thereby freeing intramitochondrial coenzyme A to participate in the B-oxidation and tricarboxylic acid cycle pathways, by which could avoid accumulation of lipids in fish body (Ozorio . 2003). According to Baker & Han (1994), lysine should be used as the reference amino acid for estimating other amino acid requirements because lysine is the only one that does not present endogenous synthesis and, unlike the sulphur amino acids, is exclusively required for body protein deposition. Furthermore, lysine is of particular concern because it is the EAA found in the highest concentration in the carcass of many fish species (Wilson & Poe 1985; Kim & Lall 2000). Therefore, it is of high priority to evaluate the dietary lysine requirement in order to formulate a more cost-e ective diet, as protein is usually the most expensive feed component.

Black sea bream (*r ro*) is an economically important food fish cultured in Japan, China and some other countries of Southeast Asia (Ma . 2007; Shao et al. 2008). It grows fast and has high market value (Hong & Zhang 2003). It is a euryhaline omnivorous species and can thrive in natural waters of salinity ranging from 4.1 to 35.0 g L^{-1}. However, traditional feed for farmed black sea bream is the limited supply of chopped or minced trash fish. This type of diet is di cult to store, has variable nutritional 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 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.

Tab 1 Composition and proximate analysis of the experimental diets (g kg^{-1} diet)

	Diets no.					
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal	280	280	280	280	280	280
Soybean protein concentrate	120	120	120	120	120	120
Gelatine	20	20	20	20	20	20
Crystalline amino acid premix[1]	90	90	90	90	90	90
Lys	0	4	8	12	16	20
Glu	20	16	12	8	4	0
Fish oil	50	50	50	50	50	50
Corn oil	80	80	80	80	80	80
Mineral premix[2]	15	15	15	15	15	15
Vitamin premix[3]	15	15	15	15	15	15
Others[4]	310	310	310	310	310	310
Total	1000	1000	1000	1000	1000	1000
Proximate analysis (g kg^{-1} dry matter)						
Crude protein	385.0	383.7	386.4	384.4	387.3	387.8
Crude lipid	148.4	150.6	149.9	152.0	148.5	149.1
Ash	120.4	120.6	120.3	120.1	120.0	120.1
Moisture	68.9	70.9	70.3	69.1	69.6	68.4
Lys	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): Na_2SiO_3, 0.4; $CaCO_3$, 350; $NaH_2PO_4{\cdot}H_2O$, 200; KH_2PO_4, 200; $MgSO_4{\cdot}7H_2O$, 10; $MnSO_4{\cdot}H_2O$, 2; $CuCl_2{\cdot}2H_2O$, 1; $ZnSO_4{\cdot}7H_2O$, 2; $FeSO_4{\cdot}7H_2O$, 2; NaCl, 12; KI, 0.1; $CoCl_2{\cdot}6H_2O$, 0.1; $Na_2MoO_4{\cdot}2H_2O$, 0.5; $AlCl_3{\cdot}6H_2O$, 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 B_{12}, 0.04; and inositol, 450.
[4] Others: zoelite, 30; α-starch, 200; carboxymethylcellulose, 50; sodium dihydrogen phosphate, 25; betaine, 5.

Tab 2 Amino acid composition (g kg^{-1} diet) of dietary ingredients (excluding tryptophan)

Amino acids	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
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.

Tab 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 ± 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.

Analytical procedures

At the end of the 8-week growth trial, based on our preliminary experiment to test the response of serum free amino acid to feeding in black sea bream, the peak level was obtained at 6 h after feeding. Therefore, collection of three fish from each tank was taken 6 h after feeding to measure serum free lysine, and the samples were deproteinized with 5-sulfosalicylic acid according to the method of Cross . (1975) and determined using an automatic amino acid analyser (Hitachi, Model L-8500A, Hitachi, Tokyo, Japan). Then remaining fish were starved for 24 h after the last feeding, and three fish from each tank were anaesthetized (MS-222, Sigma, St Louis, MO, USA at 80 mg L^{-1}) and then stored at −20 °C for subsequent whole body proximate analysis. Dorsal muscles and livers were obtained from all the remaining fish in each tank and stored at −20 °C for subsequent proximate analysis. Blood samples were drawn from the caudal vein of five fish per tank with a 27-gauge needle and 1 mL syringe. Aliquots of blood samples were used to determine blood characteristics [haemoglobin (Hb), red blood cell count (RBC) and white blood cell count (WBC)], and the remaining samples were centrifuged at 836 ***g*** for 10 min (4 °C) to obtain serum to measure nutrient levels (Ai . 2006), and serum was stored at −20 °C until use.

