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Dietary Protein Requirement for Optimising Growth Responses of Asian Cichlid, *Etroplus suratensis* (Bloch)

V. Jayaprakas and S. Sunil Kumar
Department of Aquatic Biology & Fisheries
University of Kerala, Beach P.O.
Thiruvananthapuram - 695 007, India

Supplementary diets having three levels of protein viz., 30, 35 and 40% were fed to *E. suratensis* for a period of 120 days. Growth, feed conversion efficiency, digestive enzyme activity, nucleic acid content and carcass proximate composition were evaluated. Highly significant ($p<0.01$) superior growth was obtained with 35% protein diet. High feed conversion efficiency, protein efficiency ratio (PER) and good digestibility of protein, fat and carbohydrate were obtained with the diet containing 35% protein. RNA/DNA ratio in the muscle was maximum in this group, followed by the 30% and 40% protein groups. High protein content was noticed in the carcass of fish fed on 35% protein-diet.

Key words: Dietary protein, *Etroplus suratensis*, growth, feed utilization, RNA/DNA ratio

The quality and quantity of supplementary diets are principal factors that determine the growth of fish and cost of production in aquaculture (Love, 1980). A nutritionally balanced supplementary diet can increase fish production and is one of the basic requirements of intensive fish culture. Supplementary diet should provide all elements needed for the growth of the fish to attain marketable size in short time and at low cost. Being the primary diet component for growth, protein has been given priority in evaluating nutritional requirements. The protein requirements of fish are about two to three times higher than that of mammals and hence they require much higher levels of protein in the diet than are necessary for birds and mammals (Pandian, 1987). Unlike mammals, protein acts both as a structural component and an energy source in fish (Brett & Grover, 1979). Protein has become an expensive dietary component and knowledge of the dietary protein requirements of candidate species for aquaculture is a must for formulation of efficient supplementary diet to increase fish production in aquaculture system. The protein requirements of tilapia (Jauncey, 1982) and carp (Das & Ray, 1991; Seenappa & Devaraj, 1995) have been evaluated in detail. The optimum dietary protein requirement for pearlspot (*Etroplus suratensis*) has not yet been studied. Therefore an attempt had been made to evaluate the dietary protein requirement of this species.

Materials and Methods

The growth experiment was carried out for a period of 120 days in cement tanks of size, 5x4x1m without any soil base. The tanks were drained, cleaned and dried before filling with water. The level of water in the tanks was maintained at 75±5 cm throughout the experiment. Juveniles of *E. suratensis* of uniform size (1.8-3.4 g) were collected and reared for a period of two weeks in the laboratory. The fish were randomly stocked in the experimental tanks at a density of 30/ tank. Three replicates for each treatment were maintained. Feeding was done twice daily at the rate of 5% of body weight.

Quantity of feed was adjusted after every sampling. Water samples were collected fortnightly and analyzed for temperature, pH, dissolved oxygen, alkalinity, total hardness and primary productivity following APHA (1992) procedure.

Table 1. Feed ingredients and proximate composition of various feeds used

	Protein level		
	30%	35%	40%
Rice bran (g)	32.20	17.30	11.00
Tapioca flour (g)	8.00	8.00	8.00
Fish meal (g)	28.93	33.75	46.00
Ground nut oil cake (g)	26.13	31.65	32.00
Cellulose (g)	13.74	9.30	3.00
Vegetable oil (ml)	2.50	2.00	1.50
Vitamin- mineral mixture (g)	1.00	1.00	1.00
Composition			
Moisture %	9.00	9.20	9.40
Protein (DWB)*	30.00	35.00	40.00
Fat (DWB) %	6.50	6.95	7.25
Carbohydrate (DWB) %	50.69	41.28	35.40
Fibre (DWB) %	4.20	5.63	6.20
Ash (DWB) %	8.61	11.14	11.15
Calorific value (jmg^{-1})	14.07	14.37	14.81

*DWB - Dry weight basis

Three isocaloric diets containing 30, 35 and 40% protein were prepared in pelleted form using fish meal and groundnut oil cake as the major sources of protein. The feed was formulated following the square method (Hardy, 1980). Details of feed ingredients and the proximate composition of the feed are given in Table 1. The ingredients were weighed and homogenized to a wet dough by adding sufficient water. The dough was cooked, cooled and made into pellets by pressing through a pelletizer.

