www.trjfas.org
ISSN 1303-2712
*Turkish Journal of Fisheries and Aquatic Sciences 14: 633-642 (2014)* DOI: 10.4194/1303-2712-v14_3_05

# Effects of Supplementation Coated Lysine and Methionine in Mixture Protein Diets on Growth Performance, Digestibility and Serum Biochemical Indices of Juvenile Black Sea Bream, *Acanthopagrus schlegelii*

**Jing Lu[1], Ying Hua[1], Wen-zhong Fu[1], Fan Zhou[2], Bin-bin Yang[1], Jin-xing Xiao[3], Min-hai Liu[4], Qing-jun Shao[1,3]**

[1] Zhejiang University, College of Animal Sciences, Hangzhou, China.
[2] Zhejiang Fisheries Technical Extension Station, Hangzhou, China.
[3] Zhejiang University, Ocean Research Center of Zhoushan, Zhoushan, China.
[4] Zhoushan Fisheries Research Institute, Zhejiang Province, Zhoushan, China.

* Corresponding Author: Tel.: +187.671 59275; Fax: +187.671 59275;
E-mail: qjshao@zju.edu.cn

Received 16 September 2013
Accepted 9 July 2014

**Abstract**

Four experimental diets were formulated to evaluate the effect of coated lysine (Lys) and methionine (Met) supplemented in mixture protein diets of juvenile black sea bream, *Acanthopagrus schlegelii*. Fish meal (FM) was the main protein source in control diet, and 21.2% FM was replaced by the mixture of soybean protein concentrate, meat and bone meal, and monosodium glutamate protein in replacement diets with coating Lys and Met were suppled at none (0%, 0%), low (1.7%, 1.1%) and high (5.0%, 3.4%) level (designated as NLM, LLM and HLM diet, respectively). Each diet was fed to triplicate groups of 20 fish (initial mean weight, 4.13±0.16 g) twice a day to apparent satiation for 8 weeks, in a flow-through system. Results showed that amino acid supplementation significantly improved weight gain and specific growth rate in these mixture protein diets groups, and the fish fed with HLM diet showed comparable growth with the control group. Feed efficiency ratio (FER), protein efficiency ratio (PER), and crude protein and Met content in dorsal muscle were significantly affected by diet amino acid level. The highest apparent digestibility coefficients appeared in HLM group but the values showed insignificant difference with the control group. Serum activities of aspertate aminotransferase and alanine aminotransferase in control group were lower than those of in other treatments. Fish fed with NLM and LLM diets had higher triglycerides concentration than those of fed the HLM and FM diets. Furthermore, the feed cost of the various feed treatments were evaluated, and the three test diets were all lower than the control diet. The present study indicated that 21.2% FM in black sea bream diet can be successfully replaced by mixture protein with sufficient coated Lys (5.0%) and Met (3.4%) supplemented.

*Keywords*: Lysine; methionine; mixture protein diet; black sea bream; growth performance.

## Introduction

Over the past decades, there have been a rapid increase in the global aquaculture, as well as the price of fish meal which is generally considered to be the most ideal protein source for aquatic animals (Trushenski *et al*., 2006). As the result, aquafeed manufacturers are focused on alternative proteins used in least-cost diet formulations, especially for plant proteins (Ambardekar and Reigh, 2007). However, high level of plant proteins inclusion in aquafeed usually causes severe essential amino acids (EAAs) unbalance in feed, resulting in poor growth and feed utilization rate but higher feeding cost (Abimorad *et al*., 2009). Soybean protein concentrate (SPC), meat and bone meal (MBM) are common alternative protein resource, while monosodium glutamate protein (MGP) is an potentially emerging protein resource in China, and several studies have reported the use of them in aquafeeds, however, EAAs unbalance in these proteins limit the high level of replacement (Zhao *et al*., 2010; Ngandzali *et al.*, 2011; Ye *et al.*, 2011; Zhou *et al.*, 2011a).

In recent years, many reports showed that the adverse effects could be eliminated by adding several purified limited amino acids, as proved in red sea bream (*Pagrus major*) (Takagi *et al*., 2001), rainbow trout (*Oncorhynchus mykiss*) (Davies and Morris, 1997; Gaylord and Barrows, 2009), catfish (*Ictalurus punctatus*) (Robinson and Li, 2007), and Nile tilapia (*Oreochromis niloticus* L.) (Viola and Lahav, 1991; El-Saidy and Gaber, 2007; Robinson and Li, 2007). At present, it has been accepted that fish utilized coated amino acids better than crystalline amino acids (Murai *et al*, 1982; Ambardekar and Reigh, 2007; Chi *et al*., 2011). Among all the EAAs, lysine (Lys) or methionine (Met) is often one of the most limited amino acids in ingredients of commercial aquafeeds, especially when fish meal is replaced by plant protein sources (Hardy and Barrows, 2002; Gatlin *et al*., 2007). It was found that Lys and Met both contributed to serves as a precursor to carnitine, which is involved in the transportation of long chain fatty acyl groups into the mitochondria for beta oxidation (Harpaz, 2005), and Lys played an important role in the development of nervous system (Hauler and Carter, 2001).

Black sea bream (*Acanthopagrus schlegelii*) is an economically important cultured fish in Japan, China and some other countries of Southeast Asia (Nip *et al*., 2003). With its high nutritional value and favorable biological characteristics such as fast growing, high disease resistance, euryphaline and eurphagous (Hong and Zhang, 2003), this fish species is excepted to be one of the most promising fishes in intensive aquaculture. In previous researches, the optimal Lys (Zhou *et al*., 2010a) and Met (Zhou *et al*., 2011b) level in diet for juvenile black sea bream have been estimated, respectively. Therefore, this study was aimed to evaluate the effects of supplying coated Lys and Met in mixture protein sources based diets on growth performance, digestibility and serum biochemical indices of juvenile black sea bream. The results will help to get more knowledge on EAAs utilization of this fish species as well as effectively transfer technology to the feed industries.

