



Research Article

Impact of Adding Nanopropolis and Vitamins E and C to Drinking Water on the Productive Performance, Antioxidants Status, Thyroid Hormones and Some Physiological Characteristics of Broiler Chickens

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Abstract | This study aimed to investigate the effect of adding nano-propolis, vitamins E and C to drinking water on productive traits, serum antioxidants, thyroid hormones and some physiological traits of broiler chickens. A total of 144 unsexed chicks, one day old, 40 grams weight were used in present study. The birds were divided into four different treatments, each of which had 36 birds. Each treatment consisted of three duplicates, with each replication including twelve birds. The first treatment involved consuming drinking water (RO) without any additives (control), the second treatment incorporated the addition of 150 microliters of nano-propolis per liter of drinking water, the third treatment included the addition of 3 ml (equivalent to 300 mg) of vitamin E per liter of drinking water. The fourth treatment entailed the addition of 300 mg of vitamin C per liter of drinking water. Both the overall live body weight and total weight gain of second treatment birds became significantly elevated ($P \leq 0.05$) compared to other treatments. The results indicated a significant improvement ($P \leq 0.05$) in the cumulative feed conversion efficiency for the second, third and fourth treatments compared to the control. Nanopropolis, vitamin E and C administration significantly increased ($P \leq 0.05$) thyroid hormones (thyroxin and triiodothyronine) concentrations, glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities compared to the control. On the other hand, the second, third and fourth treatments showed a significant reduction ($P \leq 0.05$) in the concentration of malondialdehyde (MDA). The results indicated that there were no significant differences in the feed intake, the catalase enzyme activity (CAT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities among the different treatments. In conclusion there was a significant improvement in the productive performance, antioxidant status, and thyroid hormones, which was observed after the addition of nanopropolis and vitamins E and C to drinking water of broiler chickens.

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Keywords | Broiler chicken, Nanopropolis, Productive traits, Serum antioxidants, Vitamins E and C.



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Introduction

The poultry sector is one of the most rapidly expanding industries in world agriculture. It plays a significant role in poorer nations as a primary source of protein from animals. The meat from poultry is distinguished by its substantial nutritional content and serves as an excellent source of high-quality protein (Bahri *et al.*, 2019). Therefore, some international companies specializing in the poultry industry have been interested in conducting breeding and genetic improvement operations to obtain a high growth rate, which resulted in obtaining commercial broiler hybrids with a higher weight than in previous decades and a shorter marketing age than before. This requires great attention to enhancing the immune aspect by increasing resistance to diseases and reducing stress resulting from rapid growth. Natural antioxidants function to impede the activity of free radicals, so mitigating the impact of oxidative stress in chickens, which negatively affects poultry production projects (Castro *et al.*, 2022; AL-Kahtani *et al.*, 2022). Plants and their extracts have been utilized to enhance the productive and physiological performance of poultry (Al-Salhi and Al-Waeli, 2019; Al-Ashoor and Al-Salhi, 2020).

Propolis is a natural compound with potent antioxidant and antimicrobial properties due to its content of flavonoids, phenolic acids and their derivatives of biologically active components (Anjum *et al.*, 2019). It enhances the growth of broilers by improving feed conversion efficiency, as the bird will enjoy high health and productive efficiency, so propolis and its extracts can be used as feed additives to broiler diets (Elsherif *et al.*, 2021). Sadarman *et al.* (2021) reported a significant increase in body weight and a significant improvement in feed conversion efficiency of broiler chicks when feeding these chicks on propolis. Khalati and Al-Salhi (2023) found an improvement in live body weight, weight gain, cumulative feed consumption and feed conversion efficiency of broiler chickens when fed different concentrations of nano-propolis. Abbas *et al.* (2020) showed in a study conducted on laying hens when adding propolis to feed a significant decrease in the malondialdehyde (MDA) compound and a significant improvement in the antioxidant status by increasing the concentration of superoxide dismutase.

