JOURNAL OF THE
ARABIAN AQUACULTURE SOCIETY
Vol. 8 No 1 **Arabian Aquaculture Conference** **June 2013**

Effect of Amino Acids (Lysine and Methionine + Cyctine) Supplementation Rate on Growth Performance and Feed Utilization of Sea Bass (*Dentrarshus laborax*) Larvae

Mohamed, Salama[2] ; Fatma, H.EL-Abed[1]* and Alaa A., El-Dahhar[2]
[1]General Authority of Fish Resources Development El-Madeia – Behera Governorate
[2]Animal and Fish Production Dept., Faculty of Agriculture (Saba Basha) Alexandria University PO Box 21531, Bolkly Alexandria Egypt

ABSTRACT

This experiment was designed to study the effect of amino acids levels (L-lysine and an equal mixture of L-methionine + cyctine) supplemented to diet1(control) (35% crude protein and 300 kcal/100g diet) at the rate of (0,0.6,1.2,1.8,2.4) g lysine/100g diet and (0,0.1,0.2,0.4,0.8) g methionine mixture/100g diet, respectively on growth performance, body composition and feed utilization of sea bass *(Dicentrarchus laborax*) larvae. The lysine and methionine+cystine requirements were estimated from the growth data using the broken line model (Zeitoun *et al.,* 1979). Using brackish water (15 ppt) and temperature 23.6ºC±0.17 for 8 weeks, fish were fed on the five basal diets containing 35% crude protein and 300 kcal/100g diet. Diets containing increasing amounts of (L-lysine and L-methionine+cystine) (diets 2-5) were compared with a practical diet (1) used as control. Sea bass fry of initial BW ± SE (0.039g ± 0.00) were stocked at the rate of 30 fish per aquarium (60*90*100cm). The frequency of feeding was maintained at 4 times daily. Fish were hand fed to apparent satiation 7 days per week. Each diet was tested by triplicates using powder diets. Final body weight (FBW), weight gain (WG) and specific growth rate (SGR) of sea bass fish were significantly affected ($P < 0.05$) by amino acids levels (lysine and methionine+cyctine). Amino acid content of sea bass larvae body increased with increasing amino acids in diet. The analyzed values for lysine in the diets were 0.216,0. 816, 1.416, 2.016 and 2.616 % of diets or 0.617, 2.33, 4.05, 5.76 and 7.47% of the dietary protein. Also, the analyzed values for methionine+cystine in the diets were 1.08, 1.18, 1.28, 1.48and 1.88% of diets or 3.09, 3.37, 3.65, 4.23 and 5.37% of the dietary protein. The lysine and methionine+cystine requirements were estimated from the growth data using the broken line model lysine level (2. % in the diet and 5.71% crude protein) with 0.4 methionine + cyctine level (1.5 % in the diet and 4.29 % crude protein). The best (FBW), WG and SGR were found when the fish were fed diet4 at 1.8g lysine level (2.016% in the diet and 5.76% crude protein) with 0.4 methionine + cyctine level (1.48% in the diet and 4.23 % crude protein). No significant difference in FBW between diets 4 and 5 was observed. Feed conversion ratio(FCR) improved with increasing lysine level to 2.016 % in the diet and 5.76% crude protein with methionine + cyctine level (1.48% in the diet and 4.23 % crude protein). this result indicated that the best growth and (SGR) of fish were obtained by using diet containing, 1.8g lysine level (2.016% in the diet and 5.76% crude protein) with 0.4 methionine + cyctine level (1.48% in the diet and 4.23 % crude protein), no difference between diet4 and diet5 was found. Feed conversion ratio improved with increase lysine and methionine + cyctine levels at diet4 than fish fed diet 1(control). Amino acids requirements of sea bass (0.039g initial weight) was found at 1.8g lysine level (2.016% in the diet and 5.76% crude protein) with 0.4 methionine + cyctine level (1.48% in the diet and 4.23 % crude protein)at 35% protein and 300kcal/100g diet.

Keywords: Sea bass (*Dicentrarchus labrax*), protein requirement, amino acids.

INTRODUCTION

Fish culture in Egypt has developed into a major industry. Sea bass (*Dicentrarchus labrax*) is one of the most important commercial fish species in Egypt and it is commonly used in aquaculture (El-Shebly, 2009).

The European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758), being a member of the recently revised family of Moronidae. It was the first marine non salmonid species to be commercially cultured in Europe and at present is the important commercial fish widely cultured in Mediterranean areas. Greece, Turkey, Italy, Spain, France and Egypt are now the biggest producers (Viale *et al*., 2006).

Fish culture in the Mediterranean is essentially based on two species, sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). European sea bass cultivated in the Mediterranean region is a euryhaline marine teleost species (Tsevis *et.al.*, 1992; Dalla Via *et. al.*, 1998 and Varsamos *et.al.*, 2001). The European sea bass are eurythermic (2-32ºc) (Barnabe 1990 and EFSA. 2008).

The common (or European) sea bass *Dicentrarchus labrax* L. (Teleost, Perciformes, Moronidea) is a marine teleost of great economic importance in the Mediterranean area able to survive under various salinity conditions. It is euryhaline species, (5- 60 ppt) (Jensen *et al*., 1998). Larvae of sea bass tolerate low salinities (5-6 ppt) and display better growth performance at (10-20 ppt) (Orhant, 2002).

There is only one breeding season per year, which takes place in winter in the Mediterranean population (December to March). Production of these species is nowadays a well-controlled process, but knowledge of their nutritional requirements is still very limited (Oliva-Teles, 2000). In aquaculture, diet is often the single largest operating cost item and can represent over 50% of the operating costs in intensive aquaculture (El-Sayed, 1999 and 2004). This cost depends on many factors such as protein level, the source, and type of ingredients that could be derived from plant or animal resources, and manufacture practices (Glencross *et al*., 2007).

Thus dietary protein requirements of European sea bass ranged from 42 to 52% depending on fish size, protein quality, protein to energy ratio and feeding management (Lim, 2003). Despite this high nitrogen requirement, only little information is available about the amino acid nutrition of this species (Tibaldi *et al.*, 1991). Peres *et al*. (2007) reported that in European sea bass, data on protein and EAA requirements are still inadequate.

Kaushik (1998a) proposed to apply the same bioenergetics principles developed for salmonids to other fish species, but information concerning the prediction of growth and digestible energy (DE) needs are still lacking for most of the marine warm-water species. Thereby, further knowledge on protein and AA nutrition of European sea bass is required for cost-effective and environmental friendly diet formulation (Tibaldi and Kaushik, 2005). There is no study available focusing the optimization of requirements for European sea bass (Peres and Oliva-Teles, 2006).

MATERIALS AND METHODS

This study was carried out in the Marine Fish Laboratory (MFL), Faculty of Agriculture (Saba-Basha); Alexandria University, Egypt.

Experimental procedure

Fish were obtained from El- Meadia Fishing Port in March from the Mediterranean Sea. Fish were acclimated in glass aquaria for 15 days on the experimental diets and environmental conditions before experiment start. Aquaria contained sea water transferred from the sea (30 ppt) with supplementary aeration contenusly. Fecal matter was removed by siphoning the water from the bottom of each aquarium one hour before giving the diet. All fish in each aquarium were weighed at the beginning of experiment and biweekly. Pooled sample of fifty hundred fish of sea bass were killed at the beginning of each experiment and kept frozen for further chemical analysis. At the end of the experiment, 15 to 20 fish were taken randomly from each aquarium (40* 60*100cm), killed and dried at 70°C for about 48 hours for final chemical analysis.

Sea water supply system

The sea water supply system consists of three components; the sea water supplies line, the sedimentation and disinfection facilities and the water storage tanks.

