

HISTOLOGICAL ANOMALIES IN GONADS OF
OREOCHROMIS NILOTICUS (CICHLIDAE) FEEDING ON
ETHYNYLTESTOSTERONE
AND ACTIVE YEAST.

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ABSTRACT

Male-population of *Tilapia nilotica* was produced with 17 α -Methynyltestosterone at 60 μ g/g diet. Subsequently fry were fed 1,2,3,4 and 5 g/kg active yeast diet for 70 days. Fish fed on dietary yeast were significantly heavier than the control. The percentage of males was 62.1. Ovotestis condition was found. Histological anomalies of the gonads of both sexes have been observed. Sterilization of the testis was complete at all levels of yeast diet. Dietary treatment had a destructive effect on the histological appearance of gonadal development.

INTRODUCTION

It has been well established that sex steroids are effective in modifying and directing the gonadal sex differentiation in teleosts fishes when administered in adequate doses and during appropriate stages of gonadal development (Yamamoto, 1969).

Tilapias are fecund and are able to reproduce at a relatively small size (Lowe; 1955). Practices and suggestion included monosex culture (Hickling, 1968; Semakula and Makoro, 1968; Shell, 1968 and Guerrero, 1975) and sex reversal (Celmens and Inslee, 1968; Chew and Stanely, 1973). Yamamoto (1986) obtained 100% sex reversal of genetic females in the medaka *Oryzias latipes*, with ethynyltestosterone. In the culture of certain species of *Tilapia*, prolific breeding results in small size at harvest, which can be avoided by monosex culture. Guerrero (1975) used ethynyltestosterone treatment in blue tilapia *Tilapia aurea* to produce stocks containing a high percentage of males. Nakamura (1975) disclosed that, in *Tilapia mossambica*, oral administration of androgen exerts a different

different influence on gonadal sex differentiation according to the dosage and duration of androgen treatment.

In the recent years, a great concern is given to fish diets in order to be economic and performed. Accordingly, attempts have been made to find out additive food which could be formulated at economic costs for fish production. For this goal, it can be shown that active yeast is used as microbial additive for protein source to improve growth performance of *Oreochromis niloticus* (Abd-Elhalim *et al.*, 1989; Kobeisy and Hussein, 1995).

Also, yeast has been used as complementary protein source in fish diets together with fish meal. However, crude protein of yeast comprises a high content of non-protein nitrogen (De Groot, 1977)

The present study was undertaken to find out the effect of oral administration of androgen or adding of active yeast *Saccharmyces serviaein* to diets on gonad development of *Oreochromis niloticus*.

MATERIAL AND METHODS

Male *Oreochromis niloticus* was produced by androgen treatment. The synthetic androgen used was α 17-ethynyltestosterone (ET60; Sigma). The fry of experimental groups were given the hormone-containing diet (44.6% crude protein) once a day for 28 days after birth. The fry of 8-10mm total length and 1.66 ± 0.1 g total weight and approximately 4-5 weeks old were divided into six groups -20 fry in each group ; Each group (duplicated) was stocked in a 120-L aquarium with aerated and dechlorinated tap water. Water temperature was thermostatically controlled at 28 ± 1 . Based on the criterion of Eckstein and Spira (1965) the fry were sexually undifferentiated.

Six diets were formulated to contain 35% crude protein. A control diet (No.1) which contained 27.1% fish meal, 28.4% soybean meal, 25% yellow corn, 3% yellow corn oil and 2% vitamin and minerals premix. While the other tested diets from NO.2 to No.6 contained 1,2,3,4 and 5 g active yeast per one kg control diet respectively. Five groups were fed yeast diet and one served as a control. Chemical analyses of the control diet and the five supplemental diets are shown in Table (1).

Fish were subsequently fed on their pelted at 5% of body weight twice daily, 6 days adjusted every week for 70 days. During experiment the water of each aquarium was changed every three days.