## Materials and Methods

### Experiment Diets

Ingredients and proximate composition of experimental diets are presented in Table 1, and amino acid compositions of experimental diets are showed in Table 2. Four isonitrogenous and isoenergetic diets were formulated and FM was used as the main protein source in the control diet (FM group). In test diets, 21.2% FM was replaced by

**Table 1.** Composition and proximate analysis of experimental diets (g $kg^{-1}$ diet)

| Ingredients | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| Fish meal | 530 | 318 | 318 | 318 |
| Fermented soybean meal | 80 | 80 | 80 | 80 |
| Monosodium glutamate protein | -- | 50 | 50 | 50 |
| Soybean protein concentrate | -- | 95 | 95 | 95 |
| Meat and bone meal | -- | 80 | 80 | 80 |
| Wheat flour | 200 | 200 | 200 | 200 |
| Fish oil | 40 | 40 | 40 | 40 |
| Corn oil | 50 | 50 | 50 | 50 |
| $Ca(H_2PO_4)_2$ | 20 | 20 | 20 | 20 |
| Betaine | 5 | 5 | 5 | 5 |
| Vatimin premix [1] | 10 | 10 | 10 | 10 |
| Mineral premix [2] | 10 | 10 | 10 | 10 |
| Zeolite flour | 54.0 | 41.0 | 38.2 | 32.6 |
| Lys | -- | -- | 1.7 | 5.0 |
| Met | -- | -- | 1.1 | 3.4 |
| $Y_2O_3$ | 1 | 1 | 1 | 1 |
| Total | 1000 | 1000 | 1000 | 1000 |
| Proximate analysis (g $kg^{-1}$ dry matter) [3] | | | | |
| Moisture | 119.0 | 101.7 | 98.2 | 115.6 |
| Crude protein | 405.4 | 417.7 | 418.5 | 424.1 |
| Crude lipid | 137.4 | 134.0 | 131.3 | 133.8 |
| Lys | 29.2 | 25.6 | 26.4 | 28.6 |
| Met | 12.1 | 8.4 | 8.8 | 11.1 |

[1] Vitamin premix (mg $kg^{-1}$ diet): retinyl acetate, 40; cholecalciferol, 0.1; a-tocopheryl acetate, 80; niacin, 168; riboflavin, 22; pyridoxine HCl, 40; thiamin mononitrate, 45; *D*-Capantothenate, 102, biotin, 0.4; folic acid, 10; vitamin $B_{12}$, 0.04; ascorbic acid, 1000 and inositol, 450.
[2] Mineral premix (g $kg^{-1}$of premix): $Na_2SiO_3$, 0.4; $CaCO_3$, 350; $NaH_2PO_4·H_2O$, 200; $KH_2PO_4$, 200; $MgSO_4·7H_2O$, 10; $MnSO_4·H_2O$, 2; $CuCl_2·2H_2O$, 1; $ZnSO_4·7H_2O$, 2; $FeSO_4·7H_2O$, 2; NaCl, 12; KI, 0.1; $CoCl_2·6H_2O$, 0.1; $Na_2MoO_4·2H_2O$, 0.5; $AlCl_3·6H_2O$, 1 and KF, 1.
[3] Values for the proximate analysis of the test diets are means of triplicate analyses.

**Table 2**. Essential amino acids (EAAs) compositions of experimental diets

| EAAs (g $kg^{-1}$ diet) | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| Phe | 17.7 | 18.1 | 17.8 | 18.0 |
| Met | 12.1 | 8.4 | 8.8 | 11.0 |
| Leu | 35.8 | 35.3 | 34.8 | 35.6 |
| Val | 23.6 | 23.2 | 22.5 | 23.5 |
| Arg | 27.3 | 27.5 | 26.8 | 26.9 |
| Lys | 29.2 | 25.6 | 26.4 | 28.6 |
| Ile | 18.5 | 18.0 | 17.3 | 18.3 |
| His | 9.4 | 9.1 | 9.0 | 9.1 |
| Thr | 17.6 | 16.7 | 16.5 | 16.6 |

mixture of SPC, MBM, MGP, and coating Lys and Met were supplied at none (0%, 0%), low (1.7%, 1.1%) and high (5.0%, 3.4%) level to formulate NLM, LLM and HLM diets, respectively. Lys and Met supplemented in diet HLM was according to these two amino acids level in FM diet. Experimental Lys and Met were slow-release coated amino acids (purity 50%, hydrogenated palm oil as the main coating ingredients) supplied by Hangzhou King Technology Feed Company, LTD (Hangzhou, China).

In preparing experimental diets, drying ingredients were ground into particles with a diameter within 180-μm, and yttrium oxide ($Y_2O_3$) was used as exogenous indicator in digestive test (Alan Ward *et al*., 2005). After weighed and mixed manually for 5 minutes, all ingredients (as well as coated Lys and Met) were transferred to a food mixer for another 15-minute mixing. During the mixing, fish oil and corn oil added slowly along with distilled water to achieve a proper consistency. Subsequently, the mixture was homogenized and extruded through a 2.5-mm die by a pelletizer (Modle HKJ-218, China). After dried at 23 °C for 72 h with air condition and electrical fan, feeds were stored in a freezer at -20°C until used.

**Feeding Trial**

Experimental juvenile black sea bream were obtained from the Research Institute of Zhejiang Marine Fisheries in Zhoushan (China). Prior to initiation of the feeding trial, all fish were fed the control diet for 2 weeks to acclimate to the experimental diet. Then 240 healthy fish with the same size (initial mean weight, 4.13±0.16 g) were allocated randomly into 12 tanks at a stocking density of 20 fish per tank (350-L water volume), tanks were supplied with sand-filtered aerated seawater at a flow rate of 2 L $min^{-1}$. The fish were cultured indoors and subjected to a natural photoperiod. During the experimental period, water quality was monitored daily, and the water temperature, dissolved oxygen, salinity and pH ranged from 27±1°C, 5.0-6.5 mg $L^{-1}$, 26-29 g $L^{-1}$, 8.1-8.3, respectively. Dietary treatments were randomly assigned to triplicate tanks, and fish were fed by hand twice a day at $8:^{00}$ h and $16:^{00}$ h to visual satiety. The feeding trial lasted for 56 days, feed consumption and mortality was recorded daily.

**Apparent Digestibility Coefficients**

During the final 3 weeks of feeding trial, faeces samples without feed losses were collected from each tank using siphon method (Sklan *et al*., 2004), at 6:00 h daily before the next feeding. After draining with filter papers, faeces samples from each tank were kept separate, freeze-dried and stored at -20°C before analysis. Dried faeces sample (0.5 g) was incinerated at 550°C for 12 h in a muffle furnace, then concentrated nitric acid (5 drops) and HCl (1:1) (10 ml) was added. The solution was transferred to a 200 mL volumetric flask and diluted to volume with distilled water. Then solution sample were analyzed by inductively coupled plasma emission spectrometer (model: IRIS Intrepid IIXSP, USA).

**Sampling Collection and Analytical Methods**

At the beginning of the experiment, 5 fish were sampled and stored at -20°C for initial whole-body composition analysis. Until the termination of the 8-week feeding trial, approximately 24 h after the last feeding, all fish were counted and measured after anaesthetized (tricane methanesulphonate MS-222, 70 ppm). And three fish from each tank were randomly selected and stored at -20 C for subsequent proximate analysis. The rest of fish were drawn blood from the caudal vein. Pooled samples grouped by tanks were centrifuged at 2500 rpm for 15 min (4°C) to obtain serum sample. Subsequently, samples of dorsal muscle, liver, intraperitoneal fat, stomach and intestinal tract were obtained, subpackaged by tanks. All samples were kept at - 20 °C before analyses.

