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THE EFFECT OF ADMINISTRATION OF 11 β-HYDROXYANDROSTENEDIONE IN FEED ON MORPHOLOGY OF SOME INTERNAL ORGANS OF WELS, *Silurus glanis* (L.)

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ABSTRACT. Studies were carried out on the effect of a natural androgen, 11 β-hyroxyandrostenedione (OHA) administrated in feed on morphology of some internal organs of wels, *Silurus glanis* (L.). No distinct masculinization or adverse impact of the hormone on gonads was observed. No pathological changes were noted in the kidneys, liver or skin. Statistically significant increase of hepatocyte size, epidermis thickness, and - in some individuals – hyperplasia and increase of circumglomerulal space in kidneys were observed only in the group treated with 50 mg of OHA per kg of feed.

Key words: WELS, 11 β-HYDROXYANDROSTENEDIONE, GONADS, KIDNEYS, LIVER, SKIN

INTRODUCTION

Androgens are commonly applied for hormonal stimulation of growth or sex reversal in fish (Reinboth 1969, Sutherland 1972, Yamazaki 1976, Higgs et al. 1982). The effects of steroids depend on fish species and age, dose, and exposure time. Short time and high doses are usually applied for sex reversal, while low doses are administered for a long time to stimulate fish growth (Yamazaki 1976, Matty and Cheema 1978, Schreck and Fowler 1982). Among the androgens, 17 α-methyltestosterone (MT) is most commonly used. It is easily absorbed, does not accumulate in fish body, and is readily excreted (Goudie et al. 1986a, b, Cravedi et al. 1989). It may, however, cause pathological changes in gonads, kidneys or liver (Ashby 1957, Hirose and Hibiya 1968, Higgs et al. 1982, Mamcarz et al. 1994). Thus, the use of other steroid hormones, such as 11 β-hydroxyandrostenedione (OHA), for fish sex reversal or growth stimulation has been studied since late 80's.

Literature data on the effect of OHA concern mainly its influence on reproductive organs of salmonid fishes, such as *Oncorhynchus mykiss* (Van der Hurk, Van Oordt 1985, Feist et al. 1994), *Oncorhynchus kisutch* and *Oncorhynchus keta* (Reeding et al. 1987), pike-perch, *Stizostedion lucioperca* (Zakes and Demska-Zakes 1997, Demska-Zakes and

Zakes 1999), and African catfish, *Clarias gariepinus* (Van der Hurk et al. 1989). The results of these studies indicate efficiency of this steroid in fish masculinization without adverse effects on the internal organs (Zakes and Demska-Zakes 1997).

The aim of the present study was to evaluate the effect of 11 β-hyroxyandrostenedione on morphology of some internal organs of wels, *Silurus glanis* (L.).

MATERIAL AND METHODS

Experimental material was obtained from the Fish Farm in Samoklęski near Lublin. Wels larvae were placed in 6 aquaria of 25 l and reared for 35 days, until the fish reached 4.2 g and total length 7.9 cm. They were then divided into 3 experimental groups (each in 2 replicates) and transferred to tanks of 200 l, supplied with recirculated water of flow rate 1.2 l min^{-1}, at 26±0.5^{o}C. Each tank was stocked with 70 fish. The fish were fed trout feed FK without (control) or else 5 or 50 mg of 11 β-hydroxyandrostenedione (OHA) per kg. This stage of rearing lasted 50 days (at the end of the experiment the fish were 85 days old).

Thirty individuals of each group were sampled for histological analyses. They were measured (TL ± 0.1 cm) and weighed (W ± 0.1 g). Pieces of skin, liver, kidneys and gonads were collected from each individual. The samples were preserved in Bouin solution, dehydrated, and embedded in paraffin. Paraffin blocks were cut into 5 μm thick slices using a microtome and stained with haematoxylin and eosin (Zawistowski 1986). Histological preparations were viewed using a light microscope, with special attention paid to morphological changes. Measurements of hepatocytes (500 cells from each individual, in 2 replicates), and epidermal cells (30 cross-sections of skin of each individual, in 2 replicates) were also performed using computer image analysis system MULTISCAN.

The results were subjected to ANOVA, and in case of significant differences ($P<0.05$) were analysed with Tuckey's or Duncans's tests.

