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# Assessment of reference dietary amino acid pattern for juvenile red sea bream, *Pagrus major*

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**Abstract.** Four semi-purified diets, containing crystalline amino acids (CAAs), were fed to juvenile red sea bream, *Pagrus major* in order to ascertain the ideal dietary amino acid pattern for this species. A control diet containing 50% casein–gelatin as protein sources, but no CAAs were fed to the fish. The other diets contained 30% casein–gelatin and 20% CAAs. CAAs were added to diets to simulate with amino acid pattern of the red sea bream eggs protein (REP), red sea bream larvae whole body protein (RLP), red sea bream juvenile whole body protein (RJP), and brown fishmeal protein (BFP). The juveniles (average initial body weight, 1.58 ± 0.01 g) were maintained in triplicate tanks and fed twice daily for 30 days. The highest weight gain was observed in juveniles fed the RJP diet. No significant difference was observed in juveniles fed the RLP and BFP diet. Feed efficiency ratio, protein efficiency ratio and amino acid retention in the whole body were significantly ($p < 0.05$) affected by the simulated dietary amino acid patterns. The essential amino acid profile and A/E ratios of the whole body after the growth trial showed little difference among the dietary treatments. The results suggest that red sea bream juveniles are able to utilize high amounts of CAA in coated form. The amino acid pattern of RJP could be used as an appropriate of reference dietary amino acid for this species.

## Introduction

Fish generally require 10 essential amino acids (EAAs) (Wilson 1989). The necessity of EAA requirements for diet preparation in semi-intensive aquaculture has been reported by De Silva and Anderson (1995). The requirement for all 10 EAAs by freshwater fish has been clarified in species such as on rainbow trout *Oncorhynchus mykiss* (Ogino 1980), catla *Catla catla* (Ravi and Devaraj 1991), channel catfish *Ictalurus punctatus*, common carp *Cyprinus carpio*, Japanese eel *Anguilla japonica* and Nile tilapia *Oreochromis niloticus* (NRC 1993). Only a small number of the EAA requirements of marine fish have been determined in species such as yellowtail *Seriola quinqueradiata* (Ruchimat et al. 1998), red drum *Sciaenops ocellatus* (Moon and Gatlin 1991) and Japanese flounder *Paralichthys olivaceus* (Alam et al. 2000). As much labor, expense, and time are needed to determine the quantitative requirements of the 10 EAAs using dose–response methods; therefore, biochemical methods using ideal protein or reference amino acid patterns have been adopted to assess the requirements of respective EAA. For example, several workers have assessed the EAA requirements of rainbow trout (Mambrini and Kaushik 1995), cherry salmon *Oncorhynchus masou* and amago salmon *Oncorhynchus rhodurus* (Ogata et al. 1983) using the ideal protein concept. Ketola (1982) suggested the possible use of amino acid patterns of fish eggs as references for EAA requirements of Atlantic salmon *Salmo salar* and rainbow trout. The concept of A/E ratio [(amount of each EAA/sum of all EAAs including cystine and tyrosine) $\times$1000], which was first introduced by Arai (1981) for formulating test diets for coho salmon *Oncorhynchus kisutch*, has been used to assess the EAA requirements of species such as the channel catfish (Wilson 1991), hybrid striped bass *Morone saxatillis* (Brown 1995).

The red sea bream is a valuable food fish species in Japan, and there are many studies on the nutritional requirements of this fish (Kanazawa et al. 1989), but there is very little information on the EAA requirements of this species. Forster and Ogara (1998) have done some work on lysine requirements. In a previous study, we investigated the relationship between the growth performance and dietary amino acid patterns of the Japanese flounder (Alam et al. 2002a) and kuruma shrimp *Marsupenaeus japonicus* (Alam et al. 2002b), according to a similar concept of Wilson (1991).

The present study is part of a larger body of work on assessing the EAA requirements of marine fish. The objective of the present study is to evaluate the reference amino acid patterns for estimating EAA requirements of the juvenile red sea bream *Pagrus major*. Feeding experiments were conducted to evaluate the effects of different dietary amino acid patterns on growth performance, retention of dietary amino acids and body amino acid profile.

