Effect of some commercial feed additives on the structure of gonads and microbiology of Nile tilapia (*Oreochromis niloticus*) fish

Abdelhamid M. Abdelhamid [1], Mehrim, A. I.[1], El-Barbary, M. I.[2] and El-Sharawy, M. A.[1]

1- Animal Production Dept., Faculty of Agriculture, Al-Mansoura University, Egypt
2- Fish pathology Lab., National Institute of Oceanography and Fisheries, Egypt

ABSTRACT

Tilapia is a popular food fish all over the world and in Egypt it has been raised as food for human consumption for a long time. A field study was conducted on brood stock Nile tilapia *Oreochromis niloticus* to improve their reproductive performance by some commercial feed additives. Both sexes were individually stocked into hapas (enclosures) and fed a basal diet supplemented with graded levels 0.0, 0.5, 1.0 and 2.0 g Therigon$^®$ kg^{-1} diet (T_1-T_4; T_1 as a control treatment, respectively); 1.0, 2.0, and 3.0 g Nuvisol Hatch P$^®$ kg^{-1} diet; (T_5-T_7); 20, 40 and 60 mg gibberllic acid kg^{-1} diet (T_8-T_{10}) for female only and 700, 900 and 1100 mg L-carnitine kg^{-1} diet for males only (T_{12}-T_{14}), respectively for 19 day. Results revealed that fish ovaries in all treatments (T_2-T_{10}) contained more advanced oocytes at final maturation stage with clear histological alterations of fish fed the highest levels of these feed additives, especially with gibberllic acid treatment. Fish treated with 60 mg gibberllic acid kg^{-1} diet (T_{10}) showed the worst histological effects on fish ovary among all treatments. Addition of 700 and 900 mg L-carnitine kg^{-1} diet caused maturation of testes better than the control treatment (T_{11}). Meanwhile, fish fed 1100 mg L-carnitine kg^{-1} diet (T_{14}) showed degeneration and severe hemolysis in seminiferous tubules. Low bacterial loads were recorded (in gills, liver, intestine and ovary / testes) at the high level of Nuvisol Hatch P$^®$. Data proved that the high bacterial load was detected in fish treated with L-carnitine in both tested media and in all tested organs. Meanwhile, low bacterial load was observed in the liver, ovary and testes in all media comparing with the other organs (gills and intestine).

Keywords: Tilapia brood stock – Gonads histology – Microbiology - Therigon$^®$ - Nuvisol Hatch P$^®$ - Gibberllic acid - L - carnitine.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) are considered as the most common and popular fish in Egypt. Egypt is a country where, arguably, the farming of tilapia has its roots (Stickney, 2006). Latest fish production statistics in Egypt revealed that tilapia constitutes 52.86% of the total fish production in 2010 (GAFRD, 2010) and occupy the 10th order concerning the world production from aquaculture (Van Hauwaert *et al*., 2000)**.** Hence, Egypt produces 20% of the world tilapia catch and 12% of the world farmed tilapia (El-Sayed, 2006). The culture of *O. niloticus* in Egypt is still growing in a remarkable way with apparent intend towards intensification that pressurizing the need of enormous number of seeds to satisfy the needs for aquaculture, so rigorous management of large numbers of brood stock are necessary for mass production of eggs and fry due to relatively small number of eggs produced per spawning.

Many limitations associated with tilapia fry production under the prevailing Egyptian conditions were described by El-Gamal (2002). Furthermore, it is widely

accepted that effective seed production demands a thorough understanding of the special husbandry and particular nutritional requirements of brood stock fish which significantly affect fecundity, survival, egg size, fertilization percentage and larval hatchability quality (Bromage, 1998). Therefore, the present research aimed to evaluate the powerful of three commercial synthetic feed additives (Therigon[®], Nuvisol Hatch P[®] and gibberllic acid) for improving the reproductive performance of females Nile tilapia brood stock, and fourth one (L-carnitine) for males from the view point of the gonadal structure characteristics and microbiological examination.

MATERIALS AND METHODS

Experimental management:
        A field study was conducted in a private earthen pond fish farm located at Alabhar belonging to Alhamol, Kafr Alshiekh governorate. Fourteen hapas (each 3 m width × 6 m length × 0.5 m water depth and 1 m total depth) were constructed in a two feddans earthen pond. The first ten hapas were stocked with *O. niloticus* females' brood stock of one year with an average body weight of 150 g from the same farm. The other four hapas were stocked with males' brood stock of (the same age as the females, but of an average body weight of 200 g) from the same farm also. Each hapa was stocked with twenty fish. They were incorporated in a feeding trial to test the effects of some commercial dietary additives on fish propagation. The experimental feeding period (19 days) began on the 20th June till the 8th of July, where the feed was offered to fish twice daily, at a daily feeding rate of 3% of the fish biomass in each hapa. The feed additives were purchased from the local market and added directly to a mash diet, which was purchased also from the local market (contained 90.31% dry matter, 80.88 % organic matter, 23.81% crude protein, 5.47% ether extract, 51.60% carbohydrate and 9.43% ash) and moistened to be pelleted via a hand mincer.

Dietary treatments:
        Fish were fed on a basal ration (BR) with or without (control) the tested feed additives as illustrated in Table 1 which were:

1- Therigon[®] powder for veterinary use, manufactured by Adwia Co., S. A. E. 10th of Ramadan city, Egypt. Each 1g contains Alpha-Amino-p-hydroxyhydrocynnamic acid, 1000 g package as GnRH stimulant (Batch No. 0601116).

2- Nuvisol Hatch P[®], imported by Khirat Alnile Co., 27 Alferdos Buildings, Flat 43, Nasr city, Egypt from Newtrix Co., Belgium, in 500 g package. Each 1 Kg contains the following vitamins (in mg): B_1 4000, B_2 5000, B_3 4000, B_6 2000, B_9 1000, B_{12} 20, PP 10000, Biotin 50, and L – carnitine 30000.

3- Gibberllic acid ($C_{19} H_{22} O_6$), type analysis, Art. 3930, M. W. 346.38, M. P. 225 °C, GA_3 content 90 %, 1g package, Batch No. 43124, imported from Lobal Chemie, Pvt. LTD, 2042 Bombay, India.

 4- L – carnitine powder from Mebaco, Egypt.

Criteria measured:
        After the 19 days feeding trial of the separate sexes of brood stock, fish samples were caught from each hapa for the histological examination of the gonads, and for microbiological tests for gills, livers, intestine and gonads.

Histological examination:
        At the end of the experiment, fish were sacrificed and the gonads were sampled. Samples were fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated by different grades of alcohol (70, 85, 96 and 99%). Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were

sectioned into six micron. The sections were stained by hematoxyline (H) and eosin (E) and then subjected to a histological examination according to Roberts (2001).

