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RESEARCH ARTICLE

Synergistic Effects of Thyroxine and Feeding Regimes on Early Survival and Biomass Gain in Asian Catfish, Magur (*Clarias batrachus*, Linn.)

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Abstract The larvae of catfish, *Clarias batrachus* (av. wt. 15 ± 2 mg) were exposed to five concentrations of thyroxine (T4) with three feeding regimes. Five media 1.3 mcg L^{-1} (T-1), 2.2 mcg L^{-1} (T-2), 3.6 mcg L^{-1} (T-3), 6.0 mcg L^{-1} (T-4), 10.0 mcg L^{-1} (T-5) of T4 and control with three feeding regimes 1 day (once a day), 2 days (twice a day) and 3 days (thrice a day) were maintained in triplicate set in 54, troughs containing 2-L water. The larvae reared in media (T1, T2, T-3, T-4 & T-5) with feeding regimes 1 day, 2 days & 3 days showed 100 % survival which was significantly high ($p < 0.05$) in comparison to control (60.5 ± 0.7, 64.5 ± 0.7 and 71.0 ± 1.4 % respectively with regimes 1 day, 2 days and 3 days). Net gain in biomass was found significantly higher ($p < 0.05$) in feeding regimes 3 days (C, 244.7 %; T-1, 305.7 %; T-2, 217.5 %; T-3, 369.9 %; T-4, 371.4 %; T-5, 298.3 %) and 2 days (C, 146.5 %; T-1, 186.6 %; T-2, 122.7 %; T-3, 243.6 %, T-4, 200 %, T5, 123.4 %) in comparison to 1 day. The synergistic effects of T4, feeding regimes and their interaction evaluated through two-way ANOVA. The color of the fish-body was more darker in the case of experimental fishes in comparison to the control groups. The findings suggest that thyroxine enriched ambient water and feeding regimes had a significant role in improved survival and growth on *C. batrachus* larvae, a prioritized fish for aquaculture.

Keywords Thyroxine · *Clarias batrachus* · Survival · Biomass gain

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Introduction

The maintenance of normal thyroid status in cold-blooded vertebrates such as fishes is a prerequisite for normal growth and development [1, 2]. Thyroid hormones (Thyroxine, T4 and Tri-iodo-thyronine, T3) are also known to play an important role in several biological processes such as oxygen consumption, metamorphosis, temperature tolerance, migration, osmoregulation, sexual development, and metabolism in a number of fish species [3]. Several studies have shown that exogenous administration of T4 and T3 enhances growth and survival in many teleosts such as tilapias (Reddy and Lam [4], salmonids [5, 6], *Cyprinus carpio* [7, 8], *Carassius auratus* [4] and *Cirrhinus mrigala* [9]). However there are reports of deleterious effects of exogenous thyroid hormones also that have shown altered body proportions and accelerated development of skin, bones, and scales [10]. The mechanism of growth stimulation in fish by T4/T3 is likely to appear through synergism with growth hormone [2, 11] or other hormones [12]. However, a direct action of T4/T3 on somatic growth, cartilage and bone development cannot be ruled out [1]. Enhancement of unit fish productivity has tremendous importance in aquaculture, particularly due to the tremendous escalation in input costs. Economic outputs can be improved through use of hormones as food additives to enhance growth rates and food conversion efficiencies, which may result in higher productivity over a shorter span

with lower inputs. Furthermore, food additives have been reported to enhance nutrient retention and lower the excretion of metabolites waste [13, 14] that could be beneficial for maintaining healthy water quality. Although, the metabolic effects of thyroid hormones have been studied in good number of tropical fish species [15], there are few reports on this work with slow growing fish species [16]. Thyroxine has been used in all researches in oral forms and there are no reports of its application in immersion form. The preliminary results were presented in 9th Indian Fisheries Forum [17]. Thus, the present study was taken up to look into the synergistic effects of thyroxine and feeding regimes on the survival and biomass gain of *Clarias batrachus* larvae.

Methods

Experimental Design

The test fish larvae, *C. batrachus* was procured from the hatchery facility of NBFGR, Lucknow. They were acclimatized in 6 plastic pools of 300 L capacity for 3 days with 5 concentrations of treatment media and a control of normal bore well water. Twenty larvae of av. wt. 15 ± 2 mg were then stocked in each of the thirty-six plastic trough of 5 L containing 3 L of treatment media and the control water in a completely randomized block design (CRBD) given in Table 1.

