Arch. Ryb. Pol.	Archives of Polish Fisheries	Vol. 5	Fasc. 2	325 - 336	1997

SEX REVERSAL IN PIKEPERCH, *Stizostedion lucioperca* (L.), BY ORAL ADMINISTRATION OF METHYLTESTOSTERONE

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A B S T R A C T. Oral administration of 17α-methyltestosterone (MT) was used to sex inverse juvenile pikeperch reared under intensive culture conditions (from D42 to D154). Fish (W=2.3 g) were stocked into six circular flow-through tanks (200 liters, 600 fish per tank). MT was mixed into the feed in dose 30 mg/kg of the diet; the treatment and post-treatment phase lasted 21 and 90 days respectively.
Administration of 30 mg MT/kg of the diet produced about 83.3% males and 10% females. Approximately 6.7% of all the examined pikeperch were classified as fish with abnormal gonads.
In hormone treated group, no morphological changes were observed in the kidney, liver and skin. The hepato-somatic index (HSI) of androgen treated fish was not significantly different from the control ($P>0.05$).
The analysis of variance showed that there were no significant differences in growth, condition factor and survival between the MT-treated group and the control, both in treatment (D63 - D84) and post-treatment (D85 - D154) phase ($P>0.05$).

Key words: *Stizostedion lucioperca* (L.), SEX REVERSAL, METHYLTESTOSTERONE, GROWTH, INTERNAL ORGANS

INTRODUCTION

In fishes sex is genetically determined, but the administration of sex steroid is known to induce sex reversal. The process of sex inversion comprises the entire sequence of differentiation, gametogenesis, behaviour and secondary sexual characteristics. The exogenous sex steroids had a sex-reversal effect depending mostly on the method of the stimulation, dose, fish size or age at which the treatment was applied, and the duration of hormone treatment (Nagy *et al.* 1981, Mirza, Shelton 1988, Demska-Zakęś, Zakęś 1996). The administration of an optimum dose of a sex steroid during the labile period produced the neofemales (genetic males inverted to phenotypic females) or neomales (genetic females inverted to phenotypic males).

Orally administered 17α-methyltestosterone can be used to control the phenotypic sex of juvenile pikeperch, *Stizostedion lucioperca* (L.), (Demska-Zakęś, Zakęś

1996). Treatment with 17α-methyltestosterone at concentrations of 60 and 90 mg/kg of the diet for 21 days in pikeperch (initially 2.2 g wet weight) reared in laboratory condition was used to produce males, neomales, females, intersex and sterile fish. Decrease in androgen dose (30 mg/kg of the diet) increased the proportion of sex-inverted females.

In mammals 17α-methyltestosterone has a nitrogen retention effect and induces muscle development. Similar growth-promoting properties have been shown in common carp, *Cyprinus carpio,* (Lone, Matty 1980) and salmonids (Schreck, Fowler 1982). In contrast to these findings, the growth depressing effect was found in channel catfish, *Ictalurus punctatus*, (Simone 1990) and Nile tilapia, *Oreochromis niloticus*, (Vera Cruz, Mair 1994). Decrease in the weight may be accompanied by an increase in size of the internal organs (Yamazaki 1976). A few studies showed that MT had a detrimental effect on these organs (Simone 1990).

The main objectives of this study were: (a) to test the effectiveness of 17α-methyltestosterone (MT) in sex reversing the pikeperch reared under intensive culture conditions, (b) to study the effect of the hormone on the gonads, liver, kidney and skin, (c) to estimate the effect of MT on the growth and survival.

MATERIALS AND METHODS

HORMONE-FEED PREPARATION

Methyltestosterone (MT, 17α-Methyl-4-androsten-17β-ol-3-one), a synthetic steroid hormone produced by Sigma Corporation, St. Louis, USA, was added to a trout feed (the proximate analysis of the feed was 54% crude protein, 13-15% fat and 17.8 MJ/kg of metabolizable energy). Stock solution of MT was prepered by dissolving weighed portion of crystalline MT in 100 ml of 95% ethanol.