Table1: Details of the experimental diets (treatments)

Treatment	Details
1, ♀	Basal ration (control femeles)
2, ♀	Basal ration + 0.5 g Therigon®/ kg diet
3, ♀	Basal ration + 1.0 g Therigon®/ kg diet
4, ♀	Basal ration + 2.0 g Therigon®/ kg diet
5, ♀	Basal ration + 1 g Nuvisol Hatch P® / kg diet
6 , ♀	Basal ration + 2 g Nuvisol Hatch P® / kg diet
٧, ♀	Basal ration + 3 g Nuvisol Hatch P® / kg diet
8, ♀	Basal ration + 20 mg Gibberllic acid / kg diet
9, ♀	Basal ration + 40 mg Gibberllic acid / kg diet
10 ,♀	Basal ration + 60 mg Gibberllic acid / kg diet
11, ♂	Basal ration (control males)
12, ♂	Basal ration + 700 mg L - carnitine / kg diet
13, ♂	Basal ration + 900 mg L - carnitine / kg diet
14, ♂	Basal ration + 1100 mg L - carnitine / kg diet

Microbiological examination:

Bacteria were identified by general and specific media and biochemical testes, the following were the cultivation media used for different purposes.
1- Nutrient agar (NA, as a general medium). 2- Salmonella / Shigella agar (SSA). 3- Brucella agar (BR). 4- Baird-parker agar (BP). 5- Trypticas soy agar (TSA). Three fish from each hapa were used for bacterial counts in fish organs (gills, liver, intestine and ovary/ testes). The fish were killed by physical destruction of the brain and the number of incidental organisms was reduced by washing the fish skin with 70% ethanol before taking the gills and opening the ventral surface with sterile scissors to expose the body cavity. From each fish, 1 g of each tested organ was removed and suspended in 10 ml of sterile saline (0.85% (w/v) NaCl). The suspension was serially diluted to 10^3, and 1 ml of the solution was spread on each medium plates. The inoculated plates were cultured as described above and the number of colonies was counted. Appropriate serial dilutions of fish and water samples were then plated onto plate count agar.

Aerobic plate count (APC):

For total heterotrophic aerobic bacterial counts of gills, liver, intestine and ovary/ testes of tilapia, all the inoculated plates were incubated at 30^oC for 48 h and colony forming units (CFU) were counted. Readings obtained within the range 30 to 300 colonies on a plate were used to calculate bacterial population numbers, recorded as CFU per g of sample. The bacterial colonies were divided into different types according to the colony characteristics shape, size, color and opacity, and the number of colonies of each recognizable type was counted. Three to five representatives of each colony type were then streaked on additional NA plates repeatedly until pure cultures were obtained.

Identification of bacteria:

All the purified isolates were observed for cell shape, spores, and gram stain reaction. The isolates were then subjected to biochemical identification following the criteria described in the Bergey's Manual of Systematic Bacteriology (1st ed.) (Holt *et al*., 1994) for identification to the genus or the species level.

Statistical analysis:

Data of the bacteriology were statistically analyzed using coefficient of variance, cross tab, and Chi-Square test, which were described by Bailey (1995).

RESULTS AND DISCUSSION

Histological examinations:
A) Ovaries

The histological studies of ovarian tissues of *O. niloticus* (Figs. 1 & 2) fed the basal ration (BR) with or without the tested feed additives (T_2-T_{10}, and T_1, respectively) showed three developmental stages of oocytes during oogenesis. These stages include cortical alveoli formation (yolk vesicle stage), vitellogenic (yolk) stage and ripe or mature stage, while few oocytes appeared at early stages.

Fish fed BR (control) showed normal structure of ovarian tissues; it showed ripe stage of oocyte with migratory nucleus and start of yolk liquefaction of cytoplasm of oocyte and large connected vacuoles of some oocytes (Fig. 1a). Ovary of fish fed the BR plus 0.5g Therigon/ kg diet (T_2) showed ripe stage of oocyte (Fig. 1b). While the ovaries of fish fed the BR plus 1.0g Therigon/ kg diet (T_3) showed oocyte in yolk vesicle stage (in normal structure) beside many oocytes in ripe stage. In addition, clear histological lesions were noticed in ovary as coagulative necrosis in yolk granules, separation of follicular layers, degeneration in oocyte (atresia) and focal area of necrosis in ovarian tissues, where the ovarian tissues were found to consist of interstitial cells, adipose cells, yolk granules and blood capillaries (Fig. 1c). Degenerative changes (atretic follicles) were observed in the ripe oocytes in ovary of fish fed the BR plus 2.0g Therigon/ kg diet (T_4) with depletion of the yolk granules (Fig. 1d). So, the ovaries in fish fed with diets supplemented with 0.5g Therigon/ kg diet were protected to be atretic comparing with those fed high levels of Therigon (1.0 & 2.0g / kg diet).

Fish fed the BR plus 1.0g Nuvisol Hatch P[®] / kg diet (T_5) showed that the ovary becomes more developed and reached to the final maturation (ripe oocytes) stage with few atretic follicles (Fig. 1e). The level of 2.0g Nuvisol Hatch P[®] / kg diet (T_6) showed different development stages of oocytes (chromatin nucleolar stage, perinucleolar stage, cortical alveoli formation stage, vitellogenic stage, ripe stage) with irregular walls of oocytes (Fig. 1f). Whereas the high level of Nuvisol Hatch P[®] (3.0g / kg diet, T_7) showed ripe oocytes stage with migratory nucleus which has an irregular outline, besides hemolysis in the ovarian tissues (Fig. 2a). Concerning the ovaries of fish fed the gibberllic acid at the 3 levels (T_8 - T_{10}, Fig. 2, b - f), T_8 and T_9 showed the ovary in ripe stage with slight histological changes of oocytes and liquefaction of cytoplasm of oocyte with nucleus loses its circularity and degeneration in oocyte wall (Fig. 2, b & c). Yet, fish fed the BR plus 60 mg /kg diet gibberllic acid (T_{10}) shows the ovary in ripe stage and vitellogenic oocyte (Fig. 2, d & e) with histological alterations including erosion in walls of some oocytes, coagulative necrosis, liquefaction of the yolk sphere with large vacuoles of ripe oocyte of *O. niloticus*, and irregular wall of oocytes (Fig. 2, d & f).

Ovaries of fish fed the two high levels of tested feed additives showed histological alterations. Furthermore, fish treated with dietary supplementation of gibberllic acid showed the worst effects on fish ovary among all treatments. In contrast, the low level of these feed additives showed no clear histological changes of ovary, especially 0.5g Therigon/ kg diet (T_2) which reflected the high level of FSH (91 mIU /ml) among all treatments (Abdelhamid *et al.*, 2010; a complementary study

of the present work). According to Nagahama *et al.* (1995 a & b), vitellogenesis and oocyte maturation are regulated primarily by FSH and LH, respectively.

The histological changes in the ovaries of *O. niloticus* observed in some treatments may be attributed to many causes, such as a mechanism to regulate fecundity may be due to the tested feed additives or to environmental stress (as water pollution), where the different pollutants such as agriculture wastes and different types of bacteria have histological effects on the reproductive tissues of fish gonads (Lye *et al.*, 1998). These effects may disturb the development of germ cells and may reduce the ability of fish to reproduce.

