowance, environmental condition, and using fish of different ages, sizes and species (strains) (Akiyama *et al.* 1997). Variations may also be attributed to differences in techniques used to calculate requirement (Kim *et al.* 1992). Some previous researches have suggested fish do not appear to utilize dietary CAAs as effectively as intact protein (Cowey and Luquet, 1983; Walton *et al.*, 1986; Wilson & Halver 1986), because CAAs added to the diet in the free form are more readily absorbed than protein-bound amino acids (Yamada *et al.*, 1981; Murai *et al.*, 1982), causing an imbalance in

amino acid profile in the tissue and diverting amino acids into catabolic rather than anabolic processes (Cowey & Sargent 1979). In our study, the WG and SGR varied from 252% to 313%, and 2.25 to 2.53, respectively. The growth performance seemed not inferior to those fed the intact-protein or precoating amino acids, which implied the black seabream might utilize CAAs more effectively than same fish species. Some previous studies reported that at least 90% or more of the dietary total amino acid should have been consumed up by the fish, which indicated that the leaching loss of lysine could be considered to be negligible and the relative poor growth was not due to leaching (Cheng *et al.* 2003; Alam *et al.* 2004 and Espe *et al.* 2007). In our present study, feeds were offered by hand carefully and slowly, in addition, the experimental black sea bream were all domesticated with high feeding activity, and the granulated feed could be scrambled and ate by fish immediately (<3 s). Thus, leaching loss of feed and crystalline lysine could be avoided almost completely. However, the total lysine concentration was partly contributed by endogenous lysine found in fishmeal, soybean protein concentrate and gelatin, when taking digestibility of the dietary ingredients into consideration, the actual lysine requirement maybe a little lower than the estimated value to formulate cost-effective and amino acid balanced diets for this fish species. Reduced growth, feed utilization, PER and PPV were observed in fish fed still higher amount of lysine (diet 5 and diet 6), as reported in other fish species such as salmonids (Anderson *et al.* 1993), Indian major carp (Murthy & Varghese 1997) and Japanese seabass (Mai *et al.* 2006). It may be due to the negative effects (lysine–arginine antagonism) of excessive amount of free lysine at this level. Dietary lysine–arginine antagonism has been well documented in poultry and rats (Jones 1964; Harper *et al.* 1970; Fico *et al.* 1982), but there are controversies in fish, further research is needed to clarify this aspect.

The negative correlation between HSI and dietary lysine level was also observed in European sea bass (Tibaldi *et al.* 1994) and gilthead sea bream (Marcouli *et al.* 2006). In our

study, protein retention decreased in fish fed lower lysine diets. Peres & Oliva-Teles (2008) pointed out as lysine deficiency limited protein synthesis, AA not used for protein synthesis might have been converted after deamination to lipids or glycogen and deposited in the liver. It also possibly explained this result and IPR reducing in present experiment.

Jeerson & Kimball (2001) suggested that lack of lysine in dietary may led to intracellular accumulation of non-aminoacyl-tRNA, the activity of protein kinase A (GCN2 general control non-derepressible protein kinase) and eIF-2 α eukaryotic initiation factor phosphatase were decreased, then eIF-2 α phosphorylation occurred accompanied by reduced activity of eIF-2 β at the same time, as the result, the peptide chain initiation inhibited. In the present study, fish fed diets with high levels of lysine showed significant increase of whole body protein when compared with that of fish fed the low lysine diets, while the highest lipid content in whole body was noted with fish fed the control diet; the variation showed an opposite trend compared to protein, which was in agreement with previous reports (Rodehutscord *et al.* 2000; Luo *et al.* 2006). The carnitine pool in fish is derived from both endogenous synthesis and diet. Carnitine is synthesized from lysine and methionine. After synthesis, the carnitine must be transported to other tissues. It is most concentrated in tissues that use fatty acids as their primary dietary fuel, such as skeletal and cardiac muscle (Harpaz 2005). Burtle & Liu (1994) reported whole body lipid content of channel catfish was reduced by supplemented dietary carnitine, lysine or both. Carnitine as well as lysine plays an important role in promoting the transport of long-chain fatty acids across the inner mitochondrial membrane, resulting in extra energy from β -oxidation. Therefore, dietary lysine supplements should enhance the oxidation of these fatty acids, thereby

