

## Effect of Nano Propolis on Productive Performance, Serum Antioxidants Status and Thyroid Hormones of Broiler Chickens

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### Abstract:

This experiment aimed to investigate the effects of different nano-propolis concentrations on productive performance, serum antioxidant and thyroid hormones of broiler chickens. A total of 144 one-day-old, unsexed broiler chickens with an average body weight of 40 g were used in this study. The birds were divided into 4 treatments, each with 36 birds, and each group include three replicates (12 birds per replicate). The first treatment consumed reverse osmosis (R.O) drinking water without additions (control), whereas the second, third, and fourth treatments consumed reverse osmosis (R.O) drinking water containing 50, 100, and 150 µl of nano-propolis per liter, respectively. Overall body weight, weight gain and feed conversion ratio were significantly ( $p \leq 0.05$ ) increase when nano-propolis add to drinking water of second, third, and fourth groups. Nano-propolis supplementation significantly ( $p \leq 0.05$ ) increased thyroid hormones concentration, including thyroxin (T4), tri-iodothyronin (T3), enzyme activity, including glutathione peroxidase (GPx) and superoxide dismutase (SOD). On the other hand, nano-propolis supplementation significantly ( $p \leq 0.05$ ) decreased malondialdehyde concentration (MDA). The results indicated no significant ( $p > 0.05$ ) difference in the cumulative feed intake, cumulative water intake and catalase enzyme among all groups. It be concluded, the addition of 50,100 and 150 µl of nano-propolis per liter of drinking water were improved productive performance, some serum antioxidant parameters that include GPx, SOD, MDA and thyroid hormones such as T4 and T3. Nano-propolis in current study without effect on the cumulative feed intake, cumulative water intake and catalase enzyme of broiler chickens.

**Keywords:** Broiler Chickens, Nano-Propolis, Productive Performance, Serum Antioxidant.

### Introduction

Propolis is a natural substance that comes from both animal and vegetable sources and has high antioxidant and antibacterial characteristics (Krocko *et al*, 2012). Flavonoids, phenolic acids, and their derivatives are propolis' bioactive components and are what give the substance its antibacterial, analgesic, anti-inflammatory, and antioxidant properties in both people and animals

(Krocko et al, 2012; Klaric et al, 2018). The biological activity of propolis is determined by the active compounds found in the polyphenolic fraction. Aromatic acids, triterpenes, lignans, carotenoids, phytosterols, and polyphenols make up the majority of this, but it also includes aromatic acids, triterpenes, lignans, carotenoids, phytosterols and polyphenols (Tatli Seven *et al*, 2009; Capcarova *et al*, 2013). Also these bioactive components available in the plants and their extracts therefore employed to improve poultry productivity and physiological performance (Al-Ashoor & Al-Salhie, 2020; Mahjar & Al-Salhie, 2022). A large number of prior studies have shown that feeding propolis to hens enhances their productivity (Tekeli *et al*, 2011). These results could be attributed to propolis extract's impact on the gut microbiota, which increases helpful bacteria while lowering pathogenic bacteria counts (Kacaniova *et al*, 2012). Several of propolis' components are found in diet additives and are generally regarded as safe, which serves as evidence of its antioxidant, antimicrobial, and antifungal properties (Burdock, 1998). Nanoscience is the science and application of small objects (less than 100 nanometers) having novel chemical and physical structures, as well as improved reactivity and solubility (Troncarelli *et al*. 2013). Because it is protected from oxidizing agents, other chemicals, and enzymes when the active component is nanostructured, the substance's stability is improved (Brandao *et al*. 2011; Troncarelli *et al*. 2013). These benefits significantly affect livestock performance, loss of income, and improved feed production. In terms of health, performance, and ongoing feed production, the natural nanomaterial nano-propolis can be helpful in veterinary medicine. Due to their smaller size, nanoparticles are more readily absorbed by the body, but nano propolis is more potent than propolis in terms of its antimicrobial properties (Afrouzan *et al*. 2012; Sahlan *et al*. 2017). Reactive oxygen species (ROS) are produced in high amounts during normal cell metabolism and are necessary for cell health. Many cell signaling processes need low levels of reactive oxygen species. Under typical physiological conditions, the levels of endogenous antioxidants that defend organs from oxidative damage and the amounts of reactive oxygen species produced during cellular metabolism are in balance. Oxidative stress is caused by an imbalance or absence of cellular redox balance, and it seriously harms biological components (Sies, 1991). Previous studies using propolis and other antioxidants have shown that it can reduce the detrimental effects of lipid peroxidation and free radical formation (Tatli Seven *et al*, 2009). Polyphenols and caffeic acid phenethyl ester, two antioxidants found in propolis, protective muscle cells from lipid oxidation (Havsteen, 2002; Hosnuter *et al*, 2004). The goal of this study was to investigate the effect of adding different levels of nano-propolis to drinking water on the productive performance, antioxidant status, and level of metabolic hormones in serum of broiler chickens.