RESULTS

THE EFFECT OF 11 β-HYDROXYANDROSTENEDIONE ON GROWTH AND REPRODUCTIVE SYSTEM OF WELS

Average body length and weight of the control fish were 22.24 cm and 78.28 g (Tab. 1). Sex ratio was 1:1 (Tab. 1). Female gonads were cylindrical and sac-shaped, transpar-

TABLE 1

Average total length body weight and sex ratio of the wels given feeds containing 0, 5 or 50 mg of 11 β-hydroxyandrostenedione per kg

Hormone dose (mg · kg^{-1})	N (fish)	Total length (cm)			Body weight (g)			Sex ratio (%)		
		range	mean*	SD	range	mean*	SD	male	female	intersex
0	30	19.5-25.7	22.24^{a}	1.64	48.6-118.6	78.28^{a}	22.24	50	50	0
5	30	19.2-27.9	23.18^{b}	1.98	51.4-156.8	90.75^{b}	25.09	47	53	0
50	30	19.5-26.1	23.16^{b}	1.50	51.2-139.8	95.90^{b}	19.15	33	57	10

**values in the same column with the same letter index do not differ significantly (P>0.05)*

ent, with numerous blood vessels and considerably fatty. Previtellogenic oocytes and dividing oogonia were observed in histological sections (Photo 1a). Histological picture of oogonia was typical zygotene and pachytene phases of meiotic division. Previtellogenic oocytes had numerous nucleoli in the nucleus. Single follicular cells were also present. Male gonads resembled thin threads with poorly visible blood vessels. Histological picture showed spermatogonial cells and primary seminal follicles with spermatocytes of the first order (Photo. 1b). Spermatogonial cells contained centrally located nucleus surrounded with a narrow rim of the cytoplasm. Spermatocytes of the first order contained one round nucleolus, chromatin was evenly distributed in the nucleus, and mitochondria were situated at the cell periphery.

Sex ratio, anatomic and histological features of ovaries and testes were similar in the wels fed feeds containing 5 mg of OHA per kg to the control ones, except slightly higher content of connective tissue in the gonads. The fish attained considerably

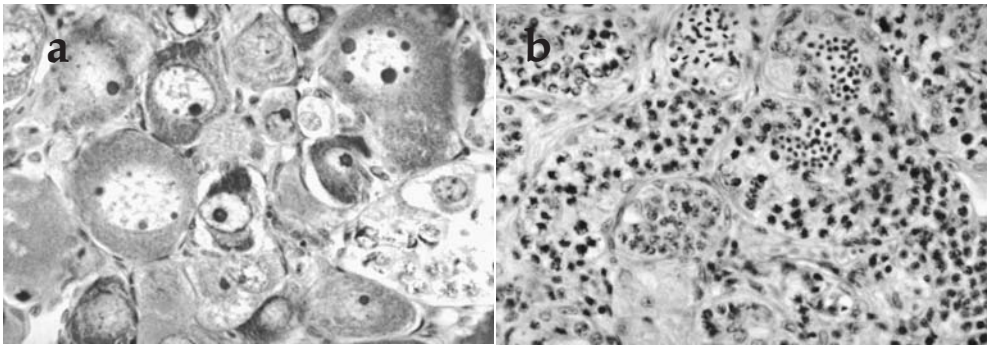

Photo. 1. Histological cross-section of gonads of control wels: (a) ovary, (b) testis. (x800)

