Fishery Technology
2000, Vol. 37(2) pp : 109 - 115

Impact of Some Hormone Combinations on Feed Utilization, RNA/DNA Ratio, Enzyme Activity, Growth and Survival of Pearlspot, *Etroplus suratensis* (Bloch)

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Juveniles of pearlspot were reared by feeding diets enriched with steroid and protein hormone combinations: Testosterone propionate (TP), Diethyl stilbestrol (DES) and Human chorionic gonadotropin (HCG) at various doses for a period of 120 days and their growth performance was evaluated. The dosages of the hormone combinations were TP 2.0 ppm+DES 1.0 ppm (T_1), TP 2.0 ppm+HCG 5.0 ppm (T_2), DES 1.0 ppm+HCG 5.0 ppm (T_3) and TP 2.0 ppm+DES 1.0 ppm+HCG 5.0 ppm (T_4). The fishes which received diets containing combinations of TP+HCG achieved the highest growth (40.10 g). Feed consumption, feed conversion efficiency and nutrient digestibility were higher in this group. Digestive enzyme activity was maximum (590.34 units/g tissue) in the intestine of fishes fed on diet containing TP+HCG. The RNA/DNA ratio and protein contents in the muscle increased concomitant with the high growth rate.

Key words: Growth promoters, hormone combinations, digestive enzyme, RNA/DNA ratio, *Etroplus suratensis*

Application of hormones, either alone or in combinations as feed additives was found to be an ideal method to improve fish growth and feed conversion efficiency in aquaculture (Sower *et al.*, 1983; Ostrowski & Garling, 1986; Lone 1989; Sindhu, 1993; Sambhu & Jayaprakas, 1997; Jayaprakas & Sambhu, 1998). Pickford *et al.* (1972) conducted the first of such studies on male hypophysectomised killifish held at 20°C and showed that mammalian thyrotropin in combination with pike pituitary growth hormone (Whilhelme F80 GH) induced a greater growth response than growth hormone alone. It was also observed that a combination of bovine growth hormone and ovine leutinizing hormone had a synergistic effect on growth. Similar findings have been noticed in mammalian studies. Scow (1959) administered T4 and growth hormone either singly or together to thyroidectomised-hypophysectomised rats and noted that in most tissues, a combination of both hormones produced superior growth than did either of the hormones. Enomoto (1964) tested the effect of several hormone combinations, namely growth hormone (GH) + thyrotropin (TSH), GH + prolactin, GH + TSG + prolactin and TSH + prolactin on growth of immature rainbow trout. Higgs *et al.* (1977) assessed the effect of hormone combinations on growth of Coho salmon. Combinations of protein and steroid hormones have been reported as promising growth promoters in cultivable fishes (Kumar, 1988; Shyama & Keshavanath, 1990; Sindhu, 1993; Sindhu & Jayaprakas, 1995). No study has hitherto been reported on the combined effect of hormones on the growth of pearlspot. Therefore, the present investigation was initiated to study the effect of protein and steroid hormones on growth, feed utilization and body composition of pearspot, *Etroplus suratensis*.

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Materials and Methods

The study was conducted in a brackishwater pond near Paravoor lake (Kerala) (84°6'N; 76°44'-76°48'E) for a period of 120 days. Juveniles of *E. suratensis* were collected from the lake using a cast net and stocked in nylon cage (1 m^2) 10 days prior to the experiment. Uniform size (3.51±0.2 g) juveniles (25 numbers) were randomly stocked in each cage in triplicate. A fish meal based supplementary diet having 40% protein (Hardy, 1980) (Table 1) was used. The dosages of hormones in the combination were determined on the basis of a series of preliminary screening experiments. The details of the hormone combinations and the dosages were: TP 2.0 ppm+DES 1.0 ppm (T_1), TP 2.0 ppm+HCG 5.0 ppm (T_2), DES 1.0 ppm+HCG 5.0 ppm (T_3) and TP 2.0 ppm+DES 1.0 ppm+HCG 5.0 ppm (T_4). The 40% protein diet without hormones served as the control (T_0). Feed was applied at the rate of 10% of the body weight daily in two rations, morning and afternoon. The quantity of the feed was readjusted fortnightly based on the growth of the fish. Parameters like temperature, dissolved O_2, pH and salinity were monitored biweekly adopting APHA (1992) procedures. The fish were sampled once in a fortnight and at least 15 fish were randomly collected for recording the total weight. On termination of the experiment, all the fish were collected and their individual weight was recorded. Ten specimens were dissected, weight of viscera, liver and gonad were recorded and Viscerosomatic index (VSI), Hepatosomatic index (HSI) and Gonadosomatic index (GSI) were calculated as follows.