Preparation of Test Media

Five freshly prepared stock test media comprised of 1.3 mcg L^{-1} (T-1), 2.2 mcg L^{-1} (T-2), 3.6 mcg L^{-1} (T-3), 6.0 mcg L^{-1} (T-4), 10.0 mcg L^{-1} (T-5) were prepared by adding thyroxine hormone (Eltroxine, GlaxoSmithkline, India) in the pre-settled bore well water and mixed well. Three litre of each of the test media was then filled in each set of treatment. The same media was used for 5 days for topping the water quantity. The test media were freshly prepared again after every 5 days and fully changed in all the troughs to maintain proper thyroxine concentration.

Table 1 Experiment design

Conc. (mcg L^{-1}) of thyroxine	Regime per day	Total observations
1.3	Once, twice and thrice	5a × 3b × 3c = 45 where 'a' = treatment media, 'b' = feeding regimes, and 'c' triplicate set + 3 × 3 = 9 controls of the three regimes in triplicate set
2.2	Once, twice and thrice	
3.6	Once, twice and thrice	
6.0	Once, twice and thrice	
10.0	Once, twice and thrice	

1 mcg L^{-1} 1 microgram L^{-1}

Feeding Strategy

The larvae were fed with freshly hatched brine shrimp larvae (BSN) ad libitum for 15 days in three regimes i.e. 1 day (feeding once a day), 2 days (feeding twice a day) and 3 days (feeding thrice a day) in all the containers (Table 1). The dead *Artemia* nauplii, fecal matter and debris accumulated were siphoned out daily to maintain hygienic condition and the volume of water was topped up with similar type of media.

Analysis of Water Quality

The water quality was analyzed for temperature, pH, dissolved oxygen, total alkalinity and electrical conductivity. The temperature, pH, DO, and electrical conductivity were measured by multi-water parameter (Thermo) and alkalinity by titration method following the standard methods [18].

Results

Survival

No mortality in any of the thyroxine treated test media was observed during the entire period of experiment. However, control media showed a survival of 60.5 ± 0.7, 64.5 ± 0.7 and 71.0 ± 1.4 % respectively with feeding regimes 1 day, 2 days and 3 days (Fig. 1).

Biomass Gain in Combination with Feeding Regimes

Net gain in biomass was found significantly higher ($p < 0.05$) in feeding regimes 2 days (C, 146.5 %; T1, 186.6 %; T2, 122.7 %; T3, 243.6 %, T4, 200.0 %, T5, 123.4 %) and regime 3 days (C, 244.7 %; T1, 305.7 %; T2, 217.5 %; T3, 369.9 %; T4, 371.4 %; T5, 298.3 %) in comparison to regime 1 day. However, the gain in weight followed a parabolic curve with increase in the concentration of thyroxine hormone. The synergic effects of thyroxine hormone, feeding regimes and their interaction evaluated through two-way ANOVA suggest that these values were significantly different ($p < 0.05$) and both thyroxine and higher feeding regimes greatly exert an effect on biomass (Table 2; Figs. 2, 3, 4). During the

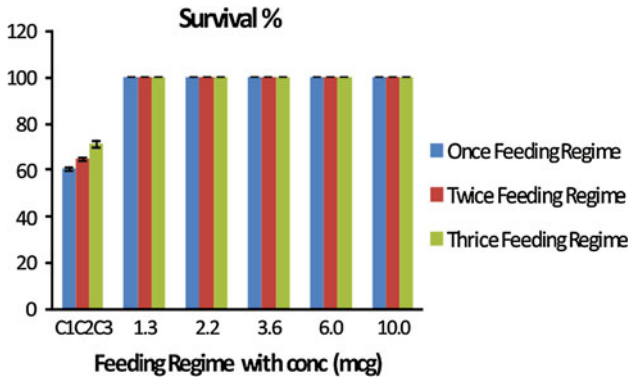

Fig. 1 Survival percentage

experimentation it was observed that the color of the fish body was more darker in the case of experimental fishes in comparison to the control groups.

Water Quality

The water quality comprises of temperature 25 ± 1 °C, pH 7.6 ± 0.5, dissolved oxygen 6.4 ± 0.4 mg L^{-1}, electrical conductivity 450 µS cm^{-1} and total alkalinity 135 ± 5 mg L^{-1}.

A two-way ANOVA was conducted that examined the effects of thyroxine concentration and feeding regimes on biomass of *C. batrachus*. The dependent variable, the biomass, was normally distributed for the groups formed by the combination of concentration of thyroxine and feeding regimes as assessed by Shapiro–Wilk test. There was homogeneity of variance between groups as assessed by Levene's test for equality of error variances. There was a significant interaction between the effects of thyroxine and feeding regime on biomass gain, $F(10,18) = 168.681$, $p = 0.005$.

Discussion

Thyroxine treated fishes showed cent percent survival in all test media and the three feeding regimes in comparison to control that showed poor survivals of 60.5 ± 0.7, 64.5 ± 0.7 and 71.0 ± 1.4 % respectively with feeding regimes 1 day, 2 days and 3 days that revealed significant ($p = 0.05$) positive effect of thyroxine hormone in improving the fish survival.