## **Materials and Methods**

### **Period and study area**

Between February and July 2022, this study was carried out on a chicken farm at the University of Basrah, College of Agriculture in Basrah, Iraq.

### **Nano-propolis synthesis**

Propolis was bought from local market. Nano-propolis was prepared in the laboratories of the ministry of science and technology / environmental and water technology research department in Baghdad. Nano-propolis extract was prepared according to (El-Ghaffar & Hashem, 2009; 2010). Scanning electron microscopy (SEM) was used to describe propolis nanoparticles (Figure 1). Energy dispersive X-ray (EDX) was used to detect the primary structures of propolis nanoparticles (Figure 2).

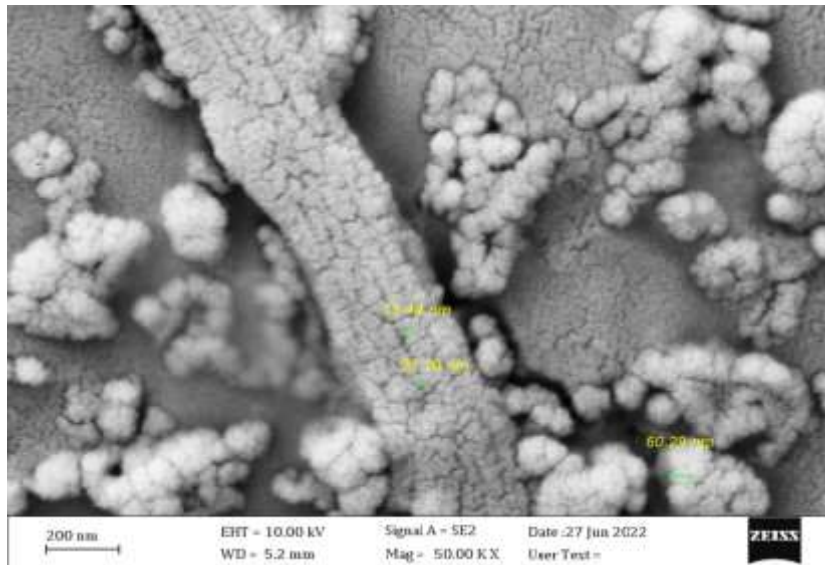


Figure (1): Detection of the size and shape of propolis nanoparticles by SEM

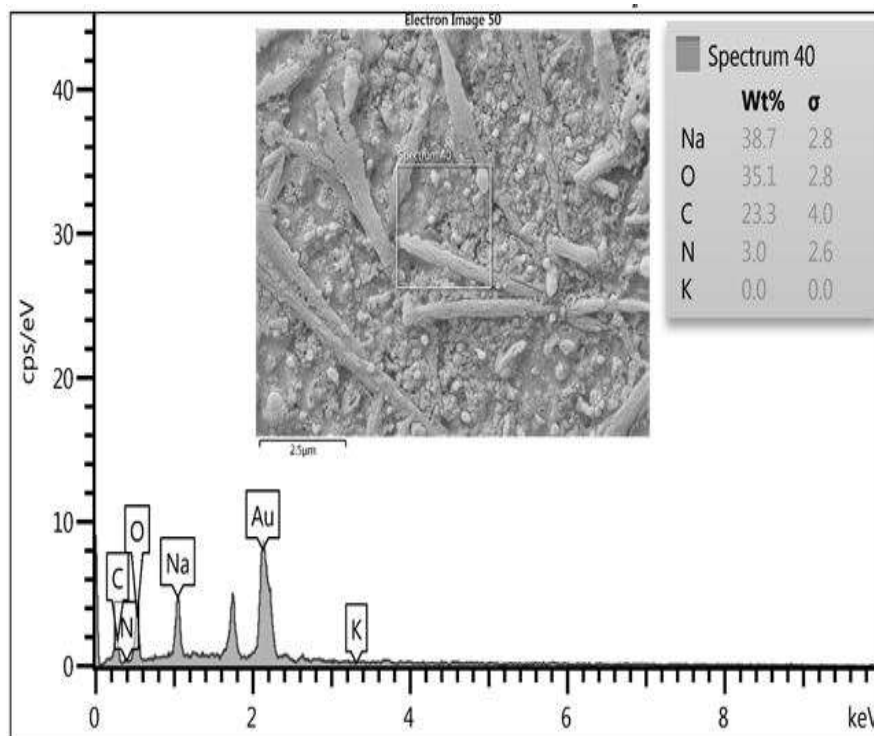


Fig (2): Detection of primary structures of propolis nanoparticles by EDX

### Animals' management

The birds were reared for thirty-five days, in accordance to the Ross 308 broiler management manual, under similar situations. The temperature was maintained at 33°C for the initial week, then descended 3°C weekly until the study end. The heat stayed at 21°C from that point on until the experiment ended. The lighting pattern was 23 hours of light and 1 hour of darkness from the first to the 35th day. The chicks were given two different basal diets, the first of which had 23.51% crude protein and 2910 kcal/kg metabolizable energy. The second diets contained 3174.5 kcal per kilogram of metabolizable energy and 20.11 percent crude protein (Table 1). The birds were fed pellet diets and *ad libitum* access to water.

**Table (1) : The ingredient and composition of the experimental diets**

Ingredient %	Starter 1-21 days	Grower 22-35 days
Maize	47	55
Wheat	13.5	10
Soybean meal (48%)	32	24.5
