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THE EFFECT OF 17α-METHYLTESTOSTERONE AND 11β-HYDROXYANDROSTENEDIONE ON THE DEVELOPMENT OF REPRODUCTIVE SYSTEM IN RAINBOW TROUT (*Oncorhynchus mykiss* Walbaum)

Krystyna Demska-Zakęś, Piotr Hliwa*, Paweł Matyjewicz*, Zdzisław Zakęś***

*Warmia and Masuria University in Olsztyn
**The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn

ABSTRACT. The effects of 17α-methyltestosterone (MT) and 11β-hydroxyandrostenedione (OHA) applied in feed for 56 days on the development of reproductive organs of rainbow trout, *Oncorhynchus mykiss* (Walbaum), were studied. It was observed that OHA was very effective in fish sex reversal. Treatment with 20 ppm of this hormone for 8 weeks resulted in 100% male population with correctly developed testes. Application of a lower dose (5 ppm) resulted in sex reversal in 30% of fish (80% of males). The same dose of MT produced lower sex reversal level (70% of males). Higher doses of this hormone or longer treatment period resulted in fish sterilization.

Key words: RAINBOW TROUT, ANDROGENS, SEX REVERSAL

INTRODUCTION

Notwithstanding development of rainbow trout rearing in Poland, the demand is still higher than production (Bontemps 1995). In 1996, rainbow trout production was about 7 thousand tons, and retail trade increased by 30% (Bontemps 1997). This resulted from increasing demand for this tasty fish and establishment of new commercial fishing grounds (Pokusa, Wołos 1997). Anglers fishing in commercial fishing grounds are usually interested in catching either many or large fish which are scarce under natural conditions. This demand is very well met by rainbow trout, a fish well known and easily reared.

Market-size fish are obtained in 16-20 months using traditional method of rearing (Steffens 1986). Fish size and weight depend on water quality, availability and quality of food, and fish health. Fish growth depends also on their sex. Thus, rearing of sterile or monosexual populations seems advantageous from the commercial point of view. Such populations may be obtained using genome engineering methods: gynogenesis, androgenesis, poliploidy induction, or manipulating with environmental conditions: temperature, salinity or geomagnetic field etc. Phenotypic sex may be also reversed

using hormonal treatments. Monosexual populations can be obtained using steroid hormones in two ways: direct or indirect (Bieniarz, Epler 1991). Direct methods involve application of androgens or estrogens in species-specific doses, before sex differentiation. Indirect methods are based on cross-breeding of these fish with "normal" individuals, this resulting in production of monosexual offspring. Sterile populations are obtained using high hormone doses before sex differentiation, or prolonged treatment with low concentrations (Van der Hurk, Slof 1981, Billard 1992). In such populations, feed conversion is more efficient since food is used for somatic growth only, and not for reproduction (Kamler 1992).

Recently, considerable progress took place in sex manipulation of salmonid fishes (Chourrout 1987), and all-female populations became popular in aquaculture (Bye, Lincoln 1986, Purdom 1993). Sex reversal was done by chromosome manipulations, or using steroid hormones, mainly 17α-methyltestosterone. The phenotypic males were used for breeding, which resulted in all-female populations without direct hormonal treatment.

In the present study the effects of two steroid hormones: synthetic 17α-methyltestosterone and natural 11β-hydroxyandrostenedione on sex reversal and sterilization of rainbow trout were compared.

MATERIAL AND METHODS

The experiment was carried out on feeding rainbow trout *Oncorhynchus mykiss* (Walbaum) fry of average body weight 0.129±0.01 g, and length 2.28±0.2 cm, obtained from trout farm in Ruś near Olsztyn. The fish were placed in 10 tanks of 30 dm^3, 400 individuals in each. Water temperature was 16.5±0.5^oC. Fish rearing started on May 9, 1997. The fish were fed commercial trout feed Kristall 3600 produced by Aller Møller. Control fish were given feed without hormones, and experimental groups – feed supplemented with 5 or 20 ppm of 17α-methyltestosterone (MT) or 11β-hydroxyandrostenedione (OHA). The feed was supplied 4-6 times a day, at the level of 5-6% of stock weight. Pellet size was changed according to fish size. Rearing lasted 56 days.

