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# Influence of dietary thyroxine in deccan mahseer (*Tor khudree* Sykes) juveniles: Effect on growth performance and feed utilization

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Thyroid hormones: 3,5,3´5´-tetraiodothyronine ($T_4$) or thyroxine and 3,5,3´-triiodothyronine ($T_3$) are widespread in the animal kingdom and these hormones in fish are reported to play vital roles either directly or indirectly in the major biological processes such as temperature tolerance, oxygen consumption, osmoregulation, migration, sexual maturation, growth and metabolism (Donaldson *et al*. 1979). Earlier studies showed that the growth of fish can be stimulated by exogenous treatment of thyroid hormones either $T_3$ or $T_4$ (Lam 1994, Devi and Vijayaraghavan 1996, Garg 2007). In view of these advantages and its function in various metabolic processes, the present study was undertaken to describe the optimum dosage of thyroxine that would be required for enhancement of juvenile growth in an otherwise slow growing fish species, *Tor khudree* and its influence on some aspects of nutritional physiology.

Experimental diets were formulated by supplementing the basal diet with thyroxine in the concentrations of 0.05, 0.075 and 0.10 mg/kg of diet. The dietary ingredients and the proximate composition of the basal diet are presented in Table 1. The deccan mahseer juveniles were obtained commercially. Trial was conducted in 20 cylindrical plastic tanks of 50 liter capacity each. The tanks were cleaned and filled up to 30 litre mark with groundwater and the water was continuously aerated. Two-third water of each tank was exchanged everyday. Uniform sized fish were randomly stocked 30 numbers in each tank after recording their initial total length and body weight. Feeding was done with the experimental diets taking 5 replicates for each dietary treatment. All the fish were fed @ 10% of body weight per day in 2 separate meals (0900 h and 1600 h) over a period of 180 days. Fish were sampled every 2 weeks for length and weight measurements. Faecal matter was removed by siphoning separately before each morning feeding, dried and subsequently analyzed for digestibility estimations. Tanks were thoroughly cleaned every 2 weeks when the fish were removed for weighing. Mortality was checked daily. The quantity of feed was readjusted fortnightly based on the growth of the fish. Prior to next feeding the uneaten feed was siphoned out collected, dried and weighed separately for calculations of feed utilization.

At the conclusion of the experimental period, approximately 18 h after the last feeding, all fish were

Table 1. Ingredient and proximate composition of the basal diet

| | |
|---|---|
| *Ingredient composition (g kg$^{-1}$)* | |
| Fish meal | 360 |
| Groundnut oil cake | 210 |
| Soybean oil cake | 120 |
| Wheat bran | 120 |
| Wheat flour | 90 |
| Vegetable oil | 60 |
| Vitamin mixture[1] | 10 |
| Mineral mixture[2] | 30 |
| *Proximate composition (%)* | |
| Dry matter | 96.96 |
| Crude protein | 37.86 |
| Crude lipid | 10.31 |
| Crude fibre | 7.65 |
| NFE | 31.03 |
| Ash | 10.09 |
| Gross energy (kJg$^{-1}$) | 17.91 |

[1]Vitamin mix: Each 100 g contains—thiamine mononitrate 3.52 g; riboflavin 3.52 g; pyridoxine hydrochloride 1.05 g; cyanocobalamin 0.004 g; folic acid 0.53 g; nicotinamide 35.15 g; calcium pentothenate 3.52 g; and ascorbic acid 52.72 g; vitamin A, 500 IU; vitamin $D_3$, 30 IU; vitamin E–81.6 IU.

[2]Mineral mix: Each 100 g contains—calcium phosphate dibasic 73.5 g; potassium phosphate dibasic 8.1 g; potassium sulphate 6.8 g; sodium chloride 3.06 g; calcium carbonate 2.1 g; sodium phosphate dibasic 2.14 g; magnesium oxide 2.21 g; ferric citrate 0.558 g; zinc carbonate 0.081 g; manganese carbonate 0.421 g; cupric carbonate 0.333 g; potassium iodide 0.00072 g; citric acid 0.702 g.