Furthermore, Abdelhamid *et al.* (2010) confirmed that dietary inclusion of 1 g Nuvisol Hatch P$^®$ / kg diet (T$_5$) and 1 g Therigon$^®$(as GnRH stimulant) / kg diet (T$_3$) realized good female's reproduction performance. Gonadotropin releasing hormone (GnRH) is a hypothalamic neuronal secretory decapeptide that plays a pivotal role in reproduction. GnRH analogue stimulates gonadotropin secretion (Van Der Kraak *et al.*, 1983) steroidogenesis (Van Der Kraak *et al.*, 1984) and ovulation (Haraldsson and Sveinsson, 1993) in salmon and in many other species. In fish, gibberllic acid (GA$_3$) at low levels improved Nile tilapia growth and gonado-somatic index (Abdelhamid *et al.*, 1998), since it is a nitrogenous compound with estrogenic effect; where it increased the percent of egg production, hatchability and ovary and oviduct relative weight significantly (El-Sebai *et al.*, 2003). So using natural GA$_3$ instead of the synthetic estrogen is safer and environmental friendly, therefore should be considered.

B) Testis

Testis of Nile tilapia brood stock fed the basal ration only (T$_{11}$, as a control) showed normal structure of seminiferous tubules with spermatocytes, spermatids and few sperms (Fig. 3 a & 3). However, dietary supplementation of L-carnitine caused histological maturation of testis of treated fish especially at level of 700 mg (T$_{12}$), which showed normal structure of seminiferous tubules filled with spermatozoa, spermatocytes and spermatids (Fig. 3 c & d). In case of the fish fed 900 mg L-carnitine / kg diet, testes showed normal structure of seminiferous tubules filled with spermatids and spermatozoa at ripe stage (Fig. 3e).

Meanwhile, fish fed the basal ration plus 1100 mg L-carnitine / kg diet (T$_{14}$) showed extensive degeneration of the seminiferous tubules, necrotic changes in the cellular elements of the seminiferous tubules and focal areas of necrosis with severe hemolysis of testes' tissue. In addition, testes of this group appeared free from spermatocytes or spermatids among all treatments (Fig. 3 f). These drastic histological alterations in testes may be related with increasing level of L-carnitine in fish diet. In addition, all of these histological alterations in testis are due to the commercial feed additives tested in the present study.

Several stages of spermatogenesis (spermatocytes, spermatid and spermatozoa) in the present study are similar to those reported by Msiska (2002). In addition, secondary spermatocytes were illustrated by darkly chromatin staining as in other teleost fish. Meanwhile, spermatozoa were concentrated in the lumen (Tyler and Sumpter, 1996). Furthermore, Abdelhamid *et al.* (2010) confirmed that dietary supplementation with 700 (T$_{12}$) and 900 (T$_{13}$) mg L-carnitine / kg diet gave better results of males' reproductive performance. L–carnitine is a naturally containing amino acid derivative (dipeptide amino acid), synthesized from methionine and lysine**.** L–carmitin, a betaine derivative of β-hydroxybutyrate, could be biosynthesized in plant and animal cells via lysine, methionine, and some vitamins like B$_6$, C, nicotinic acid and folate (Zeyner and Hameyer, 1999).

Since L–carnitine provides an energetic substrate for the spermatozoa in the epidydimis, it contributes directly in sperm motility and may be involved in the successful maturation of the sperm (Chatzifotis *et al.*, 1995). L–carnitine improved semen quality and histological characteristics of the testes (El-Damrawy, 2007). Generally, a low level of L–carnitine enrichment provides several protective effects in fish reared under intensive pond-culture conditions (Harpaz, 2005). Also, Jayaprakas *et al.* (1996) reported that supplementation with 900 mg L-carnitine / kg diet had a positive effect on the reproductive performance of male Mossambique tilapia, significantly increasing both testes weight and sperm cell concentration per ml compared with the control.

Count and identification of pathogenic bacteria in gonads and other fish organs:

The results of the total count of bacterial isolates on different media from gonads of *O. niloticuss* among different treatments are shown in Tables 2 and 3. Data exhibited absence of bacterial loads of ovaries in fish fed the diets supplemented with the three different levels of Nuvisol Hatch P$^®$, among different tested media, So, Nuvisol Hatch P$^®$ may have a protective effect against bacterial infection; in contrast, the high bacterial load appeared in testes on BR and BP media with the two high levels of L- carnitine (900 & 1100 mg / kg diet; T_{13} &T_{14}). Only T_{13} showed that SSA appeared total count 60 x 10^3 CFU/g, this media has given an appreciable count of *Salmonella* sp., *Shigella* sp., *Proteus* sp. and *Escherichia coli*. All these genera are belonging to the family *Enterobacteriaceae* which are capable of inducing gut wrenching gastroenteritis as reported by Anon. (2005). The biochemical tests (Table 4) showed that the isolated bacteria from testes were *Bacillus* sp*., Salmonella.* sp and *Brucella.* sp. which were found in T_{13} more than T_{14}. So, the histological alterations of testes in T_{14} attributed to the treatment with high level of L– carnitine (1100 mg/ kg diet) not to the contaminated bacteria.

Table 2: Bacterial count (CFU/g) of ovary /testes of *O. niloticus* isolated on different media from different experimental treatments.

	Treatment	Media				
		NA	SSA	BR	BP	TSA
1	**Control** ♀	0	0	15×10⁴	0	10×10⁴
2	**Therigon**$^®$ ♀	40×10³	0	2×10⁴	0	19×10⁴
3		2×10³	0	0	0	23×10⁴
4		5×10³	0	25×10³	0	7×10³
5	**Nuvisol Hatch P**$^®$ ♀	0	0	0	0	0
6		1×10³	0	0	0	0
7		0	0	0	0	0
8	**Gibberllic acid** ♀	0	0	6×10³	0	3×10³
9		27×10³	0	0	0	0
10		0	0	0	0	0
11	**Control** ♂	4×10³	0	0	0	0
12	**L- carnitine** ♂	11×10³	0	0	0	0
13		73×10³	60×10³	20×10⁴	9×10³	0
14		0	0	15×10⁴	8×10³	0

The typical colonial morphology on SSA medium is as follows: both *Salmonella* and *Proteus* sp. produce colorless colonies with black center, *Shigella* colony is colorless (Bopp *et al*., 1999), while *E. coli* colony is pink to red. Baird- parker agar (BP) culture medium was specially used for isolation and identification of *Staphylococcus* sp., *Micrococcus* (coccoid bacteria) and *Bacillus* (*Bacillus* is belonged to family *Bacillaceae*).

Table 3: Comparison of bacterial count obtained on different media in fish organs from different experimental treatments (means, $\times 10^3$).