To evaluate the effect of OHA and MT on sex differentiation, samples of 30 fish were taken from each group at the end of rearing. The fish were measured, weighed, and fixed in Bouin's solution (Zawistowski 1986). Then, the samples were dehydrated in ethanol solutions from 70% to 100%, immersed in xylene, and embedded in

paraffin. Preserved fish were cut into 5 μm thick sections using rotation microtome, and stained according to HE method (with haematoxylin and eosin) (Zawistowski 1986). The preparations were examined using light microscope. The results were subjected to one-way ANOVA, and Tukey's test, at significance level P = 0.05.

RESULTS

At the end of rearing, control fish reached average body length of 6.3 cm and weight 3.321 g (Table 1). Large blood vessels were observed in female gonads, and developing ovary lamellae. Gonads contained previtellogenic oocytes, often with Balbiani's nuclei (Photo. 1). Male gonads were relatively small and oblong. Main blood vessels were situated at dorsal side of the testes. Gonads contained spermatogonia and primary spermatocytes (Photo 2).

TABLE 1

Fish body length, weight, and percentage of males at the end of the experiment. Values with the same letter index in rows do not significantly differ (P<0.05)

	Experimental groups				
	Control	MT	MT	OHA	OHA
	0	5 ppm	20 ppm	5 ppm	20 ppm
Total body length (cm)					
Range	5,40 - 7,20	5,10 - 7,20	5,20 - 7,60	5,40 - 7,50	5,30 – 7,50
Mean	6,33^{a}	6,02^{a}	6,02^{a}	6,36^{a}	6,26^{a}
SE	0,09	0,10	0,14	0,09	0,13
Body weight (g)					
Range	1,98 - 4,98	1,69 - 4,99	1,65 - 5,63	2,08 - 5,88	2,15 - 5,77
Mean	3,32^{ab}	2,77^{a}	3,05^{ab}	3,57^{b}	3,51^{b}
SE	0,17	0,15	0,21	0,20	0,17
Sex ratio (%)					
Male	50	70	40	80	100
Female	50	0	0	10	0
Biseual	0	30	10	10	0
Sterile	0	0	50	0	0

Length of fish in the group fed pellets containing 5 ppm of MT did not significantly differ from the control, but average body weight was significantly lower (Table 1). This group consisted in 70% of males and 30% of bisexual individuals (Table 1). Male gonads were similar to those observed in the control, with the exception of larger amount of connective tissue. In histological sections, seminal vesicles containing sperma-

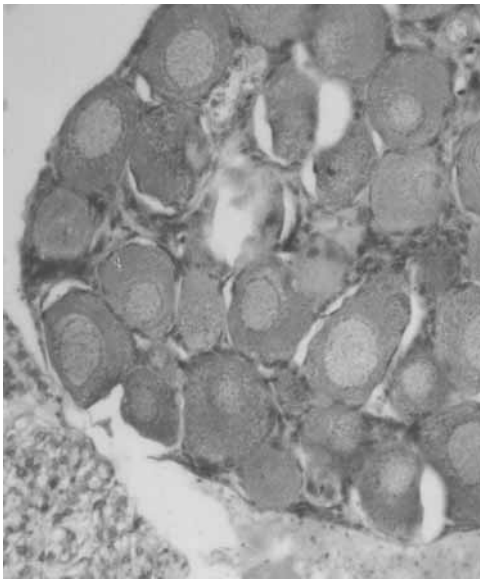

Photo. 1. Female gonad of a control individual (500 x)

