



# Evaluation the Impact of Different Levels of Propolis on Some Reproductive features in Iraqi Local Roosters

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**Abstract** | The current work designed to explore the propolis potential on some characters related with fertility in Iraqi local roosters. Thirty mature white local roosters, age between 27–28 weeks, randomly distributed into three groups (n=10). Treatment 1 (T<sub>1</sub>) group, control group, taken the standard diet during the research period (ten weeks), each rooster of treatment 2 & 3 (T<sub>2</sub> & T<sub>3</sub>) received 0.25 & 0.5 g/day of propolis, respectively. At ten weeks ending, blood samples obtained from vein of wing to evaluate the levels of cholesterol, globulin, albumin, total protein, testosterone hormone, Interstitial cell – stimulating hormone (ICSH) and Follicle- stimulating Hormone (FSH) in serum. Roosters were euthanized and testes collected to determine their length, weight, width and height. Roosters of T<sub>2</sub> and T<sub>3</sub> groups' showed a significant elevation in morphological measurements of testes compared to control group (T<sub>1</sub>) (P<0.05). In addition, the values of serum globulin, protein, testosterone hormone, ICSH and FSH raised significantly in T<sub>3</sub> and T<sub>2</sub> roosters compared to T<sub>1</sub> group (P<0.05). We can conclude that the Iraqipropolis plays a vital role in fertility efficiency because its ability to reduce oxidative stress and act as a phytoestrogens leading to improve the testicular size and weight, as well as, enhance the reproductive hormones in Iraqi local roosters.

**Keywords** | Propolis, Reproductive, Testosterone, Hormones, Poultry.

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## INTRODUCTION

In Iraq, the development of poultry industry required modern strategies to improve roosters fertility, conversion of feeding habits and production that were achieved by applying feed additives (Hassan et al., 2010). Antibiotics are widely used in several countries to promote poultry growth, but they have side effects on general health of chicken due to basic materials precipitation in the tissues and eggs of birds that will be reflected on consumers' health (Aziz, 1981; El-Ghamry et al., 2002). Al-Beitawi and El-Ghousein, 2008; Al-Khafaji, 2013a and Khafaji, 2018a resorted to apply the natural additives and herbal medical plants like celery (*Apiumgraveolens*), black cumin *Nigella sativa*, coriander (*Coriandrum sativum*), ceylon cinnamon (*Cinnamomumzeylanicum*) and *Thyme* to improve the general health, performance and reproductive activity of poultry without any harmful effects (El-Ghamry et al., 2002;

Gunal et al., 2006; Al-Khafaji, 2013b; Khafaji, 2018 b,c).

Propolis, which is a bee glue, is a natural substance, collected from flowers and trees buds, mixed with wax to paint the inner surface and seal the cracks in bees' hives to prevent the entry of any pathological agents and foreign materials (Burdock, 1998). It is rich in minerals such as Fe, Na, Ca, I, Mg, K, Cu, Mn and Zn; in addition, it contains certain vitamins including E, B2, B6, B1 and C (Banskota et al. 2001a; AL-Sirraj et al., 2018). Furthermore, it possesses various enzymes such as acid phosphatase, succinic dehydrogenase, adenosine triphosphatase and glucose-6-phosphatase (Banskota et al., 2001b).

Propolis contains many metabolites, monomers and macromolecules and other substances including Flavonoids, Flavones, aromatic acids, amino acids, fatty acids, diterpenic acids, phenolic acid and vitamins, its compo-

sition depends on harvest location, plant source and season (Greenaway et al., 1991), as well as, it is considered a good source of antioxidant, anti-microbial, immunomodulatory, anti-inflammatory and antifungal (Dobrowolski et al., 1991; Bankova, 2005), these attributed to propolis therapeutic application against many diseases such as cardiac diseases, cancer, diabetes mellitus and inflammation (Mishima et al., 2005).

Because few or no research studied the propolis's effect on local white roosters fertility, this experiment was targeted to explore the action of propolis on main reproductive traits of this animals.

## MATERIALS AND METHODS

Propolis obtained from Al-Noor market of bee, it packed in dark plastic bags in -20C until used. This experiment was done at College of Agriculture/ University of Kerbala, during the period 20/2/2018 to 7/5/2018. A thirty mature local roosters, divided randomly into three equal groups. Treatment 1 group (T<sub>1</sub>), control group, provided with the basic diet during the experimental period, each rooster of treatment 2 group (T<sub>2</sub>) received orally with 0.250 g/ day, each rooster of treatment 3 group (T<sub>3</sub>) administrated orally with 0.5 g/bird day.

Testes weight (g), width, length and height (mm) were calculated by digital balance and vernier caliper at the end of study. Blood samples drawn and put in tubs without EDTA agent to obtain serum, which were centrifuged at 3000 rpm for 10 min. The clear serum was transferred carefully into clean and dry vials and kept at -20C until analysis to determine the concentrations of globulin, cholesterol, albumin, and total protein using Tietz's, 1982 methodology.