Media	Treat. Organ	Control (♀ & ♂)	Therigon®	Nuvisol Hatch P®	Gibberllic acid	L - carnitine
NA	Gills	99.0	30.0	8.0	5.3	143.0
	Liver	0.0	1.6	0.3	4.6	9.0
	Intestine	10.0	10.0	7.6	54.0	18.0
	Gonads	1.3	9.0	0.3	9.0	120.0
SSA	Gills	100	11.0	5.3	271.0	26.0
	Liver	0.0	0.0	0.0	4.3	0.0
	Intestine	0.0	0.0	0.0	130.0	38.0
	Gonads	0.0	0.0	0.0	0.0	200.0
BR	Gills	200.0	140.0	150.0	140.0	200.0
	Liver	0.0	0.0	0.0	3.3	3.0
	Intestine	5.0	66.0	270.0	4.0	50.0
	Gonads	46.0	7.5	0.0	2.0	120.0
BP	Gills	210.0	50.0	83.0	51.0	240.0
	Liver	0.0	0.0	0.0	2.3	16.0
	Intestine	0.0	0.0	0.0	69.0	70.0
	Gonads	0.0	0.0	0.0	0.0	6.0
TSA	Gills	110.0	130.0	140.0	230.0	226.0
	Liver	0.0	0.0	7.0	13.0	0.0
	Intestine	0.0	18.0	7.5	150.0	22.6
	Gonads	29.0	140.0	0.0	1.0	0.0

Staphylococcus produces black colony on BP medium while *Micrococcus* and *Bacillus* species produce brown colony on the same medium. Brucella agar (BR) medium was used for the isolation and cultivation of *Brucella* that is coccobacilli and belonging to the family of *Brucellaceae*. Trypticas soy agar (TSA) medium was specially used for isolation of *Streptococcus*. Some biochemical testes were carried out for identification of these isolated colonies of bacteria as shown in Table 4.

Table 4: Morphological and biochemical characteristics of the isolated bacteria from fish samples which obtained on different media

Criteria	Specific morphological criteria of different bacterial genera							
Color	Pink to Red	Black center	Colorless	Black center	brown	Translucent	Brown	Yellow
Media	SSA	SSA	SSA	SSA	BP	TSA	BP	RS
Shape	Rods	Rods	Rods	Rods	Rods	Coccus (chains)	Coccus (clusters)	Rods
Gram stain	-	-	-	-	+	+	+	-
Oxidase	-	-	-	-	+	-	+	+
test	+	+	+	+	+	-	+	+
Catalase	+	-	-	+	N	N	N	+
Indole test	+	+	+	+	+	+	-	+
Glucose	+	+	-	-	N	N	N	-
Lactose	+	+	-	-	N	N	N	+
Mannitol	-	-	-	-	+/-	-	-	-
Endospores								
Suggested	*E.* coli	*Salmonella* sp.	*Shigella* sp.	*Proteus.* sp.	*Bacillus* sp.	*Streptococcus* sp.	*Micrococcus* sp.	*Aeromonas* sp.

Data in Table 5 represent the suspected bacteria found in various treatments and different organs of fish as well as their cross (Table 6, Chi square). The results

showed that the high bacterial load was observed in both gills and intestine on all media comparing with the other organs (liver and ovary /testes), this agrees with Austin (1982) who reported that most of the microorganisms in fish tissue are thought to result from surface, gills, or intestinal contamination, where microorganisms are adsorbed on the surfaces of the fish and found in their intestinal contents. So the initial microbial flora on the caught fish is dependent upon the contamination of the water, bottom sediment from the area of catch and the food entering the digestive tract which may contain microorganisms. In addition, the total count of bacteria obtained on SSA and BP media was lower in all tested organs than the other media (NA, BR and TSA). The statistical comparison of bacterial load of the tested organs among different treatments showed some observation (Table 3).

No significant differences were found among tested organs (except gills) and treatments. Generally, tabulated data proved that high bacterial load was detected in fish treated with both of gibberllic acid and L-carnitine on the tested media and in fish organs. So, there was a close correlation between histological alterations of fish gonads which fed on gibberllic acid and L-carnitine diets and the high bacterial load, especially with presence of *Salmonella* in the testis (Table 5).

Table 5: Isolated bacteria from fish organs from different treatments, according to specific media

Treatment	Bacterial isolates from different organs			
	Gills	Liver	Intestine	Ovary/Testes
Control	*E. coli* *Micrococcus* sp. *Brucella* sp. *Streptococcus* sp.		*Brucella* sp.	*Brucella* sp. *Streptococcus* sp.
Therigon® ♀	*E. coli* *Micrococcus* sp. *Bacillus* sp. *Brucella* sp. *Streptococcus* sp.		*Streptococcus* sp. *Brucella* sp.	*Streptococcus* sp.
Nuvisol Hatch P® ♀	*E. coli* *Micrococcus* sp. *Shigella* sp. *Brucella* sp.		*Streptococcus* sp. *Brucella* sp.	
Gibberllic acid ♀	*E. coli* *Micrococcus* sp. *Shigella* sp. *Brucella* sp. *Streptococcus* sp.	*E. coli* *Micrococcus* sp. *Shigella* sp. *Brucella* sp. *Streptococcus* sp.	*E. coli* *Micrococcus* sp. *Shigella* sp. *Brucella* sp. *Streptococcus* sp.	*Brucella* sp. *Streptococcus* sp.
L- carnitine ♂	*E. coli* *Micrococcus* sp. *Proteus* sp. *Brucella* sp. *Streptococcus* sp.	*Micrococcus* sp. *Brucella* sp.	*E. coli* *Bacillus* sp. *Brucella* sp. *Streptococcus* sp.	*Bacillus* sp. *Salmonella* sp. *Brucella* sp.

Table 6: Cross table of the examined organs as affected by the dietary treatments

Treatment	Organs	Media
Control(♀&♂) [b]	Gills [a]	NA [b]
Therigon® [b]	Liver [c]	SSA [b]
Nuvisol Hatch P® [b]	Intestine [b]	BRA [a]
Gibberllic acid [b]	Ovary [c]	BPA [a]
L – carnitine [a]	Testes [c]	TSA [b]

Chi-Square value = 12.32 for gills and P value = 0.015; Chi-Square value = 0 for liver and P value = 1 (not significant); Chi-Square value = 3.000 for intestine and P value = 0.558; Chi-Square value = 8.500 for gonads and P value = 0.386. a - c: Treatments, organs or media have the same letter (in the same column) are not significantly different (P ≥ 0.05).

The presence of *Enterobacteriaceae* in some fish organs in this study may be attributed to the rearing water of fish (sewage), Austin and Austin (1987) reported that

fish harvested from water polluted with human and animal wastes can contain *Salmonella, Shigella, Proteus* and *Escherichia coli* which are usually found among the most prevalent bacteria on the most rearing water of fish. *E. coli* is still the most widely used indicator for faecal pollution (Snieszko and Axelrod, 1976). Also, *Streptococcus* sp. are opportunistic pathogens in aquaculture and their pathogenicity depends on environmental stresses as low dissolved oxygen levels, high nitrite concentration (Bunch and Bejeramo, 1997), water hardness, and crowding with water temperatures exceed 20ºC (Ohnishi and Jo, 1983). All vital organs of fish stock infected with streptococci became heavily infected and mortality becomes massive (50 - 60%; Hubbert, 1989).

