Effect of thyroxin and cortisol on the hatching of eggs, larval morphometry and survival of Heteropneustes fossilis (Bloch) larvae

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ABSTRACT

Fertilized eggs of Indian catfish (Heteropneustes fossilis) were exogenously treated with L-thyroxin (T₄; 0.05 mg/l), cortisol (0.5 mg/l) and cortisol + T₄ (0.5 mg/l + 0.05 mg/l, respectively) by bath/immersion after three hours of fertilization up to 15 days of age to examine their effect on earlier development growth and survival of larvae. Growth and survival of larvae were significantly improved (P<0.001) in the treated groups as compared to controls. The experiments also suggest the advanced digestive function induced by T₄ and cortisol treated group, which was evident for the improvements in the food utilization during the critical phases of first feeding and promoted one or more vital development processes that result in uniform growth, decreased mortality, transformation of larvae to juvenile stages resulting in better survival under compromised condition. Frequency of deformities was reduced by combined treatments of hormones. The current study suggests the interactive hormonal regulation of developmental processes, working within the context of other biological variables, improve the hardiness of small larvae. Therefore, this combined therapy is emerging as an aquaculture remedy and may have practical utility in fish hatchery practice to help better larval rearing success.

Introduction

The Indian catfish Heteropneustes fossilis (Bloch) is an important commercial fish forming major bulk of the production of air-breathing fishes from the capture fishery. The bottleneck in its mass seed production is the high larval mortality during the period of transition from endogenous nutrition to exogenous feeding, or poor egg quality associated with deficiency of diverse assortment of important biological regulatory compounds (Brown and Nunez, 1994). Physiological concentrations of thyroid hormone are probably necessary for normal growth of teleosts. Poor egg quality associated with low levels of thyroid hormones, which have been involved with regulation of fish larval development, promotion of growth and survival, control of metamorphic processes at early stages of development (Lam, 1994). In addition, cortisol was shown to promote survival and stimulate the activity of mitochondria-rich cells in the yolk-sac membrane of embryos and larvae in tilapia (Ayson et al., 1995). Synergistic
effects of cortisol with thyroid hormones on the larval survival of threadfin (Brown and Kim, 1995); Catla catla (Nayak et al., 2000a); H. fossilis (Nayak et al., 2000b), and synchronized hatching in steelhead trout (Yeoh, 1993) have been reported. It appears that both T₄ and cortisol hormone interact in promoting one or more vital developmental processes, which improves survival and reduces the lapses between exhaustion of endogenous energy (in the form of yolk) and initial utilization of exogenous energy from first feeding even under compromised conditions. Bath/immersion of fertilized eggs in hormone solutions has been shown to be effective in increasing the survival rate of fish embryos and larvae (Brown and Nunez, 1994). Several experiments have been conducted in teleosts using different concentrations of T₄ and cortisol, out of which 0.05 and 0.5 mg/l respectively, gave the most consistently positive results. Therefore, the present study was undertaken to examine whether bath/immersion treatments of fertilized eggs with T₄ separately and in combination with cortisol would produce any effect on hatching of eggs, embryogenesis and post-embryonic growth or larval growth and survivability up to 15 days in H. fossilis.

Materials and methods

The brood stock was purchased locally. The fishes were maintained in the Hormonal Biotechnological Laboratory in a ferrocement tank (0.9 m dia., 0.7 m height) containing running well / pond water at 25-30°C rearing temperatures. They were acclimated to a constant overhead light fixtures of 14-h L: 10-h D photoperiod and fed daily with minced freshwater mollusc ad-libitum.

