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Effects of Dietary Propolis and Pollen on Growth Performance, Fecundity and Some Hematological Parameters of *Oreochromis niloticus*

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Abstract

This study aimed at identifying the effects of propolis and honeybee pollen (HBP) on growth performance, fecundity and some hematological indices of liver and kidney functions of Nile tilapia *"Oreochromis niloticus*" supplemented with 2.5% of propolis or HBP in diet for 21 days. The results showed that dietary propolis or HBP significantly ($P<0.05$) improved Specific Growth Rate (SGR), Average Daily Gain (ADG) and Feed Efficiency ratio (FER). Propolis significantly ($P<0.0001$) increased the percentage of *O. niloticus* with ripened eggs. Microscopically, the ovaries were seen to contain a large number of oocytes >4 mm in the treated groups. In male, HBP feeding significantly ($P<0.05$) increased testicular weight, gonadosomatic index and improved the semen quality. Nevertheless, propolis treated males showed a significant ($P<0.05$) increase in head abnormalities among all groups. Sections from the testes of HBP-fed group appeared highly active and showed accumulated sperms in seminiferous tubules. Propolis or HBP significantly ($P<0.001$) decreased the serum ALT. Concluding that, supplementation of fish diet with either propolis or honeybee pollen is promising a beneficial effect for fisheries due to its potential improving effect on the growth rate and fecundity and preserving some biochemical indices of liver and kidney functions of *O. niloticus*.

Keywords: Fecundity, growth performance, Nile tilapia, pollen, propolis.

Introduction

The intensive farming of tilapia is rapidly expanding and the need to produce sufficient quantities of quality fry is becoming crucial to meet the increasing global demands for stocking tilapia farms. Broodstock management is necessary for mass production of fry. Effective seed production needs special husbandry as well as particular nutritional requirements which significantly affect fecundity, survivability, and eggs and larval quality (Bromage, 1998). The problem in the mass production of tilapia seed is further exacerbated due to an asynchronous ovarian cycle (Rana, 1990). Therefore, its contribution demands research activities in different areas with special emphasis to improve the reproductive potential and fecundity.

Propolis (bee glue) is a resinous hive product collected by honeybees from various plant sources and is used to seal holes in their honeycombs, smooth out the internal walls and protect the entrance against intruders (El-Bassuony, 2009). Propolis has plenty of biological and pharmacological properties and its mechanisms of action have been widely investigated using different in vitro and in vivo models (Sforcin and Bankova, 2011). Studies in mammals verified that propolis decreased dead and abnormal sperm and increased testosterone in rats (Yousef and Salama, 2009) and significantly increased body weight, and the relative weight of the testes and epididymis in rabbits (Yousef *et al*., 2010). In fish, propolis has been extensively used as a growth promoter (Meurer *et al*., 2009), immunostimulant (Talas and Gulhan, 2009) and hepatoprotective agent (Deng *et al*., 2011). However, no data are available regarding the effect of propolis on fish fecundity or semen quality.

Honeybee pollen (HBP) is collected by the bee from flowers and is extracted at the hive entrance using a pollen trap. HBP is often referred to as nature's most complete food rich in proteins (25%), essential amino acids, oils (6%), more than 11 fatty acids, 12 vitamins, 28 minerals, 11 enzymes or co-enzymes and carbohydrates (Xu *et al*., 2009). It has recently gained increased attention for its antibacterial (Proestos *et al*., 2005), antifungal (García *et al*., 2001) and anticarcinogenic (Middleton, 1998) properties, treatment of some cases of benign prostatitis (Campos *et al*., 1997), improvement of semen quality and

fertility (Attia *et al*., 2011b). Nonetheless, the effect of HBP on the fecundity or semen properties has not been investigated previously in fish.

Sexually mature female Nile tilapia undergoes successive reproductive cycles at intervals of 3-6 weeks. The constituent of diet given to the brood stock in reconditioning periods before restocking in the breeding facilities is crucial to improve fertility and larval quality. Therefore, this study aimed at elucidating the potential effects of propolis and HBP enclosed in diet at 2.5% rate for 21 days on improvement of growth performance and body indices, fecundity and semen characteristics, and some biomarkers of liver and kidney functions in Nile tilapia (*O. niloticus*).