Fish were sampled every 15th day to assess their growth. A minimum of 10 fish from each tank were collected and the individual length and weight measured. Five fish from each treatment were collected monthly and RNA and DNA contents of muscle, liver and brain (Curlewis & Stone, 1987) and proximate composition of muscle (AOAC, 1984) were estimated. Final length and weight of the fish were measured on termination of the experiment.

A short-term experiment for one month was conducted in triplicate in the laboratory to study the feed intake, conversion efficiency and apparent nutrient digestibility. The experiment was carried out in nine plastic troughs, each having 50 l capacity, employing five fish each (9.2±0.25 g). Feed

Table 2. Growth and survival of *Etroplus suratensis* fed varying levels of protein

	Protein level		
	30%	35%	40%
Initial lengtn (cm)	4.6±0.44	4.61±0.44	4.61±0.44
Initial weight (g)	2.49±0.39	2.49±0.39	2.49±0.44
Final length (cm)	8.93^{a}±0.31	9.32^{a}±0.38	8.46^{b}±0.35
Final weight (g)**	11.88^{b}±0.33	13.93^{c}±0.37	10.99^{a}±0.23
Net weight gain (g)	9.39	11.44	8.5
Gain in weight (%)	73.48	78.23	70.07
Specific growth rate (%)	1.3	1.43	1.23
Survival (%)	100	95	95

**$p<0.01$; a,b,c,d - Means with the same superscript do not differ from each other (Duncan's multiple range test)

was given at the rate of 10% of body weight once daily in the morning and unconsumed feed and faecal matter were collected, dried and weighed. Pooled faecal samples were analysed and nutrient digestibility, feed conversion efficiency and protein efficiency ratio were calculated.

Digestive enzyme activities of the three regions of the intestine (foregut, midgut and hindgut) were assessed on termination of the experiment. Five fish were collected and the protease, amylase and lipase activities were determined. Amylase activity was estimated by saccharogenic assay (Henery & Chiamori, 1960), protease activity by the casein digestion method (Kunitz, 1947) and lipase activity, by Bier's titrimetric method (Bier, 1962).

ANOVA was employed to analyse the statistical significance of growth data. Monthly data on the nucleic acid content of fish muscle, liver and brain and proximate composition of fish muscle were statistically analysed using ANOVA and Duncan's multiple range test (Steel & Torrie, 1980). Regression analysis was done to dtermine the relationship between growth rate and nucleic acid content (Ricker, 1973).

Results and Discussion

During the experiments the water temperature ranged between 26.8°C and 30°C, pH between 7.0 and 7.8, dissolved oxygen between 8.41 and 8.51 ppm and total alkalinity between 1.5 and 2.00 ppm. Total hardness was in the range of 4.3 - 45.7 mg $CaCO_3$/l and primary productivity varied between 8.12 - 9.56 mg C/m^3/day.

The average length and weight attained by the fish in the various treatments are presented in Table 2 *E. suratensis* showed significantly ($p<0.01$) superior growth both in length and weight when fed on 35% protein-diet (Fig.1 & Table 2). Average net weight gain and percentage gain in weight were highest in the group given 35% protein. The specific growth rate increased with increase in dietary protein

Table 3. Feed utilization by *Etroplus suratensis* fed varying levels of protein (short term experiment)

Parameters	Protein level		
	30%	35%	40%
Initial weight (g) (W1)	9.9±0.4	9.23±1.025	7.73±0.525
Final weight (g) (W2)	10.91±0.51	10.5±1.1	8.58±0.42
Production P=(W1-W2)	1.01±0.11	1.28±0.07	0.86±0.08
Feed consumed (C)	4.53±0.715	5.54±0.82	4.13±0.59
Faecal output (F)	1.24±0.09	1.21±0.15	1.15±0.03
Relative growth rate (P/W1)	0.101±0.007	0.14±0.007	0.11±0.02
Assimilation (A=C-F)	3.3±0.62	4.34±0.665	2.98±0.56
Metabolism (R=A-P)	2.29±0.51	3.21±0.74	2.13±0.45
Net conversion efficiency (%)	31.07±2.51	29.85±2.84	29.05±1.94
Assimilation efficiency (%)	72.48±2.25	78.19±0.43	71.68±3.32
Conversion efficiency (%)	22.29	22.92	20.70
Protein efficiency ratio (PER)	0.74	0.67	0.517
Protein digestibility (%)	86.76	89.62	84.13
Fat digestibility (%)	89.41	88.64	49.61
Carbohydrate digestibility (%)	58.32	62.13	54.26

