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AN ASSESSMENT STUDY OF GROWTH PERFORMANCE AND GONADS DEVELOPMENT OF MONO SEX NILE TILAPIA IN DIFFERENT AGE STAGES DURING THE PRODUCTION PERIOD

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ABSTRACT

The objective of this study was to follow up the growth performance and gonads development of sex reversed male Nile tilapia. Fish fed commercial diet contained 30% crude protein at a rate of 3% of the biomass and 10 fish were randomly taken bi-weekly during the experimental period (16 weeks). Growth performance, feed utilization and survival rate were calculated. Histological examination of the gonads was done every two weeks to follow the growth and development of the gonads. Results showed positive interactions between growth parameters and reproductive development during different periods of fish productive cycle. Histological examination illustrated that during early ages, testicular sections of mono sex male's tilapia had abnormal architecture with deterioration of germinative tissue. Despite abnormal testis texture, fish started spermatogenesis, a step toward puberty when their body weight reached 29.16g. As fish reached 4-5 months, male start to recover their testes normal structures and at age of 5.5-6 months, testicular sections appeared normally as pointed out by firmed testicular lobules, existence of all germ cell types and the intensively stored spermatozoa in testicular lumen and testicular ducts, confirming the full maturity of males. Based on the obtained results, it can clearly conclude that there is a relationship between age, body weight and development of sexual glands. It has also been shown that the hormonal treatment of tilapia seeds in the early stages for production of mono sex (all males) leads to a marked deterioration in the testes structure, continues near the fish enters the sexual maturity. At a later age, males can overcome this deterioration, restore the histological structure of the testes and achieve full sexual maturity at the age around from 5.5 to 6 months.

Keywords: Nile tilapia, mono sex, gonads histology, growth

INTRODUCTION

Problem associated with rearing mixed sex of Nile tilapia, *Oreochromis niloticus* is the early maturation and its ability to breed at young age and small sizes. These characteristics result in the overpopulation of stocked tilapia in ponds and the stunting of growth because of the crowding of the fish **(Fashina-Bombatta and Megbowon, 2012**). Moreover, the sizes of the fish at harvest, varying from small to large due to the faster growth of males. This makes it more difficult to establish uniformity of the product. Tilapia producers lock for high yields of large-sized fish within 6 months, therefore, all male fry is preferred. Androgens are usually used to induce sex inversion, a common technique used to obtain all-male population (**Lone and Matty 1980, Mires, 1995, Gale et al 1999, Megbowon and Mojekwu, 2014)**. All-male mono sex tilapia produced by 17-α-methyltestosterone, a synthetic androgen, is an outspreading technique for the commercial aquaculture due to not only high growth of male and better feed conversion, but also to disrupt the early spawning of tilapia. Since steroid hormones also could be used as growth promoter in fish, where they enhanced the weight gain of fish and improved the rate of muscle protein accretion **(Higgs et al 1977, Donaldson et al 1979 and Ahmed et al 2002).** Sexual maturity has a major role in regulating growth and leads to significant changes in body composition. During

periods of tilapia development patterns growth vary between males as they increase in age and sexual maturation (**Bhatta et al 2012).** The present study was carried out in order to correlate body growth with puberty and sexual maturity of male Nile tilapia, *O. niloticus,* besides follow up gonadal development of sex reversed males during the production periods for 4 months.

MATERIALS AND METHODS

The experimental fish (all male Nile tilapia, *O. niloticus*) were purchased from a private hatchery at Kafr El Sheikh Governorate, Egypt and stocked in a concrete pond (32m × 13m × 1.5m depth) belongs to Fish Production Branch, Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. This concrete pond was filled with a mixture of underground water and Nile water and 30,000 fry of mono sex Nile tilapia were stocked at a density of 50 fry/m^3 during May 2017. A 2.25 HP paddle wheel and 3HP air blower were used to aerate the pond with the needed oxygen.

Experimental diet

In the present study, fish fed a floated commercial diet containing 30% crude protein (CP) three times daily (six days a week) at a rate of 3 % of their total biomass. **Table 1** showed the composition and the chemical analysis of the commercial diet used in the experiment.

