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Effect of different dietary lipid sources and arginine supplementation on body-composition and gonadal development of young Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Aiming at finding out how arginine affects fish composition and/or the reproductive performance of young Nile tilapia (*Oreochromis niloticus*), the present study was carried out. A 4 X 2 factorial design experiment was conducted to evaluate 4 dietary lipid sources, namely: canola oil, CO; soybean oil, SBO; linseed oil, LO and fish oil, FO in the presence or absence of arginine (ARG) and their interaction effect on gonadal development of young Nile tilapia. Eight practical diets were formulated to be isoproteic (30% CP) and isocaloric (20 KJ/g diet), each with one of the four mentioned oils with (0.7%) or without (zero, 0) arginine supplementation. The four 0-added ARG- diets were designated as: C, S, L, F whereas the other 4 ARG-added diets were: $C^{0.7}$, $S^{0.7}$, $L^{0.7}$, $F^{0.7}$,respectively. Following 2 months of feeding the whole-fish biochemical composition was determined and gonads (testis and ovary) were histologically examined. Results showed that dietary arginine supplementation had no beneficial effect on the protein content of fish but led to a decrease in the whole fish lipid content of both sexes.In the meantime, dietary ARG addition by 0.7% had counteracted the retardation effect on maturity stages caused by feeding fish vegetable oils. These results suggested a general positive effect of arginine supplementation on the developmental stages of maturity in both ovaries and testes in young Nile tilapia. Therefore, dietary arginine supplementation may be essential for normal development of gonadsof Nile tilapia when fed the tested vegetable oils for 2 months.

Key words: Gonadal histology - Arginine – lipid sources –Nile Tilapia

INTRODUCTION

At present, aquaculture production in Egypt accounted for 76.7% of the total fish produced in 2014 and exceeded that of overall wild fisheries (GAFRD 2014). Fresh water fish farms are currently running very successfully and contributed 80.54% of the total fish cultured in the same year (2014), with tilapias especially Nile tilapia, *Oreochromis niloticus*, as the major fish produced.Given the economic importance of feeds and feeding in aquaculture, the need to develop reliable compounded feeds for Nile tilapia with the least cost is evident. However, there is increased interest in the inclusion of vegetable oils in fish diets to partially replace and reduce the dependency on fish oil to ensure a sustainable development of the aquaculture industry. Lipids are one of the most important component of diet, both as energy and essential fatty acids sources (Sargent *et al*., 1989). From the economical point of view,the inclusion of vegetable oils

(VO) in fish diets as a substitute of fish oil(FO) has received a considerable attention worldwide. VOs are widely available and cost-effective sources for polyunsaturated fatty acids (PUFA) in aquafeeds. PUFAs of the n-3 and n-6 series cannot be synthesized in vertebrates and must be provided through diet for the maintenance and regulation of cellular structure and function (Storebakken, 2002). Fatty acid composition of the diet can also affect the overall reproductive performance in fish (Bruce *et al*., 1999; Reza *et al.*, 2013). Dietary PUFAs, are involved in gonadal development steroidogenesis and oocyte maturation in vertebrates (Sorbera *et al*., 1998; Suloma and Ogata, 2012). Previous studies performed on Nile tilapia (Gunasekara *et al*., 1996), turbot (Mourente *et al*., 1991), lake trout (Lahnsteiner *et al*., 1999), goldfish (Mercure and DerKraak, 1996) and yellow tail (Watanabe and Kiron, 1997), have demonstrated that incorporation of essential nutrients into the developing eggs depends on the availability of these nutrients in the female broodstock and consequently on the dietary input in the period preceding gonadal maturity.