Material and Methods

Preparation of Experimental Diet

Propolis-water-extract and Honeybee pollen granules (HBP) (Table 1) were provided by the Honeybee project, Faculty of Agriculture, Benha University, Egypt. Propolis-water-extract (40%) was dried at 50°C before storage at 4°C in dark sealed bottles until use.

Three experimental diets were formulated. Commercial basal diet (Table 2) (crude protein 30%) was crushed and divided into three portions. The first and second portions were thoroughly mixed with propolis and HBP at a concentration of 2.5% (w/w), respectively. The dietary level (2.5%) of pollen or propolis was determined based on a pilot study in our laboratory (data unpublished). The third portion was left as the control. Water was added to the ingredients of each diet to produce stiff dough that reformed into pellets. The moist pellets were air dried at room temperature, packed in clean plastic jars and stored at 4°C until use (Cuesta *et al*., 2005).

Experimental Design

Female and males Nile tilapia (average weight and length was 45 g and 12.5 cm, respectively) of 6-7 months age were obtained from a private fish farm, Kafer El Sheikh Governorate, Egypt (late May). The fish were stocked in fiberglass tanks (750 L capacity) and maintained in continuous aerated de-chlorinated water at the wetlab, Deptartment of Fish Diseases and Management, Faculty of Veterinary Medicine, Benha University, Egypt. Fish were left for two weeks to acclimatize the laboratory conditions and formulated pelleted diet (~3% of their body weight daily) before the experiment. Fish were health checked before they were distributed through investigation for skin and gill parasites.

At the start of the experiment, fish were distributed into three tanks (propolis, pollen and control groups), each stocked with 10 females and 5 males per tank (sex ratio 2:1), with each treatment having three replicates. Fish of the first group were fed basal diet containing 2.5% propolis; the second group was given diet incorporated with 2.5% HBP. The third group was fed additive-free diet as controls. Fish were fed at rate of 3% from the body weight twice daily for 21 days. The water temperature was maintained at ~28°C. About half of the water was changed daily, and fecal material was removed by siphoning every day. Fish were routinely checked for health and any mortality.

Determination of the Growth Performance

At the end of the experiment, the growth performance was assessed through measuring: [1]Total lengths of Fish (L) from the tip of the mouth to the tip of the caudal fin using graduated ruler to the nearest centimeter. [2]Final weight (W) using a portable digital scale to the nearest 0.1 g after scarification.

Table 1. Proximate composition of pollen (Feás *et al*., 2012) and propolis (Hegazi, 2007)

Composition of pollen	Proximate analysis (%)
Crude protein	21.8
Crude fat	5.2
Ash	2.9
Carbohydrates	67.7
Composition of propolis	Proximate analysis (%)
Resins and Balsams	55
Waxes	30
Etheric oils	10
Pollen	5

Table 2. Composition and proximate analysis of basal diet

Ingredients	(g/1000 g total diet)
Fish meal	100
Wheat bran	150
Corn	300
Soybean	407
Vegetable oil	40 ml
Mineral and Vitamin mixture[1]	3 g
Total	1000
Composition	Proximate analysis (%)
Dry matter	86.8
Crude protein	30.0
Ether extract	12.9
Crude fiber	4.8
Ash	5.2
Gross energy (kcal/kg)	4477. 7

[1]Egavet premix: Each 3 kg contain: vitamin A, 12.000.000 IU; vitamin D, 2.500.000 IU; vitamin E, 10.000 mg; vitamin K_3, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 5000 mg; vitamin B6, 1500 mg; niacin, 30.000 mg; biotin, 50 mg; folic acid, 1000 mg; pantothenic acid, 10.000 mg; Mn, 60.000 mg; Zn, 50.000 mg; Fe, 30.000 mg; Cu, 5.000 mg; Se, 100 mg; Co, 100 mg; Mn, 250.000 mg; $CaCo_3$.

[3]Condition (K) factor according to the formula:

$K = W \times 100/L^3$ (Ricker, 1975);

where W= body weight (g) and L=total length in (cm).