Tissue index =
weight of tissue/weight of fish x 100

The influence of various hormone combinations on the activity of digestive enzymes viz. amylase, protease and lipase in the stomach and intestine were assayed following the method proposed by King (1965). Unit amylase activity was calculated as mg of maltose liberated during 10 minutes at 30°C. While protease activity was expressed as the amount of tyrosine liberated in 15 minutes, one unit lipase activity was expressed as the amount of 0.025N NaOH required to neutralize the fatty acids liberated during 18 hours of incubation at 30°C. Total and specific activities of the digestive enzymes were calculated as, Total Activity = Unit enzyme activity/g tissue; Specific activity = Unit enzyme activity/mg protein.

DNA and RNA contents of the muscle and liver of the experimental fishes were estimated by following the methods of Curlewis & Stone (1987). Another experiment was conducted in fiber glass tanks (50 l) using 10 fishes (3.5±0.3 g) each in triplicate for 30 days to assess the feed intake, conversion efficiency, protein efficiency ratio, apparent protein and lipid digestibility (De Silva, 1989) of the hormone treated and control fishes. The fish were fed with the diet at 10% of the body weight daily for 30 days. The unconsumed feed and faecal matter were removed and separated every day and the pooled faecal samples were analysed for nutrients (protein and lipid). Feed conversion efficiency (FCE) and protein efficiency ratio (PER) were calculated. Apparent nutrient (protein and lipid) digestibility was also calculated.

Table 1. Diet formulation and proximate composition of the diet

Ingredients	Percentage composition	Protein contribution (% of feed)
Rice bran	13.24	1.15
Groundnut oil cake	36.76	18.49
Fish meal	36.76	20.07
Tapioca flour	3.24	0.29
Total	100.00	40.00
Proximate composition (%)		
Moisture	12.31	
Protein	40.02	
Lipid	9.14	
Carbohydrate	13.27	
Ash	5.11	
NFE*	20.15	
Total	100.00	

* Nitrogen free extract

Proximate analyses were done for feed ingredients, feed and fish muscle. The dried and powdered samples were analysed to estimate protein, lipid, glycogen, fiber and ash by standard methods (AOAC, 1990.). Analysis of variance (ANOVA) and covariance (ANCOA) were employed to find out the statistical significance in the final mean length and weight, feed utilization and digestive enzyme levels (Snedecor & Cochran, 1968). Duncan's multiple range test (Steel & Torrie, 1980) was also employed to compare the statistical difference between treatment means.

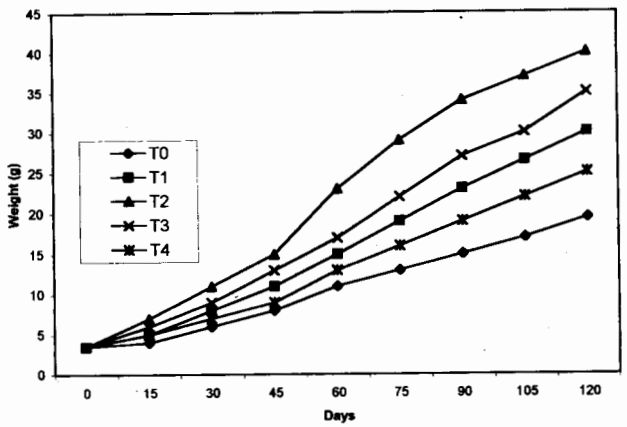

Fig. 1. Growth of *E. suratensis* treated with different hormone combinations.

Results and Discussion

The fortnightly average growth in weight attained by *E. suratensis* during the rearing period is depicted in Fig.1. Analysis of variance along with the final average weight of fishes showed that there was significant ($p<0.01$) difference in the final average weight of fishes between control and treatments (Table 2). The fishes that received the diets containing combinations of TP+HCG achieved the highest growth in weight (40.10 g). The average specific growth rate was the highest (3.64%) in T_2 and the percentage survival was also high (96.00%) in T_1 and T_2. Growth enhancement has also been reported in rohu when fed a diet containing 1.5 ppm TP+8 ppm HCG. Treatment T_3 containing DES and HCG with a dosage combination of 1 ppm and 5 ppm respectively ranked second in promoting growth. Sindhu & Jayaprakas (1995) reported that 2.5 ppm DES + 12.5 ppm HCG promoted better growth in tilapia, *O. mossambicus*. The treatment T_1 (TP, 2 ppm+DES, 1 ppm) brought higher growth than the control. A combination of androgen and estrogen has been found to be ideal for promoting maximum growth in fishes. A combination of 2 ppm 17α-methyl testosterone+2 ppm DES brought about higher growth in silver carp (Shyama & Keshavanath, 1990), while Sampath Kumar (1988) reported that 2 ppm 17α-methyl testosterone+2.5 ppm estradiol benzoate induced maximum growth in pearlspot *E. suratensis*. Ostrowski & Garling (1986) reported that 17α-methyl testosterone, alone or in combination with 5α-dihydrotestosterone (DHT), enhanced growth in rainbow trout, *S. gairdneri*. Degani (1986) reported that a combination of 0.5 ppm MT + 0.25 ppm 17β-estradiol resulted in significant increase in growth of European eel, *Anguilla anguilla*. Hence, it can be suggested that the growth promoting effect of hormones in the combination treatments are dose dependent. The better growth observed in these treatments can be attributed to the synergistic effect of hormones as reported by Shyama & Keshavanath (1990).