Furthermore, the potential of dietary arginine supplementation have been revealed in many reports, because of its ability to activate production of hormones that promote an efficient nutrient utilization (Kim *et al*., 2004; Collier *et al*., 2005; Yao *et al*., 2008; and Wu *et al.*, 2009). Arginine requirements among different fish species are generally high given its great contribution to proteins composition and body fluids and also the almost total absence of its *de novo* synthesis, as this is an essential amino acid (Li *et al*., 2009). Recently, it is shown that arginine affect also fat deposition and fatty acid synthesis in fish. The balance between dietary caloric intake and the whole-body energy expenditure affects fat deposition in both human and animals (Hill *et al*., 2003;Bell *et al*., 2001). It was found that dietary supplementation with L-arginine reduced fat mass and enhanced expression of key genes responsible for glucose and fatty acid oxidation in the diabetic fatty rats (Fu *et al*., 2005).The knowledge of the energy metabolism of fish is an indispensable tool for the preparation of suitable artificial diets during the pre-maturation period. Indeed, formulation of fish feeds must address other issues concerning fish welfare and reproduction performance besides promoting optimum growth. Supplementing fish diets with key ingredients is a strategy often used in aquaculture to improve a selected trait. Such diets (functional diets) can also be used to improve fish quality and reproductive performance and thereby, production of vital larvae. Some vegetable oils have been utilized as supplements for these diets. More recently, amino acids (AA), such as arginine, has been employed in studies of reproduction (Li *et al*., 2009), but such knowledge is still scarce. Arginine is one of the most versatile amino acids, hence it represents a good candidate for inclusion in functional diets. Therefore, the aim of this research is to investigate the influence of dietary arginine supplementation with different oil sources and their interaction on the histology of gonadal development of young Nile Tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experiment Set-up

This study was conducted in Fish Nutrition Lab, National Institute Oceanography and Fisheries (El-Qanater El-Khaireya Branch), Egypt, and lasted for 60 days from July 12th and September 10th. Nile tilapia were grown in plastic tanks (water capacity of 50 L each) supplied with well clear fresh water. The tanks were aerated by aquarium air pumps to maintain proper oxygen level in the experimental tanks. During the experimental period, water temperature ranged from 28 to 30° C.

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Experimental fish

Mixed Nile Tilapia were purchased from a commercial hatchery,and their initial body weights ranged from 6.8 to 7.5g. Fish were randomly assigned to 24 tanks (15 fish each),as three tanks for each treatment. Fish were acclimatized to experimental conditions for two weeks before initiation of the feeding trial.

Experimental Diets

Eight isoproteic (30% crude protein) and isocaloric (20 KJ/g diet), practical diets were formulated and produced in the laboratory. Dietary treatments are based on a 2 X 4 factorial design using four lipid sources (canola oil, CO; soybean oil, SBO; linseed oil, LO and fish oil, FO) with the presence or absence of arginine (0 or 0.7%). Diets were designated as: C, S, L, F (for the zero arginine) and $C^{0.7}$, $S^{0.7}$, $L^{0.7}$, $F^{0.7}$ (for the 0.7% arginine addition), respectively. Composition and proximate analyses of the experimental diets are shown in Table (1).

Table (1).Composition and proximate analyses (%DM) of experimental diets fed to Nile tilapia for 2 months.

Ingredients	Diets (g/Kg)							
	Without added ARG				With 0.7% ARG			
	C	S	L	F	$C^{0.7}$	S0.7	$L^{0.7}$	$F^{0.7}$
Yellow Corn[1]	230	230	230	230	230	230	230	230
Fish Meal %62[2]	100	100	100	100	100	100	100	100
Soybean meal (SBM) (44%)[3]	472	472	472	472	465	465	465	465
Wheat middling's[4]	100	100	100	100	100	100	100	100
NaCl (Sodium chloride)[5]	5	5	5	5	5	5	5	5
Canola Oil (CO)[6]	60				60			
Soybean oil (SBO)[7]		60				60		
Linseed oil (LO)[8]			60				60	
Fish oil (FO) [9]				60				60
L- Arginine (ARG) [10]					7	7	7	7
Ascorbic acid (Vit C)[11]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methionine – L[12]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carboxy methyl cellulose (CMC)[13]	5	5	5	5	5	5	5	5
Premix*	20	20	20	20	20	20	20	20
Crude protein	30.05	30.66	30.7	30.9	30.92	30.7	30.9	30.92
Ether Extract	7.04	7.54	7.28	7.39	7.73	7.41	7.21	7.23
Ash	5.91	6.23	6.06	6.07	6.02	6.66	6.08	6.17
NFE (calculated by difference)	57	55.57	55.96	55.64	55.33	55.23	55.81	55.68
Gross energy (kj/g diet)	30.05	19.82	20.02	19.96	20.06	20.02	19.96	20.06

