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EFFECT OF SIMULTANEOUS ADMINISTRATION OF 17 α-METHYLTESTOSTERONE AND OF 3,5,3'-TRIIODO-L-THYRONINE IN STARTER DIETS ON REARING OF WHITEFISH (*Coregonus lavaretus* L.) LARVAE

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A B S T R A C T. Whitefish (*Coregonus lavaretus* L.) larvae were rearing exclusively on dry diets in water temperature 14±0.5 °C for 20 days. Experimental groups received diets containing different levels of 17 α-methyltestosterone (MT) and 3,5,3'-triiodo-L-thyronine (T3). Group B was fed diet with addition of 5 ppm MT, group C 4 ppm MT + 0.5 ppm T3, groups D 5 ppm MT + 1.5 ppm T3 and E - 6 ppm MT + 3 ppm T3, respectively. Control group (A) was fed on diet without hormones.
At the end of rearing, larvae receiving a diet 5 ppm MT + 1.5 ppm T3 (group D) were heavier and longer ($P<0.05$) than all the remaining ones. All larvae receiving the two hormones in diets (groups C, D and E) were more advanced in larval development (LDS)($P<0.05$) than the controls. There were no statistically significant differences in mortality between the feeding groups.

Key words: WHITEFISH LARVAE, REARING, METHYLTESTOSTERONE, TRIIODOTHYRONINE

INTRODUCTION

Methyltestosterone (Matty and Cheema, 1978; Donaldson *et al.*, 1979; Higgs *et al.*, 1982; Matty and Lone, 1985) and triiodothyronine (Higgs *et al.*, 1979; Degani and Gallagher, 1986; Woo *et al.*, 1991) may be effective growth stimulators in fish. This finding was also confirmed in case of coregonid fish larvae (Poczyczynski *et al.*, 1990; Mamcarz *et al.*, 1994; Mamcarz *et al.*, 1995a, b). Despite the fact that both methyltestosterone and triiodothyronine have been recognized as being well absorbed from the intestine lumen and effective fish growth stimulators (Matty and Cheema, 1978; Donaldson *et al.*, 1979; Higgs *et al.*, 1979, 1982; Whitaker and Eales, 1993), these hormones have not been administered simultaneously in the same diet so far. The only exception was an experiment on coho salmon yearlings (Fagerlund *et al.*, 1980).

The aim of the experiment was to test the effects of simultaneous use of different levels of MT and T3 in dry diet on the results of rearing whitefish larvae.

MATERIALS AND METHODS

Whitefish (*Coregonus lavaretus*) fertilized eggs originated from spawners hatched in Hańcza Lake (Suwałki District, Northern Poland). Eggs were incubated in Weiss' jars. Newly hatched larvae (initial weight 5.6±1.3 and length 10.9±0.6 mm) after thermal acclimation were divided into 5 groups in three replicates and located in 18 tanks (volume 3 dm^3 each, at stocking density 330 larvae per tank). Tanks were supplied with filtered, recirculated water, the flow was about 0.2 dm3 per minute, temperature was 14±0.5 ^oC. Larvae were fed manually every hour from 7.00 to 20.00 hours. At the same time, the tanks were illuminated. Tanks were cleaned once a day between 17.00 and 18.00 hours. Larval mortality was registered every day. The experiment lasted for 20 days.

17 α-methyltestosterone (MT) and 3,5,3'-triiodo-L-thyronine (T3) (both produced by Sigma Corporation, St. Louis, U. S. A.) were added to the experimental diets. Their composition was the same as in the starter diet for coregonid fish used previously (Mamcarz *et al.*, 1995; Poczyczynski *et al.*, 1995). Experimental and control diets had the same composition and differed only in the levels of hormones. Group B received only methyltestosterone (5 ppm MT), group C both MT and T_3 (4 ppm MT+ 0.5 ppm T3), similar groups D and E: 5 ppm MT + 1.5 ppm T3 and 6 ppm MT + 3 ppm T3 respectively (Table).

For growth analysis the larvae were sampled every 3 days from the 5th day of rearing. After preservation in 4% formaldehyde, as determined by solution, they were weighed and measured. Larval developmental stages in accordance with Luczynski *et al.* (1987) were also recorded. 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 - average individual weight [mg] at the beginning of rearing;

W_2 - average individual weight [mg] at the end of rearing;

t - duration of rearing period in days.

