

Adding Ginkgo biloba leaf extract to the diet as an antioxidant and studying its effect on carcass traits and immune performance of Ross 308 broilers

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Abstract: The experiment was carried out in the poultry field of the Department of Animal Production/College of Agriculture/ University of Diyala during the period from 4/9/2021 to 15/10/2021 to study the effect of adding ginkgo leaf extract to broiler diets as an antioxidant and study its effect on carcass traits and immune performance of birds. The experiment was conducted using 225 unsexed 308 Ross broiler hybrid chicks at the age of one day, and the average weight of the chick was (43.62)g.

The chicks were randomly distributed since the first day of the experiment to five experimental treatments for each treatment, three replicates (15 birds/repeat). The experimental transactions were as follows, T1=(control treatment) a standard diet free of addition was provided, T2=a standard diet supplemented with 1 g/kg forage of ginkgo biloba leaf extract, T3=a standard diet supplemented with 2 g/kg forage of ginkgo biloba leaf extract, T4= a standard diet supplemented with ginkgo biloba leaf extract To it 3 g/kg of ginkgo biloba leaf extract, T5= a standard diet, to which 4 g/kg of ginkgo biloba leaf extract was added, and the results of the experiment were as follows.

The results of the experiment indicated that there was a significant effect ($P \leq 0.05$) when adding different levels of Ginkgo biloba leaf extract to the diets of Ross 308 broilers during the period 1-42 days. The fourth treatment was superior to the live weight measurement compared to the control, In addition to having a highly significant effect ($P \leq 0.01$) where the fourth and second treatment excelled when measuring the weight of the cleaned carcass and the weight of the carcass with the edible viscera compared to the control, also the second, the fourth and the fifth treatment were significantly superior ($P \leq 0.01$) when measuring the weight of the cleaned carcass without the edible entrails compared to the control, and also significantly ($P \leq 0.01$) All the addition treatments when measuring the ratio of dressing without viscera, dressing with viscera and measuring secondary cuts (neck and back) compared to the control, while no significant differences were found among all experiment treatments when measuring the main cuts.

While the results of the experiment indicated that there was a significant effect ($P \leq 0.01$) when adding different levels of Ginkgo biloba leaf extract to the diets of Ross 308 broilers during the period 1-24 days. All treatments of the experiment excelled when measuring the titer of antibodies against infectious bronchitis virus (IB) compared to the control, and also significantly ($P \leq 0.01$) the second and third treatment when measuring the titer of antibodies against Newcastle virus (ND) compared to the control.

Introduction

Poultry meat is considered one of the most important sources used to raise the rate of people's consumption of animal protein because of its high protein content, and broiler meat and eggs are mainly used for this purpose (Al-Fayyad and Naji, 2012; Al-Tamimi and Al-Tamimi, 2019). And that the production process in companies specialized in the poultry industry is affected by the increase in the world population, which prompted them to increase their production capacity, especially broilers from commercial hybrids characterized by rapid growth rates and accompanied by a high metabolic rate, which requires an increase in the rate of blood flow from the heart, which causes great stress on the heart, as a result of its small size, is therefore more susceptible to infection with ascites, oxidative stress and the formation of free radicals, and thus sudden death (Manju et al., 2010; Al-Zuhair and Al-Tamimi, 2017). In addition to the decline in the immunity of birds (Deif et al., 2007), where the Central Bureau of Statistics / Iraq (2020)

estimated that Iraq's total production of broilers for the private sector amounted to 2,148 thousand tons during the year 2020, which increased by 8.38 thousand tons of the total production of Iraq during the year 2019 Where it was 4.109 thousand tons and increased by 35.5%.

Medicinal plants are a source of many active and vital compounds and are characterized by their therapeutic value in many diseases, despite the small number of plants whose effectiveness has been tested (Hostettmann et al., 1998). Where medicinal plants have been used since ancient times in food preservation due to their anti-pollution properties in addition to being disinfectant (Hashem and Alamri, 2010), extracts of medicinal and herbal plants contain many active substances in (leaves, stems, roots, flowers, buds) With flavonoids, terpenoids, alkenes, sulfites, polyphenols, carotenoids and saponins, which are characterized by their role as catalysts for the properties of digestion and absorption and increase the activity and effectiveness of digestive enzymes and antioxidants (Alagbe, 2010), Among these plants is the Ginkgo biloba plant, which is a medicinal plant in China known for its antioxidant properties. The ginkgo plant grows in different regions of China, and the important parts that are used medically in this plant are the fresh or dried leaves and seeds, and the ginkgo leaves contain many Important and biologically active components such as flavonoids, terpenoids and polyphenols, in addition to important sugars and minerals (Simth and Luo, 2004; Defeudis and Drieu, 2000), In addition to stimulating blood circulation and the ability to reach the narrowest blood vessels in order to deliver oxygen to the heart, brain and all parts of the body, ginkgo leaves are also widely used as a stimulant for blood circulation and anti-inflammatory (Sochocka et al., 2010). Ginkgo has shown high physiological activities in the treatment of many diseases (Zhang et al., 2013; Chen et al., 2011; Shinozuka et al., 2002). Given the importance of the Ginkgo biloba plant and its content of many active substances that help increase blood flow, maintain heart health, deliver oxygen to the brain and heart, narrow blood vessels, and antioxidants and compounds that increase the body's immunity and raise the productive performance of broilers, where the study aimed to know the effect of adding Leaves extract to the diet at different levels on the productive and immune performance of Ross 308 broilers.