Table 2. Growth and survival (mean±SD) of *E. suratensis* treated with different hormone combinations

Parameters	Treatments					F value
	T_0	T_1	T_2	T_3	T_4	
Initial weight (g)	3.50±0.18	3.51±0.17	3.52±0.14	3.51±0.8	3.50±0.17	
Final weight (g)	19.36±1.89^a	29.70±2.70^c	40.10±3.11^c	34.20±2.19^d	24.80±2.91^b	37.14**
S.G.R. (%)	3.03±0.82	3.28±0.73	3.63±0.68	3.50±0.73	0.32±0.81	
Survival (%)	92.00±4.00	96.00±4.00	96.00±4.00	92.00±4.00	92.00±2.00	

** $p<0.01$ (n=20); a,b,c,d,e - means with the same superscript do not differ from each other (Duncan's multiple range test)

Table 3. Feed and nutrient utilization (mean±SD) of *E. suratensis* treated with different hormone combinations

Parameters	Treatment					F value
	T_0	T_1	T_2	T_3	T_4	
Feed consumed (g)	21.78±2.18^a	22.36±2.73^a	27.39±2.45^c	25.07±1.89^b	22.19±1.86^a	4.81**
Assimilation (g)	17.34±2.11	17.75±0.86	22.23±1.75	20.44±2.17	17.38±0.19	
FCE (%)	18.91±2.07^a	19.58±3.86^a	22.52±2.74^b	19.98±1.99^{ab}	18.92±1.74^a	11.34**
PER (%)	10.30±0.93	10.95±0.91	15.42±0.67	12.52±0.77	10.50±0.57	
APD (%)	58.33±2.73^a	63.74±3.81^b	69.33±2.98^c	66.39±3.41^b	59.31±3.07^{ab}	7.81**
ALD (%)	76.39±3.86	79.32±2.19	82.24±3.74	80.79±2.80	88.40±2.81	

** p<0.01; a,b,c - means with the same superscript did not differ from each other (Duncan's multile range test); FCE - Feed conversion efficiency; PER - Protein efficiency ratio; APD - Apparent protein digestibility; ALD - Apparent lipid digestibility.

The amount of feed consumed, feed conversion efficiency, protein efficiency ratio and apparent protein digestibility were maximum (27.39, 22.52, 15.42, 69.33) in T_2, followed by T_3 (25.07, 19.98, 12.52, 66.39) (Table 3). The present investigation showed that feed utilization of fishes improved due to the administration of hormone combinations. Sindhu (1993) also noticed an increase in feed intake and feed conversion efficiency in tilapia and rohu following supplementation of hormone combinations in the diet. Sower *et al.* (1983) reported that a combination of 5 ppm DES with 15 ppm clomiphene citrate promoted superior growth through increased protein digestibility in steel head trout. Similar observation were reported by Shyama & Keshavanath (1990) in mahseer when fed a diet containing 17α-methyl testosterone + HCG.

The changes in the various tissue indices such as VSI, HSI and GSI are shown in Fig.2. These parameters were higher in hormone treated fish over control. Studies by Saliny (1993) indicated that the hormone combinations bring about changes in tissue indices of catla and rohu and suggested that combination of TP + HCG reduced the VSI,

Table 4. Digestive enzyme activity (mean±SD) of *E. suratensis* treated with different hormone combinations

