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Effect of different levels of 3, 5, 3'-triiodo-L-thyronine Administration in a dry diet on rearing of Whitefish (*Coregonus lavaretus L., Coregoninae*) Larvae

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Abstract

Laboratory rearing of whitefish *(Coregonus lavaretus* L.) larvae was carried out in water temperature of 14±1°C. Larvae were fed exclusively on dry diet with addition of 3,5,3' triiodo-L-thyronine (T3) as a potential growth stimulator. Experimental groups received 0.5, 1.5, 3.5 and 10 ppm T3, respectively. Control group was fed with no hormone. Experiment lasted for 20 days. At the end of the experiment larvae receiving 1.5 ppm T3 in the dry diet were heavier, but no longer ($P<0.05$) in comparison with other groups (with exception of 0.5 ppm group). There was no statistical difference in mortality between experimental groups. We conclude that the best results for enhancing larval growth were obtained at dose of 1.5 ppm T3.

Keywords: *Coregonus lavaretus L*; Dry diet; Feed components; Hormone levels.

Introduction

Supplementation the feeds with small amounts of substances stimulating fish growth and decreasing the feeding coefficient (especially some hormones) is a method to increase the dry diet efficiency without changing of proportions of the major feed components. Many experiments confirmed stimulating effect of using androgens in fish feeding [1-7]. Other hormonal growth stimulators, like somatotropin [8], or triiodothyronine [9-12] were used occasionally, so far.

Using of different hormones in fish feeding in intensive aquaculture [13-17] is often considered in public opinion as possible danger for consumer's health [18-21], and restricted in different countries. In case of larval fish, reared maximally for 1-2 months before stocking to the nature, this problem should not be seen similarly. After coming to the natural life and habitat reared fish will transform to normal metabolism, so effect of stimulators (with exception of genetic therapy) would be cancelled. But, accelerating of larval development and growth with the use of stimulators may be an important tool for reducing of the most critical phases of fish ontogeny and preparing larger fish to stock.

"Division of competition" between thyroid hormones (3,5,3'-triiodothyronine - T3 and thyroxine - T4) is unclear in fish metabolism. Probably their functions have agreed to a considerable degree. The use of T3 in our experiment was connected with the fact, that in contrast to T4, this hormone may be effectively absorbed from the intestinal lumen [4,22].

The aim of the experiment was: 1) to test the effect of a dry diet supplemented with 3,5,3' triiodothyronine on larval rearing, and 2) to designate the best level of the hormone supplementation in coregonid starter diet.

Materials and Methods

3,5,3′-triiodo-l-thyronine (T3) (produced by Sigma Corporation, St. Louis, USA) was added to the experimental diet. Control feed design was the same like the starter diet for coregonid fish used previously [23,24], ie. yeast (50%), lyophilized beef liver (36%, wheat meal (4%), fish oil (2%), soya oil (2%), vitamin mixture (5%), and mineral mixture (1%). Food particle size was 0.1 mm during the first 10 days and then - 0.1-0.2 mm. There were differences in the hormone level only. T3 doses were respectively 0.5, 1.5, 3.5 and 10 ppm. Control diet was with no addition of the hormone.

Whitefish (Coregonus lavaretus) larvae with initial weight of 3.51 mg and length of 91mm, originated from Fish Farm Szwaderki (Olsztyn District, Northern Poland). After thermal acclimation, they were divided into six groups in three replicates and located in 18 tanks (of volume of three dm^3 each). The stocking densities were 330 larvae per tank.

Tanks were supplied with recirculated and filtered water with the flow rate of 0.2 dm^3per minute. Temperature of water was 14±1 °C. A constant illumination of tanks was used from7.00 to 20.00. In the same time larvae were fed manually every hour. Tanks were cleaned once daily at 18.00. Larval mortality was registered every day. Experiment lasted for 20 days.

For growth analysis the larvae were sampled every three days since 5th day of rearing.

After preservation in 4% formaldehyde solution they were weighed and measured. Larval developmental advancement (LDS) according to Luczynski *et al.* [25] was also determined. The significance of the differences between feeding groups was evaluated by Duncan's multiple test.

