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 hormones 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 (T4) and triiodothyronine (T3) treatment in telescopic eye-black goldfish, Carassius auratus larvae and fry have been described (Reddy and Lam 1992). Three intraperitoneal injection of T3 on alternate days to goldfish corrected hypocalcemia and reduction of scales (Shinobu and Mugiya 1995). The metabolism of T4 in goldfish has been reported earlier (Hoar 1958; Thornburn and Matty 1963). The enhanced survival at different growth stages of fish due to T3/T4 treatment has been reported by many earlier workers (Lam 1980; Leloup 1989; Brown et al. 1989; Ashraf and Meade 1993; Tagawa 1994; Brown and Kim 1995). 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 T3 appears to be the active thyroid hormone (Leloup 1989). T4 binds less effectively than T3 to nuclear sites and contributes modestly (<10%) to overall thyroid hormone action. T4 is regarded largely as an inactive precursor/prohormone for T3 (Higgs et al. 1982). T3 in teleosts may represent the better-buffered plasma hormone pool, while plasma T4 may undergo transitory surges in response to environmental perturbations (Brown et al. 1978).

Oral administration of T3 to fish is the most practical means of treating salmonids than T4 (Higgs et al. 1982). T4 is poorly absorbed across the intestinal wall because of binding to intraluminal proteins (Hays 1968). Most of the studies have been carried out with T3 by exposing fish via immersion or injection. The importance of ornamental fish culture and advantages of T3 application through feed were considered of interest to investigate whether feeding of T3 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 continuous provision for aeration. Part of water was changed daily to remove the egested materials. The water temperature during the experiment ranged from 28 to 32.5°C.
**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 –20°C 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>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnut oil cake</td>
<td>45</td>
</tr>
<tr>
<td>Rice bran</td>
<td>14</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10</td>
</tr>
<tr>
<td>Fish meal</td>
<td>24</td>
</tr>
<tr>
<td>Tapioca powder</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin and minerals</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1. Ingredients used in preparing the pelleted diets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy (kJ/g dry matter)</td>
<td>17.89</td>
</tr>
<tr>
<td>P/E ratio (mg protein/kJ energy)</td>
<td>21.74</td>
</tr>
</tbody>
</table>

†Consists of vitamin A- 500,000 IU, vitamin D$_3$-100,000 IU, vitamin B$_6$-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)

\[
\text{NWG} = \text{BW}_f - \text{BW}_i; \quad \text{where BW}_f \text{ and BW}_i \text{ were the average final and initial body weight (g) of fish in a tank, respectively;}
\]

2. Specific growth rate (SGR)

\[
\text{SGR} = 100 \left[ \log_e \text{final wt. (g)} - \log_e \text{initial wt. (g)} \right]/\text{time (days)} \quad \text{(Anderson et al., 1984)};
\]

3. Apparent food conversion ratio (FCR)

\[
\text{FCR} = \text{dry weight food offered (g)}/\text{wet weight gain of fish (g)};
\]

4. Protein efficiency ratio (PER)

\[
\text{PER} = \text{Wet weight gain (g)}/\text{total protein intake (g)};
\]

5. Per cent body weight increase (% BWI)

\[
%\text{BWI} = 100 \times \left( \frac{\text{BW}_f - \text{BW}_i}{\text{BW}_i} \right); \quad \text{where BW}_i \text{ and BW}_f \text{ were the average initial and final body weight (g) of fish in tank, respectively.}
\]

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 intraperitoneally with \(10^4\) live cells of *Aeromonas hydrophila* (isolated from goldfish ulcer disease) in 0.1 ml PBS/fish. The cumulative mortality (%) was recorded 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 T₃ 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 T₃ group, but the biomass of the fish was significantly greater in 1.25 ppm T₃ group than in the control. Although fish receiving higher dose of T₃ diet achieved a marginally greater mean biomass, no differences (p>0.05) in the SGR occurred between fish fed the various T₃-based diets. Comparisons of the SGR of control fish revealed a lesser value than the T₃ 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 T₃ treatments, respectively (Table 2). Similar to biomass and SGR, the FCR values did not differ significantly (p>0.05) between the two T₃ 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 T₃ feeding over the control groups (Table 2).

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 T₃-fed groups recorded mortality day 4 onwards. The least survival of 16.67% was obtained in T₃-deprived control group. However, the higher dose T₃ 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 T₃.

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet (T₃ ppm of feed or ppm)</th>
<th>Initial wt. (g)</th>
<th>Final wt. (g)</th>
<th>Net wt. gain (g)</th>
<th>Body weight increase (BWI) (%)</th>
<th>SGR (g)</th>
<th>FCR (g)</th>
<th>Total feed consumed (g)</th>
<th>Protein intake (g)</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>20.00</td>
<td>30.67</td>
<td>10.67</td>
<td>53.76</td>
<td>0.71</td>
<td>4.95</td>
<td>411.67</td>
<td>41.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.58</td>
<td>±0.67</td>
<td>±1.20</td>
<td>±7.62</td>
<td>±0.08</td>
<td>±0.84</td>
<td>±2.52</td>
<td>±423.33</td>
<td>±164.68</td>
<td></td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.25</td>
<td>19.17</td>
<td>40.50</td>
<td>21.33</td>
<td>111.18</td>
<td>2.24</td>
<td>2.52</td>
<td>423.33</td>
<td>164.88</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>±0.17</td>
<td>±1.89</td>
<td>±1.74</td>
<td>±8.18</td>
<td>±0.06</td>
<td>±0.32</td>
<td>±2.52</td>
<td>±423.33</td>
<td>±164.88</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.25</td>
<td>19.66</td>
<td>50.50</td>
<td>30.83</td>
<td>156.14</td>
<td>1.54</td>
<td>1.87</td>
<td>425.00</td>
<td>165.33</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>±0.33</td>
<td>±5.90</td>
<td>±5.94</td>
<td>±29.55</td>
<td>±0.20</td>
<td>±0.63</td>
<td>±5.77</td>
<td>±25.00</td>
<td>±164.88</td>
<td></td>
</tr>
</tbody>
</table>

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 conver-
sion 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 uti-
lization 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 hor-
mone 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 resis-
tance of young goldfish. However, further studies should be carried out with
lower variable dose and time period of feeding of T$_3$ for fu-
ture 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. Survival of goldfish against A. hydrophila (i/p) challenge fed with varying levels of T$_3$ for 60 days](image)
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.

Acknowledgments

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References


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