TABLE

Results of whitefish larvae rearing

Day of rearing	Feeding group				
	A	B	C	D	E
Hormone level (MT+T3) in ppm	0 ++ 0	5 ++ 0	4 ++ 0.5	5 ++ 1.5	6 ++ 3
Average weight (mg)					
8	11.3a	9.5a	10.7a	11.0a	12.3a
	(2.3)	(2.4)	(5.7)	(4.8)	(3.0)
14	14.1a	20.0ab	24.4c	19.6ab	17.3ab
	(7.1)	(8.3)	(14.0)	(12.3)	(6.1)
20	24.6a	26.1a	27.0a	37.0b	29.2a
	(10.9)	(17.6)	(14.8)	(18.6)	(16.9)
Average length (mm)					
8	12.8a	12.7a	12.6a	13.2a	12.6a
	(0.7)	(0.5)	(1.0)	(1.4)	(1.2)
14	14.8a	14.8a	15.8a	15.1a	14.8a
	(1.6)	(1.8)	(2.8)	(2.6)	(1.6)
20	16.1a	16.5a	16.9a	18.2b	16.7a
	(1.9)	(2.6)	(2.5)	(2.3)	(2.2)
Average larval developmental stage (LDS)					
8	1.7a	1.7a	1.7a	1.7a	1.9a
	(0.5)	(0.5)	(0.7)	(0.7)	(0.7)
14	2.2a	3.0ab	3.4b	3.0ab	2.9ab
	(0.4)	(1.3)	(1.1)	(1.1)	(0.5)
20	3.2a	3.6b	3.8b	4.1c	3.6b
	(0.9)	(0.8)	(0.8)	(0.7)	(0.7)
Specific growth rate (SGR) (% per day)					
1-20	7.40	7.69	7.87	9.44	8.26
Larval mortality (%)					
20	13.3a	20.9a	18.1a	15.5a	15.0a
	(1.8)	(0.9)	(1.7)	(2.1)	(0.6)

Standard deviation in parentheses. Means with the same superscript are not significantly different (P<0.05, Duncan's test)

RESULTS

The statistical differences (P<0.05) in average weight between the feeding groups were noted from the 11th day of rearing (Table). The final mean weight was from 24.6 mg in the control group (A) to 37.0 mg (in group D 5 ppm MT + 1.5 ppm T3). Larvae from group D were statistically heavier (P<0.05) than all the remaining ones.

In average length of the larvae, statistical differences (P<0.05) were observed from the 17th day of rearing. The final mean length was from 16.1 mm (group A) to

18.2 mm (group D)(Table). All larvae receiving MT and T3 simultaneously (groups C, D and E) were heavier and longer than the controls and the groups receiving only MT (group B). Specific growth rate values (Table) were from 7.40 (control group) to 9.44% per day (group D).

Statistical differences in larval developmental stages were observed from the 11th day of rearing (LDS - Table). The final value of LDS ranged from 3.3 (group A) to 4.1 (group D). On the last day of the experiment, all groups of larvae receiving both hormones simultaneously were more advanced in development than the controls ($P<0.05$). At the end of the experiment, the "two-hormone" larvae also exceeded group B (receiving only MT) in all parameters mentioned earlier, but no statistical differences were noted (excluding the best growing and developing group D).

The final larval mortality was from 13.3% (control group) to 20.9% (group B) (Table). In case of this parameter, there were no statistical differences between the feeding groups.

DISCUSSION

The mechanism of growth promoting effect caused by triiodothyronine and methyltestosterone is unclear so far. Thyroid hormones affect lipid, carbohydrate and protein metabolism (Narayansingh and Eales, 1974; Higgs *et al.*, 1992; Plisetskaya *et al.*, 1992). In fish larvae, thyroid hormones decide about rate and course of larval development (Inui and Miwa, 1985; Lam, 1985; Lam and Sharma, 1985; Kobuke *et al.*, 1987; Specker, 1988). The influence of methyltestosterone on fish organism is probably the most complicated. Steroid hormones act inside cell nucleus and affect DNA, influencing gene expression directly (Jensen and De Sombre, 1972; Yamamoto and Alberts, 1972, Lone and Matty, 1980). The remaining hormones (including thyroid ones) act mostly on the surface of the cell membrane changing its permeability and/or moderating the activity of the other hormones (Sutherland, 1972; Stryer, 1981). Several authors have observed interaction between androgen administration in the diet or by injection and growing thyroid gland activity (Van Overbeeke and McBride, 1971; Hunt and Eales, 1979; Fagerlund *et al.*, 1980). The question is whether methyltestosterone and triiodothyronine may act synergically, matually stimulating their activities.

Fagerlund *et al.* (1980) used in their studies on coho salmon fingerlings different diets containing: both methyltestosterone and triiodothyronine, only methyltestoste-

rone, only triiodothyronine, and no hormones. In another experiment, Higgs *et al.*, (1977) gave coho salmon fingerlings simultaneously MT in the diet and T4 (thyroxine - thyroid hormone acting similar to T3) by injection. At the same time, the other fish groups received only MT in the diet, only T4 by injection, or the control hormone-free diet. In both experiments, fish receiving the two hormones simultaneously have grown better than all the remaining groups. The order of growth responses magnitude was: MT+T3(T4)> MT> T3(T4)> control. The study on whitefish larvae has confirmed these observations. All larvae from the groups receiving simultaneously MT and T3 were heavier and longer than the larvae receiving only MT in the diet, whereas the MT-treated larvae were bigger than the control ones.