Researchers use biotechnology techniques as

environmentally friendly and cost-effective methods to manufacture nanoparticles (Pirtarighat *et al.*, 2019). In recent years, many technologies have been introduced, including nanotechnology. These technologies are promising in the poultry industry and animal production due to the importance of nanotechnology in improving the solubility, absorption and bioavailability of nanoparticles. It also provides better solutions in poultry production that can help to reduce breeding costs and improve the quality of the final product (Fesseha *et al.*, 2020).

Both vitamins C and E act as antioxidants for oxidation, and they play an influential role in reducing oxidative stress in animals. It works to protect the body from the harmful effects of free radicals, and it plays a role in improving the economic production characteristics of broiler chickens (Calik *et al.*, 2022a, b; Pecjak *et al.*, 2022). Vitamin C is considered a water-soluble antioxidant, and it has an influential role in reducing oxidative stress (Comunian *et al.*, 2022).

Therefore, the present study's purpose was to determine the impact that the addition of nano-propolis and vitamins E and C to drinking water on the productive performance, antioxidant status and metabolic hormone levels of broiler chickens.

Materials and Methods

This study was performed in the poultry field at the College of Agriculture, University of Basrah, Basrah Province, Iraq.

Propolis samples

Propolis was obtained from the local markets of Wasit Province (Kut) and was morphologically characterized. The samples were dried and ground manually using a mortar and pestle to obtain a homogeneous powder. The powder was stored in clean and sterile glass containers at 21°C until use.

Preparation of propolis powder nanoparticles

Nanoparticles have been prepared according to El-Ghaffar and Hashem (2010). The alcoholic extract of propolis was mixed at a concentration of 5% with chitosan in equivalent proportions. The collection and condensation process occurred using a Reflux device (by evaporation and reflection distillation process) in the presence of xylene until a clear solution appeared over time and separated into two layers. The chitosan

layer combined with propolis was separated using the filtration method. Then, the chitosan was washed several times using methanol, hot distilled water, and ethanol and then dried using an electric oven at 50°C. Chitosan nanoparticles with alcohol propolis were obtained through an ionic gel pathway using sodium tripolyphosphate (STPP), where 5 mg/ml of the propolis and chitosan mixture was weighed by dissolving it in 1% v/v acetic acid until the solution became clear. Then, STPP solution was added to the mixture of chitosan and alcoholic propolis extract at a ratio of 1:2.5 (weight/weight)%, with continuous stirring by a magnetic Stirrer at a warm temperature (45-50) °C for 6 hours. However, the production of chitosan nanoparticles-propolis alcoholic extract/STPP begins through the ionic gelation mechanism. Nanoparticles are initially separated through multiple washings, after which the supernatant is discarded. The precipitate is subsequently rinsed with water, dried, and stored in the refrigerator at a temperature of 4-5°C.

Vitamins E and C

Vitamin E was obtained from a private store. Its composition was that every 1000 ml contains 100,000 mg of vitamin E and 450 mg of selenium. It was in the form of a liquid solution made in India by Varsha Multi-Tech Company. Vitamin C was obtained from a private store in Basrah Province made in Vietnam by ACHAUPHARM Company, and its purity was 99%.

Study treatments

One hundred forty-four unsexed one-day-old ROSS 308 chicks, with an average weight of 40 grams, were utilized. The birds were allocated into four treatments, each consisting of 36 individuals, with three duplicates per treatment (12 replicates).

The first treatment was drinking water (R.O.) (control treatment). In contrast, the second treatment added 150 µl of the nano-propolis preparation per liter of drinking water, the third treatment added 3 ml (equivalent to 300 mg) of vitamin E per liter of drinking water, and the fourth treatment added 300 mg of vitamin C per liter of drinking water.

Birds feeding

The birds were reared for five weeks following the ROSS 308 chicken management protocols in optimal conditions. The temperature reached 33°C in the first

week, subsequently declining by two degrees each week until it settled at 25°C by the fifth week of the hens' age. The illumination consisted of 23 hours of light and one hour of darkness. Two experimental diets were used in this study. The first diet contained 23.06% crude protein and a metabolizable energy of 2946.5 kilocalories/kg. The birds were fed on it for a period of (1-21 days). The second diet contained 20.24% crude protein and metabolizable energy of 3209 kilocalories/kg. The birds were fed on it for a period of (22-35days). The experimental diets were presented in [Table 1](#).