[1]-Imported yellow corn from Argentina
[2],Important fish meal 62% from Morocco
[3]-Soy Factory, Food Technology Research Institute, Ministry of Agriculture, Giza, Egypt
[4]Local wheat meddling; Elmasrya co, Egypt
[5]- Local sodium chloride
[6]- Important canola oil , USA
[7,8,9]- zoocontrol co, Giza Egypt
[10 ,11,12,13]- El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt
NRC (2011), * supplied by a vitamin–mineral premix (Roche Vitamins Hellas).

The experimental diets are fed to fish in the form of crumbs. Fish are fed to apparent visual satiation for six days per week, three times daily (at 9:00 am, 13:00pm and 17:00 pm) throughout the experimental period.

Analytical procedures

Diets and the whole body samples were analyzed, to determine their major nutrients composition, according to the standard method of AOAC (1995). Dry matter (DM) was measured by oven drying at 105^{o}C, crude protein (N x 6.25) by the Kjeldahl method using a Kjeltech auto-analyzer (Model 1030, Tecator, Hoganas, Sweden), crude fat by Bligh and Dyer, (1959), and ash was determined according to the standard method of AOAC (1995). The dietary gross energy was calculated using the conversion factors of 23.7, 39.5 and 17.2 kJ / g protein, lipid, and carbohydrate, respectively (Brett and Groves, 1979).

Gonadal histology

At the end of the feeding trial, random male and female samples, of almost equal size each per treatment, were used to identify any effects of oil type and/or arginine on the histological structure of gonads. Three samples from each testis and ovary were taken and fixed in Bouin's solution, dehydrated in ethanol series, embedded in wax, and cross-sectioned at 5 µm, then stained with hematoxylin and eosin (Hx and E), and microscopically examined at magnifications 300X and 400X (Bancroft and Stevens, 1996). Different gonad maturity stages of Nile tilapia were first identified for ovary or testis according to the detailed description given by West (1990).

Statistical analysis

At the end of the experiment, data were subjected to factorial design analysis of variance (ANOVA) using the statistical software (SPSS 18). Duncan multiple range test was used to detect individual differences between treatment means (Duncan 1955). Data were presented as means ± standard deviation (S.D) and a rejection level of $P>0.05$ was used for significant differences.

RESULTS AND DISCUSSION

Body composition

The whole body biochemical analyses of fish from the different dietary groups are given in Tables (2 & 3), for males and females, respectively. The influence of dietary lipid sources and ARG supplementation interaction produced significant differences in fish body composition of both sexes. Arginine supplementation has led to a relative decrease in protein content for all oils tested. The highest protein contents were recorded for fish fed all tested oils, except F group, without ARG addition. In the meantime ARG supplementation has no significant effect on lipid content of fish for all dietary groups, except the $S^{0.7}$ fed fish which recorded lower lipid content as compared to that without ARGS diet. Ash content of fish was increased with dietary ARG supplementation, particularly for $S^{0.7}$ and $C^{0.7}$ fed fish. No remarkable variation in moisture content was noticed among individual dietary groups in the presence or absence of ARG, and the lowest content was that of S fed fish among all treatments.