Chemical analyses were determined by standard methods (AOAC, 1995). Moisture was assayed by oven-drying at 105°C until constant weight. Crude protein was estimated as Kjeldahl-nitrogen using factor 6.25. Crude lipid was determined by soxhlet extraction with ether for 6 h. Ash content was analyzed by incinerating samples at 550°C for 12 h in a muffle furnace.

The amino acid compositions of all samples including experimental diets, dorsal muscle and faeces were analyzed following acid hydrolysis using an automatic amino acid analyzer (Hitachi 835-50, Tokyo, Japan) equipped with a column for physiological fluid analysis by a professional laboratory. Pepsin activity in stomach, typsin activity in intestinal tract (pylorus, fore-gut, mid-gut, respectively) and liver were all measured using the diagnostic reagent kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China) according to the manufacturer's instructions. Serum total protein (TP), total cholesterol (T-CHO), triacylglycerol (TG), glucose (GLU) and alkaline phosphatase (ALP) concentration, as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed by enzymatic procedures using an automatic biochemical analyzer (Hitachi 7170, Japan) and attached kits (Daiichi Pure Chemicals Co., Japan), within 3 days.

**Statistical Analysis**

Quantitative data were expressed as means ± SE. Statistical differences were estimated by one-way ANOVA followed by Tukey's multiple range test, and a probability level of 0.05 was used to indicate significance. All statistics were performed using SPSS 16.0 (SPSS, Chicago, IL, USA).

## Results

After eight weeks of the feeding trial, survival and mean feed intake of experimental black sea bream were unaffected by dietary treatments, while growth performance and feed utilization were significantly affected (Table 3). Results showed that adding coated Lys and Met (LLM and HLM) significantly improved the weight gain (WG) and specific growth rate (SGR) of black sea bream (P<0.05). Furthermore, fish fed the HLM diet showed comparable WG and SGR with the control group (P>0.05). In terms of morphological indexes, adding high level of amino acids (Lys5.0 + Met3.4) improved the value of intraperitoneal fat ratio (IPR). The condition factor (CF) and hepotasomatic index (HSI) of fish fed diet NLM and LLM were higher than fish fed diet FM (P<0.05), and supplying amino acids narrowed this gap. With increasing supplemented coated amino acid levels, a significant increase in feed efficiency ratio (FER) and protein efficiency ratio (PER) were observed (P<0.05), but still lower than that of in the control group (P<0.05).

The whole body and dorsal muscle compositions of black sea bream is shown in Table 4. The whole body composition was not affected by dietary treatments (P>0.05). The moisture, ash and crude lipid content were also independent of dietary treatment (P>0.05), however, the lowest protein level in dorsal muscle was observed in the fish fed NLM diet (P<0.05), but no significant differences were found among the other treatments.

The essential amino acid contents in dorsal muscle of juvenile black sea bream is shown in Table 5. Met, Lys and Ile retention in muscle of experimental diets showed an increasing trend with increasing dietary amino acid levels. However, other essential amino acid contents were not affected by dietary treatments (P>0.05), even in Lys content.

The apparent digestibility coefficient (ADC) of dry matter, crude protein and crude lipid were generally high in the present study, fish fed diet the FM and HLM diets showed higher ADCs than those of fish fed NLM and LLM diets (Table 6). The ADCs of dietary EAAs showed increasing trend with dietary Lys and Met level in mixture protein source diets. The ADCs of EAAs in diet HLM were even slight higher than those of in the control group (P>0.05).

The serum biochemical parameters were presented in Table 7. The TG level of fish fed diet NLM and LLM were higher than those of HLM and FM (P<0.05). The AST activity in fish fed control diet was the lowest, and it showed an increasing trend

**Table 3**. Effects of supplementation coated lysine and methionine in diets on growth performance and feed utilization of juvenile black sea bream, *A. schlegelii*

| Index | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| MFI | 0.51±0.02 | 0.50±0.02 | 0.52±0.00 | 0.51±0.01 |
| Survival | 98.33±1.67 | 100.00±0.00 | 96.67±1.67 | 100.00±0.00 |
| WG | 673.34±4.23$^{a}$ | 599.50±4.38$^{c}$ | 640.79±3.88$^{b}$ | 662.59±2.83$^{a}$ |
| SGR | 3.65±0.01$^{a}$ | 3.47±0.01$^{c}$ | 3.58±0.01$^{b}$ | 3.63±0.00$^{a}$ |
| CF | 2.89±0.02$^{b}$ | 2.99±0.02$^{a}$ | 2.92±0.01$^{ab}$ | 2.93±0.02$^{ab}$ |
| IPR | 2.65±0.01$^{a}$ | 2.19±0.02$^{c}$ | 2.24±0.02$^{c}$ | 2.41±0.05$^{b}$ |
| HSI | 2.07±0.06$^{b}$ | 2.46±0.04$^{a}$ | 2.46±0.02$^{a}$ | 1.97±0.10$^{b}$ |
| FER | 0.97±0.01$^{a}$ | 0.88±0.02$^{c}$ | 0.92±0.01$^{bc}$ | 0.95±0.00$^{ab}$ |
| PER | 2.40±0.03$^{a}$ | 2.10±0.04$^{c}$ | 2.25±0.01$^{b}$ | 2.23±0.01$^{b}$ |

Values are presented as mean ± SE (*n*=3); values with different superscripts in the same row differ significantly (P<0.05).
Mean feed intake (MFI, g fish$^{-1}$ d$^{-1}$) = air drying diet fed in g / (fish × day); survival (%) =100 × final fish number /initial fish number; weight gain (WG, %) = 100 × (final body weight - initial body weight) / initial body weight; specific growth rate (SGR, % d$^{-1}$) = 100 × [Ln (final body weight) – Ln (initial body weight)] / day; condition factor (CF, g cm$^{-3}$) = 100 × final body weight in g / (body length in cm)$^{3}$; Intraperitoneal fat ratio (IPR, %) = 100 × intraperitoneal fat in g / body weight in g; hepatosomatic index (HSI, %) = liver weight in g / body weight in g; feed efficiency ratio (FER) = 100 × wet weight gain in g / dry diet fed in g; protein efficiency ratio (PER) = weight gain in g / protein intake in dry basis in g.