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

Ingredient %	Starter 1-21 days	Grower 22-35 days
Yellow corn	50	53
Wheat	12	12
Soybean meal (48%)	30.5	25
Protein concentrate (40%)	5	4
Plant oil	0.5	4
Limestone	1	0.5
NaCl	0.2	0.7
Premix (29%)	0.5	0.5
L-Lysine	0.2	0.2
Methionine	0.1	0.1
Total	100	100
Calculated values		
Metabolizable energy kCal/kg	2946.5	3209
Crude protein %	23.06	20.24
Ether extract %	3.51	6.97
Crude fibre %	3.55	3.21
Calcium %	0.72	0.47
Available phosphorus	0.31	0.26
Lysine %	1.29	1.12
Methionine + cysteine	0.85	0.76

Collection of data

At the finish of the experiment, the live body weight (g), total weight (g), cumulative consumption of feed (g), and total feeding efficiency of conversion (g feed intake/g weight) were assessed in accordance with [Kyakma et al. \(2022\)](#). Blood samples were obtained following a three-hour fasting period for the hens. To acquire serum, 3 ml of blood was extracted from the leg vein, and the tubes were subjected to centrifugation at

8000 rpm for 15 minutes. Following separation, the serum samples were transferred to alternative plastic tubes and maintained at a temperature of -20°C until testing was performed. A spectrophotometer set to 355 nm was utilized to measure the concentration of malondialdehyde using the protocols established by Yagii (1998); Al-Mosawy and Al-Salhie (2021). The activities of Catalase (CTA), Glutathione Peroxidase (GPx), and Superoxide dismutase (SOD) enzymes have been evaluated according to Sanjia *et al.* (2015). Kits were employed to measure the levels of total thyroxin (T4) and overall triiodothyronine (T3) in serum. The kits were employed to quantify the activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT).

Statistical analysis

The complete randomized design (CRD) was used to analyze the data using the statistical analysis program (SPSS, 2018). The Duncan (1955) multinomial test was used to test the differences between the treatments at a significance ($P \leq 0.05$) level.

Results and Discussion

The results show the effect of adding nanopropolis, vitamins E and C to the drinking water on the productive traits of broiler chickens. The second treatment demonstrated a significant enhancement ($P \leq 0.05$) in overall body weight, total weight gain, and total feed conversion efficiency. Conversely, the third and fourth treatments exhibited a statistically significant increase ($P \leq 0.05$) in these parameters relative to the control treatment (T1).

The results of Table 2 indicated no significant difference ($P \geq 0.05$) in cumulative feed intake. These findings may be attributed to the role of nano propolis, which is characterized by the small size of its particles, which are characterized by their ability to be absorbed faster and easier, as Nanopropolis exhibits superior efficacy

compared to propolis when utilized in regular form (Afrouzan *et al.*, 2012). The nano-emulsion material also improves nutrient digestibility, greatly enhancing growth and health (Kishawy *et al.*, 2023; Meligy *et al.*, 2023). There is a possibility that the beneficial effects of propolis are also associated with biologically active compounds, such as flavonoids, that possess the capacity to enhance cellular metabolic activity (Attia *et al.*, 2014).

Research has demonstrated that the incorporation of propolis in broiler diets enhances their nutritional digestion and absorption capabilities (Hosseini *et al.*, 2016). The reason for the superiority of the third and fourth treatments may be attributed to their role in vitamin E and C in protecting and maintaining the integrity of cell membranes by reducing oxidative stress and thus improving metabolic processes and reducing protein breakdown in the chickens body, ultimately leading to improved productive trials of broilers (Ahmadu *et al.*, 2016; Khalifa *et al.*, 2021; Comunian *et al.*, 2022).