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counted and weighed (total length was also recorded) to determine survival, weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and crude protein, crude fat and gross energy efficiencies (Jover *et al.* 1999). After obtaining the final total weight and total length of fish in each tank, fifteen fish per tank were randomly selected and killed by keeping on an ice tray for subsequent analysis. Of these 5 fish/tank were processed for determination of whole body composition and 10 fish/tank were dissected to obtain liver, intestine, brain and muscle samples for hepato-somatic index (HSl), viscero-somatic index (VSI), reno-somatic index (RSI) and cranio-somatic index (CSI) calculation. The intestine and liver were processed for the determination of amylase (Bernfeld 1965), protease (Bazaz and Keshavanath 1993) and lipase (Oser 1965) enzyme activity. Muscle tissue was used for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) estimation as per the methodology recommended by Schneider (1966). Proximate analysis of the formulated diets and test fish was performed according to AOAC (1995) methods. The gross energy values were calculated in terms of kilo Joules using energy values of 38.9 kJ/g for lipid, 22.6 kJ/g for protein and 17.2 kJ/g for carbohydrates (Mayes 1990). Apparent digestibility coefficient (ADC) of dry matter, protein and lipid was calculated using acid insoluble ash as internal marker. Physico-chemical characteristic of water were analyzed at fortnightly intervals in each experimental tank (APHA 1998). The water quality parameters during the experimental period were: water temperature 20.0–32.0°C; dissolved oxygen 5.2–7.7 mg/l; pH 7.94–8.47; free carbon dioxide Nil; and total alkalinity 152–280 mg/l.

The results are presented as mean + SE of 5 replicates. One-way analysis of variance (ANOVA) technique (Snedecor and Cochran 1994) was employed to test the difference between treatment means for the various parameters studied and when significant difference ($P<0.05$) was observed between treatments, further analysis was carried out employing Duncan's multiple range test at 5% level of significance (Montgomery 1991).

The growth performance in terms of percentage weight gain and SGR (Table 2) was significantly higher in fish fed with a lower dose of thyroxine (0.05 mg/kg of diet) compared to those fed with the higher doses of thyroxine (0.075 and 0.10 mg/kg of diet) and control diet. Consequently, the growth performance decreased with increase in thyroxine levels.

Table 2. Effect of dietary thyroxine on growth performance, feed utilization, apparent digestibility coefficient and digestive enzymes activities in deccan mahseer juveniles

| Parameters | | Dietary thyroxine levels (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Control | 0.05 | 0.075 | 0.10 |
| Initial live weight (g) | | 0.10±0.01 | 0.07±0.01 | 0.09±0.02 | 0.08±0.01 |
| Final live weight (g) | | 0.99±0.01 | 1.04±0.02 | 0.94±0.02 | 0.74±0.01 |
| Initial mean total length (mm) | | 18.80±0.17 | 18.24±0.15 | 18.76±0.30 | 18.68±0.45 |
| Final mean total length (mm) | | 52.44±1.20 | 52.72±0.89 | 51.48±0.79 | 49.80±1.22 |
| Weight gain (%) | | 937.72±109.49[a] | 1566.66±183.46[b] | 1110.39±236.80[c] | 831.96±71.50[a] |
| Specific growth rate (SGR) (%/day) | | 1.29±0.04[a] | 1.55±0.02[b] | 1.34±0.01[c] | 1.23±0.02[a] |
| Feed conversion ratio (FCR) | | 3.82±0.02[a] | 3.26±0.05[b] | 4.70±0.07[c] | 5.40±0.09[d] |
| Feed conversion efficiency (FCE) | | 26.17±0.01[a] | 30.70±0.49[b] | 21.29±0.32[c] | 18.55±0.32[d] |
| Protein efficiency ratio (PER) | | 0.69±0.02[a] | 0.83±0.01[b] | 0.57±0.01[c] | 0.50±0.01[d] |
| Survival (%) | | 100[a] | 99.33±0.67[a] | 98.67±0.82[a] | 96.67±2.11[b] |
| *Nutrient efficiency (%)* | | | | | |
| Crude protein efficiency (CPE) | | 10.47±0.22[a] | 12.21±0.16[b] | 8.16±0.07[c] | 6.68±0.27[d] |
| Crude fat efficiency (CFE) | | 17.42±0.37[a] | 20.20±0.24[b] | 13.11±0.15[c] | 11.30±0.45[d] |
| Gross energy efficiency (GEE) | | 8.87±0.18[a] | 10.55±0.13[b] | 7.07±0.06[c] | 5.78±0.24[d] |
| *Apparent digestibility coefficient (ADC%)* | | | | | |
| Dry matter | | 90.76±0.29[ab] | 91.52±0.38[a] | 89.94±0.79[bc] | 89.60±0.55[c] |
| Protein | | 97.49±0.06[a] | 98.25±0.09[b] | 97.07±0.15[c] | 96.76±0.13[d] |
| Lipid | | 98.79±0.07[a] | 99.24±0.06[b] | 98.29±0.14[c] | 97.17±0.14[d] |
| *Digestive enzyme activities (U/mg)* | | | | | |
| Protease | Intestine | 2.43±0.01[a] | 3.48±0.02[b] | 2.21±0.02[c] | 2.02±0.01[d] |
| | Liver | 1.68±0.004[a] | 1.77±0.01[b] | 1.56±0.01[c] | 1.51±0.002[d] |
| Amylase | Intestine | 1.20±0.01[a] | 1.77±0.02[b] | 1.43±0.01[c] | 1.28±0.01[d] |
| | Liver | 1.82±0.01[a] | 1.87±0.01[b] | 1.75±0.01[c] | 1.61±0.01[d] |
| Lipase | Intestine | 0.042±0.001[a] | 0.045±0.002[a] | 0.041±0.003[a] | 0.003±0.001[b] |
| | Liver | 0.040±0.001[a] | 0.043±0.001[a] | 0.040±0.001[a] | 0.028±0.0004[b] |

