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Hematological Parameters and Relative Weight of some Organs for Broiler Chickens Supplemented by Different Levels of Nano Propolis

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Abstract. The objective of this experiment was to examine the impact of using different levels of nano-propolis to drinking water on hematological parameters and heart, liver, gizzard, spleen and bursa gland relative weights of broiler chickens. A total of 144 one-day-old, unsexed broiler chickens with an average body weight of 40 g were used. The birds were distributed into 4 treatment groups (each with 36 birds), with three replicates in each group (12 birds per replicate). The first group drank reverse osmosis (R.O) drinking water without additions (control), whereas the second, third, and fourth groups drank reverse osmosis (R.O) drinking water containing 50, 100, and 150 µl of nano-propolis per liter, respectively. Higher some hematological variables such as RBC, Hb and PCV were observed in the 3rd and 4rd treatments compared to other treatments. Some blood parameters such as MCV, MCH, MCHC, heterophils, lymphocytes, heterophils/lymphocytes ratio and relative weight of heart, liver, gizzard, spleen and bursa gland were not affected by various amounts of nano-propolis. In conclusions, the addition of 100 and 150 µl of nano-propolis per liter of drinking water were improved some blood parameters that include RBC, Hb and PCV. Nano-propolis in current study without effect on MCV, MCH, MCHC, heterophils, lymphocytes, heterophils / lymphocytes ratio and relative weight of heart, liver, gizzard, spleen and bursa gland of broiler chickens.

Keywords: Nano-propolis, Broiler, Blood parameters

1. Introduction

Propolis is a natural compounds with strong antioxidant and antibacterial capabilities that come from both animal and vegetable sources [1]. Most of the active elements in Propolis are responsible for its antioxidant activity in both human and animal [1,2]. The biological activity of Propolis and plants is determined by the active compounds in the polyphenolic fraction. Aromatic acids, lignans, triterpenes, phytosterols, carotenoids, and polyphenols are among the constituents of flavonoids [3,4]. Plants and their extracts were therefore employed to improve poultry productivity and physiological performance [5-7]. A great number of previous research have demonstrated that feeding propolis to hens improves their production performance [8]. These effects could be linked to propolis extract's influence on the gut microbiome, which raises beneficial bacteria levels while decreasing harmful bacteria [9]. Given that some of its ingredients are found in feed and feed additives and are generally regarded as harmless, propolis' antioxidant, antibacterial, and antifungal properties make sense. [10]. The study of small things (less than 100 nm) with novel chemical and physical structures, as well as improved reactivity and solubility is known as nanotechnology. [11]. The substance's stability is increased when the active component is nanostructured because it is shielded from oxidizing agents other compounds or enzymes. [11,12]. These advantages result in improved food and feed production as well as improved livestock animal performance and financial losses. Veterinary medicine can benefit from the use of nano-propolis a natural nano-material, in terms of performance, health, and reliable food production. Nanoparticles are more readily absorbed by the body due to their smaller size but nano-propolis has greater antibacterial and antifungal action than propolis. [13,14]. Reactive oxygen species (ROS) are created in large quantities during normal cell metabolism and are required for healthy cell function. Many cell-signaling procedures require low quantities of reactive oxygen species. The quantities of reactive oxygen species created during cellular metabolism and the levels of endogenous antioxidants, which

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prevent organs from oxidative damage, are in balance under normal physiological conditions. Oxidative stress is caused by an imbalance or lack of cellular redox balance, which causes significant damage to cellular components [15]. In prior studies using propolis and other antioxidants, it was demonstrated that propolis can reduce the harmful effects of lipid peroxidation and the formation of free radicals [4]. Flavonoids and caffeic acid phenethyl ester, both of which are found in propolis, are antioxidants that protect cell membranes from lipid peroxidation [16,17].

This experiment was designed to investigate the impact of varying the amount of nano-propolis in drinking water on the hematological parameters and the relative weight of the heart, liver, gizzard, spleen and bursa gland of broiler chickens.

2. Materials and Methods

2.1. Propolis Extract and Nano-propolis Synthesis

Propolis was purchased from local markets. The alcoholic extract of propolis and nano-propolis were prepared to use in this experiment. The alcoholic extract of Propolis was prepared according to [18]. Chemical detections were made for some active compounds in the alcoholic extract of propolis, which include alkaloids, tannins, phenols, flavonoids, steroids, resins, coumarins, glycosides and saponins. Nano-propolis extract was prepared according to [19,20]. The nano extract was detected using transmission electron microscopy.