Tissue	Enzyme activity	Treatment					F value
		T_0	T_1	T_2	T_3	T_4	
Amylase							
Stomach	TA	347.31±3.47^a	389.29±2.99^c	436.71±3.33^c	400.31±4.00^d	361.34±2.97^b	21.76**
	SA	5.15±0.93	5.55±0.86	5.94±1.41	5.68±0.73	5.22±1.07	1.78^{NS}
Intestine	TA	404.29±4.31^a	464.29±3.86^c	518.36±2.93^e	491.37±3.87^d	421.36±3.97^b	13.76**
	SA	6.71±1.21	6.33±1.23	6.45±0.81	6.43±0.96	6.31±0.97	1.07^{NS}
Protease							
Stomach	TA	363.13±3.18^a	450.11±3.37^c	531.27±3.42^e	491.19±2.93^d	401.37±3.71^b	23.86**
	SA	5.40±0.07	6.41±0.01	7.23±0.07	6.97±0.71	5.80±0.13	2.78*
Intestine	TA	401.29±2.43^a	491.39±3.86^c	590.34±2.71^e	527.33±3.44^d	440.23±2.88^b	18.36**
	SA	6.66±1.86	6.70±0.93	7.35±0.74	6.90±0.81	6.59±0.74	2.42*
Lipase							
Stomach	TA	0.70±0.03^a	0.86±0.02^a	1.93±0.01^b	0.98±0.07^a	0.79±0.01^a	2.81**
	SA	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	1.23*
Intestine	TA	1.34±0.02^a	2.93±0.01^b	4.37±0.30^c	3.81±0.07^c	1.99±0.06^{ab}	3.41**
	SA	0.02±0.00	0.03±0.01	0.05±0.01	0.04±0.00	0.02±0.00	1.43*

** p<0.01, * p<0.05, NS p>0.01, a,b,c,d,e - means with the same superscript do not differ from each other (Duncan's multiple range test)
TA - Total activity; SA - Specific activity

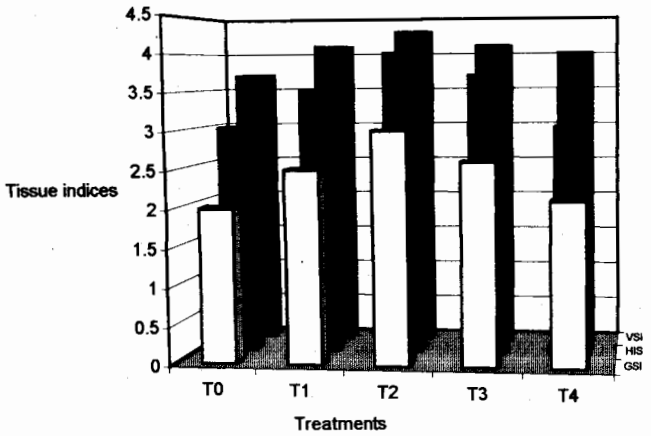

Fig. 2. Tissue indices of *E. suratensis* treated with different hormone combinations.

HSI and GSI in *L. rohita*. Sindhu (1993) noticed an increase in HSI in mrigal and common carp fed with a combination of TP + HCG. Sindhu & Jayaprakas (1995) reported higher GSI following HCG and DES administration in tilapia. The increased VSI observed in the present study could be due to the anabolic property of hormones, which enhance feed consumption, while the increased GSI can be correlated to the combined effect of estrogen and androgen on the development of gonad.

The highest amylase activity (518.36) (Table 4) was observed in the intestine of T_2 fishes, which showed maximum growth during the experiment, while lowest amylase activity was noticed in control. The activity increased in fishes treated with hormone compared to that of the control. Protease activity was maximum (590.34) in the intestine followed by the stomach (531.27) of T_2 fishes. These fishes also showed high protein digestibility and protein efficiency ratio. The activity decreased in T_4 fishes, which received combinations of 3 hormones. The highest lipase activity (4.37) was noticed in the intestine of T_2 fishes in which growth and lipid digestibility were also maximum. Lipase activity showed an increase in hormone treated fishes from that of the control. The increased digestive enzyme activity found in the alimentary canal of fishes under hormone treatment in the present study is similar to the observations in mrigal and common carp (Sindhu, 1993). The results show that combinations of exogenous hormones may have induced the endogenous enzyme activity, leading to increased nutrient digestion in pearlspot as reported by Yamazaki (1976) and Lone & Matty (1981) in carp. The increased protease and lipase activity coupled with enhanced protein and lipid digestion observed in the fast growing fishes under T_2 treatment support this assumption.