Values are expressed as mean and SD of three replicates

levels upto 35% and then slightly decreased at higher protein levels. In the present study it was found that growth rate was strongly influenced by the dietary protein levels. In the early fry of pearlspot, good growth was reported with 60 to 80% protein in the diet (Sumitra *et al.*, 1978). Diets containing 35% protein was reported as the optimum for the growth of cichlids, *Oreochromis niloticus* (Santigao *et al.*, 1982; Jauncey, 1982).

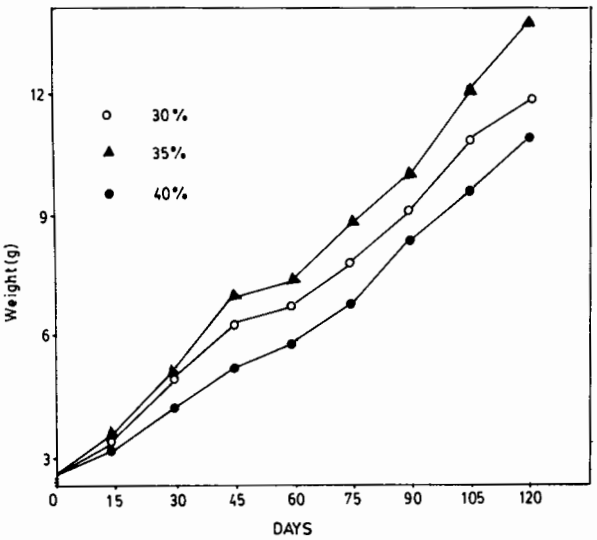

Fig. 1. Mean weight of *Etroplus suratensis* fed varying levels of protein.

Data on feed consumption, feed conversion efficiency and protein efficiency ratio of *E. suratensis* given varying levels of protein are shown in Table 3. Maximum feed intake, high conversion efficiency, assimilation efficiency and apparent protein and carbohydrate digestibility were found in the group which was given diets containing 35% protein, followed by the 30% and 40% groups. Protein efficiency ratio decreased when the protein content of the diet was more than 35%. In the present study the feed conversion efficiency increased as the protein content of the diet increased to 35% and then decreased in the 40% protein-diet. This indicates that at higher dietary protein levels the excess is catabolised to provide energy for growth with a consequent reduction in feed conversion efficiency.

Significantly high ($p<0.01$) values of viscerosomatic index, renosomatic index and cerebrosomatic index were observed in the group which was given the diet containing 35% protein. The high viscerosomatic index may be due to the increased food consumption. Hepatosomatic index was high for the group given 40% protein (Fig.2).

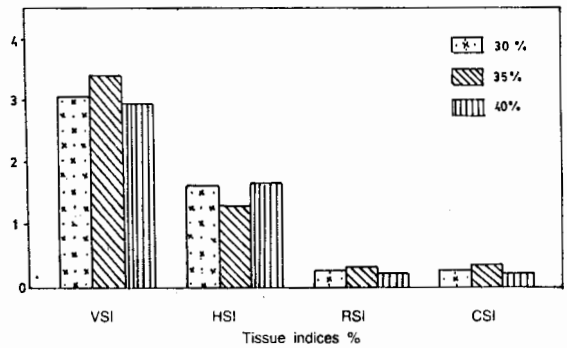

Fig. 2. Tissue indices of *Etroplus suratensis* fed varying levels of protein.