Table 1. Composition and chemical analysis of the commercial diet used in the experiment

Composition	**%**
Soy bean meal (46% CP)	28.0
Wheat middling	15.0
Wheat brane	12.0
Fish meal (60% CP)	10.0
Yellow corn	18.0
Corn gluten	10.0
Calcium diphosphate	2.0
Fish oil	2.0
Soy oil	2.7
*Premix (3451)	0.3
Chemical analysis	**on DM basis**
Crude protein (CP, %)	30.2
Ether extract (EE, %)	6.3
Crude fiber (CF, %)	4.5
Ash (%)	6.1
Nitrogen free extract (NFE, %)*	52.9
Gross energy (GE) (kcal/kg feed)	4100

*One kg premix contained:
Vitamins: $48x10^5$ I.U (A), $6x10^2$ mg (B_6), 20 mg (Biotin), $8x10^5$ I.U. (D_3), 144 mg (E), 400 mg (B_1), 1600 mg (B_2), $4x10^3$ mg (Pantothenic acid), 4 mg (B_{12}), $4x10^2$ mg (Niacin), $2x10^5$ mg (Choline chloride), and 400 mg (folic acid).

Minerals: $12x10^3$ mg Iron, $16x10^3$ mg Manganese, $12x10^2$ mg Copper, 120 mg Iodine, 80 mg Cobalt, 40 mg Selenium, and $16x10^3$ mg Zinc.

***NFE =** Nitrogen free extract (100 – [CP + Ash + CF + EE)].

***GE** = Gross Energy calculated as 5.65, 9.45, and 4.12 Kcal/ gram dry matter of protein, lipid and carbohydrates, respectively, after **(NRC, 2011).**

Water quality parameters

Water quality parameters were measured by using Nilebot® system, where water temperature, pH and dissolved oxygen (DO) were measured, recorded and sent online every 3 hours. Total ammonia concentration was measured by HACH comparison apparatus using HACK kits (Hach Co., Loveland, Colorado, USA). The percentages of unionized ammonia (NH_3) was calculated from multiplying the total ammonia value by the appropriate factor according to the following equation:

Ammonia concentration (mg/L as NH_3) = A / 100 × 1.2 × total ammonia.

Where, A is a coefficient related to water pH and temperature.

Fish sampling

Ten fish were randomly taken every 15 days throughout the experimental period that started from 26/7/2017 to 15/11/2017. Fish were weighed every two weeks to measure growth performance and to recalculate the daily feed allowances. Fish growth performance parameters such as total weight gain, total length (TL), thickness, width, average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) were measured. Gonads samples for ordinary histological examination were also taken biweekly.

Fish growth performance and feed utilization parameters were calculated according to **Cho and Kaushik (1986)** as following:

Average weight gain (AWG)= Final weight (g/fish) – initial weight (g/ fish).

Average daily gain (ADG, g / fish / day) = AWG (g) / experimental period (days).

Specific growth rate (SGR, % /day) = 100 (ln final weight - ln initial weight) / experimental period (day).

Feed conversion ratio (FCR) = Feed intake (g) / body weight gain (g).

Protein efficiency ratio (PER) = Gain in weight (g) / protein intake (g).

Protein productive value (PPV, %) = 100 [protein gain (g) / protein intake (g)].

Chemical analyses

Samples of the experimental diets and fish were chemically analyzed according to the **AOAC (2012)** Official Methods. Where, dry matter (DM) after drying in an oven at 105°C until constant weight; crude protein (N = 6.25) by Kjeldahl digestion and distillation after acid digestion; crude lipid by petroleum ether extraction in a Soxhlet extractor apparatus; ash by incineration in a muffle furnace at 550°C for 4h. Nitrogen free extract (NFE) was calculated by differences, by deducting the sum of percentages of CP, EE, CF and ash from 100.

Histological examination

The fish gonads were dissected into small pieces and fixed in formalin 10% solution for 24 hours. Later, fragments were preserved in 70 % ethanol, then dehydrated through an ascending series of alcohol, embedded and blocked in paraffin wax. Fine transverse sections of 5 µ were cut and stained with ordinary hematoxylin (H) and eosin (E) protocol according to **Genten et al. (2009).** The tissue slides were examined by light microscope and were photographed by a fluorescence Leica DM2500 Germany.

Statistical analysis

The data of growth parameters were subjected to one-way analysis of variance using SPSS-20 and expressed as means±SE. Significance was accepted at P< 0.05.