Stock solution was added to the feed at a ration to achive the dose of 30 mg MT/kg of the diet. Solvent was allowed to evaporate from the treated diet by air drying for 24 h.

SOURCE OF FISH, REARING CONDITIONS AND DATA COLLECTION

Pond-reared pikeperch, *Stizostedion lucioperca* (L.), fingerlings from Mrągowo Fish Farm (Olsztyn District, Northern Poland) were used. Fingerlings were 42-days

(D42) post-hatch when seined and transported to the experimental hatchery of the Inland Fisheries Institute in Olsztyn. Fingerlings - average weight 0.34 (±0.06) g and total length 3.58 (±0,21) cm - were randomly distributed into six 200-L fiberglass tanks at density of 1000 individuals per tank (1.70 g/L). The culture tanks were supplied with water which was filtered and re-circulated. Water temperature was 22 (±0.8) °C and the water flow rate was 5 - 8 L/min. Dissolved oxygen concentration was maintained between 82 - 93% of saturation, total ammonia and nitrite content was lower than 0.20 mg/L and pH was about 7.7. Parameters of water were measured at two-day intervals. The culture room was maintained at low light intensity (45.2 lux at the surface of the tank) for 24 h. Fish were fed to excess with trout feed over an 18-h day, continually (from 7.00 to 1.00). They were fed No. 2 granules (diametr 0.8 - 1.2 mm).

Fingerlings were reared to an average weight of 2.3 g approximately (the timing prior to sexual differentiation in pikeperch (Demska-Zakęś, Zakęś 1995, Zakęś, Demska-Zakęś 1996)) for 21 days.Then they were sorted and divided into six lots of 600 individuals each (the same tanks and culture conditions). The fish (63 days post-hatch) in three tanks were given a diet containing 30 mg MT/kg of the diet (group MT). Control diet with no hormone was used in the three other tanks (group C). Daily feed rations ranged from 7 to 5% of body weight (during MT administration - 3 weeks - from D63 to D84). After the treatment period, control and experimental groups were fed non-treated diet (5 - 3% of body weight) for 70 days.

During the experiment the growth rate and survival of fish were recorded. Measurements of length (total and standard length ±0.1 cm) and weight (wet weight 0.01 g) were taken at 1 week intervals. After the treatment and post-treatment phase 60 fish from each group were sacrificed by propiscin overdose and measured to determine weight and total length. At the end of treatment phase D84 the body cavity was opened, livers were dissected and weighed. Hepato-somatic index (HSI) was calculated for each fish as the ratio of wet liver weight (±0.001 g) to the total wet fish weight (±0.001 g) times 100. For the histological study the gonads, kidney, liver and skin were prepared for analysis. At the end of post-treatment D154 fish were sexed by gross morphological examination of the gonads. Some fish were also sexed through the histological examination. The organs were fixed in Bouin's fixative, dehydrated, embedded in paraffin, and sectioned at 5 µm using a microtome (Zawistowski 1986). The sections were stained with hematoxylin and eosin and viewed under a light microscope.

Data on growth, survival, condition factor and hepato-somatic index were analyzed by one-way analysis of variance (ANOVA) and homogeneous group testing was used to compare the mean values of the factors measured. The level of significance was accepted at $P<0.05$.

RESULTS

GROWTH AND MORTALITY

Total mortality over the 21-day treatment phase ranged from 0.83 to 1.83% (group C) and 0.50 - 3.33% (group MT). The differences in survival between the treatments were not significant ($P>0.05$). Mortality was due to mechanical failure during the three weeks of treatment rather than to the effect of the diets or disease. After the treatment phase mortalities were not observed in both group (Table 1). The losses were too low to affect the interpretation of the observed sex ratio.

Growth in length and weight of pikeperch fingerlings in both groups (control and MT supplemented diet) was very similar during the whole rearing period (Fig. 1,2).