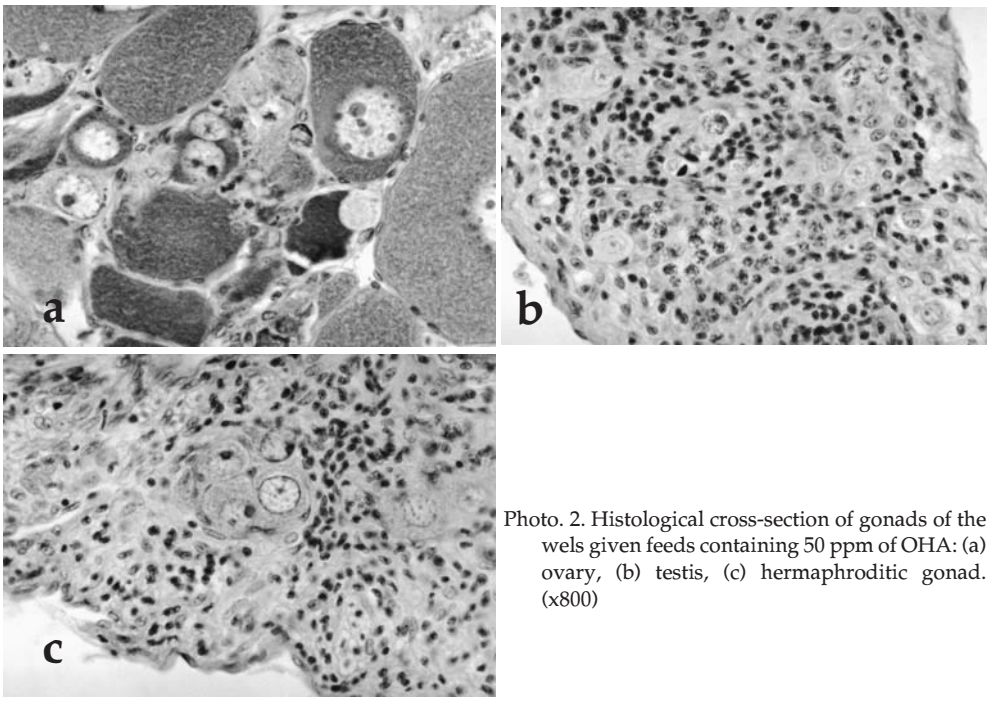

Photo. 2. Histological cross-section of gonads of the wels given feeds containing 50 ppm of OHA: (a) ovary, (b) testis, (c) hermaphroditic gonad. (x800)

higher body length and weight compared to the control ($P<0.05$, Tab. 1). Similar growth was also observed in the wels fed feeds containing the highest concentration of the hormone. Sex ratio in this group was, however, different (Tab. 1). Histological preparations showed presence of the ovaries, testes and hermaphroditic gonads. Female and male gonads did not differ much from those observed in other fish groups (Photo. 2a, b), but abnormal gonads contained a lot of connective tissue, accompanied by oogonia, oocytes at early previtellogenesis, and spermatogonial cells (Photo. 2c).

HORMONE-INDUCED CHANGES IN KIDNEYS

Kidneys of wels are long, paired organs situated extraperitoneally, extending under axial skeleton from the midriff to the caudal peduncle. No anatomical or histological changes were observed in the control fish. Histological cross-sections revealed high number of nephrons containing tubules and glomeruli, surrounded by parenchymatic tissue. Glomeruli and tubules were regular, typical of the species (Photo. 3a).

Kidneys of fish given feeds containing 5 mg OHA per kg were slightly congested, but no pathological changes were observed in glomeruli or tubules. Kidneys of fish

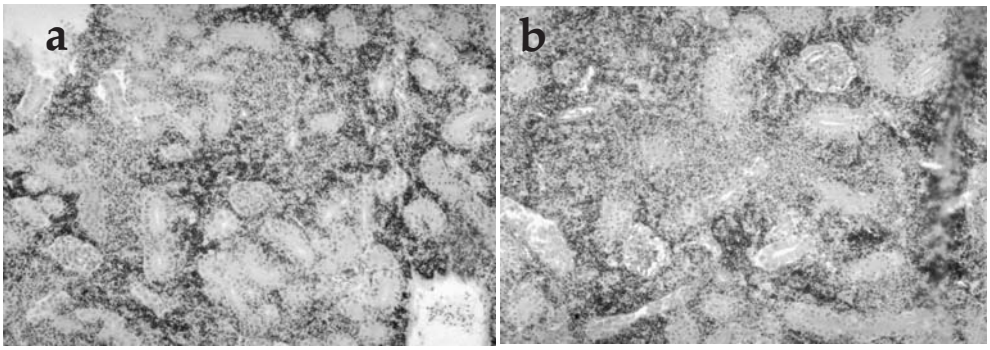

Photo. 3. Histological cross-section of kidneys of: (a) control fish, (b) fish given feeds containing 50 ppm of OHA. (x200)

treated with the highest steroid dose looked similar, except for some fish in which intense division of parenchymatic cells (hyperplasia) and increase of circumglomerular space were observed (Photo. 3b).