Diets formulation and preparation

Diets were formulated from commercial ingredients of fish meal, wheat flour, wheat bran, shrimp meal, soybean meal, yellow corn, vit and mineral mixture, fish oil, ascorbic acid and carboxy methyl cellulose. Diets composition and chemical analysis during the study are shown in Tables 1. Diets were prepared as follow: Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into the diets. Oil was emulsified with equal amount of water using 0.7 % phosphatedyl choline (lecithin) according to El-Dhhar and El-Shazly (1993), and added to the diets of the experiments. Mixtures were homogenized in a feed mixer model SNFGA (Kitchen aid St. Joseph, M 149085 USA). Boiling water then blended to the mixtures at the rate of 50% for pelleting. An autoclave was used to heat the diets for 20 min after adding boiling water at a maximum pressure of 1.2 kg/ cm^2 G. Vitamins and minerals mixture were added to the diets after heat treatments. Aquaria management, heat treating of the diet and exogenous zymogene addition were made according to El-Dahhar (1999).

This experiment was designed to study the effect of amino acids levels (L-lysine and an equal mixture of L-methionine + cyctine) supplemented to diet1(control) (35% crude protein and 300 kcal/100g diet) at the rate of (0,0.6,1.2,1.8,2.4) g lysine/100g diet and (0,0.1,0.2,0.4,0.8) g methionine mixture/100g diet, respectively on growth performance, body composition and feed utilization of sea bass *(Dicentrarchus laborax*) larvae. The analyzed values for lysine in the diets were 0.216,0. 816, 1.416, 2.016 and 2.616 % of diets or 0.617, 2.33, 4.05, 5.76 and 7.47% of the dietary protein. Also, the analyzed values for methionine+cystine in the diets were 1.08, 1.18, 1.28, 1.48 and 1.88% of diets or 3.09, 3.37, 3.65, 4.23 and 5.37% of the dietary protein. The lysine and methionine+cystine requirements were estimated from the growth data using the broken line model (Zeitoun *et al.*, 1979). Using brackish water (15 ppt) and temperature 23.6ºC±0.17 for 8 weeks, fish were fed the five basal diets containing 35% crude protein and 300 kcal/100g diet. Diets containing increasing amounts of (L-lysine and L-methionine+cystine) (diets 2-5) were compared with a practical diet (1) used as control. Sea bass fry of initial BW ± SE (0.039g ± 0.00) were stocked at the rate of 30 fish per aquarium. The frequency of feeding was maintained at 4 times daily. Fish were hand fed to apparent satiation 7 days per week. Each diet was tested by triplicates using powder diets .The whole population was weighed every two weeks. Mortality was recorded daily.

Chemical and statistical analysis

Amino acid analysis was performed following the high performance liquid chromatography (HPLC) as described by Teshima *et al.* (1986). Crude protein (total-N x 6.25) and total lipid contents of the test diets and whole bodies were determined using the Kjeldahl method and ether-extraction method, respectively. Ash and moisture contents were analyzed following the Association of Official Analytical Chemists (AOAC 1995) using two

Table 1*: Composition and chemical analysis of the test diets used (amino acids) for sea bass initial weight (0.04g).*

Ingredients (%)	Test diets				
	1	**2**	**3**	**4**	**5**
Wheat flour	6	6	6	6	6
Shrimp meal	14	14	14	14	14
Wheat bran	8.8	8.1	7.4	6.7	6
Soybean meal	15	15	15	15	15
Yellow corn	12	12	12	12	12
Fish meal	30	30	30	30	30
Fish oil	10	10	10	10	10
L- Lysine	0	0.6	1.2	1.8	2.4
L- Met+cys	0	0.1	0.2	0.4	0.8
CMC[1]	3	3	3	3	3
vit&Min.Mix[2]	0.8	0.8	0.8	0.8	0.8
Ascorbic acid	0.4	0.4	0.4	0.4	0.4
AA content from analysis(%as fed)					
Aspartic acid	1.099	1.099	1.099	1.099	1.099
Threonine	0.558	0.558	0.558	0.558	0.558
Serine	0.989	0.989	0.989	0.989	0.989
Glutamic acid	2.205	2.205	2.205	2.205	2.205
Proline	1.695	1.695	1.695	1.695	1.695
Glycine	1.442	1.442	1.442	1.442	1.442
Alanine	0.632	0.632	0.632	0.632	0.632
Lysine	0.216	0.81 6	1.416	2.016	2.616
Methionine+cyctine	1.08	1.18	1.28	1.48	1.88
Isoleusine	0.772	0.772	0.772	0.772	0.772
Leusine	1.284	1.284	1.284	1.284	1.284
Tyrosine	0.209	0.209	0.209	0.209	0.209
Phenylalanine	0.647	0.647	0.647	0.647	0.647
Proximate analysis (%)					
Moisture	9.58	9.75	9.69	9.66	9.78
Crude protein	34.89	35.03	34.88	35.01	34.95
Crude lipid	17.49	17.22	17.41	17.35	17.48
Crude fiber	5.37	5.33	5.26	5.41	5.35
Carbohydrate (NFE)[3]	33.30	33.49	33.54	33.34	33.21
Crude ash	8.95	8.93	8.91	8.89	9.01
** Gross Energy and (GE) (ME)(Kcal/100-g diet)	459.66 (300)	457.12 (300)	458.51 (300)	458.55 (300)	457.84 (300)

1-carboxy methyl cellulose.

2-vitamin and mineral mixture/kg premix: vitamin A, 4.8 million IU; D 3, 0.8 million IU; E 4g; K, 0.8g; B1 0.4g; riboflavin, 1.6g; B6, 0.6g; B12, 4mg; pantothinic acid, 4g; nicotinic acid, 8g; folic acid, 0.4g; biotin, 20 mg; choline chloride, 200 g; Cu, 4g; I, 0.4g; Iron, 12g; Mn, 22g; Zn, 22 g; selenium, 0.4 g.

3-NFE is nitrogen free extract = 100-(cp+cl+cf+ash).

- ***2% Zymogene® was added to each diet according to El-Dahar (1999a)***

**** gross energy (ge) content was calculated by using the factors 5.65, 9.4 and 4.1 kcal/g for protein, ether extract and carbohydrate, respectively (Jobling, 1983).***

replicate samples for each determination. Probabily of 0.05 was considered significant. The optimum dietary lysine and methionine + cystine levels were determined according to the broken-line method (Zeitoun *et al.* 1979; Robbins *et al.* 1979). The analysis of variance (ANOVA) test was mad according to Snedecor and Cochran (1981).

RESULTS

Growth performance

Data of final body weight (FBW), survival and specific growth rate (SGR) of sea bass are shown in Table (2). Increasing the amino acids in the fish diet increased significantly (P < 0.05) FBW, survival and SGR in response to the amino acids addition rate.

The maximum FBW was recorded in fish fed the diet containing 2.616 and 1.88% (T5) of lysine and methionine + cystine, respective whereas the minimum FBW was obtained in the group fed the diet without supplemental amino acids. It was found that FBW of sea bass larvae fed at the five diets of the third experiment were (0.239 ± 0.004g; 0.249 ± 0.002g; 0.282 ± 0.005g; 0.322 ± 0.001g and 0.324 ± 0.006g) (T1,T2,T3,T4 and T5, respectively). FBW significantly increased (P < 0.01) as the lysine and methionine+cystine levels increased up to 2.016% and 1.48 of diet (T4). However, FBW of the groups maintained at diets from (T2 to T5) (0.816 to 2.616% of lysine or 1.18 to 1.88% of methionine+cystine) were significantly higher than those of the groups fed at control diet (T1) (0.216 lysine and 1.08 % Methionine+cystine) without amino acids supplementation. In other words, sea bass fed A.A supplemented diets, exhibited FBW significantly higher (P < 0.05) than those of the group fed control (T1) (Table 2). The best FBW was found to be (0.324 ± 0.001) for the fish fed at diet (T5) (2.616 % in the diet and 7.47 % crude protein) of lysine with (1.88 % in the diet and 5.37 % crude protein) of methionine + cystine. Increasing lysine and methionine+cystine levels from 2.016 to 2.616 % and 1.48 to 1.88 respectively of diets (T4 and T5) did not affect any significant differences in FBW (Table 2).