Streptococcus has been associated with serious economic losses among cultured freshwater and saltwater fish in many parts of the world, especially among tilapia fish. El-Gawady (2002) counted up to 1.9×10^6 – 2.4×10^6 CFU/ml total aerobic bacterial in water of fish farm in winter and summer, respectively. However, the gut and skin of *O. niloticus* microbial count reached 4.3×10^8 CFU/g. Ampofo and Clerk (2003a) identified 25 species of bacteria as associated with the fish culture systems. Ampofo and Clerk (2003b) also added that maturing causes organic enrichment; it may also hasten the deterioration of the water quality making the aquatic environment favorable for the growth and multiplication of human pathogenic bacteria. Moreover, Abdelhamid *et al*. (2006 and 2007) registered the presence of pathogenic bacteria ($1.3 – 2.0 \times 10^5$) in samples of water, feed, sediments and fish, mainly in summer season. There was no difference between fish of natural resources and those of aquaculture concerning bacterial contamination. Also, Abdelhamid *et al*. (2008) found pathogenic bacteria ($\times 10^6$ CFU) in samples of water, feed, intestinal, and liver of Nile tilapia up to 113.0, 38.7, 23.33, and 25.00, respectively. However, more recently, Abdelhamid *et al*. (2012) did not find critical counts of bacteria, whether in water, diet or fish tissues. Also, there were no relations among the counts in water, diets, muscles and/or liver of the tested tilapia fish. Yet, Abd El-Shahid *et al*. (2009) found that all fish (including Nile tilapia) samples (100) collected from Alexandria fish markets were contaminated with *Enterobacteriaceae* (5.14×10^3) and coliform (1.02×10^3). Moreover, Shaltout *et al*. (2009) counted the total microbial count including fungi, yeasts and highest total bacterial counts (TBCs) as indicators for faecal pollution in Kafr El-Zayat industrial area.

Generally, it can be noted that some histopathological signs in gonads and microbiological findings in some organs of the experimental fish were observed. It may be related to fish rearing water quality, as agriculture drained water, which was polluted with different types of pesticides, fertilizers and heavy metals residues not only in this fish farm, but also in all fish farms and fish hatcheries in Egypt according to the Egyptian law No. 124/1983. Currently, this law prohibits aquaculture projects from drawing surface water, leaving more than 90% of the country's fish farms and fish hatcheries to operate on polluted agricultural drainage water.

In conclusion, the obtained results revealed that low levels of all feed additives (Therigon®, Nuvisol Hatch P® and gibberllic acid for females and L - carnitine for males) caused maturation of ovaries and testes better than the high levels. In addition, the high bacterial load was detected in fish treated with the highest levels of gibberllic acid and L-carnitine in all tested fish organs. So, it can be recommended that the Egyptian government must be mending this dishonorable law (No. 124/1983) for sustainability development in fish production sector and human health considerations.
.

REFERENCES

Abdelhamid, A.M., El-Fadaly, H.A. and Ibrahim, S.M. (2007). Studies on integrated fish/duck production system: 1- On water quality and fish production. J. Agric. Sci. Mansoura Univ., 32: 5225 – 5244.

Abdelhamid, A.M., El-Fadaly, H.A., and Ibrahim, S.M. (2008). Studies on integrated fish/duck production system: III- Microbiological concern. Egyptian Journal of Nutrition and Feeds, 11 (1): 215 – 227.

Abdelhamid, A.M., Gawish, M.M.M. and Soryal, K.A. (2006). Comparative study between desert cultivated and natural fisheries of mullet fish in Egypt, II-microbiological concern. J. Agric. Sci. Mansoura Univ., 31: 5681 – 5687.

Abdelhamid, A.M., Khalil, F.M. and El–Barbary, M.I. (1998). Effect of using graded levels of gibberellic acid in diets differing in the crude protein levels on performance and chemical composition of Nile tilapia fingerlings. Egyptian Journal of Aquaculture and Fisheries, 2: 221 – 233.

Abdelhamid, M.A., Mehrim, A.I., El-Barbary, M.I. and El-Sharawy, M.A. (2010). An attempt to improve the reproductive efficiency of Nile tilapia brood stock fish. Fish Physiolo Biochem, 36 (4):1097-1104. doi: 10.1007/s10695-010-9387-6.

Abdelhamid, M.A., Nagwa, A. Maghraby, Mehrim, A.I. and Soliman, A.A.A. (2012). Physiological, biochemical and microbiological assessment for non conventional feedstuffs by Nile tilapia fish. Egyptian J. Nutrition and Feeds, 15 (1): 359-366. Special Issue for the 13th Scientific Conference of Animal Nutrition, 14-17 February, 2012, Sharm El-Sheikh City, Egypt.

Abd El-Shahid, Y.S.Y., Khalil, H.A. and Samaha, I.A. (2009). Some enteropathogenic bacteria isolated from freshwater fish at Alexandria province. Global Fisheries & Aquaculture Research Conference, 24-26 Oct., Cairo International Conventions Center, www.fishtechexpo.org, Book of Abstracts, p: 27.

Ampofo, J.A. and Clerk, G.C. (2003a). Diversity of bacteria in sewage treatment plant used as fish culture pond in southern Ghana. Aqua Res., 34 (8): 667 - 675.

Ampofo, J.A. and Clerk, G.C. (2003b). Short communication: Bacterial flora of fish feeds and organic fertilizers for culture ponds in Ghana. Aqua Res., 34 (8): 677 - 680.

Anon. (2005). Foodborne Illness: Food Poisoning. Outbreak, Inc.

Austin, B. (1982). Taxonomy of bacteria isolated from costal-marine fish rearing unit. J. Appl Bacteriol, 53:253-268.

Austin, B and Austin, D.A. (1987). Bacterial Fish Pathology: Disease in Farmed and Wild Fish. Ellis Harwood, Chichester.

Bailey, N.T.J. (1995). Statistical methods in biology. 3rd ed. The press syndicate of the University of Cambridge, Cambridge, UK., P.272.

Bopp, C.A., Brenner, F.W., Wells, J.G. and Strockbine, N.A. (1999). *Escherichia, Shigella,* and *Salmonella*. In: Murray, P.R., Pfaller, M.A., Tenover, F.C., Baron, E.J. and Yolken, R.H. (editors). Manual of Clinical Microbiology, 7th ed. Washington, DC: ASM Press; P. 459 - 474.

Bromage, N. (1998). Broodstock management and the optimisation of seed supplies. Suisan Zoshoku, 46: 395 – 401.

Bunch, E.C. and Bejerano, I. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *O. niloticus* and *O. aureus* to streptococcosis, Isr. J. of Aquaculture, 49:67-76.

Chatzifotis S, Takeuchi, T. and Seikai, T. (1995). The effect of dietary L – carnitine on growth performance and lipid composition in red sea bream fingerlings. Fish Sci., 61: 1004 –1008.

El-Damrawy, S.Z. (2007). L-carnitine supplementation for age - induced reproductive criteria in male pigons. J. Agric. Sci. Mansoura Univ., 32:   8915 – 8929.

El-Gamal, A.A. (2002). Basic Management techniques and reproductive performance of brood tilapia *Oreochromis niloticus* kept under recycling water systems. The 1st Annual Conference of the Egyptian Aquaculture Society. 13th - 15th Dec., Al-Arish, Egypt.

El-Gawady, M.M.M. (2002). Study on microbial pollution types in Manzal Lake. M.Sc. Thesis, Fac. of Agric., Mansoura Univ.

El-Sayed, A.M. (2006). Tilapia Culture. CABI Publishing, U.K.

El-Sebai, A., Abaza, M. and Elnagar, S.A. (2003). Physiological effects of gibberellic acid (GA$_3$) on female Japanese quail production and reproduction. Egyptian Poultry Science, 23: 977 – 992.