At the end of the experiment, the fish were collected, weighed and measured. Fishes were dissected, gonads and liver of experimental and control fish were extirpated and the gonado-somatic (GSI) and hepatosomatic (HSI) indices were calculated as follows:

$$\text{GSI} = \text{gonad weight} \times 100 / \text{gutted weight.}$$

$$\text{HSI} = \text{liver weight} \times 100 / \text{gutted weight.}$$

Gonads were fixed in Bouin's, dehydrated, sectioned and stained with Hematoxylin and Eosin.

RESULTS

Survival and growth

No gross abnormalities were observed in treated fish. The survival of experimental fish after 70 days was not significantly different ($P > 0.05$) from that of controls. The overall mean survival rate for the experimental fish was 78.3%.

The mean weights of experimental fish at the end of the experiment were generally higher than that of the control (Table 2). Significant difference ($P < 0.05$) between the mean weights of fish fed on yeast treated and controls indicating that treated fish had better growth than controls.

Total length did not differ ($P > 0.05$) between the control and yeast-treated fish during the period of experiment.

Effectiveness of Androgen for Inducing Sex Reversal

The combined group consisted of 62.1% males, 20.7% females and 17.2% ovotestis.

Gonads

The ovaries of control females were in no case furnished with any trait of testicular structure in any region of ovaries. The testis of *O. Niloticus* belongs to the radial type.

Control

The different germ cells of spermatogenesis were identifiable. The central part of the testis was loaded with spermatozoa. The spermatogonia were small, mostly peripheral and spermatocytes and spermatids were found in fair amounts (Fig. 1). The mean GSI was 0.7.

The control females (100days old) had ovaries with oocytes developing to ripe stage (Fig.2). Most oocytes were normal in aspects. The ripe ova contained abundant yolk materials being surrounded with a sheath of follicular cells. The interfollicular space appeared clear. Atretic oocytes were rare. The mean GSI was 1.2.

Histological anomalies

The testis

The spermatogenesis in the testes of fish fed on yeast for 70 days were notably affected. Definite testicular injury was revealed by marked discoloration and abnormal size and divided into two unequal portions. The upper portion was small and tapering, while the lower portion was blunt. Germ cells including spermatogonia were clearly reduced in number and mostly disappeared from the gonads.

Histological examination of testis sections (1g/kg diet yeast) showed marked changes, the most prominent of which were marked proliferation of the connective tissues, extensive haemorrhage necrosis and pyknosis. Inside the seminiferous lobule, complete necrotic and degenerated sperm follicles were seen (Fig. 3). Many blood vessels were packed with erythrocytes were visible between the testes lobules. The wall of blood vessel had collapsed in places and many erythrocytes had aggregated to invade the lobules causing the lobules boundary cells to disintegrate and the nuclei to become pyknotic. Germ cells were absent (GSI= 1 Fig. 3).

In fish fed on 2g/kg diet yeast (GSI= 2) the interlobular connective tissue was packed with erythrocytes which surrounded the lobules causing some sort of hypertrophy and inter-lobular edema was observed. The lobules had disrupted sheaths and appeared empty having some pyknotic nuclei and spermatocytes were ruptured (Fig. 4).

Examination of fishes fed on 3g/kg diet yeast showed remarkable malformation and disruption of their testicular lobules. The lobules lost their membranes. Malformation of spermatogonia which became enlarged in size and their cytoplasm degenerated. Also, dense chromatin materials were seen. Dislocation of nuclei of spermatogonia was observed. The spermatozoa in the seminiferous lobules lost their normal appearance with short tails. Necrotic appearance was noticed as indicated by the presence of numerous pyknotic nuclei (GSI= 1.2 Fig. 5).

Microscopic examination of the testes of fish fed on 4g/kg diet yeast (GSI= 1.5) displayed an abnormal increase of pyknosis, which lead to necrosis, arrest of spermatogenesis. The lobular wall

degenerated completely. The germ cell cysts of early spermatogenetic stages including the spermatogonia were, however, evidently absent and those of more advanced stages comprising transforming spermatids and spermatozoa were abnormal in feature. Pyknosis of primary and secondary spermatocytes was observed (Fig.6).

