Vet.Med.J.,Giza. Vol.50, No.1. (2002) :47-59.

UTILIZATION OF AMINO ACIDS IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FRY.
1-UTILIZATION EFFICIENCY OF SYNTHETIC AMINO ACID BY NILE TILAPIA FRY

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

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

** National Institute of Oceanography and Fisheries.

Received: 31-7-2001.
Accepted: 10-10-2001

SUMMARY

The effect of replacing different levels of synthetic amino acids (15%, 35%, 50%, 65% and 85%) in the same proportions as in dietary casein protein on growth performance and nitrogen retention (NR) of Nile tilapia fry was investigated.

The fry fed the experimental diets containing synthetic amino acids at levels 15% and 35% of dietary crude protein of casein, were significantly (P<0.05) higher in the final body weight than the other treatments. The same trend was shown in average daily gain and specific growth rate. Feed conversion ratio was improved by increasing replacement of synthetic amino acid up to 35% of dietary protein casein compared to other treatments.

The percentage of whole body moisture, ether extract and ash content increased significantly (P<0.05) as the level of dietary synthetic amino acid level increased, while inverse trend was observed with percentage of whole crude protein and gross energy.

The data confirm that increasing synthetic amino acids level more than the replacement level of 35% from casein in the tilapia diet affected negatively on growth and feed utilization of tilapia fry.

Key word: synthetic amino acids, nitrogen retention, Nile tilapia (*Oreochromis niloticus*) fry .

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) was one of the first cultured fish species. Positive aquacultural characteristics of tilapia are their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms (Watanabe, 1991). Knowledge of the protein and amino acid requirements of an aquaculture species is essential for the formulation of cost effective diets. The nutritive value of a dietary protein is influenced primarily by the amino acid composition. Since protein is the largest and most expensive component in aquaculture diets, the choice of protein to be used in practical diet formulations should be based on the quantitative amino acid requirements of the cultured species. Using this information, feeds can be formulated on an amino acid rather than on a protein basis, thus potentially reducing feed costs (Millamena *et al.*, 1997). Some experiments diets with large amounts of crystalline amino acids have given lower growth rates than diets containing whole protein (Robinson *et al.*, 1981), while, more recently both Cho *et al.* (1992) and Kim *et al.* (1992 a and b) have obtained good growth rates with diets containing high levels of crystalline amino acids. Dupree and Halver (1970) reported that the supplementation of diets deficient in amino acids with synthetic amino acids results in a return to optimum growth depends on the ability of the culture animal to absorb and metabolize the supplemental amino acid. However, when a diet containing only free amino acids is utilized, absorption appears to be acceptable although, the growth generally is not as good as that demonstrated by fish on practical diets .No such data are available on tilapia . Lopez- Alvarado and Kanazawa (1994) reported that incorporation of free amino acid (FAA) into formulated diets has proven difficult due to the small free particle size and the problem of rapid leaching to the environment. The problem with supplementation of practical diets with purified amino acids may be related to the immediate availability for absorption as compared to practical protein sources. Absorption sites may become saturated, whereupon much of the amino acid component of purified diet would pass through the intestine and be evacuated from the fish gut. Amino acids present in practical feed ingredients are released much more slowly through enzymatic activity; thus only a small amount of any particular amino acid is available for absorption at one time (Stickney, 1979).

The aim of the present study was to elucidate the effect of varying dietary synthetic amino acids levels on performance and carcass nutrients retention of Nile 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. The study was designed to determine the effect of varying dietary synthetic amino acids levels on performance and carcass nutrients retention of Nile tilapia, (*Oreochromis niloticus*) fry.

Feed ingredients:

Experimental diets were formulated as semi-purified diets. A mixture of casein-vitamin free and gelatin were used as the sole protein source, corn oil and cod liver oil used as the fat source; dextrin, cellulose and carboxymethyl cellulose as digestible carbohydrate, fiber and binder, respectively. All synthetic amino acid 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 proximate analysis of the amino acid content of protein sources (casein and gelatin).

Experimental fish:

Nile tilapia fry was purchased from Nawa hatchery, Shebien EL-Kanater, Kaluobia Governarate. Fishes were healthy, free from parasites with initial average weight of 1.24 ± 0.01g. The experimental fish were kept for a conditional period of 14 days, afterwhich period, the actual experimental period followed directly for one month.

Table (1): The amino acid composition of casein and gelatin (g / 100 g) 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	16.79
Serine	5.56	3.12

*According to NRC (1993).