TABLE 1

Effects of 17 α-methyltestosterone (0, 30 mg/kg diet) on growth, survival and sex distribution in pikeperch. Values are means from three repetitions; weight, total lenght, HSI index, condition factor and mortality values are means ±SD.

Parameter	Sample time (day after hatching)					
	D63 (begining of treatment)		D84 (end of treatment)		D154 (end of the post-treatment)	
	Control	MT	Control	MT	Control	MT
Total length (cm)			9.51 (±0.34)	9.48 (±0.49)	16.06 (±1.01)	16.21 (±1.31)
Weight (g)	6.50 (±0.41)	6.47 (±0.32)	7.33 (±0.62)	7.51 (±1.24)	34.25 (±7.24)	35.42 (±8.08)
Specific growth rate (% d^{-1})*	2.33 (±0.41)	2.30 (±0.44)	5.46	5.63	2.95	3.00
Condition factor K**			1.41 (±0.07)	1.43 (±0.05)	1.29 (±0.06)	1.33 (±0.04)
Hepato - somatic index (HSI)	1.47 (±0.07)	1.44 (±0.08)	2.80 (±0.63)	2.93 (±0.51)		
Survival (%)			98.56 (±0.43)	97.72 (±1.26)	98.56 (±0.43)	97.72 (±1.26)
Sex ratio			(n=60)	(n=60)	(n=60)	(n=60)
male			48.3	88.3	46.7	83.3
female			51.7	6.7	53.4	10.0
abnormal				5.0		6.7

**SGR = [(ln final wt - ln initial wt) / 21 or 91 days)] x 100.*

***Condition factor [K = weight in g / (standard length in cm)^3]*

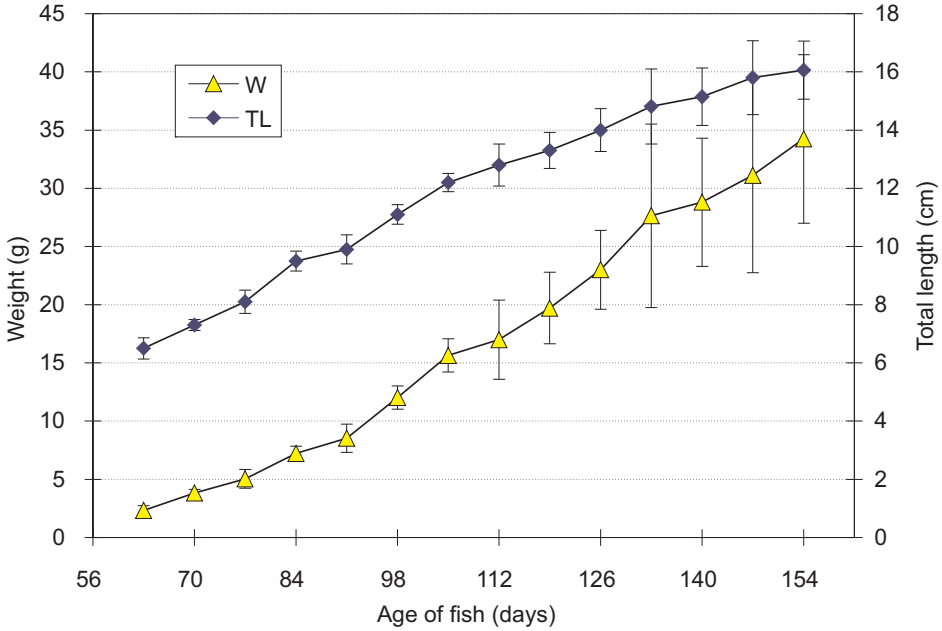

Figure 1. Growth of pikeperch fingerlings fed non-treated diet. Vertical bars indicate standard deviation.