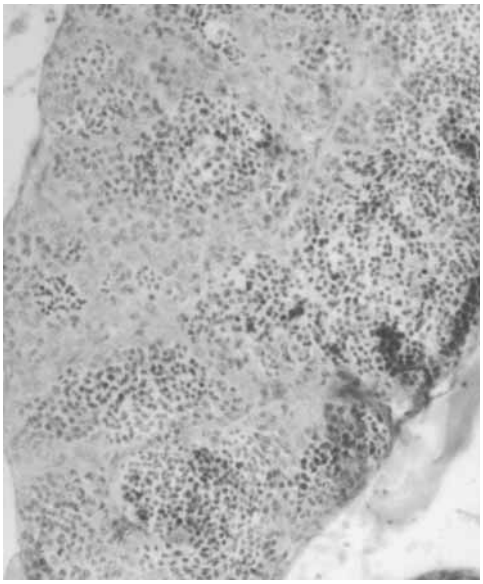

Photo. 2. Male gonad of a control individual (500 x)

togonia and spermatocytes primary were clearly visible. In bisexual individuals, the gonads showed ovary-like or testis-like shape. They contained numerous previtellogenic oocytes and structures typical for testes – seminal vesicles (Photo 3).

In the group treated with 20 ppm of MT, male and bisexual gonads were similar; males comprised 40% of fish, bisexual individuals – 10%, and 50% were sterile fish.

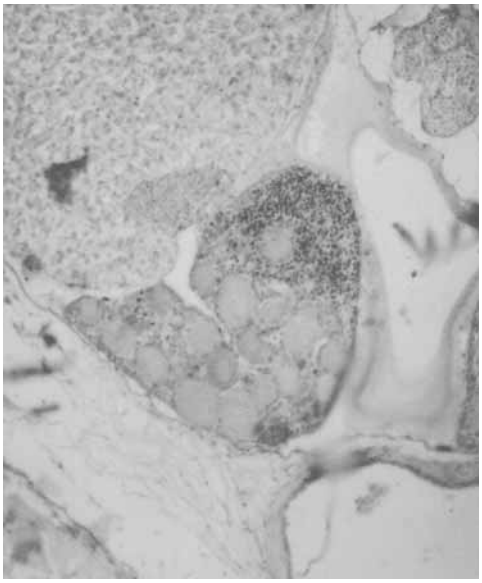

Photo. 3. Gonad of bisexual individual fed the feed containing 5 ppm of MT (300 x)

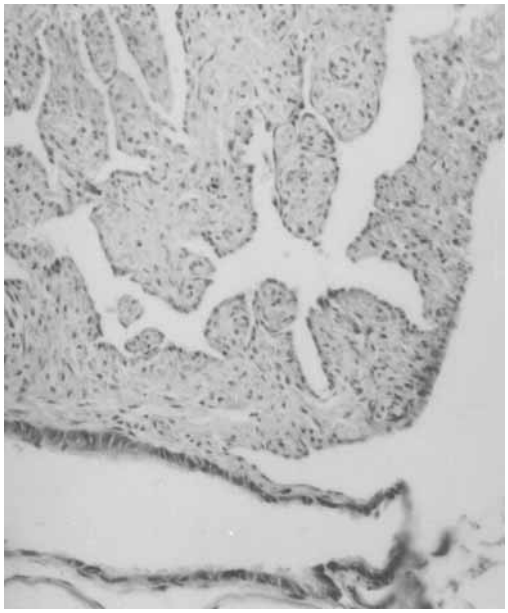

Photo. 4. Gonad of sterile individual fed the feed containing 20 ppm of MT (500 x)

Gonads of sterile fish were of various shapes and with different somatic cells, sometimes individual sexual cells were present (Photo. 4). Average length of these fish did not significantly differ from the group treated with 5 ppm of MT. Body weight did not differ from the control, but was significantly higher than in 5 ppm of MT group (Table 1).