H. fossilis female and male (1:1 ratio, 100 and 60 g respectively) were induced to spawn with a single injection of Ovaprim (at 0.8 ml/kg). Eggs were manually stripped 10-12 h after the injection and fertilized with milt from the sacrificed male. The fertilized eggs were washed several times and incubated in three separate identical plastic troughs (48 cm dia. and 16.5 cm height) containing one litre fresh pond water each. Pond water was supplied to the troughs from above drop-wise and drained with an overflow drainage tube after 24 h of bath/immersion. Experiments were conducted using concentration of T₄ -(0.05 mg/l), cortisol (0.5 mg/l), T₄ + cortisol (0.05mg/l and 0.5 mg/l respectively) and untreated control. The treatment of eggs was started three hours after fertilization. Fertilized eggs from a single brood fish was treated by bath/immersion in one litre fresh pond water containing 0.05 mg/l T₄ (Eltroxin tab - Glaxo) for Experiment-1, 0.5 mg/l cortisol for Experiment-2, 0.05 +0.5 mg/l T₄ and cortisol (hydrocortisone, Sigma Co; St. Louis), respectively for Experiment-3, along with untreated batch as a control group. Eggs in each treatment were slowly washed with dropping pond water after 24 hours of immersion. Synchronized hatching occurred at 7.30 p.m. in both T₄ and T₄ + cortisol troughs, whereas in control, it was at around 11 pm. It was allowed to complete hatching up to next day morning for the control group only. Newly hatched larvae were maintained with gentle aeration at an average density of 25 larvae per litre. One replicate and one reserve troughs were used for each treatment. After the start of egg hatching, any remains of unhatched eggs in the trough were replaced suitably with the newly-hatched larvae from the respective reserve treatment troughs, for ensuring the presence of equal number of larvae at the start of the larval growth and survival experiments. Finely sieved zooplankton was provided from the third day at a density...
Thyroxin and cortisol hormones promoting growth

of 20-25 No./ml. Water was exchanged daily. Dead larvae were siphoned daily from the bottom screen and daily mortality pattern recorded. Furthermore, potassium permanganate and vitamin B-complex applications in requisite concentrations were also done during the experiment. Newly hatched larvae were immersed in pond water containing requisite concentration of hormones for 15 days. The experiment was terminated on day 15. Survival was calculated from the difference between the initial number of larvae stocked and the final number remaining up to the 15th day. Results were analysed using one-way ANOVA followed by multiple comparisons of means test. After hatching and termination of the experiment, larvae from each treatment were immediately sampled, blotted dry, and total length (from the tip of the snout to the tip of the caudal fin) and body wet weight were determined.

**Results**

An influence of T4 was found on the first day after hatching (Table 1). All larvae in the control troughs, except five in trough 2 were lying on their sides at the bottom of the trough with only slight occasional body movement. In the T4, cortisol, and T4 + cortisol troughs, more than 50, 70, and 78% of the larvae respectively, were swimming upright, although they returned to the bottom to rest periodically. They rested upright on their yolk sacs instead of on their sides. The larvae appeared comparatively bigger with a smaller yolk sac both in cortisol and T4 + cortisol treatments than the T4 and controls.

On the third day of the experiment, differences among the control, T4, cortisol and T4 + cortisol treated larvae were even more obvious (Table 2). More than 75% of the larvae in T4-treated, 80% in cortisol-treated and almost all larvae in T4 + cortisol-treated troughs showed little or no yolk sac and were swimming actively, whereas the yolk sac was clearly visible in the control larvae. The T4, cortisol and T4 + cortisol treated larvae also appeared bigger with an enlarged head. The treatment was found to have a very significant impact on the yolk absorption period in treated concentration and different ontogenic development from untreated larvae. Prior to mouth opening, advanced eye development was observed among larvae from T4, cortisol and T4 +

**Table 1.** Number of *H. fossilis* larvae swimming upright in the control, thyroxin (T4), cortisol and T4 + cortisol treated groups on the first day of experiment

<table>
<thead>
<tr>
<th>Trough</th>
<th>Control</th>
<th>T4</th>
<th>Cortisol</th>
<th>T4 + Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/25</td>
<td>10/50</td>
<td>16/25</td>
<td>17/25</td>
</tr>
<tr>
<td>2</td>
<td>5/25</td>
<td>17/50</td>
<td>20/25</td>
<td>23/25</td>
</tr>
<tr>
<td>Total</td>
<td>5/50</td>
<td>27/50</td>
<td>36/50</td>
<td>40/50</td>
</tr>
</tbody>
</table>

**Table 2.** Number of *H. fossilis* larvae actively swimming with little or no yolk sac in the control, T4, cortisol and T4 + cortisol-treated groups on the third day of experiment

