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EFFECT OF PARTIAL SUBSTITUTION OF DIETARY PROTEIN BY NON-ESSENTIAL AMINO ACID ON GROWTH INDICES AND FEED UTILIZATION OF COMMON CARP, *CYPRINUS CARPIO* (L.) LARVAE

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SUMMARY

The present study was conducted to investigate the effect of partial substitution (25%) of dietary protein by three non-essential amino acids (aspartic acid, glutamic acid and serine) as energy sources on growth indices and feed utilization of common carp, *Cyprinus carpio* (L.) larvae. Six isocaloric 17.0 MJ kg^{-1} diets including two basal control diets (high control, and low control) were formulated. All tested diets were isonitrogenous of 30% crude protein except for low control diet (20% CP). The two basal-control diets were prepared with fish meal (FM) as the main protein source. Diets 3-6 each, 25% of the dietary high control FM protein content, were substituted by aspartic acid (diet 3), glutamic acid (diet 4), serine (diet 5) and a mixture of equal amount proportion form this three non-essential amino acids (diet 6). A total of 1,800 common carp larvae with an average initial weight of 0.2 ± 0.1 g were equally divided into the 18 glass aquaria (100 L each) at stocking density of 100 larvae $aquaria^{-1}$. The experiment was conducted for 10 wks (66 days) using three replicate for each treatment. The highest significant ($P \leq 0.05$) final body weight, feed intake, weight gain, specific growth rate and feed conversion ratio were recorded for common carp fed the high control diet. Fish fed the diet with serine showed poorest ($P \leq 0.05$) values of growth indices. The highest values for protein efficiency ratio (PER) and protein productive value (PPV) were observed for fish fed the low control diet. The present results showed that the supplementation with either glutamic acid or a mixture of equally amount of three non-essential amino acid (aspartic acid, glutamic acid and serine) as energy sources (equivalent to 25% of high control FM protein diet), had slightly reduced growth performance and feed efficiency of common carp, *Cyprinus carpio* (L.) larvae, meanwhile using serine has a negative effect on growth performance and feed utilization of fish.

***Keywords**: aspartic acid; glutamic acid; serine; energy sources; common carp, larvae, growth*

INTRODUCTION

In fish as in other animals, the major factors influences maximum fish growth are dietary protein requirement, essential (EAA) and non-essential amino acid (NEAA) profile and the protein quality (Benakappa and Varghese, 2003). Fish require the same ten EAA in their diets like terrestrial animals. Meanwhile, fish appear to oxidize largest amount of dietary amino acid (AA) to release energy and exhibit a lower protein turnover rate in the muscle compared to mammals that use dietary AA more efficiently to synthesis their body protein (Fauconneau and Arnal, 1985). Therefore, the balance between dietary EAA profile and that required for growth and maintenance of fish are the critical point for increasing the efficiency of dietary protein utilization and affects overall fish performance. Although, definition of the best EAA/NEAA ratio of the diets is also important for maximization of animal growth performance and overall efficiency of protein utilization and therefore reducing N waste (Heger *et al.*, 1998). This is particularly important in low protein diets where NEAA concentration may become a limiting factor (Peres and Oliva-Teles, 2006).

The provirus assessment studies for the relationship between dietary protein and their AAs composition revealed that, fish like other animals have a requirement for a well–balance mixture of AAs rather than a true protein requirement (Wilson, 1989). Schwarz (1998) reported that an important aspect in fish feed formulation is to maximize nitrogen retention and to minimize nitrogen excretion into the culture systems. Recently, most of studies have been focused on the optimum dietary EAA profiles for maximum growth and protein utilization of several fish species (Ng and Hung, 1995; Akiyama *et al.*, 1997; Kaushik, 1998; Gomez-Requeni *et al.*, 2003; Rollin *et al.*, 2003; Peres and Oliva-Teles 2005; 2007). Meanwhile, few series of studies have been conducted to investigate the importance of the dietary NEAA composition for fish growth (Mambrini and Kaushik, 1994; Cowey, 1995; Schuhmacher *et al.*, 1995; Peres and Oliva-Teles 2006). Concern on the adequate dietary EAA to NEAA ratio for promoting maximum growth and protein deposition in higher vertebrates such as chicken, pig and rat has been extensively studies (Heger, 2003, Corzoa *et al.*, 2005). In fact, diets including EAA as the only N source or with very low levels of NEAA are used less efficiently than diets with adequate levels of both EAA and NEAA in chickens, pigs and fish (Mambrini and Kaushik, 1994; Schuhmacher *et al.*, 1995; Heger *et al.*, 1998).

Gaye-Siessegger *et al.* (2007) reported that while the EAA must be provided with the food, the NEAA can be synthesized by the fish from other amino acids e.g. from glutamate, aspartate and serine (which are further on termed precursor amino acids). Hughes (1985) investigated the influence of dietary NEAA on the growth of lake trout, *Salvelinus namaycush* and rainbow trout, *Oncorhynchus mykiss* using semi-purified diets containing either glutamate or glycine as the only source of NEAA and found that trout use glutamate more efficiently than glycine when no other source of NEAA was

presented. Mambrini and Kaushik (1994) reported that for Nile tilapia, *Oreochromis niloticus* the substitution level of 25% from dietary protein (30% CP) by alanine, aspartic and glutamic acid given as single or as a mixture, irrespective of the nature of the NEAA, has slightly reduced Nile tilapia growth (10%), while, nitrogen retention and excretion were unaffected. Schuhmacher *et al.* (1995) determined the effect of dietary glutamate, glycine and glutamine as sources of NEAA as well as the different dietary EAA/NEAA ratios in rainbow trout, *Oncorhynchus mykiss* and found that glutamine was superior to glycine, meanwhile glycine superior to glutamate as NEAA source. Recently, Gaye-Siessegger *et al.* (2006, 2007) used the application of stable isotope analysis to investigate the importance of the metabolic utilization of different dietary non-essential amino acid composition for the growth performance of Nile tilapia and the experiment shows the importance of the dietary NEAA composition for the growth performance of Nile tilapia.

Nowadays, fish meal is the main protein source in practical diets for fish, the reduction of dietary fish meal inclusion level is a priority, due to the scarcity of this feed ingredient in the world and consequent rising cost. Compared to fish meal, alternative protein sources are usually deficient in certain EAA. However, the dietary EAA unbalances may occur when these ingredients are used in the diet formulation. Therefore, the efficient use of alternative protein sources depends on an adequate estimation of EAA requirements of the cultured species. In this regard, Santiago and Lovell (1988) cited that the essential amino acid (EAA) needs for Nile tilapia *O. niloticus* fry represents only 10 % of the diet. Kim *et al.* (1991) reported that, if NEAA were provided as an energy source (equivalent to 10% dietary protein), the dietary protein level of 25% would be necessary instant to 35% for protein rainbow trout *Oncorhynchus mykiss*. From nutritional point of view, since, the dietary excess of NEAA can be used as energy sources, these will save the dietary EAA by reducing the need to EAA used in the synthesis of NEAA and, may help to spear all dietary EAA in quantity sufficient to meet fish requirement. Therefore, if present guess success maybe these basic information results can help the nutritionist to formulation suitable plant protein-based diets for fish?.

