Vet.Med.J.,Giza. Vol.50, No.1. (2002):-77-85.

UTILIZATION OF AMINO ACIDS IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FRY.
3-UTILIZATION OF NON-ESSENTIAL AMINO ACIDS AS ENERGY SOURCE.

A. M. A.-S, GODA*, O. M., EL-HUSSEINY and A.M. SULOMA****

* National Institute of Oceanogrophy and Fisheries.

** Anim. Prod. Dept., Fac. Agric., Cairo Univ.

Received: 31-7-2001.

Accepted: 30-10-2001.

SUMMARY

An experiment was designed to find out the effect of the replacement of essential amino acids by non-essential amino acids as energy source at seven ratios (45%: 55% as a control, 50%: 50%, 40%: 60%, 35%: 65%, 30%: 70%, 25%: 75% and 20%: 80%, essential amino acid: non-essential amino acid,) respectively. The higher significant ($P \leq 0.05$) values of average final live body weight, average daily body gain, specific growth rate were observed for Nile tilapia fry fed diets containing ratios (50: 50), (45: 55) and (40: 60) (essential: non-essential amino acids) than the other treatments. Nile tilapia fry fed the diet containing ratios of (50: 50), (45: 55) and (40: 60) (essential: non-essential amino acids) had the better-feed conversion ratio than other treatments. Protein efficiency ratio and nitrogen retention among different experiments decreased significantly ($P \leq 0.05$) with increasing dietary ratio of non-essential amino acids. This effect was moderate when 60% of dietary protein was substituted by non-essential amino acids, while ratio over than 65%, the values were further reduced. Carcass crude protein was significantly ($P \leq 0.05$) lowest when fish fed diet containing more than 60% non-essential amino acid. Carcass lipid content and gross energy content followed the opposite tendency.

Depending on the present data it is of interest to point out that, the dietary crude protein requirement for tilapia can be reduced to the range of 12.2- 15.7% when appropriate energy source that have metabolizable energy values equivalent to protein are used (for example, non-essential amino acids) to substitute dietary protein to levels of 30%, with metabolized energy density of 13.71 MJ and had a P/E ratio (CP/ME) of 22.40 g./MJ

The data confirmed that substitution of dietary protein nitrogen by non-essential amino acids

should not exceed 60% of the total dietary amino acid requirement.

Key word: Non-essential amino acids, essential amino acids, energy source, Nile tilapia, growth performance.

INTRODUCTION

Tilapia are widely cultured in different regions of the world and constitute the third largest group of farmed finfish, only after carps and salmonids, with an annual production of about 11.5%. Global production of farmed tilapia has increased more than three-fold since 1984, from 186.544 m.t. to 659.000 m.t., representing about 4.48 % of the total farmed finfish in 1995, with a value of US$ 925 million (El-Sayed , 1999)

Since the nitrogen of essential amino acids can also be used in the synthesis of non-essential amino acids, a protein with essential amino acids in surplus relative to the non-essential amino acids may still support maximum N retention (NR) (Cowey, 1994). Kim et al., (1991) showed that the dietary protein requirement of rainbow trout is not more than 30% on dry matter basis (the lowest protein level used in their experiment). Protein requirement is probably best expressed in terms of dietary energy because, at dietary protein levels necessary for optimal growth, much of it is, in fact, utilized as an energy source. The 30% protein diets actually supplied 16.1 MJ digestible energy /kg diet with 17.6 g digestible protein/MJ digestible energy (DP/DE ratio), a value in line with currently accepted values. However, in a second experiment the author gave a diet containing 25% crude protein (and supplying all essential amino acid in quantities sufficient to meet requirement) together with a non-essential amino acid mixture equivalent to 10% protein (in conventional terms a 35% protein diet) to rainbow trout. The growth showed equal to that obtained with a diet containing 35% crude protein. The results concluded that the protein requirement of trout is 25% when appropriate energy source (that have ME values equivalent to protein) are used to substitute protein. No such data are available for tilapia .

Mambrini and Kaushik (1994) fed tilapia diets containing 30% protein and replaced dispensable amino acid (DAA) alanine, glutamic acid and glycine at a level of 25% and 50% from dietary crude protein in the free form, given singly or as a mixture. They reported, that at the 25% substitution level, irrespective of the nature of the DAA, growth was slightly reduced (10%), while, glycaemia, nitrogen retention and excretion were unaffected a 50% substitution with the DAA mixture resulted in a marked reduction in growth (50%), a modified pattern of glycaemia and lower nitrogen retention associated with a higher nitrogen excretion. Results suggest that DAA should not account for more than 60% of the total amino acid supply in tilapia diets.

