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EFFECT OF COMBINED THYROXINE AND CORTISOL TREATMENT ON HATCHING OF EGGS, POST-EMBRYONIC GROWTH AND SURVIVAL OF LARVAE OF *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

In order to record the effects of thyroxine and cortisol (individual/ combined) on hatching, post-embryonic growth and survival of larvae of *Heteropneustes fossilis*, newly fertilized eggs were given bath immersion treatments of L-thyroxine (T_4; 0.05 mg/l), cortisol (0.50 mg/l) and T_4+ cortisol (0.05 mg/l+0.50 mg/l) for 15 days. Hatching of eggs, growth and survival of the larvae improved significantly ($P < 0.001$) in the hormone treated groups as compared to those of control. The frequency of deformities was reduced in the combined hormone treatment group. The present observations suggest that the advanced digestive function probably induced by T_4+cortisol treatment might have resulted in improvement in food utilization during the critical phases of first feeding and promoted vital developmental processes resulting in uniform growth, decreased mortality, better survival and transformation of larvae to juveniles. This combined hormone therapy appears to have practical utility in fish hatchery practice for better success in larval rearing.

Keywords: Thyroxine (T_4), cortisol, larvae, survival, growth, *Heteropneustes fossilis*

INTRODUCTION

Heteropneustes fossilis is a commercially important air-breathing catfish suitable for culture in waters quite low or deficient in dissolved oxygen such as swamps, wetlands and oxidation ponds due to accessory respiratory organs (Dehadrai and Kamal, 1993). This species commands good consumer preference because of nutritional as well as medicinal values. Though the fish has been bred successfully through pituitary (Khan and Mukhopadhyay, 1975), ovaprim (Singh *et al.*, 2002) and ovatide administration (Pandey and Koteeswaran, 2004), mass-scale seed production has not yet been achieved due to high mortality rate during early developmental period (Nayak *et al.*, 2000). Recent studies have demonstrated that growth harmone, thyroxine and cortisol (either alone or in combination) enhance growth and survival during early stages of development in fishes. Thyroid hormones (thyroxine T_4 and

triiodothyronine T_3) play important role in regulation of metabolic rate of the whole body as well as reproduction (Matty, 1985; Evans, 1998). Growth promoting effect of thyroid hormones has attracted wide attention because of its potential applications in aquaculture (Higgs *et al.,* 1982; Lam, 1994; Sawant and Belsare, 1994; Pandey *et al.,* 2002). Thyroid hormones dissolved in water or supplemented in diets enhanced larval development, growth and survival in *Sarotherodon (Tilapia) mossambicus* (Lam, 1980), *Tilapia niloticus* (Nabario, 1983), *Siganus guttatus* (Ayson and Lam, 1993), *Cyprinus carpio* (Lam and Sharma, 1985), *Chanos chanos* (Lam *et al.,* 1985), *Catla catla* (Pandey *et al.,* 2002), *Labeo rohita* (Pandey *et al.,* 2004a) and *Cirrhinus mrigala* (Pandey *et al.,* 2004b). Cortisol, a hormone responsible for hydromineral regulation of fish, has been demonstrated to enhance survival of *Lates calcarifer* larvae (Sampath Kumar *et al.,* 1993) as well as stimulated activity of mitochondria-rich cells in the yolk-sac membrane of embryos and larvae in tilapia (Ayson *et al.,* 1995). There are indications that thyroid hormones affect growth and survival of the larvae of fishes through their interactions with steroid and growth hormones (Leatherland, 1982). There exist reports that cortisol acts synergistically with thyroid hormones in survival of threadfin larvae (Brown and Kim, 1995; Kim and Brown, 1997) and synchronized hatching in steelhead trout (Yeoh, 1993). Bath immersion of the fertilized eggs in hormone solutions is an effective and practical means for studying their effects on fish embryos and larvae (Nugegod and Lam, 1994). An attempt has been made to record the effect of thyroxine and cortisol (alone or in combination) on hatching of eggs, post-embryonic growth and survival of larvae of *H. fossilis.*