Protein concentrate (40%)	4.5	4.5
Plant oil	0.5	3.5
Limestone	1	1
NaCl	0.2	0.2
Premix (29%)	1	1
L-Lysine	0.2	0.2
Methionine	0.1	0.1
Total	100	100
Calculated values		
Metabolizable energy kCal/kg	2910	3174.5
Crude protein %	23.51	20.11
Ether extract %	3.40	6.57
Crude fibre %	3.63	3.17
Calcium %	0.70	0.68
Available phosphorus	0.30	0.27
Lysine %	1.31	1.12
Methionine + cysteine	0.85	0.77

**Study treatments:**

Four treatments were employed in this study. 144 unsexed, day-old broiler chickens with an average body weight of 40 g were used in this research. Three replicates were used in each of the four treatments, which each included 36 chicks (twelve chicks per replicate). The first treatment consumed reverse osmosis (R.O) drinking water with no additions (control), whereas the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> treatments consumed reverse osmosis (R.O) drinking water containing 50, 100, and 150 µl of nan-propolis per liter, respectively.

**Data collection**

At the end of the study, all replicates were measured for live body weight (g), average weight gain (g), cumulative feed intake (g), feed conversion ratio (g feed intake / g weight gain), and cumulative water consumption (ml). At the end of the experiment, blood samples were taken after the broiler chickens had fasted for three hours. To obtain serum, three ml of blood was drawn from a shank vein and centrifuged for 10 minutes. A spectrophotometer with a 353 nm wavelength was used to measure the concentration of malondialdehyde (MDA) in according to (Yagi, 1998; Al-Mosawy & Al-Salhie, 2021). The activities of the enzymes catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) were assessed according to (Sanja *et al.*, 2015). Commercial ELISA kits (VIDAS Company) were used to measure serum concentrations of total thyroxin (T4) and total tri-iodothyronin (T3).

