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Effect of L-Lysine Supplementation with Different Protein Levels in Diets on Growth, Body Composition and Protein Metabolism in Pearl Spot *Etroplus Suratensis* (Bloch)

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Abstract

Experimental diets with 0.5% L-lysine (HCl) having 25, 30, 35 and 40% protein were formulated with purified ingredients except soya meal. At each protein level a diet devoid of L-lysine was used as control. Performance parameters were assessed by conducting feeding trail experiments in pearl spot (*Etroplus suratensis*) for a period of 41 days. Irrespective of the dietary protein levels, growth of *E. suratensis* was higher in fish received L-lysine supplemented diets when compared to those reared on control diets. The data on feed conversion ratio (FCR) revealed that the effect of L-lysine supplementation was significantly higher ($P<0.05$) at low protein diets (25 and 30%) fed fish than the others. The influence of L-lysine supplementation on Specific Growth Rate (SGR) of *E. suratensis* was more pronounced at low protein levels than those reared at high protein levels. The protein consumption rate of *E. suratensis* fed with diets having 25 and 35% protein was higher than that of fish reared on control diet at the same protein level. Similarly the protein production rate was 20.37, 19.74, 3.55 and 3.64% more than those received diets devoid of L-lysine respectively at 25, 30, 35 and 40% protein levels. The protein utilization efficiency ratio ranged between 73.73 ± 1.35 to 85.95±0.72% and 76.49±0.99 to 86.21±0.51% for the control and the experimental diets fed fishes, respectively. The Protein Production Value (PPV) was greater in the experimental diets fed fish than those fed with control diets and the variation between them was statistically significant ($P < 0.05$) for those fish reared on low protein diets.

Key words: L-lysine, protein diets, *Etroplus suratensis*, SGR, FCR.

Introduction

In intensive fish culture practice, manufactured feed are essential for economic use of time, space and aquacultural facilities. Any balanced formula for fish diets must include an energy source plus sufficient indispensable amino acids, essential fatty acids, specific vitamins and minerals to support life and to promote growth (Halver *et al*., 1958). The quantitative essential amino acids are determined by feeding graded levels of each amino acid with an amino acid test diet so as to elicit a dose-response curve (Ketola, 1982; Cowey and Lequet, 1983; Shaik Mohamed and Ibrahim, 2001; Reigh *et al*., 2002).

For several fish species, lysine is one among the ten indispensable amino acids required in the dietary protein, because lysine is generally the most limiting amino acid in the plant proteins and it is the most critical amino acid in fish feed. In addition to meet the basic metabolic requirements for maximum growth, dietary lysine supplementation has been shown to have other positive effects on various animals (Borlongan and Benitez, 1990; Cheng and Hardy, 2003). The dietary protein requirement to supply the necessary nitrogen and amino acid has been determined for several species of fish reared in ponds and in laboratory under controlled condition, using either partial or purified diets (Page and Andrews, 1973; Lovell, 1975; Robinson, 1992). These studies indicated that the optimum protein requirement ranges from 20 to 60%. These differences in apparent protein requirement may attributed to the differences in the fish size, water temperature, natural feed availability, daily feed allowance, total amount of energy in the feed, quality of the dietary proteins etc. Since protein must be supplied to the fish with sufficient amount of essential amino acids, the lower the protein content in the diet, the higher must be the concentration of these amino acids in the protein. In view of this, the present study was undertaken to investigate the effect of L-lysine supplemented diet with different protein level on growth responses, body composition and protein metabolism in cichlid *Etroplus suratensis*.

Materials and Methods

Diet Preparation

Experimental diets were prepared with 25, 30, 35 and 40% dietary protein using casein, gelatin, defatted soya meal, dextrin, carboxy methyl cellulose, α-cellulose, cod liver oil, vitamin-mineral mix and L-lysine (0.5%). At each protein level, a diet devoid of L-lysine served as the control diet. In the test diets having 25.20±0.40 to 40.10±0.86% protein, the carbohydrate, lipid contents and energy levels ranges from 25.40±0.42 to 38.50±0.90%, 9.00±0.22 to 9.40±0.38% and 1.41±0.64 to 14.46±0.26 J/mg,

respectively (Table 1).