Values are means (± SE); means within a row having different superscripts are significantly different ($P<0.05$).

Accordingly, survivability of fish too significantly ($P<0.05$) reduced at higher dose of thyroxine 0.10 mg/kg of diet. Lower dose of thyroxine was effective in accelerating growth, whereas, higher doses reduced growth and survival. Variations occur in the effective dose of thyroxine due to species specificity or environmental conditions such as temperature, salinity, pH, etc. and apart from these different juvenile stages have different sensitivities to thyroxine action (Reddy and Lam 1987).

The values of FCR, FCE and PER at lower level of thyroxine (0.05 mg/kg of diet) were significantly ($P<0.05$) better than that for higher doses of thyroxine (0.075 to 0.10 mg $kg^{-1}$ of diet) and control (Table 2). The administration of thyroid hormone in low dose stimulated appetite, increased voluntary food intake and food utilization, which in turn resulted in better FCR and FCE values (Garg 2007). Likewise, the ADC of dry matter, protein and lipid decreased significantly ($P<0.05$) at higher dietary thyroxine levels of 0.075 to 0.10 mg/kg of diet compared to that of the control and 0.05 mg/kg of diet thyroxine. In fish fed with low dose of thyroxine (0.05 mg/kg of diet) and control diet, ADC of dry matter and lipid were statistically similar, and that of protein was significantly higher ($P<0.05$) in 0.05 mg/kg of diet thyroxine. The crude protein, crude fat and gross energy efficiencies as well were significantly ($P<0.05$) higher in fish fed with lower supplemental level of thyroxine (0.05 mg/kg of diet) and were significantly ($P<0.05$) less in fish fed with higher concentrations of thyroxine (0.075 and 0.10 mg/kg of diet) than that of the fish fed with control diet.

The digestive enzymes activities of both intestine and liver were observed to be highest in fish fed diet supplemented with low dose of thyroxine (0.05 mg/kg of diet) compared to those fed with higher doses of thyroxine (0.075 and 0.10 mg/kg of diet) and control diet (Table 2). With increase in dietary levels of thyroxine digestive enzyme activities decreased to values less than that for control diet. For all the treatments in the present study, protease and lipase activities were higher in intestine and amylase activity was higher in liver. Increased proteolytic activity of alimentary canal suggested higher protein turnover and active mobilization, which advantageously influenced growth and food utilization by fish (Hidalgo *et al.* 1999). Hence, thyroxine in low doses improved digestion and absorption and enhanced proper utilization of nutrients (Devi and Vijayaraghavan 1996).

The values for body indices VSI, HSI and RSI were higher in fish receiving low dose of thyroxine (0.05 mg/kg of diet) and declined in fish fed with higher doses of thyroxine (0.075 and 0.10 mg/kg of diet) in reference to fish fed with the control diet (Table 3). Garg (2007), as well, accounted that the values of VSI and HSI increased in fish receiving lower dietary levels of thyroxine and decreased in fish receiving higher dietary doses compared to control group and envisaged that the decrease in HSI may be due to the depletion of hepatic glycogen. On the contrary, CSI values increased significantly ($P<0.05$) with increase in dietary thyroxine (0.075 and 0.10 mg/kg of diet) and were less in fish fed small dose of thyroxine (0.05 mg/kg of diet) and control diet. Similarly, Chaudhry *et al.* (1990) observed significant increase in the weight of brain relative to body weight in fish juveniles receiving higher dose of thyroxine and concluded that this increase in relative weight of the brain may not be due to an increase in the total weight of brain *per se*, but due to a decrease in the total weight of fish. As a result, CSI displays inverse relationship to growth and it decreased with increase in fish size. For this reason the higher values of CSI in fish fed with higher doses of thyroxine may be due to reduced growth of fish at higher levels of dietary thyroxine.