**Statistical analyses:**

The SPSS program (SPSS, 2016) was used to analyze experimental data using a completely random design (One-way ANOVA). Additionally, significant differences between means were evaluated using Duncan's multiple range tests (Duncan's, 1955) at a 0.05 level of significance.

**Results and Discussion**

Productive performance of broiler chickens supplemented by different water levels of nano-propolis is presented in Table 2. Overall body weight, weight gain and feed conversion ratio were significantly ( $p \leq 0.05$ ) improved in the fourth, third and second treatments compared to first treatment (control). The results indicated no significant ( $p > 0.05$ ) difference in cumulative feed intake and cumulative water intake. The beneficial effects of propolis could well be linked to bioactive substances including flavonoids, which may enhance the metabolic function of broiler chickens (Attia *et al.* 2014). As a result, the flavonoid concentration and palatable characteristics of the propolis diet could be attributed with improved productivity. Supplementing broilers with propolis has been shown to improve their ability to digest and absorb nutrients (Hosseini *et al.* 2016), which could help explain the improvement in performance seen in this study. Our findings are in agreement with those of Attia *et al.* (2014), who found that using propolis on a continuous or intermittent schedule improved broiler chicken productivity during the entire trial compared to the control group. Roodsari *et al.* (2004) found that supplementing the diet with propolis enhanced productivity compared to those fed a basal diet. These findings are consistent with those of Zeng *et al.* (2004), who found that when broilers were fed a 2.5:1 ratio of flower pollen and propolis, their body weights increased by 10% when compared to the control group. In addition, quails fed propolis at a rate of 0.5 to 1.5 g kg<sup>-1</sup> had considerably larger body weights compared to control group (Denli *et al.*, 2005). The findings were also consistent with those of (Seven *et al.*, 2010 ; Hassan & Abdulla, 2011), who found that propolis supplementation led to an improvement in body weight gain. It is possible that the antibacterial activity of the propolis extract components resulted in enhanced gut health (Denli *et al.*, 2005). The use of nano-propolis in broiler drinking water enhanced weight gain and improved feed conversion ratios significantly. The addition of propolis to the quail diets produced results that were comparable (Denli *et al.*, 2005) or drinking water (Tayeb & Sulaiman, 2014). The consumable components found in propolis diets, like as resin, wax, honey, and vanillin, may be responsible for these beneficial effects on broiler performance (Shalmany & Shivazad, 2006). Propolis beneficial properties reduce protein degradation by partially inhibiting oxidative protein denaturation (Sahin *et al.*, 2003). As a result, the presence of nano-propolis in broiler chicken drinking water is estimated to enhance feed conversion ratios and nutrient digestibility, resulting in high broiler chicken performance.

**Table (2): Productive performance of broiler chickens supplemented by different levels of nano-propolis (Mean± SE)**

Treatments Parameters	T1	T2	T3	T4
Overall body weight (g)	1801.53 <sup>d</sup> ± 14.25	1899.57 <sup>c</sup> ± 14.44	2027.93 <sup>b</sup> ± 15.13	2101.93 <sup>a</sup> ± 15.71
Final weight gain (g)	1761.53 <sup>d</sup> ± 16.77	1859.57 <sup>c</sup> ± 15.78	1987.93 <sup>b</sup> ± 17.64	2061.93 <sup>a</sup> ± 17.34
Cumulative feed intake(g)	2881.07 <sup>a</sup> ± 13.69	2891.29 <sup>a</sup> ± 13.07	2898.71 <sup>a</sup> ± 14.60	2905.17 <sup>a</sup> ± 11.63
Feed conversion ratio (g.g)	1.64 <sup>a</sup> ± 0.02	1.55 <sup>b</sup> ± 0.04	1.46 <sup>c</sup> ± 0.03	1.41 <sup>d</sup> ± 0.03
Cumulative water intake (L)	6.13 <sup>a</sup> ± 0.14	6.28 <sup>a</sup> ± 0.15	6.30 <sup>a</sup> ± 0.13	6.40 <sup>a</sup> ± 0.12

There are significant differences when there are different letters in the same row ( $p \leq 0.05$ ).