The aim of the present study was to find out the effect of the replacement of essential amino acids by non-essential amino acids as energy source by NNile tilapia, (*Oreochromis niloticus)* fry.

MATERIALS AND METHODS

This study was carried out in the fish Laboratory of Animal and Poultry Nutrition Department, Faculty of Agriculture, Cairo University.

Feed ingredients:

Experimental diets were formulated as semi-purified diets. A mixture of casein vitamin free and gelatin were used as sole protein source, corn oil and cod liver oil were used as the fat source; while dextrin, cellulose and carboxymethyl cellulose were included as digestible carbohydrate, fiber and binder, respectively. All synthetic amino acids used in this study were in DL form. The optimum ratio between casein and gelatin was 5: 1 to balance amino acids pattern according to Murai *et al.*, (1984). Table (1) illustrate the amino acids composition of casein and gelatin.

Experimental fish :

Nile tilapia fish fry was purchased from Nawa hatchery, Shebien EL-Kanater, Kaluobia Governorate. Fishes were healthy, free from parasites with an average initial weight of 1.24 ± .01g. The experimental fish fry were kept 14 days for acclimatization, thereafter , the actual experimental period followed directly (from 15 July 2000 to 15 August 2000).

Technique of culture:

The experiment was conducted in 7 glass aquaria of (80 x 50 x 40 cm) 160 liters capacity, representing 7 nutritional treatments. Aquaria were filled with dechlorinated tap water and supplied with aeration. Water was changed three times weekly, while the temperature adjusted at 27-28°C, and ammonia concentration was maintained at less than 0.3 mg /l. with pH of 8.00.

A total of 280 tilapia fry, were stocked and equally divided into 7 aquaria of 40 fish in each. To find out the effect of the replacement of essential amino acids by non-essential amino acids as energy source at seven ratios (45%: 55% as a control, 50%: 50%, 40%: 60%, 35%: 65%, 30%: 70%, 25%: 75% and 20%: 80%, essential amino acids: non-essential amino acids, respectively) according to Santiago and Lovell (1988) ,Mambrini and Kaushik (1994) and Cowey, (1994) . Seven experimental diets are listed in Table (2). Tables (3 & 4) present the mixture of amino acids added (calculated according to El-Husseiny et al., 2001) and amino acid composition of different experimental diets, recpectivly.

Chemical composition analysis of diets and fish:

Samples from the fry at the beginning and end of the experimental period were randomly collected

Table (1): The amino acid composition of casein and gelatin (g/100g) used in the experimental diets.

Amino Acid	Casein	Gelatin
Arginine	3.59	7.12
Histidine	2.73	0.95
Isoleucine	4.30	1.24
Leucine	8.54	2.81
Lysine	7.31	3.52
Methionine	2.69	0.78
Cysteine	0.37	0.15
Phenylalanine	4.89	1.94
Tyrosine	5.39	0.63
Threonine	3.87	1.61
Tryptophan	(1.19)*	(0.05)*
Valine	5.28	2.24
Alanine	3.14	10.17
Aspartic acid	6.62	5.45
Glutamic acid	21.95	9.58
Glycine	1.82	31.95
Proline	10.85	16.97
Serine	5.56	3.12

*According to NRC (1993)

Table (2): Chemical composition of the different experimental diets.

EAA: NEAA	45:55	50:50	40:60	35:65	30:70	25:75	20:80
Ingredient %							
Mixture EAA[1]	10.64	11.37	8.20	6.62	5.03	3.44	1.86
Mixture NEAA[1]	9.36	8.63	11.80	13.38	14.97	16.56	18.14
Casein	5.32	5.32	5.32	5.32	5.32	5.32	5.32
Gelatin	5.12	5.12	5.12	5.12	5.12	5.12	5.12
Dextrin	39.28	39.28	39.28	39.28	39.28	39.28	39.28
α-Cellulose	17.78	17.78	17.78	17.78	17.78	17.78	17.78
Corn oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Cod liver oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
(Min-Vit) Mix.	5.50	5.50	5.50	5.50	5.50	5.50	5.50
C.M.C.[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Nutrient Composition (% on DM basis)							
Dry matter	89.78	89.87	89.57	89.65	89.83	89.67	89.73
Metabolizable Energy (Mj/kg)[3]	13.74	13.73	13.71	13.73	13.70	13.71	13.73
Crude protein%	30.92	30.87	30.63	30.71	30.55	30.84	30.67
P/E ratio (CP/ME)	22.49	22.48	22.34	23.37	22.30	22.50	22.34
Ether-Extract %	5.77	5.73	5.64	5.81	5.67	5.57	5.79
Carbohydrate[4] %	57.20	57.22	57.22	57.27	57.59	57.46	57.36
Ash%	6.11	6.18	6.14	6.21	6.19	6.13	6.18