RESULTS AND DISCUSSION

Water quality

During the experimental periods water quality parameters were within the normal levels suitable for tilapia fish (**El-Sayed, 2006**), where, water temperature was maintained at 28±2ºC; pH was 8 ± 0.2; dissolved oxygen (DO) was 5.5 ± 0.5 mg/L and total ammonia was 0.0009 ± 0.0001 mg/L.

Growth performance

To investigate the state of growth, the differences between fish were analyzed every two weeks started from two months' age till fish reached six months' age. The body weight was found to be increased throughout the experimental period, where fish were able to double their weigh every 4 weeks. The initials fish TL were 9.30 ± 0.14 cm; BW1 6.33 ± 0.71 g; thickness 1.50 ± 0.0 cm and width 3.16 ± 0.06 cm, while the final fish TL, BW, thickness and width were 19.71 ± 0.48 cm, 173 ± 8.58 g, 3.21 ± 0.09 cm and 7.03 ± 0.15 cm, respectively **(Table 2).**

Table 2. Mean ± SE of body measurements of mono sex male Nile tilapia throughout the experimental periods

Sample No.	Body weight (g/fish)	Total length (cm)	Width (cm)	Thickness (cm)
Initial	16.33 ±0.71	9.30 ±0.14	3.16 ±0.06	1.50 ±0.00
1	23.33 ±1.08	10.06 ±0.14	3.31 ±0.10	1.51 ±0.06
2	29.16 ±2.38	11.11 ±0.41	3.31 ±0.14	1.51 ±0.06
3	48.16 ±3.33	13.31 ±0.39	4.46 ±0.15	2.05 ±0.03
4	54.66 ±1.68	14.00 ±0.08	4.70 ± 0.08	2.10 ±0.04
5	62.83 ±4.11	14.13 ±0.23	4.70 ± 0.20	2.15 ±0.05
6	68.33 ±11.06	14.23 ±0.74	4.81 ±0.33	2.20 ±0.12
7	107.66 ±6.46	16.08 ±0.25	5.65 ± 0.15	2.45 ±0.09
Final	173.16 ±11.06	17.23 ± 0.74	7.03 ±0.15	3.21 ± 0.09

Values within each column (between periods) are statistically deifferent (*P < 0.05*).

Data of fish growth rate and feed utilization throughout the experimental periods are presented in **Table (3).** These results are corresponding to those reported by **Marjani et al (2009)**, who revealed that, all male *O. niloticus* recorded higher growth performance in terms of final BW, standard length, weight gain, SGR, feed utilization parameters and survival rate.

Table 3. Growth performance and feed efficiency of mono sex male Nile tilapia throughout the whole experimental period (4 months)

Item	Initial weight (g/fish)	Final weight (g/fish)	Total gain (g/fish)	ADG	SGR	Feed intake (g/fish)	FCR	PER	PPV	Survival rate (%)
Mean	16.33 ± 0.71	173.16 ± 8.58	156.83 ± 7.87	1.31	1.97	283.84	1.63	2.47	44.45	98

Growth patterns of male Nile tilapia was changed **(Figure 1)** with increasing age and sexual maturity. The males began to grow rapidly at three months age, with the onset of spermatogenesis. The results showed that SGR of male Nile tilapia was 1.9, 2.1, 2.06 and 3.11 for the first, second, third, and fourth intervals (every four weeks), respectively **(Figure 2).**

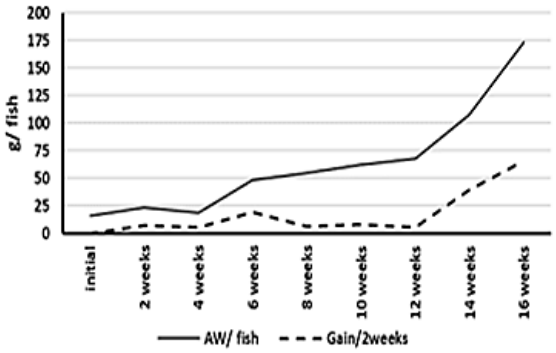

Fig. 1. Average weight (AW) and weight gain every two weeks intervals of mono sex male *O. niloticus*.

