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Effects of Feeding Triiodothyronine on Growth, Food Conversion and Disease Resistance of Goldfish, *Carassius auratus* (Linn.)

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Abstract

The effect of inclusion of 3,3',5-triiodo-L-thyronine (T_3) at 0, 1.25 and 6.25 ppm in the diet was examined in goldfish *Carassius auratus* young ones. The 60-day feeding trial brought about significant ($p<0.05$) increase in specific growth rate, per cent body weight gain, food conversion efficiency and protein efficiency ratio at both the levels of incorporation of T_3 than the control (0 ppm feed). The final weight and net weight gain were significantly raised at the higher level of T_3 incorporation in the diet only than the control. However, there was no difference in these parameters at two different levels of T_3 incorporation. The survivability obtained against intraperitoneal challenge with pathogenic bacteria *Aeromonas hydrophila* on day 60 were 16.67, 66.67 and 33.33% in 0, 1.25 and 6.25 ppm T_3 inclusions, respectively. The result suggests that in the goldfish T_3 stimulated growth and disease resistance. High doses of T_3 had less beneficial effect on survival than the lower dose (1.25 ppm) thus indicating the lower level of inclusion in the diet for growth and disease resistance.

Introduction

The maintenance of adequate thyroidal status in fish is a prerequisite for normal growth. Thyroid hormones are thought to play a permissive role in the growth process, enhancing the effects of other anabolic hormones, mostly growth hormone (Donaldson et al. 1979; Eales 1979; Higgs et al. 1982). The growth promoting effect of thyroid hormones has been attributed to enhanced

appetite, improved food conversion efficiency, digestive and absorptive actions (Etheridge 1993; Sherly and Jayaprakas 1995), increased protein synthesis, digestive enzymes activity and nutrient digestibility (Sambhu and Jayaprakas 1997). Thyroid hromones are known to exert profound effects on lipid, carbohydrate and protein metabolism (Donaldson et al. 1979; Higgs et al. 1982; Pisetskaya et al. 1983). The differentiation and growth of fins due to thyroxine (T_4) and triiodothyronine (T_3) treatment in telescopic eye-black goldfish, *Carassius auratus* larvae and fry have been described (Reddy and Lam 1992). Three intraperitoneal injection of T_3 on alternate days to goldfish corrected hypocalcemia and reduction of scales (Shinobu and Mugiya 1995). The metabolism of T_4 in goldfish has been reported earlier (Hoar 1958; Thornburn and Matty 1963). The enhanced survival at different growth stages of fish due to T_4/T_3 treatment has been reported by many earlier workers (Lam 1980; Leloup 1989; Brown et al. 1989; Ashraf and Meade 1993; Tagawa 1994; Brown and Kim1995). Most thyroid hormone action is believed to depend on stimulation of DNA-dependent RNA synthesis and subsequent protein synthesis (Higgs et al. 1982). In all the developmental stages T_3 appears to be the active thyroid hormone (Leloup 1989). T_4 binds less effectively than T_3 to nuclear sites and contributes modestly (<10%) to overall thyroid hormone action. T_4 is regarded largely as an inactive precursor/prohormone for T_3 (Higgs et al. 1982). T_3 in teleosts may represent the better-buffered plasma hormone pool, while plasma T_4 may undergo transitory surges in response to environmental perturbations (Brown et al. 1978).

Oral administration of T_3 to fish is the most practical means of treating salmonids than T_4 (Higgs et al. 1982). T_4 is poorly absorbed across the intestinal wall because of binding to intraluminal proteins (Hays 1968). Most of the studies have been carried out with T_3 by exposing fish via immersion or injection. The importance of ornamental fish culture and advantages of T_3 application through feed were considered of interest to investigate whether feeding of T_3 is involved in growth and survival of goldfish against one common bacterial pathogen attack. Positive results could have practical implications for the culture and production of goldfish.