1. Mixture of essential and non essential amino acid supplementation calculated according to El-Husseiny *et al.*, (2002). See Table (3).
2. Carpoxymethyl Cellulose.
3 .Using values of 5.65, 4.2 and 9.45 Kcal/g for protein ,carbohydrate and fat ,respectively According to Hepher *et al*, (1983).
4.Calculated by differences.

Table (3): Mixture of essential and non-essential amino supplementation for different experimental diets.

EAA: NEAA	45:55 Control	50:50	40:60	35:65	30:70	25:75	20:80
Arginine	0.764	0.878	0.589	0.455	0.311	0.166	0.022
Histidine	0.580	0.561	0.473	0.389	0.314	0.229	0.145
Isoleucine	0.916	0.928	0.655	0.533	0.502	0.370	0.137
Leucine	1.820	1.937	1.418	1.143	0.880	0.615	0.351
Lysine	1.556	1.645	1.191	0.948	0.716	0.483	0.252
Methionine	0.576	0.646	0.480	0.398	0.315	0.233	0.151
Cysteine	0.076	0.086	0.063	0.051	0.040	0.028	0.017
Phenylalanine	1.004	1.044	0.738	0.585	0.433	0.280	0.127
Tyrosine	1.148	1.184	0.865	0.704	0.534	0.374	0.214
Threonine	0.824	0.927	0.685	0.564	0.343	0.221	0.200
Tryptophan	0.252	0.282	0.212	0.177	0.143	0.108	0.073
Valine	1.124	1.166	0.836	0.668	0.502	0.336	0.170
Alanine	0.668	0.561	0.810	0.935	1.060	1.185	1.310
Aspartic acid	1.408	1.247	1.622	1.810	1.997	2.185	2.370
Glutamic acid	3.404	3.531	4.695	5.278	5.860	6.443	7.025
Glycine	0.388	0.220	0.611	0.806	1.001	1.196	1.392
Proline	2:308	2.011	2.702	3.048	3.394	3.739	4.085
Serine	1.184	1.055	1.356	1.507	1.657	1.808	1.959

EAA: Essential amino acids.
NEAA: non-essential amino acid

Table (4): The total amino acids and the ratio between essential and non-essential amino acids content of different experimental diets.

EAA: NEAA	45:55	50:50	40:60	35:65	30:70	25:75	20:80	* Amino acid requirement (% diet)
Arginine	1.319	1.433	1.144	1.010	0.866	0.721	0.577	1.18
Histidine	0.774	0.845	0.667	0.583	0.508	0.423	0.339	0.48
Isoleucine	1.206	1.218	0.945	0.823	0.792	0.660	0.427	0.78
Leucine	2.414	2.532	2.012	1.737	1.474	1.209	0.945	0.95
Lysine	2.122	2.211	1.757	1.514	1.282	1.049	0.818	1.43
Methionine	0.755	0.825	0.659	0.577	0.494	0.412	0.330	0.75
Cysteine	0.105	0.115	0.092	0.080	0.069	0.057	0.046	-
Phenylalanine	1.389	1.429	1.123	0.970	0.818	0.665	0.512	0.51
Tyrosine	1.464	1.500	1.181	1.020	0.850	0.690	0.530	-
Threonine	1.110	1.213	0.971	0.850	0.629	0.507	0.486	1.05
Tryptophan	0.318	0.348	0.278	0.243	0.209	0.174	0.139	0.28
Valine	1.518	1.560	1.229	1.062	0.896	0.730	0.564	0.78
Alanine	1.355	1.248	1.497	1.622	1.747	1.872	1.997	-
Aspartic acid	2.038	1.877	2.252	2.440	2.627	2.815	3.000	-
Glutamic acid	4.886	5.189	6.353	6.936	7.518	8.101	8.683	-
Glycine	2.120	1.952	2.343	2.538	2.733	2.928	3.124	-
Proline	3.752	3.455	4.146	4.492	4.838	5.183	5.529	-
Serine	1.637	1.508	1.809	1.960	2.110	2.261	2.412	-