Survival rate was found to range between 74.44 to 83.33% (Table 2). Survival rate of the groups maintained at diets (T2 and T5) 0.816 to 2.616% of lysine or 1.18 to 1.88% of methionine+cystine were significantly higher than those of the groups fed at diet (T1) (control) (0.216 lysine and 1.08 % methionine+cystine). The best survival rate was found to be (83.33 ± 0.00%) for the fish fed at diet (T4) (2.016 % in the diet and 5.76 % crude protein) of lysine with (1.48 % in the diet and 4.23 % crude protein) of methionine + cystine. SGR of sea bass fed the five diets of the third experiment were found to be (3.239 ± 0.028; 3.312 ± 0.015; 3.530± 0.029; 3.768 ± 0.006 and 3.781 ± 0.030 %/d) (T1, T2, T3, T4 and T5, respectively). SGR of the fish fed on control diet (T1) (3.239 ± 0.028%/d) was significantly less (P<0.05) than SGR of the other fish fed all other diets (Table 2). SGR increased significantly with increasing the supplementation rate of amino acids to the control diet up to 2.016 % of lysine with (1.48 %) of methionine + cystine. The best SGR was found to be (3.781± 0.030) for the fish fed at diet T5 (2.616 % in the diet and 7.47 % crude protein) of lysine with (1.88 % in the diet and 5.37 % crude protein) of methionine+cystine. Increasing the lysine or methionine+cystine levels from 2.016 to 2.616 % or 1.48 to 1.88, respectively of diet (T4 and T5) got no significant differences in SGR (Table 2). Data of weight gain (WG), feed consumption and feed conversion ratio (FCR) of sea bass maintained at the five treatments of the experiment are shown in Table (3).

WG of sea bass was found to be (0.201 ± 0.003; 0.209 ± 0.002; 0.242 ± 0.006; 0.283 ± 0.002 and 0.285 ± 0.005 g/fish) (T1, T2, T3, T4 and T5, respectively). WG of the fish fed diet (T1) (control) was significantly less (P < 0.05) than all groups fed the other diets (Table 3).

Table 2: ***Mean ± standard error (SE) of final weight, survival and specific growth rate (SGR) of sea bass (Decentrarshus laborax) (0.039g initial BW) fed five diets supplemented with (lysine and methionine+cyctinet for eight weeks.***

(Met+cys) level	Lysine level	Treatment	Final weight	Survival (%)	SGR(%/d)
%diet (%protein)	%diet (%protein)		(g/fish)		
1.08 (3.09)	0.216 (0.617)	T1	0.239d ± 0.004	74.44d ± 0.82	3.239d ± 0.028
1,18 (3.37)	0.816 (2.33)	T2	0.249c ± 0.002	81.11c ± 0.82	3.312c ± 0.015
1.28 (3.65)	1.416 (4.05)	T3	0.282b ± 0.005	82.22b ± 0.82	3.530b ± 0.029
1.48 (4.23)	2.016 (5.76)	T4	0.322a ± 0.001	83.33a ± 0.00	3.768a ± 0.006
1.88 (5.37)	2.616 (7.47)	T5	0.324a ± 0.006	82.22b ± 0.82	3.781a ± 0.030

*** Means in the same column not sharing the same letter are significantly different P < 0.05.**

WG was increased significant with increasing lysine and methionine + cyctine levels up to (T4) (2.016 % in the diet and 5.76 % crude protein) and (1.48 % in the diet and 4.23 % crude protein), respectively of diet. But no statistical difference was occurred between (T4 and T5) where the lysine or methionine + cystine levels increased from 2.016 to 2.616 % or 1.48 to 1.88, respectively of diets. Weight gain data subjected to broken-line model indicated optimum dietary lysine and methionine + cystine levels for the larvae sea bass were of 2.0 and 1.5% of diet or 5.71 and 4.29% of protein.

Also, feed consumption of sea bass fed the five diets of this experiment were found to be (0.598 ± 0.012 g; 0.605 ± 0.007 g; 0.614 ± 0.014 g; 0.652 ± 0.011 g and 0.658 ± 0.004 g/fish), (T1,T2,T3,T4 and T5, respectively). Feed consumption of the fish fed diet (T1 and T2) (0.598 ± 0.012 g and 0.605 ± 0.007 g) were significantly less (P < 0.05) than all groups fed the other diets. Feed consumption was lowest for the groups fed the 0.216% of lysine and 1.08% of methionine+cystine and highest for the group fed 2.016% of lysine and 1.48 of methionine + cystine (T4). Feed consumption was increased significant with increasing lysine and methionine + cyctine levels at (T4) (2.016 % in the diet and 5.76 % crude protein) and (1.48 % in the diet and 4.23 % crude protein diet), respectively. No significant difference in feed consumption between T4 and T5 was observed (Table 3).

Likewise, FCR exhibited the same trend of weight gain and feed consumption. FCR improved with the use of amino acids (lysine and methionine + cyctine) in the diets. It improved as the fish fed diets (T2, T3, T4 and T5) with increasing (lysine and methionine + cyctine levels) than fish fed T1. The values of these groups were (2.964 ± 0.053; 2.883 ± 0.039; 2.534 ± 0.011; 2.304 ± 0.042 and 2.311 ± 0.051) (T1, T2, T3, T4 and T5, respectively). Feed consumption was improved significantly with increasing lysine and methionine + cystine levels at (2.016 % in the diet and 5.76 % crude protein) and (1.48 % in the diet and 4.23 % crude protein diet), respectively (diet 4). No significant differences (P < 0.05) in FCR between diets (T4 and T5) were observed (Table 3).

Body Composition

Table 4 shows the chemical composition of sea bass (*Decentrarshus laborax*) body as affected by the five diets. The results showed that the body moisture content of fish fed different levels of dietary amino acids decreased (P < 0.05) with increasing lysine and methionine

Table (3): ***Mean ± standard error (SE) of weight gain (WG), feed consumption and feed conversion ratio (FCR) of sea bass (Decentrarshus laborax) (0.039g initial BW) fed five diets supplemented with(lysine and methionine+cyctine) for eight weeks.***

(Met+cys) level	Lysine level	Treatment	Weight Gain g/fish	Feed consumption g/fish	FCR
%diet (%protein)	%diet (%protein)				
1.08 (3.09	0.216 (0.617)	T1	0.201d ± 0.003	0.598c ± 0.012	2.964a ± 0.053
1,18 (3.37)	0.816 (2.33)	T2	0.209c ± 0.002	0.605bc ± 0.007	2.883a ± 0.039
1.28 (3.65)	1.416 (4.05)	T3	0.242b ± 0.006	0.614b ± 0.014	2.534b ± 0.118
1.48 (4.23)	2.016 (5.76)	T4	0.283a ± 0.002	0.652a ± 0.011	2.304c ± 0.042
1.88 (5.37)	2.616 (7.47)	T5	0.285a ± 0.005	0.658a ± 0.004	2.311c ± 0.051

*** Means in the same column not sharing the same letter are significantly different P < 0.05.**

+ cyctine in the diets up to (2.016% and 1.48 % in the diet, 5.76% and 4.23 % crude protein), respectively in T4 and thereafter an increase in body moisture content was observed with decreasing dietary lysine and methionine + cyctine levels (T1). At the same time, the difference in moisture contents of the fish body fed T4 and T5 containing (2.016 and 2.616% lysine and 1.48 and 1.88% methionine + cyctine in the diet) was not significant (P > 0.05). The lowest lipid value was obtained with T1 (1.52 ± 0.03%). The highest lipid content of sea bass was found to be (2.12 ±0.04%) for the fish fed control (T4), which content of lysine and methionine + cyctine levels up to 2.016g/100g diet and 1.48 g/100g diet, respectively.