Materials and Methods

Fish

The goldfish fry (average weight 3.28±0.13 g) were procured from Ornamental fish section of Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, India and brought to the laboratory. Six numbers of fish were maintained in each 10 l capacity circular glass jar. They were randomly distributed into three groups of 36 fish each. The experiment was run in 6 sets per each group. The fish were maintained in ground water (pH 7.42±0.212 -7.52±0.200). There was continuos provision for aeration. Part of water was changed daily to remove the egested materials. The water temperature during the experiment ranged from 28 to 32.5ºC.

Feed

One control diet was formulated using locally available ingredients. The ingredient and proximate composition of the diet are depicted in Table 1. (AOAC, 1990). The fish were acclimatized with the control diet before 15 days from the start of the experiment.

T_3 (3,3',5-triiodo-L-thyronine sodium salt - Sigma, USA-T-2752) of required quantity was initially dissolved in 4:1::ethanol:0.1 M hydrochloric acid. T_3 was incorporated into the basal control diet ingredients before hand pelletization @ 1.25 and 6.25 ppm feed mixture. After pelletization, the air-dried pellets were preserved in –20ºC for further feeding. The feed was prepared on weekly basis.

Experimental design

A (3 x 6) factorial design was followed. Group A received the control diet, group B was provided with T_3 incorporated diet @ 1.25 ppm feed and group C received 6.25 ppm T_3/kg feed mixed in the diet. The feeding trial was continued up to 60 days.

The fish were fed at a rate of 5% of their wet biomass per day in two equal installments at 10.00 and 17.00 h. The fish (pooled fish of each tank) were weighed at the start and end, and at 2-weeks intervals during the trial. Feed residue, if any, was removed after 1 h of feeding the fish, thus recording feed consumed and fecal matter excreted. Dry weight of feed residue was noted.

Table 1. Ingredients used in preparing the pelleted diets.

Ingredients	Percentage
Groundnut oil cake	45
Rice bran	14
Soybean meal	10
Fish meal	24
Tapioca powder	5
Vitamin and minerals[1]	2
Proximate composition (% on dry matter basis)	
Moisture	3.12
Crude protein	38.9
Crude lipid	6.82
Ash	9.67
Gross energy (kJ/g dry matter)	17.89
P/E ratio (mg protein/kJ energy)	21.74

[1]Consists of vitamin A- 500,000 IU, vitamin D_3-100,000 IU, vitamin B_2-0.2 g, Vitamin E-75 Units, vitamin K-0.1 g, Calcium Pantothenate 0.25 g, Nicotinamide –1.0 g, Vitamin B_{12}-0.6 mg, Choline chloride 15 g, Calcium-75 g, Manganese –2.75 g, Iodine 0.1 g, Iron-0.75 g, Zinc –1.5 g, Copper-0.2 g, Cobalt-0.045 g.

Growth and food conversion

The following parameters were measured :

(1) Net weight gain (NWG)

NWG = BW_f-BW_i; where BW_f and BW_i were the average final and initial body weight (g) of fish in a tank, respectively;

(2) Specific growth rate (SGR)

SGR =100 [$\log_e$ final wt.(g) – $\log_e$ initial wt. (g)]/time (days) (Anderson et al., 1984);

(3) Apparent food conversion ratio (FCR)

FCR = dry weight food offered (g)/wet weight gain of fish (g);

(4) Protein efficiency ratio (PER)

PER = Wet weight gain (g) /total protein intake (g);

(5) Per cent body weight increase (% BWI)

%BWI = 100 x (BW_f-BW_I)/BW_I, where BW_I and BW_f were the average initial and final body weight (g) of fish in tank, respectively.

Challenge experiment

At the end of the trial, twelve fish were randomly selected from each group and were maintained in three glass jars. The fish were challenged intra-peritoneally with 10^4 live cells of *Aeromonas hydrophila* (isolated from gold fish ulcer disease) in 0.1 ml PBS/fish. The cumulative mortality (%) was re-corded up to 10 days. The cause of mortality was determined by reisolating the bacteria from 10% of dead fish kidney of each group.