Technique of culture:

Experiment was conducted in 6 glass Aquarius of (80 x 50 x 40) cm3 with capacity of 160 liters representing 6 nutritional treatments. Aquaria were filled with dechlorinated tap water and supplied with aeration. Water was changed three times weekly, temperature was maintained at 28°C by using heater, and ammonia concentration was maintained at less than 0.3 mg /l. with pH of 8.00.

A total of 240 tilapia fry was stocked and equally divided into 6 aquaria of 40 fish in each. To study the level of synthetic amino acids in the same proportions as in casein that gives similar rate of nitrogen retention (NR) to intact protein without significant difference by replacing 15%, 35%, 50%, 65% and 85% from casein and gelatin protein with synthetic amino acid (according to Santiago and Lovell, 1988; Wang and Fuller, 1989). Tables (2&3) illustrate the six test diets fed and the amino acid composition of different diets, respectively .The six diets were isocaloric of 19.50 MJ GE /Kg and isonitrogenous of 30% crude protein according to (NRC, 1993). Fish were fed at a level of 5% of body weight twice a day at 9 a.m. and 12 a.m.

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

Synthetic amino acid level (% of CP)	0 %	15 %	35 %	50 %	65 %	85 %
Ingredients %						
Casein	26.57	21.25	15.94	10.63	5.32	--
Gelatin	5.12	5.12	5.12	5.12	5.12	5.12
Dextrin	38.03	38.35	38.66	38.97	39.28	39.60
α-Cellulose	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
Cod liver oil	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
C.M.C.[1]	2.00	2.00	2.00	2.00	2.00	2.00
Mixture A-A.[2]	--	5.00	10.00	15.00	20.00	25.00
Nutrient Composition (%DM basis).						
Dry matter	89.71	88.95	89.69	90.05	89.78	86.89
Gross Energy (MJ/Kg).[3]	19.51	19.47	19.70	19.54	19.64	19.69
Crude protein %	29.95	30.72	30.84	30.65	30.92	30.67
Ether-Extract %	5.12	5.14	5.34	5.22	5.77	6.20
Carbohydrate %[4]	59.23	57.94	58.66	58.26	57.20	56.89
Ash %	5.70	6.20	5.16	5.87	6.11	6.24

[1] Carpoxymelhyl Cellulos. [2] Mixture A-A see Table (3).

[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.

Chemical composition analysis of diets and fish:

Samples of fry at the beginning and at the end of experimental period were randomly chosen from each experimental treatment for proximate composition analysis. Samples were analyzed by standard methods A.O.A.C. (1980) for dry matter (oven dried at 105°C for 24 h). Crude protein (N-Kjeldahl X 6.25), fat (solvent extraction with petroleum ether, bpt 40-60 oC 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 for 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:

Statistical analysis was done for data form each experiment 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 ($P<0.05$) (Duncan,1955).

Table (3):The total dietary amino acid content and synthetic amino acid added to the experimental diets.

Amino acid	Casein control (0 %)		15 %		35 %		50 %		65 %		85 %	
	C+G[1]	SAA[2]	C+G[1]	SAA[2]	C+G[1]	SAA[2]	C+G[1]	SAA[2]	C+G[1]	SAA[2]	C+G[1]	SAA[2]
Arginine	1.319	-	1.128	0.191	0.937	0.382	0.746	0.573	0.555	0.764	0.364	0.955
Histidine	0.774	-	0.629	0.145	0.484	0.290	0.339	0.435	0.194	0.580	0.049	0.725
Isoleucine	1.206	-	0.977	0.229	0.748	0.458	0.519	0.687	0.290	0.916	0.063	1.143
Leucine	2.414	-	1.959	0.455	1.504	0.910	1.049	1.365	0.594	1.820	0.144	2.270
Lysine	2.122	-	1.733	0.389	1.344	0.778	0.955	1.167	0.566	1.556	0.180	1.942
Methionine	0.754	-	0.611	0.144	0.467	0.288	0.323	0.432	0.179	0.576	0.039	0.716
Cysteine	0.105	-	0.086	0.019	0.067	0.038	0.048	0.057	0.029	0.076	0.007	0.098
Phenylalanine	1.398	-	1.138	0.251	0.887	0.502	0.636	0.753	0.385	1.004	0.099	1.290
Tyrosine	1.464	-	1.177	0.287	0.890	0.574	0.603	0.861	0.316	1.148	0.032	1.432
Threonine	1.110	-	0.904	0.206	0.698	0.412	0.492	0.618	0.268	0.824	0.082	1.028
Tryptophan	0.318	-	0.255	0.063	0.192	0.126	0.129	0.189	0.066	0.522	0.002	0.316
Valine	1.518	-	1.237	0.281	0.956	0.562	0.675	0.843	0.394	1.124	0.115	1.403
Alanine	1.355	-	1.188	0.167	1.021	0.334	0.854	0.501	0.687	0.668	0.521	0.834
Aspartic acid	2.038	-	1.686	0.352	1.334	0.704	0.982	1.056	0.630	1.408	0.279	1.759
Glutamic acid	6.322	-	5.154	0.851	3.988	1.702	2.333	2.553	1.658	3.404	0.490	4.255
Glycine	2.120	-	2.023	0.097	1.926	0.194	1.829	0.291	1.732	0.388	1.636	0.484
Proline	3.752	-	3.175	0.577	2.598	1.154	2.021	1.731	1.444	2.308	0.869	2.883
Serine	1.637	-	1.341	0.296	1.045	0.592	0.749	0.888	0.453	1.184	0.160	1.477
Ratio	100	-	85	15	65	35	50	50	35	65	15	85

