Impact of Adding *Eucalyptus camaldulensis* Leaves Phenolic Extract to Drinking Water on Productive performance, Blood Parameters and Antioxidant Status of broiler Chickens

Duha S. A. Al-Ashoor and Khalid C. K. Al-Salhie

Department of Animal Production, College of Agriculture, University of Basrah, Iraq.

Corresponding author Email: Khalid.chillab@uobasrah.edu.iq

DOI: https://doi.org/10.36077/kjas/2026/v18i2.14721

Received date: 8/1/2024

Accepted date: 22/4/2024

Abstract

The current study aimed to determine the effect of adding different levels of *Eucalyptus camaldulensis* leaves phenolic extract to drinking water on the productive performance, blood parameters and antioxidant status of broiler chickens. A total of 144 unsexed birds, one day old, 42 g weight, were used in current study. The birds were distributed into four experimental treatments, with three replicates (12 birds each replicate). Treatments were T1: control without addition, T2, T3 and T4 were add 50, 75 and 100 mg/liter of eucalyptus leaves phenolic extract to drinking water respectively. Results showed that eucalyptus leaves phenolic extract in the T3 and T4 had greater final body weight and cumulative weight gain, with an improvement in the overall feed conversion ratio compared to other treatments. Red blood cells (RBC), hemoglobin concentration (Hb), packed cell volume (PCV) and lymphocytes percentage were significantly ($P \leq 0.05$) increased in the T4 compared to other treatments. Heterophil percentage and lymphocyte to heterophil ratio were significantly ($P \leq 0.05$) decreased in the T4 compared to the other treatments. The superoxide dismutase and glutathione peroxidase activities were significantly ($P \leq 0.05$) increased in the T3 and T4 compared to other treatments. The catalase activity was significantly ($P \leq 0.05$) increased in the T2 compared to the control treatment. Malondialdehyde (MDA) was significantly ($P \leq 0.05$) decreased in the T4 compared to control treatment. Feed intake, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were unaffected by add different levels of eucalyptus leaves phenolic extract to drinking water. It can be concluded that eucalyptus leaves phenolic extract was improved the productive performance, blood parameters, and antioxidant status of broiler chickens.

Keywords: Antioxidant status, Broilers, Eucalyptus, Productive performance.

Introduction

For thousands of years, nature has provided medicinal agents, 50% of medications have their origins in plants. Since ancient times, medicinal plants have been used. Knowledge regarding the applications of therapeutic plants has been transmitted from one generation to the next (20). Medicinal plants and their extracts were used in the poultry industry, because of its positive impact on health, productive performance, and physiological status (4 and 26).

There are roughly 700 species of eucalyptus trees, which are native to Australia. The eucalyptus tree is strong, grows quickly and is quickly able to adapt to its surroundings. It is also a financially viable tree and its leaves have medicinal value (5). The most significant active ingredients in eucalyptus leaves include 1,8 cineole, p-cymene, α-pinene, terpinen-4-ol, aromadendrene and α-terpineol, which are essential for preventing a variety of diseases (22). Nasr *et al*. (30) concluded that the acetone extract of eucalyptus leaves contains phenolic compounds, while the aqueous extract contains a high amount of tannins, these compounds act as antioxidants that suppress free radicals. It was discovered that *Eucalyptus camaldulensis* essential oil has a strong ability to inhibit free radicals (25). Mohebodini *et al*. (28) indicated that adding eucalyptus leaves oil to broiler diets led to a significant increase in live body weight and an improvement in feed conversion ratio. Li *et al.* (24) found that broiler chickens' diet containing a polyphenolic extract of eucalyptus leaves significantly increased the glutathione peroxidase activity, but had no effect on superoxide dismutase activity or malondialdehyde concentration.

Because the leaves of the eucalyptus tree contain a lot of phenolic compounds therefore, the current study aimed to determine the effect of adding different levels of eucalyptus leaves phenolic extract to drinking water on the productive performance, blood parameters and antioxidant status of broiler chickens.

Materials and Methods

The current study was conducted at the poultry farm, College of Agriculture, University of Basrah from 19/11/2022 to 23/12/2022.