Data analysis

The data were analyzed by one-way analysis of variance (Snedecor and Cochran, 1968). Difference between the means were calculated by Duncan's multiple range test (Ducan, 1955).

Results

The growth of the goldfish in different concentrations of T_3 exposure after 60 days of treatment is summarized in Table 2 and the growth pattern of fish

observed at 2 weeks intervals is depicted in Fig.1. The fish in 6.25 ppm T_3 group were significantly ($p<0.05$) larger than the control in final body weight, net weight gain and per cent body weight increase. There was no significant difference ($p<0.05$) in growth (final weight, net weight gain and % body weight increase) between the two hormone - treated groups. There was also no significant difference in final body weight and net weight gain between the control group and 1.25 ppm T_3 group, but the biomass of the fish was significantly greater in 1.25 ppm T_3 group than in the control. Although fish receiving higher dose of T_3 diet achieved a marginally greater mean biomass, no differences ($p>0.05$) in the SGR occurred between fish fed the various T_3-based diets. Comparisons of the SGR of control fish revealed a lesser value than the T_3 fed fish (Table 2).

The food conversion ratio of 4.95 g of fish per kg of feed in control group was markedly reduced to 2.52 and 2.95 g in 1.87 and 6.25 ppm of T_3 treatments, respectively (Table 2). Similar to biomass and SGR, the FCR values did not differ significantly ($p>0.05$) between the two T_3 treatment groups. There was no change in the total feed consumption and protein intake values among all the groups. However, the protein efficiency ratio revealed an increasing trend ($p<0.05$) with the increase in T_3 feeding over the control groups (Table 2).

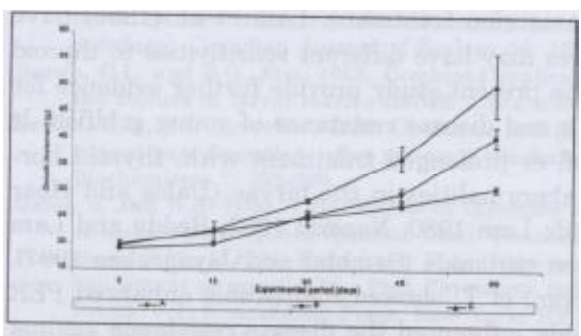

Fig. 1. Growth performance of goldfish fed with varying levels of T_3

No mortality was marked in any of the groups during the 60-day trial. On challenge with *A. hydrophila*, mortality in control group was started from day 2 onwards; whereas T_3-fed groups recorded mortality day 4 onwards. The least survival of 16.67% was obtained in T_3-deprived control group. However, the higher dose T_3 fed group showed 33.33% survival in

Table 2. Growth parameters, food conversion, protein intake and efficiency of *C. auratus* fed supplementary diets containing different levels of T_3.

Group	Diet (T_3 ppm of feed or ppm)	Initial wt. (g)	Final wt. (g)	Net wt. gain (g)	Body weight increase (BWI) (%)	SGR (g)	FCR (g)	Total feed consumed	Protein intake (g)	PER
A	0	20.00^{a} ±0.58	30.67^{a} ±0.67	10.67^{a} ±1.20	53.76^{a} ±7.62	0.71^{a} ±0.08	4.95^{a} ±0.84	411.67^{a} ±6.01	160.14^{a} ±2.34	0.07^{a} ±0.001
(Control)										
B	1.25	19.17^{a} ±0.17	40.50^{ab} ±1.89	21.33^{ab} ±1.74	111.18^{b} ±8.18	1.24^{b} ±0.06	2.52^{b} ±0.32	423.33^{a} ±7.26	164.68^{a} ±2.82	0.13^{b} ±0.002
C	6.25	19.66^{a} ±0.33	50.50^{b} ±5.90	30.83^{b} ±5.94	156.14^{b} ±29.55	1.54^{b} ±0.20	1.87^{b} ±0.63	425.00^{a} ±5.77	165.33^{a} ±2.24	0.19^{c} ±0.003