Fish and Rearing Conditions

Experimental fish *Etroplus suratensis* were collected from a local fish farm, M. M. Aquapark, Rajakkamangalam, South India. The collected fish were well acclimatized in ambient laboratory condition for ten days. After acclimatization, healthy fish weighing about 15.0±1.50 g were reared in FRP aquaria (Cap. 500 L) in three replicates for a period of 41 days at the rate of 10 fishes/tank. Optimum environmental conditions (salinity 15±2‰, temperature 28±1ºC, DO 5.5±0.5 mg/L and pH 7.5–8.0) were maintained throughout the rearing period. The fish were fed to satiation with formulated diets three times a day at 9.00 A.M., 12.00 noon and 3.00 P.M.

Sample Collection and Analysis Methods

After the set duration of feeding, the uneaten feeds were siphoned out from individual tank in tested condition with utmost care to avoid the disintegration, oven dried and weighed. Considering the amount of uneaten feeds with that of the total feed provided, the amount of feed consumed by individual fish was calculated. During the experimental period, 30 to 40% water exchange was made daily. Before water exchange, the fecal matter egested was carefully collected daily and were oven dried, weighed and used for further analysis. At the end of the experiment, the fishes were weighed separately in all the treatments. Then they were sacrificed and the muscle tissue was removed under low temperature of 10–12°C at aseptic condition and used for protein, carbohydrate and lipid analysis following the standard procedure described below.

Performance parameters such as weight gain, specific growth rate and feed conversion ratio of *E. suratensis* at the end of the experimental period were calculated as follow:

Specific growth rate (%) = 100 x (Ln W_2–Ln W_1)/t (SGR)

Where,
W_1 = Initial weight of the fish (g)
W_2 = Final weight of the fish (g)
t = duration of the experiment (days)

Feed Conversion Ratio (FCR) = Amount of dry food consumed / wet weight gain

The biochemical constituents such as protein, carbohydrate and lipid in experimental diets, fish muscle and also in feces were analysed using the procedure of Lowry *et al*. (1951), Seifter *et al*. (1950) and Bligh and Dyer (1959), respectively. For the scheme of mass budget, the IBP formula of Petruzewic and McFadyen (1970) was used. Considering the mass budget value with that of the respective protein value, the protein metabolism of *E. suratensis* was calculated.

Protein Consumption Rate (mg g^{-1} day^{-1}) = Protein consumed (mg dry wt.) / [Initial wet weight of the fish (g) x Experimental duration]

Table 1. Proportions of various feed ingredients (g/100 g) used for the formulation of test diets and biochemical composition (%) of individual diets

Ingredients	25% protein		30% protein		35% protein		40% protein	
	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
Casein	9.6	9.6	14.9	14.9	20.3	20.3	25.6	25.6
Gelatin	6	6	6	6	6	6	6	6
Soya meal (Fat free)	21	21	21	21	21	21	21	21
Dextrin (Hi media)	37.5	37.5	33.5	33.5	28.5	28.5	23.5	23.5
α - Cellulose	11.9	11.4	10.6	10.1	10.2	9.7	9.9	9.4
Carboxy methyl cellulose (Sodium salts of high viscosity)	2	2	2	2	2	2	2	2
Cod liver oil	8	8	8	8	8	8	8	8
Vitamins and Minerals (Multi Vitamin – Rovigon, Mumbai)	4	4	4	4	4	4	4	4
L-lysine (HCl)	-	0.5	-	0.5	-	0.5	-	0.5
Biochemical Composition								
Protein*	25.2±0.40	25.4±0.62	30.1±0.55	30.0±1.0	35.88±1.20	35.15±1.15	40.1±1.40	40.1±0.86
Carbohydrate*	38.5±0.90	38.4±1.05	35.4±0.76	35.1±0.84	30.15±0.66	30.25±0.76	25.75±0.52	25.4±0.42
Lipid*	9.2±0.20	9.15±0.34	9.05±0.26	9.15±0.42	9.4±0.38	9.15±0.34	9.2±0.28	9.0±0.22
Energy J/mg **	14.1±0.64	14.1±0.42	14.35±0.56	14.3±0.48	14.46±0.26	14.37±0.38	14.45±0.44	14.33±0.58