The reproductive hormones: Follicle stimulating hormone (FSH), Interstitial cell – stimulating hormone (ICSH) and Testosterone hormone were estimated in serum by ELIZA technique following Elias and Franey's, 1968 methodology.

### STATISTICAL ANALYSIS

Data of this study were calculated by using Complete Random Design (C.R.D). Statistical analysis was done by using system of (SAS, 2001). Statistical significant value was determined at P<0.05 .

## RESULTS

Table 1 explained the impact of Propolis on width (mm), length (mm) and height (mm) and weight (g) of local rooster testes. The mean values of weight, width, length and height were increased significantly (P<0.05) in T<sub>2</sub> with mean values (13.105± 0.543, 21.492± 1.645, 34.282

± 1.645, 17.93 ±1.662) respectively, in comparison with control roosters, as well as T<sub>3</sub> roosters were influenced significantly (P<0.05) by propolis with means (15.369±0.548, 27.496±0.831, 46.2±1.274, 24.078±0.241) respectively, in comparing with T<sub>1</sub> roosters at means (9.184±0.542, 16.888±1.155, 23.212±2.766 , 13.034±0.615) respectively.

**Table 1:** Impact of propolis on weight, length, width and height of testes in Local roosters.

Parameters/Groups	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Weight of testes (g)	0.542 c± 9.184	0.543 b± 13.105	0.548 a± 15.369
Width of testes (cm)	1.155 c±16.888	0.585 b± 21.492	0.831 a± 27.496
Length of testes (cm)	2.766 c± 23.212	1.645 b± 34.282	1.274 a± 46.2
Height of testes(mm)	0.615 c± 13.034	1.662 b± 17.93	0.241 a± 24.078

\*Data set as mean ± SD.

\*The dissimilar letters in the same rows are significantly differences at P<0.05.

The results of propolis activity on mean values of total protein, globulin, albumin and cholesterol of local roosters are demonstrated in Table 2. The statistical analysis revealed that the serum globulin and protein in T<sub>2</sub> (2.76±0.167 and 5.64±0.31) and T<sub>3</sub> treated groups (3.34±0.167 and 6.6±0.39) respectively, increased significantly (P<0.05) in comparison with T<sub>1</sub> group.

On other hand, the concentrations of cholesterol and albumin were not noted significant levels (P>0.05) in comparison among the three groups of roosters.

**Table 2:** Role of propolis on biochemical parameters in Local rooster.

Parameters/Groups	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Total protein(g/100ml)	0.29 c± 4.36	0.31 b± 5.64	0.39 a± 6.6
Albumin (g/100ml)	0.29 a± 3.12	0.29 a± 3.36	0.29 a± 3.44
Globulin (g/100ml)	0.178 c± 2.32	0.167 b± 2.76	0.167 a± 3.34
Cholesterol (g/100ml)	4.192 a±126.62	5.283 a± 128.92	7.649 a± 133

\*Data set as mean ± SD.

\*The dissimilar letters in the same rows are significantly differences at P<0.05.

Table 3 clarified the concentrations results of Interstitial cell – stimulating hormone (ICSH), Follicle stimulating hormone (FSH) and Testosterone hormone under effect Propolis. The statistical analysis indicated significant raise (P<0.05) in concentration of FSH, ICSH and Testosterone

hormone in  $T_2$  ( $3.788 \pm 0.041$ ,  $3.544 \pm 0.034$ ,  $2.965 \pm 0.047$ ) and  $T_3$  with values ( $4.860 \pm 0.129$ ,  $4.432 \pm 0.044$ ,  $4.064 \pm 0.243$ ) respectively, when compared with mean value of  $T_1$  rooster ( $02.89 \pm 0.083$ ,  $2.526 \pm 0.054$ ,  $1.925 \pm 0.086$ ).

**Table 3:** Effect of Propolis on the levels of some Reproductive hormones of Local roosters.

Parameters/Groups	$T_1$	$T_2$	$T_3$
FSH(miu/ml)	0.083 c± 02.89	0.041 b±3.788	0.129 a± 4.860
ICSH (miu/ml)	0.054 c±2.526	0.034 b±3.544	0.044 a± 4.432
Testosterone (ng/ml)	0.086 c± 1.925	0.047 b± 2.965	0.243 a±4.064

\*Data set as mean ± SD.

\*The dissimilar letters in the same rows are significantly differences at  $P < 0.05$ .

## DISCUSSION

The results of this study indicate that the oral administration of propolis to local roosters at individual dose 0.250 and 0.5 g/day for ten weeks- generated beneficial effects on animals health and improved male sexual organ physical and biomarkers parameters related with fertility characteristic. The valuable actions of propolis were characterized by high measurements testes' characteristics and high serum protein, globulin and reproductive hormones values. These effects may be arbitrated to numerous mechanisms of the active constituents of propolis.