Addition of T4 also showed immediate spurt in biomass gain in all the treatments, however, T4 had a better stimulatory effect in T-4 treatment than that observed in chronically order in the case of T-5, T-1, T-2 and T-3. These results are in agreement with those of Singh and Eales [10], Arul [19] and Kumar et al. [8] who also reported faster growth in brook trout, *Channa striatus* and *Cyprinus carpio* in T4 treated fish with that of control and showed better SGR.

The significantly ($p < 0.05$) higher survival and higher biomass gain in T4 treated fish in the present case could be well correlated to improved metabolism in fish. According to Farbridje and Leatherland [11], thyroid hormones have been found to stimulate appetite or food utilization which, in turn, results in higher food conversion efficiencies and lower FCR values. Higgs [20] suggested that thyroid hormones may influence gastrointestinal function in fish. In mammals, thyroid hormones are known to stimulate gastric and intestinal mobility, enzyme synthesis, and substrate absorption rate in the small intestine and synthesis of bile salts [21]. Perhaps thyroid hormones are likely to exert similar actions on the gut of teleosts also. Woo et al. [22] observed increased appetite and food consumption efficiency (FCE) as a result of higher intestinal enzyme activity in the yearlings of red sea bream, after oral administration of tri-iodo-thyronine. Better FCR following T4 administration has also been reported in *C. carpio* [8]

Table 2 Effect of thyroxine and feeding regimes on biomass production of *Clarias batrachus*, results of two-way ANOVA

Feeding regime	Thyroxine concentration (mcg L^{-1})						Total
	Control	1.3	2.2	3.6	6.0	10.0	
	Weight of fish (g)						
Once	0.57 ± 0.014	0.66 ± 0.007	0.73 ± 0.007	0.78 ± 0.007	0.97 ± 0.028	0.87 ± 0.007	0.766^{3}
Twice	1.40 ± 0.012	2.16 ± 0.028	2.20 ± 0.021	2.25 ± 0.070	2.28 ± 0.035	1.98 ± 0.014	2.047^{2}
Thrice	1.96 ± 0.021	3.08 ± 0.014	3.12 ± 0.035	3.18 ± 0.035	3.46 ± 0.021	3.48 ± 0.007	3.050^{1}
Mean	1.31^{e}	1.96^{d}	2.02^{c}	2.07^{b}	2.24^{a}	2.11^{b}	

The numbers in superscript in last row indicate the significance levels amongst the feeding regimes. Alphabets superscript in last row indicate the significance levels amongst the concentration of thyroxine