Data are expressed as mean ± standard error (N=6). Means within the same column not sharing a common superscript are significantly different ($P<0.05$)

comparison to 66.67% in 1.25 ppm T_3 fed group (Fig. 2). At 1.25 ppm of T_3 in corporation, significantly higher protection against *A. hydrophila* was obtained in comparison to other two groups.

Discussion

The results showed that T_3 markedly accelerated the growth, food conversion and disease resistance of the young goldfish. The growth-promoting effect of T_3 observed in goldfish in the present study is consistent with the reports of other workers on larvae and fry of goldfish (immersion treatment of T_3) (Reddy and Lam 1992) and other fish species (Lam 1980; Nacario 1983; Lam et al. 1985; Lam and Sharma 1985; Reddy and Lam 1987, 1992). The administration of thyroid hormone promotes growth of teleosts by increasing voluntary food intake (Higgs et al. 1979; Muniandi 1989) and gross feed conversion efficiency (Konda Reddy 1990). T_3, particularly may potentiate appetite and (or) food utilization directly, or indirectly by, for example, stimulating growth hormone secretion (Markert et al. 1977; Higgs et al. 1982).

In the present study, 1.25 and 6.25 ppm T_3 were found to be effective in growth promotion while 6.25 ppm was found to confer poor resistance to pathogen challenge, resulting in 33.33% survival of fish. Similarly, in goldfish fry, Reddy and Lam (1992) found the effective concentration of T_3 to be 0.01 ppm for growth in a 40 days immersion treatment. Lam et al. (1985) have also suggested that different stages may have different sensitivities to thyroid hormone action. The results of the present study provide further evidence for a role of T_3 feeding in the growth and disease resistance of young goldfish. It is well known that high doses of, or prolonged treatment with, thyroid hormone inhibit growth and cause abnormalities in the larvae (Dales and Hoar 1954; Honma and Murakawa 1955; Lam 1980; Nacario 1983; Reddy and Lam 1992) and 100% mortality in green chromide (Sambhu and Jayaprakas 1997). Although the higher dose (6.25 ppm) of T_3 showed comparable enhanced PER with lower dose of T_3, it negatively influenced the disease resistance against one common pathogen *A. hydrophila*. Thus the results suggested the addition of lower (1.25 ppm) level of T_3 into feed for better growth and disease resistance of young goldfish. However, further studies should be carried out with lower variable dose and time period of feeding of T_3 for future practical application in goldfish farming. Should this disease resistance effect of T_3 be further confirmed in other species as well as for other pathogens, the hormone would find important applications as an aid to raise healthy stock, which has remained a major problem in aquaculture

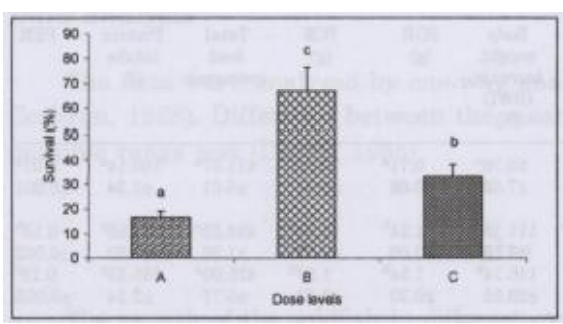

Fig. 2. Surviaval of goldfish against *A. hydrophila* (i/p) challenge fed with varying levels of T_3 for 60 days

growth. As already reported, thyroid hormones shorten the larval rearing period, accelerate growth and development of larvae and fry of goldfish, increase length of fins and tail (Reddy and Lam 1992); it may have wide practical application in goldfish farming.

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