[1] C+G = Casein + Gelatin .

[2]SAA = Synthetic amino acid.

RESULTS AND DISCUSSION

Effect of varying dietary synthetic amino acids levels on growth performance :

The Nile tilapia fry apparently utilized the different test diets, which contained up to 85% of the protein as crystalline amino acids without any mortality. The results in Table (4) showed the Nile tilapia fry growth performance. Average of initial live body weight of Nile tilapia fry ranged between 1.25 to 1.27 ±0.1 gm. No significant difference in initial weight of fish among the experimental treatments was observed; indicating the accuracy of randomization process between and within the experimental treatment.

Analysis of variance for final body weight (Table, 4) indicated that the difference between treatments were significant ($P<0.05$). Fry fed the experimental diets containing synthetic amino acid at levels 15% and / or 35% of dietary crude protein casein were significantly higher in final body weight than the other treatments. The data revealed that increasing the percentage of replacing casein by mixture of synthetic amino acids than 35% of dietary crude protein, had a negative response in final body weight of fish. The same trend was shown in relative growth rate, average daily gain and specific growth rate. Feed conversion ratio was increasing replacement level of synthetic amino acid up to 35% of dietary protein casein compared to the other treatments. The worst feed efficiency was recorded for tilapia fed 85% synthetic amino acid of dietary protein casein. The data disagreed with Santiago and Lovell (1988) who reported that the young Nile tilapia relatively well utilized the test diets which contained up to 82% of the protein as crystalline amino acids, may be due to the gain in body weight per unit of protein consumed (or amino acid) was not refined criteria for evaluating the utilization efficiency or dietary amino acid levels as proveisly reported befor by Hepher (1988) who reavealed that protein efficiency ratio as evaluation critoria for protein does not consider differences in the composition of the weight gained and the accumulation of fat in fish tisses. Whereas, protein productive value is a more refined criterion for the evaluation of dietary protein since it takes into account the transformation of the dietary protein into body protein rather than the overall increase in body weight.

Table (4): The growth performance of tilapia fed the different experimental diets.

Synthetic amino acid level (% of CP)	0 % Control	15 %	35 %	50 %	65 %	85 %	SE ±
Initial body weight (g)	1.25	1.27	1.25	1.23	1.24	1.25	--
Final body weight (g)	2.650^{a}	2.670^{a}	2.623^{a}	2.417^{b}	2.307^{c}	2.210^{d}	0.056
Average daily gain							
(mg./ fish /day)[1]	46.66^{a}	46.68^{a}	46.17^{a}	39.58^{b}	35.78^{c}	31.96^{d}	1.23
Relative growth rate (%)[2]	112.00^{a}	110.2^{a}	109.84^{a}	96.50^{b}	86.05^{c}	81.15^{c}	5.28
Specific growth rate (% day)[3]	2.503^{a}	2.497^{a}	2.483^{a}	2.253^{b}	2.077^{c}	1.890^{d}	0.056
Feed consumption (g. / fish)							
Feed efficiency (%)[4]	1.96	1.98	1.97	1.81	1.95	1.52	--
Feed conversion ratio	71.43^{a}	70.71^{a}	69.70^{a}	65.58^{b}	54.72^{c}	57.56^{c}	3.38
per fish[5].	1.40^{c}	1.41^{c}	1.42^{c}	1.52^{b}	1.53^{b}	1.58^{a}	0.055

[1]Average daily gain (ADG) = 1000 X |(Final body weight (g.) - Initial body weight(g.))| / period (day).