**Table 4**. Effect supplementation coated lysine and methionine in diets on proximate compositions of whole body and dorsal muscle of juvenile black sea bream, *A. schlegelii* (% on wet matter basis)

| Index | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| Whole body | | | | |
| Crude protein | 17.25±0.15 | 17.50±0.20 | 17.12±0.66 | 16.99±0.12 |
| Crude lipid | 14.12±0.24 | 12.64±0.35 | 12.39±1.15 | 11.94±0.37 |
| Ash | 4.61±0.12 | 4.50±0.17 | 4.62±0.11 | 4.41±0.08 |
| Moisture | 66.51±0.20 | 66.17±0.57 | 64.94±0.64 | 65.33±0.38 |
| Dorsal muscle | | | | |
| Crude protein | 19.69±0.16$^{ab}$ | 18.34±0.27$^{b}$ | 19.93±0.24$^{a}$ | 20.01±0.20$^{a}$ |
| Crude lipid | 5.18±0.20 | 4.42±0.38 | 5.46±0.39 | 4.18±0.25 |
| Ash | 1.55±0.05 | 1.43±0.02 | 1.45±0.02 | 1.45±0.01 |
| Moisture | 74.59±0.02 | 74.87±0.06 | 74.34±0.25 | 74.82±0.23 |

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

**Table 5**. Effect of supplementation coated lysine and methionine in diets on essential amino acid contents in dorsal muscle of juvenile black sea bream, *A. schlegelii* (% on dry matter basis)

| Index | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| EAAs | | | | |
| Met | 2.29±0.03$^{ab}$ | 2.19±0.05$^{b}$ | 2.22±0.02$^{ab}$ | 2.36±0.03$^{a}$ |
| Lys | 6.63±0.19 | 6.56±0.14 | 6.82±0.14 | 7.05±0.11 |
| Phe | 3.04±0.05$^{b}$ | 3.11±0.01$^{ab}$ | 3.09±0.02$^{ab}$ | 3.19±0.04$^{a}$ |
| Leu | 6.26±0.13 | 6.47±0.10 | 6.27±0.06 | 6.71±0.14 |
| Val | 3.77±0.10 | 3.87±0.08 | 3.73±0.04 | 4.05±0.09 |
| Arg | 4.35±0.09 | 4.45±0.09 | 4.33±0.07 | 4.63±0.08 |
| Ile | 3.37±0.06$^{b}$ | 3.50±0.03$^{ab}$ | 3.39±0.01$^{ab}$ | 3.61±0.08$^{a}$ |
| His | 1.63±0.04 | 1.68±0.02 | 1.64±0.01 | 1.74±0.02 |
| Thr | 2.88±0.16 | 3.10±0.03 | 2.90±0.13 | 3.19±0.07 |
| ∑EAA[1] | 34.13±0.80 | 35.29±0.47 | 34.13±0.19 | 36.63±0.63 |

Values are presented as mean ± SE (*n*=3); values with different superscripts in the same row differ significantly (P<0.05). [1]∑EAA, total essential amino acid.

**Table 6**. Effect of supplementation coated lysine and methionine in diets on apparent digestibility coefficients (ADCs) of juvenile black sea bream, *A. schlegelii*

| Index | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| Dry matter | 82.06±2.41$^{a}$ | 67.59±1.54$^{b}$ | 72.16±1.08$^{b}$ | 86.73±0.86$^{a}$ |
| Crude protein | 91.73±1.09$^{a}$ | 85.34±0.75$^{b}$ | 87.57±0.40$^{b}$ | 94.17±0.20$^{a}$ |
| Crude lipid | 90.55±1.60$^{ab}$ | 81.73±1.95$^{b}$ | 82.08±1.22$^{b}$ | 91.42±0.84$^{a}$ |
| EAAs | | | | |
| Met | 93.26±1.11$^{ab}$ | 88.15±1.13$^{c}$ | 88.93±0.32$^{bc}$ | 94.74±1.05$^{a}$ |
| Lys | 94.87±0.93$^{ab}$ | 91.86±0.80$^{b}$ | 92.18±0.43$^{b}$ | 96.74±0.69$^{a}$ |
| Phe | 91.37±0.87$^{ab}$ | 87.05±1.14$^{c}$ | 87.53±0.27$^{bc}$ | 94.03±2.13$^{a}$ |
| Leu | 92.94±2.07$^{ab}$ | 89.39±0.90$^{b}$ | 90.08±0.12$^{b}$ | 96.13±0.90$^{a}$ |
| Val | 91.73±1.63$^{ab}$ | 86.34±1.31$^{b}$ | 86.77±0.38$^{b}$ | 93.96±1.20$^{a}$ |
| Arg | 92.78±1.52$^{ab}$ | 89.68±1.04$^{b}$ | 89.93±0.04$^{b}$ | 95.13±1.08$^{a}$ |
| Ile | 91.68±0.63$^{a}$ | 86.23±1.38$^{b}$ | 86.46±0.46$^{b}$ | 93.97±1.19$^{a}$ |
| His | 91.67±1.83$^{ab}$ | 89.17±0.93$^{b}$ | 89.33±0.17$^{b}$ | 94.98±1.10$^{a}$ |
| Thr | 89.67±0.60$^{ab}$ | 84.23±1.56$^{b}$ | 84.88±0.37$^{b}$ | 92.49±1.65$^{a}$ |

Values are presented as mean ± SE (*n*=3); values with different superscripts in the same row differ significantly (P<0.05).
EAAs, essential amino acids. ADC of dry matter (%) = 100 × [1 - (Y diet) / (Y faeces )];ADC of nutrients (%) = 100 × [1 - (Y diet / Y faeces) × (N faeces / N diet)], where Y is percent of yttrium oxide and N is percent of nutrients.

**Table 7**. Effect of supplementation coated lysine and methionine in diets on serum biochemical indices of juvenile black sea bream, *A. schlegelii*

| Index | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| TP (g $L^{-1}$) | 54.67±1.45 | 57.67±1.45 | 59.00±1.00 | 57.00±1.52 |
| ALP (g $L^{-1}$) | 28.00±2.00 | 28.67±1.33 | 25.67±2.73 | 27.00±2.52 |
| T-CHO (mmol $L^{-1}$) | 16.10±0.14 | 17.07±0.58 | 17.32±0.81 | 15.31±0.62 |
| TG (mmol $L^{-1}$) | 5.10±0.55$^{b}$ | 8.60±0.66$^{a}$ | 8.77±0.61$^{a}$ | 6.16±0.14$^{b}$ |
| GLU (mmol $L^{-1}$) | 6.21±0.14 | 6.21±0.42 | 5.47±0.21 | 6.27±0.18 |
| ALT (mmol $L^{-1}$) | 21.67±1.67$^{b}$ | 29.67±1.85$^{ab}$ | 29.33±1.67$^{ab}$ | 33.00±2.08$^{a}$ |
| AST (mmol $L^{-1}$) | 254.67±14.10$^{b}$ | 364.00±21.66$^{a}$ | 403.00±8.14$^{a}$ | 412.67±14.77$^{a}$ |

Values are presented as mean ± SE (*n*=3); values with different superscripts in the same row differ significantly (P<0.05).
TP, total protein; ALP, alkaline phosphatase; T-CHO, total cholesterol; TG, triacylglycerol; GLU, glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

with increasing dietary Lys and Met levels, as well as ALT. However, the TP, ALP, T-CHO and GLU concentrations were independent by dietary treatments, and no significant differences were detected among the various groups (P>0.05).