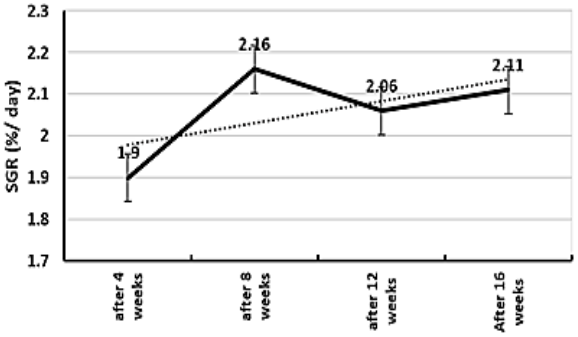

Fig. 2. Specific growth rate (%/d) of mono sex male *O. niloticus* every 4 weeks intervals during the experimental period.

Similar values were recorded by **Workagegn et al (2014)** who found that SGR of Nile tilapia was 3.2%/day. The results of **Alhassan et al (2018)** showed that SGR of *O. niloticus* fingerlings weighed (17.43–24.75 g/fish) was 2.51%/day, however, with smaller fish (2.03–7.26 g/fish) value of SGR was higher than that of larger fish.

Chemical composition analyses of the experimental fish are presented in **Table (4),** where moisture at the end of the experiment was 75.4%, body protein content was increased with increasing age, body lipid was changing with age, the highest lipid content was recordrd for three months aged fish while the lowest lipid content was recorded in fish aged six months. Ash content was increased with incerasing age, the highest ash percentage was recorded in six months aged fish.

Table 4: Chemical composition (% on dry matter basis) of the whole fish body at the end of the experiment

Item	Moisture (%)	Crude Protein (%)	Lipid (%)	Ash (%)
Initial	76.10	66.35	25.13	6.45
Final	75.40	72.90	21.70	7.50

Gonads development

The examined testicular sections were classified into several distinct spermatogenic stages according to the most developed germ cells according to **Kosai et al (2011)** as follow: **Stage I:** Immature testes were recognized by the absence of spermatogenic activity in the germinative tissue compartment and the presence of primarily spermatogonia. **Stage II:** Early spermatogenesis was characterized by germ cell proliferation and differentiation (spermatocytes to spermatids). **Stage III:** mid-spermatogenesis, the germinative tissue compartment was moderately thick and active germ cell differentiation could be observed; spermatocytes, spermatids and spermatozoa were present

in roughly equal proportion. **Stage IV:** Late spermatogenesis, the germinative tissue compartment was thick, although all germ cell types were existing, spermatozoa predominate in this stage. Stages from **II** to **IV** were characteristic of pubertal and sexually mature fish, with the least activity occurring in immature or offseason (stage II) and the most activity taking place immediately prior to and during the spawning season (stage IV).

The current study was carried out to follow-up the development of gonads of mono sex male tilapia in relation to body growth during the productive periods. At two months' age gonads histological observations of *O. niloticus* were detected as shown in **Figure 3,** where the appearance of testicular architecture was not firmed with deterioration of germinative layer of some testicular lobules, these findings could be due to the hormonal sex reversal applied during the fry stage (**Abdelhamid et al 2009**). The authors mentioned that the histological examination of testis of 17-α-methylt estosterone treated *O. niloticus* showed slight atrophy, abnormal structure and depression of seminiferous tubules, degeneration of spermatogenetic layer of some seminiferous tubules and disappearance regions of some seminiferous tubules. Nevertheless, in the present study the lobules of testicular section **(Figure 3)** appeared to contain some spermatogenic cysts at different stages of development, which mean spermatogenesis was started. Each spermatogenic cyst enclosed only one type of germ cells at the same stage of development. At this age fish body weight and fish body length were 16.3g and 9.3cm/fish, respectively**.**

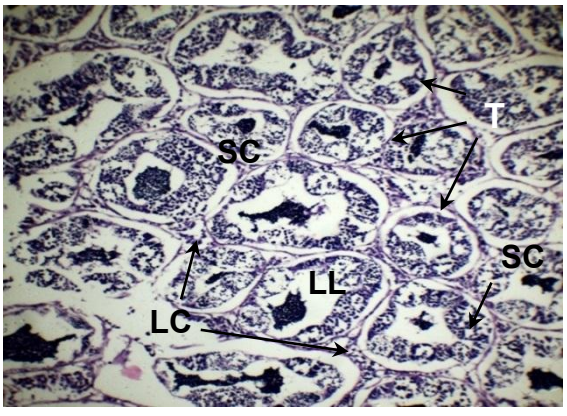

Fig. 3. Testicular transverse section of two months age mono sex *O. niloticus* males stained with H&E (10X), illustrates an abnormal overview of testicular formation, deterioration of testicular lobules and germinative epithelium. Testicular lobules (TL), spermatogenic cysts (SC), lobular lumen (LL) and Leydig cells (LC).