Higher enzyme activity in all the three regions of the intestine *viz.*, foregut, midgut and hindgut was observed in the group given 35% protein than in the 30% - and 40% - protein groups. Enzyme activity was highest in the midgut in all the three groups (Fig. 3). Enzyme activity in the gut is species

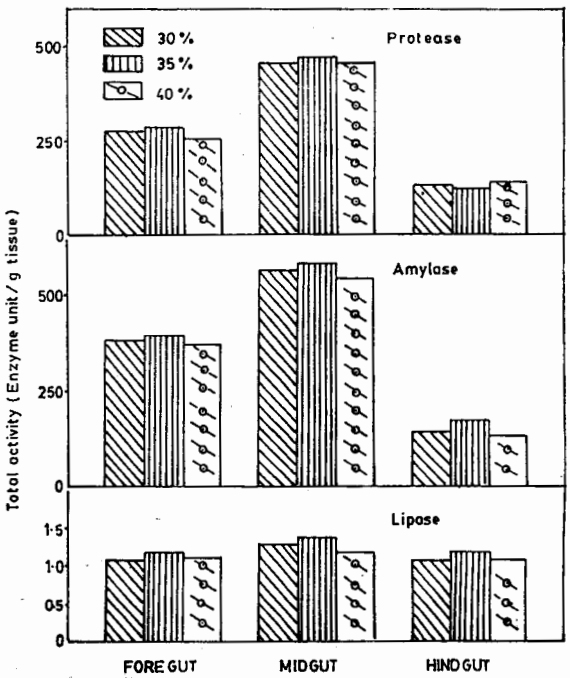

Fig. 3. Enzyme activity of *Etroplus suratensis* fed varying levels of protein.

Table 4. Nucleic acid content (mg/g tissue) of *Etroplus suratensis* fed varying levels of protein (Mean ± SD)

Culture Period (days)	Protein level	MUSCLE** DNA	MUSCLE** RNA	MUSCLE** RNA/DNA	LIVER** DNA	LIVER** RNA	LIVER** RNA/DNA	BRAIN** DNA	BRAIN** RNA	BRAIN** RNA/DNA
0	Initial	0.16 ±0.01	0.618 ±0.03	3.71	0.256 ±0.04	0.43 ±0.02	1.7	0.23 ±0.02	0.43 ±0.03	1.85
30	30%	0.15 ±0.01	0.89 ±0.021	5.91^{a}	0.24 ±0.01	0.53^{a} 0.01	2.19	0.24 ±0.02	0.58 0.02	2.42^{a}
	35%	0.16 ±0.02	0.97 ±0.02	6.12^{a}	0.27 ±0.01	0.6^{a} ±0.01	2.22	0.25 ±0.01	0.68 ±0.02	2.68^{a}
	40%	0.19	0.80	4.24^{ab}	0.28	0.51^{b}	1.82	0.28	0.61	2.17^{ab}
60	30%	0.16 ±0.01	1.10 ±0.10	6.63^{a}	0.25 ±0.01	0.67^{a} ±0.01	2.69	0.24 ±0.01	0.74 ±0.01	3.08^{a}
	35%	0.17 ±0.01	1.20 ±0.02	7.18^{a}	0.25 ±0.01	0.76^{b} ±0.02	2.97	0.25 ±0.01	0.77 ±0.02	3.01^{a}
	40%	0.19 ±0.02	0.10 ±0.02	5.61^{ab}	0.26 ±0.08	0.7^{a} ±0.02	2.68	0.21 ±0.01	0.59 ±0.02	3.81^{ab}
90	30%	0.16 ±0.01	1.30 ±0.10	7.96^{a}	0.25 ±0.01	0.75^{a} ±0.01	2.92	0.24 ±0.01	0.76 ±0.01	3.05^{a}
	35%	0.17 ±0.01	1.36 ±0.02	8.08^{a}	0.27 ±0.01	0.85^{a} ±0.02	3.16	0.22 ±0.02	0.87 ±0.01	3.83^{ab}
	40%	0.19 ±0.01	1.18 ±0.02	6.19^{b}	0.28 ±0.01	0.78^{b} ±0.02	2.77	0.24 ±0.01	0.82 ±0.01	3.46^{a}
120	30%	0.15 ±0.00	1.32 ±0.07	8.68^{a}	0.28 ±0.02	0.95^{a} ±0.07	3.41	0.23 ±0.02	0.97 ±0.09	4.07^{a}
	35%	0.15 ±0.02	1.50 ±0.07	9.83^{b}	0.30 ±0.02	1.05^{b} ±0.09	3.49	0.17 ±0.02	1.07 ±0.07	4.18^{a}
	40%	0.18 ±0.03	1.25 ±0.09	6.85^{b}	0.28 ±0.008	0.88^{a} ±0.02	3.1	0.27 ±0.03	0.73 ±0.01	3.75^{b}