Lipid contents of sea bass fed T2, T3, T4 and T5 (lysine and methionine + cyctine levels) were significantly (P < 0.05) higher than that of the fish fed control T1 without amino acids supplementation. At the same time, the difference in lipid content of the fish body fed (T4) and (T5) containing (2.016 and 2.616 g/100g diet lysine and 1.48 and 1.88 % methionine + cyctinein the diet) was not significant (P > 0.05) Table (4). The lowest protein content was found to be (11.32 ± 0.02%) for the fish fed control diet (T1).

The highest protein content of sea bass was found to be (12.11 ± 0.02) for the group fed T4 containing lysine and methionine + cyctine in the diets up to (2.016 % and 1.48 % diet, 5.76 % and 4.23 % crude protein), respectively. At the same time, the difference in protein content of the fish body fed (T4) and T5 containing (2.016 and 2.616 g / 100g diet lysine and 1.48 and 1.88% methionine + cyctine in the diet) was not significant (P > 0.05) (Table 4). The lowest protein content was found to be (11.32 ± 0.02%) for the fish fed control diet (T1).

Amino acids determination

Table 5 shows the chemical analysis of 14 amino acids in the carcass of sea bass. Lysine and cystine contents in the carcass of sea bass fed on T1, T2, T3,T4 and T5 increased significantly (P<0.05) with the increase of amino acids in control diet (T1). But, the increase of methionine in the fish body was found significant (P<0.05). The lowest content of lysine and cyctine in the carcass of sea bass were found to be (4.60% and 9.09%) respectively, in the fish fed T1 (control), but the lowest content of methionine was found in the fish fed T2 with the increase of methionine in the control.

The highest amino acids (lysine and cyctine) content of sea bass was found to be for the fish fed T4 (Table 5). Methionine + cyctine contents in carcass of sea bass fed on T1, T2, T3, T4 and T5 were increased significantly (P < 0.05) with increasing the amino acids in the diet.

Table 4. ***Mean ± standard error (SE) of moisture, protein and lipid content (% dry matter content) in the carcass of sea bass (Decentrarshus laborax) (0.039 g initial BW) fed five diets supplemented with (lysine and methionine+cystine) for eight weeks.***

(Met+cys) level %diet (%protein)	Lysine level %diet (%protein)	Treatment	Moisture	Lipid	Protein
Initial			80 ± 0.01	1.34 ± 0.014	10.5 ± 0.21
1.08 (3.09	0.216 (0.617)	T1	78.48a ± 0.05	1.52d ± 0.03	11.32d ± 0.02
1,18 (3.37)	0.816 (2.33)	T2	77.19b ± 0.04	1.65c ± 0.02	11.59c ± 0.04
1.28 (3.65)	1.416 (4.05)	T3	75.77c ± 0.08	1.87b ± 0.03b	11.89b ± 0.02
1.48 (4.23)	2.016 (5.76)	T4	74.89d ± 0.04	2.12a ± 0.04	12.11a ± 0.02
1.88 (5.37)	2.616 (7.47)	T5	74.76d ± 0.09	2.11a ± 0.01	12.08a ± 0.03

****Means in the same column not sharing the same letter are significantly different P < 0.05.***

Table 5: ***Mean of amino acids content in the carcass of sea bass (Decentrarshus labrax) (0.039 g initial BW) for eight weeks.***

Amino Acids (as% of body protein)	Lysine / methionine+cystine level in the diets					
	Initial	o.216/1.08	0.816/1.18	1.416/1.28	2.016/1.48	2.616/1.88
		T1	T2	T3	T4	T5
Aspartic acid	8.04	7.83	8.20	8.27	7.80	8.27
Threonine	3.34	3.58	4.07	4.53	4.40	4.57
Serine	3.06	3.40	3.91	3.90	3.74	3.86
Glutamic acid	8.37	8.62	8.69	7.65	8.75	7.18
Proline	15.47	14.29	14.13	13.92	13.54	13.23
Glycine	8.05	7.76	7.66	7.31	7.39	6.61
Lysine	**3.67**	**4.60c**	**5.25b**	**5.37b**	**7.01a**	**6.97a**
Methionine	**1.43**	**2.14ab**	**1.86b**	**2.3a**	**2.19ab**	**2.35a**
Cystine	**7.59**	**9.09d**	**9.49c**	**9.87b**	**10.95a**	**9.73b**
Isoleucine	4.61	4.68	5.23	5.82	5.62	5.72
Leucine	6.95	7.90	8.65	9.34	9.13	9.55
Tyrosine	2.001	2.20	2.38	2.10	2.20	2.22
Phenylalanine	4.97	5.50	5.62	5.47	5.43	4.96
Alanine	3.17	2.85	3.09	3.30	2.92	3.27

****Means in the same row not sharing the same letter are significantly different P < 0.05.***

Protein and energy utilization

Values of PER, PPV% and ER% were shown in Table 6 and Figs. (7, 8 and 9). PER, PPV% and ER% of sea bass fed the five diets of the experiment increased significantly (P < 0.05) with the diet (T2, T3, T4 and T5) Containing (lysine and methionine + cyctine) than T1 (without AA supplementation). However, the lowest significant values of PER, PPV% and ER% were found with T1 (control).

PER (gain/protein fed) increased significantly (P < 0.05) when larvae given diets containing amino acids levels up to T4 and no significant difference was found between T4 and

Table 6. ***Mean ± standard error (SE) of protein efficiency ratio (PER), protein productive value (PPV %) and energy ratio (ER %) of sea bass (Decentrarshus labrax) (0.039g initial BW) fed five diets with amino acids level (lysine and methionine+cyctine) for eight weeks.***

(Met+cys) level %diet (%protein)	Lysine level %diet (%protein)	Treatment	PER	PPV%	ER%
1.08 (3.09	0.216 (0.617)	T1	0.958c ± 0.032	10.92d ± 0.41	8.90d ± 0.376
1,18 (3.37)	0.816 (2.33)	T2	0.991c ± 0.014	11.65c ± 0.16	14.42c ± 0.226
1.28 (3.65)	1.416 (4.05)	T3	1.128b ± 0.054	13.64b ± 0.54	56.69b ± 2.26
1.48 (4.23)	2.016 (5.76)	T4	1.236a ± 0.022	15.22a ± 0.35	64.76a ± 1.22
1.88 (5.37)	2.616 (7.47)	T5	1.240a ± 0.028a	15.18a ±0.40	63.30a ± 1.67

***Means in the same column not sharing the same letter are significantly different P < 0.05.**

T5. The highest PER value (1.236 ± 0.022) was obtained in fish fed on T4 which content lysine and methionine + cyctine levels (2.016% and 1.48 % in the diet, 5.76% and 4.23 % crude protein) Table 6 and Fig.7. The lowest PER of larvae (0.958 ± 0.032) was found with the T1 which contained lysine and methionine + cyctine levels (0.216% and 1.08 % in the diet, respectively) (Fig. 8).