[4]Average daily gain (ADG) = [Average final weight (g) - average initial weight (g)]/ feeding period (days).

[5]Feed conversion ratio (FCR) = F/(Wf-Wi);

where F is the weight of feed offered to fish, Wf is the final weight of fish and Wi is the weight of fish at stocking (Hopkins, 1992).

[6]Feed efficiency ratio (FER) = Weight gain (g) / dry feed fed (g) (Ricker, 1979).

[7]Specific growth rate (SGR) (% g day^{-1}) = 100 × (ln final body weight (g)) – ln initial body weight (g)) / feeding period (day).

[8]Spleensomatic index (SSI) = (weight of spleen (g) / total body weight (g)) ×100.

[9]Hepatosomatic Index (HSI) = (weight of liver (g) / total body weight (g)) ×100. [10]Survival rate (SR) (%) = 100× (final fish number / initial fish number) (Yun *et al*., 2012).

Determination of Fecundity

Soon after dissection, ovaries were excised from all females (n=10 in triplicate $group^{-1}$), weighed (to the nearest milligram) and from which the egg mass was carefully removed with a spatula. The egg mass was teased apart and individual eggs were counted. Ten eggs were examined under a calibrated binocular stereomicroscope to measure the diameter (Coward and Bromage, 1999). Since eggs were ellipsoid-shaped, both axes (long and short) were measured in order to calculate mean egg size [(long axis length + short axis length)/2] and mean egg volume [= π/6 × long axis × short axis/2]. Total egg volume per ovarian weight (cm^3 gm^{-1}) was calculated according to the formula: mean egg volume × number of eggs/weight of the ovaries. The relative fecundity (i.e. the number of eggs per length unit (cm) or body weight (g) were calculated according to Bagenal (1967). The gonadosomatic indexes (GSI) of both sexes were separately determined as

GSI=GW×100/BW-GW;

where GW=gonad weight and BW=body weight (De VIaming and Chapman, 1982).

Semen Analysis

Semen (milt) samples were stripped by gentle pressure on the abdomen of males (n=5 in triplicate $group^{-1}$) on day 21 after anesthetization with immersion in water containing Mepecaine (Mepivacaine HCl 36 mg 1.8 ml^{-1}, Alexandria Company for Pharmaceuticals and Chemical Industries, Egypt). Special care was paid to collect all the available semen and to avoid any contamination by fecal matter, urine, blood, or scales.

Semen samples were assessed by one observer as described previously for hydrogen ion concentration (pH), individual sperm motility (Morita *et al*., 2003), sperm viability and abnormalities in stained film with eosin-nigrosin stain (Crespo Garcia, 1991) and sperm cell concentration by using a hemocytometer (Tvedt *et al*., 2001).

Serum Samples and Chemical Analysis

At the end of the experiment, blood samples were collected from 10 fishes of each treatment (5 males and 5 females) and sera were harvested by centrifugation at 3000 g for 15 min. The whole blood was centrifuged at 1400 ×g for 15 min and the separated sera were pooled together and used to estimate aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (Huang *et al*., 2006), urea and creatinine content (Halk *et al*., 1954) using E-Merck's kit (Germany) according to the manufacturer's instructions.

Tissue Sampling Preparation and Histopathological Examination

Gonads of experimentally treated and control *O. niloticus* were collected at the end of the experiment, fixed in Bouin's solution overnight and processed for histological evaluation according to Zaroogian *et al*. (2001). Ripened (mature) oocytes were identified by the enlargement of both cortical alveoli and yolk granules, marked increased size, peripheral migration of the nucleus, clearly visible zona radiate and cuboidal or low cuboidal follicular cells surrounded by thin thecal layer (Srijunngam and Wattanasirmkit, 2001). The appearance of actively dividing spermatogonia A (SGA) cells in gonads was considered as the earliest signal of the onset of maturation. An active (spawning) testis was characterized by filling the lumen of lobules with free spermatozoa and presence of cysts with spermatids at the end of spermiogenesis next to the lobule walls (Dziewulska and Domagała, 2002).