Antioxidant status and thyroid hormones of broiler chickens supplemented by different levels of nano-propolis were showed in Table 3. Nano-propolis supplementation significantly ( $p \leq 0.05$ )



increased enzyme activity, such as GPx and SOD. On the other hand, nano-propolis supplementation significantly ( $p \leq 0.05$ ) reduced in malondialdehyde. Thyroid hormones concentration, including thyroxin (T4) and tri-iodothyronin (T3) were significantly ( $p \leq 0.05$ ) increased when nano-propolis supplemented. The results indicated no significant ( $p > 0.05$ ) difference in catalase enzyme. Nano-propolis significantly increased SOD and GPx while decreasing MDA in the current study. Propolis contains flavonoids and essential fatty acids, which may be the cause of these results. Flavonoids have been shown to have beneficial impacts on the immune systems of several species (Hegazi *et al.*, 1995). Additionally mentioned were aluminum and calcium, as well as aromatic oils, protein, amino acids, vitamins, and flavonoids (Burdock 1998; Hassig *et al.*, 1999; Lee *et al.*, 2001). Propolis contains flavonoids, phenolic acids, and terpenes, which have antioxidant and antitumor properties (Prytyk *et al.*, 2003; Wang *et al.*, 2004; Seven *et al.*, 2010). The present finding, it's possible that nano-propolis dosages reduced the formation of lipid peroxidation radicals. The level of MDA in blood and tissues rises as lipid peroxidation rises (Okutan *et al.*, 2005). Antioxidant enzyme activities, such as SOD, can either decrease or increase in lipid peroxidation (Wohaieb & Godin, 1987; Huang *et al.*, 1999; Ozkaya *et al.*, 2002; Aliciguzel *et al.*, 2003). In the current study, it was discovered that elevated antioxidant enzyme activities including those of SOD and GPx serve as a defense against induced reactive oxygen species (ROS). Superoxide dismutase and glutathione peroxidase are antioxidant enzymes that protect cells from the damaging effects of reactive oxygen species (ROS) (Altan *et al.*, 2003). Thyroid hormones regulate metabolic heat production, which is required for animals to maintain a steady body temperature (Beyzai & Adibmoradi, 2011). Our results are consistent with those of Mahmoud *et al.* (2014), who discovered that adding 250 mg/kg of propolis, vitamin E and C to broilers' diets can reduce the oxidative damage caused by heat stress. In compared to non-supplemented birds bred under heat stress, these favorable effects were demonstrated by lower serum corticosterone levels and improved thyroid hormone levels. Mahmoud *et al.* (2017) found that the 250 mg kg<sup>-1</sup> propolis treatment group had a significantly higher T3/T4 ratio than the control group. The results indicated no significant ( $p > 0.05$ ) difference in catalase enzyme activity. Our findings were on the contrary with Tatli Seven *et al.* (2016) who indicated the Liver catalase enzyme activity of propolis fed quail were higher than those of the other groups.

**Table (3): Antioxidant status and thyroid hormones of broiler chickens supplemented by different levels of nano-propolis (Mean± SE)**

Treatments Parameters	T1	T2	T3	T4
MDA Micromole.L <sup>1</sup>	6.73 <sup>a</sup> ± 0.61	6.16 <sup>ab</sup> ± 0.59	4.78 <sup>bc</sup> ± 0.43	3.44 <sup>c</sup> ± 0.29
SOD U.ml. <sup>1</sup>	1.72 <sup>c</sup> ± 0.04	2.05 <sup>b</sup> ± 0.05	2.56 <sup>a</sup> ± 0.12	2.76 <sup>a</sup> ± 0.09
Gpx U.L. <sup>1</sup>	0.89 <sup>c</sup> ± 0.06	1.21 <sup>b</sup> ± 0.06	1.67 <sup>a</sup> ± 0.08	1.89 <sup>a</sup> ± 0.08
Catalase U.ml. <sup>1</sup>	39.16 <sup>a</sup> ± 1.59	41.23 <sup>a</sup> ± 1.52	42.34 <sup>a</sup> ± 1.44	44.19 <sup>a</sup> ± 1.32
T3 ng.ml. <sup>1</sup>	0.11 <sup>c</sup> ± 0.02	0.17 <sup>bc</sup> ± 0.01	0.24 <sup>ab</sup> ± 0.02	0.28 <sup>a</sup> ± 0.05
T4 ng.ml. <sup>1</sup>	6.12 <sup>c</sup> ± 0.74	8.14 <sup>bc</sup> ± 0.99	10.09 <sup>ab</sup> ± 0.89	11.93 <sup>a</sup> ± 0.69

There are significant differences when there are different letters in the same row ( $p > 0.05$ ).