PPV% (gained protein/protein fed) increased significantly (P < 0.05) in larvae fed on diets T2, T3,T4 and T5 which contain lysine and methionine + cyctine at level (0.816, 1.416, 2.016, 2.616 and1.18,1.28 ,1.48 ,1.88%), respectively followed by T1 (control). But the best value of PPV% (15.22 ± 0.35 %) was obtained with T4 at (2.016 % lysine and 1.48 % methionine + cyctine in the diet) with no significant (P > 0.05) difference between T4 and T5. However, the lowest PPV% was found when the fish fed T1 (10.9 2 ± 0.41) which contained lysine and methionine + cyctine levels (0.216 % and 1.08% respectively) (Table 6 and Fig. 8).Also, ER% (retained energy/energy fed) significantly increased when the fish fed on T2, T3, T4 and T5 which contain lysine and methionine + cyctine (Table 6). But, the highest ER% (64.76 ±1.22) was obtained with T4 (2.016% of Lysine and 1.48% of methionine + cyctine) with no significant (P > 0.05) differences between T4 and T5. The lowest ER% (8.90± 0.37) (Table 6 and Fig. 9) was observed with the larvae fed T1 (control), which contains (0.216 of Lysine and 1.08% of methionine + cyctine).

From the data presented in this study it is recommended that amino acids (lysine and methionine+cyctine) should be, added to a diet low in protein level to get the significantly best growth and feed utilization of sea bass(*Decentrarshus laborax*) of (0.039 g) initial BW. The best lysine and methionine+cyctine level were obtained to be 2.016 and 1.48% of diet (5.76 and 4.23 g/100 g protein). Broken-line model indicated that the optimum dietary lysine and methionine+cyctine levels were 2.0% and 1.5% of diet (5.71% and 4.285% of protein), respectively. These values were close to the value by weight gain data.

DISCUSSION

This study was carried out to investigate the effect of amino acid supplementation levels (lysine and methionine + cystine) rate on weight gain, feed utilization and body composition of sea bass (*Dicentrarchus labrax*). Only little information is available about the amino acid nutrition of this species (Tibaldi *et al*., 1991). Peres *et al*., (2007) reported that in European sea bass, data on EAA requirements are still inadequate.

In the present study, low feed intake recorded for fry fed the diet deficient of amino acids (lysine and methionine + cystine) resulted in low growth as related to sea bass(initial BW 0.039g) in this experiment. This result was found by Ravi and Devaraj (1991) on *C. catla*. Also, Essential-amino-acid deficiency has been widely demonstrated to reduce feed intake, where normal intake values are reached only when the amino-acid concentration in the diet meets the requirements of the fish, as reviewed by De la Higuera (2001).

The present study found that FBW,WG, SGR, survival and feed efficiency increased with increasing levels of dietary lysine up to1.8g (2.016% in the diet and 5.76% crude protein) with methionine+cystine level 0.4g (1.48 % in the diet and 4.23 % crude protein) at 35% crude protein of sea bass (initial weight 0.039g). Also, Berge *et al*. (1998) determined the dietary lysine requirement of Atlantic salmon and found that fish fed diets containing low levels of lysine showed reduced feed utilization. Increasing lysine level in the diet improved the protein efficiency ratio (PER) and the feed conversion ratio (FCR). Higher level of lysine than the suggested requirement did not improve feed utilization. An indispensable amino acid deficiency may cause reduced growth and poor feed conversion (Wilson and Halver, 1986);

therefore, satisfying the indispensable amino acid requirements of a species is outmost importance in preparing well-balanced diets.

Also, Small and Soares (2000) reported that 2.01% dietary lysine increased weight gain of fingerling striped bass (1.5g). This result is in agreement with found in this study.

In the present study, increasing levels of lysine and methionine + cystine in the diet improved the protein efficiency ratio, PPV and the feed conversion ratio (FCR). Mai *et al*. (2006b) reported that PER values were reduced when juvenile Japanese sea bass fed lysine deficient diets (1.28 to 1.86%), while growth response and diet utilization were improved with supplementation of crystalline lysine at 2.46%. Likewise, WG %, SGR% and feed efficiency increased with increasing levels of dietary lysine up to the level of 5.27% protein and remained nearly constant thereafter. The same trend was observed with dietary methionine up to the level of 3.51% protein of sea bream (initial BW 3.5g) (Tibaldi and Kaushik, 2005).

On the other hand, methionine deficiency resulted in reduced growth and feed efficiency, as well as in cataract of salmon (Walton *et al*., 1982; Rumsey *et al.*, 1983 and Cowey *et al*., 1992). Luo *et al*. (2005) from their study on methionine requirement of juvenile grouper, they observed low PPV % when fish fed the diets containing 0.55% methionine. Significant improvement in PPV % was observed when level of methionine supplementation increased at ranging from 1.34 to 1.81 % to be 28.8 and 31.6 % PPV %, respectively. Also, Kim *et al.* (1992) observed in their study on rainbow trout, significant increase in nitrogen retention (19.2 to be 35.9%) with increasing the levels of methionine in the diets from (0.23% to 0.60%, respectively).

The optimal dietary lysine levels requirement based on maximum weight gain (%) was determined to be 2.016 % of the diet or 5.76 % of the dietary crude protein. Dietary lysine requirements of 13 fish species (% of protein) as presented by Akiyama *et al.* (1997) rang from 3.8 % to 6.2 %, the estimated a value of 6.2 % is in a close agree with the value of 5.76 % determined in the present study. Also, lysine requirements of fish range from 5.0 to 6.8 % of dietary protein, the highest values ordinarily related to nutritional requirements of carnivorous fish (NRC, 1993). However, when the requirements are expressed as a percentage of dietary protein, the estimated value for sea bass (5.76 %) falls within the same range (5 and 6.8 %). Ravi and Devaraj (1991) reported that lysine requirement of catla is 6.2 %, or rainbow trout 6.1% (Ketola, 1983) and Atlantic salmon 6.1% (Rollin,1999).

As a matter of fact, Coyle *et al.* (2000) reported that lysine requirement of largemouth bass is 2.8 % of the diet or 6.0% of the dietary protein. Concerning gilthead sea bream requirement level, Luquet and Sabaut (1974) estimated a value of 5%, while Vergara (1992) based on the IAA profile of sea bream carcass, calculated a value of 5.49%. A value (4.8%) was determined by Tibaldi and Lanari (1991a) for sea bass (initial BW 0.85g). Dairiki *et al.* (2007) determined largemouth bass *Micropterus salmoides* (1.29 g) require (2.1/43) of dietary lysine or (4.9%) dietary protein for final weight, WG and SGR. Adequate dietary lysine contents improve survival and growth rate (Halever 1988 and 1989 and Keembiyehetty and Gatlin, 1992).

Also, Keembiyehetty and Gatlin (1992) reported that lysine requirement for striped bass (8g BW) was found as (1.41/35) of dry diet (4.03%) of dietary protein. On the other hand, Brown *et al*. (1988) reported lysine requirement of red

drum to be 4.6-5.7% of dietary protein(1.6-2.0/35).The wide variation observed in the requirements for lysine among fish species may be due to the differences in dietary protein sources, the reference protein which amino acid pattern is being imitated (Forster and

Ogata, 1998), diet formulation, size and age of fish, genetic differences, feeding practices and rearing conditions (Ruchimat *et al.*, 1997), dietary protein level and lysine availability (Wilson, 1985), initial fish size and method of feeding (fixed rate vs. satiation feeding) (Ronald *et al*., 2000).

The optimal dietary methionine + cystine levels requirement based on maximum weight gain (%) was determined to be 1.48 % in the diet or 4.23 % of the dietary protein. Regarding methionine+cystine requirement, values for several fish species as reviewed by Akiyama *et al.* (1997) vary from 2.2 to 4%. Sea bream requirement level for (Met+Cys) as determined by Luquet and Sabaut (1974) is 4% , while according to the estimations of Vergara (1992) and Kaushik (1998b) the Met+Cys requirement level of sea bream is 2.7% and 2.4%, respectively. But, from data in our study, it is in agreement with 4.4% Met+Cys for sea bass had found (http:// www. Malawi cichlid hompage.com) estimated, a value of 4.4% is in a close agreement with the value of 4.23 % determined in the present study.