To our knowledge, there is no study available focusing on the optimization of dietary NEAA for common carp *Cyprinus carpio*. Also, evaluating the importance of dietary NEAA composition for a possible improves growth of common carp is scarce. Generally, common carp, *Cyprinus carpio* L. are not able to use synthetic amino acids efficiently for growth. Low palatability, poor EAA profile and complex synergistic interactions among many nutritional factors were held responsible by the authors for the reduced carp growth (Plakas and Katayama, 1981). Yan and Qiu-Zhou (2006) showed that for juvenile Jian carp (*Cyprinus carpio* var. Jian) fed the diet supplemented with glutamine (as 1.5%) improved weight gains, feed intake, gain: feed ratio, intestinal weight, fold height and digestive enzyme activities.

The present study was conducted based on the concept that since the nitrogen of EAA could be used in the synthesis of NEAA, the dietary NEAA, will saves dietary energy and helps to promote fish growth by reducing the need for dietary EAA as energy source (Cowey, 1994). Therefore, the present work was investigated to determine the growth indices and feed utilization of common carp *Cyprinus carpio* (L.) larvae in response to three NEAA substituted (aspartic acid, glutamic acid and serine or by a mixture of equal amount proportion for these three amino acids) for 25% of dietary protein diets as energy sources.

MATERIALS AND METHODS

Experimental Fish and Culture Technique

The experiment was carried out in the Fish Nutrition Laboratory, at El-Kanater El-Khayria, National Institute of Oceanography and Fisheries (NIOF), Kalubiya Governorate, Egypt. The larvae of common carp *Cyprinus carpio* (L.) were purchased from the Fish Research Station Farm, NIOF, Kalubiya Governorate, Egypt. Six different experimental treatments were assigned in triplicate. A total of 1,800 common carp larvae with an average initial weight of 0.20 ± 0.1 g were equally divided into the 18 glass aquaria (100 l each, 30× 45×60 cm) at stocking density of 100 larvae aquaria^{-1} for 10 wks (66 days). All aquaria were filled with dechlorinated tap water and supplied with a source of aeration. The turnover rate of water was 100% aquaria^{-1} day^{-1} and fish were held under natural light (14:10h light: dark schedule). Prior to start the trial, the larvae were acclimated to experimental condition for two wks. During this period, larvae were fed a high control diet (30% CP), at a level of 5% of body weight daily. The daily ration was divided into three equal amounts and offered three times a day at 09.00, 12.00 and 15.00 h.

Water temperature, dissolved oxygen, pH and ammonia were monitored during the trial; to ensure maintain water quality at recommended range for common carp larvae. Water temperature was recorded daily at 13.00 h using a mercury thermometer suspended at 30-cm depth. Dissolved oxygen (DO) was measured at 05.00 h using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH at 09.00 h by using a pH meter (Orion pH meter, Abilene, Texas, USA). Ammonia and alkalinity were measured three times a wk according to APHA, AWWA, WPCF (1985).

Experimental diets

Six test diets (Table 1) were formulated including two basal control diets. All tested diets were isocaloric 17.0 MJ DE kg^{-1} and isonitrogenous of 30% crude protein except for low control diet (20% CP). The two basal-control diets were prepared with fish meal (FM) as the main protein source. Diets 3-6 each, the amount of 25% from the high control dietary protein was replaced by an amount in proportion either aspartic acid (diet 3), glutamic acid (diet 4) and serine (diet 5) or by a mixture of equal amount proportion for these three amino acids (diet 6). The overall EAA profile

Table (1). Formulation and proximate composition of the experimental test diets (as-fed basis).

	Experimental diets					
	High control	Low control	Asp. acid	Glu. aci	Ser.	Asp. + Glu. + Ser.*
Diet assign	D1	D2	D3	D4	D5	D6
Ingredient (g/kg diet)						
Fish meal	200	100	100	100	100	100
Soybean meal	270	150	170	170	170	170
Yellow corn	236	306	358	358	358	358
Wheat bran	200	350	200	200	200	200
Soybean oil	50	50	40	40	40	40
Agar	-	-	30	30	30	30
Vit-Min.[1]	30	30	30	30	30	30
Aspartic acid	-	-	72	-	-	24
Glutamic acid	-	-	-	72	-	24
Serine	-	-	-	-	72	24
Composition (%) [2]						
Moisture	9.2	9.8	9.7	9.7	9.7	9.7
Crude protein %	29.3	20.3	29.0	29.0	29.0	29.0
Crude fat %	7.9	8.6	7.2	7.2	7.2	7.2
Carbohydrate %[3]	44.0	53.4	46.0	46.0	46.0	46.0
Ash %	9.6	7.9	8.1	8.1	8.1	8.1
Gross energy (MJ/kg diet)[4]	17.6	17.4	17.6	17.6	17.6	17.6
P/E ratio (mg CP/kJ GE g)	16.7	11.7	16.5	16.5	16.5	16.5

[1] Vitamin and mineral mixture each 1-kg of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D) , 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium, 1.2 mg folic acid; 12 mg niacin; 26 mg d-calcium pantothenate; 6 mg pyridoxine HCl; 7.2 mg riboflavin; 1.2 mg thiamin HCl; 3077 mg sodium chloride (NaCl, 39% Na, 61% Cl); 65mg ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe); 89 mg manganese sulfate ($MnSO_4$, 36% Mn); 150 mg zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn); 28 mg copper sulfate ($CuSO_4.5H_2O$, 25% Cu); 11 mg potassium iodide (KI, 24% K, 76% I); 1000 mg Celite AW521 (acid-washed diatomaceous earth silica).

[2] Chemical composition % on DM basis.

[3] Total carbohydrates calculated as [100- (Moisture+ protein+ fat+ ash)].

[4] Calculated using gross calorific values of 23.63, 39.52 and 17.15 kJ g^{-1} for protein, fat and carbohydrate, respectively according to Brett (1973).

of high control diet was adjusted to that of common carp requirements as estimated by (NRC 1993). Before mixing crystalline amino acid with the other dietary ingredients, an attempt was made to reduce the rate of their release and consequently improve their utilization, by coated the crystalline-AA mixture with agar. The findings and instructions published by Cho *et al.* (1992); with minor modification of Mambrini and Kaushik (1994); Peres and Oliva-Teles (2006) were followed. Agar (30 g) was dissolved in boiled water (10% of the diet) and cooled to 40°C before adding the crystalline-amino acids. Then, all dietary ingredients were thoroughly mixed and dry pelleted in a laboratory mincing machine through a 2-mm die. The pellets were air dried at room temperature (38 ° C) and stored in a refrigerator (-4 °C) before used.

The ingredients and proximate composition of the experimental diets are presented in Table (1). The amino acid profiles of the experimental diets are shown in Table (2). All diets formulated mainly by fish meal and soybean meal, yellow corn and wheat bran. Fish meal (999 Con-Kix Fish Meal, Triple Nine Fish Protein a.m.b.a., Thyborøn, Denmark), soybean meal, wheat bran, yellow corn vitamin and mineral premix were purchased from a commercial feed manufacturer (Animal Production Islamic Company (APICO), Dokki-El-Giza, Egypt). Soybean oil was purchased from local markets.