2.2. Study Treatments

Four treatment groups were employed in this study. In this study, 144 one-day-old, unsexed broiler chicken (ROSS 308) chicks with an average body weight of 40 g were used. The birds were distributed into 4 treatment groups (each with 36 birds), with three replicates in each group (12 birds per replicate). The first group drank reverse osmosis (R.O) drinking water without additions (control), whereas the second, third, and fourth groups drank reverse osmosis (R.O) drinking water containing 50, 100, and 150 µl of nano-propolis per liter, respectively.

2.3. Animal Management

The chicks were raised for five weeks (35 days) under identical conditions. For the first week, the temperature was set at 33°C, then steadily reduced by 3°C each week until the ending of the fifth week. The temperature thereafter stayed at 21°C throughout the remainder of the trial. From the first to the 35th day, there were 23 hours of light and 1 hour of darkness. Two different basal diets were fed to the chicks, the first diet containing 23.51 % (C.P.) and 2910 kcal\kg (M.E.). The second diets, were included 20.11 % (C.P.) and 3174.5 kcal\kg (M.E.). Pellet diets and *ad libitum* water were supplied to the chicks.

2.4. Data Collection

After the broiler chickens had fasted for 3 hours, blood samples were taken at the end of the study. Using a 3ml syringe (23G needle) and an EDTA-filled tube, blood was extracted 2-3 ml from a shank vein that had been cleaned with alcohol. Red blood cells (RBC $x10^6.mm^3$) Hb (g.dl), PCV(%), MCV (fl), MCH (pg), MCHC ($g.l^{-1}$), heterophils (%), lymphocytes (%), and heterophils/lymphocytes ratio were measured. All blood parameters were determined according to [21].

2.5. Relative Weight of some Organs

Five identically sized birds from each group were used to measure the relative weights of the heart, liver, gizzard, spleen and bursa gland after the 35-day experiment. The birds were chosen at random, weighed separately, and then slaughtered. Organ weights were collected, calculated in proportion to live body weights, and expressed.

2.6. Statistical Analysis

A completely randomized design (One –way ANOVA) was applied to analyze experimental data using the program SPSS [22]. Duncan test [23] were used to assess significant differences between means at a 0.05 percent level of significance

3. Results and Discussions

3.1. Chemical Analysis of Active Ingredients of Propolis Extract

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Qualitative chemical detection of the active ingredients of propolis extract were shown in table 1. The results of the qualitative detection of the active compounds in the Propolis extract indicated the presence of glycosides, tannins, phenols and flavonoids with a very high intensity, while the alkaloids, resins and terpenes appeared with a lower intensity. On the other hand, the results indicated the absence of saponins, coumarins and steroids in the alcoholic extract of propolis. Plants contain phenolic compounds such as phenolic acids, flavonoids, stilbenes, tannins, coumarins, curcuminoids, and quinines. These substances provide propolis its anti-inflammatory, anti-carcinogenic, anti-mutagenic, and antioxidant characteristics. [24]. Polyphenols, including flavonoids, are found in propolis. Flavonoids are utilized to assess the quality of propolis [25]. Aluminum and calcium, as well as aromatic oils, protein, amino acids, vitamins, and flavonoids, were mentioned [10,26,27]. Because of its high flavonoid, phenolic acid, and terpenoid content, propolis has antioxidative, antimutagenic, and immunomodulatory properties [28-30].

Table 1. Qualitative chemical detection of the active compounds in the Propolis extract.

Type of active compounds	Result
Glycosides	+++
Alkaloids	++
Tannins	+++
Resins	++
Saponins	-
Coumarins	-
Phenols	+++
Flavones	+++
Terpenes	+
Steroids	-

+++high intensity available , ++ highly available, + available , - unavailability

3.1. Hematological Variables

The effect of various amounts of nano-propolis on RBC, Hb, PCV, MCV, MCH and MCHC are shown in Table 2. The results showed significantly increased ($p \leq 0.05$) RBC, Hb and PCV in the 3rd and 4rd treatments compared to other treatments. MCV, MCH and MCHC were not affected by different levels of nano-propolis. Increased RBC, hemoglobin, and PCV levels could suggest improved broiler health, which could be linked to increased nutritional consumption, particularly iron. Furthermore, propolis can enhance iron utilization and, as a result, hemoglobin regeneration[31,32] . Our findings are in agreement with [33] , who found that using 100 and 150 mg propolis in diets resulted in high packed cell volume in laying hens. As a result, [34] discovered that including propolis in diet enhanced hemoglobin levels, packed cell volume, and erythrocyte counts. [35] indicated that continuous administration of bee pollen and Proplis, alone or in combination, increased RBC count compared to the control treatment. Our findings are consistent with those of [36], who observed that adding propolis to the broiler diet had no influence on MCV and MCH when compared to broilers fed a control diet.