Testes of fish fed on 5g/kg diet yeast showed sever damage where the spermatogonia became enlarged in size with dark chromatin dense. The sperms exhibited varying degrees of malformation, remarkable degeneration of sperm tails and collapse of sperm's heads which have dark pyknotic nuclei. Disappearance of all intermediate stages between spermatogonia and sperms. Misshapen and damaged lobular walls and blood vessels were noticed (GSI= 2.2, Fig. 7).

The ovary

The ovaries of experimental groups had no trait of testicular structure in any region. On the other hand, females of the same age fed on yeast diets examination of ovaries sections revealed normal structure, but marked increase of atretic oocytes, hypertrophy of granulosa layer abnormal yolk formation as evidenced by non-fusion of yolk droplets and rapture of epithelial follicles. The nuclear envelope disappeared and the chromatin disintegrated. The very thin zona radiate (ZR) was thrown into folds and penetrated the degenerated cytoplasm (Fig. 8). Simultaneously, the number and size of granulosa cells increased and they began to invade the shrunken nucleoplasmic mass. The steady progress of atresia resulted in the loss of cytoplasm, and the degenerating oocytes were occupied by phagocytic derived from the follicular cells and granulose layer. The yolk mass exhibited various degrees of liquefaction and a number of different sized vacuoles appeared (Fig. 9). Zona radiata had disappeared and the nucleus was no longer discernible as a separate structure.

The small oocytes in the previtellogenic stage showed nuclear malformations, where their chromatin bodies became separated from the nucleoplasm, also edema and the interfollicular tissue contained leukocytes, and red blood cells (Fig. 10). In general, all the examined ovaries revealed signs of severe damage and different stages of atresia.

The histological observations showed that younger germ cells such as oogonia and synaptic oocytes were undetected through the entire gonadal region. The ripe oocytes were very few in number.

Examination of sections of spent female fed on yeast revealed a complete degeneration of all oocytes, loss of cytoplasm and rupture of oocyte walls (Fig. 11).

The GSI values of ripe females fed on 2, 3 and 4g/kg yeast were very high (4.5, 9.7 & 8.4 respectively), while the GSI value of control ones were 1.2 (Table 2).

An interesting feature detected in the testes was the occurrence of oviform germ cells intermingled among male germ cells. These cells were rounded in contour and appeared mostly in the central region of the testes singly or in a few numbers. It closely resembled the oocytes in the period of the first growth phase. Sometimes the nucleus of the oviform cell was provided with several nucleoli arrayed in close contact with nuclear membrane (Fig. 12).

Examinations of gonad sections revealed that ovotestes condition was observed among specimens examined and its percentage was 17.2%. The histological observations showed that, their gonads were in complete degeneration, quite similar to those of males (Fig. 13).

The hepatosomatic index (HSI) of the groups fed active yeast were significantly different ($P < 0.05$) from that of the control group. It was found that fish fed on 2 and 3g/kg diet yeast had the highest values of HSI (Table 2).

DISCUSSION

The fish gonad, a possible indicator of physiological disturbances, has rarely been studied with relation to the possible histopathologic effects of food.

In the present study histopathological changes were observed in the gonads of *O. niloticus* fed with different levels of active yeast (*Saccharomyces servisiaein*) in diet.

In the present experiment on *O. niloticus*, the mortality rate was low. This result agrees closely with that of Yashouv and Eckstein (1965) who indicated that tilapia treated with male hormone during the first month of life had low mortality rate.

In this study, the percentage of males treated with ET-60 was 62.1%. This result differs apparently from that obtained by Guerrero (1975) in which 100% males of *Oreochromis aureus* was produced with the same androgen.

In the present study, according to Eckstein and Spira (1965) the fry of *O. niloticus* at 4-5 weeks old were sexually undifferentiated at the beginning of feeding with yeast diets.

Androgens, especially when given to fry or juvenile fishes at high dosage levels and for long duration of days, occasionally lead to complete sterility of the gonad (Takahashi, 1977). In the rainbow trout *salmo gairdneri* Jalabert *et al* (1975) stated that the oral administration of androgen diet for 5 months to juvenile fish could produce some sterile adults.