## Material and methods

### *Experimental diets*

Four semi-purified diets containing crystalline amino acids (CAAs) were formulated. The ratio of CAAs in each diet simulated the amino acid patterns of fertilized red sea bream egg protein (REP), larval red sea bream whole body (18 days after hatching) protein (RLP), juvenile red sea bream whole body (90 days after hatching) protein (RJP) and brown fish meal (Nippon Suisan Co., Ltd., Japan) protein (BFP). A control diet containing intact protein sources only, {a mixture of casein–gelatin (about 2: 1, w/w)} was also formulated (Tables 1 and 2). The major nutrient components were maintained according to recent nutrient requirement information for red sea bream. The simulation of the dietary amino acid pattern was achieved by adding 20% CAAs to the 30% casein–gelatin mixture. The amino acid composition of the reference proteins and the control diet are given in Table 3, and the dietary ingredients and the supplementation of CAA are shown in Table 4. In order to reduce leaching of CAAs from the diets to the surrounding water, pre-coated

*Table 1.* Composition of control and CAA based diets (g per 100 g dry diet) fed to juvenile red sea bream.

| Ingredients | Control diet | CAA based diet[a] |
|---|---|---|
| Casein | 34.0 | 20.0 |
| Gelatin | 16.0 | 10.0 |
| Amino acid mix[b] | 0 | 20.0 |
| Squid liver oil[c] | 5.0 | 5.0 |
| Soybean lecithin[d] | 5.0 | 5.0 |
| Vitamin mix[e] | 6.0 | 6.0 |
| Mineral mix[e] | 5.0 | 5.0 |
| α-Starch | 14.0 | 14.0 |
| CMC[f] | 4.0 | 4.0 |
| κ-Carrageenan | 2.5 | 2.5 |
| α-Cellulose | 7.5 | 7.5 |
| Attractants[g] | 1.0 | 1.0 |

[a]For REP, RLP, RJP and BFP diet.
[b]See Table 3.
[c]Feed oil squid, Riken Vitamin, Tokyo, Japan.
[d]Kanto Chemical Co., Inc., Tokyo, Japan.
[e]According to Alam et al. (2000).
[f]Carboxymethyl cellulose.
[g]Taurine 0.5, betaine 0.4 and inosine-5′ monophosphate 0.1.

*Table 2.* The proximate composition of five diets (%) fed to juvenile red sea bream.

| | Control | REP | RLP | RJP | RFP |
|---|---|---|---|---|---|
| Crude protein | 50.1 | 49.2 | 49.2 | 49.6 | 49.5 |
| Total lipid | 7.7 | 7.8 | 7.4 | 8.0 | 7.5 |
| Ash | 7.4 | 7.3 | 7.3 | 7.2 | 7.7 |

CAA-based diets were prepared. The CAA mixture (20 g) was pre-coated with cooked carboxymethylcellulose (CMC; 1.50 g). Casein and gelatin were mixed with water to form a paste in a hot water bath. The bound CAA mixture and the dry ingredients (vitamin mixture, mineral mixture, attractants, α-starch and α-cellulose) were added to the casein–gelatin paste. Blended oil and lecithin were added to this and mixed continuously with a spoon. Sufficient water was added to produce a dough-like consistency. To improve the water stability, an addition of 2.5 g CMC per 100 g diet was then mixed into the dough and 2.5 g κ-carrageenan was gelatinized at 85 °C in a water bath to form a homogenous gel and added to the mixture. The pH of the diets was adjusted to 7.0–7.5 by gradually adding 4 N sodium hydroxide. The dough was then passed through a pelletizer to obtain 2 mm diameter pellets and dried at 40 °C in a constant temperature oven (DK 400, Yamato Scientific Co., Ltd., Japan) for two hours. The dry pellets were ground, sieved and stored at − 30 °C (Alam et al. 2000).