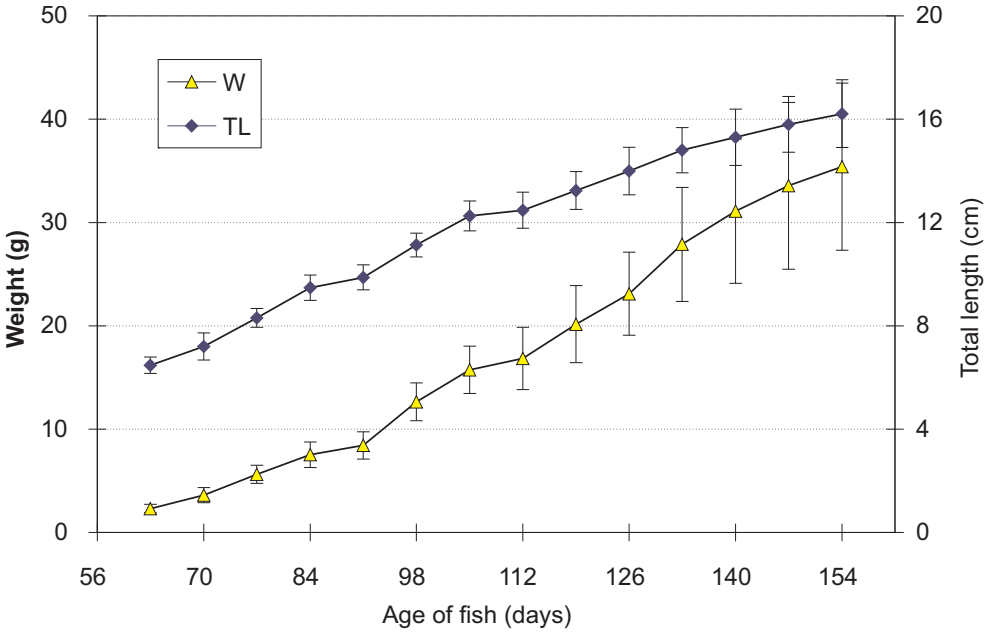

Figure 2. Growth of pikeperch fingerlings fed MT-treated diet (30 mg MT/kg diet). Vertical bars indicate standard deviation.

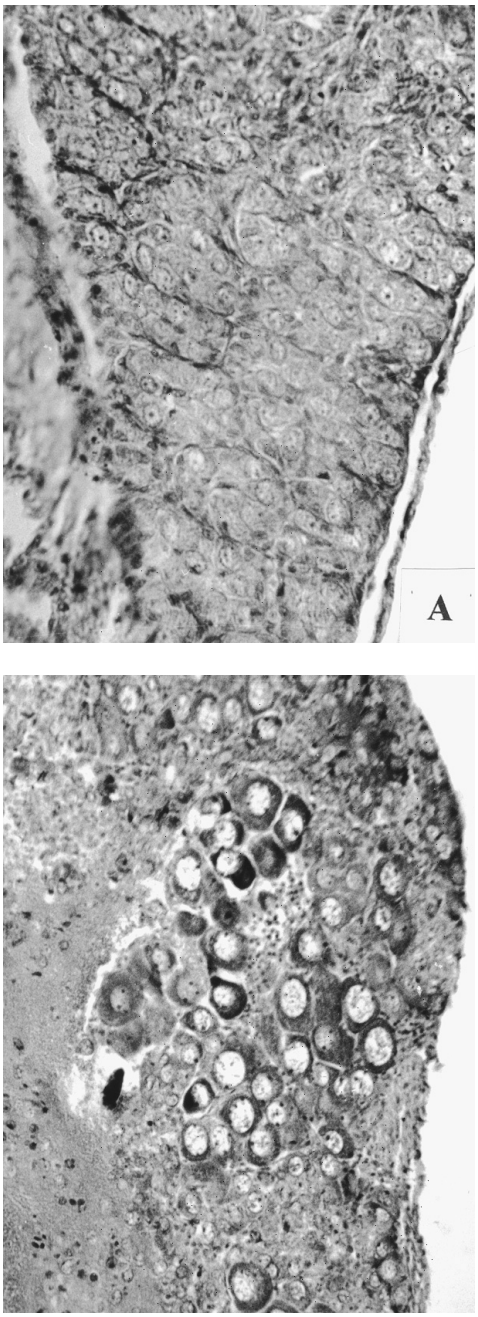

Figure 3. Cross sections of MT-treated gonads from 154-day-old pikeperch. (A) Testes from 34.9 g wet weight male; (B) ovary from 47.7 g wet weight female; (C) abnormal gonad from 35.4 g wet weight fish.

