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RESEARCH ARTICLE

Effect of sex reversal in rainbow trout (*Oncorhynchus mykiss* Walbaum) using 17α-methyltestosterone and 11β-hydroxyandrostenedione

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Abstract. The aim of the experiment was to compare the effectiveness of sex reversal in mature female rainbow trout using 17α-methyltestosterone (6 ppm-MT) and 11β-hydroxyandrostenedione (20 ppm-OHA). Hormones were administered with the first exogenous feed for up to 60 days. Among the fish treated with OHA, 95% females were reversed into mature neo-males and 1.6% to bisexual individuals. Among the fish treated with MT only, 59.7% females were reversed to neo-males and 1.8% to bisexual individuals. Functional neo-males were 1.6% and 5.3%, respectively. The rest of the neo-males had obstructed seminal vesicles in various stages. The gonadsomatic index was 3.9% at age 3+ in both groups of mature fish. The shape of the testes ranged from elongated to stunted. The bisexual fish had immature oocytes and mature testes with viable semen that was used for egg fertilization.

Keywords: sex control, GSI, rainbow trout, sex reversal

Introduction

There has been growing demand in recent years for the culture of mono-sex female fish populations for the consumer market. Rainbow trout females can be supplied through gynogenesis as salmonids have an XY sex determination system with male heterogamety. In subsequent generations, this is achieved by fertilizing eggs with milt obtained from masculinized females (XX). Although it is also possible to obtain a female phenotype population through hormonal therapy (Johnstone et al. 1979, Goryczko et al. 1991), the application of hormones in the production of food is generally forbidden in most countries. Traditionally, sex reversal from female to male in rainbow trout has been achieved by treating the fish with small doses of methyltestosterone in the starter feed from days 60 to 90 following the beginning of exogenous feeding. However, the application of 11β-hydroxyandrostenedione is more effective and safer in terms of overdose risk (Demska-Zakęś et al. 1999). This was proved in the current research, in which mono-sex female rainbow trout were masculinized. In the most recent research, scientists are trying to disrupt the process of the aromatization of estradiol from testosteron through the application of aromatase inhibitors to produce sex-reversed females (Afonso et al. 2000, Lee et al. 2006, Vizziano et al. 2008). The inhibition of the male differentiation process with cytochrome P450 is one of the steps required to do this (Govorum et al. 2001), but this technology has not yet been developed for rainbow trout.

The aim of the current study was to compare the effectiveness of 6 ppm 17α-methyltestosterone and

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20 ppm 11-β-hydroxyandrostenedione for sex reversal in mature female rainbow trout.

Materials and Methods

The study material comprised mono-sex female rainbow trout fry produced at the Inland Fisheries Institute in Olsztyn, Department of Salmonid Research in Rutki. The great-grandparents of the fry were obtained through gynogenesis, while subsequent generations were achieved through fertilizing eggs with milt obtained from masculinized females.

In the current experiment, the fish were treated with a dose of 6 ppm 17α-methyltestosterone (MT) and 20 ppm 11β-hydroxyandrostenedione (OHA) delivered with the feed. Hormones were given to the fry from the time they began exogenous feeding for a period of 60 days. The appropriate amount of hormone (diluted in 96% ethanol) was added to the Bio-Optimal trout starter (BioMar, Denmark) using the spray method. The fish were fed *ad libitum*. Control fish were fed without the hormone treatment. The initial number of reared fish was 150 specimens per group. The sex ratio was examined during the spawning season at age 3+. Testicular milt was used to fertilize the eggs. The gonadsomatic index (GSI) was calculated as the ratio of gonadal weight (Wtestes) to the total fish weight (Wfish):

$$GSI = 100\% \times Wtestes \times Wfish^{-1}$$

The weights of the whole fish, gutted fish, and testes were measured using samples of 20 sex-reversed neo-males from each group. Statistical differences among groups were tested with ANOVA analyses using the Statistica 7 computer program (StatSoft Inc., Tulsa, OK, USA)

Results

From the group of rainbow trout treated with 6 ppm MT, 61.5% neo-males were obtained, including 1.8% bisexual individuals at age 3+. Mature females comprised 36.7%, and sterile females 1.8% of the population. The application of 20 ppm OHA resulted in the masculinization of 96.6% of the trout including, 1.6% bisexual individuals. Females and sterile fish comprised 1.7% each (Table 1).