* Values are in percentage (%) of dry weight basis
** Energy density was calculated considering the protein, carbohydrate and lipid level and their standard oxycalorific coefficient

Protein Assimilation Rate (mg g^{-1} day^{-1}) = Protein assimilation (mg dry wt.) / [Initial wet weight of the fish (g) x Experimental duration]

Protein Production Rate (mg g^{-1} day^{-1}) = Protein production (mg dry wt.) / [Initial wet weight of the fish (g) x Experimental duration]

Protein Utilization Efficiency (%) = [Protein assimilation (mg dry wt.) / Protein consumed (mg dry wt.)] x 100

Protein Productive Value (%) = [Protein production (mg dry wt.) / Protein consumption (mg dry wt.)] x 100

Statistical Analysis

The data obtained were subjected to statistical analysis (student 't' test and SNK test) described by Daniel (1987).

Results

Growth Response

E. suratensis fed with 25% protein diet supplemented with L-lysine had higher growth compared to those fed with control diet devoid of L-lysine. In fish fed with 30, 35 and 40% protein levels also higher growth was recorded when fed with L-lysine supplemented diets when compared with those fed with control diet. The Specific Growth Rate (SGR) of fish received L-lysine supplemented diets was more than those received control diet at all the tested protein levels. Except in fish fed with 45% protein diet, the variation in SGR between control and experimental diets fed group was statistically significant ($P<0.05$; student's 't' test). The food conversion ratio of the experimental diets fed fish ranged from 2.09±0.09 to 2.52±0.07. Student 't' test revealed that, influence of L-lysine supplementation on FCR of *E. suratensis* fed with control and experimental diets having 40% protein was not statistically different ($P>0.05$). On the other hand, it was significantly different ($P<0.05$) for the FCR values of control and experimental diets fed fish at 25 and 30% protein densities (Table 2).

Biochemical Composition

The overall results on variation in carcass biochemical composition inferred that, it was much influenced by both variation in dietary protein density and also L-lysine supplementation. The carcass protein, carbohydrate and lipid contents of fish fed on control and test diets with high protein levels (35 and 40%) was higher than those fish fed with low protein diets (25 and 30%). Among the biochemical constituents analysed, carbohydrate and lipid contents of fish fed with 25 to 35% protein diets varied much between control and experimental diets fed groups. On the other hand, at these same dietary protein levels, the protein content was not differed much between fish fed with control and L-lysine supplemented diets. Also in those fish fed with 40% protein diets, protein and lipid constituents were not varied much between those fed control and L-lysine supplemented diets (Table 3).

Protein Metabolism

Protein metabolism of *E. suratensis* fed with control and test diets showed an enhancing trend with raise in protein density in the diet. But at the respective tested protein level, the protein metabolic indices showed higher values in those fish fed with L-lysine supplemented diets than those fed control diet, devoid of L-lysine. For instance, in fish fed 25% protein diet had higher protein consumption, assimilation and production when compared with those fed with control diet. At this same protein level, protein utilization efficiency and protein production value were also more in fish fed on L-lysine supplemented diet than those fed on control diet. A similar trend was also noticed for those fish fed with 30% protein level. Yet the effect of L-lysine supplementation was not much effective in fish fed with high protein diets (35 and 45%). In fish fed with 35% protein diet, the protein production rates recorded were 1.210±0.013 and 1.253±0.021 $mg.g^{-1}.day^{-1}$ respectively in control and experimental diets fed groups. At this same protein level, the protein utilization efficiency and protein production values registered for fish fed with control and L-lysine added diets were not varied significantly ($P>0.05$). Likewise in fish fed with 45% protein diet, the protein production recorded were 1.346±0.052 and 1.395±0.082 $mg.g^{-1}.day^{-1}$ respectively for control and L-lysine added diet fed groups. At this protein level, the protein utilization efficiency and protein productive value between control and test diets fed groups were not statistically differed ($P>0.05$; Table 4).