GAFRD (2010). General Authority for Fish Resources Development. The 2010 Statistics Yearbook. Ministry of Agriculture and Land Reclamation Publications, Cairo, Egypt, P. 96.

Haraldsson, H. and Sveinsson, T. (1993). Effects of LHRHa treatments upon the timing of ovulation and upon egg and offspring quahty in Arctic charr *Safoelinus alpinus* (L) Aquacul. Fish. Manag., 24: 145-150.

Harpaz S. (2005). L-carnitine and its attributed functions in fish culture and nutrition – a review. Aquaculture, 249: 3 – 21.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, Baltimore.

Hubbert, R.M. (1989). Bacterial diseases in warm water aquaculture. Shilo, M. and Sarig, S. (Eds.) Fish Culture in Warm Water System: Problems and Trends, CRC Press. Boca Rotan, Florida. P.179-194.

Jayaprakas, V., Sambhu, C. and Sunil Kumar, S. (1996). Effect of dietary l-carnitine on growth and reproductive performance of male *Oreochromis mossambicus* (Peters). Fish Technol., 33: 84 – 90.

Lye, C.M., Fred, C.L.J. and Gill, M.E. (1998). Seasonal reproductive health of flounder *Platichthys flesus* exposed to sewage effluent. Marine Ecology Program, 170: 249 - 260.

Msiska, O.V. (2002). The histology of mature gonads of *Oreochromis (Nyasalapia) karongae* (Trewavas). Afr. J. Ecol., 40: 164-171.

Nagahama,Y., Yoshikuni, M., Yamashita, M., Tokumoto, T. and Katsu, Y. (1995a). Regulation of oocyte growth and maturation in fish. Curr Top Dev Biol., 30: 103–145.

Nagahama, Y., Yoshikuni, M., Yamashita, M., Tokumoto, T. and Katsu, Y. (1995b). Regulation of oocyte growth and maturation in fish. In: Pederson, R.A., Schatten, G.P. (Eds.), Current Topics in Developmental Biology. Academic Press Inc., New York.

Ohnishi, K. and Jo, Y. (1983). Studies on streptococcal infection in pond- cultured fishes: 1, isolated from cultured ayu and among in 1977-1978. Fish Pathol., 16:63-67.

Roberts, R. J. (2001). Fish Pathology. 3rd edition, W.B. Saunders.

Shaltout, K.H., Mahmoud, Y.A.G., Rabeh, S.A. and El-Samagawy, W. (2009). Ecomicrobiological study on River Nile at Kafr El-Zayat industrial area. 3rd

Inter. Conf. on Aquatic Resources "Prospects and Challenges". 17th – 20th Nov., Alexandria, NIDF, P. 39.

Snieszko, S.F. and Axelrod, H.R. (1976). Chemical factors in diseases. In: Diseases of Fishes. T.F.H. Publication, Inc. Itd. Washington, USA.

Stickney, R.R. (2006). Foreword. In: El-Sayed, A.M. (author) Tilapia Culture, CABI Publishing, U.K.

Tyler, C.R. and Sumpter, J.P. (1996). Oocyte growth and development in teleost. Rev Fish Biol Fisher, 6: 287 - 318.

Van Der Kraak, G., Dye, H.M. and Donaldson, E.M. (1984). Effects of LH-RH and des Gly i" [BAla LH-RH ethyhunide on plasma sex steriod profiles in adult female Coho salmon (*Oncorhynchus kisutch*). Gen Comp Endocr, 55: 36 - 45.

Van Der Kraak, G., Lin, H.R., Donaldson, E.M., Dye, H.M. and Hunter, G.A. (1983). Effects of LH-RH and des-Gly10 [D-Ala6] LH-RH ethylarnide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon *(Oncorhynchus kisutch).* Gen Comp Endocr, 49: 470-476.

Van Hauwaert, A., Melard, C., Verstraete, P. and Berzak, R. (2000). Development of a cold season diet for tilapia (*Oreochromis niloticus*). Proc. Inter. Conf. AQUA 2000, Nice, France, 2nd – 6th May, P. 728.