Pooled samples of liver, dorsal muscle and whole fish in each tank were analysed in triplicate for proximate composition. Moisture, ash, protein and lipid were determined following methods of the Association of O cial Analytical Chemists (AOAC 1984). Moisture concentration was determined by drying minced samples for 6 h in a forced-air oven maintained at 105 °C. Ash content was analysed by incinerating samples at 600 °C overnight in a mu e furnace; protein was estimated as Kjeldahl-nitrogen using factor 6.25 and lipid was determined by Soxhlet extraction with petroleum ether for 6 h. The serum protein, total cholesterol, triacylglycerol, glucose concentration (GLU) in juvenile black sea bream were all measured within 3 days, using the diagnostic reagent kit purchased from Nanjing Jiancheng Bioengineering Institute (China) according to the manufacturer's instructions. Haematological characteristics of juvenile black sea bream were determined using automated hematology analyzer (CELL-DYN, 3200, System, USA). The diets and dorsal muscle of fish used for analysis of amino acid content were freeze-dried at 55 °C for 48 h, and then hydrolyzed with 6 N HCl at 110 °C for 24 h and the chromatographic separation and analysis of the amino acids was performed after orthophthaldehyde (Sigma) derivation using reverse-phase high performance liquid chromatography (HPLC, HP1100, USA) followed the mod d proc Gardner & Miller (1980). While for methio e and the samples were oxidized with performic a t −1 3 h to obtain methionine sulfone and cy aci freeze-dried twice with deionized water. T eez ingredients were analysed as the process of ot ino by a commercial laboratory using an automat o analyser (Hitachi 835–50) equipped with a colu itac custom ion exchange resin no. 2619). Tryptopha d not be detected after acid hydrolysis and it was excl om analysis at the present experiment.

Calculation and statistical analysis

The following variables were calculated:

Weight gain rate (WGR %) = 100 × (final mean weight − initial mean weight) in g/initial mean weight in g.

Specific growth rate (SGR) (% day^{-1}) = 100 × (ln final mean weight − ln initial mean weight)/day.

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

Protein e ciency 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 × (live weight, g)/(body length, cm)^3.

Hepatopancreas index (HSI) = 100 × (liver weight, g)/(body weight, g).

Intraperitoneal fat ratio (IPR) = 100 × (intraperitoneal fat weight)/(body weight).

All data were subjected to analysis of variance and regression analysis where appropriate using SPSS for windows (version 16·0, USA). Di erences between the means were tested by Tukey's multiple range test. Di erences were considered significant at < 0.05. The optimum dietary lysine requirement based on SGR, which was estimated by second-order polynomial regression analysis (= + + ^{2}) (Zeitoun . 1976).

Results

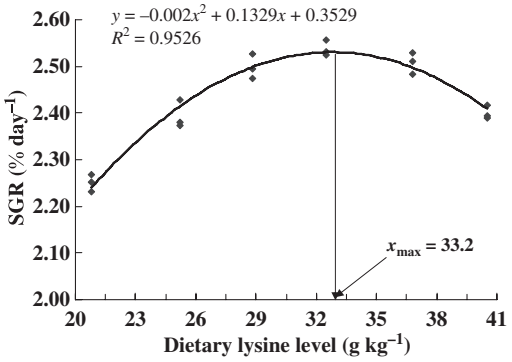
Growth performance, body-organ indices and feed utilization for juvenile black sea bream given graded levels of L-lysine for 56 days are shown in Table 4. No evidence of outward pathological signs was noted in fish given low levels of dietary lysine in our experiment. Survival of fish fed diets with lysine from 20.8 to 40.5 g kg^{-1} was above 90% and showed no significant di erence. The growth rates of juvenile

Tab 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

	Diets (lysine)					
Parameters	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 di ered 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% (< 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 (< 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 e cient 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



F 1 The relationship between specific growth rate (SGR, % day^{-1},) and dietary lysine levels (g kg^{-1},) in juvenile black sea bream.