MATERIAL AND METHODS

Broodstock of *Heteropneustes fossilis* (Bloch) were procured from the local market and maintained in ferro-cement tank (0.9 m diameter, 0.7 m height) containing constantly running pond water at 25-30°C. They were acclimatized to the constant overhead light fixtures of 14 L:10 D photoperiod and fed daily with minced freshwater molluscs ad libitum. The catfish (Female and male 1:1 ratio, body weight 100 gm and 60 gm, respectively) were induced to ovulate with single intraperitoneal injection of ovaprim (0.8 ml/kg). Eggs were manually stripped after 10-12 hrs of the injection and fertilized with milt from sacrificed males. Newly fertilized eggs (5 a.m.) from a single brood were washed thoroughly with the water and incubated in four different groups: Group I - eggs in this group were maintained in pond water and served as control (C), Group 2 - eggs were given immersion treatment in pond water containing T_4 at concentration of 0.05 mg/l (Eltroxin tablets, Glaxo, India) (T_4), Group 3- eggs were given immersion treatment in pond water containing cortisol at the concentration of 0.50 mg/l (Hydrocortisone, Sigma Chemicals Company, St. Louis, USA) (Cor), and Group 4 - eggs were given immersion treatment in pond water containing T_4+ cortisol at concentration of 0.05 mg/l+0.50

mg/l (T_4+Cor). All the plastic troughs were of same colour, shape and size (48 cm diameter and 16.5 cm height) each containing 1 litre pond water which was supplied from above (drop-by-drop) and drained with an out-fitted overflow tube after 24 hrs of immersion.

In these experiments, there were differences in time of hatching of eggs in treated and control groups. Synchronized hatching occurred in both T_4 as well as T_4+cortisol treated groups at 7.30 p.m. whereas in control group, it was initiated at around 11 p.m. of the same day but completed only by the next day morning. Newly hatched larvae were provided with gentle aeration. The larvae and eggs were kept at the temperature ranging from 25°C-28°C and pH between 7.0-7.8 throughout the experiment period of 15 days. Number of the larval density was maintained at 25 larvae/litre. One replicate as well as one reserve troughs were maintained to replace the unhatched eggs with the newly hatched ones to keep the equal number of larvae at beginning of the experiment. Finely sieved zooplankton (20-25 number/ml) were given as feed from day 3 onwards. Dead larvae were siphoned off from the bottom to avoid the microbial infection and daily mortality was recorded. Results were evaluated for statistical significance using students 't' test as well as one way ANOVA followed by multiple comparisions of means test. At the termination of the experiments larve from each treatment group were sampled, blotted dry and total length (from tip of the snout to tip of the caudal fin) as well as wet body weight were recorded.

RESULTS AND DISCUSSION

Effect of hormonal treatments on *H. fossilis* was observed on day 1 of the treatment (Table 1). In the control trough, all larvae except 5 in trough 2 were lying on their side at the bottom with only brief occasional movement. In the T4, cortisol and T4+ cortisol treated troughs, correspondingly 54%, 72% and 80% larvae were swimming upright but returned to the bottom to rest periodically. They rested upright on yolk sac instead of their side. The larvae looked slightly bigger with smaller yolk sac both in cortisol and T_4+ cortisol treated groups than those of T_4 and control.

Table 1: Number of larvae of *Heteropneustes fossilis swimming upright in different* treatment groups on day 1 of the experiment.

Trough	Number of larvae swimming upright/total number			
	Control	T_4	Cortisol	T_4 + Cortisol
1	0/25	10/25	16/25	17/25
2	5/25	17/25	20/25	23/25
Total	5/50 (10%)	27/50 (54%)	36/50 (72%)	40/50 (80%)

On day 3 of the treatment, difference among the control, T_4, cortisol and T_4+ cortisol treated larvae were more pronounced (Table 2). 76% larvae in T_4, 82% in cortisol and 90% in T_4+ cortisol treated groups showed little or no yolk sac and were swimming actively whereas the yolk sac was clearly visible in 90% larvae of the control group. Also, the T_4, cortisol and T_4+ cortisol treated larvae looked bigger with an enlarged head than control. The treatments were found to reduce the period of yolk absorption as well as ontogenetic development. Prior to mouth opening, advanced eye development was observed among larvae from T_4, cortisol and T_4+ cortisol groups while the larvae from the control group retained yolk beyond the onset of feeding. In the next few days, T_4 and T_4+ cortisol treated larvae exhibited juvenile features while the control and cortisol-treated larvae were still in the post-larval stage. The hatchability of the viable eggs were better in the treatment groups and followed the order T_4+ cortisol > cortisol > T_4 > control (Table 3).