The statistical analysis proved a significant elevation in weight and all physical measurements of testis because the propolis regarded as growth promoter (Meurer et al., 2009; Yousef et al., 2010) due to its contents of flavonoids and 4-hydroxybenzoic acid and benzoic, that promoted animal's feeding habit, feed conversion ratio and could enhance nutrient digestibility like ash and proteins (Tatli Seven, 2008; Seven et al., 2012; Attia et al., 2014) that reflected on current results of testis size, weight and body condition. Numerous studies showed a beneficial correlation among weight and size of testis, general body health and fertility (Hocking, 1990), the current results concur with Yousef and Salama (2009), Yousef et al. (2010) and El-Metwally et al. (2017).

Propolis regarded as a testicular protective agent because it is rich in phenolic and flavonoids contents that protect against oxidative stress and testicular tissues apoptosis by preventing free radicals' actions on testicular cells proteins, DNA and membranes through raising the potential concentration of Glutathione-S-transferase, Catalase, Super-oxide dismutase and Glutathione, which enhance the metabolic functions and subsequently increase testicular size

as reported in current results (Yousef and Salama, 2009; Shalaby and Saleh, 2011; Rizk et al., 2014).

In addition, the present results revealed that serum protein and globulin elevated significantly due to the ability of propolis components to improve functions of liver by increasing the generated protein which accompanied with actions of antioxidant agents (Sulaiman et al., 2011). As well as, the changes in our results may be attributed to propolis efficacy to induce immunological proteins synthesis due to high levels of amino acids and flavonoids (Tayeb and Sulaiman, 2014). The current results agree with Galal and his colleagues's (2008) findings who reported an increment in globulin and protein concentration when broiler fed with 198 and 188 mg/kg of propolis extract. Furthermore, propolis possesses anabolic activity because it has vitamins, essential amino acids and minerals that induce liver proteins production that associated with general health improvement via potential immunostimulatory activity of propolis which increased IgG and IgM levels (Çetin et al., 2010; Shihab and Ali, 2012; Abdel-Rahman and Mosaad, 2013). The statistical analysis of our work recorded significant raise in testosterone level (Table 3), this may be attributed to lignans and phenolic compounds—which are regarded as phytoestrogens—that metabolized in cell into enterolactone and enterodiol, they have estrogen hormone like effects (Branham et al., 2002; Zingue et al., 2017), could be influence donhypothamus and adenohipophysis that lead to produce of LH and FSH which in turn promote gonads to increase testosterone generation and the process of spermatogenesis, respectively (Pineda and Dooley, 2003).

The increment in testosterone values in our investigation are required for spermatogenesis and normal seminiferous tubules function that may be attributed to the ability of propolis to increase mRNA expressions of major enzymes is responsible for androgenesis process in testes including  $17\beta$ -hydroxysteroid dehydrogenase ( $17\beta$ -HSD), and  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ -HSD) (Sharpe et al., 1992; Jana et al., 2006). The current results support by Rizk and his colleagues's (2014) findings that showed an elevation in testosterone concentration in normal rats injected with extract of propolis that due to an increase in the expression of  $17\beta$ -HSD and  $3\beta$ -HSD gens. As well as, propolis possesses the ability to improve testicular proteins and 17-ketosteroid reductase (17-KSR) enzyme that are necessary for testosterone synthesis, Shalaby and Saleh (2011) revealed that the application of propolis could protect DNA from damage and increase LH, testosterone hormone, 17-KSR enzyme and proteins levels due to propolis contents of phenolic acid and flavonoids that prevent and delete the harmful effects of free radicles because it acts as scavenger of free radicles (Baykalir et al., 2016; El-Metwally et al., 2017), consequently increases testicular 17-KSR enzyme level which is required to produce



testosterone from androstenedione that leads to high level of testosterone hormone (Shalaby and Saleh, 2011) such found in present results.

Several studies established that propolis could enhance semen quantity and quality and spermatogenesis via influencing fertility hormones, as supported by Ghazi et al. (2013) who revealed the augmentation action of propolis on reproductive feature of rats treated with acrylamide agent, as improvement LH, testosterone hormone, FSH and production of sperm (Yousef et al., 2010; ElMazoudy et al., 2011). The present results are agreement with Husien's (2011) suggestion who investigated the enhancement actions of propolis on LH, testosterone and FSH in the weaning period of lambs. According to our finding, this work investigated the potential role of propolis at dose 0.250 and 0.5 g/day on general health and the reproductive features of Iraqi local roosters via utilizing physical testicular metrics, as well as, its progressive relationship between propolis dosage and fertility hormones such testosterone hormone, FSH and LH that improving spermatogenesis process and semen characters. In future, several researches required to determine the action of propolis on general performance and gene expression of liver enzymes of local roosters.

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## CONFLICT OF INTEREST

This work is personal noncommercial research and there is no conflict of interest.

## AUTHORS CONTRIBUTION

Sura Safi Obayes Khafaji, Thamer Kareem ALjanabi and Salam Merza SuhailAltaie designed the experiment. Salam Merza SuhailAltaie and Thamer Kareem ALjanabi conducted the statistical analysis, as well as, Sura Safi Obayes Khafaji, Salam Merza SuhailAltaie and Thamer Kareem ALjanabi supported the manuscript via conducting the experimental measurement, writing the article and the serious review of article before submission.

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