<table>
<thead>
<tr>
<th>Trough</th>
<th>Control</th>
<th>T4</th>
<th>Cortisol</th>
<th>T4 + Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/25</td>
<td>18/50</td>
<td>20/25</td>
<td>23/25</td>
</tr>
<tr>
<td>2</td>
<td>2/25</td>
<td>20/50</td>
<td>21/25</td>
<td>22/25</td>
</tr>
<tr>
<td>Total</td>
<td>5/50</td>
<td>38/50</td>
<td>41/50</td>
<td>45/50</td>
</tr>
</tbody>
</table>
cortisol groups. Larvae from the control groups retained most yolk beyond the onset of feeding. In the next few days of treatment, the T₄ and T₄ + cortisol-treated larvae had become juveniles with distinct features. However, the control and cortisol larvae were post-larval in form. The hatchability of the viable eggs appeared to be better in all treated groups: T₄ + cortisol > cortisol > T₄ > control. Besides this, treatments of T₄, cortisol and T₄ + cortisol were found to reduce the hatching period (Table 3).

By 15th day when the experiment was terminated, the difference was marked in terms of body weight and length. The mean body weight was significantly (P < 0.001) greater in larvae treated with T₄ + cortisol, cortisol or T₄ than in control (Fig. 1). The total length were longer in size (Fig. 2). The treated larvae were broader dorso-ventrally and thicker, particularly in the combined group than the control. By 15th day of treatment, differences among the three groups of larvae were obvious. Not only the treated larvae (both combined and T₄) were longer and heavier, but also had better developed structural forms (in fact a juvenile stage, specifically in the T₄ + cortisol groups), whereas the control and cortisol-treated larvae were still not like those in the other treatments in terms of body depth and width.

A treatment-dependent

<table>
<thead>
<tr>
<th>Trough</th>
<th>Control</th>
<th>T₄</th>
<th>Cortisol</th>
<th>T₄ + Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28/50</td>
<td>34/50</td>
<td>38/50</td>
<td>42/50</td>
</tr>
<tr>
<td>2</td>
<td>24/50</td>
<td>37/50</td>
<td>37/50</td>
<td>38/50</td>
</tr>
<tr>
<td>Total</td>
<td>52/100</td>
<td>71/100</td>
<td>75/100</td>
<td>80/100</td>
</tr>
</tbody>
</table>

* Excluding nonviable eggs

**Table 3. Effect of T₄, cortisol and T₄ + cortisol on hatching rate in H. fossilis**

![Fig. 1. Effect of T₄, cortisol and cortisol + T₄ on the total length of H. fossilis larva after 15 days of exposure](image1)

![Fig. 2. Effect of T₄, cortisol and cortisol + T₄ on the total weight of H. fossilis larva after 15 days of exposure](image2)
Thyroxin and cortisol hormones promoting growth

Thyroxin and cortisol hormones promoting growth

The present experiments showed that administration of T4 alone and in concert was effective in enhancing growth and survivability and have beneficial effect on the eggs by improving hatching and yolk absorption of H. fossilis larvae. The influence has further confirmed it as an important application or as an aid to larval rearing. The used concentration of T4 and cortisol was found to stimulate growth in these fish larvae. Thus, it appears that T4 not only promotes growth in fishes, but also accelerates the development of fish larvae and fry. Thyroxin did accelerate hatching in chum salmon (Dales and Hoar, 1954) suggesting that it may stimulate embryonic development and/or the hatching mechanism. A marked synergism between T4 and cortisol has been seen in the subse-

to the combined treatment had less pronounced mortality during the first week in comparison to T4 and cortisol. The synergistic effect of cortisol with T4 on the larvae subsequently enhanced the absolute percentage of survivability at the end of the experiments.

Larvae grew at different rates resulting in larger size variation in the treated experiments. T4 + cortisol-treated larvae grew more uniformly. Some of the larvae showed morphological deformities in non-treated group and consequently had reduced body length than those of T4 + cortisol group.

Discussion

The present experiments showed that administration of T4 alone and in concert was effective in enhancing growth and survivability and have beneficial effect on the eggs by improving hatching and yolk absorption of H. fossilis larvae. The influence has further confirmed it as an important application or as an aid to larval rearing. The used concentration of T4 and cortisol was found to stimulate growth in these fish larvae. Thus, it appears that T4 not only promotes growth in fishes, but also accelerates the development of fish larvae and fry. Thyroxin did accelerate hatching in chum salmon (Dales and Hoar, 1954) suggesting that it may stimulate embryonic development and/or the hatching mechanism. A marked synergism between T4 and cortisol has been seen in the subse-
quent survival rate of post-hatchling larvae than those from eggs treated with T₄ or cortisol alone. The amelioration of episodic mortality events around the third and 10 days were subsequently reduced in combined application than in single treatment of T₄ or cortisol. Probably, these were the critical periods of the larvae in which we can illustrate a point about the consistency of the actions of a combination of T₄ + cortisol which had a substantial beneficial effect on survival.