By day 15 of treatment, differences among the three groups of larvae were more marked (Table 4). The mean final

Table 2: Number of larvae actively swimming with little or no yolk in different treatment groups of *Heteropneustes fossilis* on day 3 of the experiment.

Trough	Number of larvae with little or no yolk/total number			
	Control	T_4	Cortisol	T_4 + Cortisol
1	3/25	18/25	20/25	23/25
2	2/25	20/25	21/25	22/25
Total	5/50 (10%)	38/50 (76%)	41/50 (82%)	45/50 (90%)

Table 3 : Effect of T_4, cortisol, and T_4+ cortisol on hatching rate of *Heteropneustes fossilis*.

Trough	Number of larvae with little or no yolk/total number of eggs* (%)			
	Control	T_4	Cortisol	T_4 + Cortisol
1	28/50	34/50	38/50	42/50
2	24/50	37/50	37/50	38/50
Total	52/100 (52%)	71/100 (71%)	75/100 (75%)	80/100 (80%)

* Excluding non-viable eggs.

length (Fig. 1) and wet weight (Fig. 2) of larvae of *H. fossilis* were significantly ($P < 0.001$) greater in T_4+ cortisol treated group than cortisol, T_4 or control. The treated larvae from both T_4+ cortisol and T_4 groups were not only larger and gained more weight but also looked better developed in the form (juvenile stage) specifically in the former group as compared to the control as well as cortisol treated larvae (in terms of body depth and width). Since there was no obvious difference among the larvae in the replicate troughs either for the control or the T_4, cortisol and T_4+ cortisol treated groups by day 15, data of the corresponding troughs were combined together for statistical analysis (Table 4).

Besides these, treatments with T_4, cortisol and T_4+ cortisol were found to reduce the hatching period as compared to the control. After one week of treatment, the dorsal and anal fins were clearly differentiated and appeared longer in T_4 and T_4+ cortisol treated larvae as compared to those immersed in cortisol and control. Further, a treatment-dependent deformity

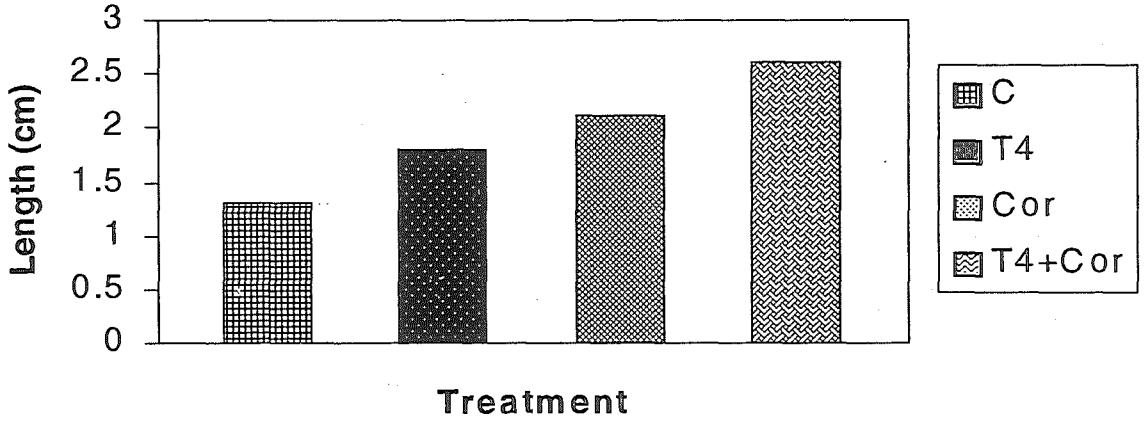

Fig. 1 : Effect of T_4, cortisol and T_4+cortisol on total length of Heteropneustes fossilis larvae after 15 days of treatment.