Statistical Analysis

Data obtained from fishes (n=10 females, 5 males in three replicates for each treatment) were tabulated and statistically analyzed, where appropriate, by the Statistical Package for the Social Sciences (SPSS) version 14. Mean ± SEM, ANOVA and Duncan's multiple range tests were calculated for

all traits under investigation.

Results

Influence of Propolis or Pollen Feeding for Three Weeks on Somatic Indices and Growth Performance of *O. niloticus*

Females: Supplementation of propolis to the diet of female Nile tilapia significantly ($P<0.05$) affected their growth performance exhibited by an increase in the final weight, length, specific growth rate (SGR), average daily gain (ADG) and feed efficiency ratio (FER). Propolis significantly ($P<0.05$) lowered the feed conversion ratio (FCR) when compared to control. The HBP inclusion in diet resulted in an improvement in the final weight, length and SGR of female *O. niloticus*, but failed to affect ADG and FER. In contrast, both treatments had no impact on condition (CF) factor, spleno-somatic index (SSI) and hepato-somatic index (HSI) when compared to control after 21 days from start feeding (Figure 1).

Males: Male tilapia fed on diet enriched with propolis showed a significant increase in SGR, ADG and FER in association with a substantial enhancement of the growth performance in the form of the final weight and total length, but did not affect K-factor compared to pollen and control fed groups. However, FCR, HSI, SSI were significantly lower in the propolis fed group. Inclusion of HBP in the fish diet had no effect on body indices or the growth performance except for HSI which was lower than control but higher than propolis groups (Figure 1).

Influence of Propolis or Pollen Feeding for Three Weeks on the Fecundity and Reproductive Functions of *O. niloticus*

Females: Feeding of female Nile tilapia on diet containing propolis resulted in an increase in the number of females with large sized egg populations in