During the 10-wks experimental period, all fish were fed their respective diets at a level of 5% of body weight. From each experimental treatment aquaria replicate, fish were weighed collectively every two weeks, average fish weight was calculated and the amount of daily diet was adjusted accordingly. The daily ration was divided into three equal amounts and offered three times a day at 09:00, 12:00 and 15:00 h.

Growth indices

Mean final body weight (FBW) of each experimental fish was determined by dividing total fish weight in each aquaria by the number of fish. Survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), fat retention (FR) and energy retention (ER) were calculated using the following equations:

SR = (final number of fish larvae /initial number of fish larvae) ×100.

WG = Final body weight (g) - Initial body weight (g).

SGR = [(ln FBW - ln IBW)/t] ×100; where: FBW is final body weight (g); IBW is initial body weight (g); In= natural logarithmic; t = time in days.

FCR = Feed intake (g)/weight gain (g).

PER = weight gain (g)/protein intake (g).

PPV = (protein gain (g)/protein intake (g))× 100.

FR = (fat gain (g)/fat intake (g)) ×100.

ER = (energy gain (kJ)/energy intake (kJ)) ×100.

Analytical methods

At the beginning of the trial, a random pooled sample of 50 fish larvae was collected, anaesthetized with t-amyl alcohol and sacrificed for determine the initial whole body proximate composition. Also, at the termination of the feeding trial, 20 fish

Table (2). The amino acid composition of different experimental diets (g 100 g diet $^{-1}$).

	High control	Low control	Asp. acid	Glu. aci	Ser.	Asp. + Glu. + Ser.*	NRC (1993) Requirement**
Diet assign	D1	D2	D3	D4	D5	D6	
Arginine	1.93	1.30	1.45	1.45	1.45	1.45	1.6
Histidine	0.89	0.60	0.67	0.67	0.67	0.67	0.8
Isoleucine	1.40	0.99	1.05	1.05	1.05	1.05	0.9
Leucine	2.03	1.50	1.51	1.51	1.51	1.51	1.3
Lysine	2.14	1.54	1.61	1.61	1.61	1.61	2.2
Methionine+ Cysteine	1.18	0.84	0.89	0.89	0.89	0.89	0.84
Phenylalanine + Tyrosine	2.48	1.76	1.86	1.86	1.86	1.86	2.5
Threonine	1.54	1.10	1.16	1.16	1.16	1.16	1.5
Tryptophan	0.22	0.15	0.16	0.16	0.16	0.16	0.3
Valine	1.52	1.07	1.14	1.14	1.14	1.14	1.4
Alanine	1.25	0.88	0.94	0.94	0.94	0.94	-
Proline	1.45	1.01	1.00	1.00	1.00	1.00	-
Glycine	1.49	1.04	1.12	1.12	1.12	1.12	-
Aspartic acid	3.14	2.22	9.56	2.36	2.36	4.76	-
Glutamic acid	4.59	3.00	3.44	10.64	3.44	5.84	-
Serine	1.53	1.04	1.15	1.15	8.35	3.55	-
Others	0.72	0.26	0.15	0.15	0.15	0.15	
EAA/NEAA* ratio	51 : 49	51 : 49	40 : 60	40 : 60	40 : 60	40 : 60	36 : 64

* Asp. : Aspartic acid, Glu. : Glutamic acid, Ser. : Serine EAA: Essential amino acid, NEAA: Non-essential amino acid

**Data of dietary amino acids requirement for common carp was determined using diet content 38.5 % protein (NRC, 1993).

were randomly selected from each aquaria replicate and anaesthetized with t-amyl alcohol, sacrificed and homogenized in a blender, to determine the final proximate whole body composition. The fish were pooled for each aquaria, oven-dried, ground and stored at –20 °C for subsequent analysis. The chemical composition of fish and diet samples were determined according to the procedures of AOAC (1995). Moisture was determined after drying the samples in an oven (105 °C) for 24 h. Ash by incineration at 550 °C for 12h. Crude protein was determined by micro-Kjeldhal method, N% ×6.25 (using Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by soxhlet extraction with diethyl ether (40-60 °C).

Statistical analysis

Data were statistically analyzed by ANOVA using MSTAT-C version 4 software (MSTAT-C, 1987). Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed (Duncan 1955), at $P \leq 0.05$ level. All percentage data were arc-sign transformed prior to analysis (Zar, 1984). However, data are presented untransformed to simplify comparisons.

RESULTS AND DISCUSSION

Over the duration of the present study, no significant differences ($P \leq 0.05$) were observed in the water quality indices between the experimental treatments. The water temperature ranged from 27 to 28 ± 0.9 °C, dissolved oxygen from 5.9 to 6.5 ± 0.8 mg L^{-1}, pH from 6.8 to 7.8 ± 0.3, ammonia (NH_3) from 0.23 to 0.28 ± 0.1 mg L^{-1} and alkalinity from 175 to 184 ± 5.1 mg L^{-1}. These water quality indices were showed within the acceptable range for common carp (Hepher & Pruginin, 1981).

All conditions of the experimental evaluation were apparently satisfactory, and fell under the optimal standards defined for nutritional evaluations in common carp *Cyprinus carpio* (L.) larvae. No significant difference ($P< 0.05$) was observed for common carp larvae survival rate during the experimental period (Table 3); indicating that the natural dietary substations of NEAA did not affect the apparent experimental fish health.

The average final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed intake and feed conversion ratio (FCR) are presented in Table (3). The results showed that, the highest significant ($P \leq 0.05$) FBW, WG and SGR were observed for the fish fed the high control diet (30%). The same trend was observed for the changed in weight gain the over all experimental period (Fig. 1). No significant differences ($P \leq 0.05$) in fish growth indices were observed between fish fed the low control diet (20%) and the diets replaced with either glutamic acid or mixture of three NEAA (Asp. + Glu. + Ser.). The lowest values of FBW, WG and SGR ($P \leq 0.05$) for common carp larvae were observed when fish fed the diet containing serine.