In this concern, previous studies have reported that supplementation of arginine and linoleic acid (the tested VOs in the present study have high-linoleic acid content) individually

have been shown to reduce adiposity in many animal species and modulate lipid metabolism (Smith *et al*., 2002; Adams *et al.*, 2005; Park *et al*., 1999; Jobgen *et al*., 2009; Nall *et al*., 2009).

Table (2).Body composition (%DM)of Male Nile tilapia at end of the experiment.

Dietary	Crude protein (CP) (%)	Crude lipid (EE) (%)	Ash (%)	Moisture (%)
C	64.48±1.46^{ab}	15.39±3.40^{ab}	^{ab}17.97±1.93	80.25±0.32
S	65.15±0.26^{a}	17.44±0.89^{ab}	15.83±1.15^{bc}	78.46±1.65
L	64.48±0.07^{ab}	18.79±0.44^{a}	15.15±0.37^{c}	79.29±0.06
F	60.93±1.31^{bc}	19.62±0.83^{a}	17.97±0.48^{ab}	82.61±1.20
$C^{0.7}$	61.06±1.46^{b}	15.71±0.13^{ab}	21.05±1.59^{a}	80.31±0.00
$S^{0.7}$	62.34±1.08^{b}	14.84±3.54^{b}	22.24±3.66^{a}	81.43±1.09
$L^{0.7}$	60.39±1.47^{bc}	17.66±0.56^{ab}	20.17±2.02^{ab}	82.98±0.88
$F^{0.7}$	59.38±1.46^{c}	18.54±0.71^{b}	20.59±2.17^{ab}	79.76±1.84
Two-Way Anova				
Oils	p<0.01	p<0.06	p<0.49	p<0.70
Arginine	p<0.001	p<0.18	p<0.003	p<0.31
Interaction	p<0.56	p<0.53	p<0.45	p<0.11
^{2}R	0.86	0.67	0.75	0.58

Table (3).Female Nile tilapia body composition (% DM)at the end of the experiment.

Dietary	Crude protein (CP) (%)	Crude lipid (EE) (%)	Ash (%)	Moisture (%)
C	63.38±3.05^{ab}	18.57±0.76^{ab}	17.37±2.29 ^{cd}	78.99±1.89
S	64.85±0.45^{a}	18.71±0.32^{ab}	14.97±0.78^{d}	81.33±1.22
L	63.14±0.65^{ab}	19.92±0.92^{a}	15.63±0.28^{d}	79.53±3.16
F	61.40±0.43^{ab}	16.20±0.60^{b}	21.79±0.14^{ab}	81.83±2.14
$C^{0.7}$	63.02±3.67^{ab}	13.84±0.72^{c}	23.56±2.96^{ab}	81.24±2.29
$S^{0.7}$	57.12±0.15^{c}	17.00±0.15^{ab}	25.84±0.00^{a}	81.91±2.2
$L^{0.7}$	62.42±1.24^{ab}	16.50±1.82^{ab}	18.96±2.66^{bcd}	81.42±1.09
$F^{0.7}$	60.89±1.66^{b}	16.3±0.45^{bc}	21.84±0.35^{abc}	79.93±0.97
Two-Way Anova				
Oils	p<0.12	p<0.008	p<0.08	p<0.57
Arginine	p<0.03	p<0.001	p<0.001	p<0.36
Interaction	p<0.02	p<0.006	p<0.03	p<0.36
^{2}R	0.82	0.91	0.86	0.47

Gonadal histology

Testes of fish fed the F, C,S,L diets without ARG showed abnormal morphology, retarded maturity and their semniferous lobules with relatively law amounts of spermatozoa, (H&E×400). However, testes in fish fed the ARG-added diets showed normal maturity stages with lobules filled of spermatozoa in their central portion. The spermatogenic activity is further evidencedat the periphery of the testis (Fig. 1).