Increasing dietary lysine content elevated protein concentration in the serum, cholesterol and triacylglycerol declined by and large among different diets, however GLU was variable and not related to dietary treatments as pointed out in groupers (Luo *et al.* 2006). According to Regost *et al.* (1999) the decrease in whole body fat content along with the decrease in plasma triacylglycerol concentrations suggests lipid mobilization in those groups exhibiting very poor growth, which was not observed in the present investigation. Tissue cholesterol concentrations are known to vary depending on the nutritional status of fish (Kaushik *et al.* 1995; Regost *et al.* 1999). Little information is currently available on the effect of lysine on blood characteristics, and more investigations are needed. To support the requirement estimated by growth and feed utilization, levels of free lysine in serum were analysed. Serum-free lysine taken after 6 h remained at a relatively low and constant level up to fish fed diet 3. However, a notably increased free lysine level was observed in fish fed diet 4 and diet 5 ($P < 0.05$), thereafter the values returned to relatively low level in fish fed diet 6 similar to the first three groups. Robinson *et al.* (1981) pointed out that serum levels of free arginine depended not only on dietary arginine concentration, but also on interrelations with other dietary amino acids. Berge *et al.* (1998) suggested the decreased level of lysine in plasma following high dietary supplementation may be caused by an increased oxidation of this amino acid. Oxidative enzymes do not allow the concentrations of EAA to rise in blood (Millward & Rivers 1998). The findings of present experiment indicated there may be a homeostatic mechanism for the regulation of free serum lysine in this fish species.

To our knowledge, there is little information on the effects of dietary lysine on haematological characteristics of fish. The paramount function of the RBC is generally reckoned to be oxygen carriage. RBC are both mechanical and biochemical barriers against infections, bacteria, and blood parasites. Immune reactions are regulated to ensure harmony between the RBC and WBC populations. Gray (1963) reported the lysine deficiency could result in 22% serum white blood cells decrease of rats. Hb in aquatic animals operates over wide and independent variations in oxygen at the sites of loading and unloading and shows adaptations both to environmental conditions and to metabolic requirements, which govern oxygen availability and transport to tissues (Weber & Wells 1989). In our study, the Hb concentration was significantly ($P < 0.05$) higher at 32.5 g kg⁻¹ dietary lysine inclusion compared to the other dietary levels, excepting 36.8 g kg⁻¹ lysine which showed no difference with that of 32.5 g kg⁻¹. WBC presented the similar trend as Hb but

without significant difference ($P > 0.05$). The RBC declined from diet 1 to diet 6 ($P > 0.05$). In the study on lysine requirement of yellowtail, Hb and RBC were not influenced by dietary lysine, however, haematocrit was significantly different between fish fed different diets (Ruchimat *et al.* 1997). The oppression of growth performance and feed efficiency may be likely to relate with the RBC and Hb in serum, because when fish fed with excessive lysine dietary, the transport of oxygen and nutrient substance were affected by the decrease in the concentration of RBC and Hb in serum.

In conclusion, results of the present investigation indicate that the lysine requirement of juvenile black sea bream is little higher than other species. Second-order polynomial regression analysis based on SGR showed that L-lysine requirement for juvenile black sea bream (initial average weight, 9.13 ± 0.09 g) was 33.2 g lysine kg⁻¹ of the dry diet or 86.4 g lysine kg⁻¹ dietary protein. The level is also suitable for black sea bream protein and EAAs accretion as well as physiological parameter. The data generated in the present study would be useful in developing lysine-balanced practical diets for the intensive culture of this species. Evaluation of other EAAs requirements, the effect of coating EAAs and the interaction among EAAs should be conducted in future.

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