Specific growth rate (SGR) was calculated from the equation: $SGR = 100[(\ln W_2 - \ln W_1)/t]$,

Where

W_1- is average individual weight in mg at the beginning of rearing,

W_2- is average individual weight in mg at the end of rearing, and t - is duration of rearing in days.

Results

At the end of the experiment the statistical differences ($P<0.05$) between feeding groups both in average weight and length of larvae were observed (Table 1). Larvae fed dry diet with 1.5 ppm T3 reached mean weight of 41.4 mg and length of 18.0 mm, while those fed with 10 ppm T3 had 25.2 mg and 16.8 mm, respectively. Fish in the control group reached size of32.3 mg and 17.6 mm. Specific growth rate values ranged from 9.85% per day in larvae fed with 10 ppm T3 to 12.35% per day in group fed with 1.5 ppm T3 in the diet (Table 1).

Table 1. Results of *Coregonus lavaretus* larvae rearing on dry diet with T3 supplementation. Standard deviations in parentheses, means with the same superscript letter are not significantly different ($P<0.05$, Duncan's multiple test).

Day of rearing	Feeding groups (T3 level in ppm)					
	0	0.5	1.5	3	5	10
Average body weight in mg						
8	10.5 (4.0)^{a}	15.7 (3.1)^{a}	16.7 (6.2)^{b}	12.3 (3.3)^{ab}	10.0 (5.1)^{a}	9.0 (2.6)^{a}
14	26.8 (9.4)^{a}	25.6 (7.4)^{a}	25.9 (3.8)^{a}	23.1 (8.1)^{a}	29.7 (11.5)^{a}	22.5 (5.8)^{a}
20	32.3 (15.5)^{bc}	36.6 (13.6)^{cd}	41.1 (18.5)^{d}	28.9 (12.1)^{ab}	28.6 (14.3)^{ab}	25.1 (9.5)^{a}
Average total length in mm						
8	13.0 (1.6)^{a}	13.9 (1.6)^{a}	13.8 (1.1)^{a}	13.3 (1.0)^{a}	12.4 (1.5)^{a}	12.7 (1.1)^{a}
14	16.2 (1.5)^{a}	16.4 (1.4)^{a}	16.2 (0.6)^{a}	15.7 (1.6)^{a}	16.5 (1.4)^{a}	15.5 (0.9)^{a}
20	17.6 (2.4)^{ab}	17.7 (2.2)^{ab}	18.0 (2.1)^{b}	17.1 (2.3)^{a}	17.0 (2.0)^{a}	16.8 (4.6)^{a}
Average larval development stage - LDS						
8	2.5 (0.1)^{a}	2.6 (0.2)^{ab}	2.8 (0.3)^{ab}	2.9 (0.2)^{b}	2.7 (0.3)^{ab}	2.8 (0.3)^{b}
14	4.3 (0.3)^{a}	4.4 (0.2)^{ab}	4.7 (0.3)^{c}	4.6 (0.2)^{bc}	4.6 (0.2)^{c}	4.5 (0.1)^{c}
20	5.4 (1.0)^{ab}	5.6 (0.8)^{bc}	5.8 (0.7)^{c}	5.5 (1.0)^{bc}	5.3 (0.9)^{ab}	5.2 (0.8)^{a}
Final mortality in %						
20	21.2 (2.0)^{a}	26.2 (3.2)^{a}	22.7 (1.9)^{a}	24.7 (2.3)^{a}	26.7 (4.9)^{a}	42.2 (3.4)^{a}
Specific growth rate – SGR (% per day)						
1-20	11.11	11.74	12.35	10.55	10.50	9.85

From 5th day of rearing statistically significant differences in larval development stages (LOS) were also observed (Table 1). The final LDS value ranged from 5.2 (group of 10 ppm T3) to 5.8 (group of 1.5 ppm T3). Larvae receiving the dose of 1.5 ppm T3 exceed the others in all studied parameters, with exception group fed with 0.5 ppm T3.

Final larval mortality ranged from 21.2% in control group to 42.3% in group fed dry diet with 10 ppm T3 (Table 1). There were no statistical differences in mortality between feeding groups.