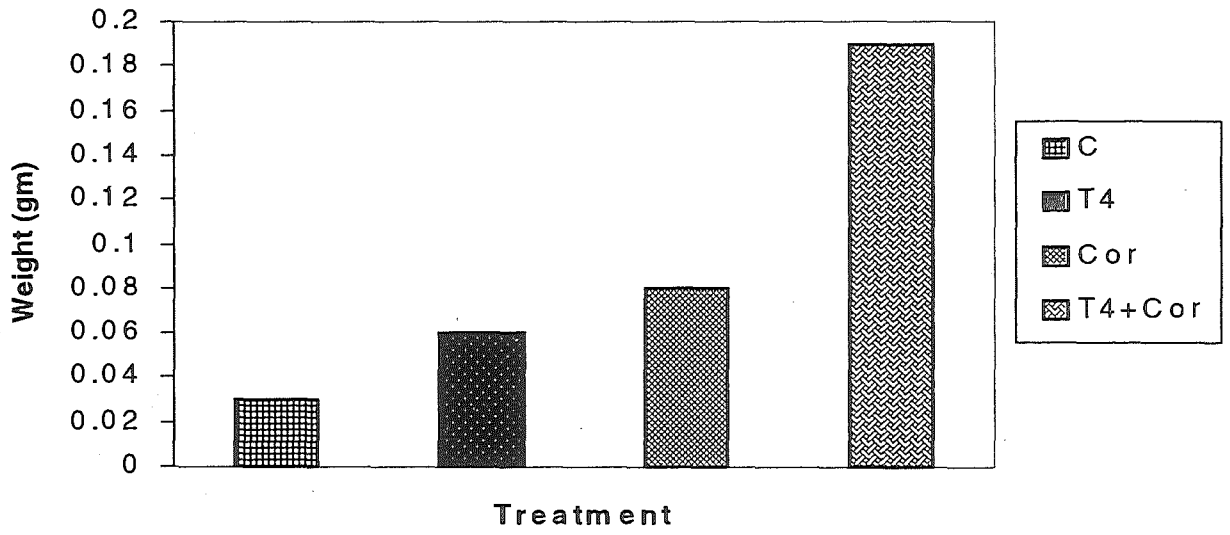

Fig. 2 :Effect of T_4, cortisol and T_4+cortisol on total wet weight of Heteropneustes fossilis *larvae after 15 days of treatment.*

was also observed as bent vertebral column and curved tail were consistently observed in the control group and fewer in cortisol as well as T_4 treated groups but none in the T_4+ cortisol group. The final length and wet weight were significantly greater ($P<0.001$) in T_4+cortisol treated larvae than cortisol and T_4 treated ones as well as control cohort.

Significant difference was noticed in the larval survival rate of *H.fossilis* among the various treatment groups (Table 4). Highest survival rate (90%) occurred in T_4+cortisol group followed by T_4 (74%), cortisol (64%) and control group (44%) (Fig.3). While an episodic increase in mortality was observed in control group during the first week of the experiment, larvae of the T_4+cortisol group exhibited less mortality during this period in comparison to those subjected to T_4 and cortisol treatments (Fig.4). The synergistic effect of cortisol with T_4 on the larvae subsequently enhanced absolute survival percentage at end of the experiment.

Despite the equality in size at the time of hatching, larvae of *H. fossilis* grew at different rates resulting in marked size variations in the treated groups. In T4+cortisol treated group, the larvae were

Table 4: The mean total length, wet body weight and cumulative mortality of the larvae of *Heteropneustes fossilis* in the different treatment groups on day 15.

Treatment	Trough	No of larvae survived in both troughs	Mean ± Standard Error (SE)				Cumulative Mortality (Number)
			Length (cm)		Wet weight (gm)		
			Initial	Final	Initital	Final	
Control	1	11	0.273	1.169 ± 0.077	0.00043	0.029 ± 0.006	14
	2	11	0.271	1.167 ± 0.076	0.00078	0.033 ± 0.001	14
	1+2	22/50	0.272	1.168 ± 0.053	0.00036	0.031 ± 0.008	28/50 (56%)
T_4	1	19	0.280	1.771 ± 0.051	0.00084	0.060 ± 0.0008	6
	2	18	0.282	1.771 ± 0.050	0.00044	0.056 ± 0.0007	7
	1+2	37/50	0.281	1.771±0.035**	0.00040	0.058±0.0006*	13/50 (26%)
Cortisol	1	17	0.299	2.143 ± 0.005	0.00084	0.066 ± 0.001	8
	2	15	0.301	2.135 ± 0.007	0.00041	0.061 ± 0.001	10
	1+2	32/50	0.300	2.139 ± 0.004**	0.00042	0.063±0.001**	18/50 (36%)
T_4+ Cortisol	1	23	0.296	2.606 ± 0.071	0.00104	0.189 ± 0.0006	2
	2	22	0.294	2.602 ± 0.071	0.00052	0.187 ± 0.0006	3
	1+2	45/50	0.295	2.604 ± 0.050**	0.00052	0.188 ± 0.0004**	5/50 (10%)