THE EFFECT OF 11 β-HYDROXYANDROSTENEDIONE ON THE LIVER OF WELS

Bi-lobed liver of wels is situated at the ventral side of the digestive tract. It contains polyhedral cells, concentrically surrounding bile ducts. Basic structural unit of the liver, so-called hepatic lamella, contains 5-6 cells. Cell size in the control fish was 11.09 μm (Tab. 2). Nucleus was round, central or excentrically located (Photo. 4a).

Liver anatomy in wels given feeds containing 5 or 50 mg of OHA per kg was similar to that observed in the control fish, except for small haemorrhages in some

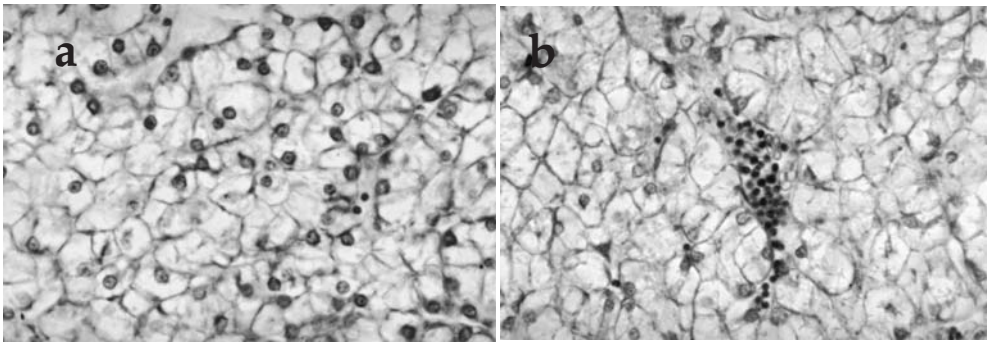

Photo. 4. Histological cross-section of liver of: (a) control fish, (b) fish given feeds containing 50 ppm of OHA. (x800)

individuals (Photo. 4b). Hepatocytes of the fish treated with the highest steroid doses were significantly larger compared to cells of the control fish and of 5 mg group ($P<0.05$, Tab. 2).

TABLE 2

Diameter of hepatocytes and thickness of epidermis in wels given feeds containing 0, 5 or 50 mg of 11 β-hydroxyandrostenedione per kg

Hormone dose (mg · kg^{-1})	N (fish)	Hepatocyte diameter (μm)			Epidermis thickness (μm)		
		range	mean*	SD	range	mean*	SD
0	30	3.74-22.51	11.09^{a}	2.77	72.43-184.50	135.82^{a}	20.99
5	30	3.90-24.41	10.98^{a}	2.75	74.66-203.10	139.79^{a}	21.35
50	30	4.54-29.04	13.04^{b}	2.91	69.57-231.12	148.03^{b}	26.91

**values in the same column with the same letter index do not differ significantly (P>0.05)*

HORMONE-INDUCED CHANGES IN SKIN

All layers of skin of the control fish were regular (Photo. 5a). Average epidermis thickness was 135.82 μm (Tab. 2). Skin of the fish treated with 5 mg of OHA per kg of feed did not show any pathological changes, their epidermis was slightly thicker compared to the control (139.79 μm) but the difference was not significant ($P>0.05$, Tab. 2). Epidermis was 12.21 μm thicker in the fish fed 50 mg of OHA per kg of the feed, and it differed significantly from the other groups (Tab. 2). Additionally, epidermis was more compact, with elongated glands (Photo. 5b).