The present results showed that partial substitution (25%) of dietary protein with glutamic acid as NEAA led to reduce weight gain by 5% compared

to high control diet (30%). This effect was moderate when the dietary protein was replaced by a mixture of Asp. + Glu. + Ser. (9%). Meanwhile, more growth retarded were observed when common carp larvae fed the diets containing either aspartic acid (14%) or serine (18%). These results indicated that, the utilization of synthetic non-amino acids of aspartic acid or serine seemed to be poor by common carp larvae. Souba *et al.* (1985) reported that glutamine is an important fuel which can yield 30 mol of adenosine triphosphate (ATP), comparable to the 36 mol of ATP produced from glucose, and serves as a precursor for nucleotide biosynthesis in rapidly replicating cell populations. Newsholme *et al.* (1985) have pointed out that the important pathway of glutamine metabolism by enterocyte is the conversion of alpha-ketoglutarate to oxaloacetate, which is subsequently converted to pyruvate. High rates of glutamine utilization provide optimal conditions for regulating the use of TCA cycle intermediates for the synthesis of purine and pyrimidine nucleotides during the cell cycle. In this regard, Wu *et al.* (1995; 1996) reported that glutamine is the most abundant free amino acid in blood, and glutamine is a major fuel for pig enterocytes and an essential nutrient for the proliferation of intestinal intraepithelial lymphocytes. Therefore, the supply of glutamine from the diet is likely to support the high metabolic activity of enterocytes as well as the proliferation, maturation, and migration of crypt cells or to maintain the function of intestinal lymphocytes (Alverdy *et al.*, 1992). Moreover, Wu *et al.* (1994) reported that glutamine is an abundant free amino acid in the plasma of animals and serves as an essential precursor for the synthesis of proteins, purines and pyrimidines nucleotides, NAD and amino sugars. Recently, Yan and Qiu-Zhou (2006) reported that dietary glutamine supplementation promotes intestinal development in carp and digestion ability was correlated with digestive enzyme activity, as these enzymes digested nutrients, also the intestinal enzyme activities were positively correlated with dietary glutamine supplementation level.

In fact, the intestine is a major organ of glutamine utilization and converts glutamine into other amino acids. Walton (1985) found that rainbow trout seem to utilize free amino acids in their diets more efficiently than different warm water species such as common carp, *Cyprinus carpio* and tilapia, *Tilapia zillii*. Ng *et al.* (1996) argued that the absorbed amino acids in the plasma are catabolized and excreted faster in warm water species which may explain the poor utilization of free amino acid diets. Kaushik and Dabrowski (1983) observed retarded in growth of juvenile common carp when fed a mixture of free amino acids instead of the entire protein. Silk *et al.* (1985) suggested that carp may absorb small peptides more efficiently than free amino acids. Similarly, Carvalho *et al.* (2004) showed the poorest larval common carp growth indices when fed the diets containing higher levels of free amino acids (soluble hydrolysed casein) compared with water-insoluble casein or soluble non-hydrolysed casein. Yan and Qiu-Zhou (2006) reported that in stomachless fish such as carp, the intestine plays a key role as the site of nutrient digestion and absorption, and digestive function correlates with

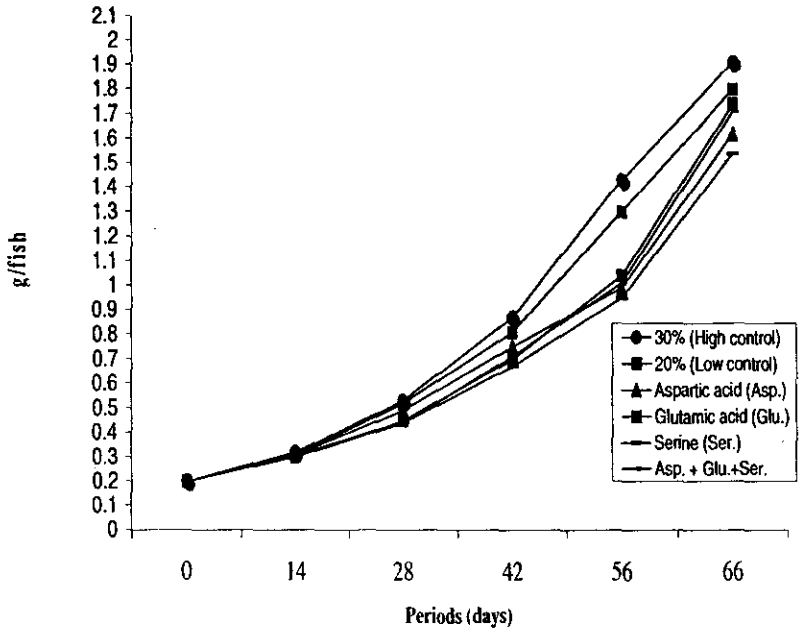

Fig.1 Effect of dietary aspartic acid , glutamic acid and serine supplementatin on the changing in weight gain of common carp, *Cyprinus carpio* L. larvae.

Table (3). Growth performance of common carp larvae fed the different experimental diets.

	High control	Low control	Aspartic acid	Glutamic acid	Serine	Asp. + Glu. + Ser.*
Initial body weight (g fish^{-1})	0.20 ± 0.1	0.20 ± 0.1	0.20 ± 0.1	0.20 ± 0.1	0.20 ± 0.1	0.20 ± 0.1
Final body weight (g fish^{-1})	2.11 ± 0.4^{a}	1.94 ± 0.3^{ab}	1.82 ± 0.3^{b}	2.00 ± 0.1^{ab}	1.74 ± 0.1^{c}	1.91± 0.1^{ab}
Weight gain (g fish^{-1})	1.91 ± 0.3^{a}	1.74 ± 0.3^{ab}	1.62 ± 0.4^{b}	1.80 ± 0.2^{ab}	1.54 ± 0.2^{c}	1.71 ± 0.8^{ab}
Relative weight gain to control diet (%)	100.0	91.1	84.8	94.2	80.6	89.5
Specific growth rate (% day)	3.57 ± 0.1^{a}	3.44 ± 0.3^{ab}	3.35 ± 0.1^{ab}	3.49 ± 0.1^{ab}	3.28 ± 0.1^{b}	3.42± 0.1^{ab}
Feed intake (g fish^{-1})	3.36 ± 0.3^{c}	3.77 ± 0.1^{a}	3.54 ± 0.2^{b}	3.44 ± 0.2^{b}	3.69 ± 0.2^{b}	3.49 ± 0.3^{b}
Feed conversion ratio	1.76 ± 0.3^{c}	2.17 ± 0.1^{ab}	2.19 ± 0.2^{ab}	1.91 ± 0.3^{b}	2.40 ± 0.2^{a}	2.04 ± 0.2^{ab}

-Means values (± SD) in the same row sharing the same subscript are not significantly different (P ≤0.05).
*Asp. : Aspartic acid, Glu. : Glutamic acid, Ser. : Serine.

Table (4). The amino acid intake of different experimental diets (g fish^{-1}).

	High control	Low control	Aspartic acid	Glutamic acid	Serine	Asp. + Glu. + Ser.*
EAA/NEAA ratio	51 : 49	51 : 49	40 : 60	40 : 60	40 : 60	40 : 60
Arginine	69	45	49	52	48	50
Histidine	32	21	23	23	22	23
Isoleucine	50	34	35	37	34	36
Leucine	73	52	51	53	50	52
Lysine	76	53	54	56	53	55
Methionine+ Cysteine	42	29	30	31	29	30
Phenylalanine + Tyrosine	89	61	62	66	61	64
Threonine	55	38	39	41	38	40
Tryptophan	7	5	5	6	5	6
Valine	54	37	38	40	37	39
Alanine	45	30	31	33	31	32
Proline	52	35	34	35	33	34
Glycine	53	36	38	39	37	38
Aspartic acid	112	76	320	82	77	163
Glutamic acid	164	103	115	37	113	200
Serine	55	36	39	40	273	121
Others	26	9	5	5	5	5
EAA*	547	322	386	405	377	395
NEAA*	507	325	582	607	570	593

- Data of dietary amino acids requirement for common carp was determined using diet content 38.5 % protein (NRC, 1993).