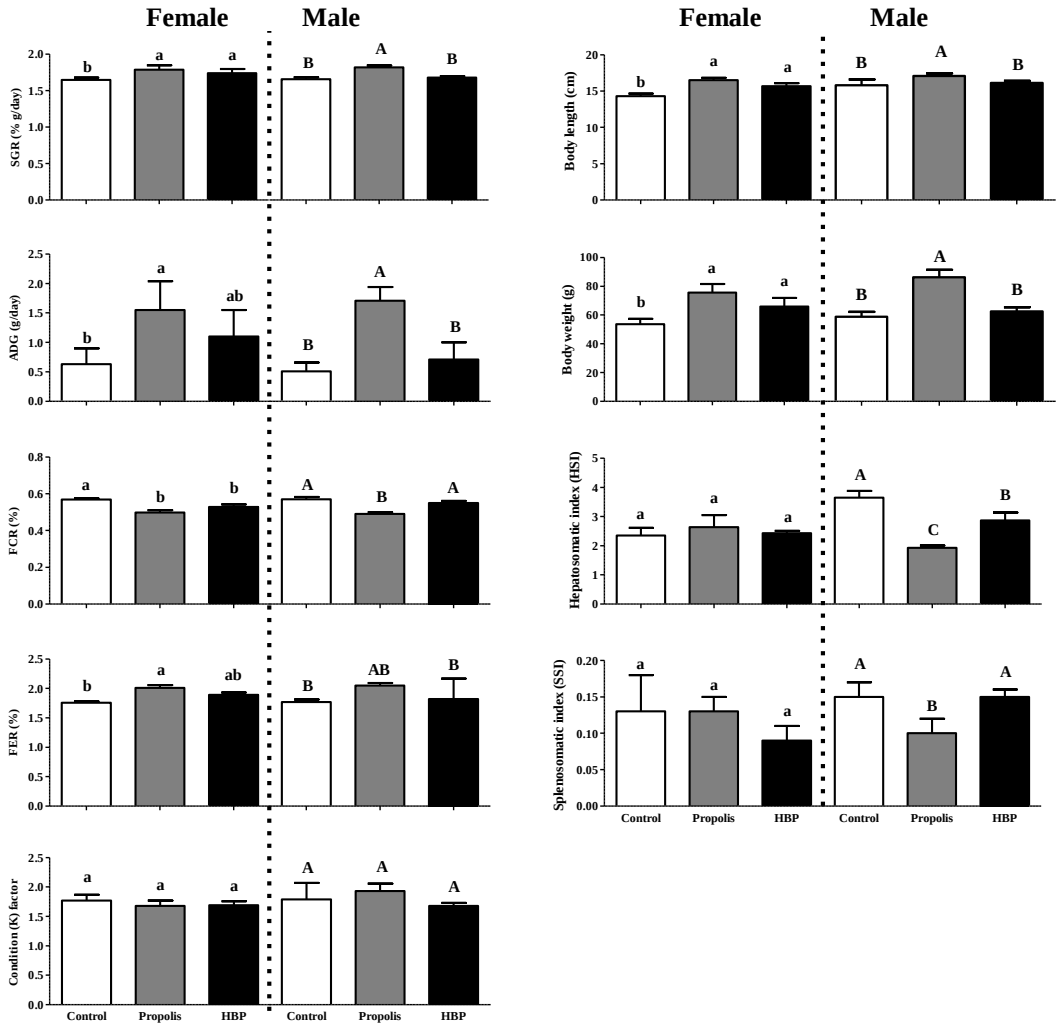

Figure 1 Growth performance parameters of the female and male Nile tilapia (*Oreochromis niloticus*) fed on propolis and pollen incorporated diets for three weeks. Specific growth rate (SGR; % g day^{-1}), average daily gain (ADG; gm day^{-1}), feed conversion rate (FCR), condition factor (K factor), Hepatosomatic index (HSI), Splenosomatic index (SSI), Total length (TL; cm) and body weight (BW; gm) in control (□), propolis (▨) and honeybee pollen (■) groups. Values (means ±SEM; n=5/group) with different letters in the same body index were significantly different at $P<0.05$.

their ovaries that reflect an increase in the total egg volume/ovary (cm^3/g). However, no significant differences in the gonadal indices (gonadosomatic index (GSI), gonadal weight (g), relative gonadal weight and fecundity) were observed among the dietary treatments. Treatment with HBP under the present experimental conditions did not have any effect neither on the gonadal indices or fertility parameters when compared to the control. However,the percentage of female tilapia had large sized (>4 mm in diameter) egg population on their ovary was comparatively higher in pollen-fed animals than control (Table 3).

Males: Addition of propolis to the diet changed the semen quality with a tendency (P=0.08) to significantly improve the sperm livability though a high rate of head abnormalities (P<0.01) as compared with controls. Inclusion of fish with HBP in the diet of male Nile tilapia improved semen characteristics represented in a significant (P<0.05) increase in sperm motility, besides, a numerical increase in sperm count and low tail abnormalities (Table 3).

Influence of Propolis or Pollen Feeding for Three Weeks on Serum Biochemical Parameters

Although propolis or HBP showed a tendency to increase serum creatinine levels (P=0.08), there was no change in level of urea when compared to control. However, while ALT activity was significantly (P<0.001) lower in propolis or HBP treated groups, AST did not show any significant change among the three fish groups (Figure 2).

Influence of Propolis and Pollen Feeding for Three Weeks on Gonadal Histology in *O. niloticus*

Females: Histological examination of gonads in control and treated fish groups confirmed the same pattern i.e. ovaries showed numerous oocytes of different sizes and stages embedded in the ovarian interstitial tissues and enclosed with thin connective tissue capsule composed of germinal epithelium and tunica albuginea. However, ovaries of fish receiving pollen or propolis incorporated diet showed a large number of ripe oocytes when compared to control (Figure 3).

Males: Sections in testes of *O. niloticus* illustrated the presence of thin tunica albuginea with numerous seminiferous tubules (S.T.) contained different spermatogenic stages; spermatogenia, spermatocytes, spermatids and sperms; as well as interstitial connective tissues in between S.T. In the group which received propolis, the testes had smaller S.T. lumens and high replication of sperm producing cells than those of the controls. Testes of males fed pollen supplemented diet were highly active, showing accumulated sperms in S.T. with increased size of the interstitial cells when compared to control (Figure 3).