Materials and methods

This study was conducted in the poultry field of the Department of Animal Production - College of Agriculture - University of Diyala for the period from 4-9 to 15-10-2021, With the aim of adding ginkgo leaf extract to the diet as an antioxidant and studying its effect on carcass traits and immune performance of Ross 308 broilers, In the experiment, 225 unsexed Ross 308 hybrid broiler chicks were used at one day old, obtained from Al-Sadiq hatchery - Canaan district, with an average initial weight of 43.62 g/chick, The chicks were bred in ground enclosures with dimensions of 2 x 1.5 m. The chicks were randomly distributed from the first day into five experimental treatments with three replicates for each treatment (15 birds/repeat).

The study included five treatments, in the following order:

T1: control treatment without addition

.T2: Add ginkgo leaf extract at a ratio of 1g/kg of feed

.T3: Add ginkgo leaf extract at a rate of 2g/kg feed

.T4: Add ginkgo leaf extract at a ratio of 3g/kg of feed

T5: Add Ginkgo leaf extract at a rate of 4g/kg of feed.

The birds were fed a starter diet from the age of 1-14 days, and a growth diet from the age of 15-28 days, and a final diet from the age of 29-42 days. Ginkgo leaf extract was added to the diets by mixing it manually with a small amount of feed, then the quantity was increased with mixing. It was added to the quantities of feed allocated weekly for each treatment, and after the mixing was completed, it was filled in sealed bags and marked according to each treatment for the purpose of maintaining the effectiveness of the additives. Table 1 shows the components of the feed used in the experiment and the chemical analysis of the feeds used in the experiment.

Table 1. Shows the components (%) and chemical analysis of the feeds used in the experiment

Feed material (%)	Starter ration (1-14 days)	Growing ration (15-28 days)	Final ration (29-42 days)
Yellow Corn	52	55	57.5
Soybean meal*	41	36	33

Premix**	2.5	2.5	2.5
Sunflower oil	3	4.5	5
Dicalcium Phosphate	1.5	1	1
Limestone	0	1	1
grand total	100	100	100
Calculated Chemical Analysis ***			
Crude protein (%)	23.16	21.5	20.1
Energy represented kilocalories/kg	2983	3095	3168
methionine (%)	0.56	0.58	0.52
Methionine and cysteine (%)	0.94	0.92	0.86
Lysine (%)	1.39	1.40	1.19
Calcium (%)	0.87	0.87	0.8
Phosphorous (%)	0.44	0.49	0.38

*Soybean meal of Argentine origin contains 44% of crude protein and 2230 kcal/kg of energy.

**Ingredients of premix 2.5 AH produced by WAFI International Company, containing protein 29.50%, energy 1817 kcal/kg, lysine 11.70%, methionine 10.40%, methionine + cysteine 10.46%, available phosphorous 12.90%, sodium 5.30%, calcium 6.40 %, with a group of vitamins and minerals.

*** According to the chemical composition according to the analyzes of the feed materials contained in the reports of the US National Research Council N.R.C (1994).

The extract of ginkgo leaves produced from the US Department of Agriculture was obtained and packed in sealed bags weighing 227 gm per bag. It is a powdered powder where 5 bags were purchased through the global Amazon website.

Statistical analysis was conducted using the Complete Randomized Design (CRD) as indicated by Al-Rawi and Abdel Aziz (1980), and to test the significance of differences between the averages of the transactions, Tukey's test was used at the level of significance ($0.05 \geq P$), and the ready-made statistical analysis program SPSS (2011) was used. for data analysis.