* Asp. : Aspartic acid, Glu. : Glutamic acid, Ser. : Serine EAA: Essential amino acid, NEAA: Non-essential amino acid

intestinal development and the dietary glutamine supplementation improved weight gain, feed intake, gain: feed ratio, intestinal weight and digestive enzyme activities of juvenile carp (*Cyprinus carpio* var. Jian).

In the present study, all fish were observed to feed actively throughout the experimental period. However the differences in AA absorption rates are one of the major factors reduced the efficacy of crystalline-AA as compared to protein-bound-AA (Cowey, 1995). This factor affects the AA availability in the AA pool and consequently affects the protein synthesis. The rapid absorption of crystalline-AA compared to protein-bound-AA has been demonstrated in fish (Zarate *et al.*, 1999), resulting in a faster increase and earlier peak levels of plasma AA concentration (Schuhmacher *et al.*, 1997). However, for the effective utilization of crystalline-AAs its absorption must occur simultaneous with that of protein-bound AAs (Zarate *et al.*, 1999; Peres and Oliva-Teles, 2007). Encapsulation or covalent-binding of crystalline-AA can improve the effectiveness of dietary crystalline-AA utilization by reducing its solubility in water and the absorption rate of crystalline-AA (Schuhmacher *et al.*, 1997; Zarate and Lovell, 1997; Segovia-Quintero and Reigh, 2004). In the present study, coating crystalline-NEAA with agar seemed to be not suitable method to delay crystalline-NEAA absorption for common carp due to that carp is stomachless fish and the absorption of AA was done in intestine. Such strategy has been used in other studies and was reported to improve crystalline- AAs utilization (Mambrini and Kaushik, 1994; Fournier *et al.*, 2002; Rollin *et al.*, 2003; Peres and Oliva-Teles, 2005).

A depression in fish performance and decrease dietary protein utilization efficiency associated with lower effectiveness of dietary crystalline-amino acids (AAs) inclusion has been reported in some previous fish studies (Mambrini and Kaushik, 1994; Rodehutscord *et al.*, 1995; Zarate and Lovell, 1997; Zarate *et al.*, 1999; Watanabe *et al.*, 2001; Peres and Oliva-Teles, 2005) and attributed to poor diet palatability, the leaching of crystalline-AAs, the differences between protein bound-AAs and crystalline-AAs in gut absorption time and also to the simultaneous availability of all dietary AAs in the AA-pool (Peres and Oliva-Teles, 2006, 2007). In the present study, the detrimental effects on the common carp larvae growth when fed higher dietary non essential free amino acids levels may be due to a huge quantity of these molecules which would be available simultaneously for intestinal absorption causing saturation and competition for transport mechanisms (Plakas and Katayama, 1981). Furthermore, as free amino acids are absorbed faster than protein-bound amino acids, the precocious absorption of some free amino acids comparatively to those protein-bounded may lead to amino acid imbalances and consequent decrease the protein utilization and larval performance (Rønnestad *et al.*, 2000). Similarly, Kolkovski and Tandler (2000) showed that a surplus of free amino acids provided by high dietary levels of squid meal hydrolysates may explain the observed depressed growth of gilthead sea bream *Sparus aurata* larvae. Lo´pez-Alvarado and Kanazawa (1995) obtained lower

growth indices of larval red sea bream when fed the diet was replaced by above 10% of free amino acids. Additionally, Espe and Lied (1994) found the optimal replacement level of intact protein by free amino acids in diets for pre-smolt Atlantic salmon, *Salmo salar* at 20–30%. Whiteman and Gatlin (2005) compared the gain, feed efficiency and protein efficiency ratio of red drum, *Sciaenops ocellatus* and hybrid striped bass, *Morone chrysops* × *Morone saxatilis* fed crystalline amino acids with and without neutralization relative to an intact protein diet. They found that, both species fed the intact protein diet had significantly higher body mass gain, feed efficiency and protein efficiency ratio values than those fed the crystalline amino acid diets.

Significantly highest feed intake was observed for the fish fed low control diet (20%), while the lowest value ($P \leq 0.05$) was observed for the fish fed high control diet (30%) associated with the better feed conversion ratio (FCR). The total feed intake was influenced by the dietary NEAA substitution to the different diets. Irrespective of dietary NEAA supplementation, feed intake increased as dietary EAA decreased (Tables 3, 4), suggesting that fish tried to adjust feed intake to a suitable EAA and NEAA intake. Cho and Kaushik (1990) reported that fish as other animals, adjust feed intake to satisfy energy requirements, within limits they are also able to adjust intake to meet specific nutrient requirements. A similar result was observed in the present study (Tables 3). Similar effect was observed regarding protein intake for European sea bass, *Dicentrarchus labrax* (Oliva-Teles and Cerqueira, 1997; Peres and Oliva-Teles, 1999). In these studies, fish adjusted feed intake to a constant protein intake rather than to a constant digestible energy intake.

The optimum dietary protein contents depend on several important factors, including the digestibility and the AA composition of the protein. Since, the importance of EAA in the diets of fish has already been investigated, (Green *et al.*, 2002; Peres and Oliva-Teles 2007), the ratio of EAA/NEAA and the influences of the dietary NEAA content on fish growth indices and feed efficiency have been recently considered (Mambrini and Kaushik 1994; Goda *et al.*, 2002; Peres and Oliva-Teles 2006). Goda *et al.* (2002) found that, NEAA should not account for more than 60% of the total dietary AAs supply for Nile tilapia fry diets. Peres and Oliva-Teles (2006) suggested that dietary EAA/NEAA ratio of 50/50 is necessary to promote maximum growth performance of juvenile European sea bass, *Dicentrarchus labrax*. However, this ratio should be increased to 60/40 if maximization of feed, protein and energy utilization is to be considered. No such data are available for common carp. However, irrespective of dietary NEAA the result of present study reveled that the ratio of 50/50 seems to be suitable to promote maximum growth indices of common carp larvae.