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

Body compositions were significantly a ected by dietary lysine level (< 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 a ected by dietary lysine levels (< 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 was 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 (< 0.05). Increasing dietary lysine level enhanced EAAs contents of muscle (< 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 (< 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

Tab 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)

	Diets (lysine g kg^{-1})					
Parameters	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 l 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 e ect on WBC and RBC, however, Hb was significantly di erent among treatments

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

	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)
	0 ± 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
	18	4.44 ± 0.40	4.88 ± 0.49	5.18 ± 0.32	4.84 ± 0.21	4.78 ± 0.21
		5.28 ± 0.16	5.20 ± 0.22	5.18 ± 0.19	5.08 ± 0.16	4.92 ± 0.17

d blood cell count.
3); values with different superscripts in the same row differ significantly ($P < 0.05$).

hen fish fed diet

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 e ectively than same fish species. Some previous studies reported that at least 90% or e of the dietary total amino acid should have been con-up by the fish, which indicated that the leaching loss ould be considered to be negligible and the relative was not due to leaching (Cheng . 2003; 4 and Espe . 2007). In our present study, by hand carefully and slowly, in addition, sea bream were all domesticated with nd the granulated feed could be mediately (<3 s). Thus, leaching ine could be avoided almost lysine concentration was sine found in fishmeal, latin, when taking onsideration, the than the esti-o acid bal-th, feed still in

(4.5%, Montes-Girao & Fracalossi 2006); grouper (5.56%; Luo . 2006), Japanese seabass (5.80–6.07%, Mai . 2006); gilthead seabream (5.04%, Marcouli . 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), di erent diet formulation and digestible energy (Encarnação . 2004), di erent feeding regime and allowance, environmental condition, and using fish of different ages, sizes and species (strains) (Akiyama . 1997). Variations may also be attributed to di erences in techniques used to calculate requirement (Kim . 1992). Some previous researches have suggested fish do not appear to utilize dietary CAAs as e ectively as intact protein (Cowey and Luquet, 1983; Walton ., 1986; Wilson & Halver 1986), because CAAs added to the diet in the free form are more readily absorbed than protein-bound amino acids (Yamada ., 1981; Murai ., 1982), causing an imbalance in

other fish species such as salmo
Indian major carp (Murthy & Varghe
seabass (Mai . 2006). It may be due to
(lysine–arginine antagonism) of excessive an
lysine at this level. Dietary lysine–arginine antago
been well documented in poultry and rats (Jones 1
Harper . 1970; Fico . 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 . 1994) and gilthead sea bream (Marcouli . 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.

Je erson & 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-2α eukaryotic initiation factor phosphatase were decreased, then eIF-2α phosphorylation occurred accompanied by reduced activity of eIF-2β 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 . 2000; Luo . 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 di erent diets, however GLU was variable and not related to dietary treatments as pointed out in groupers (Luo . 2006). According to Regost . (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 . 1995; Regost . 1999). Little information is currently available on the e ect 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 (< 0.05), thereafter the values returned to relatively low level in fish fed diet 6 similar to the first three groups. Robinson . (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 . (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 e ects 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 bloods 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 (< 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 di erence with that of 32.5 g kg^{-1}. WBC presented the similar trend as Hb but without significant di erence (> 0.05). The RBC declined from diet 1 to diet 6 (> 0.05). In the study on lysine requirement of yellowtail, Hb and RBC were not influenced by dietary lysine, however, haematocrit was significantly di erent between fish fed di erent diets (Ruchimat . 1997). The oppression of growth performance and feed e ciency 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 a ected 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 e ect of coating EAAs and the interaction among EAAs should be conducted in future.

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