The effects of the individual hormones were less consistent than those of combined application and therefore, it may be concluded that T₄ + cortisol interact to promote one or more vital developmental processes, which enhance survival even under compromised conditions. The effects of individual hormones may be disguised by other variables when applied alone. The results also showed that continuous T₄ and T₄ + cortisol treatments for 15 days was effective in H. fossilis larvae, with an acceleration of the transformation of the larvae to juvenile stage and the possibility of improved larval survival. Development of the digestive tracts was advanced to some extent by cortisol treatment and was slightly more evident in the combined treatment of T₄ + cortisol, which possibly reduces the lapses between exhaustion of endogenous energy, in the form of yolk and initial utilization of exogenous energy from first feeding (Specker, 1988). Multiple hormonal regulators are known to be involved in the control of development of gastrointestinal tract during amphibian metamorphosis (Norris and Dent, 1989) and indirect actions of these hormones on gut developments having peripheral interactions on developing target tissues in the Japanese flounder Paralichthys olivaceus (de Jesus et al., 1990). The influence of cortisol evidently prevented the increased rate of deformities in the combined treatment. Cortisol might have aided in the provision of nutrients needed for skeletal and other organ differentiation.

Cortisol has been found to stimulate growth in post-hatchling larvae of greasy grouper during embryonic development (Tay et al., 1997), in C. catla and H. fossilis (Nayak et al. 2000 a, b). T₄ did not produce a significant effect on the length by the time of hatching than that of control, which suggests that T₄ may stimulate pre-hatching embryonic development. Yolk resorption was quicker in cortisol and combined treatments than in T₄ and control ones which suggests that the effect of T₄ on yolk resorption may be different, at least in efficacy during pre- and post-hatching period. Growth in terms of total mean length observed in T₄ + cortisol and cortisol, T₄ -treated larvae on 15th day were 2.60, 2.13 and 1.76 cm respectively, whereas in the control group it was 1.16 cm, approximately. This investigation suggests that T₄ and cortisol did improve length and larval survival but to a lesser extent compared to the combined treatment.

Better swimming capability was noted among the larvae treated with the combination than T₄ -treated ones. Peripheral development of neuromuscular systems may be influenced by cortisol and T₄ interaction, which may be an important development promoting action of thyroid hormones or involving in the process of triggering action of secondary hormone systems or other indirect mechanisms of developmental actions (McNabb and King, 1993). The onset of free-swimming habit in the larvae was also accelerated by the cortisol treatment probably due to accelerated reduction of the yolk sac, a sizable load that impedes swimming. Cortisol might have enhanced thyroid hormone activity in at least two
Thyroxin and cortisol hormones promoting growth

...Thyroxin and cortisol hormones promoting growth ways: cortisol may increase the conversion of $T_4$ to the more potent $T_3$ (Galton, 1990); enhanced developmental effects of $T_4$ and cortisol may improve binding of the thyroid hormones to nuclear receptors in target tissues.

Apparently, these are the tests of effectiveness of combined hormone treatment in hatchery-scale $H$. fossilis larval rearing trials, which would offer a practical means of improving the hardness of small larvae rather than conventional methods. From the results, we may assume that the treatments enhanced the embryogenesis and therefore, less time was required for hatching than control. Requirement of exogenous hormone provided at a requisite concentration to a particular stage fulfills the minimal imbalances of maternal investment in eggs significantly improving the survivability and reducing the mortality rates particularly at the critical phases like central nervous system formation, gastrointestinal tract differentiation, swimbladder formation and inflation and metamorphosis-like events imparting a sequence of behavioural and physiological changes associated with growth and survivability. The present study suggest that treatment of $H$. fossilis eggs with $T_4$ + cortisol in concert is useful and is an effective practical way to promote growth and larval survival, which may be adopted in fish hatchery practice to improve larval rearing success.

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References


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