Humoral Immunity

Newcastle disease and bronchitis are among the most common viral diseases that affect broilers in Iraq, The enzyme-linked immunosorbent assay (ELISA) was used as reported by Al-Mayah (2009), using a special measuring kit to measure the volumetric criterion of antibodies to bronchitis antibody titer, The TEST-KIT for measuring the volumetric criterion of Newcastle Disease Antibody Titer, and all these standard kits produced by the American company SYNBIOTICS, which consist of:

- 1- Microtiter tray coated with antigens.
- 2- Positive Controller
- 3-Negative control
- 4- Horseradish peroxidase
- 5- Substrate
- 6- Substrate diluent
- 7- Stop solution

Results

Carcass quality:

Table (2) indicates through the results of the statistical analysis that adding different levels of ginkgo leaf extract to the diet leads to a significant effect ($P \leq 0.05$) when measuring the live weight of carcasses before slaughter, where the fourth treatment (T4) with the addition of (3) g / kg feed, which recorded a value of (2932.25) g/fowl compared to the control treatment (T1), which recorded a value of (2637.50) gm/fowl, while no significant effects appeared between (T4) and the rest of the addition treatments.

Table 2. Effect of adding Ginkgo biloba leaf extract to the diet on the dressing ratio and carcass weight (g/fowl) for broilers for the period (1-42) days (mean ± standard error)

Adjectives	Treatment					Morale
	T1	T2	T3	T4	T5	
live weight g/bird	2637.50 ± 89.84 b	2915.00 ± 80.46 ab	2752.50 ± 47.67 ab	2932.25 ± 63.98 a	2821.25 ± 40.33 ab	*
carcass weight g/bird	2420.00 ± 69.88 b	2771.25 ± 82.77 a	2615.00 ± 42.18 Ab	2798.75 ± 61.45 a	2686.50 ± 39.35 ab	**
The weight of the carcass with giblets is g/bird	2189.50 ± 77.54 b	2541.50 ± 81.06 a	2386.25 ± 43.17 Ab	2547.50 ± 57.64 a	2432.00 ± 40.15 ab	**
Carcass weight without giblets, g/fowl	2039.50 ± 51.61 b	2442.00 ± 97.15 a	2268.25 ± 45.03 Ab	2406.50 ± 47.84 a	2305.75 ± 36.77 a	**
dressing with viscera	83.00 ± 0.37 b	87.15 ± 0.62 a	86.69 ± 0.13 A	86.87 ± 0.27 a	86.19 ± 0.26 a	**
giblets without giblets	77.39 ± 0.71 b	83.69 ± 1.11 a	82.40 ± 0.31 A	82.08 ± 0.33 a	81.72 ± 0.13 a	**

*There is a significant effect of the treatments at the level of probability ($P \leq 0.05$) in the analysis of variance table.

**There is a highly significant effect of the coefficients at the probability level ($P \leq 0.01$) in the analysis of variance table.

T1 control (without addition), T2, T3, T4, T5 (1,2,3,4) g/kg of ginkgo leaf extract.

The table indicates that there is a significant effect ($P \leq 0.05$) of the fourth treatment (T4) and the second (T2) on carcass weight, which recorded values of (2771.25, 2798.75) g/bird compared to the control treatment (T1) which recorded a value of (2420.00) g/bird, while there were no significant effects between them and the rest of the addition treatments when measuring the weight of the cleaned carcass, From the table, it is noted that there is a significant effect ($P \leq 0.05$) for the fourth treatment (T4) and the second (T2), which recorded values of (2547.50, 2541.50) g/bird, respectively, compared to the control treatment (T1), which recorded a value of (2189.50) g/ birds, while no significant differences were found between them and the rest of the addition treatments when measuring the weight of the cleaned carcass with the edible entrails, The table indicates that there is a significant effect ($P \leq 0.05$) for the second treatment (T2), the fourth (T4) and the fifth (T5), where the values of (2442.00, 2406.50, 2305.75) g/bird were recorded, respectively, compared to the control treatment (T1), where the value of (2039.50) g/bird, while there were no effects with the addition treatment (T3) when measuring the weight of the cleaned carcass without the edible entrails, and from the table it is noted that there is a significant effect ($P \leq 0.05$) for all the treatments of adding ginkgo leaf extract (T2, T3, T4). , T5), where the values of (87.15, 86.69, 86.87, and 86.19) were

recorded, respectively, compared to the control treatment (T1), where the value of (83.00) g/bird was recorded when measuring the percentage of dressing with edible offal. In addition to the presence of a significant effect ($P \leq 0.05$) for all the treatments of adding ginkgo leaf extract (T2, T3, T4, T5) where the values of (83.69, 82.40, 82.08, 81.72) g/bird were recorded, respectively compared to the control treatment (T1) which The value of (77.39) g/bird was recorded when measuring the percentage of dressing without the edible entrails.