A summary of mean length, weight, condition factor at the begining, after the treatment phase and at the end of the experiment are presented in Table 1. Analysis of variance of this data showed that there were no significant differences in growth between the MT-treated group and the control ($P>0.05$). After 21 and 91 days of the experiment the average weights of fish in the androgen treatment were 7.51 and 35.42 g respectively, and the average weights of fish in the control were 7.33 and 34.25 g.

There were no significant differences in condition between the fish treated with MT and the control ($P>0.05$)(Table 1).

EFFECT ON THE TISSUES

Gonads. In the 84-day-old (D84) pikeperch from the control group the sex ratio of male to female was 48.3:51.7. The ovaries were characterized by a medially located ovary cavity (ovocoell) and contained oogonia, meiotic oocytes and oocytes in previtellogenesis stage. Males had relatively smaller lobate gonads containing primordial germ cells, gonocytes and spermatogonia. Sex distribution of 154-day-old control fish was similar to those of D84 (Tab.1). At this time the ovary was larger, with well-developed blood vessels. Longitudal sections of some ovaries showed previtellogenic oocytes with a large vesicular nucleus with multiple nucleoli that was surrounded by a strongly basophilic cytoplasm. These oocytes were surrounded by numerous primary follicles that were arranged in layers called lamelle. In some oocytes the Balbiani's vesicles were observed. The gross morphology of the testicular tissue consisted of short lobules fused into a single large mass. Histological sections of the tissue revealed spermatogonia, primary spermatocytes and tubules lined with simple columnar epithelium. The septa found between the tubules were composed of fibrous connective tissue.

Three types of the gonads were observed in the MT-treated pikeperch after the phase of androgen treatment (D84): testes, ovaries, and abnormal gonads (Tab. 1). Histological examination revealed that testes and ovaries resembled those of the control. Abnormal gonads contained small spaces resembling the ovocoell of ovaries, well-developed connective tissue resembling that of testes, and undifferentiated germ cells. The histological examination of D154 fish showed three types of gonads too: typical testes (83.3% of all specimens) and ovaries (10.0%), and gonads with abnormal histology (6.7%) (Fig. 3). These gonads (morphologically testes) contained