A least requirement level (4%) was determined by Thebault *et al.* (1985) for juvenile sea bass in comparison to the level of 4.23 % determined for larvae sea bass (initial BW 0.039) in this study. juvenile sea bass (35g) is 1.0%. Luo *et al.* (2005) determined that juvenile grouper, *Epinephelus coioides* (13.25g) require 1.31% dietary methionine. Dietary methionine requirement for optimal specific growth rate, feed efficiency, and conversion and protein efficiency ratio of the yelo croaker, *Pseuosciaena croacea,* is 1.41% (Mai *et al.*, 2006a). Many factors have been identified that may affect optimum levels or amino acid requirements, including species and age, dietary protein sources, crystalline amino acids, environmental conditions and experimental design (Tacon and Cowey1985 and Moon and Gatlin, 1991). Some differences between results were found to be due to different fish species and different experimental conditions e.g. fish size.

Changes in the amino acid profile of the whole fish along developmental stages have been described in different species, such as gold fish (Ostrowski and Divakaran, 1989), sturgeon(Ng and Hung, 1994), turbot (Con1.48 and 1.88% methionine +ceicao *et al.*, 1997), dentex (Tulli and Tibaldi, 1997), African catfish (Conceicao *et al.* 1998a) or Senegal sole (Araga *et al.* 2004). Such variations are usually explained as the result of changes in the proteins synthesized at different moments of the larval development (Conceic e*t al.* 1998b and Mente *et al.* 2001).

In the present study, the results showed that the body moisture and lipid content of fish fed different levels of dietary methionine decreased ($P < 0.01$) with increasing lysine and methionine+cyctine but protein content was increased in the diets up to (2.016% and 1.48 % in the diet, 5.76% and 4.23 % crude protein) at level 1.8 g/100g diet and 0.4 g/100g diet respectively. Luo *et al*. (2005) from their study on methionine requirement of juvenile grouper, they observed that carcass protein content showed an increasing trend with increasing dietary methionine levels ($P < 0.05$) but moisture content decreased ($P < 0.05$). Ash content kept relatively constant among these treatments.

On the other hand, Mai *et al.* (2006b) found in their study on juvenile Japanese sea bass that, the whole body protein and lipid were significantly affected by dietary lysine levels ($P < 0.01$). The whole body protein (range from 18.8% to 21.2%) was positively correlated with dietary lysine level, while lipid (range from 8.4% to 7.3%) was negatively correlated with it. Also, Rodehutscord *et al.* (2000) found that the crude protein (CP) in trout body increased linearly with increased dietary lysine supplementation disregarding dietary CP levels, and fat decreased linearly with increased lysine supplementation.

Likewise, Cheng *et al.* (2003) from their study on rainbow trout (*Oncorhynchus mykiss*) found that the whole body moisture content, CP, fat and ash among fish fed different diets. Lysine supplementation in the plant protein-based diets increased CP and reduced fat in fish body. Akiyama *et al*. (1997) reported that the variations in the essential amino acid requirements of different species possibly reflect the true differences between phylogenetically distinct families or species.

Fish mortality for this study was high because of the small stocking size (0.08, and 0.039g) of the fish. This agrees with that found by (Cotton and Walker, 2002) who observed thatfish mortality of black sea bass was high because of the small stocking size (0.82 ± 0.02 g) of the fish. Also, Cannibalism is a problem that has been previously observed among black sea bass and other serranids.

REFERENCES

Akiyama, T., Oohara, I. and Yamamoto, T. (1997). Comparison of essential amino acid requirements with A/E ratio among fish species. Fish. Sci., 63: 963-970.

AOAC (1995). Official Methods of Analysis, th16 ed. AOAC, Arlington, VA.USA.

Aragao, C., Conceic¸a˜ o, L.E.C., Fyhn, H. and Dinis, M.T. (2004) Estimated amino acid requirements during early ontogeny in fish with different life styles: gilthead sea bream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*). Aquaculture, 242, 589–605.

Barnabe, G. (1990). Rearing bass and gilthead bream. In: G.Barnabe (Ed.), Aquaculture, Vol. II. Ellis Harwood, London; pp 647-687.

Berge, G. E., Sveier, H and Lied, E. (1998). Nutrition of Atlantic salmon (*Salmo salar*): the requirement and metabolic effect of lysine. Comp. Biochem. Physiol., 120A, 447 – 485.

Brown, P. B., Davis, D. A. and Robinson, E. H.(1988). An estimate of the dietary lysine requirement of juvenile red drum, *Sciaenops ocellatus*. J. World Aquaculture, Soc., 19: 109-112.

Cheng, Z. J., R. W. Hardy and J. L. Usry. 2003. Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. Aquaculture, 215, 255 – 265.

Conceicao, L.E.C., Dersjant-Li, Y. and Verreth, J.A.J. (1998a) Cost of growth in larval and juvenile African catfish (*Clarias gariepinus*) inrelation to growth rates, food intake and oxygen consumption. Aquaculture, 161; 95–106.

Conceicao, L.E.C., Ozo´ rio, R.O.A., Suurd, E.A. and Verreth, J.A.J. (1998b) Amino acid profiles and amino acid utilization in larval African catfish (*Clarias gariepinus*): effects of ontogeny and temperature. Fish Physiol. Biochem., 19, 43–57.

Conceicao, L.E.C., van der Meeren, T., Verreth, J.A.J., Evjen, M.S.,Houlihan, D.F. and Fyhn, H.J. (1997) Amino acid metabolism and protein turn over in larval turbot (*Scophthalmus maximus*) from first feeding until metamorphosis, fed natural zooplankton or Artemia. Mar. Biol., 129; 255–265.

Cotton, C. F. and Walker, R.L. (2002). Optimal commercial diet and feeding ration for black sea bass (*Centropristis striata*) fingerlings. To be submitted to the Journal of Applied Aquaculture.

Cowey, C.B., Cho, C.Y., Sivak, J.G., Weerheim, J.A. and Stuart, D.D. (1992). Methionine intake in rainbow trout (*Oncorhynchus mykiss*), relationship to cataract formation and the metabolism of methionine. J. Nutr., 122, 1154 – 1163.

Coyle, S.D., Tidwell, J.H. and Webster, C. (2000). Response of largemouth bass (*Micropterus salmoides*) to dietary supplementation of lysine, methionine, and highly unsaturated fatty acids. Journal of Worled Aquaculture Society, 31:89-95.

Dalla Via, J., Villani, P., Gasteiger, E. and Niedersta°tter, E. (1998). Oxygen consumption in sea bass fingerlings (*Dicentrarchus labrax*) exposed to acute salinity and temperature changes: metabolic basis for maximum stocking density estimation. Aquaculture 169, 303– 313.

Dairiki, J.K., Dias, C.T.D.S. and Cyrino, J.E.P (2007). Lysine Requirements of Largemouth Bass, *Micropterus salmoides*: A Comparison of Methods of Analysis of Dose-Response Trials Data. Journal of Applied Aquaculture, 19(4): 2007.

De la Higuera, M. (2001). Effects of nutritional factors and feed characteristics on feed intake. In: Food intake in fish, Ed. Houlihan, D., Boujard, T. and Jobling M. Blackwell Science- COST Acction 827, Oxford. pp 250-268.

El-Dahhar, A. A. (1999a). Effect of Heat Treated feed and Exogenous Zymogene on survival and Growth of Grey Mullet Larvae. *Liza Ramada* (Risso) in Egypt. Aquaculture Research. 30, 165-173.