Eucalyptus leaves phenolic extract:

Eucalyptus leaves were collected directly from the trees, then cleaned and dried. The leaves were ground in an electric grinder. Phenolic compounds were extracted according to (33), by taking 20 gm of eucalyptus leaves powder. Add 200 ml of 2% acetic acid solution to it. The samples were placed in a reflux condenser in a water bath at a temperature of 70°C for 15 minutes. Nominated mixture with the filter paper. The upper layer (phenols), containing the phenolic compounds was separated using the separation funnel. Concentrate using a rotary evaporator at a temperature of 50°C until it reaches the lowest

concentration. The extract was dried and stored in dark containers in the refrigerator until use.

Antioxidant activity of eucalyptus leaves phenolic extract:

The antioxidant activity of the phenolic extract was estimated by using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition method according to (40). Butylated hydroxytoluene (BHT) was used for comparison. The BHT concentrations were 50, 75 and 100 mg/50 ml of dimethyl sulfoxide (DMSO).

Birds management:

The birds were reared at a poultry field, equipped with double-floor iron cages. The dimensions of each cage were 90 ×90 ×70 cm. A total of 144 Ross-308 broiler birds, one day old, unsexed, 42 g weight were used. It was distributed into four treatments with three replicates (12 birds each replicate). The temperature at the first week was 33°C, then reduced by two degrees for each week and kept at 25°C until the end of the experiment. The birds were fed on two diets. The first diet contained 23% protein and 2900 kcal per kilogram of metabolizable energy (from 1-15 days of age), then gradually replaced with a grower diet to the end of the experiment, contained 21% protein and 3100 Kcal metabolizable energy per kilogram feed. Water and feed were provided to the birds *ad libitum*.

The experiment included four experimental treatments: T1: control without addition. T2, T3 and T4 were adding 50, 75 and 100 mg/liter of eucalyptus leaves phenolic extract to drinking water, respectively.

Production performance:

Final body weight (g), cumulative weight gain (g), total feed intake (g), overall feed conversion ratio were calculated according to (2).

Blood Parameters:

Blood was collected from shank vein, from three males of each treatment. Blood was placed into tubes containing EDTA anticoagulant. Red blood cells (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), heterophil, lymphocyte percentage, heterophil to lymphocyte ratio (H\L), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were estimated according to (10).

Antioxidant status:

Blood was placed in tubes devoid of anticoagulants. Blood was separated by a centrifuge at 8000 rpm for 15 minutes to obtain serum. The superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) activities were measured by kits according to (17). The concentration of malondialdehyde (MDA) was estimated according to (39).

Statistical analysis:

Data were analyzed by use the statistical analysis program SPSS (37). Using a completely randomized design (CRD). Duncan (14) test was used to estimate the differences between the treatments at a significance level (P≤0.05) according to the following equation:

$$Y\ i\ j = \mu + T\ i + eij$$

Results and discussion

Figure (1) shows the antioxidant activity of eucalyptus leaves phenolic extract ability to scavenge free radicals (DPPH) compared to BHT. The results indicated that there were significant differences (P≤0.05) among the concentrations of eucalyptus leaves phenolic extract and butylated hydroxytoluene. The concentration of 100 mg/50 ml DMSO demonstrated the highest antioxidant activity of the extract, with concentrations of 75 and 50 mg/50 ml DMSO following closely behind. These findings might be explained by the eucalyptus tree's leaves abundance in phenolic compounds, which have antioxidant properties. Consequently, they prevent free radicals from acting because they have hydroxyl groups, which can give a free radical an electron (35). The results of the current study were agreed with (36), who found that eucalyptus leaves oil has the ability to inhibit free radicals with an ability that reached 63.85 and 32.78 micrograms/ml at concentrations of 100 and 50 micrograms/ml. The results were contrary with (8), who obtained a low effectiveness in inhibiting free radicals (18.9 µg/ml) for the aqueous extract of eucalyptus leaves. The findings showed that T2 (75 mg/50 ml DMSO) demonstrated the highest effectiveness of the antioxidant butylated hydroxytoluene, followed by T3 and T1.