*Table 3.* Amino acid composition (g per 50 g protein) of reference protein in each of the five diets fed to the red sea bream.

| Amino acids | REP | RLP | RJP | BFP | Control[a] |
|---|---|---|---|---|---|
| *EAA*[b] | | | | | |
| Arginine | 3.67 | 3.17 | 3.50 | 3.18 | 2.73 |
| Histidine | 1.47 | 1.34 | 1.31 | 1.79 | 0.81 |
| Isoleucine | 2.46 | 1.87 | 2.60 | 2.06 | 1.97 |
| Leucine | 3.56 | 4.03 | 3.88 | 3.78 | 3.39 |
| Lysine | 4.33 | 3.83 | 5.38 | 4.82 | 3.89 |
| Methionine | 1.14 | 1.61 | 1.30 | 1.25 | 1.02 |
| Phenylalanine | 2.10 | 2.14 | 2.41 | 2.28 | 2.43 |
| Threonine | 2.31 | 2.31 | 2.25 | 2.19 | 1.82 |
| Tryptophan | 1.07 | 1.02 | 1.31 | 1.35 | 0.85 |
| Valine | 2.91 | 2.40 | 2.71 | 2.46 | 2.47 |
| *NEAA*[c] | | | | | |
| Aspartic acid | 3.52 | 4.63 | 4.57 | 4.72 | 3.17 |
| Glutamic acid | 7.08 | 7.38 | 7.00 | 6.04 | 8.77 |
| Serine | 2.17 | 2.49 | 1.53 | 1.59 | 1.53 |
| Proline | 3.33 | 2.85 | 2.76 | 2.60 | 5.78 |
| Glycine | 1.43 | 2.76 | 1.76 | 2.66 | 3.89 |
| Alanine | 3.03 | 4.00 | 2.59 | 2.88 | 2.30 |
| Tyrosine | 2.86 | 1.78 | 2.06 | 1.99 | 2.28 |
| Cystine | ND | ND | ND | ND | ND |

ND = not determined.
[a]Mixture of casein–gelatin.
[b]Essential amino acids.
[c]Non-essential amino acids.

### *Experimental fish and rearing methods*

Juvenile red sea bream were obtained from Matsumoto Suisan, Miyazaki, Japan, and adjusted to a commercial pellet diet (Higashimaru Feeds, Japan) under standardized environmental conditions for two weeks in the wet laboratory of the Faculty of Fisheries, Kagoshima University. After acclimatization, the fish were sorted and placed in polycarbonate tanks (30 l) containing 15 juveniles (mean weight of 1.58 ± 0.01 g) each. The fish were given the respective test diets in triplicate tanks at a rate equaling 5% of body weight per day for 30 days. The fish were fed twice a day at 0830 and 1630 h. Uneaten food was removed 1 h after feeding and dried to quantify feed intake. Faecal matter was removed by siphoning the water from the bottom of each tank 1 h before feeding. Every 10 days the fish were weighed and the ration was adjusted accordingly. The water temperature was 25.6 ± 1.58 °C (mean ± S.D.) during the feeding period. The pH and salinity of the water during the study period were 8.00 ± 0.15, and 33.5 ± 0.39 ppt, respectively. The water flow of the tank was 1.2 l/min and a 12:12 h light–dark photoperiod was maintained.

*Table 4*. Amino acid composition (g per 100 g dry diet) of ingredients and supplementation of CAA in different diets to simulate the amino acid pattern of reference protein.

| Amino acids | Casein, 20% | Gelatin, 10% | CAA supplemented[a] | | | |
|---|---|---|---|---|---|---|
| | | | REP | RLP | RJP | BFP |
| Arginine | 0.69 | 0.98 | 2.00 | 1.50 | 1.83 | 1.51 |
| Histidine | 0.45 | 0.03 | 0.99 | 0.86 | 0.83 | 1.32 |
| Isoleucine | 0.96 | 0.21 | 1.29 | 0.70 | 1.43 | 0.89 |
| Leucine | 1.65 | 0.37 | 1.54 | 2.01 | 1.86 | 1.76 |
| Lysine | 1.80 | 0.52 | 2.01 | 1.51 | 3.06 | 2.50 |
| Methionine | 0.53 | 0.08 | 0.53 | 1.00 | 0.69 | 0.64 |
| Phenylalanine | 1.19 | 0.26 | 0.65 | 0.69 | 0.96 | 0.83 |
| Threonine | 0.71 | 0.20 | 1.40 | 1.40 | 1.34 | 1.28 |
| Tryptophan | 0.49 | 0.01 | 0.57 | 0.52 | 0.81 | 0.85 |
| Valine | 1.19 | 0.28 | 1.44 | 0.93 | 1.24 | 0.99 |
| Aspartic acid | 1.25 | 0.66 | 1.61 | 2.72 | 2.66 | 2.81 |
| Glutamic acid | 4.03 | 1.21 | 1.84 | 2.15 | 1.76 | 0.81 |
| Serine | 0.66 | 0.25 | 1.26 | 1.58 | 0.62 | 0.68 |
| Proline | 1.92 | 1.57 | 0 | 0 | 0 | 0 |
| Glycine | 0.26 | 2.16 | 0 | 0.34 | 0 | 0.25 |
| Alanine | 0.48 | 0.92 | 1.63 | 2.60 | 1.19 | 1.47 |
| Tyrosine | 1.24 | 0.11 | 1.51 | 0.43 | 0.71 | 0.64 |