** $p<0.01$; a,b,c - Mean followed by the same letters do not differ from each other (Duncan's multiple range test)

Table 5. Least square regression for RNA, DNA and RNA/DNA against % growth/day for *Etroplus suratensis* fed varying levels of protein

Parameters	Protein level %	Regression	r	Correlation(p)
Growth/day(%)	30	log DNA=-0.92 log gr/day -0.355	-0.491	$p<0.05$
DNA	35	log DNA=-0.708 log gr/day-0.405	-0.273	NS*
	40	log DNA=-01805log gr/day -0.653	-0.149	NS
growth/day(%)	30	log RNA=-1.4819log gr/day+0.776	0.413	NS
RNA	35	RNA=-0.615gr/day -0.913	0.552	$p<0.05$
	40	RNA=-0.4928gr/day -0.1365	0.767	$p<0.01$
growth/day(%)	30	logRNA=-0.559log gr/day +1.130	0.47	NS
RNA/DNA	35	RNA/DNA=5.714gr/day -12.302	0.6115	$p<0.01$
	40	logRNA/DNA=3.24log gr/day +0.1926	0.719	$p<0.01$

*NS - Not significant

Table 6. Proximate composition of carcass of *Etroplus suratensis* fed varying levels of protein

Culture Period (days)	Protein level %	Moisture**	Protein** (%) (a)	Lipid** (%) (a)	Ash (%) (a)	NFE (%) (a)
0	Initial	68.67	51.16	1.05	0.93	46.86
30	30	70.42	52.18	1.46	1.04	45.32
	35	72.02	55.17	1.33	1.12	42.38
	40	70.87	51.88	1.33	1.03	45.76
60	30	71.11	54.11	2.15	1.19	42.55
	35	73.21	57.18	2.04	1.31	39.47
	40	72.01	55.11	1.67	1.24	41.98
90	30	71.41	57.83	2.10	1.46	38.61
	35	74.11	60.11	2.69	1.61	35.89
	40	72.81	58.21	2.75	1.41	37.63
120	30	72.19	60.11	3.73	1.84	34.32
	35	74.81	64.13	3.39	2.04	30.84
	40	73.17	61.21	2.86	1.84	34.09

**$p<0.01$ (Highly significant); a = Dry weight basis

specific. Variation in enzyme activity may be related to protein structure and duration of feed retention in the digestive tract (Venkatesh *et al.*, 1986).

Nucleic acid content of muscle, liver and brain of the fish in the various treatments are shown in Table 4. RNA content was higher in all the three tissues of the fish fed 35% protein. RNA/DNA, ratio increased in all the three tissues with significantly higher values ($p<0.01$) in the case of muscle and liver of the group which received 35% protein. RNA and RNA/DNA ratio showed significant positive correlation with growth rate per day in the 35 and 40% protein groups (Table 5). RNA/DNA ratio serves as a sensitive index of growth rate of fish (Love, 1980). Similar results were found in rainbow trout (Matty & Cheema, 1978) and in *C. batrachus* (Khan & Jafri, 1991). Protein synthesis is accompanied by an increase in RNA and decrease in DNA concentration and hence the increase in RNA concentration observed in the present study is an indication of more efficient utilization of dietary protein upto 35% level. A fall in RNA in fish fed more than 35% dietary protein established that there were limits to the amount of protein that a fish could convert to its body materials (Love, 1980). Positive correlation between RNA/DNA ratio and growth rate had also been observed for adult golden shiners (Bulow, 1970), small mouth bass and carp (Haines, 1973).

The increased protein content in the muscle of the fish fed diet containing 35% protein indicated better protein synthesis and growth. The proportion of ash, fibre etc. were not affected by variations in protein levels in the diet. It can be recommended that a diet with 35% protein is optimum for maximum growth and high feed utilization in *E. suratensis*.

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