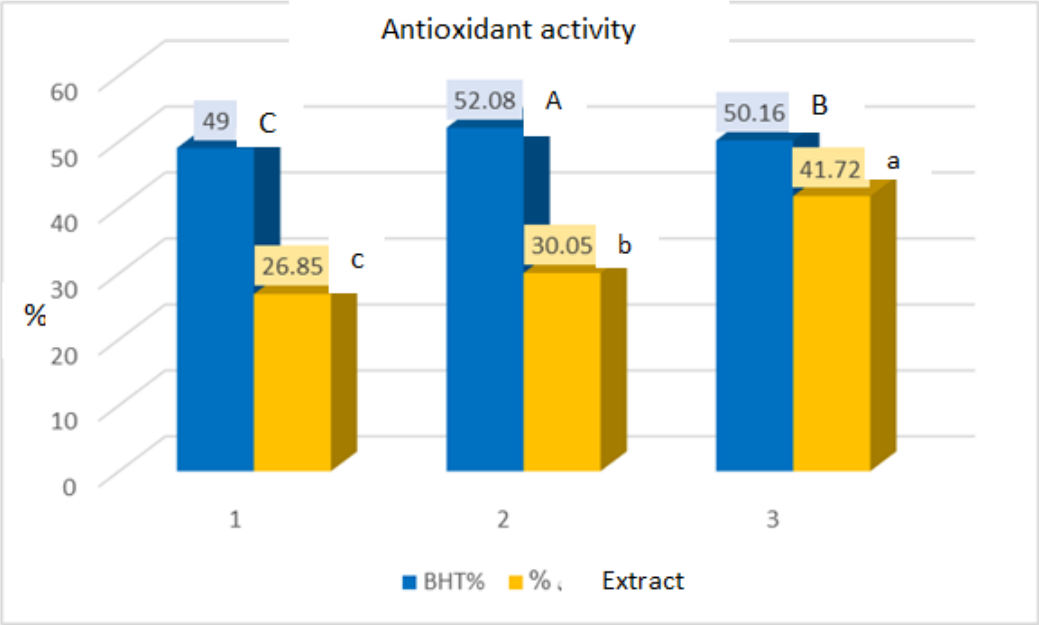

Figure 1. Antioxidant activity eucalyptus leaves phenolic extract and BHT

Butylated hydroxytoluene (BHT).

Phenolic extract of eucalyptus leaves.

1: T1 (50 mg/50 ml DMSO) of BHT and eucalyptus leaves phenolic extract 2: T2 (75 mg/50 ml DMSO) of BHT and eucalyptus leaves phenolic extract 3: T3 (100 mg/50 ml DMSO) of BHT and eucalyptus leaves phenolic extract.

Results of table (1) were indicated the effect of adding different levels of eucalyptus leaves phenolic extract on final body weight, cumulative weight gain, total feed intake and overall feed conversion ratio. The T3 and T4 showed a significant ($P \leq 0.05$) increase in final body weight, cumulative weight gain, and an improvement in the overall feed conversion ratio when compared to the control. The increased broiler chickens productivity may be attributed to the active compounds found in eucalyptus leaves such as 1,8-cineole is the most important of these because it has antioxidant properties (38 and 13). Enhance nutrient absorption, increase of digestive enzymes activity and slow the growth of pathogenic bacteria in the intestines. All these reasons led to improve the health of birds and raising productive performance. The productive performance of broiler was enhanced by the addition of eucalyptus leaves powder and oil. The leaves' active ingredients improved the body's immunity, causing body weight and weight gain to increase dramatically (20 and 15). The results of the study agreed with (29), who found an improvement in feed conversion ratio, weight gain and an increase in body weight when adding eucalyptus leaves powder and oil to the rations of broiler chickens. The results agreed with (3), who noticed that adding aqueous extract of eucalyptus leaves to the drinking water of broiler chickens led to a significant increase in body weight, weight gain, and an improvement in feed conversion ratio. The results were contrary with (6), who indicated that there were no significant differences in live body weight and weight gain when adding aqueous extract of eucalyptus leaves to the drinking water of broiler chickens. The results were contrary with (31), who found that there were no significant differences in body weight, weight gain and feed conversion ratio when adding of eucalyptus leaves oil to the drinking water of broiler chickens. Total feed intake was unaffected by add different levels of eucalyptus leaves phenolic extract to drinking water. Consistent with the findings of (27), who also observed no significant differences in feed intake when broiler chickens were fed eucalyptus leaves powder. The findings disagreed with those of (3), who demonstrated that when broiler chicken drinking water was supplemented with an aqueous

eucalyptus leaves extract, there were significant differences in feed intake between treatments.

Table 1. The effect of adding different levels of eucalyptus leaves phenolic extract to the drinking water on productive performance (mean ± standard error).