Average length of fish fed pellets with an addition of 5 ppm of OHA was very similar to the control, but final body weight was significantly higher (Table 1). This group consisted in 80% of males, 10% of females, and 10% of bisexual individuals (Table 1). Male gonads were similar to testes of the control fish and contained well developed seminal vesicles, small blood vessels, and slightly more connective tissue. Correctly developed previtellogenic oocytes were observed in the ovaries. Such oocytes were also noted in the gonads of bisexual fish, but presence of seminal vesicles indicated developmental anomalies.

In the group treated with 20 ppm OHA, all-male population was obtained, with well developed testes. Average body length was similar as in other groups, but the fish were significantly heavier compared to the control (Table 1).

DISCUSSION

Sex reversal in fish is usually achieved using 17α-methyltestosterone – a synthetic testosterone analogue (Ostrowski 1988). Steroid hormones are applied in implants, immersion, injection, or with feed (Billard 1992).

In our experiment 5 ppm of MT was applied with feed. After 56 days of rearing, 70% of males were obtained and 30% of bisexual individuals. Appearance of the latter might have resulted from aromatization of 17α-methyltestosterone or natural testosterone in the gonads to estradiol (Norris, Jones 1987). Similar observations were made by Johnstone et al. (1979), who treated rainbow trout fry with 3 ppm of MT for 90 days. Feist et al. (1994) treated rainbow trout with MT used in immersion (40 ppm dm^{-3} of water), in food (3 ppm of feed for 60 days), or both methods together. Percentage of males varied in immersed fish, and in those treated using both methods – almost all-male population was obtained. The best results were observed in the group immersed in the hormone solution one week after hatching of 50% of the fish. Repeated treatments produced adverse effects. In fish subjected to immersion only, well developed seminal ducts were observed, while in groups treated using either both methods or repeated immersions – seminal ducts were occluded. Higher percentage of males (82%) was obtained by Baker et al. (1988) who treated *Oncorhynchus tshawytscha* with 2-20 ppm MT in immersion. So successful sex reversion probably resulted from high sensitivity of this species to low MT concentration.

Application of 20 ppm produced in rainbow trout considerable alteration in sex proportions: 40% of males and 10% of bisexual individuals with testes containing am-

poules, but also oogonia and previtellogenic oocytes – immature female gametes. The remaining 50% of fish were sterile. This might have resulted from too long treatment. Further feeding of fish with hormone-containing pellets would probably cause sterilization of all individuals. On the other hand, shorter period of treatment might produce all-male population. Similar conclusion was formulated by Van den Hurk and Slof (1981) who treated rainbow trout fry with about 10 ppm of MT for over 8 weeks, and 30 ppm for 4 weeks. They obtained sterile fish in both cases. The authors supposed that application of lower hormone doses, or shorter period of treatment, would have produced higher share of males, and excessive dose or time of application resulted in sterilization of all fish.

Fagerlung and McBride (1975) reared *Oncorhynchus kisutch* for 72 weeks using 10 ppm of MT. This resulted in hypertrophy and degradation of spermatogonia in all individuals. Goetz et al. (1979), who immersed *Oncorhynchus kisutch* embryos in hormone solution and then fed the larvae feeds containing MT for 10 weeks did not observe normally developed gonads.

Due to difficulties in setting appropriate MT dose, that would produce successful sex reversal without adverse effect on gonad development, this substance is often replaced with11β-hydroxyandrostenedione (Higgs et al. 1982). It is a natural hormone belonging to the androgens, and produced by secretory cells of testes. According to available data, it does not produce such a strong effect on gonads and reproductive cells as MT (Redding et al. 1987, Feist et al. 1994).