Zeyner, A. and Hameyer, J. (1999). Metabolic functions of L-carnitine and its effects as feed additive in horses, a review. Arch Anim Nutr, 52: 115 – 138.
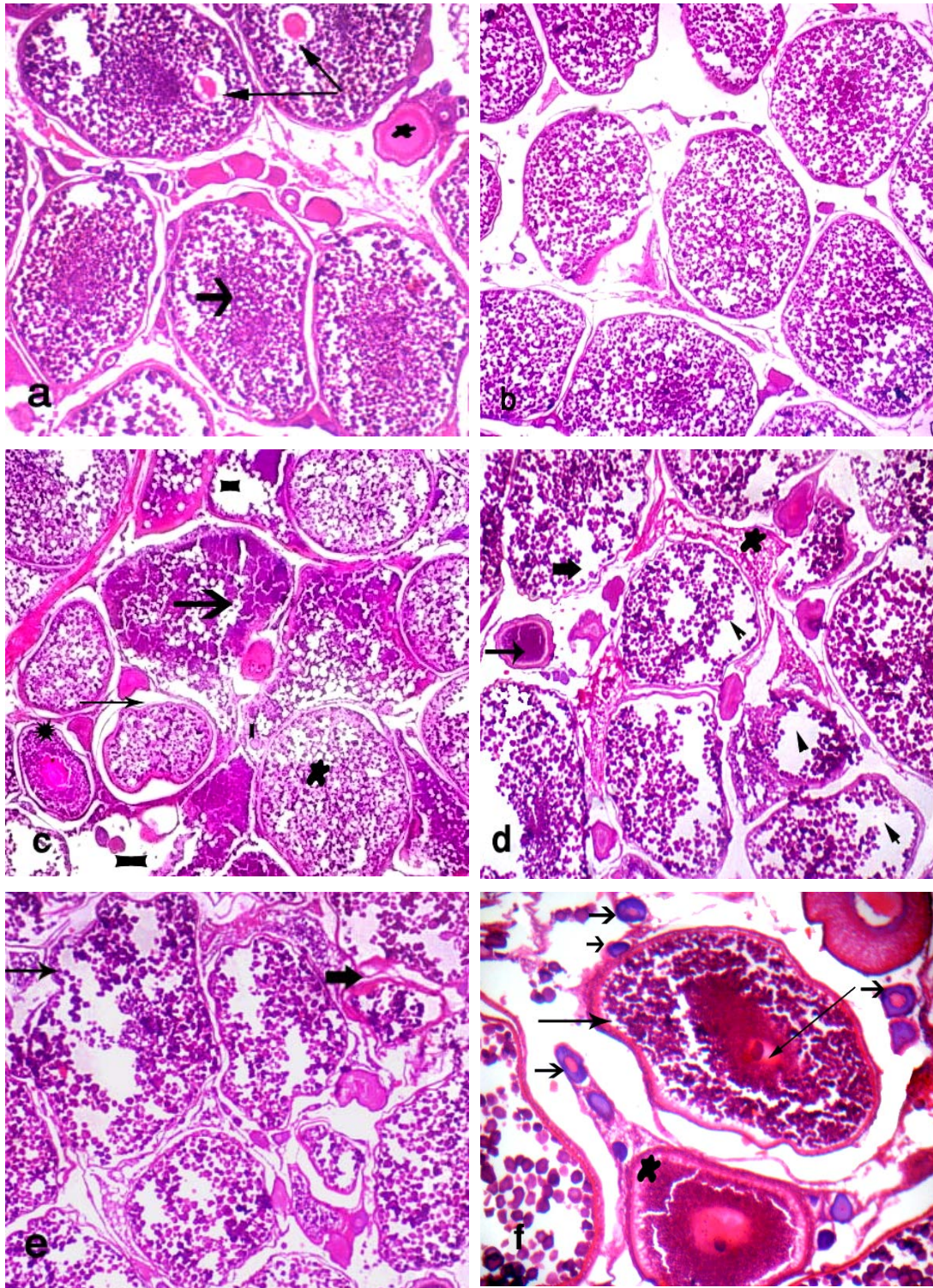
Fig. 1 (a-f): Cross sections of ovaries of *O. niloticus* fish stained with H & E; (a) ovary of control fish showing normal ovary tissue (x250); (b-d) ovaries of fish fed BR plus 0.5,1.0 &2.0g Therigon®/ kg diet (T_2-T_4, respectively); (b) showing normal structure of ripe stage of oocyte (x200); (c) showing coagulation necrosis in yolk granules (stars) and atresia of ripe oocytes (arrows) (x200); (d) showed depletion in yolk granules (arrows and arrowheads), and atresia of oocytes (star) (x200); (e & f) ovaries of fish fed BR plus 1.0& 2.0g Nuvisol Hatch P® / kg diet (T_5&T_6, respectively); (e) showing ovary in ripe oocytes stage with some atretic follicles (arrows) (x200); (f) different developmental stages of oocytes with irregular walls (arrows) (x 300).
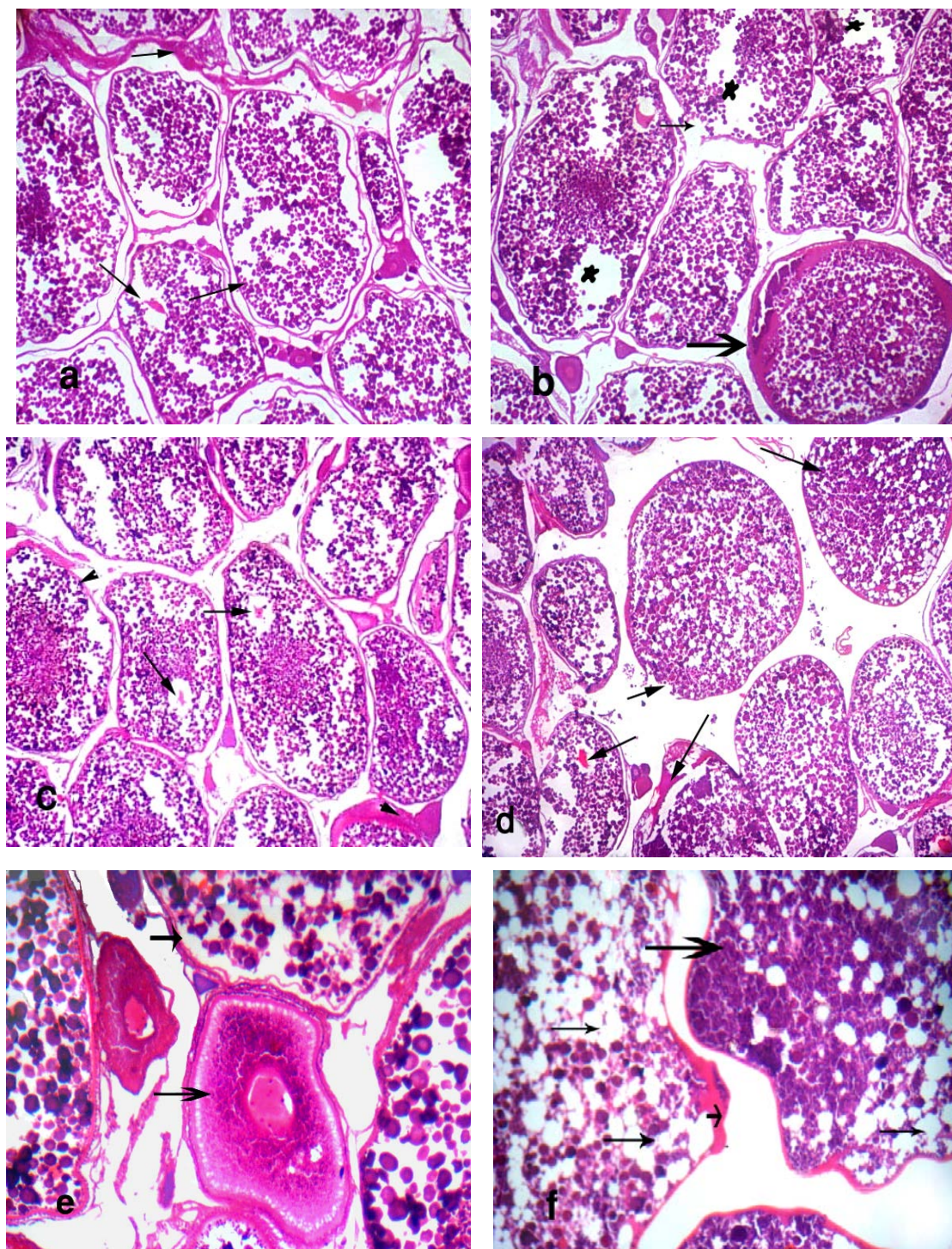
Fig. 2 (a): Cross sections of ovary of *O. niloticus* fish fed BR + 3.0g Nuvisol Hatch P$^{®}$ / kg diet (T$_7$) showing ovary in ripe oocytes stage with migratory nucleus which loses its circularity besides hemolysis in ovarian interstitial tissues (arrows) (x250). Fig. 2 (b, c & d-f): Sections of ovaries of *O. niloticus* fed BR + 20, 40 and 60 giberllic acid mg /kg diet (T$_8$- T$_{10}$, respectively); (b) T$_8$ showing ovary in ripe stage with slight histological changes of oocytes as liquefaction of cytoplasm of oocyte (stars) with nucleus loses its circularity and degeneration in wall of oocyte (arrows) (x200); (c) T$_9$ showing ovary in ripe stage with slight histological changes of oocytes as liquefaction of cytoplasm of oocyte nucleus loses its circularity (arrows) and degeneration in wall of oocyte (arrowheads) (x200); (d-f) T$_{10}$ showing the ovary in ripe stage and vitellogenic oocyte (Figs. 2d, x 200 & 2e, x 250) with erosion in margin of some oocytes wall (arrows), coagulative necrosis and liquefaction of the yolk sphere with large vacuoles in ripe oocyte and irregular oocytes wall (arrows) (Figs. 2d & 2 f; x 200 & x300, respectively).
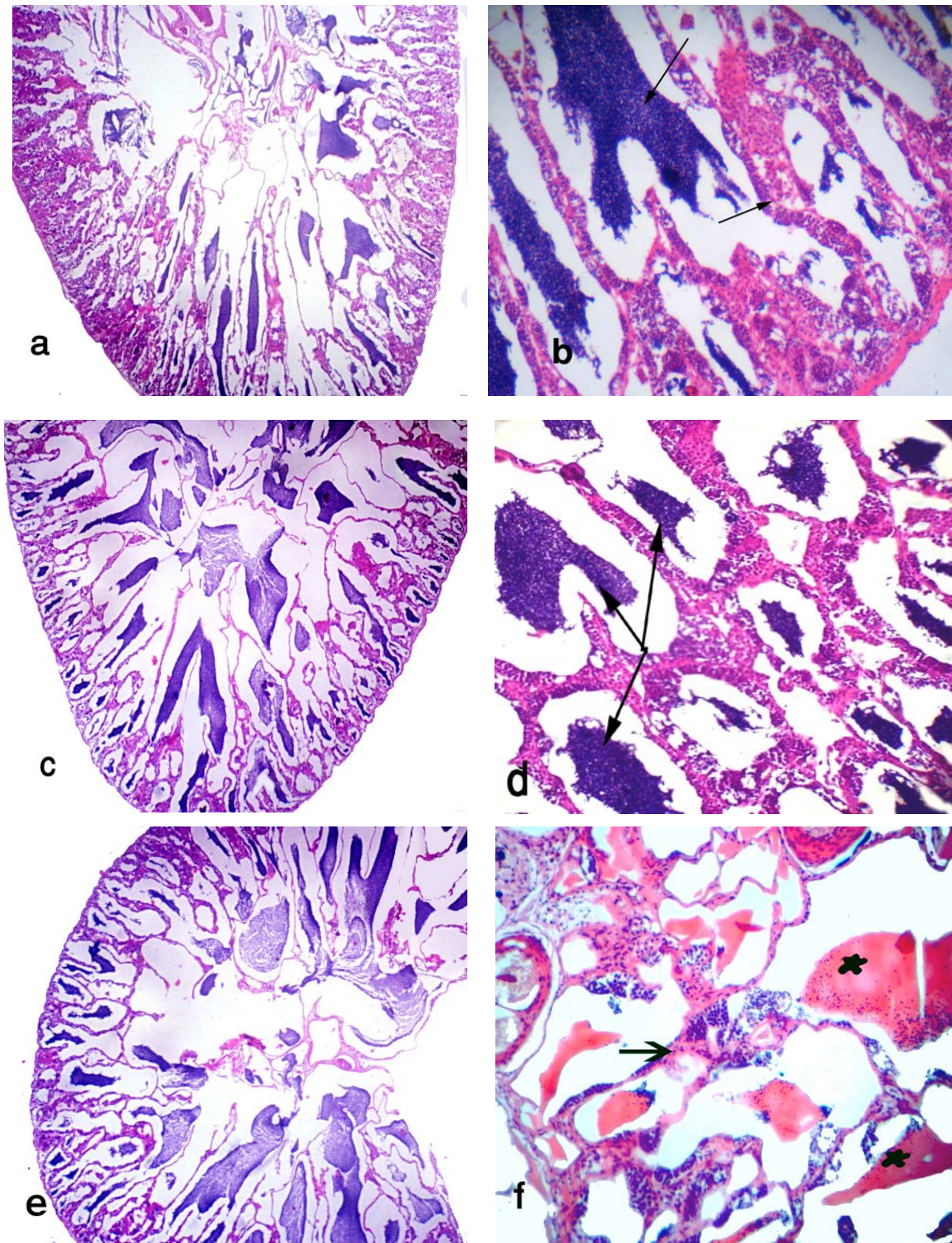
Fig. 3 (a-f): Sections of testes of *O. niloticus* fish stained with H & E, (a & b) testes of control fish (T_{11}) showing seminiferous tubules with few sperms (x100), which were observed only in spermatic duct (arrows) (b; x 200); (c & d) testes of fish fed BR plus 700 mg L - carnitine / kg diet (T_{12}) showing seminiferous tubules filed with sperms and the testes appeared in ripe stage (arrows) (x100 & 200, respectively). (e); testes of fish fed BR plus 900 mg L - carnitine / kg diet (T_{13}), testes showing seminiferous tubules filed with sperms in ripe stage (x100). (f); testes of fish fed on BR plus 1100 mg L - carnitine / kg diet (T_{14}) showing some degenerative changes in the cellular elements of seminiferous tubules (arrows) and necrotic areas with severe hemolysis and disorganization of the intertubular tissue (stars) (x 200).