The protein efficiency ratio (PER), protein productive value (PPV), fat retention (FR) and energy retention (ER) are illustrated in Table (5). The highest PER and PPV ($P \leq 0.05$) values were observed for the fish fed low control diet, while the highest ($P \leq 0.05$) value of fat retention was recorded for

Table (5). **Protein intake, energy intake, protein efficiency ratio, protein productive value, fat retention and energy retention for common carp *Cyprinus carpio* L. fed different experimental diets.**

	High control	Low control	Aspartic acid	Glutamic acid	Serine	Asp. + Glú. + Ser.*
Protein intake (g fish^{-1})	1.05 ± 0.3^{a}	0.70 ± 0.1^{c}	0.97 ± 0.2^{ab}	1.01 ± 0.1^{a}	0.95 ± 0.1^{b}	0.99 ± 0.2^{ab}
Gross energy intake (kJ fish^{-1})	591.0 ± 4.1^{d}	654.5 ± 3.0^{a}	622.7 ± 2.3^{c}	605.1 ± 1.0^{cd}	649.1 ± 3.0^{ab}	613.9 ± 2.1^{c}
Protein efficiency ratio	1.95 ± 0.2^{b}	2.30 ± 0.12^{a}	1.58 ± 0.14^{d}	1.80 ± 0.10^{bc}	1.49 ± 0.09^{e}	31.45 ± 1.5^{b}
Protein productive value	27.20 ± 0.2^{ab}	30.02 ± 0.13^{a}	21.46 ± 0.12^{bc}	24.56 ± 0.17^{b}	17.87 ± 0.13^{c}	24.28 ± 0.46^{b}
Fat retention	31.21 ± 1.7^{b}	27.77 ± 1.06^{c}	31.78 ± 1.96^{b}	35.92 ± 2.11^{a}	26.45 ± 1.66^{d}	31.45 ± 1.5^{b}
Energy retention	15.46 ± 0.10^{a}	12.44 ± 0.18^{ab}	12.38 ± 0.63^{ab}	14.06 ± 0.18^{ab}	10.28 ± 0.13^{b}	13.26 ± 0.05^{ab}

- Means values (± SD) in the same row sharing the same subscript are not significantly different ($P \leq 0.05$).

* Asp. : Aspartic acid, Glu. : Glutamic acid, Ser. : Serine.

Table (6). The whole body composition of common carp *Cyprinus carpio* L fed different experimental diets (wet weight basis).

	High control	Low control	Aspartic acid	Glutamic acid	Serine	Asp. + Glu. + Ser.*
Moisture, %	76.6 ± 0.2	77.0 ± 0.9	75.7 ± 0.5	76.1 ± 0.4	77.5 ± 1.3	75.7 ± 0.8
Crude protein, %	14.0 ± 1.0^{a}	13.0 ± 0.8^{ab}	13.6 ± 0.4^{ab}	13.6 ± 1.9^{ab}	12.4 ± 1.3^{b}	14.4 ± 0.2^{a}
Ether extract, %	4.8 ± 1.2	5.2 ± 1.2	5.0 ± 1.3	4.9 ± 1.7	4.6 ± 1.2	4.8 ± 0.7
Ash, %	4.6 ± 2.2^{b}	4.8 ± 1.9^{ab}	5.7 ± 1.8^{a}	$5.4 \pm .9^{a}$	5.5 ± 1.4^{a}	5.1 ± 1.2^{ab}
Energy (kJ 100g fish^{-1})	520.5 ± 5.1	512.7 ± 7.1	519.0 ± 6.8	515.0 ± 6.1	474.8 ± 5.1	529.9 ± 3.3

-Means values (± SD) in the same row sharing the same subscript are not significantly different ($P \leq 0.05$).

* Asp. : Aspartic acid, Glu. : Glutamic acid, Ser. : Serine.

fish fed the high control diet. The same trend was observed for energy retention.

The proximate body composition of common carp larvae are illustrated in Table 6. No significant differences were showed in the whole body moisture and lipid contents as affected by different experimental diets, this may be related to the lower growth rate of these fish, as whole-body lipid levels tend to increase as fish grow, or to conversion of excess dietary NEAA to depot lipids. The data agree with the findings of Mambrini and Kaushik (1994). In terrestrial animals, it was also found that increasing NEAA in low protein diets also decreases body fat content (Han *et al.,* 1992; Kerr and Kiss, 1999).

The whole body protein contents was significantly lowest ($P \leq 0.05$) when fish fed the diet with serine. The same tendency was observed for PER, PPV and ER which associated with the lower whole body gross energy content. This result may be indicated the lower efficiency of dietary protein, when fish fed the diet containing serine compared to different experimental diets. In fact, metabolic energy losses increased as N retention decreased, leading to lower E retention. Similar results were reported by Rodehutscord *et al.* (1997) for rainbow trout, *Oncorhynchus mykiss* and El- Husseiny *et al.* (2002) for Nile tilapia, *Oreochromis niloticus* and also previously reported in terrestrial animals (Kerr and Easter, 1995). They reported that among traits performance monitored of fish, protein deposition is the most sensitive indicator of a sub-optimal supply of dietary amino acid.

CONCLUSION

Results of this study demonstrate that substitution of 25% from the high control FM crude protein diet (30% protein) with glutamic acid or a mixture of equally amount of three NEAA, had slightly reduced growth indices and feed efficiency of common carp *Cyprinus carpio* (L.) larvae, meanwhile substitution with serine has a negative effect on growth performance and feed utilization. More information is needed to clearly the influences and roles of non-essential amino acids nature on their specific metabolic pathway. Another studies were also, need to determine the effect of dietary NEAA supplementation for plant protein based-diets on growth performance and feed utilization of common carp.

REFERENCES

Alverdy, J.A., E. Aoys, P. Weiss-Carrington and D.A. Burke (1992). The effect of glutamine-enriched TPN on gut immune cellularity. Journal of Surgical Research, 52: 34–38.

Akiyama, T., I. Oohara and T. Yamamoto (1997). Comparison of essential amino acid requirements with A/E ratio among fish species (review paper). Fisheries Science, 63: 963–970.

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

APHA, AWWA, WPCF (1985). Standard Methods for the

Examination of Water and Wastewater.19 th ed. American Public Health Association , American Water Works Association AND Water Pollution Control Federation ,Washington, DC. 1268pp

Benakappa S. and T.J. Varghese (2003). Isoleucine, leucine, and valine requirement of juvenile Indian major carp, *Cirrhinus cirrhosus* (Bloch, 1795). Acta Ichthyologica Et Piscatoria, 33 (2): 161–172.

Brett J. R. (1973). Energy expenditure of Sockeye salmon, *Oncorhynchus nerka* during sustained performance. Journal of Fish Research Board of Canada, 30 (12/1): 1799 – 1809.

Carvalhoa A.P., R. Sa´, A. Oliva-Telesa and P. Bergot (2004). Solubility and peptide profile affect the utilization of dietary protein by common carp (*Cyprinus carpio*) during early larval stages. Aquaculture, 234: 319–333.

Cho, C.Y. and S.J. Kaushik (1990). Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Ver. Nutr. Diet., 61: 132–172.

Cho, C.Y., S.J. Kaushik and B. Woodward (1992). Dietary arginine requirement of young rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology, 102A: 211-216.

Corzoa, A., C.A. Frittsa, M.T. Kidda and B.J. Kerrb (2005). Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Animal Feed Science and Technology, 118: 319–327.

Cowey, C.B (1994). Amino acid requirement of fish: a critical appraisal of present values. Aquaculture, 124: 1-11.

Cowey, C.B. (1995). Protein and amino acid requirements—a critique of methods. Journal of Applied Ichthyology, 11: 199–204.

Duncan D.B. (1955). Multiple range and Multiple F test. Biometric, 11: 1-112.

El-Husseiny, O.M. ;Goda, A.M.A-S and Suloma, A, M (2002). Utilization of amino acids in Nile tilapia *Oreochromis niloticus* fry .2- Response of Nile tilapia fry to dietary essential amino acids deficiency. Vet. J. Giza, 50(1): 61-75.