Treatment Traits	T1	T2	T3	T4	Sig.
Final body weight(g)	2125.64^{b}±12.64	2108.55^{b}±33.26	2296.88^{a}±21.77	2341.31^{a}±26.73	*
Cumulative weight gain(g)	2083.64^{b}±12.64	2066.55^{b}±33.26	2254.88^{a}±21.77	2299.31^{a}±26.73	*
Total Feed intake(g)	3143.95±19.43	3111.04±30.65	3094.19±19.74	3107.09±33.89	N.S
Overall feed conversion ratio	1.50^{a}±0.017	1.50^{a}±0.028	1.37^{b}±0.018	1.35^{b}±0.014	*

*Horizontally different letters there was a significant difference at a significance level of (P≤0.05). NS: no significant differences. T1: control (without addition), T2, T3 and T4 were add 50, 75 and 100 mg/L of eucalyptus leaves phenolic extract to drinking water, respectively.

Table (2) indicates a statistically significant (P≤0.05) increase in packed cell volume (PCV), red blood cells (RBC) and hemoglobin concentration (Hb) in the T4 when compared to the control treatment. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were unaffected by add different levels of eucalyptus leaves phenolic extract to drinking water. Heterophil percentage and heterophil to lymphocytes ratio were significantly decreased (P≤0.05) in the T4 compared to control. On the other hand, lymphocyte percentage was significantly increased (P≤0.05) in the T4 compared to the control treatment. Eucalyptus leaves contain many mineral elements, the most important of which is iron (23). According to (12) hemoglobin concent rations are correlated with iron percent ages.

One possible explanation for these find ings is that the eucalyptus tree's leaves are rich in phenolic chemicals, which h ave the ability to lower oxidative stress (figure1). One of the most important substances that act as antioxidants is eucalyptol, thus protecting cell membranes from damage (34). The results of the current study were contrary with (7), who noted that there

was no significant difference in RBC and Hb of broilers supplemented with eucalyptus leaves powder. The results were contrary with (27), who indicated that RBC, Hb and PCV were not significantly affected when adding eucalyptus leaves powder to broiler diets. Heterophil to lymphocyte ratio is a biological indicator of stress in birds. Low heterophil to lymphocyte ratio in the blood is evidence of the positive effect of the phenolic extract of the leaves on supplementation treatments. The leaves extract has the ability to suppress free radicals (figure 1). Therefore, the extract acts as an antioxidant and protects cells from damage (18). The results were agreed with (1) who indicated a significant decrease in the heterophil to lymphocyte ratio when adding eucalyptus leaves to the diets of laying hens. The results disagreed with those of (31), who demonstrated that adding and spraying broiler chicken drinking water with eucalyptus leaves oil did not significantly change the number of lymphocyte or heterophil. The results were contrary with (9), who showed that there were no significant differences in heterophil percentage when adding eucalyptus leaves to Japanese quail diets. The results were agreed with (31), who showed that there were no significant differences in MCV and MCHC, when adding eucalyptus leaves oil to the drinking water of broiler chickens. Kaur *et al.* (21) observed that when eucalyptus leaves powder was added to the diets of laying hens, there were no apparent changes in MVC, MCH, or MCHC. Bello (9) demonstrated that feeding Japanese quail eucalyptus leaves resulted in a significant increase in MCV, MCH, and MCHC.

Table 2. The effect of adding different levels of eucalyptus leaves phenolic extract on some blood parameters of broiler chickens (mean ± standard error).

Treatment Traits	T1	T2	T3	T4	Sig.
PCV (%)	28.14^{b}±0.73	27.83^{b}±1.58	28.00^{b}±1.15	31.66^{a}±0.33	*
RBC (10^{6}/ml^{3})	3.24^{b}±0.09	3.20^{b}±0.19	3.23^{b}±0.14	3.68^{a}±0.04	*
Hb (gm/100 ml)	9.38^{b}±0.24	9.27^{b}±0.52	9.33^{b}±0.38	10.55^{a}±0.11	*
MCV (Fi)	86.66±0.18	86.78±0.42	86.71±0.30	85.85±0.06	N.S
MCH (pg/ cell)	28.88±0.06	28.92±0.14	28.90±0.10	28.61±0.02	N.S
MCHC (gm/ L)	33.33±0.00	33.33±0.00	33.33±0.00	33.33±0.00	N.S
Heterophil (H%)	27.69^{a}±2.24	27.29^{a}±1.38	21.67^{ab}±1.21	18.99^{b}±3.51	*
Lymphocytes (L%)	53.33^{c}±1.66	60.66^{b}±1.76	65.33^{ab}±1.20	66.66^{a}±1.20	*
Heterophil Lymphocytes ratio (H/L)	0.52^{a}±0.05	0.44^{ab}±0.01	0.33^{bc}±0.02	0.28^{c}±0.05	*