Discussion

In aquaculture, nutrition is critical because feed represents 40-50% of the production costs (Abowei and Ekubo, 2011). The honeybee products of pollen

Table 3. Gonadal response and semen characteristics in Nile tilapia (*Oreochromis niloticus*) supplemented with propolis or honeybee pollen (HBP) in diet for three weeks

Item	Control	Propolis	HBP
a. Female gonadal response			
Gonadosomatic index (GSI)	5.30 ± 1.35^{a}	3.68 ± 0.86^{a}	3.84 ± 0.64^{a}
Gonadal weight (gm)	2.80 ± 0.78^{a}	2.62 ± 0.50^{a}	2.44 ± 0.30^{a}
Number of eggs	289 ± 50^{a}	307 ± 76^{a}	350 ± 95^{a}
Size of eggs (mm)	3.668 ± 0.357^{b}	4.464 ± 0.535^{a}	3.663 ± 0.359^{b}
Ratio of female tilapia has eggs > 4 mm in diameter	20%	80%	40%
Relative gonadal weight	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
Total egg volume/ovary ($cm^3\ gm^{-1}$)	0.0030 ± 0.0010^{b}	0.0050 ± 0.0010^{a}	0.0030 ± 0.0004^{b}
Relative fecundity:			
In relation to length	20.54 ± 4.21^{a}	18.73 ± 4.69^{a}	22.15 ± 6.04^{a}
In relation to weight	5.88 ± 1.39^{a}	4.05 ± 0.91^{a}	5.42 ± 1.67^{a}
b. Semen characteristics			
Hydrogen ion conc. (pH)	6.96 ± 0.07^{a}	6.88 ± 0.05^{a}	6.98 ± 0.09^{a}
Sperm cell conc. ($\times10^6$)	1195.2 ± 201^{a}	1788 ± 685^{a}	1871 ± 319^{a}
Sperm motility (%)	58 ± 9^{b}	67 ± 5^{ab}	85 ± 7^{a}
Sperm livability (%)	82 ± 8^{a}	$96\pm2^{a*}$	88 ± 5^{a}
Sperm normality (%)	55 ± 3^{a}	51 ± 3^{a}	61 ± 7^{a}
Head abnormalities (%)	6 ± 1^{b}	11 ± 2^{a}	5 ± 1^{b}
Tail abnormalities (%)	37 ± 2^{a}	38 ± 5^{a}	31 ± 3^{a}

Value; mean±S.E. (n=30 females and 15 males/group) within the same row with different alphabetic superscript are significantly different (P<0.05).

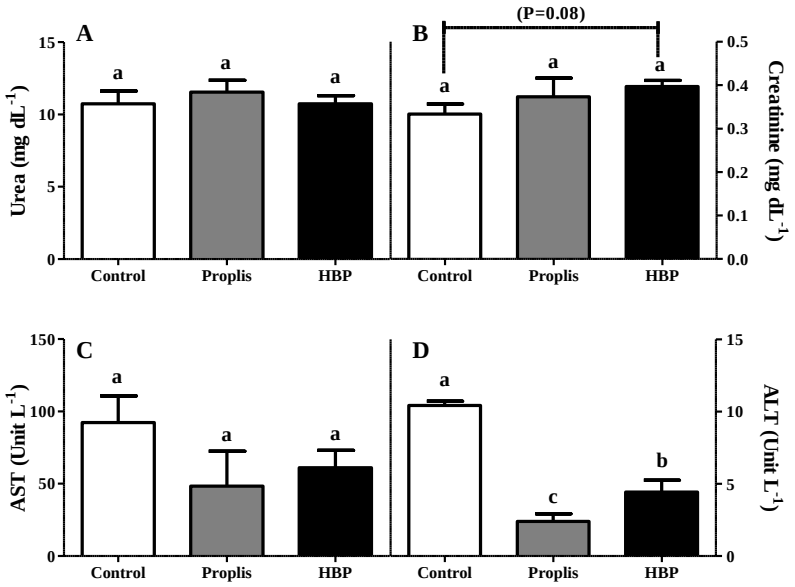

Figure 2. Urea (A), creatinine (B) levels and Aspartate aminotransferase (AST) (C) and alanine aminotransferase (ALT) activity (D) in the serum of mixed sampled (male and female) Nile tilapia (*Oreochromis Niloticus*) of control (□), propolis (▯) and honeybee pollen (■) groups. Values (means ±SEM; n=5/group) with different letters in the same body index were significantly different at P<0.05.