The experimental feed cost was showed in Table 8, and the cost price per kg feed were: control diet was ¥ 8.21 Ren Min Bi(¥, RMB), NLM diet was ¥ 7.36, LLM diet was ¥ 7.47 and HLM diet was ¥ 7.68, respectively. The highest and lowest ratio of feed cost / weight gain were obtain in the control group and NLM group, respectively. However, among the

**Table 8**. Feed cost of the experiment (RMB $kg^{-1}$ diet) [1]

| Ingredients | Diets | | | |
|---|---|---|---|---|
| | FM | NLM | LLM | HLM |
| Cost contribute of protein source [2] | | | | |
| Fish meal | 5.57 | 3.34 | 3.34 | 3.34 |
| Fermented soybean meal | 0.40 | 0.40 | 0.40 | 0.40 |
| Monosodium glutamate protein | 0.00 | 0.10 | 0.10 | 0.10 |
| Soybean protein concentrate | 0.00 | 0.78 | 0.78 | 0.78 |
| Meat and bone meal | 0.00 | 0.50 | 0.50 | 0.50 |
| Cost contribute of coated amino acid [3] | | | | |
| Lys | 0.00 | 0.00 | 0.08 | 0.23 |
| Met | 0.00 | 0.00 | 0.03 | 0.09 |
| Cost contribute of others [4] | 2.24 | 2.24 | 2.24 | 2.24 |
| Cost of feed (RMB $kg^{-1}$) | 8.21 | 7.36 | 7.47 | 7.68 |
| Feed cost - weight gain ratio[5] | 9.58±0.10$^{a}$ | 9.31±0.16$^{ab}$ | 9.00±0.03$^{b}$ | 9.16±0.02$^{b}$ |

The value of feed cost - weight gain ratio is presented as mean ± SE (n=3); values with different superscripts in the same row differ significantly (P<0.05).
[1] RBM 6.13 = US $ 1.00
[2] Actual purchase price: fish meal, 10500 RMB (1000 kg)$^{-1}$; fermented soybean meal, 5000 RMB (1000 kg)$^{-1}$; monosodium glutamate protein, 1900 RMB (1000 kg)$^{-1}$; soybean protein concentrate, 8200 RMB (1000 kg)$^{-1}$; meat and bone meal, 6300 RMB (1000 kg)$^{-1}$.
[3] Actual purchase price: Lys, 45.5 RMB $kg^{-1}$; Met, 26.5 RMB $kg^{-1}$. Brought from Hangzhou King Technology Feed Company, LTD (Hangzhou, China).
[4] Actual purchase price: fish oil, 12000 RMB (1000 kg)$^{-1}$; corn oil, 8840 RMB (1000 kg)$^{-1}$; wheat flour, 3200 RMB (1000 kg)$^{-1}$; $Ca(H_2PO_4)_2$, 3400 RMB (1000 kg)$^{-1}$; zeolite flour, 260 RMB (1000 kg)$^{-1}$; betaine, 10 RMB $kg^{-1}$; vatimin premix, 40 RMB $kg^{-1}$; mineral premix, 14 RMB $kg^{-1}$; $Y_2O_3$, 12 RMB $kg^{-1}$.
[5] feed cost-weight gain ratio (RMB $kg^{-1}$) = feed cost in RMB / (final body weight - initial body weight) in kg.

mixture alternative protein resources treatments, no significant differences were found.

## Discussion

Amino acid balance in diets is necessary for optimal growth of fishes, and most authors reported that some amino acids or their metabolites are important regulators of key metabolic pathways which are necessary for feed intake, maintenance, growth, nutrient utilization, immunity as well as resistance to environmental stressors and pathogenic organisms (Moon and Gatlin, 1991; Davies and Morris, 1997; Wilson, 2002; Li *et al*., 2009; Nwanna *et al*., 2012). For example, individual supplementation of Lys, Met or some other EAAs in plant source diets significantly improved the growth rate, feed efficiency and protein digestibility of fish (Forster and Ogata, 1998; Espe *et al*., 2007; Nwanna *et al*., 2012). However, some studies showed that significant improvement of growth was observed in fish fed diets with additions of several amino acids together, while no significant effect existed on the growth performance when supplied with the same amino acids individually (Rumsey and Ketola, 1975; Mukhopadhyay and Ray, 2001). Those may indicate that supplementation of any deficient amino acid alone can not significantly improve the nutritional quality of the alternative protein. Based on the observations from previous researches in our lab (Zhou *et al*. 2010a; 2011b), black sea bream has been testified to intake and assimilate the exogenous amino acids in feed well, and the growth improving effect were found as EAA adding. In present study, black sea bream fed amino acids deficient diets (NLM and LLM) showed poor growth performance (WG and SGR) and feed utilization (FER and PER), while these indexes were all improved when exogenous coated Lys and Met were adequate supplement (HLM). However, differed with previous reports which proved amino acids to be a powerful feeding stimulant for fish (Martin *et al*., 2003; Vilhelmsson *et al*., 2004; Gómez-Requeni *et al*., 2005), however MFI in present experiment showed no obvious improvement as dietary Lys and Met supplementation increased. The probably reason may be that juvenile black sea bream could utilize coated amino acids relatively well. Researchers have reported that some fish species have the special digestive process in the organ of stomach, which could ease the contradiction of different forms of amino acids absorption unsynchronized from free ones and protein-hydrolyzed ones occurred in stomach fish such as black sea bream (Segovia-Quintero and Reigh, 2004). In present study, improved growth performance was observed in fish fed amino acid added diets accompanied with increases in both feed and protein utilization efficiencies. One possible reason might be that Lys and Met are vital in keeping the balance of diet nutrition (Wilson, 2003; Wacyk *et al*., 2012). On the other hand, supplement of deficient amino acids up to levels of FM group improved the utilization rate of other amino acids, and reduced the degradation rate at the same time (Bender, 2012). However, Hansen *et al*. (2007) reported that with Lys and Met supplemented in plant proteins based diet according to amino acid profile of diet FM, there was not any positive effects on growth performance of Atlantic cod (*Gadus morhua* L.). Furthermore, the growth rate of Nile tilapia (*O. niloticus*) fed diets adding enough Lys and

Met still can not catch up with those of fish fed diet FM (Thompson *et al*., 2012). The reason may be that some factors other than the shortage of some EAAs may be responsible for the inferior nutritive value of the mixture protein diets, not only containing some anti-nutrients, but also lacking some nutrition factors compared with FM, such as taurine (Gaylord *et al*., 2007; Chatzifotis *et al*., 2008) and possibly steroids (Hardy, 2010).