Similar histological findings were also observed in testicular sections of males aged 2.5, 3- and 3.5-months **(Figures 4 A; B and C,** respectively). Despite the abnormal testicular architecture, photomicrographs showed that after starting the meiosis, the spermatocytes were subjected to produce some scattered spermatid cysts **(Figure 4).** At this histological phase, mean of fish BW and TL were 48.16 g and 13.31 cm, respectively**.**

With increasing fish BW and TL reaching mean values of 68.33 g and 14.23 cm, respectively, mono sex *O. niloticus* males aged 4, 4.5 and 5 months began to recover their normal testes architectures, where testicular lobules and spermatogenic cysts appeared normally **(Figures 5 A; B and C).** All germ cell type cysts are existed including spermatogonia, spermatocyte and spermatids in addition to spermatozoa meaning the active spermatogenesis stage and spermiogenesis process**.**

When fish reached an averages BW of 173.16 g and TL of 17.23 cm, photomicrographs of their testicular sections appeared normally in texture, indicating that mono sex *O. niloticus* males after sex reversal at fry stage by 17-α- methyltestosterone can reach full sexual maturity at 5.5 to 6 months of age **(Figures 6 A and B)** as indicating by the intensively existence of spermatozoa in testicular lumen and testicular ducts. The expansion of the efferent and testicular ducts and its filled with spermatozoa were observed when males reached 5.5 - 6 months indicating full gonad maturation and milt could be acquired by hand-stripping **(Figure 6).** Thus, as fish BW increases with the advancing of fish age, gonads of mono sex *O. niloticus* males completely developed, and fish reaches the full sexual maturity, hence gonadal development is positively correlated with BW gain as previously mentioned by **Abdelhamid et al (2010).**

The stage of development in which the individual is able to reproduce sexually is called puberty, it is the developmental period which includes the transition from an immature state to a mature state of the reproductive system (**Kosai et al 2011)**). It is characterized by the activation of the brain-pituitary gonadal axis (BPG). In vertebrates, including non-mammals, germ cell maturation and development appear in a similarity **(Pudney, 1995).** Spermatogenesis can be divided into distinct sequential stages as follows: mitotic renewal of stem cells, mitotic proliferation of spermatogonia and supporting cells (e.g., Leydig and Sertoli cells), meiosis of germ cells to haploid spermatids and spermiogen-