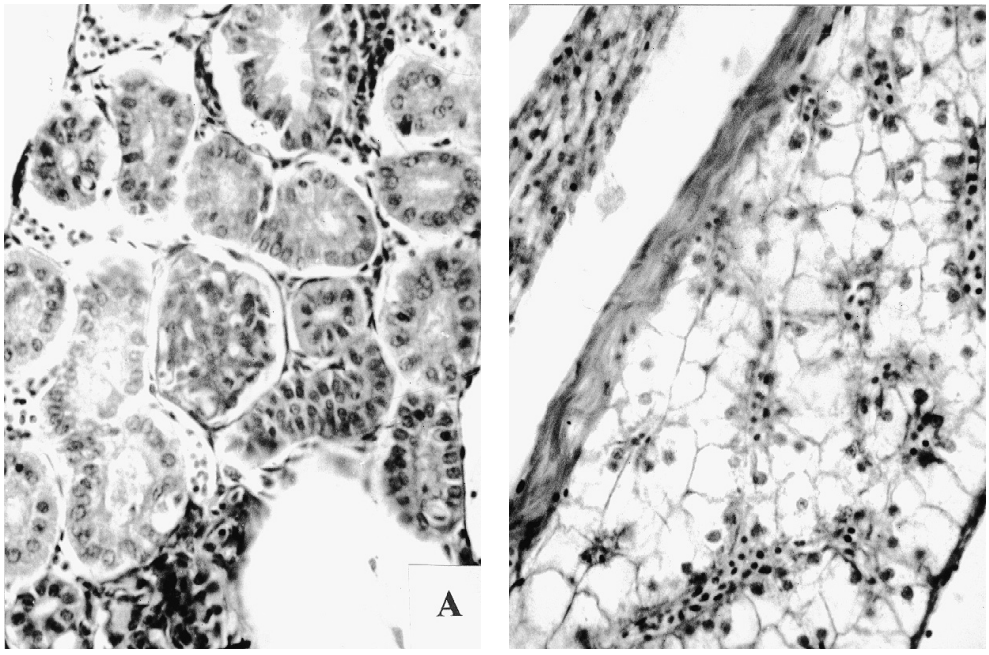

Figure 4. Cross section of MT-treated internal organs from 84-day-old pikeperch. (A) Kidney; (B) liver.

well-developed connective tissue that is also formed in testes, however ovocoell and oocytes in previtellogenesis stage were present as well.

Kidney. Histologically there was no deviations from the normal morphology in kidney from the MT-treated pikeperch. Both the epithelium of the renal tubules and renal corpuscles did not show any sings of cellular hypertrophy (Fig. 4A).

Liver. The livers of all examined fish had the characteristic pattern of vacuolated parenchymal cells arranged as anastomosing laminae which surround central veins (Fig. 4B). Results for the hepatosomatic index are given in Table 1. The index HSI (range 2.2 - 3.9) of MT treated group was not significantly different from the control (range 1.9 - 3.9) ($P>0.05$).

Skin. The integument of the pikeperch is composed of two parts: the epidermis and the dermis. No distinct alterations in the thickness and the structure of the two skin layers were noted in any of the treated fish. The cuboidal cells of the germinal layer, mucous cells and connective tissue had normal histology without signs of hypertrophy and hyperplasia.

DISCUSSION

Our results showed that methyltestosterone administrated in food, at a dose 30 mg MT/kg of the diet, did not influence the growth and survival rate, both in treatment and post-treatment phase. However, our first laboratory study (Demska-Zakęś, Zakęś 1996) showed a decrease in growth rate during the time of androgen administration at all levels of androgen tested (30, 60, 90 mg MT/kg diet). It is difficult to explain the reason for these differences in growth. It seems probable that it could have been due to different rearing conditions (laboratory study and intensive culture conditions). Similarly, Bieniarz *et al.* (1991) have shown that methyltestosterone administred in food did not influence the growth of rainbow trout, *Oncorhynchus mykiss*, (dose 3 mg MT/kg of the diet), except during the treatment phase. By contrast, McBride and Fagerlund (1973) showed significant increases in weight and length of juvenile coho salmon, *Oncorhynchus kisutch.* Panadian and Varadaraj (1987) indicated that MT - treated tilapia, *Oreochromis mossambicus*, weighed more and provided a higher yield than control fish at equivalent densities. A similar trend was also reported Macintosh *et al.* (1985) and Guerrero (1979).

The results obtained with MT at 30 mg/kg are in agreement with those of Demska-Zakęś and Zakęś (1996) - a large masculinization was achieved. They showed that testes development in the MT-treated fish was similar to that of the control. Because the morphology and histology of both gonads and ductus defferens were typical, the males were functional. The study did not produce complete sex reversal. Approximatelly 17 % of all examined fish had typical ovaries or ovaries with well-developed connective tissue. Probably these fish were larger than 2.3 g body weight when the MT was distributed. The sex differentiation and the effect of 17α-methyltestosterone on gonad development in allied species - walleye, *Stizostedion vitreum*, were described by Malison *et al.* (1990) and Malison (1995). The author reported that in walleye, treatment of fish (initially 50 mm TL) for 60 days with MT at 50 mg/kg of diet induced only partial sex inversion in females.