[2]Relative growth rate (%) = (Final body weight (g) - Initial body weight (g.)) / Initial body weight (g).

[3]Specific growth rate (SGR) = 100 X ((ln Final body weight - ln Initial body weight) / period (days)).

[4]Feed efficiency (%)= (Weight gain (g.) /Dry weight of feed (g.) X 100.

[5]Feed conversion ratio (FCR) = Dry weight of feed (g.) / Weight gain (g).

a,b,c,..... etc. means in same raw with different subscripts are significantly ($P \leq 0.05$).

Nutritionists have paid a great deal of attention to use synthetic amino acids in fish diets, particularly, when diets are deficient in one amino acid or more. Some attempts were done with a significant success, while many faced a noticeable failure. A number of explanations have been suggested to explain the poor growth of fish fed amino acid based diets. Tanaka *et al.*, (1977) explained the low growth rate of common carp fed synthetic amino acid test diet by the short retention time of the amino acids in the intestine. These acids are absorbed into blood and serum to excrete at a higher rate than they are metabolized. Wang and Fuller (1989) suggested that the use of synthetic amino acids in a conventional diet to estimate amino acid requirements could lead to an overestimate, especially when animals were fed only once a day. Yamada *et al.*, (1981) tried to improve the efficiency of test diets containing crystalline amino acids for common carp by increasing the frequency of feeding. However, feeding frequency increased with the same total amount of feed, weight gain and feed efficiency increased proportionally.

Chiu *et al.*, (1987) showed that the level of dietary electrolytes markedly influences the metabolism of amino acids and that the transport of amino acids through the intestinal wall is often closely associated with Na^+ and K^+ present. Murai *et al.*, (1984) tried to separate the effect of pH from that of the electrolytes. They found that both play a role in improving amino acids utilization. The adding k+ to the diet to equimolar level of the Cl^- from supplemented amino acid Hcl, improved utilization, but not in case of excess supplements of K^+ and Na^+. In this connection, Hepher (1988) reported that the differences in the absorption rate observed in some fishes my affect the availability of the amino acids at the proportions required for biosynthesis and thus reduce their utilization for growth. Cowey and Walton (1988) explained the poor growth of fish fed amino acid based diets due to the more rapid appearance and higher concentration of the amino acids in plasma which is thought to enhance the activities of amino acid catabolizing enzymes leading to greater amino acid degradation and lower levels of protein synthesis. Although, Cho et al., (1992); Kim *et al.*, (1992 a and b); Rodehutscord et al., (1995a and b) ; Rodehutscord *et al.*, (1997) and Davies and Morris (1997) have obtained good growth rates with diets containing high levels of crystalline amino acids.

From the nutrition point of view, the errors arising from the use of synthetic amino acids in the present experiments are some what small due to (1) casein is one of the most fully and rapidly digested amino acids; (2) all the essential amino acids are used to balance the amino acids mixture in each experimental diet; (3) instead of feeding the animals once daily, fish in experiment reported herein were fed twice a day.

The results indicated that the synthetic amino acid mixture could replace casein protein up to 35% without significant difference in growth performance of Nile tilapia (*Oreochromis niloticus*).

Effect of synthetic amino acids on feed utilization:

Amount of feed consumed expressed as, gm dry matter/fish (Table,4); nitrogen intake and energy intake expressed as gm/fish and Kcal/fish, respectively (Table,5) followed a similar trend to those recorded for the growth performance parameter of tilapia. The amount of dry matter consumed, protein intake and energy intake decreased with increasing dietary level of synthetic amino acid added in diet.

The protein utilization expressed in terms of pro-

Table (5) : The protein efficiency ratio , protein intake , nitrogen retention, energy intake and energy retention of tilapia fed the different experimental diets.