[a]To simulate amino acid pattern of reference protein (Table 3).

### *Biochemical analysis*

To obtain the protein fraction of the reference protein sources, wet samples were treated with 10% trichloroacetic acid (TCA) to precipitate proteins. The protein precipitates were washed successively with 7% TCA, ethanol, chloroform–methanol (3:1), and di-ethyl ether by centrifugation and collection of precipitate. The sample proteins, ingredients of the test diets and the whole body of the juveniles after the feeding trial, were analysed for amino acid content according to Teshima et al. (1986), using high performance liquid chromatography (HPLC), (Shimadzu LC-6A, Japan). Crude protein and lipid contents of the test diets and whole body were determined by Kjeldahl (total-N $\times$6.25) with a Tecator Kjeltec System (FOSS Tecator, Sweden) and Bligh and Dyer (1959) methods, respectively. Ash and moisture contents were analysed using the method of the Association of Official Analytical Chemists (AOAC 1990).

### *Statistical analyses*

Data were subjected to statistical verification using one-way analysis of variance (package super-ANOVA, ver. 1.11, Abacus Concepts, Berkeley, California, USA). Significant differences between means were tested using the Tukey Kramer test (Kramer 1956). Probabilities of $p < 0.05$ were considered significant.

## Results

Table 5 shows the mean weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), apparent protein utilization (APU) and survival of the red sea bream juveniles fed diets containing different amino acid patterns. The highest weight gain (562%) was observed in juveniles fed the RJP diet which simulated the amino acid pattern of juvenile whole body protein. The lowest (489%) weight gain was observed in juveniles fed the REP diet. Weight gain among the juveniles fed the REP, RLP, BFP diet and the control diet did not show any significant differences. Significantly higher FER and PERs were seen in juveniles fed the RJP diet compared to those fed the REP diet. The juveniles fed the BFP and control diet showed significantly higher APU than those fed the REP diet, however, APU did not show any statistical difference among the fish fed RLP, RJP, BFP and control. Percentage survival was not significantly affected between the dietary treatments.

Crude protein, crude lipid, dry matter and ash contents of the whole body after the feeding trial were slightly affected by the different patterns of amino acids in the diets (Table 6). Although higher protein content was observed in juveniles fed the control diet but there were no statistical differences between the juveniles fed the RLP, BFP and RJP diets. Crude lipid content in the whole bodies of juveniles fed the control diet was significantly lower than those fed the REP diet. Ash content was higher in the juveniles fed the control diet than the juveniles fed other diets. The total amino acid composition of the whole body after the feeding trial is shown in Table 7. Only leucine, glycine and methionine showed significant difference between treatments. The leucine and methionine contents were statistically lower in juveniles fed the REP diet compared to those fed the other diets.

Significantly lower retention for individual dietary EAA was found in juveniles fed the REP diet (Table 8). The highest histidine, methionine and threonine retention were observed in the juveniles fed the control (intact