Table (3) shows through the results of the statistical analysis that adding different levels of ginkgo leaf extract to the diet showed that there were no significant effects among all experiment treatments (T1, T2, T3, T4, T5) in the main parts of the carcass (thighs, chest and wings), It is also evident from the results of the statistical analysis that there is a significant effect ($P \leq 0.05$) for all the addition treatments (T2, T3, T4, T5) (1,2,3,4) g/kg of feed, where the values of (5.58,5.40, 5.58, 5.36) respectively compared to the control treatment (T1) which recorded the value of (4.40) when measuring the neck piece, It also appears in the table that there is a significant effect ($P \leq 0.05$) on the level of measurement of the back piece, where the second treatment (T2) with the addition of (1) g/kg, the fourth (T4) with the addition of (3) g/kg, and the fifth (T5) with the addition outweighed (4) g/kg, where the following values were recorded (19.25, 19.20, 19.38) respectively compared to the control treatment (T1) where the following value was recorded (17.82), while no significant differences appeared between them and the third treatment (T3) in measuring the weight of a piece noon.

Table 3. Effect of adding Ginkgo biloba leaf extract to the diet on carcass cuts (gm/fowl) for broilers for a period of (1-42) days (mean \pm standard error)

adjectives	Treatment					Morale
	T1	T2	T3	T4	T5	
Thighs	26.72 \pm 0.80	23.66 \pm 1.85	24.30 \pm 1.85	23.68 \pm 1.81	23.49 \pm 1.80	N.S
chest	37.84 \pm 1.12	38.53 \pm 0.36	37.89 \pm 0.60	39.48 \pm 0.16	38.99 \pm 0.19	N.S
Wings	10.32 \pm 0.81	9.54 \pm 1.21	9.85 \pm 1.10	9.47 \pm 1.07	9.42 \pm 1.17	N.S
Neck	4.40 b \pm 0.16	5.58 a \pm 0.06	5.40 a \pm 0.20	5.58 a \pm 0.02	5.36 a \pm 0.10	**
back	17.82 b \pm 0.41	19.25 a \pm 0.29	18.97 ab \pm 0.31	19.20 a \pm 0.10	19.38 a \pm 0.04	**

*There is a significant effect of the treatments at the level of probability ($P \leq 0.05$) in the analysis of variance table.

**There is a highly significant effect of the coefficients at the probability level ($P \leq 0.01$) in the analysis of variance table.

T1 control (without addition), T2, T3, T4, T5 (1,2,3,4) g/kg of ginkgo leaf extract.

Humoral Immunity:

Table (4), through the results of the statistical analysis of adding different levels of ginkgo leaf extract to the diet, indicates a significant effect ($P \leq 0.05$), where all ginkgo leaf extract treatments outperformed the control treatment in the antibodies directed against infectious bronchitis disease, where the treatment excelled The fifth (T5) on all addition and control treatments of antibodies directed against infectious bronchitis disease recorded the highest value of (9787.25), while the rest of the addition treatments T2, T3,

T4 recorded the following values (5753.25, 6880.75, 7566.75), respectively in the values of antibodies directed against infectious bronchitis disease compared to the control treatment, which recorded the lowest antibody value (1169.00), It appears from the table that there is a significant effect ($P \leq 0.05$), where the second treatment (T2) with the addition of (1) g / kg of feed outperformed all the addition and control treatments, where the value of (7229.50) was recorded, and it is noted that there is a significant effect ($P \leq 0.05$).) for the third treatment (T3) with the addition of (2) g/kg of feed in the antibodies directed against Newcastle disease compared to the two treatments (T4, T5), which had no significant differences between them and the control treatment.

Table 4. The effect of adding Ginkgo biloba leaf extract to the diet on the humoral immunity of broilers for a period of (1-42) days (mean \pm standard error)

treatment	adjectives	
	I.B	N.D
T1	1169.00 c ± 357.00	1155.50 c ± 105.50
T2	5753.25 b ± 503.06	7229.50 a ± 90.70
T3	6880.75 b ± 311.11	5248.00 b ± 272.72
T4	7566.75 b ± 171.38	2918.50 c ± 404.80
T5	9787.25 a ± 514.68	1455.75 c ± 183.77
Morale	**	**

**There is a highly significant effect of the coefficients at the probability level ($P \leq 0.01$) in the analysis of variance table.

T1 control (without addition), T2, T3, T4, T5 (1,2,3,4) g/kg of ginkgo leaf extract.