1 mcg 1 microgram

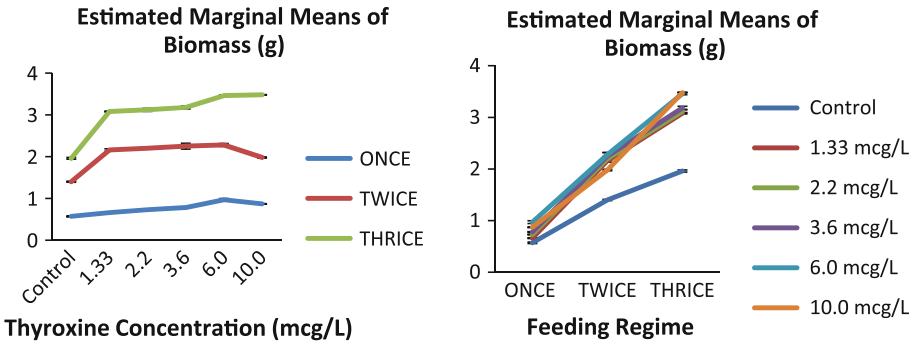

Fig. 2 Gain in biomass in different treatments and feeding regimes

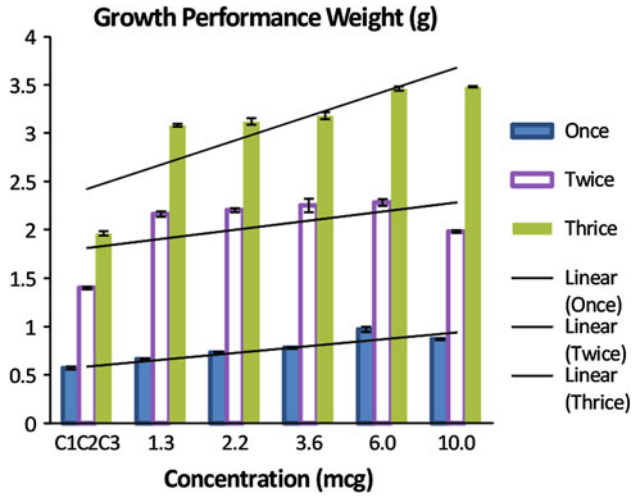

Fig. 3 Trend in weight gain in *Clarias batrachus* fry

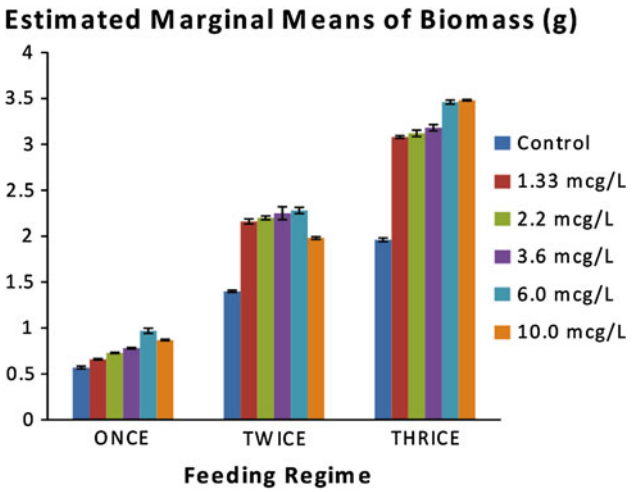

Fig. 4 Overall weight gain in thyroxine concentrations and feeding regimes

and *C. striatus* [19]. The results of several studies also suggest that T4 administration might promote release of growth hormone from pituitary somatotrophs [1, 6, 11, 23, 24].

In coho salmon, oral administration of T4 is known to increase food consumption efficiency [25]. However, there are contradictory reports also that of Matty and Lone [26] and Moon et al. [27], who reported no increment in growth in cases of Coho salmon and red drum, when the fish was given oral administration of T4. However, the immersion methods of providing T4 in the present case showed significantly better results in all the treatments in the present case. This may perhaps be the better way of absorption of T4 in fish body; however, this needs to be studied further as there is dearth of such literature.

Thyroxine needs to be used in optimum quantity for survival and growth as excessive doses result either in growth inhibition or in excessive mortality [28]. This was partly evident in the present case as although the fish survival improved in all the treatments in the present study, the fish reared in high T4 levels in T-5 treatment did not show better growth than T-4 treatment indicating that T4 requirement of this fish is optimum at T-4 concentration level. In the present study, the treatment of fish with T4 increased the voluntary food intake capacity and weight gain. These observations are similar to those reported by Gross et al. [29] and Higgs et al. [24]. Smith and Thorpe [30] also observed that administration of 0.25–5.0 mg kg^{-1} of T4 reduced nitrogen excretion in rainbow trout. Such studies needs to be carried out on this species also as catfishes like *C. batrachus* are better suitable for intensive farming where nitrogen byproducts in the form of nitrite and unionized ammonia are released in higher concentrations and sometime cause fish mortality.

Feeding frequency was found to have significant ($p < 0.05$) impact on biomass gain as all treatment and control groups showed better performance of growth with highest in 3 days feeding regime followed by 2 days and 1 day respectively. Thus, feeding this fish three times a day gave better performance in terms of biomass gain. The combined effects of T4 and feeding regimes evaluated through two-way ANOVA also indicated significant ($p < 0.05$) variations in terms of biomass gain, with highest growth in 3 days followed by 2 days and 1 day respectively.

The survival and growth-acceleration technology in aquaculture is facing regulatory hurdles particularly with respect to larval rearing of many prioritized fishes including catfishes. The treatment of such fishes with hormones that rapidly metabolized and/or excreted makes their use for food fish production feasible. Many such studies have revealed that hormone treated fish did not contain any residual hormones and thus are quite safe for human consumption as hormone clearance takes place well before marketing [31].

In general, regardless of the means of administration, exogenous hormones such as thyroxine are cleared in a matter of days as they are made up of small molecules of modified amino acids with a half-shelf life of 7 days. Furthermore, the use of hormones as anabolic agents or for sex control in fish has been wrongly compared to the use of hormones to fatten livestock. These two situations are entirely different as the level of this hormone subsided to 1 % level in a matter of few days or weeks [32] and there is no risk that the fish marketed months or years later will contain any significant amount of the parent compound or its metabolites absorbed from ambient water system.

Thus, the present study suggests that Thyroxine (T4), has a direct role in improving the survival of larvae and the synergic effects of T4, feeding regimes and their interaction performed altogether better and result in better survival and higher net weight gain. The performance of *C. batrachus* larvae was found best at T4 level of 6.0 mcg L^{-1} with regimes 3 days. The application of this study is useful for the hatchery operators to rear the juveniles of this fish for initial days (up to fry/advance fry stage) to reduce the mortality by using Thyroxine enriched ambient water.

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