ARABIC SUMMERY

تأثير استخدام بعض الإضافات الغذائية التجارية على تركيب المناسل والميكروبيولوجي لأسماك البلطى النيلى

عبد الحميد محمد عبد الحميد¹ ، أحمد إسماعيل محرم¹ ، منال إبراهيم البربرى² ومحمد عبد الفتاح الشعراوى¹

١- قسم إنتاج الحيوان ـ كلية الزراعة ـ جامعة المنصورة – مصر.
٢- معمل أمراض الأسماك ـ المعهد القومى لعلوم البحار والمصايد – مصر.

تعتبر أسماك البلطى غذاء شعبى فى كل أنحاء العالم ، وفى مصر أرتفع استهلاكها كغذاء للإنسان منذ زمن بعيد. لذلك أجريت تجربة حقلية على أمهات البلطى النيلى لزيادة تناسلها، فخُزنت الأسماك من كلا الجنسين منفصلين فى هابات فى حوض ترابى، وغُذيت لمدة ١٩ يوما على عليقة أساسية مزودة بإضافات مختلفة وبمستويات متدرجة من كل منها (٠,٥ – ١,٠ – ٢,٠ جم ثيريجون ، ١,٠ – ٢,٠ – ٣,٠ جم نيوفيسول هاتش ب ، ٢٠ – ٤٠ – ٦٠ مجم حمض جبريليك للإناث فقط ، ٧٠٠ – ٩٠٠ – ١١٠٠ مجم ل– كارنتين/ كجم عليقة للذكور فقط). تم تقييم النتائج المتحصل عليها من التركيب النسيجى للمناسل والميكروبيولوجى. وقد أشارت النتائج إلى أن التركيزات المنخفضة من كل من الإضافات الغذائية كان لها تأثير إيجابي علي كل من المبايض والخصي ، بينما كان التركيز العالي من حمض الجبرليك تأثيراته السيئة على المبايض، وكذلك التركيز الأقصى (١١٠٠ مجم/كجم) من ل– كارنتين على الخصى من حيث الصفات التشريحية والنسيجية. ومن الناحية الميكروبيولوجية فقد خفّض التركيز الأقصى من النيوفيسول هاتش ب من الحمل البكتيرى ، بينما ل– كارنتين رفع الحمل البكتيرى فى الأعضاء الداخلية للأسماك، وأن الكبد والمناسل كان حملهما البكتيرى أقل من الخياشيم والأمعاء. ومن خلال هذه الدراسة يتضح أن التركيزات المنخفضة من هذه الإضافات أدت إلى تحسن نضج المبايض والخصى مقارنة بالتركيزات الأعلى، كما أظهرت هذه الدراسة أن أرتفاع تركيز حمض الجبرليك وكذلك ل– كارنتين أدى إلى زيادة الحمل الميكروبى فى الأعضاء الداخلية للأسماك المختبرة.