Discussion

L-lysine requirement of different fish species exhibits wide variation, being 20 g/kg diet (5.0% of protein) for Chinook salmon (Halver *et al*., 1958); 22 g/kg diet (5.7% of protein) for common carp (Nose, 1979); 20 g/kg diet (4.8% of protein) for Japanese eel (Nose, 1979); 19 g/kg diet (4.2% of protein) for rainbow trout (Walton *et al*., 1984; Cheng and Hardy, 2003); 1.3 g/kg diet (4.6% of protein) for Nile tilapia (Santiago and Lovell, 1988) and 1.62 g/kg diet (4.1% of protein) for *Oreochromis mossambicus* (Jackson and Capper, 1982). Such variations among species are attributed to differences in metabolic requirements of the species and in daily protein consumption by fish,

Table 2. Growth response and food conversion efficiency of *E. suratensis* fed with 25-40% protein diets supplemented with either 0 or 0.5% L-lysine

Experimental Protein diets	Initial Wet Weight (g)	Final Wet Weight (g)	Growth (g)	Total Dry Food Consumed (g)	Food Conversion Ratio	Specific Growth Rate (%)
25% (F1)	15.59±0.32	19.57±0.55	3.98±0.08	12.79±0.24	3.21±0.08	0.439^{a}±0.01
25% (F2)	15.52±0.41	20.68±0.53	5.16±0.07	13.02±0.31	2.52±0.07	0.707^{b}±0.014
30% (F3)	14.99±0.26	19.61±0.47	4.62±0.02	12.99±0.26	2.81±0.14	0.659^{c}±0.020
30% (F4)	14.52±0.20	19.98±0.60	5.64±0.09	13.23±0.30	2.34±0.04	0.756^{d}±0.018
35% (F5)	15.74±0.34	21.18±0.52	5.44±0.10	13.62±0.38	2.50±0.06	0.707^{b}±0.019
35% (F6)	15.25±0.25	21.28±0.68	6.03±0.14	12.61±0.27	2.09±0.09	0.829^{e}±0.024
40% (F7)	14.95±0.32	20.79±0.74	5.84±0.08	13.70±0.20	2.35±0.10	0.805^{e}±0.028
40% (F8)	15.32±0.38	21.58±0.84	6.26±0.12	13.57±0.16	2.16±0.05	0.829^{e}±0.031

Each value is a mean of three observations
Means in a column having the same letter are not statistically significant
(P>0.05; Students't' test)

Table 3. Biochemical composition of *E. suratensis* fed with 25-40% protein diets supplemented with either 0 or 0.5% L-lysine. Each value is the mean of three estimates

Experimental Diets	Biochemical Parameters *			
	Protein (%)	Carbohydrate (%)	Lipid (%)	Moisture (%)
Initial value	46.65±0.58	11.80±0.08	10.65±0.06	79.86±1.46
25% (F1)	43.14±0.42	11.84±0.09	8.20±0.07	77.60±1.24
25% (F2)	43.50±0.53	13.95±0.13	9.60±0.09	78.60±1.46
30% (F3)	46.35±0.38	12.95 ±0.08	8.72±0.11	78.10±1.02
30% (F4)	46.45±0.26	14.80±0.17	9.90±0.14	77.80±1.44
35% (F5)	47.95±0.37	13.30±0.16	10.15±0.06	77.70±2.10
35% (F6)	48.70±0.26	16.60±0.21	11.20±0.12	76.80±2.40
40% (F7)	48.80±0.19	14.64±0.19	11.7±0.08	77.20±1.32
40% (F8)	48.95±0.38	16.87±0.08	11.85±0.14	75.51±1.40