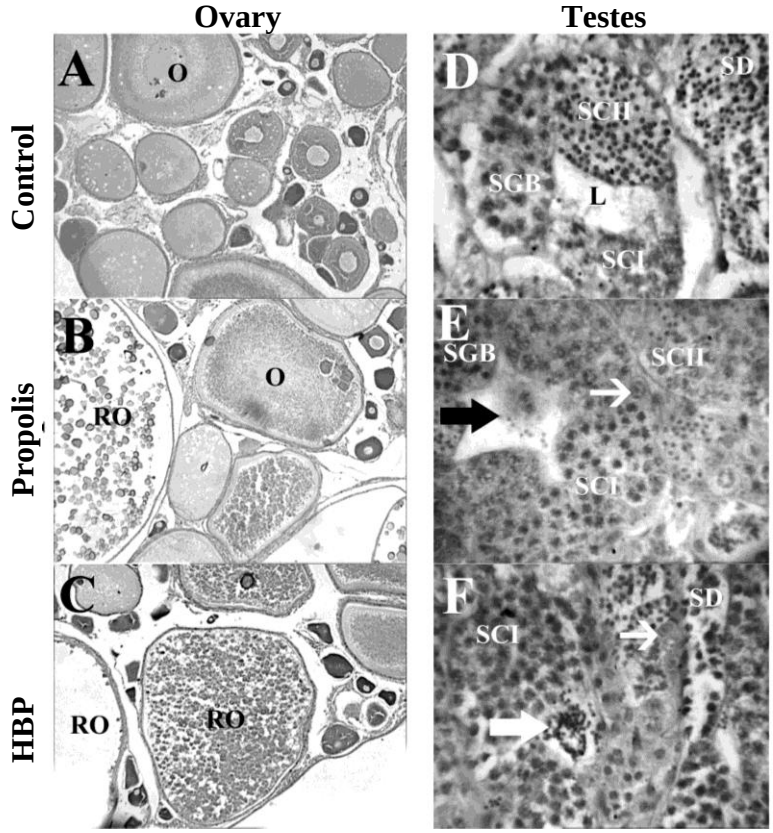

Figure 3. Histomorphological changes of the female (A, B, C) and male (D, E, F) gonads (H&E stain; × 40) of Nile tilapia (*Oreochromis niloticus*) fed on propolis and pollen incorporated diets. Note, the ovaries of propolis (B) or pollen (C) groups showed ripe oocytes (RO) as compared with control group (A). In the testes, all spermatogenetic cells were observed in testicular lobules: type A spermatogonia (narrow arrrow); cysts with type B spermatogonia (SG B), primary spermatocytes (SC I), secondary spermatocytes (SC II), spermatids (SD); and spermatozoa (broad arrow) released into the lobule lumen (L). Testes of propolis group (E) had smaller S.T. lumens and high replication of sperm producing cells than those of control group. Testes of males fed pollen inclusion diet (F) appeared highly active, showing accumulated sperms in S.T. with increased size of the interstitial cells as compared with control.

and propolis characterize by having nutritionally valuable substances that can be used to improve fish farming (Velotto *et al*., 2010). In the present study, adding of propolis, in the diet of Nile tilapia, seems to have noticeable increase in the SGR, ADG and FER in addition to improvement of the final weight and length of both females and males. This finding indicates the presence of a potential effect for propolis on brood stock growth performance as shown from the significant lowered feed conversion ratio (FCR). It has been reported that dietary propolis supplement, regardless of the inclusion level, decreased the whole body moisture and ash contents, but increased the whole body protein and lipid contents (Deng *et al*., 2011). Propolis extract (Meurer *et al*., 2009) or crude propolis (Abd-El-Rhman, 2009) decreased the feed conversion ratio and increased the growth performance (Abd-El-Rahman 2009; Meurer *et al*., 2009), improved the specific growth rate (Deng *et al*., 2011), decreased the tonus and amplitude of the peristaltic movements in rats (Cristina *et al*., 2007) as well as improved the growth performance in poultry (Seven, 2008). On the other hand, while HBP inclusion in diet improved SGR, final weight and length of female *O. niloticus*, it had no effect on body indices or the growth performance in males when compared to control. This finding is reflected on the lowered HSI only in males. Pollen feeding in mammals increased the intestinal absorptive capacity through the longer and thicker villi (Wang *et al*., 2007) in association with a significant improvement in body weight gain due to higher protein anabolism (Attia *et al*., 2011a).