Significant response : * $P < 0.01$, ** $P < 0.001$

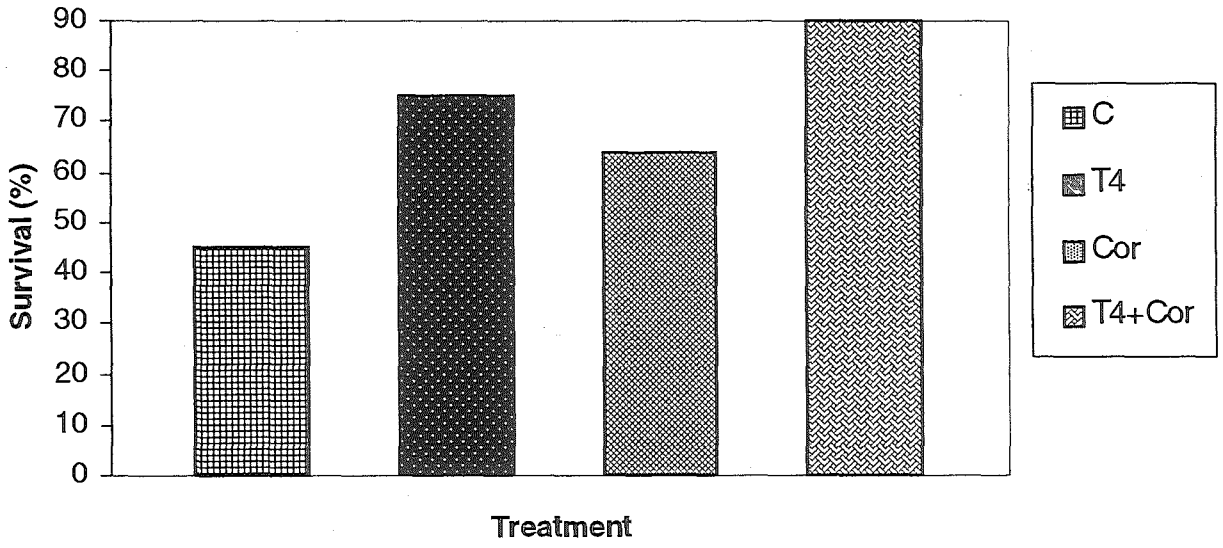

Fig. 3 : Effect of T_4, cortisol and T_4+cortisol on survival of Heteropneustes fossilis larvae after 15 days of treatment.