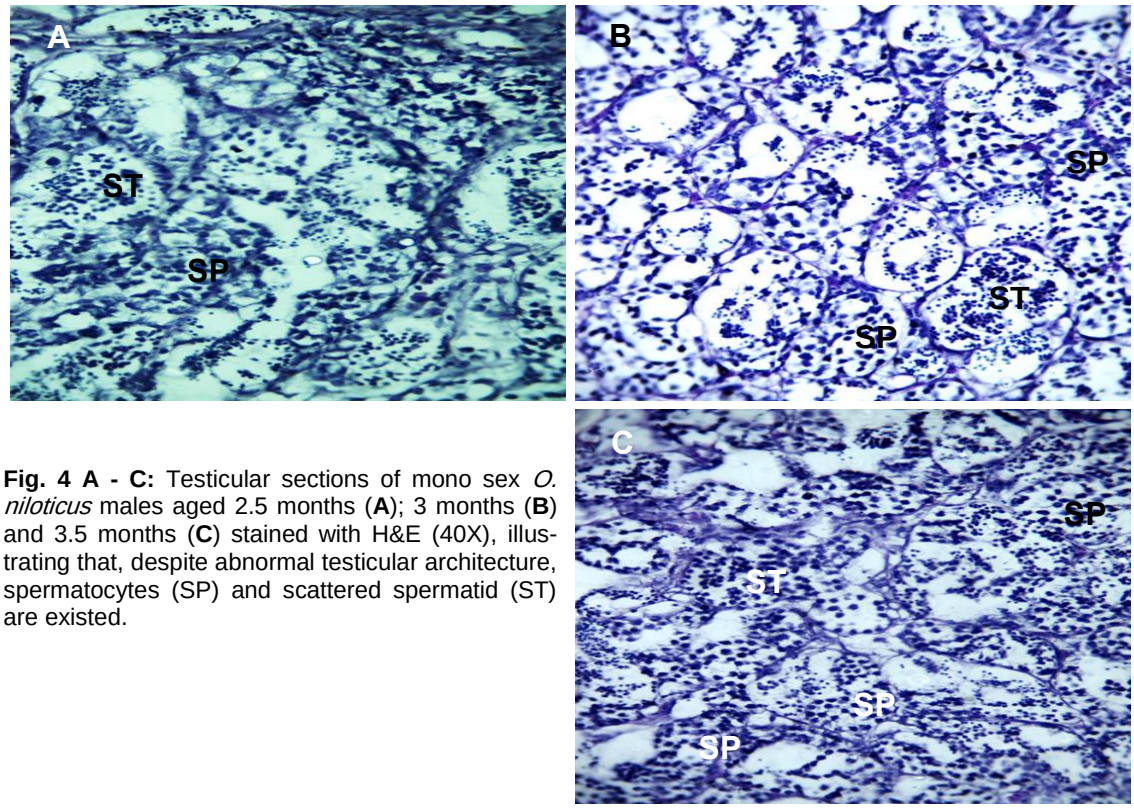

Fig. 4 A - C: Testicular sections of mono sex *O. niloticus* males aged 2.5 months (**A**); 3 months (**B**) and 3.5 months (**C**) stained with H&E (40X), illustrating that, despite abnormal testicular architecture, spermatocytes (SP) and scattered spermatid (ST) are existed.