## Conclusions

In conclusions, the addition of 50,100 and 150 µl of nano-propolis per liter of drinking water were improved productive performance, some serum antioxidant parameters that include GPx, SOD, MDA and thyroid hormones such as T4 and T3. Nano-propolis in current study without effect on the cumulative feed intake, cumulative water intake and catalase enzyme of broiler chickens.

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## Contributions of authors

R.H.K.: Sample and data collection.

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## تأثير العكبر النانوي في الاداء الانتاجي وحالة مضادات الاكسدة وهرمونات الغدة الدرقية لفروج اللحم

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### الملخص:

هدفت الدراسة الحالية لمعرفة تأثير استخدام مستويات مختلفة من العكبر النانوي لمياه الشرب على الأداء الإنتاجي ومضادات الأكسدة وهرمونات الغدة الدرقية في الدم لفروج اللحم. أُستخدم في الدراسة 144 فرخاً من افراخ فروج اللحم غير المجنسة بعمر يوم واحد بمتوسط وزن جسم 40 جرام. وُزعت الافراخ عشوائياً على 4 معاملات (كل منها تحتوي على 36 طائر) بواقع ثلاثة مكررات للمعاملة الواحدة ولكل مكرر 12 فرخاً. استهلكت طيور المعاملة الأولى مياه الشرب بالتناضح العكسي بدون إضافات (معاملة سيطرة) ، بينما استهلكت طيور المعاملات الثانية والثالثة والرابعة مياه شرب بالتناضح العكسي تحتوي على 50 و 100 و 150 ميكرو لتر من العكبر النانوي لكل لتر على التوالي. اشارت النتائج الى ارتفاع معدلات وزن الجسم والزيادة الوزنية الكلية وتحسن كفاءة التحويل الغذائي معنوياً ( $p \leq 0.05$ ) عند إضافة العكبر النانوي لمياه شرب المعاملات الثانية والثالثة والرابعة. أدت إضافة العكبر النانوي الى زيادة معنوية ( $p \leq 0.05$ ) في تركيز هرمونات الغدة الدرقية المتمثلة بهرمون الثايروكسين (T4) ، والثريونين ثلاثي اليود (T3) فضلاً عن زيادة في نشاط الانزيمات المضادة للأكسدة المتمثلة بأنزيم الجلوتاثيون بيروكسيديز (GPx) وديسموتاز الفائق (SOD). من ناحية أخرى ، أدت إضافة العكبر النانوي الى انخفاض معنوي ( $p \leq 0.05$ ) في تركيز مركب المألون ثنائي الالديهيد (MDA). اشارت النتائج إلى عدم وجود فرق معنوي ( $p > 0.05$ ) في كمية العلف التراكمي ، وكمية الماء التراكمية وإنزيم الكاتليز بين جميع المجموعات. نستنتج مما تقدم أن إضافة 100، 50 و 150 ميكرو لتر من العكبر النانوي لكل لتر من مياه الشرب قد أدى إلى تحسين الأداء الإنتاجي ، وبعض معايير مضادات الأكسدة في الدم التي شملت GPx و SOD و MDA وهرمونات الغدة الدرقية مثل T4 و T3، ولم تؤثر إضافة العكبر النانوي في الدراسة الحالية على كمية العلف التراكمي وكمية الماء التراكمية وإنزيم الكاتليز لفروج اللحم.

**الكلمات المفتاحية:** فروج اللحم ، العكبر النانوي، الاداء الانتاجي ، مضادات الاكسدة