In the present study, treatment with propolis showed an increase in the number of females with over-ripened egg population and the total egg volume per ovary, in spite of the short course of treatment; 3 weeks. However, GSI, gonadal weight and relative gonadal fecundity in the treated groups did not vary from that in the control. It has been suggested that the therapeutic activities of propolis depend mainly on the presence of flavenoids (Marcucci, 1995) that modulate steroid hormones (phytoestrogen activity) and consequently hormone-dependent ovarian activity (Oršolić, 2010) through their capacity to interact with estrogen receptors-ß (Matsumoto *et al*., 2004) in the reproductive organs. After propolis treatment, a gradual reduction in the mortality of fish eggs *in vitro* (1.2-2% compared to untreated eggs) has been emphasized (Velotto *et al*., 2010). On the other hand, treatment with HBP revealed an increase in percentage of female tilapia which had ovarian over-ripened (>4 mm in diameter) egg population, while gonadal indices did not change when compared to control. Moon *et al*. (2006) mentioned that the main active ingredients of bee pollen are primarily phytoestrogens which may lead to changes in hormonal levels and/or ovarian sensitivity. In rabbits, bee pollen feeding improved conception rate in does (Attia *et al*., 2011b). In vitro studies showed that bee pollen regulates the insulin like growth factor-1, released by mammalian ovarian granulosa cells, which is important for regulation of ovarian functions (Kolesarova *et al*., 2011).

Male Nile tilapia, fed propolis containing diet, showed an improved milt quality represented by the increased sperm count and high sperm livability in spite of the increased head abnormalities when compared to control, a finding which was accompanied, histologically, with smaller S.T. lumen and high replication of sperm producing cells. In mammals, It has been noticed that propolis extract containing phenol compounds significantly increase testosterone level, semen characteristics and seminal plasma enzymes (Yousef *et al*., 2010) and protect sperm membrane from the deleterious action of oxidative attack (Russo *et al*., 2006). On the other hand, feeding of male Nile tilapia with HBP in the diet improved semen characteristics exhibited by the numerical increase in sperm count, noticeable increase in sperm motility and lower tail abnormalities, a finding which came in accordance with the high activity of ST suggesting that bee pollen has an androgenic effect in fish. This finding came in agreement with some previous studies indicating that bee pollen has a remarkable improvement in semen quality, increase in the sperm count and the testosterone level (Attia *et al*., 2011a; Selmanoğlu *et al*., 2009).

In the present study, propolis or HBP tended to have an increase in the serum creatinine level and did not provoke changes in the serum urea level when compared to control; a finding which might suggest that pollen provides an additional protective effect against kidney injury (Nagyova *et al*., 1994). Feeding of either propolis or HBP incorporated diets showed a significant ($P<0.001$) lower the serum ALT activity, contrary to the serum AST level when compared to control. These findings suggested the presence of a hepato-protective activity for propolis and HBP in *O. niloticus as* indicated by reducing AST, ALT and alkaline phosphatase activities in liver damage induced in mice by allyl alcohol (Wojcicki *et al*., 1987; Deng *et al*., 2011) and confirmed histopathologically. Previous studies demonstrated that quinic acid derivatives naturally present in propolis have strong liver-protective effects and promote healing of toxic liver cells (Seo *et al*., 2003).

From the present study, it can be concluded that keeping brood stock Nile tilapia on diet with 2.5% propolis or pollen before restocking into the breeding results in the highest rate of hatchability in female and fertilizing capacity in males, beside the improvement of their growth performance and some function indices of liver and kidney.

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