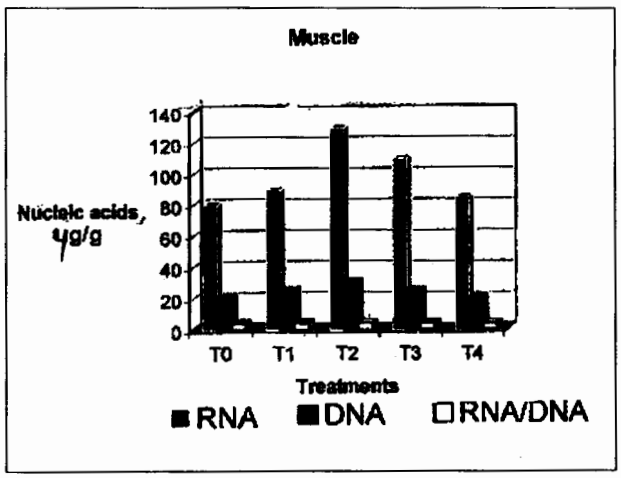

Fig. 3. Nucleic acid content in the muscle of *E. suratensis* treated with different hormone combinations.

Table 5. Carcass composition (% mean ± SD) of *E. suratensis* treated with different hormone combinations

Parameters	Treatment					F value
	T_0	T_1	T_2	T_3	T_4	
Moisture	64.21±2.81	61.23±3.45	56.33±2.86	59.61±2.41	63.27±2.23	4.11**
Dry matter	33.79±1.32^a	38.77±1.91^b	43.67±1.39^d	40.39±1.86^c	36.73±2.19^b	7.81**
Protein	63.27±3.91^a	68.39±2.86^b	72.13±2.72^c	70.20±2.17^{cb}	67.33±1.89^b	9.71**
Lipid	3.82±0.87	3.36±0.93	3.96±0.71	3.44±0.68	3.79±0.74	1.72^{NS}
Ash	3.61±0.23	3.98±0.13	4.73±0.18	4.31±1.34	4.51±1.50	2.71*
NFE	29.30±1.21	24.27±1.35	19.18±1.33	22.04±1.34	24.37±1.56	3.12*

** $p<0.01$, * $p<0.05$, NS $p>0.01$, a,b,c,d - means with the same superscript do not differ from each other (Duncan's multiple range test)

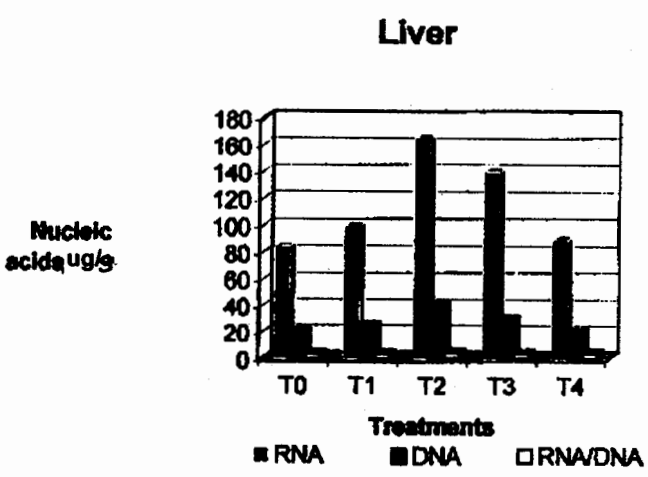

Fig. 4. Nucleic acid content in the liver of *E. suratensis* treated with different hormone combinations.

The values of the nucleic acid content in the muscle and liver of fishes under various treatments are presented in Figs. 3 and 4. High RNA and DNA contents and RNA/DNA ratio were observed in the muscle of fast growing T_2 fishes and low values were recorded in slow growing control fishes. The nucleic acid values were comparatively low in T_4 fishes, which received a combination of three hormones, viz. TP+DES+HCG. Reports regarding the effect of hormone combinations on nucleic acid contents in fishes are scanty. However, Lone (1989) and Sindhu (1993) suggested that hormone combinations might induce nucleic acid synthesis leading to increase in protein content in common carp and mrigal. In the present study also, a positive effect on nucleic acid contents was noticed.

In the entire hormone treated fishes, total dry matter content was found to be higher than that of the control (Table 5). Crude protein, glycogen and ash contents were higher in the hormone treated fishes when compared with the control. The difference in lipid content was not significant. High protein and lipid contents found in the muscle of pearlspot agree with the findings of Sampath Kumar (1988), following the administration of 17α-methyl testosterone + 17β-estradiol. Sindhu (1993) observed higher levels of protein and ash in the muscle of common carp and mrigal treated with HCG+DES. Increased protein content was also observed in catla and rohu after HCG + TP administration (Sindhu, 1993).

The authors wish to record their sincere thanks to Prof. Dr. P. Natarajan, Head, Department of Aquatic Biology and Fisheries. One of the authors (C. Sambhu) is greatly thankful to CSIR., Govt. of India for financial assistance in the form of Research Associateship.

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