* According to Santiaho and Lovell (1988).
EAA: Essential amino acids.
NEAA: non-essential amino acid

from each experimental treatment for proximate chemical analysis. The samples were analyzed the by standard methods of A.O.A.C. (1980) for dry matter (oven dry 105°C for 24 h), Crude protein (N-Kjeldahl X 6.25), fat (Solvent extraction with petroleum ether, b p t 40-60°C for 10-12 h), ash (Oven ashed at 550°C) and crude fiber (acid 1.25% and alkali 1.25% digestion), respectively. Nitrogen free extract was calculated by difference. The gross energy was calculated according to Hepher *et al.*, (1983). Determination of amino acids in casein and gelatin was carried out according to the methods described by Moore (1963). The oxidized samples were analyzed using a LKAB Alpha-Plus amino acid analyzer.

Statistical analysis:

Data for each experiment was statistically analyzed using classification one way MSTAT version 4 (1987). Significant differences among the mean of different treatment were carried out by Duncan's multiple range test ($P \leq 0.05$) Duncan (1955) .

RESULTS AND DISCUSSION

The tilapia growth performance

The average final body weight, average daily gain body, specific growth rate, feed consumption and conversion ratio are presented in Table (5). Nile tilapia fed the diets containing ratios (50: 50), (45: 55) and (40: 60) (essential: non-essential amino acids) had the highest significant values ($P \leq 0.05$) of growth performance parameters than other treatments. The total feed consumption data were influenced by the dietary non-essential amino acid added to the different diets. The feed consumption per fish decreased with increasing dietary graded levels of non-essential amino acids. The reverse reaction was observed for feed conversion ratios, the fish fed diet containing ratios of (50: 50), (45: 55) and (40: 60) (essential: non-essential amino acids) had better feed conversion ratio than the other treatments.

The results indicated that partial replacement of dietary protein by dispensable amino acids led to reduce growth if the substitution level was more than 60% of the total amino acids in Nile tilapia diets. This effect was moderate when 70% of dietary protein was substituted, whereas, when the substitution level was more than the 70%, the growth was further reduced. The data agreed with findings of Mambrini and Kaushik (1994) who replaced dietary crude protein (30% dry matter) at a level of 25% and 50% by dispensable amino acids in the free form and found that, at the 25% substitution level, irrespective of the nature of the dispensable amino acids, growth of tilapia was slightly reduced (10%), while nitrogen retention and excretion were unaffected. The 50% substitution with the dispensable amino acids mixture resulted in a marked reduction in growth (50%). Dispensable amino acids should not account for more than 60% of the total amino acids supply in tilapia diets. In this connection, Davies and Morris

(1997) cited that the experiments of Kim *et al.*, (1991) suggested that the essential amino acids requirement of trout could be met with a balanced protein source (casein/ gelatin) amounting to only 25% of the diet associated with 10% of the diet as a mixture of non-essential amino acids to provide sufficient digestible energy and nitrogen for maintenance and growth. This implies that a diet containing 35% crude protein is adequate for trout when sufficient non-protein energy is made available.

The utilization of non-essential amino acids as energy source:

Results of protein efficiency ratio, nitrogen retention and energy retention are illustrated in Table (6). Significant differences among the means of different experimental treatments were observed. Protein efficiency ratio and nitrogen retention among different treatments decreased significantly ($P \leq 0.05$) with increasing dietary ratio of non-essential amino acids. This effect was moderate when 60% of dietary essential amino acids were substituted by non-essential amino acids, whereas, when the substitution level was more than 65%, the protein efficiency ratio and nitrogen retention was further reduced. The observed reverse tendency noticed for energy retention may be explained by the increasing in carcass fat content Table (7) with decreasing carcass crude protein.

Carcass crude protein (Table, 7) was significantly ($P \leq 0.05$) lowest when fish fed diet containing more than 60% non-essential amino acid. Carcass lipid content and gross energy content followed the opposite tendency of crude protein, the highest significant ($P \leq 0.05$) values of carcass lipid and energy were observed for fish fed diet contained more than 60% non-essential amino acid.