On the other hand, ovaries in fish fed the S and L diets showed degenerated oocyte follicles (DF)(H&E ×400). Fish fed C diet showed severely destructed oocytes or atretic follicles (AT), while fish fed the F diet showed fatty degeneration (FD), fat droplets and marked development of recruitment stock of ova (RO). In the meantime, ovaries in fish fed the ARG-added diets showed different types of follicle with marked development of recruitment stock of ova (Fig. 2).

It seems that the effect of arginine on the developmental stages of maturity is more obvious in testes than in ovary of Nile tilapia. It is established that arginine is the dominant compound of promatines, which are important components of spermatozoa formation (Lewis *et al.*, 2004). The present results were in the line with those of earlier studies which reported that arginine contents were significantly higher in the testis than in the ovary of Goldlined seabream (Qari *et al*., 2013). Arginine is very important for energy metabolism in aquatic animals (Tsuchiya, 1962) particularly fish. Mommsen *et al*.(2001) documented that the higher amount of arginine in the testis than in the ovary suggested that spermatozoa need arginine as an energy medium more than the ovary does, since spermatozoa swim actively at fertilization. Arginine can stimulate the release of various hormones such as insulin, growth hormone, and glucagon in fish (Mommsen *et al*., 2001). Jobgen *et al*. (2006) and Yao *et al*. (2008) reported that arginine plays a crucial role in regulating endocrine and reproductive functions.

Conclusion

It could be concluded that supplementation of arginine to the pre-matured tilapia is essential, when fed all vegetable oil lipid source. In order to develop functional pre maturity diets for young Nile tilapia ARG supplementation has to be considered.

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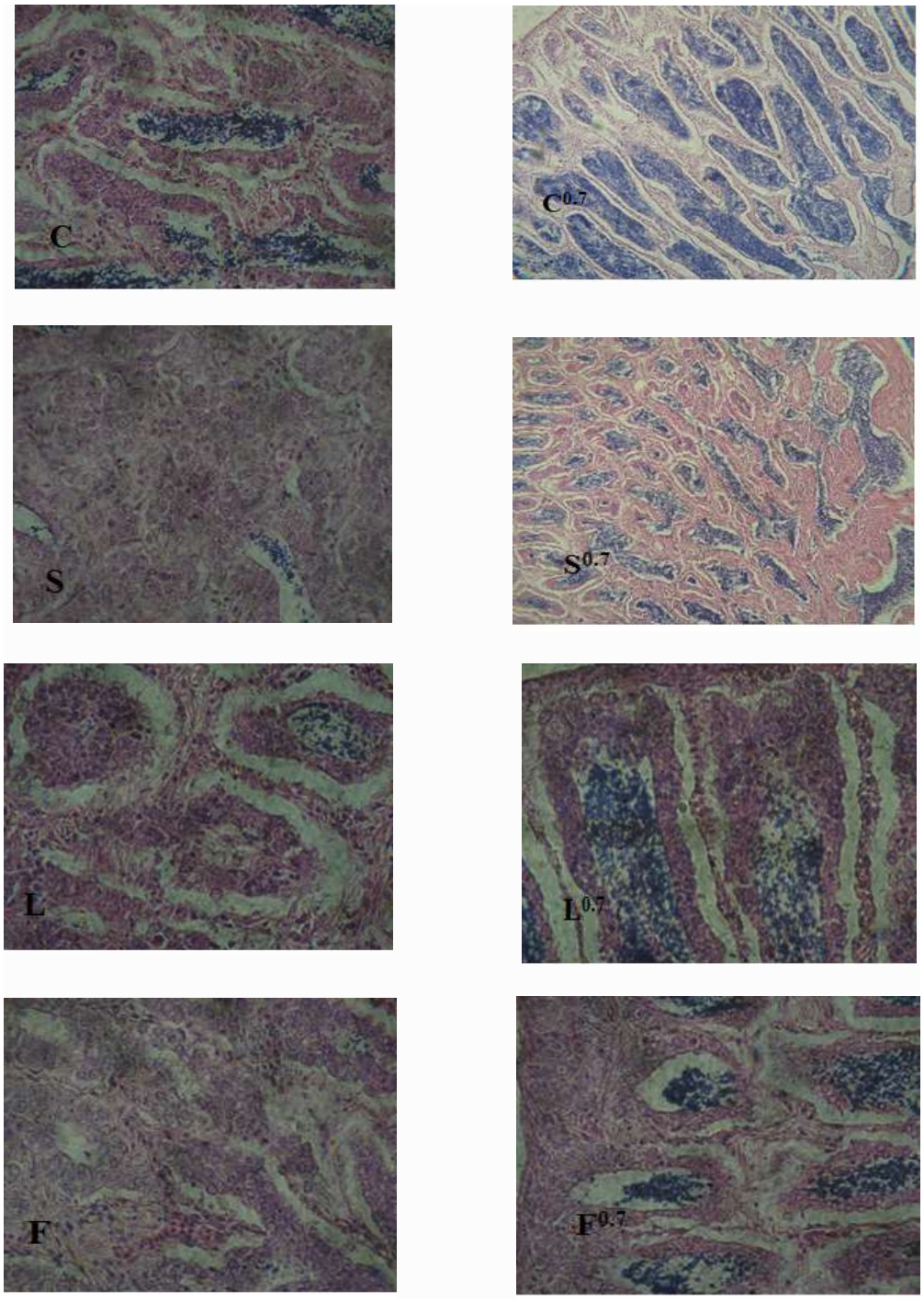