*Horizontally different letters there was a significant difference at a significance level of (P≤0.05). NS: no significant differences. T1: control (without addition), T2, T3 and T4 were add 50, 75 and 100 mg/L of eucalyptus leaves phenolic extract to drinking water, respectively.

Table (3) indicates a significant increase (P≤0.05) in antioxidant status indicators. The superoxide dismutase and glutathione peroxidase activities were increased in the T2, T3 and T4 compared to the control. Catalase enzyme activity was significantly (P≤0.05) increased in the T2 compared to the control. Malondialdehyde was significantly (P≤0.05) decreased in the T4 compared to control treatment. One potential reason for these results could be the high level of antioxidant acting compounds found in eucalyptus leaves. Antioxidant enzymes are caused by phenolic extracts and subsequently suppress free radicals. Eucalyptus leaves contain the compound eucalyptol (1,8-cineol), which has the ability to suppress free radicals and inhibit the effectiveness of peroxide hydrogen (32 and 11).

The results of the study agreed with (28), who found an increase in the superoxide dismutase activity and a decrease in the concentration of malonidedehyde when adding the essential oil of eucalyptus leaves to the diet of broiler chickens. The results agreed with the findings of (24), who found a significant increase in the glutathione peroxidase activity of broiler chickens when adding the polyphenolic extract of eucalyptus leaves to the feed. The results of the current study were contrary with (16), who showed that there were no significant differences in the concentration of malondialdehyde among treatments when add to Japanese quail diets eucalyptus leaves powder. The results were contrary with (24), who found that superoxide dismutase activity and malondialdehyde concentration were not significantly affected when adding eucalyptus leaves polyphenolic extract to the broiler chickens diets.

Table 3. The effect of adding different levels of eucalyptus leaves phenolic extract to drinking water on antioxidant status of broiler chickens (mean ± standard error).

Treatment Traits	T1	T2	T3	T4	Sig.
Superoxide dismutase(µmol\L)	2.53^{b}±0.33	2.92^{ab}±0.35	3.69^{a}±0.22	3.74^{a}±0.24	*
Clathione peroxidase(µmol\L)	567.68^{c}±16.24	667.99^{b}±53.96	699.25^{b}±21.65	805.21^{a}±11.19	*
Catalase(µmol\L)	3.89^{ab}±0.01	5.10^{a}±0.13	4.10^{ab}±0.81	3.12^{b}±0.48	*
Malonidedehyde(µmol\L)	0.66^{a}±0.07	0.60^{ab}±0.06	0.49^{ab}±0.01	0.45^{b}±0.02	*

*Horizontally different letters there was a significant difference between the coefficients at a significance level of (P≤0.05). T1: control (without addition), T2, T3 and T4 were add 50, 75 and 100 mg/L of eucalyptus leaves phenolic extract to drinking water, respectively.

Conclusion

It can be concluded that eucalyptus leaves phenolic extract has the ability to suppress free radicals. When broiler chickens were given phenolic extract from eucalyptus leaves at a concentration of 100 mg/L in their drinking water, their productivity increased. Blood parameters PCV, RBC, and Hb improved with the use of phenolic extract from eucalyptus leaves. Eucalyptus leaves phenolic extract led to an improvement in the antioxidant status of broiler chickens.

Acknowledgements

The authors are very much grateful to the staff of the poultry farm, College of Agriculture, the University of Basrah for their assistance in the study.

Conflict of interest

The authors declare no conflict of interest.

References

1- **Abd El-Motaal, A.M., Ahmed, A.M.H., Bahakaim, A.S.A. and Fathi, M.M. 2008.** Productive performance and immunocompetence of commercial laying hens given diets supplemented with eucalyptus. International Journal of Poultry Science, 7(5): 445-449. https://doi.org/10.3923/ijps.2008.445.449

2- **Akomah, C., Sogunle, O.M., Adeyemi, O.A. and Bamgbose, A.M. 2021.** Growth performance and haematological characteristics of pullet chickens fed different feed forms supplemented with or without Oyster mushroom (*Pleurotus ostreatus*). Nigerian Journal of Animal Production, 48(5): 203-212. DOI: https://doi.org/10.51791/njap.v48i5.3202

3- **Al Salman, N.T.S. and Al-Gharawi, J.K. 2019.** Effect of eucalyptus leaves water extract on some productive traits of broilers. Plant Archives, 19(1): 920-923.