Discussion

Determination the best hormone level in the diet is probably the most important problem in studies on hormonal stimulation of growth. But results reported by different authors are difficult to compare. Different experiments were taken on various fish species and in various age classes. Moreover, time of rearing lasted from 20 (this study) to 112 days (coho salmon - 9). It seems to be typical in case of using of hormonal growth stimulators in fish diets [5,9,26,27].

Designed in our experiment the best level of triiodothyronine in coregonid starter diet was about 1.5ppm. This level was unexpectedly low, but practically has decided about growth of larvae. Nearing value of stimulation in coho salmon (increase of growth by 35%) and American eel fry (increase by 21%) was observed after long term administration, but doses were of 100 and 60 ppm T3 respectively [9,10]. In other our experiment the use of 1.5 ppm T3 enhanced of *Coregonus albula* larval growth gain by 11-12% in relation to control [28].

Administration of five ppm T3 improved specific growth rate, protein conversion ratio and appetite in Red Sea bream fingerlings [12]. However, the same level of T3 improved only by 3% weight of channel catfish fry [11]. Optimal stimulating level of T3 in the diet is probably a species' attribute. This initially optimal level changes in course of ontogenesis too.

Administration of T3 affected more on advancement of development in larval *Coregonus lavaretus* than on the size parameters (weight and length). Larvae receiving 10 ppm T3 in the diet were more advanced in development until 17th day of rearing than control fish. By the whole experiment they were smaller than controls. It was rather symptomatic, because Luczynski *et al.* [25] found strong mathematic correlation between length of larvae and LDS in four species of coregonid fish. Kobuke *et al.* [29] and Specker [30] pointed out that thyroid hormones have exerted deciding influence on rate and course of fish larval development. The administration of thyroxine by immersion has accelerated metamorphosis in flounder and carp larvae [31,32,33]. Lack of correlation between size of *Coregonus lavaretus* larvae and their LDS have confirmed above observations.

Higgs*et al.* [9] and Woo et al. [12] observed decrease of feeding coefficient in fish fed T3 supplemented diet. Furthermore, Woo *et al.* [12] noted an increase of activity of some digestive enzymes like lipase, α-amylase and disaccharases in T3 treated fish. Other authors reported about influence of thyroid hormones administration on lipid [34] and protein [35]. Different data were very contradictory [4,6,36]. So the mechanism of stimulating T3 effect on fish growth has been unclear yet.

This experiment showed that the use of 3,5,3′-triiodothyronine may effectively stimulate growth of white fish larvae. In experimental group fed with 1.5 ppm T3 average weigh to fish was 28% higher than in control group. Similar results were also recorded by other authors in fry of cohosalm on [9], and in American eel [10].

Stimulating effect of T3 on growth of larval coregonids is less visible in comparison to methyltestosterone [14,23,37]. However, long term administration of androgens (including methyltestosterone) in the diet may lead to histopathological changes of many organs, like gonads, liver, alimentary tract and kidney [6,11,38,39]. Gannam & Lovell [39] reported that pathological changes causing T3 administration in channel catfish fry were very slight in comparison with those caused by androgens. This confirms also our earlier results obtained for other coregonids. Administration of 5ppm methyltestosterone caused pathological changes in liver and kidney of *Coregonus albula* larvae [40]. *Coregonus albula*and *Coregonus lavaretus* are very close species, Stimulation of *Coregonus albula* larval growth by T3 did not caused any changes in the internal organs [41]. But not yet similar detailed histological studies for *Coregonus lavaretus* larvae. After visual observations of larval body we suppose that the effect is similar. In general, it may also suggest that triiodothyronine is more harmless growth stimulator for fish' organism than androgens [42], but stimulation with T3 is less effective than male sex steroids. Further comparative studies on this problem are needed.

Conclusions

We conclude that the best results for larval growth enhancing were obtained at dose of 1.5 ppm T3. Supplementation of dry starter diets for whitefish larvae with other T3 doses used in the experiment also enhances moderately larval growth and development (with exception of maximal dose 10 ppm T3).

Conflict of interest

The authors have declared no conflict of interest.

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