The results agreed with Sadarman *et al.* (2021), who indicated a significant increase in the improvement of productive characteristics of broilers after feeding them propolis at 1.66 and 2.13 g/kg. On the other hand, the results agreed with del Barrio *et al.* (2020), who indicated improving the productive characteristics of broilers when adding 200 g/1000 L of vitamin C to drinking water. The results were consistent with Calik *et al.* (2022a) when using vitamin E at 250 mg/kg in the diets of broiler chickens exposed to stress led to an improvement in the productive characteristics of broiler chickens. The presence of nano-propolis in the drinking water of broiler chickens enhances the feed conversion rates and digestibility of nutrients, which leads to high performance of broiler chickens. Recently, Eldiasty *et al.* (2024) clarified that adding nano-propolis liposomes (NPRL) at 100, 250 and 400

Table 2: Effects of nano-propolis and vitamins E and C on the productive performance of broiler chickens (mean \pm SE)

Treatment parameters	T1	T2	T3	T4
Initial body weight (g)	40	40	40	40
Overall body weight (g)	1779.53 ^c \pm 11.99	2231.93 ^a \pm 12.62	2130.27 ^b \pm 11.46	2104.60 ^b \pm 13.87
Final weight gain (g)	1739.53 ^c \pm 14.49	2191.93 ^a \pm 13.59	2090.27 ^b \pm 16.37	2064.60 ^b \pm 16.14
Cumulative feed intake(g)	3035.95 ^a \pm 11.15	3079.98 ^a \pm 11.95	3046.13 ^a \pm 11.53	3040.10 ^a \pm 11.44
Feed conversion ratio (g/g)	1.74 ^a \pm 0.03	1.40 ^b \pm 0.04	1.45 ^b \pm 0.03	1.47 ^b \pm 0.03

Different letters within a row indicate significant differences ($P \leq 0.05$) between the means.

mg/kg diets supplemented with NPRL significantly improved the growth indices.

The reason for the superiority of the third and fourth treatments compared to the control in these parameters may be associated with the antioxidant activities of both vitamins C and E. They can maintain the integrity of cell membranes by inhibiting the action of free radicals in the cellular environment and reducing the level of malondialdehyde (Adenkola and Angani, 2017; Hashem *et al.*, 2019). The findings of the present study agree with the results reported by Mahmoud *et al.* (2014), which demonstrated that the inclusion of 250 mg/kg of Propolis, vitamins E and C in broiler diets mitigates oxidative stress induced by heat stress, in comparison to control birds subjected to heat stress. These results agree with those of Eldiasty *et al.* (2024). Malondialdehyde levels considerably decreased with the addition of nano-propolis liposomes (NPRL) at dietary concentrations of 100, 250, and 400 mg/kg; in contrast, the levels of SOD and GSH-PX enzymes increased.

In Table 3, they provide the results of an experiment that investigated the impact of nano-propolis and vitamins E and on the amount of antioxidant activity of broiler chickens. A significant increase was observed in the level of activity of SOD and GSH-PX enzymes in the second, third and fourth treatments compared to the control treatment. On the other hand, the first treatment recorded the highest levels

of malondialdehyde concentration compared to other treatments. Based on the findings, it was determined that there were no significant variations in the activity of the CAT enzyme between the various experimental treatments. The incorporation of nano-propolis and vitamins E and C into the drinking water of broilers resulted in a considerable reduction in MDA relative to the control group. The findings may be attributed to the presence of propolis, which contains active compounds that suppress free radicals responsible for oxidative stress and cellular damage. Propolis comprises natural antioxidants, such as flavonoids, phenolic acids, and their derivatives, which are biologically active constituents that safeguard against oxidative stress (Tatli Seven *et al.*, 2012; El-Guendouz *et al.*, 2017).