In our experiment, sex reversal of rainbow trout using OHA was satisfactory. In case of 5 ppm applied in feed, 80% of males were obtained, and appearance of 10% of bisexual fish and 10% of females might have resulted from too low hormone dose. Feist et al. (1994) immersed rainbow trout in 40 ppm dm^{-3}, fed the fish pellets containing 3 ppm of OHA, and used both methods together. In the fish subjected to immersion only, low percentage of males was obtained, while in the group treated with OHA in immersion and feed simultaneously – about 70% of males were observed. Males of both experimental groups had well developed seminal ducts, their sperm fertilized the eggs, and all-female offspring was obtained. Redding et al. (1987) applied OHA for *Oncorhynchus kisutch* and *O. keta* masculinization. The fish were treated with 0.5 ppm in immersion for 4 hours 3 times a week, and fed pellets containing 5 or 50 ppm for 4-8 weeks. In 98% of immersed fish testis-like gonads developed. Treatment with 5 ppm for 4 weeks was not satisfactory, but the same dose applied for 8 weeks resulted in sex reversal in

about 14% of fish (64% of males). Higher dose (50 ppm) of OHA applied for the same time produced 90-96% of males.

In the present study, application of 20 ppm of OHA in feed resulted in all-male population. No considerable anomalies in gonad development were observed. This suggests that the dose of 20 ppm, and treatment duration of 8 weeks are optimum for production of monosexual, all-male population. In both experimental groups no sterile individuals were observed, contrary to the groups given MT-supplemented feed. Lack of sterile fish indicates that OHA is not useful for fish sterilization.

OHA was also used in sex reversal of pike, *Esox lucius* (Demska-Zakęś et al. 1999), and African catfish, *Clarias gariepinus* (Van den Hurk et al. 1989). In case of pike, high percentage of males was obtained using 20 and 30 ppm of OHA in feed (80% and 87% respectively). In African catfish only 77% of males were produced with 30 ppm of the hormone. These results confirm that OHA is very useful for production of all-male populations of various fish species.

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STRESZCZENIE

WPŁYW PODAWANIA W PASZY 17α-METYLOTESTOSTERONU I 11β-HYDROKSYANDROSTEDIONU NA ROZWÓJ UKŁADU ROZRODCZEGO PSTRĄGA TĘCZOWEGO (*Oncorhynchus mykiss* Walbaum)

Pstrąg tęczowy jest gatunkiem odgrywającym znaczącą rolę w akwakulturze, a wśród jego hodowców istnieje stałe zainteresowanie tworzeniem populacji monopłciowych lub sterylnych, które efektywniej przyswajają pokarm, ograniczając jednocześnie tworzenie produktów płciowych. Drogą do uzyskania takich populacji są metody stosowane w inżynierii genomowej (gynogeneza, zwiększanie ploidalności), czy też sterowanie czynnikami środowiskowymi lub hormonalnymi. W niniejszej pracy opisano wpływ podawania w paszy dwóch hormonów steroidowych: 17α-metylotestosteronu (MT) i 11β-hydroksyandrostedionu (OHA) w dawkach 0; 5 i 20 ppm, na układ rozrodczy pstrąga tęczowego.

Wyniki badań dowodzą, że obydwa androgeny nadają się do przeprowadzania maskulinizacji pstrąga. "Lepszym" z zastosowanych hormonów okazał się być 11β-hydroksyandrostedion, ponieważ podawanie 20 ppm OHA w paszy przez okres 8 tygodni, pozwoliło uzyskać w grupie doświadczalnej 100% samców. W przedstawionych badaniach nie zaobserwowano też istotnych zmian w budowie morfologicznej i cytologicznej gonad ryb. 17α-metylotestosteron podawany w wyższych dawkach niż 20 ppm lub przez okres dłuższy niż 8 tygodni, może być natomiast z powodzeniem stosowany do sterylizacji pstrąga tęczowego.

ADRESY AUTORÓW

Dr Krystyna Demska-Zakęś
Mgr inż. Piotr Hliwa
Mgr inż. Paweł Matyjewicz
Katedra Ekologii Ewolucyjnej
Uniwersytet Warmińsko-Mazurski
10-718 Olsztyn, ul. Oczapowskiego 5

Dr Zdzisław Zakęś
Instytut Rybactwa Śródlądowego
10-719 Olsztyn, ul. Oczapowskiego 10