Synthetic amino acid level (% of CP)	0 % Control	15 %	35 %	50 %	65 %	85 %	SE ±
Protein consumption (g)	0.587	0.608	0.607	0.552	0.510	0.464	--
Protein efficiency ratio	2.380[a]	2.297[b]	2.280[b]	2.147[c]	2.103[c]	2.140[c]	0.08
N-Retention %	46.20[a]	45.95[a]	45.41[a]	34.71[b]	34.50[b]	31.97[c]	1.06
Energy Intake (Kcal/ fish)	9.14	9.22	9.30	8.45	7.75	7.16	--
Energy Retention %	22.83[ab]	23.29[a]	22.08[ab]	18.29[c]	19.47[b]	18.34[d]	0.801

SE : standard error. Calculated from residual mean square in the analysis of variance.
a,b,c,.... etc. means in same raw with different superscripts are significantly (P≤0.05).

tein efficiency ratio (PER) for tilapia fed different levels from synthetic amino acid is presented in Table (5). The higher significant differences (P<0.05) value was recorded for fish fed control diet (no synthetic amino acid added), followed by the group of fish fed diet containing 15% and/or 35% synthetic amino acid in a decreasing order. These results revealed that increasing synthetic amino acid level in tilapia diet negatively affected the protein efficiency ratio (PER).

Data for nitrogen and energy retention are presented in Table (5). Amount of nitrogen and energy retained among experimental fish were the highest in the group fed diet containing a level of 15% and/or 35% synthetic amino acid added without significant differences with the control. The data followed the same findings of Robinson *et al.*, (1981) and Walton *et al.*, (1982 and1986) who reported that many fish species, (catfish and rainbow trout), grew well when fed diets containing optimum amounts of synthetic amino acid, while, when the diets contain large amounts of synthetic amount acid the growth being inferior to diets of similar amino acid composition in which the nitrogen component in protein. In this connection, Hepher (1988) cited that when a diet containing only synthetic amino acids, the amino acids absorption and utilization appear to be unacceptable; as the growth is generally less than that demonstrated by fish on practical diets. Lopez-Alvarado and Kanazawa (1994) reported that incorporation of free amino acid (FAA) into formulated diets has proven difficult, due to the small free particle size and the problem of rapid leaching into the environment. The problem with supplementation of practical protein diets with purified amino acid may be related to the immediate availability for absorption as compared to practical protein sources (Dupree and Halver , 1970). Absorption sites may become saturated, whereupon much of the amino acid component of purified diet would pass though the intestine and be evacuated from the fish gut. Amino acids present in practical feed ingredients are released much more slowly through enzymatic activity; thus only a small amount of any particular amino acid is available for absorption at one time. (Stickney, 1979).

The data supported the finding of Rodehutscord *et al.*, (1997) who reported that among traits performance monitored of fish, protein deposition is the most sensitive indicator of a sub-optimal supply of an amino acid. They added that at least part of the differences found in recommendations for amino acid supply in the literature must be due to different response criteria chosen in individual studies.

The data confirm the conclusion that, increasing a replacement of synthetic amino acids mixture level up to 35% from dietary protein (casein) in the tilapia's diets would negatively affect the dietary protein and energy utilization.

The whole body composition of tilapia:

The whole body proximate analyses of experimental fish are presented in Table (6). The results showed that the percentage of whole body moisture, ether extract and ash content increased significantly ($P<0.05$) as the level of dietary synthetic amino acid increased, while an inverse trend was observed with percentage of whole body crude protein and gross energy content. The highest level of carcass fish lipid and the lowest level of carcass crude protein were recorded with excess dietary synthetic amino acid than the level of 35% (Table, 6), not only for the limiting dietary energy but also for the limiting of dietary amino acids pattern required for optimal growth and protein retention. The results agreed with finding of Jackson and Copper (1982) for tilapia, *O.mosambicus*, Lovell *et al*, (1974) and Hepher (1988) who reported that amino acids flow in the blood can undergo metabolism in either of two directions: the anabolic direction (biosynthesis of new proteins such as hormones and enzymes or formation new tissues) or the replacement of the protein molecules could produces carbon skeletons (catabolic route) which my be utilized for either energy or lipogenesis.

Table (6): The gross body composition of tilapia fed different experimental diets.

Synthetic amino acid level (% of CP)	0% control	15 %	35 %	50 %	65 %	85 %	SE ±
Moisture	74.80^{c}	74.65^{c}	74.71^{c}	76.31^{ab}	75.50^{bc}	76.44^{a}	0.88
Crude protein % (DM)	62.15^{a}	62.25^{a}	62.20^{a}	58.50^{b}	56.74^{c}	56.98^{c}	0.87
Ether extract % (DM)	16.96^{c}	17.11^{c}	16.89^{c}	18.24^{b}	19.40^{a}	19.76^{a}	0.90
Ash % (DM)	20.74^{b}	20.43^{b}	20.78^{b}	22.86^{a}	23.76^{a}	23.25^{a}	1.53
Gross energy (Kcal/100g fish)	512.05^{ab}	514.28^{a}	511.86^{ab}	504.57^{d}	504.33^{d}	508.71^{c}	1.60

SE : standard error. Calculated from residual mean square in the analysis of variance.
a,b,c,.... etc. means in same raw with different superscripts are significantly ($P \leq 0.05$).