Table 4 showed the effect of adding nano-propolis, vitamins E and C to drinking water on thyroid hormones, and AST and ALT enzyme activity (IU/L). The results indicated a significant increase ($P \leq 0.05$) in the concentration of thyroxine (T4) and triiodothyronine (T3) in the second, third and fourth treatments compared to the control treatment. The significant increase in the rate of T3 and T4 hormones in the second treatment may be due to the flavonoids of propolis, which are antioxidant compounds that improve the level of metabolic hormones (Haro *et al.*, 2000).

Table 3: Impact of nano-propolis and vitamins E and C on the antioxidants status of broiler chickens (Mean \pm SE)

Treatments parameters	T1	T2	T3	T4
MDA Micromole.L ¹	5.91 ^a \pm 0.60	3.20 ^b \pm 0.35	3.35 ^b \pm 0.57	3.41 ^b \pm 0.02
SODU.ml. ¹	2.08 ^b \pm 0.01	2.88 ^a \pm 0.07	2.80 ^a \pm 0.09	2.78 ^a \pm 0.09
GpxU.L. ¹	1.20 ^b \pm 0.01	1.75 ^a \pm 0.07	1.70 ^a \pm 0.06	1.68 ^a \pm 0.06
CatalaseU.ml. ¹	41.16 ^a \pm 1.72	46.19 ^a \pm 1.89	44.34 ^a \pm 1.46	43.23 ^a \pm 1.51

Different letters within a row indicate significant differences ($P \leq 0.05$) between the means.

MDA: Malondialdehyde, SOD: Superoxide dismutase, Gpx: Glutathione Peroxidase

Table 4: Effect of nano-propolis and vitamins E and C on thyroid hormones and AST and ALT activities of broiler chickens (IU/L) (mean \pm SE)

Treatments parameters	T1	T2	T3	T4
T3ng.ml. ¹	0.12 ^c \pm 0.04	0.30 ^a \pm 0.02	0.23 ^{ab} \pm 0.01	0.21 ^b \pm 0.03
T4ng.ml. ¹	5.45 ^c \pm 0.09	13.27 ^a \pm 0.36	10.75 ^b \pm 0.51	10.08 ^b \pm 0.53
AST(IU/L)	158.00 ^a \pm 2.89	150.44 ^a \pm 2.66	153.00 ^a \pm 2.65	155.00 ^a \pm 2.08
ALT(IU/L)	28.10 ^a \pm 1.48	23.00 ^a \pm 2.08	25.28 ^a \pm 1.46	26.00 ^a \pm 1.73

Different letters within a row indicate significant differences ($P \leq 0.05$) between the means.

T3: Triiodothyronine, T4: Thyroxine, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.

of the amino acids phenylalanine and tyrosine, which The increase of these hormones in the third and fourth treatments relative to the control may be attributed to the influence of vitamins E and C on the synthesis are critical for the production of thyroid hormones; they also work act as antioxidants and have a positive role in increasing metabolic activity in birds' bodies (Cinar *et al.*, 2014). The findings did not reveal any statistically significant changes in the levels of AST and ALT activities among the various experimental treatments. The results of the current study agreed with Khalati and Al-Salhie (2023) regarding a significant improvement in the T3 and T4 hormones of broiler chickens after consuming nano-propolis.

Conclusions and Recommendations

The study shows that the including of vitamins E, C, and nano-propolis in the drinking water of broiler chickens enhanced their productive performance, antioxidant indices, and thyroid hormone levels. We advise incorporating the nano-propolis formulation into the drinking water of broilers at a concentration of 150 µl/l.

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Novelty Statement

This work demonstrates how to prepare nanoparticles of propolis powder and give it to broiler chickens with drinking water, as it acts as a natural antioxidant and thus prevents the action of free radicals and thus reduces the effect of oxidative stress in poultry.

Author's Contribution

Rasool H. Khalati: Write the manuscript and statistical analysis, Helped in field experiment sample, data collection and analysis.

Khalid C.K. Al-Salhie: Editing and review.

Generative AI and AI-assisted technology statement

The authors have declared there is no generative AI

and AI-assisted technology used in this manuscript.

Conflict of interest

The authors have declared that there is no conflict of interest.

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