Body protein, lipid and gross energy contents declined

Table 3. Effect of dietary thyroxine on body indices and carcass composition in deccan mahseer juveniles

| Parameters | Dietary thyroxine levels (mg/kg) | | | |
|---|---|---|---|---|
| | Control | 0.05 | 0.075 | 0.10 |
| *Body indices* | | | | |
| Viscero-somatic index (VSI) | 4.38±0.18[a] | 4.64±0.36[a] | 4.35±0.41[a] | 3.52±0.16[b] |
| Hepato-somatic index (HSI) | 1.58±0.03[a] | 1.71±0.04[a] | 1.57±0.05[a] | 1.54±0.04[a] |
| Reno-somatic index (RSI) | 0.35±0.01[a] | 0.52±0.02[b] | 0.50±0.02[b] | 0.45±0.03[b] |
| Cranio-somatic index (CSI) | 2.56±0.04[a] | 2.51±0.05[a] | 2.64±0.08[b] | 2.76±0.04[b] |
| *Carcass composition (% wet weight)* | | | | |
| Moisture | 76.90±0.27[a] | 76.69±0.41[a] | 77.66±0.24[a] | 77.70±0.17[a] |
| Crude protein | 14.18±0.05[a] | 14.21±0.09[a] | 13.42±0.02[b] | 13.19±0.05[c] |
| Crude fat | 6.28±0.02[a] | 6.30±0.07[a] | 6.07±0.02[b] | 6.01±0.05[b] |
| Ash | 2.63±0.02[a] | 2.76±0.04[b] | 2.83±0.02[b] | 3.09±0.02[c] |
| Gross energy (kJ/g) | 5.65±0.02[a] | 5.66±0.03[a] | 5.40±0.004[b] | 5.22±0.02[c] |
| *Nucleic acid content (mg/g)* | | | | |
| RNA | 0.20±0.002[a] | 0.21±0.001[a] | 0.18±0.002[b] | 0.17±0.0003[c] |
| DNA | 0.13±0.002[a] | 0.13±0.0004[a] | 0.13±0.0004[a] | 0.14±0.001[b] |
| RNA/DNA | 1.59±0.02[a] | 1.61±0.003[a] | 1.38±0.01[b] | 1.18±0.01[c] |

Values are means (±SE); means within a row having different superscripts are significantly different ($P<0.05$).

significantly (P<0.05) and ash content increased significantly (P<0.05) with increase in thyroxine concentration and at lower concentration of 0.05 mg/kg of diet these values were statistically similar to that of the control (Table 3). The higher growth concomitant with higher muscle RNA/DNA ratio of fish fed varied levels of thyroxine in the present study revealed that the supplemental thyroxine improved growth by increasing protein synthesis and to a greater extent in fish fed at the level of 0.05 mg/kg of diet compared to those fed with higher doses. The inhibitory effect of thyroxine at higher dietary doses may be attributed to the fact that the excessive exogenous supply of thyroid hormone interfered with feed back mechanism and caused decreased synthesis and release of thyroid stimulating hormone from pituitary (Donaldson *et al*. 1979).

Thyroxine supplementation to the deccan mahseer diet, at a lower dose, improved the growth performance and feed utilization. Thyroxine with its role in hastening the growth and development of juvenile stages may provide ample possibility and impetus to the intensification of hatchery rearing practices of deccan mahseer.

## SUMMARY

The present study was conducted to investigate the effects of dietary thyroxine levels on growth performance and feed utilization of deccan mahseer juveniles. Under the experiment 5 replicate groups were fed 4 diets with increasing levels of thyroxine (0, 0.05, 0.075 and 0.10 mg/kg of diet) for 180 days. The weight gain (%), specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), nutrient and gross energy efficiency, apparent digestibility coefficient (ADC) of protein and lipid were significantly higher in fish fed with lower level of thyroxine (0.05 mg/kg of diet) and significantly decreased in response to the increasing levels of dietary thyroxine (from 0.075 to 0.10 mg/kg of diet). The survivability of fish too at higher dose was significantly lower. In fish fed on low dietary thyroxine level digestive enzyme activity, viscero-somatic index (VSI), hepato-somatic index (HSI) and reno-somatic index (RSI) values were high and cranio-somatic index (CSI) value was low. Whole body protein, lipid and gross energy content and muscle RNA/DNA ratio followed a similar pattern as growth parameters in relation to dietary thyroxine levels. Suggesting that thyroxine at a lower dosage accelerated growth performance and feed utilization in deccan mahseer juveniles.

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