REFERENCES

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

Chiu, Y.N., Austic, R.E. and Rumsey, G.L. (1987): Interaction among dietary minerals, arginine and lysine in rainbow trout (*salmo gairdneri*). Fish. Biochem. Physiol. 4:45-55.

Cho, C.Y., Kaushik, S.J. and Woodward, B. (1992): Dietary arginine requirement of young rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol*, F-tests. Biomet., 102A: 211-216.

Cowey, C.G., Walton, M.J., (1988): Studies on the uptake of (^{14} C) amino acids derived from both dietary (^{14}C) protein and dietary (^{14} C) amino acids by rainbow trout, *Salmo gairdneri*, Richardoson. J. Fish.

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.

Dupree, H. K. and Halver, J. E. (1970): Amino acids essential for the growth of channel catfish, *Ictaluru spunctatus*. Trans. Am. Fish. Soc. 99: 90-92.

Hepher, B. (1988): Nutrition of pond fishes. Cambridge: Cambridge University Press.

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.

Jackson, A. J. and Copper, B. S. (1982): Investigations into the requirements of the tilapia, *Sarotherodon mossambicus* for dietary methionine, lysine and arginine in semi-synthetic diets. Aquaculture, 29: 289-297.

Kim, K.I., Kayes, T.B and Amundson, C.H. (1992a): Requirement for sulfur amino acids and utilization of D-methionine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 101: 95-105.

Kim, K.L., Kayes, T.B and Amundson, C.H. (1992b): Requirement for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 106: 333-344.

Lopez-Alvarado, and J. and Kanazawa, A., (1994): Effect of dietary arginine levels on growth of red sea bream larvae fed diets supplemented with crystalline amino acids. Fish. Sci. 60, 435-439.

Lovell, R. T.; Prather, E. E., Tres-Dick, G. and Lim, C. (1974): Interrelationships between quality and quantity of protein in feeds for channel catfish in intensive pond culture. Proc. Annu. Conf. Southeast. Assoc. Game Fish Comm., 28: 456-461.

Millamena, O.M., Bautista, M.N., Reyes, O.S. and Kanazawa, A.(1997): Threonine requirement of Juvenile marine shrimp Penaeus monodon. Aquaculture, 151:9-14.

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 Gakkaishi 50, 893-897.

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

Robinson, E.H., Wilson, R.P. and Poe, W.E. (1981). Arginine requirement and apparent absence of lysine-arginine antagonism in fingerling channel catfish. J. Nutr. 111: 46-52.

Rodehutscord, M., Becker, A., Pack, M. and Pfeffer, E. (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. J. Nutr. 127, 1166-1175.

Rodehutscord, M., Jacobs, S., Pack, M. and Pieffer, E (1995a): Response of rainbow trout growing from 50 to 150 g to supplements of DL-methionine in a semipurified diet containing high or low levels of cystine.J.Nutr,125:964-969.

Rodehutscord, M., Jacobs, S., Pack, M. and Pieffer,-E (1995b): Response of rainbow trout growing from 50 to 170 g to supplements of either L-arginine or L-methionine in a semipurified diet. J. Nutr. 125:970-975.

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

Stickney, R. R. (1979): Principles of worm water aquaculture book. pp.187.

Tanaka, Y., Hokasano, S., Katayama, T., Simpson, K.L. and Chichester,C.O., (1977): Metabolism of amino acids in aquatic animals. I. The effect of addition of phosphate salts, indigestible materials and algae to the diets of carp and the relationship of intestinal retention time to their growth rate. Mem. Fac. Fish. Kagoshima Univ. 26: 39-43.

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

Walton, M.J., Cowey, C.B., Coloso. R.M and Andron, J.W. (1986). Dietary requirements of rainbow trout for tryptophan, lysine and arginine. Determined by growth and biochemical measurements. Fish. Biochem. Physiol. 2:161-169.

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

Watanabe, W.O. (1991): Saltwater culture of tilapia in the Caribbean.World Aquaculture 22, 49-54.

Yamada,S., Tanaka, Y. and Katayama, T. (1981): Feeding experiments with carp fry fed an amino acid diet by increasing the number of feedings per day. Bull. Jap. Soc. Sci. Fish., 47. 1247-1258.