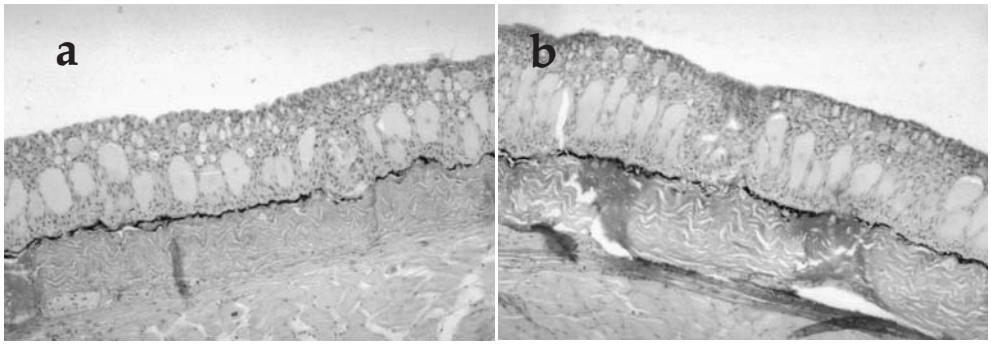

Photo. 5. Histological cross-section of skin of: (a) control fish, (b) fish given feeds containing 50 ppm of OHA. (x200)

DISCUSSION

The effects of steroid hormones used for growth stimulation depend on fish species, type of hormone and its dose (Higgs et al. 1982). Simone (1990) observed that increase of length and weight of channel catfish, *Ictalurus punctatus*, fed feeds containing 5 or 10 ppm of MT were lower than in the control fish. On the other hand, Guerrero (1975) observed 43.6% increase of body weight in *Tilapia aurea* given a feed containing 30 ppm of MT compared to the control. Similar reaction was observed in common carp, *Cyprinus carpio* (Lone and Matty 1980), and in *Oncorhynchus kisutch* (McBride and Fagerlund 1976). Anabolic action of 11 β-hyroxyandrostenedione was observed only in grayling, *Thymallus thymallus* (Poczyczyński, unpublished), and rainbow trout, *Oncorhynchus mykiss* (Demska-Zakęś et al. 1999). No effect of this steroid on body weight and length increase was observed in pikeperch, *Stizostedion lucioperca* (Zakes and Demska-Zakes 1997). In the present study, addition of 5 or 50 mg of 11 β-hydroxyandrostenedione per kg of feed produced anabolic effect. The differences in wels growth were statistically significant and dose-dependent.

Steroid hormones are applied mainly for sex reversal, to obtain monosexual or sterile populations, to reduce or eliminate reproductive potential, or to increase it (Yamazaki 1976). The use of 17 α-methyltestosterone in various fish species resulted in all-male populations irrespective of the way of administration (Yamazaki 1976). Our own data on wels (Demska-Zakes, unpublished) fed MT-containing feed indicate feminization (72% of females in the population). This result was quite surprising taking into consideration masculinizing properties of this steroid. Similar result was, however, obtained by Van den Hurk et al. (1989) who observed that treatment of African catfish, *Clarias gariepinus*, with high doses of MT induced feminization. The authors suggested so called "paradoxical feminization" caused by aromatisation of androgens to estrogens. Similar reactions were observed in channel catfish (Goudie et al. 1983), *Tilapia mossambica* (Nakamura 1975), and a cichlid *Hemihaplochromis multicolor* (Reinboth 1969, Hackmann and Reinboth 1974).

The effect of 11 β-hydroxyandrostenedione on fish reproductive organs was studied on few species. Van den Hurk et al. (1989), who immersed African catfish in 300 μg OHA per dm^3 of water, obtained 77% of males and 23% of females. Similarly, in pikeperch treated with 60 mg of the hormone per kg of feeds, 93% of males were obtained and 7% of hermaphrodites (Demska-Zakes and Zakes 1999). The studies on rainbow trout and pike, *Esox lucius*, confirmed masculinizing effect of 11 β-hydroxyandrostenedione (Demska-Zakes et al. 1999, Demska-Zakes et al. in press).

No masculinization occurred in wels fed feeds containing 5 or 50 mg of 11 β-hydroxyandrostenedione per kg. Presence of bisexual individuals in 50 mg per kg group indicates the effect of the hormone on sexual organs. Lack of masculinization might have resulted from a too late hormone treatment. It should be stressed that considerable differences of fish size occurred at the beginning of the experiment, and this might have affected the final results.