Similar to the previous studies conducted by Zhou *et al*. (2010a; 2011b), protein deposition in dorsal muscle of juvenile black sea bream was increased markedly by supplement of coated Lys and Met in present study, even slightly higher than those of diet FM. Nwanna *et al.* (2012) also reported that the protein content of the common carp (*Cyprinus carpio* L.) fed the DL-Met supplemented diet had significantly higher protein level in muscle than those of fish fed the Met-deficient diet. The possible reason for protein deposition increased was that balanced amino acid profile promoted protein synthesis rates in muscle (Furuya *et al*., 2004). In present study, there was no significant difference in dorsal muscle Lys deposition with increasing levels of coated amino acid supplementation. This response has also been described by other fish feeding diets with high levels of Lys, such as juvenile Japanese flounder *Paralichthys olivaceus*, juvenile red sea bream *P. major* (Forster and Ogata, 1998) and fingerling *Catla catla* (Hamilton) (Zehra and Khan, 2012). On the contrary, significant increase in dorsal muscle Met deposition was observed in HLM group in present study, similar result was found by in black sea bream (Zhou *et al*., 2011b). The difference dorsal muscle deposition of Lys and Met might due to the difference of free amino acid in the amino acid pool of the muscle, or Lys is probably superior to be used by black sea bream compared with Met. Besides, there was another possibility that the Lys (28.6 g $kg^{-1}$) and Met (11.1 g $kg^{-1}$) content in diet HLM were both under the estimated requirement of black sea bream were 33.3 and 17.1 g $kg^{-1}$, respectively (Zhou *et al*., 2010a; Zhou *et al*., 2011b), while Met content (11.1 g $kg^{-1}$) may reached its maintenance requirement. Maintenance needs were generally thought to be highly dependent on body size and temperature, thus were proportional to the metabolic body weight, while the needs for growth were governed by the amount and composition of the added body weight gain (Lupatsch *et al*., 2010; He *et al*., 2013).

Results of EAAs retention in muscle in present study showed an increasing trend with increasing dietary amino acid levels, which probably reflected the high availability of coated amino acids and efficiency of utilization by juvenile black sea bream. As dietary restriction of one or more EAAs might led to an oxidation increase of other EAAs (Rønnestad *et al*., 2000), so the supplement of coated Lys and Met alleviated the amino acids imbalance of diets, and decreased the deamination effect in fish metabolism.

Apparent crude protein digestibility of feedstuffs for commercially cultured fishes such as channel catfish, red drum (*Sciaenops ocellatus*), and rohu (*Labeo rohita ranges*) ranged from 65% to 92% among plant and animal protein sources (Ambardekar and Reigh, 2007). The ADCs of crude protein was significantly higher in black sea bream fed diet FM when compared to those fed diet NLM and LLM in the present study. This indicates supplemental amino acids improved the quality of the alternative protein sources. Consistent with this argument, Nwanna *et al*. (2012) reported better protein digestion of carp fed diets with 0.4% DL-Met supplement compared with the control diet. Tang *et al.* (2009) attributed the similar phenomenon observed in juvenile Jian carp to met supplementation which improved hepatopancreas and intestine weight, hepatosomatic and intestine index, intestinal γ-glutamyltransferase and creatine kinase activity. It has reported that coated amino acids can improve the utilization efficiency by reducing its solubility in water, and also help to slow the rate of release of amino acids to transport sits in the intestinal mucosa of fish (Alam *et al*., 2004).

The ALT and AST levels were both important amino transferase in mitochondria of fish, and their activity is closely associated with the metabolism of amino acids (Chien *et al*., 2003). In present study, serum ALT and AST activity in fish fed mixture protein were obviously higher than those fed the control diet. It indicated that transamination were obviously active in diet NLM, LLM and HLM. It could be inferred that even adding Lys and Met, the amino acid balance in mixture protein is still worse than in FM, and may lead to a series of amino acid metabolic disorders, however, it may not serious because the survival rate was relatively higher in all dietary treatments without significant difference. In addition, a significant lower TG concentration was observed in fish fed HLM diet than thoes of fish fed NLM and LLM diets. It is possibly showed that supplement of amino acids reduced the fat metabolism as an energy consumption, which is beneficial to weight gain (Regost *et al*., 1999). However, serum GLU levels, as well as ALP activity were not significant differences among dietary groups, all the experimental fish appeared healthy and actively feeding which seems to suggest that any nutrient imbalance in the test diets did not cause a serious metabolic or immune system malfunction during the experimental period. High survival (>96%) and acceptable growth performance (WG>599%) of the fish observed in the present study seems to further support this observation that the fish were relatively healthy.

Besides feed utilization by aquatic animals and growth performance, feed cost was another important consideration before a new feed formula applied in aquaculture practices, because feed is the major operational cost for most aquaculture enterprises. Through the production economics analysis, LLM and

HLM diets showed better feed cost-weight gain ratio (FWR) (9.00 and 9.16 RMB $kg^{-1}$, respectively) than the control and NLM diets (9.58 and 9.31 RMB $kg^{-1}$, respectively). So it can be inferred that functional amino acids hold great promise for development of balanced aquafeeds to enhance the efficiency and profitability of aquaculture production in the practice.

In conclusion, the present results demonstrated that adding enough Lys (5.0%) and Met (3.4%) in mixture alternative protein sources diets had positive effects on performance and feed utilization of juvenile black sea bream, 21.2% of FM in diet can be successfully replaced and the feed cost could also decreased. The result generating from currest study is beneficial to formulate effective and nutritionally balanced practical diet for this fish species.

## Acknowledgements

The study was supported by Scientific Fund Programme for Mariculture and Demonstration in the East China Sea (The Ministry of Science and Technology of the People's Republic of China, 2011BAD13B08), Zhejiang Provincial scientific and technological innovation team program (2010R50025), Scientific Fund Programme for Research and Demonstration of Efficient Sustainable Feed for Black Sea Bream (Science Technology Department of Zhejiang Province, 2011C12054) and Marine Scientific Crossover Fund of Zhejiang University (2012HY014B). We thank the Zhoushan Fisheries Research Institute of Zhejiang Province (Zhoushan, Zhejiang Province) for supplying the experimental rearing system and for providing logistical support during the feeding trial. Thanks to Zhejiang Kefeng Biotechnology Co. Ltd. (Zhejiang) for their supplying the fermented soybean meal. We are grateful to H. Z. Luo, S. Gao, H. Li and J. Zhou for their assistance with fish husbandry and sampling.

## References

Abimorad, E.G., Favero, G.C., Castellani, D., Garcia, F. and Carneiro, D.J. 2009. Dietary supplementation of lysine and/or methionine on performance, nitrogen retention and excretion in pacu (*Piaractus mesopotamicus*) reared in cages. Aquaculture, 295: 266-270. doi: 10.1016/j.aquaculture.2009.07.001.

Alam, M., Teshima, S., Koshio, S. and Ishikawa, M. 2004. Effects of supplementation of coated crystalline amino acids on growth performance and body composition of juvenile kuruma shrimp *Marsupenaeus japonicus*. Aquaculture Nutrition, 10: 309-316. doi: 10.1111/j.1365-2095.2004.00316.x.