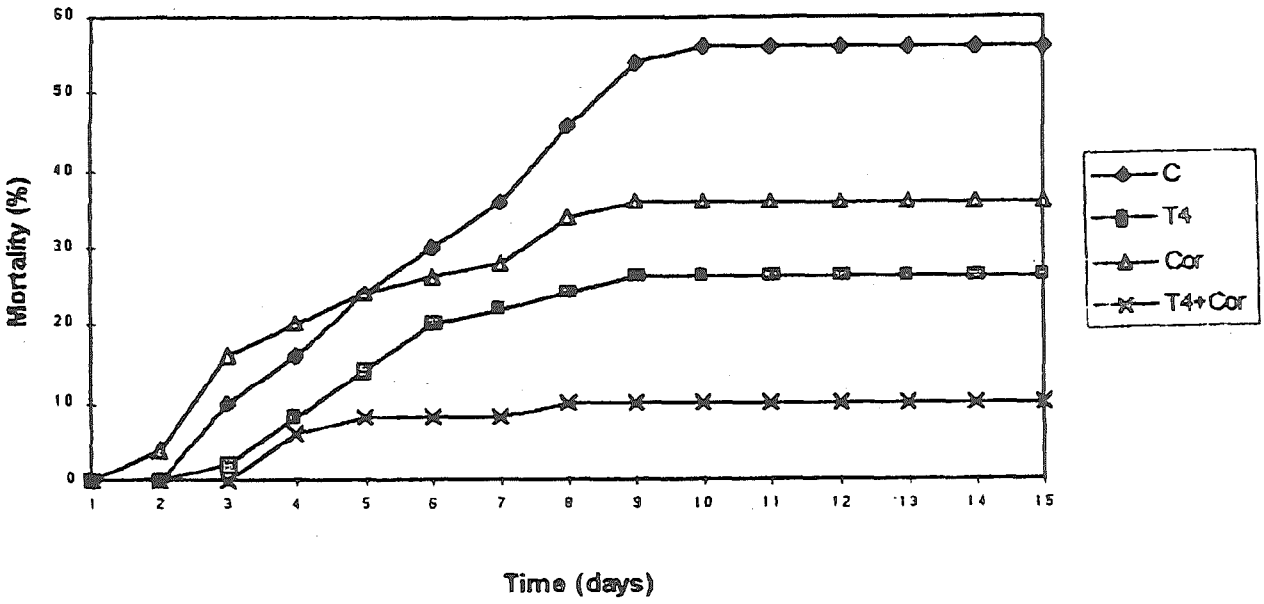

Fig. 4 : Effect of T_4, cortisol and T_4+cortisol on mortality of Heteropneustes fossilis larvae after 15 days of treatment.

more uniform with regard to their total length. Some of the larvae showed morphological deformities in other treatment groups resulting in reduced body length.

DISCUSSION

The present experiments showed that bath treatment of T_4 alone or in combination with cortisol was effective in improving hatching and yolk absorption

in eggs as well as enhancing growth and survival of larvae of *H. fossilis*. The concentration of T_4 and cortisol used in the present study was found to stimulate growth in the larvae. Thus it appears that T_4 not only promotes growth but also accelerates development of fish larvae and fry. Thyroxine accelerates hatching in chum salmon (Dales and Hoar, 1954) by stimulating embryonic development and/or the hatching mechanism. Thyroid hormone generally enhances growth through calorigenic action by modulating lipid, protein and carbohydrate metabolism (Lee and Laycock, 1978). A marked synergism between T_4 and cortisol was seen in the subsequent survival rate of post-hatching larvae than those eggs treated with T_4 and cortisol alone. The episodic mortality around 3rd and 10th day was subsequently reduced by combined T_4+cortisol application than single treatment of T_4 or cortisol. Probably these were the critical periods of the larvae in which one can illustrate a point about the consistency of the actions of a combination of T_4+cortisol which had a substantial effect on survival.

Our observations in the catfish indicate that the effects of individual hormone was less consistent than those of combined application suggesting that T_4+cortisol interact to promote one or more vital development processes resulting in survival advantage even under compromised conditions than the effect of the individual hormone. The results also showed that continuous treatment with T_4 and T_4+cortisol for 15 days was effective in *H. fossilis* larvae with acceleration of the transformation to juvenile stage and improved larval survival. Though immersion treatment on a large-scale basis would be impractical, administration of the hormone to brood fishes prior to induced breeding may solve the practical problem. The enhanced viability and hatchability of the fertilized eggs in T_4 and T_4+cortisol treated groups probably reflect increased resistance of the treated embryos against microbial infection.

A preadaptive role of thyroid hormones in preparation of larval intestinal tissues for exploitation of new food sources has been suggested (Specker, 1988). Development of the digestive tract was advanced to some extent by cortisol treatment and was slightly more so by the combination of T_4+cortisol, thus probably reducing the lapse between exhaustion of endogenous energy (yolk) and utilization of exogenous energy through the first feeding. Multiple hormonal regulators are known to operate in the control of gastrointestinal tract changes during amphibian metamorphosis (Norris and Dent, 1989). Dimethylaminostyryl-methylpyridinium iodide (DASPEI) fluorescence has been reported in the developing gut of some species suggesting the presence of functional mitochondria for nutrient and/or ion transport (Bereiter-Hahn, 1976). It appears that T_4+cortisol treatment might have accelerated intestinal differentiation of *H. fossilis* larvae that facilitated nutrient uptake resulting in enhanced survival (Cornell *et al.*, 1994). Though we lack evidence which would enable us to discriminate between possible direct or indirect actions of these hormones on gut development, there exist reports that these hormones have direct