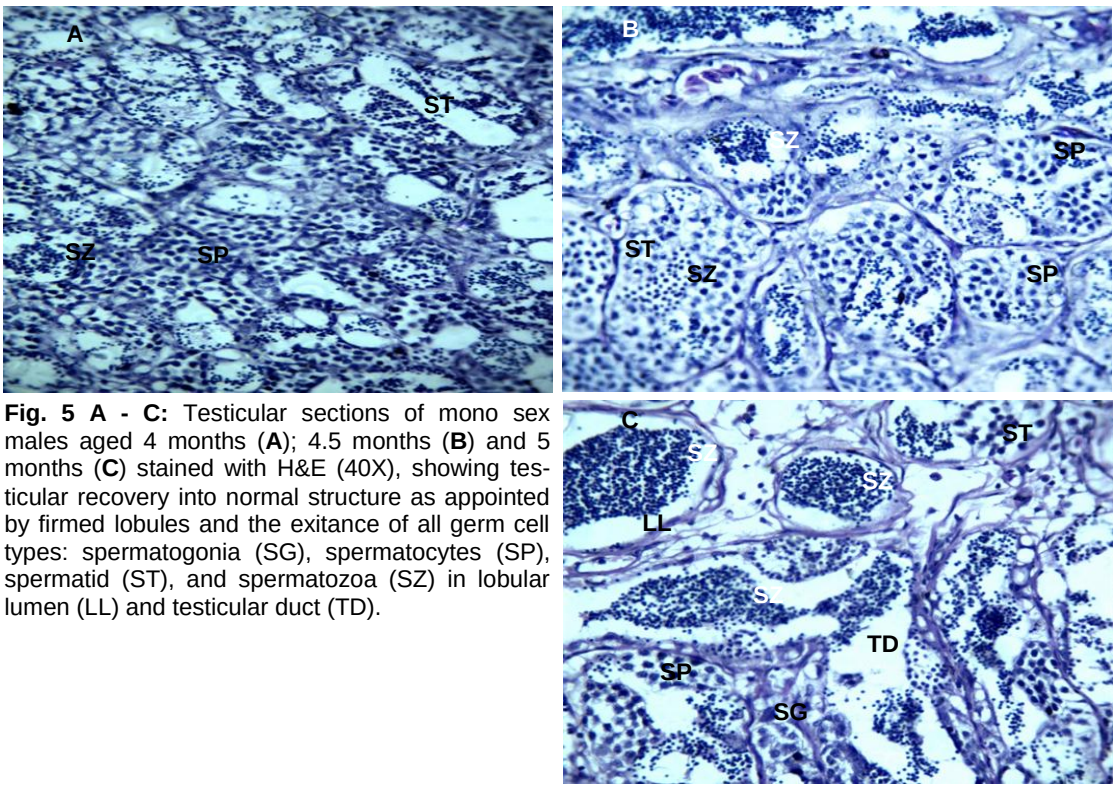

Fig. 5 A - C: Testicular sections of mono sex males aged 4 months (**A**); 4.5 months (**B**) and 5 months (**C**) stained with H&E (40X), showing testicular recovery into normal structure as appointed by firmed lobules and the exitance of all germ cell types: spermatogonia (SG), spermatocytes (SP), spermatid (ST), and spermatozoa (SZ) in lobular lumen (LL) and testicular duct (TD).

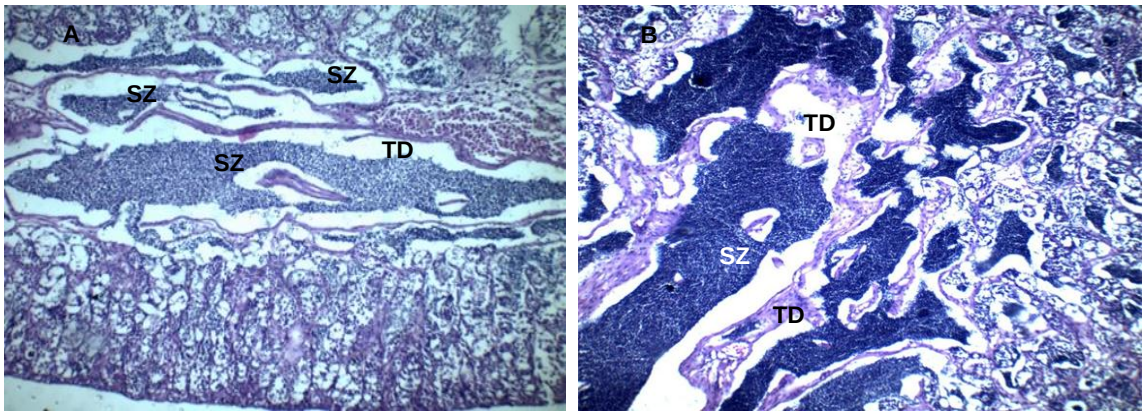

Fig. 6 A - B: Testicular transverse sections of mono sex male aged 5.5 months (**A**) and 6 months (**B**) stained with H&E (10X), showing full recovery of testicular structure, testicular lumens and ducts (TD) are intensively filled with spermatozoa (SZ).

esis (the transition of spermatids into fully functional spermatozoa) **(Miura et al 1991 & 2001 and Loir, 1999 and Schulz et al 2010)**.

In tilapia fry stage, undifferentiated gonads contained only somatic cells and primordial germ cells which later developed into spermatogonia. With the onset of spermatogenesis, spermatogonia stem cells proliferate for self-renewal and to multiply their numbers. Spermatogonia type A and type B are located on the edge of testicular lobules and a part of their Sertoli cells were attached to the basement membrane forming spermatogenic cyst. Concomitantly, multiplication of germ cell within each cyst takes place, and this development is paralleled by the elongation of testicular lamellae and rapidly differentiate into spermatocytes that entranced to meiotic divisions, which observed around age of two months **(Figure 3).** After the initiation of meiosis, cells rapidly underwent to spermatids, then processed through spermiogenesis, a morphological metamorphosis process to form spermatozoa. Around 3 months age, asynchronously all forms of spermatogenic cells spermatogonia (SG), spermatocyte (SC), spermatids (ST) and a few cysts of spermatozoa (SZ) were presented in testis **(Figure 4),** the same results were mentioned by **Msiska (2002).**

Present observations of histological study revealed that testis of hormonal reversed mono sex male Nile tilapia during early stages of development appeared abnormal in its architectures with abnormal testicular lobule and spermatogenic cysts. These findings agree with **Magouz et al (2000) and Abdelhamid et al (2009)** who found that 17-α-methyltestosterone hormonal treatment resulted in abnormal structure of the tilapia fish testis including seminiferous tubules degeneration and atrophy of the interstitial tissue. In the present study, histological observations reveled that mono sex male Nile tilapia recovered their normal testicular structure with increasing body weight and by the advancing of age a full maturity was reached at age of 5.5 to 6 months as indicating by the intensive existence of spermatozoa in testicular lumens and testis ducts. Reversely, **Ebada (2004)** reported that the use of 17-α-methyltestosterone with tilapia fry caused sterility, in term of separation of the spermatogenetic layers from the basement membrane of the seminiferous tubules of the testes. Moreover, the author mentioned that seminiferous tubules are free from spermatogenetic layers and the interstitial was almost free from Leydig cells. The last author added that there were no spermatozoa in the lumen of the seminiferous tubules in Nile tilapia sex reversed male fish.