Espe, M. and E. Lied (1994). Do Atlantic salmon (*Salmo salar*) utilize mixtures of free amino acids to the same extent as intact protein sources for muscle protein synthesis?. Comparative Biochemistry and Physiology, 107A: 249– 254.

Fauconneau B. and Arnal M. (1985). Leucine metabolism in trout (*Salmo gairdnerii* R.). Influence of temperature. Comparative Biochemistry and Physiology, 82: 435–445.

Fournier, V., M.F. Gouillou-Coustans, R. Metailler, C. Vachot, J. Moriceau, H. Le Delliou, C. Huelvan, E. Desbruyeres and S.J. Kaushik (2002). Nitrogen utilization and ureogenesis as affected by dietary nucleic acid in rainbow trout (*Oncorhynchus*

mykiss) and turbot (*Psetta maxima*). Fish Physiology and Biochemistry, 26: 177–188.

Gaye-Siessegger, J., U. Focken and K. Becker (2006). Effect of dietary protein/ carbohydrate ratio on activities of hepatic enzymes involved in the amino acid metabolism of Nile tilapia, *Oreochromis niloticus* (L.). Fish Physiology and Biochemistry, 30(4): 275-282.

Gaye-Siessegger, J., U. Focken, H.J. Abel and K. Becker (2007). Influence of dietary non-essential amino acid profile on growth performance and amino acid metabolism of Nile tilapia, *Oreochromis niloticus* (L.). Comparative Biochemistry and Physiology, 146 A: 71–77

Goda, A.M.A-S; O.M. El-Husseiny and A.M. Suloma (2002). Utilization of amino acids in Nile tilapia *Oreochromis niloticus* fry. 3- Utilization of non-essential amino acids as energy source. Vet. J. Giza, 50(1): 77-85 .

Gomez-Requeni, P., M. Mingarro, S. Kirchner, J.A. Calduch-Giner, F. Medale, G. Corraze, S. Panserat, S.A.M. Martin, D.F. Houlihan, S.J. Kaushik and J. Perez-Sanchez (2003). Effects of dietary amino acid profile on growth performance, key metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream (*Sparus aurata*). Aquaculture, 220: 749–767.

Green, J.A., R.W. Hardy and E.L. Brannon (2002). The optimum dietary essential: nonessential amino acid ratio for rainbow trout (*Oncorhynchus mykiss*), which maximizes nitrogen retention and minimizes nitrogen excretion. Fish Physiology and Biochemistry, 27: 109–115.

Han, Y., H. Suzuki, C.M. Parson and D.H. Baker (1992). Amino acid fortification of a low-protein corn and soybean meal diet for chicks. Poultry Science, 71: 1168–1178.

Heger, J. (2003). Essential to non-essential amino acid ratio. In: D'Mello, J.P.F. (Ed.), Amino Acids in Animal Nutrition, 2nd ed. CABI Publ., Oxon, United Kingdom, pp. 103–123.

Heger, J., S. Mengesha and D. Vodehnal (1998). Effect of essential: total nitrogen ratio on protein utilization in the growing pig. British Journal of Nutrition, 80: 537–544.

Hepher B. and Y. Pruginin (1981). Commercial fish farm. John Wiley and Sons Inc. Press New York Toronto.

Hughes, S.G. (1985). Evaluation of glutamic acid and glycine as sources of nonessential amino acids for lake trout (*Salvelinus namaycush*) and rainbow trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology, 81 A: 669–671.

Kaushik, S.J. and K. Dabrowski (1983). Nitrogen and energy utilization in juvenile carp (*Cyprinus carpio* L.) fed casein, amino acids or a protein-free diet. Reproduction Nutrition Development, 23: 741–754.

Kaushik, S.J. (1998). Whole body amino acid composition of

European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. Aquatic Living Resources, 11: 355–358.

Kerr, B.J. and R.A. Easter (1995). Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. Journal of Animal Science, 73: 3000–3008.

Kerr, B.J. and M.T. Kiss (1999). Amino acid supplementation of low protein broiler diets: 1. Glutamic acid and indispensable amino acid supplementation. Journal of Applied Poultry Research, 8:298–309.

Kim, K.L., T.B Kayes and C.H. Amundson (1991). Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout. Aquaculture, 96: 57-67.

Kolkovski, S. and A. Tandler (2000). The use of squid protein hydrolysate as a protein source in micro-diets for gilthead seabream, *Sparus aurata* larvae. Aquaculture Nutrition, 6: 11–15.

Lo´pez-Alvarado, J. and A. Kanazawa (1995). Optimum levels of crystalline amino acids in diets for red sea bream (*Pagrus major*). International Council for the Exploration of the Sea, ICES Marine Science Symposium, 201: 100– 105.

Mambrini, M. and S.J. Kaushik (1994). Partial replacement of dietary protein nitrogen with dispensable amino acids in diets of Nile tilapia, *Oreochromis niloticus*. Comparative Biochemistry and Physiology, 109A (2): 469-477.

Mambrini, M. and S.J. Kaushik (1995). Indispensable amino acid requirements of fish—correspondence between quantitative data and amino acid profiles of tissue proteins. Journal of Applied Ichthyology, 11: 240–247.

MSTAT-C version 4 (1987). Software program for the design and analysis of agronomic research experiments. Michigan St. Univ., M.S., USA.

Newsholme, E.A., B. Crabtree and M.S. Ardawi (1985). The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells. Bioscience reports, 5: 393–400.

Ng, W.K. and S.S.O. Hung (1995). Estimating the ideal dietary indispensable amino acid pattern for growth of white sturgeon, *Acipenseer transmontanus* (Richardson). Aquaculture Nutrition, 1: 85–94.

Ng, W.K., S.S.O. Hung and M.A. Herold (1996). Poor utilization of dietary free amino acids by white sturgeon. Fish Physiology and Biochemistry, 15:131–142.

NRC (1993). National Research Council, Nutrition Requirement of fish Washington, D.C.

Oliva-Teles, A. and A.L. Cerqueira (1997). Effect of the dietary protein level on metabolic utilization of diets by sea bass (*Dicentrarchus labrax*). In: Proc. III Int. Symp. on Research for Aquaculture and Fundamental and Applied Aspects, Barcelona, Spain, Poster n° 187.

Peres, H. and A. Oliva-Teles (1999). Influence of temperature on protein utilization in juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture, 170: 337–348.

Peres, H. and A. Oliva-Teles (2005). The effect of dietary protein replacement by crystalline amino acid on growth and nitrogen utilization of turbot, *Scophthalmus maximus* juveniles. Aquaculture, 250:755–764.

Peres, H. and A. Oliva-Teles (2006). Effect of the dietary essential to non-essential amino acid ratio on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture, 256 (1-4): 395-402.

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

Plakas, S.M. and T. Katayama (1981). Apparent digestibilities of amino acids from three regions of the gastrointestinal tract of carp (*Cyprinus carpio*) after ingestion of a protein and a corresponding free amino acid diet. Aquaculture, 24: 309– 314.