Discussion

It was found from the results of the productive characteristics that the improvement of broilers when adding ginkgo leaf extract may be due to the active substances found in the leaves of Ginkgo biloba rich in many different compounds that play an important role in regulating growth and metabolism in animals (Wu et al., 2015). Flavonoids, which are one of the most important active substances in ginkgo with antioxidant activity, enhance the secretion of growth hormone (GH) and increase the receptor of hepatic growth hormone, thus increasing the concentration of insulin-like growth hormone-1, which works to regulate and improve growth performance and increase the digestion of nutrients.(Ouyang et al., 2016;Muqier et al., 2017), In addition to its ability to enhance the synthesis of proteins in the muscles, thus promoting growth (Kamboh et al., 2013), and this improvement is related to the active components of Ginkgo biloba because it contains a lot of essential oils that improve digestion as well as improve the balance of the microbial ecosystem in the intestine. And thus stimulating the secretion of digestive enzymes that lead to improving growth performance and increasing the utilization of digested food in domestic birds (Lovkova et al., 2001), Ginkgo biloba also contains polyphenolic compounds that have growth stimulating properties (Graikou et al., 2011), in addition to its antimicrobial activity by breaking down the walls and membranes of these microbes cells (Graikou et al., 2011;Dkhil et al., 2013), as some studies indicated. The addition of ginkgo leaves increased the utilization of nutrients and energy in a manner dependent on the amount of added dose (Zhang et al., 2012), The dried leaves of Ginkgo biloba contain flavonoids, terpenes and polyphenols in addition to sugars and other compounds, and these compounds perform many biological functions such as improving growth performance, digestion of nutrients and antioxidant activities in animals (Van beek and Montoro, 2009), as explained by Srividya et al (2010) Flavonoids are phytochemicals that have antibacterial

activity against a wide range of microbial species, including undesirable bacteria found in the gastrointestinal tract. It reduces the absorption of nutrients. Given that the ginkgo plant contains many vitamins, and this was explained by Sahin et al (2001) that the effect of vitamins on the rate of body weight is through the increase in the appetite of birds and the increase in the rates of digestion, metabolism, absorption and metabolism of nutrients, especially proteins necessary for the health and building of the body. This explains the results obtained, and that the improvement that appears on the percentage of dressing, carcass weight and some carcass pieces when adding ginkgo leaf extract to the main diet for broilers is due to the high average weights of carcasses and their ratio to live body weight, as this shows the positive role of ginkgo leaf extract. In the metabolism process in the cells of the bird's body, growth and construction in the muscular and skeletal structure, and consequently the increase in body weight, which is positively reflected on the percentage of dressing and carcass weight of broilers raised in the experiment. This was explained by Al-Fayyad et al (2012), where they did not notice a direct relationship between body weight and the percentage of dressing on the one hand, and the percentage of carcass cuts on the other hand. As for the humoral immunity, the results obtained indicate that the improvement in the humoral immunity when measuring the volumetric criterion not against the Newcastle virus and infectious bronchitis is due to the active compounds found in the ginkgo leaves such as polyphenols and flavonoids, which are characterized by their immune-stimulating and anti-inflammatory activities and inhibiting the production of cytokines. inflammatory and reduce its receptors (Kemburaj, 2005), Yang et al. (2009) found that flavonoids are natural inhibitors of iNos and are very useful in the treatment of inflammatory diseases, which explains the anti-inflammatory property of ginkgo biloba, and Boligon et al. (2012) showed that flavonoids showed protective effects against the damage caused by hydrogen peroxide. In lymphocytes by reducing oxidative stress due to their antioxidant nature, the flavonoids and terpenoids present in ginkgo leaves are the main compounds responsible for effects on small intestine morphology and immune responses without any adverse effects in broilers (Zhang et al., 2013), Where studies indicated that fermented ginkgo can increase the levels of immunoglobulins (IgM, IgG, IgA) in broilers due to the effect on the growth of pathogenic microorganisms in the gastrointestinal ecosystem due to the active substances contained in ginkgo (Zhang et al., 2013), as Studies conducted on flavonoids indicated that they have functions that differ according to their chemical structure and have anti-tumor and anti-inflammatory effects, thus stimulating the body's immunity, and most importantly, they are considered safe (Galati and O'Brien, 2004), The improvement in the humoral immunity may be due to the ginkgo containing the amino acid methionine, which plays a vital role in improving the health status of broilers by stimulating the humoral immunity, increasing the proliferation of lymphocytes, increasing the activity of the Fabricia gland, and raising the levels of immune globulin, nitric oxide and cytokines (Swain and Johri, 2000)

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