Takahashi (1977) indicated that all affected testes guppy *Poecilia reticulata*, 35 days after birth were improved and liberated after hormone withdrawal from a severe development suppression to assume eventually a nearly normal structure of the testes. Sterilization of guppy *P. reticulata* was complete only when the androgen treatment was protracted over 70 days following birth. Donaldson and George (1982) concluded that low dose of androgen caused male differentiation, while a high dose of androgen effectively sterilized both male and female. Guerrero (1975) reported that gonads of *O. aureus* treated with ET-60 μ g/g diet were comparable with those of the control, androgen had no gross morphological and histological effect on the gonads. Also, he stated that treated males spawned with females.

In the present experiment on *O. niloticus* small dose of androgen was given to fry for a short period (28 days after birth). Also, degeneration of the gonads of both males and females were not observed in the group of fish fed on control diet for 70 days. So, from the above results, it can be concluded that ET-60 μ g/g diet had no effect on the gonad development.

Guerrero (1975) found that no ovotestis condition was observed among the specimens examined of *O. aureus* treated with ethynyltestosterone. On the contrary, the results with the same androgen in the present study indicted that ovotestis condition was noticed among 17.2% of the gonads examined of *O. niloticus*.

Yamamoto, (1958) observed the appearance of oviform cells in the testes of the medaka, *Oryzias latipes*, subjected to the androgen treatment, but he regarded them as degenerated enlarged gonia, but not as true oocytes because there were no follicle cells, and the cells showed a strong affinity for dye. Also, Takahashi (1977) found this oviform cell in the testes of guppy. The oviform cells existing in the affected testes of *O. niloticus* truly resemble the young oocyte in the

first growth phase. They were too scanty and too spars in distribution to be regarded as spermatogonia forced to undergo degeneration by the treatment. A disturbance of testicular stromal tissue influenced by the androgen treatment in the present study might have some significance in the occurrence of oviform cell in the affected testes of *O.niloticus* (Takahashi, 1975).

In the present study, although the administration of ET-60 was commenced prior to the stage of ovarian differentiation, it could not completely prevent the appearance of oocytes in affected gonads of genetic females during gonadal development. As described in *Tilapia mossambica*, treated with androgen, some germ cells in the gonad of possible genetic females under went oogenesis even under the influence of androgen (Nakamura, 1975).

Yeast had a high nutritive value not only because of its protein content (15%), but also because of simultaneous presence of protein, vitamins and enzymes (DeSilva *et al.* 1989). The present study revealed that fry of *O.niloticus* fed on dietary yeast were significantly ($P<0.05$) higher in body weight than those fed on control diet. This finding agrees closely with those of Abd-El-halim *et al.* (1989) and Hussein (1998) on the same species. In this study, body length did not differ ($P>0.05$) between the control and yeast –treated fish during the experiment, indicating that yeast did not affect early growth.

Tacon and Cook (1980) stated that yeast has high content of nucleic acids (6-11% DNA &RNA) which accumulated in the blood. Feeding experiments have shown that yeast have high lysine which has a role with fish reproduction (Edwards and Hassall, 1980). In the present experiment on *O.niloticus* histopathological changes and complete sterilization and malformation of both testes and ovary were realized only when the fry (30 days old) were fed on the different levels of yeast. This might be explained by the high protein content in dietary yeast given during the maturation cycle of the gonad in *O.niloticus* as indicated by Washburn *et al.* (1990). Also, yeast is a source of vitamin B6 which is essential cofactor for the transaminases enzymes which is also known as amino transferases (Edwards and Hassall, 1980). Moreover, the metabolism of both methionine and cysteine, which are important in protein anabolism, dependent on the availability of vitamin B6 (Tryfiates, 1986). For this reason, the extreme physiological disturbance or even death in animals is thought to arise by an increasing or a deficiency of vitamin B6 (Edwards and Hassall, 1980).

Contrasting to the dietary protein requirement (28%) for young tilapia, weighing 1.5g (De-Silva *et al.* (1989), the dietary protein for young tilapia weighing 1.6g at the beginning of the experiment was 44%, then it decreased to 35% in the present study. It is widely accepted that too much energy in relation to protein can prevent fish from consuming enough protein.