Alan Ward, D., Carter, C. and Townsend, A. 2005. The use of yttrium oxide and the effect of faecal collection timing for determining the apparent digestibility of minerals and trace elements in Atlantic salmon (*Salmo salar*, L.) feeds. Aquaculture Nutrition, 11: 49-59. doi: 10.1111/j.1365-2095.2004.00323.x.

Ambardekar, A.A. and Reigh, R.C. 2007. Sources and utilization of amino acids in channel catfish diets: a review. North American Journal of Aquaculture, 69: 174-179. doi: 10.1577/A06-007.1.

AOAC 1995. Official Methods of Analytical of the Association of Official Analytical Chemists (16th edn). AOAC, Arlington,VA.

Bender, D.A. 2012. Amino Acid Metabolism (3th edn). Wiley-Blackwell, West Sussex, UK, 46 pp.

Chatzifotis, S., Polemitou, I., Divanach, P. and Antonopoulou, E. 2008. Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet. Aquaculture, 275: 201-208. doi: 10.1016/j.aquaculture.2007.12.013

Chi, S., Tan, B., Lin, H., Mai, K., Ai, Q., Wang, X., Zhang, W., Xu, W. and Liufu, Z. 2011. Effects of supplementation of crystalline or coated methionine on growth performance and feed utilization of the pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition, 17: e1-e9. doi: 10.1111/j.1365-2095.2009.00710.x.

Chien, Y.-H., Pan, C.-H. and Hunter, B. 2003. The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. Aquaculture, 216: 177-191. doi: 10.1016/S0044-8486(02)00056-X.

Davies, S. and Morris, P. 1997. Influence of multiple amino acid supplementation on the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed soya based diets. Aquaculture Research, 28: 65-74.

El-Saidy, D.M. and Gaber, M. 2002. Complete Replacement of Fish Meal by Soybean Meal with Dietary L-Lysine Supplementation for Nile Tilapia *Oreochromis niloticus* (L.) Fingerlings. Journal of the World Aquaculture Society, 33: 297-306. doi: 10.1111/j.1749-7345.2002.tb00506.x.

Espe, M., Lemme, A., Petri, A. and 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.

Forster, I. and Ogata, H.Y. 1998. Lysine requirement of juvenile Japanese flounder (*Paralichthys olivaceus*) and juvenile red sea bream (*Pagrus major*). Aquaculture, 161: 131-142. doi: 10.1016/S0044-8486(97)00263-9.

Furuya, W.M., Pezzato, L.E., Barros, M.M., Pezzato, A., Furuya, V.R. and Miranda, E.C. 2004. Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research, 35: 1110-1116. doi: 10.1111/j.1365-2109.2004.01133.x.

Gómez-Requeni, P., Calduch-Giner, J., de Celis, S., Médale, F., Kaushik, S.J. and Pérez-Sánchez, J. 2005. Regulation of the somatotropic axis by dietary factors in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition, 94: 353-361. doi: dx.doi.org/10.1079/BJN20051521.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å. and Nelson, R. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research, 38: 551-579. doi: 10.1111/j.1365-2109.2007.01704.x.

Gaylord, T.G. and Barrows, F.T. 2009. Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. Aquaculture, 287: 180-184. doi: 10.1016/j.aquaculture.2008.10.037

Gaylord, T.G., Barrows, F.T., Teague, A.M., Johansen, K.A., Overturf, K.E. and Shepherd, B. 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 269: 514-524. doi: 10.1016/j.aquaculture.2007.04.011

Hansen, A.C., Rosenlund, G., Karlsen, Ø., Koppe, W. and Hemre, G.I. 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I-Effects on growth and protein retention. Aquaculture, 272: 599-611. doi: 10.1016/j.aquaculture.2007.08.034

Hardy, R.W. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research, 41: 770-776. doi: 10.1111/j. 1365-2109.2009.02349.x.

Hardy, R.W. and Barrows, F.T. 2002. Diet formulation and manufacture. Fish nutrition, 3: 505-600.

Harpaz, S. 2005. L-carnitine and its attributed functions in fish culture and nutrition-a review. Aquaculture, 249: 3-21. doi: 10.1016/j.aquaculture.2005.04.007.

Hauler, R.C. and Carter, C.G. 2001. Reevaluation of the quantitative dietary lysine requirements of fish. Reviews in Fisheries Science, 9: 133-163. doi: 10.1080/20016491101735.

He, J.Y., Tian, L.X., Lemme, A., Gao, W., Yang, H.J., Niu, J., Liang, G.Y., Chen, P.F. and Liu, Y.J. 2013. Methionine and lysine requirements for maintenance and efficiency of utilization for growth of two sizes of tilapia (*Oreochromis niloticus*). Aquaculture Nutrition, 19: 629-640. doi: 10.1111/anu.12012.

Hong, W. and Zhang, Q. 2003. Review of captive bred species and fry production of marine fish in China. Aquaculture, 227: 305-318. doi: 10.1016/S0044-8486 (03)00511-8.

Li, P., Mai, K., Trushenski, J. and Wu, G. 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids, 37: 43-53. doi: 10.1007/s00726-008-0171-1.

Lupatsch, I., Deshev, R. and Magen, I. 2010. Energy and protein demands for optimal egg production including maintenance requirements of female tilapia *Oreochromis niloticus*. Aquaculture Research, 41: 763-769. doi: 10.1111/j.1365-2109.2009.02229.x.

Martin, S., Vilhelmsson, O., Médale, F., Watt, P., Kaushik, S. and Houlihan, D. 2003. Proteomic sensitivity to dietary manipulations in rainbow trout. Biochimica et Biophysica Acta (BBA)-Proteins & Proteomics, 1651: 17-29. doi: 10.1016/S1570-9639(03)00231-0.

Moon, H.Y. and Gatlin, III D.M. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture, 95: 97-106. doi: 10.1016/0044-8486(91)90076-J.

Mukhopadhyay, N. and Ray, A. 2001. Effects of amino acid supplementation on the nutritive quality of fermented linseed meal protein in the diets for rohu, *Labeo rohita*, fingerlings. Journal of Applied Ichthyology, 17: 220-226. doi: 10.1046/j.1439-0426.2001.00287.x.

Murai, T., Akiyama, T. and Nose, T. 1982. Effects of casein coating on utilization of dietary amino acids by fingerling carp and channel catfish (*Ictalurus pumetatus*). Bulletin of the Japanese Society of Scientific Fisheries, 48: 787-792.

Ngandzali, B.O., Zhou, F., Xiong, W., Shao, Q.J. and Xu, J.Z. 2011. Effect of dietary replacement of fish meal by soybean protein concentrate on growth performance and phosphorus discharging of juvenile black sea bream, *Acanthopagrus schlegelii*. Aquaculture Nutrition, 17: 526–535. doi: 10.1111/j.1365-2095. 2010.00835.x

Nip, T.H., Ho, W.Y. and Wong, C.K. 2003. Feeding ecology of larval and juvenile black seabream (*Acanthopagrus schlegeli*) and Japanese seaperch (*Lateolabrax japonicus*) in Tolo Harbour, Hong Kong. Environmental Biology of Fishes, 66: 197-209. doi: 10.1023/A:1023611207492.