Proteins are ultimately degraded into amino acids that are utilized either as an energy source or for somatic protein synthesis. Cowey (1994) reported that protein requirement is probably best expressed in terms of dietary energy because, at dietary protein levels necessary for optimal growth, much of it, in fact, is utilized as an energy source. Current estimates of protein requirement for trout are 22-25% protein, this is usually supplied in diets with a digestible energy of 15-17 MJ/ Kg (Cho and Woodward, 1989) and 16.6 MJ DE/ Kg (Kim *et al.*, 1991) with a non-essential amino acids in quantities sufficient to meet 10% proteins. With regard to this last finding of Cowey (1994), it is of interest to point out that depending on the data present, the dietary crude protein requirement for tilapia can be reduced to the range of 12.2-15.7% when appropriate energy source that have metabolizable energy values equivalent to protein are used (for example, non-essential amino acids) to substitute dietary protein to levels of 30%, with metabolized energy density of 13.71 MJ and had a P/E ratio of 22.40 g./MJ (CP/ME) (Table, 2).

The data answer the question of why fat is not an "appropriate energy source " in the diet of tilapia as reported in previous results by Viola and Arileli (1983); Hanley(1991) and Chou and Shiau (1996) when they found several implications with supplementation of graded levels and sources of oils in tilapia diets.

In conclusion, the substitution of dietary protein nitrogen by non-essential amino acids should not exceed 60% of the total dietary amino acid requirement. This must be considered when essential amino acids are replaced by non-essential amino acids in isonitrogenous diets for Nile tilapia. Indeed, the non- protein energy sources used to spare dietary protein in tilapia diets must have metabolizable energy values equivalent to protein. Moreover, non-essential amino acid can be used as appropriate equivalent energy source to dietary protein, but more information is needed to show influences of non-essential amino acids nature on growth of tilapia and their specific metabolic pathway.

Based on the optimum ratio of essential: non-essential amino acid (40: 60), the calculated optimum balance among the essential amino acids (g\ 16g protein) was thus: lysine 5.74, methionine+cystine 2.45, therionine 3.17, tryptophan 0.91, valine 4.01, isoleucine 3.08, leucine 6.57, phenylalanine+tyrosine 7.87, histidine 2.18 and arginine 3.74.

REFERENCES

AOAC (Association of Official Analytical Chemists) (1980). Official methods of analysis, 12th edition. A.O.A.C., Washington, DC.

Cho, C.Y. and Woodward, B (1989). Studies on the protein-to-energy ratio in diets for rainbow trout (*salmo gairdneri*) .PP.37-40 in proceedings of the Eleventh symposium on energy metabolism,

Chou, B. S. and Shiau, S. Y. (1996). Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus* X *Oreochromis aureus*. Aquaculture. 143:184-195.

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

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

Duncan,D.B. (1955). Multiple ranged and multiple F-tests. Biomet., 11:1-42.

El-Husseiny, O. M.; Goda, A. M. E-S. and Suloma, A. M. (2002).

Utilization of amino acids in Nile tilapia (*Oreochromis niloticus*) diets. 1-Utilization efficiency of synthetic amino acid by Nile tilapia fry .In press.

El-Sayed, A.F.M. (1999). Alternative dietary Protein sources for farmed tilapia, *Oreochromis spp*. Aquaculture 199, 149-168

Hanley, F. (1991). Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion and body composition of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture. 93:323-333.

Hepher, B.; Liao, I. C.; Cheng, S. H. and Haseih, C. S. (1983): Food utilization by red tilapia. Effect of diet composition, feeding level and temperature on utilization efficiency for maintenance and growth. Aquaculture, 32: 255-272.

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

Mambrini.M. and Kaushik.S.J. (1994). Partial replacement of dietary protein nitrogen with dispensable amino acids in diets of Nile tilapia, *Oreochromis niloticus*. COMP.-BIOCHEM.-PHYSIOL., A . Vol. 109A, No. 2. pp. 469-477.

Moore, S. (1963). On the determination of cystine as acid. J. Biol. Chem. 238, 235-237.

MSTAT Version 4 (1987): Software program for the design and analysis of agronomic research experiments. Michigan St. Univ., M.S. U.S.A.

Murai, T., Akiyama, T., Nose, T. (1984): Effect of amino acid balance on efficiency in utilization of diet by fingerling carp. Nippon Suisan Gallaishi 50, 893-897.I

Research Council (NRC), (1993): Nutrient Requirements fish. National Acasemy of Science , Washington, DC, USA.

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

Viola, S. and Arileli, Y. (1983): Nutrition studies with tilapia. *Sarotherodon*, 1. Replacement of fish meal by soybean meal in feeds for intensive tilapia culture . Bamidgeh, 35 (1): 9-17.

Wang, T.C. and Fuller, M.F. (1989): The optimum dietary amino acid pattern for growth pigs. 1. Experiments by amino acid deletion. Br.J. Nutr. 62: 77-89.