Table 2. Effect of various amounts of nano-propolis on some hematological variables (Mean±SE).

Treatments Parameters	T1	T2	T3	T4
PCV (%)	20.5 c± 0.12	21.65 bc± 0.66	22.65 b± 0.64	24.52 a± 0.12
Hb (g.dl)	6.83 c± 0.04	7.22 bc± 0.22	7.55 b± 0.22	8.17 a± 0.04
RBC ($x10^6.mm^3$)	2.12 c± 0.01	2.26 bc± 0.08	2.38 b± 0.08	2.61 a± 0.01
MCV (fl)	96.69± 2.41	95.79± 2.32	95.16± 2.15	93.94± 1.66

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MCH (pg)	32.21± 0.83	31.94± 0.96	31.72± 0.98	31.30± 0.98
MCHC ($g.l^{1}$)	33.31± 1.59	33.34± 1.60	33.33± 0.99	33.31± 0.99

Dissimilar letters are mean there are significant different at $p \leq 0.05$.

The results of heterophils ratio, lymphocytes ratio and H/L are shown in Table 3. Various amounts of nano-propolis had no significant effect ($p > 0.05$) on heterophils ratio, lymphocytes ratio and H/L. Our findings are in agreement with [37] who indicated that the use of propolis in diet of Muscovy broiler ducks did not affect leukocytes counts. According to [38] , adding 0.2 g/kg of ethanol extract of propolis to the diet had no influence on the H/L ratio of broiler chicks. Inclusion of 1% to 4% of propolis extraction waste in broiler chickens did not impact the heterophil:lymphocyte (H:L) ratio, according to [39]. Our results are in contrast those of Omar et al. [34], who found that supplementing diets with propolis enhanced the number of heterophils in broiler chickens. According to [31], the alcoholic extract of propolis exhibited an immunostimulant effect and helped to maintain good health. Sforcin, [40] reported the high heterophils proportion may be linked to propolis ability to activate macrophages.

Table 3. Effect of various amounts of nano-propolis on Heterophils %, Lymphocytes % and H/L (Mean±SE)

Treatments Parameters	**T1**	**T2**	**T3**	**T4**
Heterophils (%)	34.23± 4.08	33.69± 3.18	22.64± 3.64	22.22± 3.53
Lymphocytes (%)	72.34± 2.96	72.61± 3.56	63.78± 3.27	63.06± 1.73
H/L ratio	0.47± 0.04	0.46± 0.02	0.35± 0.04	0.35± 0.05

3.2. Organs Relative Weight

The results showed the various amounts of nano-propolis had no significant effect ($p > 0.05$) on relative weight of heart, liver, gizzard, spleen and bursa gland. Our findings on relative organ weights are also similar with those of [38], who found no significant effect of an ethanol extract of propolis on the relative weights of liver and heart in broilers at slaughter age. There were no significant variations in the relative weight of the liver and gizzard of quails fed diets supplemented with varied doses of propolis, according to [41]. Eyng [39] found that varying doses of propolis in the feed (1%, 2%, 3%, and 4%) had no influence on the relative weight of the cloacal bursa and spleen of male Cobb-Vantress chicks. Dietary propolis supplementation had no influence on the weights of the liver, heart, gizzard, and spleen, according to [42]**.**

Table 4. Effect of various amounts of nano-propolis on some relative weight of organs (Mean±SE).

Treatments Parameters	**T1**	**T2**	**T3**	**T4**
Heart weight (%)	0.45± 0.03	0.45± 0.03	0.51± 0.03	0.57± 0.04
Liver weight (%)	2.41± 0.19	2.55± 0.18	2.92± 0.21	2.97± 0.04
Gizzard weight (%)	1.59± 0.13	1.7± 0.15	1.83± 0.14	1.97± 0.07
Spleen weight (%)	0.11± 0.02	0.13± 0.02	0.14± 0.02	0.16± 0.02
Bursa weight (%)	0.20± 0.03	0.18± 0.02	0.15± 0.03	0.12± 0.02

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Conclusions

In conclusions, the addition of 100 and 150 µl of nano-propolis per liter of drinking water were improved some blood parameters that include RBC, Hb and PCV. Nano-propolis in current study without effect on MCV, MCH, MCHC, heterophils, lymphocytes, heterophils/lymphocytes ratio and relative weight of heart, liver, gizzard, spleen and bursa gland of broiler chickens.

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