Table 1

Sex ratio in a mono-sex female population of rainbow trout masculinized with 17α-methyltestosterone (MT) and 11β-hydroxyandrostenedione(OHA) at age 3+

	MT – 6 ppm		OHA – 20 ppm	
Sex	n	(%)	n	(%)
Dysfunctional males	31	54.4	57	93.4
Functional males	3	5.3	1	1.6
Total males	34	59.7	58	95.0
Bisexual	1	1.8	1	1.6
Females	21	36.7	1	1.7
Sterile	1	1.8	1	1.7
Total	57	100.0	61	100.0

Functional males, with properly developed and unobstructed seminal vesicles (Photo 1a), comprised 5.3% of the population in the MT group and 1.6% in the OHA group. The dysfunctional males had well-developed testes containing sperm capable of fertilization, but the spermatic ducts were obstructed and in various stages of reduction (Photo 1b, 1c). The mean weight of the testes was 3.9% of total fish weight (Table 2).

Table 2

Body examinations of rainbow trout sex-reversed neo-males (n = 20) using 17α-methyltestosterone (MT) and 11β-hydroxyandrostenedione (OHA) at age 3+. Different letter indexes indicate statistically significant differences among groups ($P < 0.05$), * – gonadsomatic index (GSI)

Weight	T – 6 ppm		OHA – 20 ppm	
	(g/pcs.)	(%)	(g/pcs.)	(%)
Whole fish	975.3[a]	100.0	958.5[a]	100.0
(SD)	(156.8)	-	(130.3)	-
Gutted fish	850.0[b]	87.1	837.8[b]	87.4
(SD)	(140.5)	-	(115.1)	-
Testes	38.4[c]	3.9*	37.8[c]	3.9*
(SD)	(12.2)	-	(8.6)	-

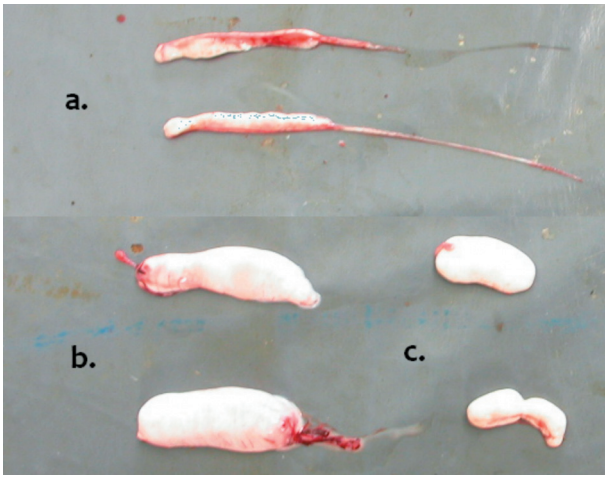

Photo 1. Testes in sex-reversed rainbow trout females: a – elongated with properly developed and unobstructed seminal vesicles in functional males; b and c – stunted shape with obstructed spermatic ducts in various stages of reduction in dysfunctional males.

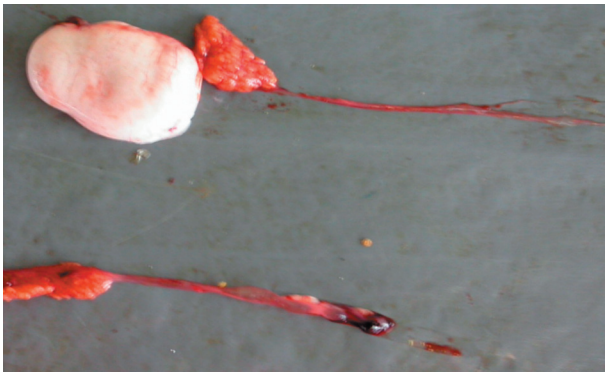

Photo 2. Gonads of bisexual rainbow trout with well-developed left testicles (upper) and immature oocytes. Right testicle is undeveloped (lower).

ANOVA analyses did not indicate any statistical significant differences ($P < 0.05$) between the application of MT or OHA in these two groups of reversed females to neo-males, or between the weight of testes and whole or gutted fish. The shape of the testes ranged from elongated to stunted (Photo 1). Bisexual fish had immature oocytes that comprised up to 0.3% of the fish weight, and mature testes with semen capable of fertilizing eggs (Photo 2). The control fish ($n = 69$) were all females. Survival at age 3+ in the groups treated with MT and OHA, and the control group was 38.0, 40.7, and 46.0%, respectively.