peripheral interactions on developing target tissues of Japanese flounder, *Paralychthys olivaceus* (de Jesus *et al.,* 1990). Though the catfish larvae of T_4 treated group exhibited some deformities similar to those reported in other fish (Higges *et al.,* 1982), the application of T_4+cortisol prevented such deformities. While we lack sufficient data to explain the mechanism(s) of interaction of both the hormones during larval differentiation and development, it appears that the promotion of absorptive function by cortisol might have aided in transduodenal movement of nutrients and ions needed for skeletal as well as other tissues differentiation.

Cortisol has been found to stimulate growth in post-hatching larvae of *Oreochromis mossambicus* (Mathiyalagan *et. al.,* 1996) and greasy grouper during embryonic development (Tay *et al.,* 1997). T_4 did not produce significant effect on the length by the time of hatching than that of control suggesting that T_4 may stimulate pre-hatching embryonic development. Yolk resorption was quicker in cortisol and combined experiment than that of T_4 and control groups which suggests that effect of T_4 on yolk resorption may be different, at least in efficacy during pre-and post-hatching periods. Growth in length observed in T_4+cortisol, cortisol, T_4 treated larvae on 15th day were 2.60 cm, 2.13 cm and 1.76 cm, respectively, in comparison to the control group (1.16 cm). This investigation suggests that T_4+cortisol did improve length and larval survival as compared to the individual hormone treatment.

The enhanced swimming capability observed among the T_4+cortisol treated larvae than those immersed in T_4 suggests that peripheral development of neuromuscular systems might have been influenced by cortisol and T_4 interaction which may be the important development promoting action of thyroid hormones by involving secondary hormone systems or other indirect mechanisms of action (McNabb and King, 1993). The onset of free-swimming in the larvae was also accelerated by the cortisol treatment probably due to the accelerated reduction of the yolk sac. Cortisol might have enhanced thyroid hormones activity in at least two ways - (i) cortisol may increase the conversion of T_4 to the more potent T_3 (Galton, 1990) thus enhancing the developmental effects of T_4 and (ii) cortisol may enhance binding of the thyroid hormones to nuclear receptors in target tissues (Frieden and Naile, 1955; Kaltenbach, 1958; Kikuyama *et al.,* 1983). Cortisol have a greater enhancing effect on T_4 than T_3 in larval toad *(Bufo boreas)* suggesting that an increase in monodeiodinase activity by cortisol was a possible mechanism for at least some of the effects of the hormone (Hayes, 1995).

The observed effectiveness of combined hormone (T_4+cortisol) treatment in larval rearing of *H. fossilis* under hatchery trials offer a practical means of improving the hardiness of small larvae over the conventional methods. From results we may assume that the treatment enhanced the embryogenesis reducing the time required for hatching. It also delineates the requirement of exogenous hormone in a requisite concentration to a particular stage to fulfill the minimal imbalances of

maternal investment in eggs. This treatment significantly improved the survivability and reduced the mortality rates particularly at the critical phases like central nervous system and gastrointestinal tract differentiation, swim-bladder formation and inflation as well as metamorphic events which impart a sequence of behavioural as well as physiological changes closely associated with growth and survivability. The present study suggests that the combined treatment of the fertilized eggs of *H. fossilis* with T_4+cortisol is useful and effective practical means to enhance growth and survival which may be adopted in fish hatchery practice to improve larval rearing success. Since *H. fossils* is declining fast from its natural habitats and expected to feature in the threatened list in this country (Pandey *et al.*, 2004), the combined T_4+cortisol treatment would play an important role in conservation of this species for mass rearing of larvae with better survival and enhanced growth for their ranching in depleted water bodies. Current basic research into the molecular mode of action, receptor dynamics and multihormonal interactions will help us in elucidating the multitude of biological effects of the hormones and their potential applications in sustainable development of aquaculture.

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