* Protein, carbohydrate and lipid contents are in percentage (%) of dry matter basis. The moisture content is expressed as wet weight (%) basis

Table 4. Protein metabolism of *E. suratensis* fed with 25–40% protein diets supplemented with either 0% or 0.5% L-lysine. Each value is the mean of three individual observations

Experimental Diets	Protein Consumption Rate (mg/g/day)	Protein Assimilation Rate (mg/g/day)	Protein Production Rate (mg/g/day)	Protein Utilization Efficiency (%)	Protein Production Value (%)
25% (F1)	5.04±0.14	3.71± 0.09	0.756±0.016	73.73±1.35	15.00^{a}±0.38
25% (F2)	5.20±0.11	3.98± 0.08	0.910 ±0.018	76.49±0.99	17.50^{b}±0.42
30% (F3)	6.36±0.17	4.86± 0.13	0.927±0.021	76.47±1.45	14.58^{ca}±0.39
30% (F4)	6.67± 0.18	5.29±0.17	1.11±0.014	79.38±1.28	16.64^{d}±0.66
35% (F5)	7.57±0.24	6.08±0.22	1.210± 0.013	80.37±1.14	15.98^{ea}±0.62
35% (F6)	6.91±0.31	5.63±0.18	1.253±0.021	81.46±0.90	18.13^{f}±0.57
40% (F7)	8.96±0.26	7.70±0.34	1.346±0.052	85.95±0.72	15.02^{ga}±0.49
40% (F8)	8.66±0.33	7.47±0.37	1.395±0.082	86.21±0.51	16.10^{gad}±0.36

Note : Means in the row with the same letters are not significantly different (P>0.05; SNK test)

caused by the variation, dietary formulations (type and amount of protein used), and feeding regimes used in the classical dose response experiments (Cowey, 1993; Fagbenro *et al*., 1998). Critical analysis of the data in the present study showed a significant (P<0.05) interacting effect of dietary protein level and L-lysine supplementation on growth performance and protein metabolism of *E. suratensis*. At low dietary protein levels (25 to 30%), the influence of L-lysine on growth was comparatively more obvious than that at high protein level (40%). The same trend was also observed in FCR and SGR. These results were supported by the findings of Bai and Galtin (1994) for channel catfish *Ictalurus punctatus*. They reported that fish fed with 0.5% L-lysine supplemented soy diet containing 25% protein level showed 24% increase in weight gain compared to 11% in those fed at 30% protein level. It has also been reported that channel catfish can utilize supplemental synthetic lysine in practical cotton seed meal based diets (Robinson, 1992); peanut meal based diet (Robinson *et al*., 1980) and methionine in a

soybean meal based diet (Murai *et al*., 1982), when the basal diets are deficient in the respective amino acid. Less growth variation of *E. suratensis* at 40% protein diet may be due to the availability of needed level of L-lysine and other amino acids in control diet devoid of L-lysine. Furthermore, presence of other amino acids at optimum level may have sparing effect for dietary protein deficit.

The results on the muscle biochemical composition of *E. suratensis* fed with test diets having nil or 0.5% L-lysine inferred note worthy information. Among the constituents tested, protein content of *E. suratensis* fed with respective control and test diets at the tested protein densities was not varied much; whereas, the carbohydrate and lipid contents registered marked variation. It may be inferred that in the present study, addition of L-lysine at 0.5 g/100 g altered the growth responses of *E. suratensis* through carbohydrate and lipid synthesis. Here the protein sparing action of dietary carbohydrate and lipid along with supplemented L-lysine was high at low dietary protein levels of 25 and 30% than high dietary protein levels (35 and 40%). As the results, the former nutrients availability may increase and this in turn influence the deposition of dietary carbohydrate and lipid through lipogenesis and glyconeogenesis. This conclusion is consistence with the earlier report of Adron *et al*. (1976) and Brauge *et al*. (1994).