*Table 5.* Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER), apparent protein utilization (APU) and percentage survival of juvenile red sea bream fed diets containing different patterns of amino acids.[1]

| Diet | Weight gain (%) | FER[2] | PER[3] | APU[4] (%) | Survival |
|---|---|---|---|---|---|
| REP | 489 ± 25[a] | 1.02 ± 0.06[a] | 2.08 ± 0.12[a] | 23.4 ± 1.0[a] | 89.3 ± 5.9[a] |
| RLP | 546 ± 11[ab] | 1.12 ± 0.06[ab] | 2.28 ± 0.12[ab] | 25.8 ± 0.7[ab] | 93.0 ± 0[a] |
| RJP | 562 ± 31[b] | 1.17 ± 0.02[b] | 2.37 ± 0.04[b] | 27.4 ± 1.2[ab] | 95.0 ± 2.3[a] |
| BFP | 551 ± 23[ab] | 1.13 ± 0.02[ab] | 2.29 ± 0.04[ab] | 28.0 ± 1.0[b] | 95.3 ± 6.2[a] |
| Control | 490 ± 24[a] | 1.08 ± 0.05[ab] | 2.16 ± 0.09[ab] | 28.3 ± 0.4[b] | 90.0 ± 2.9[a] |

[1]Values are means ± SEM of triplicate groups. Means with different letter in the same column differ significantly ($p < 0.05$).
[2]Feed efficiency ratio = weight gain (g)/total feed intake in dry weight basis (g).
[3]Protein efficiency ratio = weight gain (g)/protein ingested (g).
[4]Apparent protein utilization = protein retained (g) × 100/protein ingested (g).

*Table 6.* Effects of different amino acid patterns on body composition (% dry matter basis) of red sea bream.[1]

| Diet | Dry matter | Crude protein | Crude lipid | Ash |
|---|---|---|---|---|
| REP | 21.1 ± 0.45[a] | 56.7 ± 0.35[a] | 22.4 ± 0.15[b] | 12.5 ± 0.15[a] |
| RLP | 22.3 ± 0.32[ab] | 57.4 ± 0.34[ab] | 21.5 ± 0.50[ab] | 12.8 ± 0.34[a] |
| RJP | 23.4 ± 0.50[b] | 57.3 ± 0.77[ab] | 21.7 ± 0.61[ab] | 12.3 ± 0.14[a] |
| BFP | 23.6 ± 0.35[b] | 57.6 ± 0.68[ab] | 21.5 ± 0.17[ab] | 12.4 ± 0.62[a] |
| Control | 22.3 ± 0.32[ab] | 58.6 ± 0.58[b] | 19.8 ± 1.26[a] | 13.9 ± 0.32[b] |

[1]Values are means ± SEM of triplicate groups. Initial body composition was 20.69% dry matter, 57.2% protein, 20.9% lipid and 12.9% ash. Means with different letters in the same column differ significantly ($p < 0.05$).

*Table 7.* Amino acid composition (g per 100 g dry sample) of the whole body after feeding trial.[1]

| Amino acids | REP | RLP | RJP | RFP | Control |
|---|---|---|---|---|---|
| Arginine | 2.68 | 2.98 | 2.90 | 3.04 | 3.07 |
| Histidine | 1.11 | 1.12 | 1.16 | 1.13 | 1.17 |
| Isoleucine | 1.16 | 1.66 | 1.77 | 1.69 | 1.74 |
| Leucine | 2.09[a] | 3.04[b] | 3.10[b] | 3.14[b] | 3.15[b] |
| Lysine | 3.35 | 3.53 | 3.85 | 3.63 | 3.43 |
| Methionine | 1.02[a] | 1.34[b] | 1.18[ab] | 1.35[b] | 1.43[b] |
| Phenylalanine | 1.58 | 1.75 | 1.79 | 1.75 | 1.86 |
| Threonine | 1.44 | 1.64 | 1.50 | 1.72 | 1.68 |
| Tryptophan | 0.43 | 0.38 | 0.59 | 0.57 | 0.49 |
| Valine | 1.76 | 1.88 | 1.96 | 1.90 | 2.01 |
| Aspartic acid | 3.34 | 3.56 | 3.51 | 3.63 | 3.68 |
| Glutamic acid | 6.55 | 6.96 | 6.89 | 7.02 | 7.39 |
| Serine | 1.01 | 1.13 | 0.89 | 1.25 | 1.11 |
| Proline | 2.01 | 2.18 | 2.05 | 2.15 | 2.32 |
| Glycine | 2.40[ab] | 2.50[b] | 2.02[a] | 2.48[b] | 2.39[ab] |
| Alanine | 2.24 | 2.43 | 2.18 | 2.48 | 2.44 |
| Tyrosine | 2.18 | 2.08 | 2.01 | 2.25 | 2.18 |
| Taurine | 0.98 | 1.08 | 1.04 | 0.83 | 0.76 |

[1]Values are means of triplicate groups. Means with different letters in the same column differ significantly ($p < 0.05$).

protein sources only) diet. The retention of individual dietary EAA in the whole body among the juveniles fed the RLP, RJP, BFP and control diet were not significantly different. A/E ratios did not show a great variation among the dietary groups except lysine, isoleucine and tryptophan (Table 9).