EL-Dahhar, A.A. and El-Shazly, K. (1993). Effect of essential amino acids (Methionine and lysine) and treated oil in fish diet on growth performance and feed utilization of nile *tilapia, Tilapia* nilotica (L.). Aquaculture, 24: 73-739.

El-Sayed, A.-F. M. (1999). Alternative protein sources for farmed tilapia, *Oreochromis spp*. Aquaculture, 179: 149-168.

El-Sayed, A.-F.M. (2004). Protein nutrition of farmed tilapia: searching for unconventional sources. Pages 364-378 in R. Bolivar, G. Mair, and K. Fitzsimmons, eds. Sixth International Symposium on Tilapia in Aquaculture, Manila, Philippines.

El-Sayed, A.-F. M. (1999). Alternative protein sources for farmed tilapia, *Oreochromis spp*. Aquaculture, 179: 149-168.

El-Sayed, A.-F.M. (2004). Protein nutrition of farmed tilapia: searching for unconventional sources. Pages 364-378 in R. Bolivar, G. Mair, and K. Fitzsimmons, eds. Sixth International Symposium on Tilapia in Aquaculture, Manila, Philippines.

El-Shebly, A.A. (2009). Aquaculture Potential of Sea Bass (*Dicentrarchus Labrax)* in Brackish Water Fish Farms in Egypt. Journal of Applied Sciences Research, 5(7): 765-769, 2009, INSInet Publication.

European Food Safety Authority (EFSA) (2008). Animal welfare aspects of husbandry systems for farmed European sea bass and gilthead sea Prepared by Working Group on sea bass /sea bream welfare Annex I to the EFSA Journal (2008) 844,1-49.

Forster, I. and Ogata, Y. (1998). Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea bream *Pagrus major*. *Aquaculture,* 161: 131-142.

Gatlin, D.M. (1995). Review of red drum nutrition, pp. 41-49. In C. Lim and D.J. Sessa (eds.).Nutrition and utilization Technology in Aquaculture. AOCS Press, Champaign, Illinois.

Glencross, B. D., M. Booth and G. L. Allan. (2007). A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition, 13: 17–34.

Halver, J.E. (1988). Fish Nutrition, 2nd edition. Academic Press, London, England.

Jensen, M.K., Madsen, S.S and Kristiansen, K. (1998). Osmoregulation and salinity effects on the expression and activity of Na^+/K^+-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). J. Exp. Zool. 282, 290-300.

Jobling, M. (1983). The influences of feeding on the metabolic rate of fishes: a short review. J. Fish Biol., 18, 385 – 400.

Kaushik, S.J. (1998a). Nutritional bioenergetics and estimation of waste production in non-salmonids. Aquat.Living Resour., 11: 211–217.

Kaushik, S.J. (1998b). Whole body amino acid composition of European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. Aquat. Living Resour., 11: 355-358.

Kim, K. I., Kayes, T. B. and Amundson, C. H. (1992). Requirements for lysine and arginine by rainbow trout *Oncorhynchus mykiss*. Aquaculture, 106, 333 – 344.

Keembiyehetty, C.N. and Gatlin III. D.M. (1992). Dietary lysine requirement of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). Aquaculture 104: 271-277.

Ketola, H. G. (1983). Requirement for dietary lysine and arginine by fry of rainbow trout. Anim. Sci., 56, 101 – 107.

Lim, C.E (2003). Nutrient requirements of European sea bass (*Dicentrarchus labrax*) and Gilthead Sea bream (*Sparus aurata*).United States Department of Agriculture Technical Bulletin.

Luo, Z., Liu, Y., Mai, K., Tian, L., Yang, H., Tan, X. And Liu, D. (2005). Dietary L-methionine requirement of juvenile grouper *Epinephelus coioides* at a constant dietary cystine level. Aquaculture, 249: 409-418.

Luquet, P. and Sabaut, J.J. (1974). Nutrition azotée et croissance chez la daurade et la truite. In: Actes Colloq. CNEXO, 1: 243-253.

Mai, K., Wan, J., Ai, Q., Xu, W., Liufu, Z., Zhang, L., Zhang, C. and Li, H. (2006a). Dietary methionine requirement of large yellow croaker, *pseudosciaena crocea* R. Aquaculture, 253:564-572.

Mai, K., Zhang, L., Ai, Q., Duan, Q., Zhang, C., Li, H., Wan, J. and Liufu, Z. (2006b). Dietary lysine requirement of juvenile Japanese sea bass, *Lateolabrax japonicus*. Aquaculture, 258, 535 - 542.

Mente, E., Houlihan, D.F. and Smith, K. (2001) Growth, feeding frequency, protein turnover, and amino acid metabolism in European lobster *Homarus gammarus*. L. J. Exp. Zool., 289: 419–432.

National Research council (NRC), (1993). Nutrient Requirements of Warm Water Fishes and Shell fishes. National academy press, Washington, DC.,USA.

Ng, W.K. and Hung, S.S.O. (1994). Amino acid composition of whole body, egg and selected tissues of white sturgeon (*Acipenserransmontanus*).Aquaculture, 126: 329–339.

Ng, W.K. and Hung, S.S.O. (1994). Amino acid composition of whole body, egg and selected tissues of white sturgeon (*Acipensertransmontanus*).Aquaculture, 126:329–339.

Oliva-Teles.A. (2000). Recent advances Walton, M. J., in European sea bass and gilthead sea bream nutrition. Aquaculture International, 8.(6).

Orhant T. E (2002). Growth performance, Body Traits and Fillet composition of the European sea bass (*Dicentrarchus labrax*) Reared in various salinities and freshwater. Turk. J. Vet. Anim sci., 26: 993- 1001.

Ostrowski, A.C. and Divakaran, S. (1989). The amino acid and fatty acid composition of selected tissues of the dolphin fish (*Coryphaena hippurus*) and their nutritional implications. Aquaculture, 90: 285–299.

Peres, H and Oliva- Teles, A. (2006). Effect of the dietary essential amino acid pattern on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture, 256: 395-402.

Peres, H and Oliva- Teles, A. (2007). Effect of the dietary essential amino acid pattern on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture, 267(Issues 1-4) 3: 119-128.

Ravi, J. and Devaraj, K. V. (1991). Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). Aquaculture, 96: 281 – 291.

Rodehutscord, M., F. Borchert, F.., Gregus, Z., Pack, M. and Pfeffer, E. (2000). Availability and utilization of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. Aquaculture, 187: 163 – 176.

Rollin, X. (1999). Critical study of indispensable amino acids requirements of Atlantic salmon (*Salmo salar* L.) fry. PhD thesis, Universite catholique de Louvain, Belgium.

Ronald, G. Twibell., Keith, A. Wilson and Paul, B. Brown. (2000). Dietary Sulfur Amino Acid Requirement of Juvenile Yellow Perch Fed the Maximum Cystine Replacement Value for Methionine. *Journal of Nutrition,* 130: 612-616.

Ruchimat, T., Masumoto, T., Hosokawa, H., Itoh, Y. and Shimeno, S. (1997). Quantitative lysine requirement of yellowtail (*Seriolaquin queradiata*). Aquaculture, 158: 331 – 339.

Rumsey, G. L., Page, J. W. and Scott, M. L. (1983). Methionine and cystine requirements of rainbow trout. Prog. Fish-Cult., 45: 139 – 143.

Small, B.C. and Soares, Jr. J.H. (2000). Quantitative dietary lysine requirement of juvenile striped bass *Morone saxatilis*. Aquaculture Nutrition, 6: 207-212.

Snedecor, G, W. and Cochran, W.G.(1981). Statistical Methods-lowa Stat Univ., Ames, J.O., USA. 341pp.

Tacon, A. G. and Cowey, C.B. (1985). Protein and amino acid requirements. In: Fish Energetics: and New Perspectives (Tytler, P. and P. Calow, eds.). Croom Helm, London, UK. pp. 155-183.