4- **Al-Ashoor, D.S. and Al-Salhie, K.C. 2020.** Effect of adding broccoli leaves (*Brassica oleracea* L. *var. italica*) extract to drinking water on eggs production and intestinal microflora of Japanese quail *Coturnix japonica* Temmink & Schlegel, 1849. Basrah Journal of Agricultural Sciences, 33(2):42-51. https://doi.org/10.37077/25200860.2020.33.2.04

5- **Alemu, M.M. 2016.** Eucalyptus Tree Production in Wolayita Sodo, Southern Ethiopia. Open Access Library Journal, 3(12): 1-10. http://dx.doi.org/10.4236/oalib.1103280

6- **Al-Fataftah, A.R. and Abdelqader, A. 2013.** Effect of *Salix babylonica*, *Populus nigra*

and *Eucalyptus camaldulensis* extracts in drinking water on performance and heat tolerance of broiler chickens during heat stress. American-Eurasian Journal of Agricultural & Environmental Sciences, 13(10): 1309-1313. DOI: 10.5829/idosi.aejaes.2013.13.10.76143

7- **Ayoob, A., Memon, A., Rajput, N., Arain, M.B., Lanjar, Z., Qureshi, M.H. and Muneir, P. 2023.** Growth Performance, Blood Components, Immune Response, and Carcass Traits in Broiler Chickens Fed with *Eucalyptus globulus*. Iranian Journal of Applied Animal Science, 13(2): 313-319.

8- **Bencheikh, D., Gueddah, A., Soualat, K., Ben-aissi, H., Benslama, A., Harrar, A. and Khennouf, S. 2021.** Polyphenolic contents, antioxidant and antibacterial activities of aqueous extracts of *Eucalyptus globulus* L. and *Trigonella foenum-greacum* L. Journal of Applied Biological Sciences, 15(1): 53-63.

9- **Bello, A. U. 2015.** The effects of blue gum (*Eucalyptus globulus*) leaves as feed additive on hematological traits of Japanese quail. International Journal of Life Sciences Research, *3*, 85-89.

10- **Campbell, T.W. 1995.** Avian hematology and cytology (No. Ed. 2). Iowa State University Press,176-198.

11- **Chograni, H., Riahi, L., Dhahri, S., Ezzine, O., Chakroun, H. and Messaoud, C. 2020.** Interspecific variability of 1, 8-cineole content, phenolics and bioactivity among nine Eucalyptus taxa growing under the sub-humid bioclimate stage. Journal of Complementary and Integrative Medicine, 17(4):20190159. https://doi.org/10.1515/jcim-2019-0159

12- **Dakheel, M.H., Mohammed, W.A., Murad, H.M. and Fadhil, I.A. 2017.** Effect of Ethanolic Extracts leaves of *Eucalyptus camaldulensis* on heamatological parameters of Males wistar rats. International Journal of ChemTech Research, 10(4):448-452.

13- **Di, Y., Cao, A., Zhang, Y., Li, J., Sun, Y., Geng, S. and Zhang, L. 2022.** Effects of Dietary 1, 8-Cineole Supplementation on Growth Performance, Antioxidant Capacity, Immunity, and Intestine Health of Broilers. Animals, 12(18): 2415. https://doi.org/10.3390/ani12182415

14- **Duncan, D.D. 1955.** Multiple range and Multiple F-Test. Biometrics.Vol.11:1 – 42. https://doi.org/10.2307/3001478

15- **Farhadi, D., Karimi, A., Sadeghi, G., Sheikhahmadi, A., Habibian, M., Raei, A. and Sobhani, K. 2017.** Effects of using eucalyptus (*Eucalyptus globulus* L.) leaves powder and its essential oil on growth performance and immune response of broiler chickens. Iranian journal of veterinary research, 18(1): 60.