Rodehutscord, M., A. Becker, M. Pack and E. Pfeffer (1997). Response of rainbow trout, *Oncorhynchus mykiss* to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement for essential amino acids. Journal of Nutrition, 127:1166–1175.

Rodehutscord, M., S. Mandel, M. Pack, S. Jacobs and E. Pfeffer (1995). Free amino acids can replace protein-bound amino acids in test diets for studies in rainbow trout (*Oncorhynchus mykiss*). Journal of Nutrition, 125: 956–963.

Rollin, X., M. Mambrini, T. Abboudi, Y. Larondelle and S.J. Kaushik (2003). The optimum dietary indispensable amino acid pattern for growing Atlantic salmon (*Salmo salar* L.) fry. British Journal of Nutrition, 90: 865–876.

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

Santiago, C.B. and T. Lovell (1988). Amino acid requirements for growth of Nile tilapia. Journal of Nutrition, 118: 1540-1546.

Schuhmacher, A., M. Münch and J.M. Gropp (1995). Non-essential amino acid sources in crystalline amino acid diets for trout (*Oncorhynchus mykiss*). Journal of Applied Ichthyology, 11: 317–321.

Schuhmacher, A., C. Wax and J.M. Gropp (1997). Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or a crystalline amino acid diet. Aquaculture, 151: 15–28.

Schwarz , F.J., M Kirchgessner and U. Deuringer (1998). Studies on the methionine requirement of carp *Cyprinus carpio* (L.). Aquaculture, 161: 121–129.

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

Silk, D.B.A., G.K. Grimble and R.G. Rees (1985). Protein digestion and amino acid and peptide absorption. Proceedings of the Nutrition Society, 44: 63–72.

Souba, W.W., R.J. Smith and D.W. Wilmore (1985). Glutamine metabolism by the intestinal tract. Journal of Parenteral and Enteral Nutrition, 9: 608–617.

Whiteman, K.W. and D.M. Gatlin III (2005). Evaluation of crystalline amino acid test diets including pH adjustment with red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops*×*Morone saxatilis*). Aquaculture, 248: 21–25.

Walton, M.J. (1985). Aspects of amino acid metabolism in teleost fish. pp. 47–68. In: Cowey C.B., Mackie A.M., Bell J.G., (eds.) Nutrition and feeding in fish. Academic Press, London.

Watanabe, T., H. Aoki, K. Watanabe, M. Maita, Y. Yamagata and S. Satoh (2001). Quality evaluation of different types of non-fish meal diets for yellowtail. Journal of Fish science, 67: 461–469.

Wilson, R. P. (1989). Amino acids and proteins. In: J. E. Halver, (ed.), Fish Nutrition. Academic Press London. Pp.111 – 151.

Wu, G., D.A. Knabe and N.E. Flynn (1994). Synthesis of citrulline from glutamine in pig enterocytes. Biochemical Journal, 299: 115–121.

Wu, G., N.E. Flynn, W. Yan and D.G. Barstow (1995). Glutamine metabolism in chick enterocytes: absence of pyrroline-5-carboxylase synthase and citrulline synthesis. Biochemical Journal, 306: 717–721.

Wu, G., S.A. Meier and D.A. Knabe (1996). Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. Journal of Nutrition, 126: 2578–2585.

Yan, L. and X. Qiu-Zhou (2006). Dietary glutamine supplementation improves structure and function of intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture, 256 (1-4) :389-394.

Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall, Englewood Cliff, NJ, USA.

Zarate, D.D. and R.T. Lovell (1997). Free lysine (L-lysine/HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (*Ictalurus punctatus*). Aquaculture, 159: 87–100.

Zarate, D.D., R.T. Lovell and M. Payne (1999). Effects of feeding frequency and rate of stomach evacuation on utilization of dietary free and protein-bound lysine for growth by channel catfish, *Ictalurus punctatus*. Aquaculture Nutrition, 5:17–22.

تأثير الاحلال الجزئى لبروتين العليقة بالاحماض الامينية الغير اساسية على الأداء الانتاجى وكفاءة الاستفادة من الغذاء ليرقات المبروك العادى

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أجريت هذه الدارسة لبيان أثر استخدام الإحلال الجزئى (25%) من بروتين العليقة بثلاثة احماض غير اساسية (الاسبارتك، الجلوتامك، السرين او مخلوط متساوى من الثلاث الاحماض الامينية الغير اساسية السابقة) على الأداء الإنتاجى و الاستفادة من الغذاء ليرقات اسماك المبروك العادى المرباه فى أحواض زجاجية.

تم إعداد 6 علائق تجريبية تشمل على عليقتين قياسيتين(كونترول)، حيث كان مسحوق السمك المصدر الرئيسى للبروتين. العليقة القياسية رقم 1 كانت تحتوى على 30% بروتين وحين العليقة القياسية 2 كانت تحتوى على 20% بروتين. العلائق المختبرة من 3- 6 كانت تحتوى على 30% بروتين. تم احلال 25% من بروتين العليقة القياسية المرتفعة فى البروتين (عليقة رقم 1) منفردا بواحد من الأحماض الأمينية الغير أساسية ، الاسبارتك (عليقة 3)، الجلوتامك (عليقة 4)، السرين (عليقة 5) او مخلوط متساوى من الثلاث الأحماض الأمينية الغير اساسية السابقة (عليقة 6). وقد كان محتوى جميع العلائق متساوى فى الطاقة (17 ميجا جول طاقة ممثلة / كجم عليقة).

تم تسكين 1800 يرقة من سمك المبروك العادى ذات وزن ابتدائى 0.2 ±0.1 جرام فى 18 حوض زجاجى ذو سعة 100 لتر . وقد استخدم لكل معاملة 3 احواض مكررات وقد استمرت هذة التجربة لمدة 66 يوم و أظهرت نتائج التجربة ما يلى:

- أن أعلى قيمة معنوية لمعدل وزن نهائى للأسماك ، الغذاء المأكول، معدل الوزن النوعى و كفاء التحويل الغذائى قد سجلت للأسماك التى تغذت على العليقة القياسية المرتفعة فى البروتين (كونترول 1)، بينما اقل القيم المعنوية قد سجلت للاسماك التى تغذت على عليقة تحتوى على الحامض الأمينى السرين.
- كان أعلى قيمة معنوية لكفاء الاستفادة من البروتين ومعدل ترسيب البروتين للأسماك التى تغذت على العليقة القياسية المنخفضة فى البروتين (كونترول 2) .
- وقد خلصت التجربة إلى أن الإحلال الجزئى بنسبة 25% من بروتين عليقة تحتوى على 30% بروتين بالحامض الأمينى الغير أساسى الجلوتامك او مخلوط متساوى من الأحماض الأسبارتك، الجلوتامك والسرين يؤدى إلى إنخفاض طفيف فى الأداء الإنتاجى وكفاءة الاستفادة من الغذاء ، فى حين أن الإحلال بحامض السرين يؤثر سلبيا على الأداء الإنتاجى وكفاءة الأستفادة من الغذاء ليرقات المبروك العادى .