## Discussion

The EAA pattern of the whole body of a given species of fish has been considered to be representative of the EAA requirement profile of that species

*Table 8*. The retention of total dietary EAA in the whole body of the juveniles fed different diets.[1]

| Amino acids | EAA retention (%)[2] | | | | |
|---|---|---|---|---|---|
| | REP | RLP | RJP | BFP | Control |
| Arginine | $14.89 \pm 1.04^{a}$ | $21.17 \pm 0.24^{b}$ | $20.19 \pm 1.06^{b}$ | $23.22 \pm 0.63^{b}$ | $26.36 \pm 1.31^{b}$ |
| Histidine | $14.86 \pm 0.24^{a}$ | $17.83 \pm 1.12^{ab}$ | $20.84 \pm 1.50^{ab}$ | $14.53 \pm 1.41^{a}$ | $32.17 \pm 2.10^{b}$ |
| Isoleucine | $8.84 \pm 0.20^{a}$ | $22.07 \pm 1.08^{b}$ | $16.75 \pm 0.54^{b}$ | $19.77 \pm 1.46^{b}$ | $20.66 \pm 1.44^{b}$ |
| Leucine | $9.01 \pm 0.40^{a}$ | $21.51 \pm 0.91^{b}$ | $18.76 \pm 0.95^{b}$ | $20.02 \pm 1.24^{b}$ | $21.57 \pm 1.10^{b}$ |
| Lysine | $15.90 \pm 0.06^{a}$ | $14.54 \pm 0.67^{a}$ | $17.71 \pm 1.05^{b}$ | $18.24 \pm 1.28^{b}$ | $20.41 \pm 1.24^{b}$ |
| Methionine | $18.02 \pm 1.24^{a}$ | $24.34 \pm 1.25^{ab}$ | $22.37 \pm 1.27^{ab}$ | $26.82 \pm 2.21^{ab}$ | $33.75 \pm 1.51^{b}$ |
| Phenylalanine | $15.03 \pm 1.50^{a}$ | $21.28 \pm 1.41^{a}$ | $18.01 \pm 1.11^{a}$ | $18.27 \pm 2.00^{a}$ | $17.78 \pm 0.81^{a}$ |
| Threonine | $12.45 \pm 0.57^{a}$ | $20.91 \pm 0.54^{b}$ | $15.91 \pm 0.91^{b}$ | $18.91 \pm 1.12^{b}$ | $25.38 \pm 1.34^{c}$ |
| Tryptophan | $8.93 \pm 0.81^{ab}$ | $8.72 \pm 0.32^{ab}$ | $11.90 \pm 0.69^{b}$ | $11.03 \pm 0.67^{b}$ | $6.77 \pm 0.33^{a}$ |
| Valine | $12.15 \pm 1.01^{a}$ | $22.20 \pm 0.35^{b}$ | $17.50 \pm 1.15^{b}$ | $18.38 \pm 0.99^{b}$ | $18.88 \pm 1.09^{b}$ |

[1]Values are means ± SEM of triplicate groups. Means with different letters in the same column differ significantly ($p < 0.05$).
[2]% Dietary each EAA retention = {(mg of each EAA in final whole body per fish – mg each EAA in initial whole body per fish)/mg each EAA intake per fish} $\times 100$.