Since histological anomalies were observed in the tilapia gonads the question remains as to the mechanism and pathway by which yeast and androgen or yeast only disrupt the normal gonadal cycle. The present experiment gives conclusive data that yeast directly affects the gonad development and lead to complete sterilization of both sexes. However, the specific function of yeast in endocrine tissues is still not fully understood and need further investigations.

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Table 1: Chemical composition of the control and active yeast diets.

Component (%)	Control diet	Active yeast				
		1g/kg	2g/kg	3g/kg	4g/kg	5g/kg
Dry matter	92.37	92.41	92.51	92.74	92.85	92.93
Crude protein	34.86	35.25	35.53	35.78	35.94	36.33
Ether extract	5.11	5.14	5.16	5.19	5.21	5.24
Crude fiber	4.75	4.76	4.81	4.84	4.86	4.89
Ash	8.83	8.88	8.93	8.98	9.03	9.08
Nitrogen free extract	46.45	45.97	45.57	45.21	44.96	44.46

Table 2: The average body weight, hepatosomatic and gonadosomatic indices of *Oreochromis nilotica* fed different levels of active yeast.

Treatment Parameter	Control diet	Active yeast				
		1g/kg	2g/kg	3g/kg	4g/kg	5g/kg
Initial Body Weight(g/Fish)	1.67	1.65	1.64	1.66	1.67	1.65
Final body weight (g/fish)	16.8 ^d	18.90 ^d	22.20 ^{bc}	26.16 ^a	22.80 ^b	21.25 ^{bc}
Hepatosomatic index(HIS)	1 ±0.27	2.1 ±0.23	1.6 ±0.25	2.6 ±0.21	1.4 ±0.35	1.7 ±0.41
Male (GSI)	0.7 ±0.04	1 ±0.11	2 ±0.05	0.8 ±0.09	1.5 ±0.14	1.7 ±0.16
Female (GSI).	1.2	0.3	4.5	9.7	8.4	notfound

EXPLANATION OF FIGURES

- Fig. 1:** Photomicrograph of a testis, control group, showing the normal mature lobules and the sperm duct distended with spermatozoa (S). X 36
- Fig. 2:** Photomicrograph of a control ovary showing ripe oocytes in normal 3 yolk stage. X 90
- Fig. 3:** Photomicrograph of a testis showing marked proliferation of the connective tissue (ct), many blood vessels packed with erythrocytes were visible between lobules (L) and extensive hemorrhagic necrosis. Atrophy of interstitial cells X 360
- Fig. 4:** Photomicrograph of a testis showing invasion of the blood vessel (bv) which were packed with erythrocytes (E). Many erythrocytes invaded the lobules (L) causing the atrophy of testis. X 360
- Fig. 5:** Photomicrograph of a testis showing enlargement of spermatogonia (g) which had abnormal appearance indicted by dense chromatin and degenerated cytoplasm. Pyknosis of nuclei of spermatocytes (P) and sperm's head (S). Disappearance of lobule wall. X 900
- Fig. 6:** Photomicrograph of a testis showing the degeneration of lobule wall (L), Pyknosis and necrosis (P) of all germ cells. X 900
- Fig. 7:** Photomicrograph of a testis showing, the enlargement of spermatogonia (g), disintegration of spermatozoa (S) whose nuclei became pyknotic (P) and disappearance of nest membrane (M). X 900
- Fig. 8:** Photomicrograph of the ovary showing degenerated oocytes, hypertrophy of granulosa layer (gl). X 36
- Fig. 9:** Photomicrograph of the ovary showing the invasion of blood vessel and hypertrophy of granulose (arrow). X 360

Fig. 10: Photomicrograph of the ovary showing edema and the interfollicular tissue contained leukocytes, and red blood cells and nuclear deformation (N). X 225.

Fig. 11: Photomicrograph of the ovary showing spent ovary with completely degenerated oocytes (DC). X 36

Fig. 12: Photomicrograph of a testis showing the appearance of oviform cell inside the testis (arrow). X 900

Fig. 13: Photomicrograph of the ovotestis showing the presence of oocytes at the outer region of the testis (arrow). X 36