In the previous laboratory studies on whitefish larvae, the optimal (for growth promoting effect) levels of separately administered MT and T3 in coregonid starter diet have been determined. The doses of MT which have effectively promoted whitefish larvae growth were from 4 to 6 ppm, whereas the doses of T3 were from 0.5 to 1.5 ppm. The optimal level has been designated as 5 ppm for MT and 1.5 ppm for T3. The use of those optimal levels has caused that MT-stimulated larvae were 44%, and T3-stimulated 28% heavier than the controls after 20 days (Poczyczyński, 1992; Poczyczyński data unpublished). After 20 days of this experiment, whitefish larvae from the best growing group D (receiving 5 ppm MT+1.5 ppm T3) were 50% heavier than the control ones. Therefore, simultaneous administration of MT and T3 has resulted in smaller growth promoting effect in comparison to simple algebraic sum of possible individual stimulating potential of these hormones in whitefish (50% *versus* 44+28%). The same phenomenon was observed in the above mentioned experiments on coho salmon (Higgs *et al.*, 1977; Fagerlund *et al.*, 1980).

If synergic positive interaction between methyltestosterone and triiodothyronine occured, then the cumulative effect of this hormone administration might have been anticipated; more visible growth could be expected stimulation with comparison to the separate use. However, it may be also possible that the hormone-caused growth promoting effect has the upper limit which could be connected with possible maximum of growth rate potential of a given species in given conditions (water temperature, growth stimulator(s), diet digestibility, feeding coefficient).

The optimal hormone levels for whitefish growth in the "two hormone stimulated larvae" were 5 ppm MT and 1.5 ppm T3. Synergic positive interaction between MT and T3 might have probably reduced the optimal level of individual hormone in the diet. This would be caused by mutual activity stimulation. However, the optimal

level of both MT and T3 has been the same, independently of whether the hormones were administrered simultaneously (this experiment) or separately (Poczyczynski, 1992; Poczyczynski unpublished data).

All the above mentioned data have excluded positive synergic interaction between methyltestosterone and triiodothyronine in whitefish larvae. The two hormones have probably acted relatively independently and have not stimulated each other. Probably either of these hormones "has made itself": MT (as a stronger growth stimulator) in the first place has stimulated fish growth, whereas T3 has accelerated larval development.

It has been symptomatic that the larvae from all groups receiving simultaneously MT and T3 have been more advanced in larval development (measured as mean LDS) than both the control ones and those that received only MT. The strong connection between T3 administration in the diet and acceleration of larval development has been noted in one former experiment too (Poczyczynski data unpublished).

Simultaneous methyltestosterone and triiodothyronine treatment, despite a possible lack of synergic positive interaction of these hormones, has produced more visible growth promoting effect in whitefish larvae with comparison to separate use of these hormones. Moreover, the other positive effect of the "two-hormone" stimulation has been the acceleration of the larval development. The phenomenon suggests potential reduction of the larval period, one of the most critical phases of fish ontogeny.

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STRESZCZENIE

WPŁYW JEDNOCZESNEGO PODAWANIA W PASZACH STARTOWYCH RÓŻNYCH DAWEK 17 α - METYLOTESTOSTERONU I 3,5,3'-TRÓJJODO - L TYRONINY NA WYNIKI PODCHOWU LARW SIEI (***Coregonus lavaretus*** L.)

Przeprowadzono dwudziestodniowy podchów larw siei (*Coregonus lavaretus* L.) na paszach sztucznych w temp. 14±0,5°C. Grupy doświadczalne żywiono paszami zawierającymi różny poziom 17 α-metylotestosteronu (MT) i 3,5,3'-trójjodo-L-tyroniny (T_3). Grupa B była żywiona paszą z dodatkiem 5 ppm MT, grupa C 4 ppm MT + 0,5 ppm T_3, grupa D 5 ppm MT + 1,5 ppm T_3, zaś grupa E 6 ppm MT + 3 ppm T_3. Grupa kontrolna (A) otrzymywała paszę bez hormonów.

Na zakończenie doświadczenia larwy otrzymujące w paszy 5 ppm MT + 1,5 ppm T_3 (grupa D) były cięższe i dłuższe ($P.< 0,05$) od wszystkich pozostałych. Wszystkie larwy otrzymujące w paszach oba hormony równocześnie (grupy C, D i E) były bardziej zaawansowane w rozwoju larwalnym (LDS) ($P.< 0,05$) niż kontrolne. Nie odnotowano natomiast statystycznie istotnych różnic pomiędzy grupami w śmiertelności larw.

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