Chinu *et al*. (1986) reported that the addition of crystalline lysine significantly improved the biological value of corn gluten meal as a protein source for milk fish fry. Andrews and Page (1974) have shown supplementation of synthetic methionine, cystine or lysine has no effect on growth of channel catfish diet containing soybean meal. The less response to supplemental amino acids was probably due to the basal diets not being deficient in these amino acids. This may be attributed to the present observation that the less difference in the growth responses of *E. suratensis* fed at high protein diet (40%).

The results on protein utilization efficiency yield note worthy information on the interacting effect of dietary protein level and L-lysine supplementation on protein metabolism. In those fish fed with 25 and 30% protein diets added with L-lysine registered 3.74% and 3.78% increase in protein utilization efficiency over those fed with diets devoid of L-lysine at the same protein levels. This differences in protein efficiency is also reflected in protein production and the increase recorded was 20.37% and 19.74% in *E. suratensis* fed with 25% and 30% protein added with L-lysine when compared the values recorded in control diet fed group at the respective protein level. The poor protein production observed in the present study by *E. suratensis* fed with diets devoid of L-lysine agrees with the report of Arai *et al*. (1972); who noticed the increased mortality in European eel fed with lysine free diet. Halver (1957) and Dupree and Halver (1970) were also recorded poor growth rate with nil mortality in Chinook salmon and channel catfish fed with lysine free diets.

In contradict to this, in those fish fed with 35 and 40% protein diets, the variation in protein utilization efficiency was not marked between control and test diet fed groups. Obviously the protein production of *E. suratensis* fed on control and test diets at these protein levels (35 and 40%) was also not deviated much. This present study inferred that supplementation of L-lysine at low dietary protein levels (25 and 30%) involved in protein sparing effect and thereby altered the protein utilization and protein production. But at the higher protein levels (35% and 40%), the fish could able to meet their protein requirement even on control diets devoid of L-lysine. These tested higher dietary protein levels have reported as the optimum protein level for variety of fish species (Delong *et al*., 1958; Lim *et al*., 1979; Jauncey and Ross, 1982). Hence it is obvious that, *E. suratensis* is satisfied their protein requirement even without addition of L-lysine at 35% and 40% protein level and at these level addition of L-lysine is mere a raise in particular amino acid level, which is unwarranted.

Deshimaru and Kuroki (1975) reported that increase in the rate of protein to amino acids in the protein source improved both growth and feed intake and lowered mortality in prawn. In a subsequent study, Deshimaru (1976) showed that poor performance of prawn fed diets with supplemented amino acids was probably due to rapid absorption and uncoordinated assimilation in prawn tissue. The efficiency of supplemented individual amino acids decreased with increasing dietary concentration of the respective amino acid, resulting in plateaus that could be described by exponential functions, reduction in either absorption rate or intermediary utilization of absorbed amino acids or both (Rodehutscord *et al*., 1997). The protein utilization efficiency of *E. suratensis* in the present study was lower in low protein diets and this variation may be attributed to the variation in level of protein in the basal diet.

Hepher (1988) also reported that the lower the digestibility of the protein, the higher the concentration of the essential amino acid must be supplied. The true value of amino acid requirement as a percentage of the dietary protein will therefore be obtained only when the latter is fed at the optimum amount required to maintenance and maximal growth. In the present study, though the protein sources are same for all the diets, the levels are varied. Thus variation in protein levels lead to utilize the supplemental L-lysine more efficiently at low protein diets.

Conclusion

Results from the present study clearly demonstrated that a diet having 35% protein supplemented with 0.5% L-lysine was found to be the optimum for maximizing the growth responses of *E. suratensis*. However, more detailed experiments on L-

lysine supplementation in different concentrations at varying protein levels are needed to fully understand its efficiency on growth response of *E. suratensis* and on synthesis of biochemical constituents.

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