Discussion

In research on sex reversal in rainbow trout, Demska-Zakęś et al. (1999) used 20 ppm OHA in a starter diet and obtained 100% males, while with 5 ppm MT, they obtained 70% males and 30% bisexual individuals. Since this experiment was based on a two-sex population consisting of 50% males and 50% females (in the control group), it can be concluded that after female masculinization, the authors obtained 100% neo-males in the OHA group, and about 40% neo-males plus about 60% bisexual individuals from the MT group. In this study, the sex ratio of the MT group was verified through microscopic analysis of gonad samples taken from fish that were 56 days old.

The current study employs the same sex reversal method as that presented in Demska-Zakęś et al. (1999); however, the sex ratio was checked after the fish had matured at age 3+. The application of 20 ppm OHA in the starter feed delivered to mono-sex female fry of rainbow trout for a period of 60 days resulted in a 96.6% sex reversal into neo-males, including 1.6% bisexual individuals (Table 1). A slightly higher dosage of 6 ppm was used for female sex reversal with MT, and this resulted in a 61.5% share of sex-reversed neo-males, including 1.8% bisexual individuals. The rest of the fish were either mature females (36.7%) or sterile individuals (1.8%). Similar sex reversal ratios were obtained by Bieniarz et al. (1991) when 3 ppm of MT were used. Initiating hormone treatment before the start of exogenous feeding could probably improve the sex reversal ratio. The combination of MT immersion (0.4 mg dm^{-3} per 2 h) before feed intake (1-2 weeks after hatching), followed by oral administration (3 mg kg^{-1} feed) produced a nearly 100% male population (Feist et al. 1995). In the current study, the same result was obtained using OHA (20 ppm) administrated only with the feed. A lower dosage of OHA in the feed (3 ppm), even combined with the immersion treatment before feed intake, produced up to 70% of sex-reversed neo-males (Feist et al. 1995). Therefore, it was confirmed that the administration of 20 ppm

OHA with the starter diet for 60 days (Demska-Zakęś et al. 1999) is a very effective method for female sex reversal. However, the absence of spermatic ducts in neo-males means they can only be used once for reproduction (Bye and Lincoln 1986). Survival in the MT and OHA groups was similar at 38.0 and 40.7%; these small differences were caused by unknown factors. However, the highest survival was noted in the control groups (46.0%) which comprised only females. The gonadsomatic index in sex-reversed females (neo-males) with MT and OHA was the same at 3.9%. The GSI value has a direct influence on the total number of spermatozoa, which is very important for the reproductive cycle. The concentration of spermatozoa in the testes of rainbow trout males is $5.8x10^{10}$ g^{-1} of gonad, and the GSI is usually within the range of 6-8% (Billard 1992).

Conclusions

1. Using OHA is much more effective than MT for sex reversal in rainbow trout.
2. Testis weight and body index do not differ significantly among the sex-reversed neo-males from either the groups treated with OHA or MT.

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Streszczenie

Efekt odwracania płci u pstrąga tęczowego (*Oncorhynchus mykiss* Walbaum) przy użyciu 17α-methyltestosteronu oraz 11β-hydroxyandrostenedionu

Porównano wyniki odwrócenia płci u samic pstrąga tęczowego za pomocą 17α-methyltestosteronu (MT) oraz 11β-hydroksyandrostenedionu (OHA) podawanego wraz z paszą w dawce odpowiednio 6 ppm i 20 ppm przez pierwsze 60 dni żywienia. Po osiągnięciu dojrzałości płciowej w wieku 3+ u ryb poddanych działaniu OHA uzyskano 95% neo-samców, 1,6% ryb obupłciowych oraz 1,7% samic i 1,7% osobników sterylnych. W pełni funkcjonalne neo-samce stanowiły 1,6% populacji. Charakteryzowały się one w pełni funkcjonalnymi, drożnymi, nasieniowodami oraz możliwością wielokrotnego pozyskiwania nasienia. Natomiast większość neo-samców, stanowiących 93,4% populacji, były rybami z dobrze wykształconymi jądrami oraz w różnym stopniu zredukowanymi nasieniowodami, których wspólną cechą był brak ich funkcjonalnej drożności. Efektywność maskulinizacji pstrągów za pomocą MT była niższa – uzyskano 59,7% neo-samców, 1,8% ryb obupłciowych, 36,7% samic oraz 1,8% osobników sterylnych. Kształt jąder neo-samców w obu grupach był podobny, zawierał się od wydłużonych do kulistokształtnych. W wieku 3+ przeciętna masa jąder wynosiła 37,8-38,4 g, a GSI stanowił 3,9%. U osobników obupłciowych nie było dojrzałej ikry, zaś występujące oocyty były w stadium poprzedzającym i/lub początkowym wzrostu protoplazmatycznego. Ryby w grupie kontrolnej były wszystkie samicami.