CONCLUSION

Based on the above obtained results, it could be clearly concluded that there is a strong relationship between age, BW, growth and development of *O. niloticus* sexual glands. It has been also shown that the hormonal treatment of tilapia seeds in the early stages for single sex production (all-males) leads to a marked deterioration in testicular structure that continues until the fish begin to enter sexual maturity, then and at a later age, males can overcome this deterioration and restore the testicular structure and achieve full sexual maturity at the age around from 5.5 to 6 months**.**

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دراسة تقييمية لأداء النمو وتطور الغدد التناسلية للبلطي النيلي آحادي الجنس في المراحل العمرية المختلفة خلال فترة الإنتاج

[37]

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الموجـــــز

تم تصميم هذه الدراسة لتقييم أثر التغذية بعليقة تجارية خلال دورة الإنتاج على تطورِ الغدد التناسلية لذكور البلطي النيلي المحولة جنسيا. حيث تم تربية الأسماك في حوض أسمنتى (32×13×1,5م) تابع لقسم الإنتاج الحيوانى – كلية الزراعة – جامعة عين شمس. وتم تغذية الأسماك على عليقة تجارية (30 % بروتين خام) بمعدل 3 % من الكتلة الحية. تم أخذ 10 أسماك كعينة عشوائية كل أسبوعين طوال الفترة التجريبية (16 أسبوع)، حيث تم وزن الأسماك لضبط نسب التغذية وحساب مقاييس النمو. كذلك تم إجراء الفحص النسيجي للغدد التناسلية كل أسبوعين لتتبع نموها وتطورها.

أظهرت النتائج وجود علاقة بين نمو الأسماك وتطور الغدد التناسلية خلال الفترات العمرية المختلفة على مدار دورة الإنتاج. أوضحت النتائج أن ذكور البلطى النيلى المحولة جنسيا استطاعت مضاعفة وزنها كل 4 أسابيع. كما بينت الدراسة الهستولوجية أن الذكور بدأت عملية تكوين الحيوانات المنوية عند وزن جسم 29,16جم. أثناء تلك المرحلة العمرية، أظهرت القطاعات الهستولوجية للخصية تدهور واضمحلال واضح فى وحدات النسيج الجرثومى، وبرغم هذا التدهور فبزيادة الوزن والعمر استمرت عملية نمو وتطور الخلايا المنوية. أظهرت القطاعات الهستولوجية أن الخصيتين بدأت تسترد تركيبها الطبيعى عندما وصلت الأسماك لعمر 4–5 شهور (54,66–68,33 جم/سمكة). وعند عمر 5,5–6 أشهر، استعادت الأسماك البنية الطبيعية لخصيتيها بشكل كامل واتضح ذلك من تماسك تركيب فصيصات الخصية ووجود جميع أنواع الخلايا الجرثومية وظهور الحيوانات المنوية مخزنة بكثافة في التجويف الخصوى والقنوات الخصوية مما يؤكد وصول الذكور إلى النضج الجنسى.

استنادا إلى النتائج المتحصل عليها، يمكن استنتاج وجود علاقة بين العمر ووزن الجسم ونمو وتطور الغدد الجنسية. وأن المعاملة الهرمونية لزريعة البلطي في المراحل المبكرة لإنتاج البلطى وحيد الجنس (الكل ذكور) سبب تدهور ملحوظ في بنية الخصية استمر إلى بداية الدخول فى مرحلة البلوغ الجنسى، بعدها تمكنت الذكور التغلب على هذا التدهور واستعادة التركيب الطبيعي للخصية وتحقيق النضج الجنسي التام عند عمر يتراوح من 5.5 إلى 6 شهور

الكلمات الدالة: البلطي النيلي، وحيد الجنس، النمو، قطاعات هيستولوجية في الغدد الجنسية

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