Literature data on the effect of steroid hormones on fish internal organs is scarce. No pathological changes in the kidneys of *O. kisutch* (Sinnhuber et al. 1979) and pike-perch (Zakęś et al. 1997) treated with 17 α-methyltestosterone were observed. Adverse effect of MT was, however, noted in kidneys of channel catfish (Simone 1990), including hypertrophy of the epithelium of renal tubules and glomeruli, and increased reno-somatic index. Similar changes were observed by McBride and Van Overbeeke (1971) in *Oncorhynchus nerka*, and by Demska-Zakęś (unpublished) in wels. In the present experiment no adverse effect of 11 β-hydroxyandrostenedione on excretory organs was observed. Renal tubules and glomeruli were unaffected. In some individuals slight congestion, intense proliferation of parenchymal cells, and increase of circumglomerular space were observed, indicating accelerated excretion of the metabolites. Anabolic steroids, even if administrated under full control, may cause hypertrophy (Ashby 1957, Hirose and Hibiya 1968, McBride and Van Overbeeke 1971, Higgs et al. 1982) or liver degeneration (Hirose and Hibiya 1968, Higgs et al. 1982). Simone (1990) did not observe any changes in hepatic lamellae in channel catfish administered MT-supplemented feed. Also Demska-Zakęś (unpublished) did not find any greater changes in wels liver. Only size of hormone-treated fish significantly differed from the control. In wels fed feeds containing either 5 or 50 ppm of 11 β-hydroxyandrostenedione, no changes were observed in the structure of hepatocytes. Small haemorrhages occurred in some individuals treated with the highest hormone dose. Hepatocytes of this group of fish had the longest diameter (13.04 μm), significantly different from the control and 5 ppm group.

Fish skin consists of two tissues: epithelium (epidermis) and connective tissue (dermis). Treatment with 17 α-methyltestosterone may result in an increase of epidermis thickness. McBride and Fagerlund (1973, 1976) observed dose-dependent thickening of epidermis in *Salmo*, and Higgs et al. (1982) noted similar reaction in steelhead trout. The data on the effect of OHA on fish skin are scarce. Only Zakes and Demska-Zakes (1997) reported that 30, 60 and 90 ppm of OHA did not induce any

changes in the structure or thickness of pikeperch epidermis. In wels, however, treatment with 50 ppm of OHA resulted in significant thickening of the epidermis. The epidermis of fish was also more compact, with elongated glands.

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STRESZCZENIE

WPŁYW PODAWANIA W PASZY 11 β-HYDROKSYANDROSTEDIONU NA BUDOWĘ NARZĄDÓW WEWNĘTRZNYCH SUMA EUROPEJSKIEGO *Siluris glanis* L.

Od końca lat 80. prowadzone są eksperymenty z zastosowaniem naturalnego steroidu 11 β-hydroksyandrostedionu (OHA) jako środka do zmiany płci lub stymulacji przyrostów u ryb. Wyniki tych prac dowodzą wyraźnie przydatności tego androgenu do przeprowadzania maskunilizacji, a jednocześnie braku niekorzystnych zmian w budowie narządów wewnętrznych. W niniejszej pracy starano się przeanalizować wpływ OHA podawanego w paszy w dawkach 0; 5 i 50 mg steroidu na kg paszy na budowę wybranych narządów wewnętrznych u suma europejskiego, *Silurus glanis* (L.). Materiał do badań stanowił narybek o średniej masie ciała 4,16 g i długości całkowitej 7,89 cm. Podchów trwający 50 dni prowadzono w obiegu zamkniętym, w temperaturze 26 ±0,5°C. W grupach doświadczalnych nie stwierdzono wyraźnego efektu maskulinizacyjnego oraz negatywnego oddziaływania hormonu na budowę gruczołów płciowych. Zaobserwowano jedynie nieco większą ilość tkanki łącznej w gonadach, a w grupie poddanej działaniu 50 mg OHA na kg paszy pojawiły się osobniki obojnacze (10% badanych ryb).

Również w przypadku narządów miąższowych (nerek, wątroby) oraz skóry nie zaobserwowano zmian patologicznych. Jedynie u ryb karmionych paszą z dodatkiem 50 mg OHA na kg paszy stwierdzono zwiększoną istotnie statystycznie wielkość hepatocytów, większą średnią grubość naskórka oraz u nielicznych osobników - hiperplazję i powiększenie przestrzeni okołokłębuszkowej w nerkach.

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