Nwanna, L., Lemme, A., Metwally, A. and Schwarz, F. 2012. Response of common carp (*Cyprinus carpio* L.) to supplemental DL-Methionine and different feeding strategies. Aquaculture, 357: 365-370. doi: 10.1016/j. aquaculture.2012.04.044.

Rønnestad, I., Conceição, L.E., Aragão, C. and Dinis, M.T. 2000. Free amino acids are absorbed faster and assimilated more efficiently than protein in postlarval Senegal sole (*Solea senegalensis*). The Journal of nutrition, 130: 2809-2812.

Regost, C., Arzel, J. and Kaushik, S. 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*). Aquaculture, 180: 99-117. doi: 10.1016/S0044-8486(99)00026-5.

Robinson, E.H. and Li, M.H. 1994. Use of plant proteins in catfish feeds: replacement of soybean meal with cottonseed meal and replacement of fish meal with soybean meal and cottonseed meal. Journal of the World Aquaculture Society, 25: 271-276. doi: 10.1111/j.1749-7345.1994.tb00190.x.

Rumsey, G. and Ketola, H. 1975. Amino acid supplementation of casein in diets of Atlantic salmon (*Salmo salar*) fry and of soybean meal for rainbow trout (*Salmo gairdneri*) fingerlings. Journal of the Fisheries Board of Canada, 32: 422-426.

Segovia-Quintero, M.A. and Reigh, R.C. 2004. Coating crystalline methionine with tripalmitin-polyvinyl alcohol slows its absorption in the intestine of Nile tilapia, *Oreochromis niloticus*. Aquaculture, 238: 355-367. doi: 10.1016/j.aquaculture.2004.05.023.

Sklan, D., Prag, T. and Lupatsch, I. 2004. Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* × *Oreochromis aureus* (Teleostei, Cichlidae). Aquaculture Research, 35: 358-364. doi: 10.1111/j.1365-2109.2004.01021.x.

Takagi, S., Shimeno, S., Hosokawa, H. and Ukawa, M. 2001. Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. Fisheries Science, 67: 1088-1096. doi: 10.1046/j.1444-2906.2001.00365.x.

Tang, L., Wang, G.X., Jiang, J., Feng, L., Yang, L., Li, S.H., Kuang, S.Y. and Zhou, X.Q. 2009. Effect of methionine on intestinal enzymes activities, microflora and humoral immune of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture Nutrition, 15: 477-483. doi: 10.1111/j.1365-2095.2008.00613.x.

Thompson, K.R., Velasquez, A., Patterson, J.T., Metts, L.S., Webster, C.D., Brady, Y.J., Gannam, A.L., Twibell, R.G. and Ostrand, S.L. 2012. Evaluation of plant and animal protein sources as partial or total replacement of fish meal in diets for Nile Tilapia fry and juvenile stages. North American Journal of Aquaculture, 74: 365-375. doi: 10.1080/15222055.2012.675999.

Trushenski, J.T., Kasper, C.S. and Kohler, C.C. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture, 68: 122-140. doi: 10.1577/A05-006.1.

Vilhelmsson, O.T., Martin, S.A., Médale, F., Kaushik, S.J. and Houlihan, D.F. 2004. Dietary plant-protein substitution affects hepatic metabolism in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition, 92: 71-80. doi: dx.doi.org/10.1079/BJN 20041176.

Viola, S. and Lahav, E. 1991. Effects of lysine supplementation in practical carp feeds on total protein sparing and reduction of pollution. Israeli Journal of Aquaculture/Bamidgeh, 43: 112-118.

Wacyk, J., Powell, M., Rodnick, K., Overturf, K., Hill, R.A. and Hardy, R. 2012. Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. Aquaculture, 357: 223-234. doi: 10.1016/j.aquaculture.2012.05.013.

Wilson, R. 2003. Amino acid requirements of finfish and crustaceans. In: J.P.F. D'Mello (Ed.), Amino Acids in Animal Nutrition CABI Publishing, Cambridge, USA: 427-449.

Wilson, R.P. 2002. Amino acids and proteins. In: E. John and R.W.H. Halver (Ed.), Fish Nutrition Elsevier Science, Florida USA*:* 144-175.

Ye, J.D., Wang, K., Li, F.D., Sun, Y.Z. and Liu, X.H. 2011. Incorporation of a mixture of meat and bone meal, poultry by-product meal, blood meal and corn gluten meal as a replacement for fish meal in practical diets of Pacific white shrimp *Litopenaeus vannamei* at two dietary protein levels. Aquaculture Nutrition, 17: 337-347. doi: 10.1111/j.1365-2095.2010.00768.x.

Zehra, S. and Khan, M.A. 2012. Dietary lysine requirement of fingerling *Catla catla* (Hamilton) based on growth, protein deposition, lysine retention efficiency, RNA/DNA ratio and carcass composition. Fish Physiology and Biochemistry, 9: 1-10. doi: 10.1007/s10695-012-9715-0.

Zhao, H., Jiang, R., Xue, M., Xie S., Wu, X. and Guo L. 2010. Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) less than 2 g. Aquaculture Nutrition, 16: 648-654. doi: 10.1111/j.1365-2095.2009.00708.x.

Zhou, F., Shao, J., Xu, R., Ma, J. and Xu, Z. 2010a. Quantitative l-lysine requirement of juvenile black sea bream (*Sparus macrocephalus*). Aquaculture Nutrition, 16: 194-204. doi: 10.1111/j.1365-2095. 2009.00651.x.

Zhou, F., Xiong, W., Xiao, J.X., Shao, Q.J., Bergo, O.N., Hua, Y. and Chai, X. 2010b. Optimum arginine requirement of juvenile black sea bream, *Sparus macrocephalus*. Aquaculture Research, 41: 418-430. doi: 10.1111/j.1365-2109.2009.02474.x.

Zhou, F., Song, W., Shao, Q., Peng, X., Xiao, J., Hua, Y., Owari, B.N., Zhang, T. and Ng, W.K. 2011a. Partial replacement of fish meal by fermented soybean meal in diets for black sea bream, *Acanthopagrus schlegelii*, juveniles. Journal of the World Aquaculture Society, 42, 184-197. doi: 10.1111/j.1749-7345.2011.00455.x.

Zhou, F., Xiao, J., Hua, Y., Ngandzali, B. and Shao Q. 2011b. Dietary l-methionine requirement of juvenile black sea bream (*Sparus macrocephalus*) at a constant dietary cystine level. Aquaculture Nutrition, 17: 469-481. doi: 10.1111/j.1365-2095.2010.00823.x.