*Table 9*. A/E ratios of the essential amino acid composition of whole body tissue of the red sea bream fed different reference amino acid pattern diets.

| Amino acids | REP | RLP | RJP | BFP | Control | Forster and Ogata[a] |
|---|---|---|---|---|---|---|
| Arginine | 135 | 139 | 133 | 137 | 138 | 129 |
| Histidine | 56 | 52 | 53 | 51 | 53 | 51 |
| Isoleucine | 59 | 78 | 81 | 76 | 78 | 84 |
| Leucine | 156 | 142 | 142 | 143 | 142 | 157 |
| Lysine | 169 | 165 | 177 | 164 | 154 | 165 |
| Methionine | 52 | 63 | 54 | 61 | 64 | 84[b] |
| Phe + Tyr[c] | 190 | 179 | 174 | 180 | 182 | 152 |
| Threonine | 73 | 77 | 69 | 78 | 76 | 67 |
| Tryptophan | 22 | 18 | 27 | 26 | 22 | 21 |
| Valine | 89 | 88 | 90 | 86 | 90 | 92 |

[a]Forster and Ogata (1998).
[b]Methionine and cystine.
[c]Phenylalanine and tyrosine.

(Wilson and Poe 1985). The highest weight gain (562%) was observed in juveniles fed the RJP diet, which simulated the amino acid pattern of juvenile whole body protein. Mambrini and Kaushik (1995) recorded similar results for rainbow trout. In earlier studies, whole hen egg (Halver et al. 1959) and fish eggs (Ketola 1982) were chosen to be used as reference amino acid pattern in the diet of fish. The present study shows that significantly lower weight gain occurred in juveniles fed the diet that simulated red sea bream egg protein (REP) compared to those fed the RJP diet (Table 5). The levels of methionine, threonine, phenylalanine, lysine and tryptophan were a little lower in the diet REP than in the RJP diet. The low growth rate in juveniles fed the REP diet could be due to lower levels of EAAs being present compared to the requirement levels (Table 3). It has been reported that the imbalance of amino acids causes low availability of one or more EAAs in the diet together with low growth rate in carp (Murai et al. 1989). The higher growth rate of juveniles in the RJP groups may be due to the balanced amino acid pattern in the diet or the levels of EAAs may be close to the requirement levels for the species. Growth performance data from this study indicates that the amino acid pattern of the red sea bream juvenile whole body protein diet could be used as a reference amino acid pattern in the diet of the juvenile red sea bream.

Supplementation of CAAs did not always correlate with good growth amongst the fish. This was probably due to the leaching of CAAs from diets into the surrounding water. Cowey and Luquet (1983) reported on low growth performance of fish fed CAA-based diets compared to diets containing intact protein. The pre-coating of CAAs in the present study is thought to improve the utilization of CAAs by minimizing their leaching into water and improving absorption from the intestine.

The high percent of crude protein in the whole body of juveniles fed the control diet could be due to high protein synthesis. The amino acid composition of the whole body after the feeding trial did not show marked differences among the dietary treatment except leucine, glycine and methionine (Table 7). Similar results were found in our previous study carried out on the Japanese flounder (Alam et al. 2002a). Kaushik (1998) found no significant differences in total whole body amino acids between two different sizes of European sea bass, gilthead sea bream and turbot.

Assessment of EAA requirements of rainbow trout (Ogino 1980) and kuruma shrimp (Teshima et al. 2002) has been studied using the levels of amino acids retained in the carcass. The retention of individual dietary EAA (Table 8) in the whole body among the juveniles fed the RLP, RJP, BFP and control diet were not markedly different. These differences in EAA retention probably reflect differences in their availability and efficiency of utilization by juvenile red sea bream, as observed in our previous study for Japanese flounder (Alam et al. 2002a) and also for white sturgeon by Ng and Hung (1995). Brown (1995) has listed to determine the requirements of EAA for striped bass using A/E ratio after determining the lysine requirement through dose–response trials. In the present study, the A/E ratios did not show a great variation among the dietary groups except lysine, isoleucine and tryptophan. Our calculated A/E ratios for the red sea bream were closely related with the previously reported values for the same species by Forster and Ogata (1998).

Since the ideal amino acid profile for fish may correspond to that of whole body protein, red sea bream juvenile whole body protein could be the most appropriate dietary amino acid pattern for optimum growth. Data on the EAA pattern of whole body protein could be used as one of the reference dietary amino acids when developing diets in which at least part of the dietary fishmeal is replaced with other plant or animal protein sources until the requirements of all the EAAs for red sea bream are established. This study also confirms that juvenile red sea bream are able to utilize high amounts of CAAs in coated form for growth.

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