Simone (1990) reported that the channel catfish, *Inctalurus punctatus* treated with MT showed increased kidney weight and elevated reno-somatic index. The increase in kidney weight resulted in edematous action of the renal tubules and renal corpuscles. Kidney edema was reported in sockeye salmon, *Oncorhynchus nerka* (McBride,

Van Overbeeke 1971). The hypertrophy and the highly vacuolated appearance that is characteristic of an edematous state were not observed in pikeperch kidneys.

The liver weights of MT-treated pikeperch were insignificantly greater than in the control; however the hepatotrophic response and hyperplasia were not observed. Similar observation was noted in carp, *Cyprinus carpio* (Lone, Matty 1980). In contrast, Bulkley and Swihart (1973), and Simone (1990) observed an increase in HSI when the channel catfish, *Ictalurus punctatus*, were fed MT. It is possible that the hepatotrophic response to MT administration depend both on fish species and on dose level.

Our observation that the dermis from the MT-treated pikeperch had a typical histology was is not confirmed by observations made in other reports. McBride and Fagerlund (1973), in studies with salmon, *Oncorhynchus tshawytscha*, noted a thickening of epidermis and degeneration of the germinal and gland cells. According to Yamazaki (1976) methyltestosterone increased the thickness of the skin and reduced bacterial infections in fish.

ACKNOWLEDGMENT

We extend our thanks to Mrs. Elżbieta Ziomek and Mrs. Ewa Bednarska, Olsztyn University of Agriculture and Technology, Poland, for their help in the preparation of the histological sections. The photographs were taken by Mr. Jacek Sztorc, Olsztyn University of Agriculture and Technology, Poland.

This manuscript is the result of research supported by the Grant No. 5 S 311 003 05 financed in 1993-1996 by the Scientific Research Committee (KBN), Poland.

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STRESZCZENIE

ODWRACANIE PŁCI U SANDACZA EUROPEJSKIEGO, *Stizostedion lucioperca* (L), POPRZEZ PODAWANIE PASZY Z DODATKIEM METYLOTESTOSTERONU

Metylotestosteron (MT), syntetyczny hormon sterydowy podawano rybom w komercyjnym granulacie pstrągowym. Materiałem doświadczalnym był narybek sandacza podchowany w stawach do wielkości 3.58 cm Lt i masy ciała 0.34 g W. Po osiągnięciu przez ryby (po 21 dniach intensywnego podchowu w warunkach kontrolowanych) wielkości, w której rozpoczyna się proces dyferencjacji płci (W=2.3 g) obsadzono sześć 200 l cyrkulacyjnych zbiorników podchowowych (600 sztuk narybku na zbiornik). Czas podawania MT (30 mg/kg paszy) ustalono na 21 dni. Po tym okresie prowadzono dalszy, 70 dniowy podchów (ryby z grupy doświadczalnej i kontrolnej karmiono paszą bez dodatku hormonu).

Trzy typy gonad obserwowano w grupie ryb karmionych paszą z dodatkiem MT: jądra (83.3% badanych osobników), jajniki 10.0% oraz gonady o anormalnej budowie (6.7%). Rozwój gonad sandacza z grupy kontrolnej przebiegał w sposób typowy dla tego gatunku, a stosunek płci (samców do samic) wyniósł 46.7 : 53.4.

Badania histologiczne nie wykazały żadnych istotnych zmian w wątrobie, nerce i skórze ryb karmionych paszą z MT. Indeks wątrobowy przyjął wyższe wartości dla ryb z tej grupy, jednak różnice te, w porównaniu z grupą kontrolną, nie okazały się istotne statystycznie ($P > 0.05$).

Analiza statystyczna uzyskanych wyników nie wykazała istotnych statystycznie różnic w tempie wzrostu, kondycji i przeżywalności pomiędzy grupą doświadczalną a kontrolą ($P > 0.05$)

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