Fig. (1). Transverse sections of Nile tilapia testes exposed to C,S,L,F , $C^{0.7}$,$S^{0.7}$,$L^{0.7}$,$F^{0.7}$ treatments. Stained HX-E, Mag. X 300

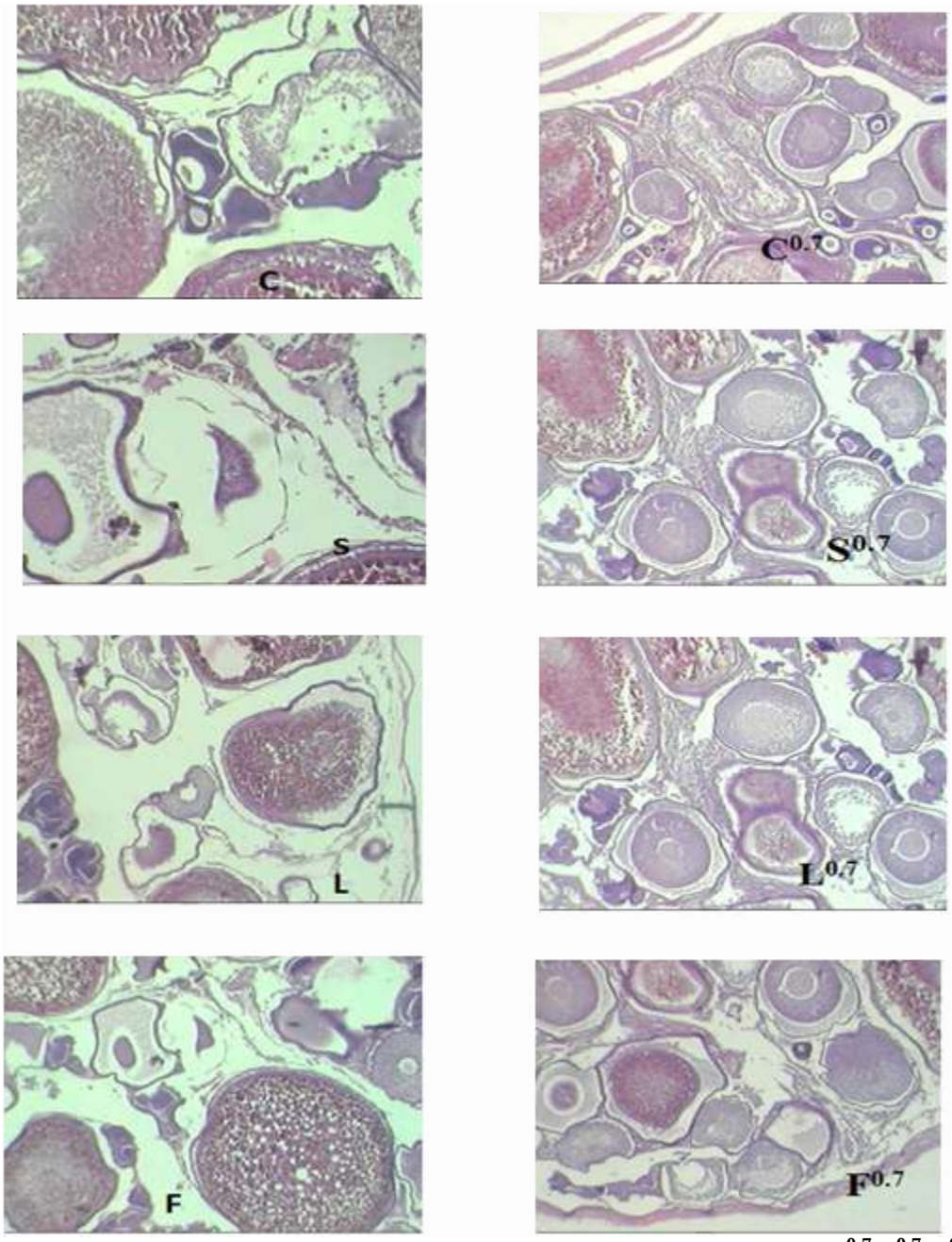

Fig. (2). Transverse sections of Nile tilapia ovaries exposed to C,S,L,F , $C^{0.7}$,$S^{0.7}$,$L^{0.7}$,$F^{0.7}$ treatments. Stained HX-E, Mag. X 400

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تأثير التغذية على اللبيدات و الأرجنين في إحداث تغيرات في التركيب الهيستولوجي للغدد الجنسية و في التركيب الكيماوي لجسم أسماك البلطي النيلي أثناء مرحلة النضج

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المستخلص

لدراسة مدى تأثير إستخدام مصادر مختلفة من الزيوت و الحمض الأميني الأرجينين مجتمعآ في علائق البلطي النيلي على تطور الغدد الجنسية لصغار أسماك البلطي النيلي. تم إستخدام 8 علائق متساوية في محتوى البروتين (30% بروتين خام) و الطاقة (20 كيلو جول/ جرام) لكل المعاملات ولكن تختلف في محتواها من مصادر الزيوت وتركيز الأرجينين . وكانت المعاملات ثنائية العوامل 2×4 . لإثنين مستوى من الأرجينين(0, 0.7 %) بالإضافة إلى 4 زيوت : زيت الكانولا و زيت الصويا و زيت الكتان وزيت السمك وكانت الإختصارات الدالة على المعاملات كالتالي $C,S,L,F, C^{0.7},S^{0.7},L^{0.7},F^{0.7}$ على التوالي . لوحظ إنخفاض محتوى الجسم من الدهون وتتطور خلايا المبيض في الأسماك التي غذيت على علائق الأرجينين مقارنتآ بالعلائق الأخرى و ظهرت تشوهات في الفحص الهيستولجي بالإضافة لإنخفاض تطور الخلايا المكونة للحيوانات المنوية بالخصية التي غذيت على العلائق بدون إضافة الأرجينين . يوصى البحث بإستخدام الأرجينين كمكمل غذائي في علائق صغار أسماك البلطي إثناء إعدادها قبل النضج الجنسي و قبل مرحلة التفريخ في المفرخات السمكية.