Teshima, S., Kanazawa a, A. and Yamashita, M.(1986). Dietary value of several proteins and supplemental amino cids for larvae of the prawn, *Penaeus japonicus*. Aquaculture, 51: 225-235.

Thebault, H., Alliot, E and Pastoureud, A (1985). Quantitative methionine requirement of juvenile sea bass (*Dicentrarchus labrax*). Aquaculture, 50: 75-87.

Tibaldi, E. and Kaushik, S. (2005). Amino acid requirements of Mediterranean fish species. In: Montero, D., Basurco, B., Nengas, I., Alexis, M., Izquierdo, M. (Eds.), Proceeding of the Workshop on Mediterranean Fish Nutrition, Rhodes (Greece), 1–2 June, 2002. Cahier Options Méditerranéennes, 63: pp. 59–65.

Tibaldi, E. and Tulli, F. (1999). Dietary threonine requirement of juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture, 175: 155-166.

Tibaldi, E. and Lanari, D. (1991). Optimal dietary lysine levels for growth and protein utilization of fingerling sea bass (*Dicentrarchus labrax L.*) fed semi purified diets. Aquaculture, 95: 297-304.

Tsevis, N., Klaoudatos, S. and Conides, A. (1992). Food conversion budget in sea bass *Dicentrarchus labrax,* fingerlings under two different feeding frequency patterns. Aquaculture, 101, 293– 304.

Tulli, F. and Tibaldi, E. (1997) Changes in amino acids and essential fatty acids during early larval rearing of dentex. Aquac. Int., 5,229–236.

Varsamos, S., Connes, R., Diaz, J., Barnabe´, P., and Charmantier, G. (2001). Ontogeny of osmoregulation in the European sea bass *Dicentrarchus labrax* L. Mar. Biol, 138: 909–915.

Vergara, J.M. (1992). Studies on the utilization of dietary protein and energy by gilthead sea bream (*Sparus aurata*). PhD thesis, University of Stirling, UK.

Viale, I., Cubadda, C., Angelucci, G. and Zalati, F. (2006). Immunization of European Sea bass, *Dicentrarchus labrax* L. 1758, Fingerlings with a Commercial Vaccine against Vibriosis: A One Year Survey on Antibody Level, Diseases and Growth. Aquaculture,18 (3).

Walton, M. J., Cowey, C.B. and J. W. Adron, J.W. (1982). Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr., 112: 1525 – 1535.

Walton, M. J., Cowey, C.B. and J. W. Adron, J.W. (1982). Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr., 112: 1525 – 1535.

Wilson, R. P. and Halver, J.E. (1986). Protein and amino acids requirements of fishes, Ann. Rev., Nutr, 6: 225 – 244.

Wilson, R. P. and Poe, W. E. (1985). Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in catfish, *Ictalurus punctatus.* Comp. Biochem. Physiol. 80B, 385 -388.

Wilson, R. P. and Halver, J.E. (1986). Protein and amino acids requirements of fishes, Ann. Rev., Nutr, 6: 225 – 244.

Wilson, R. P. and Poe, W. E. (1985). Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in catfish, *Ictalurus punctatus.* Comp. Biochem. Physiol. 80B, 385 -388.

Zeitoun, I, H., UIIrey, D.E., Magee, W.T., Gill, J.L. and Bergen, W.G. (1979). Quantifying nutrient requirements of fish. J. Fish. Res. Board Can., 33: 167-172.

تاثير اضافة الاحماض الامينية (ليسين وميثايونين+ سستين) على كفاءة النمو والاستفادة من الغذاء ليرقات اسماك القاروص

محمد السيد سلامة *؛ فاطمة حمدى العابد ** وعلاء عبد الكريم الدحار*

***قسم الانتاج الحيوانى و السمكى - كلية الزراعة (سابا باشا) جامعة الاسكندرية**

**** الهيئة العامة لتنمية الثروة السمكية**

أجريت هذه الدراسة في كلية الزراعة (سابا باشا) جامعة الإسكندرية ، بهدف دراسة تاثير مستويات من الأحماض الأمينية على كفاءة النمو والاستفادة من الغذاء على أسماك القاروص في مراحل النمو الأولى. في هذه التجربة تم استخدام مستوى بروتين 35% مع أعلى مستوى من الطاقة 300 ك كالورى/ 100جم من العليقة والتى تعتبر العليقة الكنترول وتم إضافة مستويات من الأحماض الأمينية إلى العلائق من 2 إلى 5 وقد كان معدل إضافة الليسين هى (2.4,1.8,1.2,0.6) كما أن إضافة حامض الميثايونين و السستين (0.8,0.4,0.2,0.1) على التوالى حيث تم استخدام خمس علائق كل معاملة تم تكرارها ثلاث مرات بعد فترة أقلمة استمرت أسبوعين ، بمعدل تخزين 30 سمكة بكل حوض . استمرت هذه التجربة 8 أسابيع بوزن ابتدائي (0.039 جم) تتم التغذية حتى الشبع حيث تقدم التغذية أربع مرات يوميا لمدة سبعة أيام أسبوعيا وقد تم اجراء التحليل الكيماوى لأسماك القاروص بعد انتهاء التجربة ،وايضا العلائق لمعرفة محتواها من الأحماض الأمينية بمدينة مبارك للأبحاث العلمية والتطبيقات التكنولوجية وحدة الخدمات العلمية والتكنولوجية

وقد أظهرت النتائج ما يلي :

1. توجد اختلافات معنوية في معدل نمو وبقاء الأسماك التي تم تغذيتها بالعلائق من 2- 5 والتي تحتوي على مستويات الأحماض الأمينية مقارنة بالعليقة الكنترول رقم (1) التى أظهرت انخفاض فى معدل نمو وبقاء الاسماك.
2. لوحظ زيادة معدل النمو النوعى بزيادة مستويات الاحماض الامينية حتى العليقة 4 والتى تحتوى على ليسين (بنسبة مئوية 2,016من العليقة و5.76 من البروتين) مع ميثايونين+سستين (بنسبة مئوية 1.48 فى العليقة و 4.23 من البروتين) ولا يوجد اختلاف معنوى بين العليقة 4 و العليقة 5 .
3. لوحظ زيادة معدل النمو النوعى بزيادة مستويات الاحماض الامينية حتى العليقة 4 والتى تحتوى على ليسين (بنسبة مئوية 2,016من العليقة و5.76 من البروتين) مع ميثايونين+سستين (بنسبة مئوية 1.48 فى العليقة و 4.23 من البروتين) ولا يوجد اختلاف معنوى بين العليقة 4 و العليقة 5 .
4. أمكن من خلال الرسم البيانى وعند انكسار المنحنى تحديد الإحتياجات من الأحماض الأمينية (ليسين) (بنسبة مئوية 2, من العليقة 5.71 من البروتين) مع الميثايونين+ السستين (بنسبة مئوية 1.5فى العليقة و 4.23 من البروتين).

من هذه النتائج نستنتج ما يلي

1. أنه بإضافة الأحماض الأمينية يظهر أعلى معدل نمو و بقاء واستفادة من الغذاء بالعلائق لاسماك القاروص (بوزن ابتدائى0.039جم)عند العليقة 4 والتى تحتوى على الليسين (بنسبة مئوية 2,016 من العليقة و5.76 من البروتين) مع الميثايونين+ السستين (بنسبة مئوية 1.48 فى العليقة و 4.23 من البروتين).
2. بإضافة الأحماض الأمينية أظهر تحسن في معدل التحويل الغذائى لأسماك القاروص بالعلائق بزيادة مستوى الليسين والميثايونين + السستين وذلك مقارنة بالعليقة الكنترول(1) وذلك حتى العليقة 4.