16- **Fathi, M.M., Al-Homidan, I., Ebeid, T.A., Abou-Emera, O.K. and Mostafa, M.M. 2020.** Dietary supplementation of Eucalyptus leaves enhances eggshell quality and immune response in two varieties of Japanese quails under tropical condition. Poultry science, 99(2): 879-885. https://doi.org/10.1016/j.psj.2019.09.001

17- **Flohe, L. and Günzler, W.A. 1984.** Assays of glutathione peroxidase. In Methods in enzymology Vol. 105, pp: 114-120. https://doi.org/10.1016/S0076-6879(84)05015-1

18- **Ganesan, D., Al-Sayed, E., Albert, A., Paul, E., Singab, A.N.B., Govindan Sadasivam, S. and Saso, L. 2018.** Antioxidant activity of phenolic compounds from extracts of *Eucalyptus globulus* and *Melaleuca styphelioides* and their protective role on D-glucose-induced hyperglycemic stress and oxalate stress in NRK-49Fcells. Natural product research, 32(11): 1274-1280. https://doi.org/10.1080/14786419.2017.1343324

19- **Hassan, M.S.H., El-Sanhoury, M.H., Ali, W.A.H. and Ahmed, A.M.H. 2011.** Effect of using eucalyptus leaves as natural additives on productive, physiological, immunological and histological performance of laying Japanese quail. Egyptian Poultry Science Journal, 31(2): 305-329.

20- **Jamshidi-Kia, F., Lorigooini, Z. and Amini-Khoei, H. 2017.** Medicinal plants: Past history and future perspective. Journal of herbmed pharmacology, 7(1): 1-7. doi: 10.15171/jhp.2018.01

21- **Kaur, M., Kumar, R., Mondal, B. C., Siddiqui, R., Kumar, A., Palod, J. and Kumar, S. 2022.** Effect of supplementation of eucalyptus (*Eucalyptus globulus*) leaves powder in diet containing phytase enzyme on performance of commercial laying hens. Indian Journal of Veterinary Sciences & Biotechnology, 18(1): 28-33.

22- **Kheder, D.A., Al-Habib, O.A., Gilardoni, G. and Vidari, G. 2020.** Components of volatile fractions from *Eucalyptus camaldulensis* leaves from Iraqi–Kurdistan and their potent spasmolytic effects. Molecules, 25(4): 804. https://doi.org/10.3390/molecules25040804

23- **Kusumaningtyas, D.A., Khoirudin, H., Tami, M., Sari, M.U., Nirsatmanto, A., Nugraheni, A.D. and Nugroho, F. 2022.** Eucalyptus Leaves as Potential Indicators of Gold Mine in Indonesia. Jurnal Penelitian Pendidikan IPA, 8(1): 45-50. https://doi.org/10.29303/jppipa.v8i1.1092

24- **Li, W., Zhang, X., He, Z., Chen, Y., Li, Z., Meng, T. and Cao, Y. 2020.** In vitro and in vivo antioxidant activity of eucalyptus leaves polyphenols extract and its effect on chicken meat quality and

cecum microbiota. Food Research International, 136: 109302. https://doi.org/10.1016/j.foodres.2020.109302

25- **Limam, H., Jemaa, M.B., Tammar, S., Ksibi, N., Khammassi, S., Jallouli, S. and Msaada, K. 2020.** Variation in chemical profile of leaves essential oils from thirteen Tunisian Eucalyptus species and evaluation of their antioxidant and antibacterial properties. Industrial Crops and Products, 158: 112964. https://doi.org/10.1016/j.indcrop.2020.112964

26- **Mahjar, N.T. and Al-Salhie, K.C. 2022.** The effects of in ovo injection of Garlic (*Allium sativum* L.) extract on hatchability, liver enzymes and antioxidant status of broiler chickens. Basrah Journal of Agricultural Sciences, 35(1): 61-70. https://doi.org/10.37077/25200860.2022.35.1.05

27- **Mashayekhi, H., Mazhari, M. and Esmaeilipour, O. 2018.** Eucalyptus leaves powder, antibiotic and probiotic addition to broiler diets: effect on growth performance, immune response, blood components and carcass traits. Animal, 12(10):2049-2055. https://doi.org/10.1017/S1751731117003731

28- **Mohebodini, H., Jazi, V., Ashayerizadeh, A., Toghyani, M. and Tellez-Isaias, G. J. P. S. 2021.** Productive parameters, cecal microflora, nutrient digestibility, antioxidant status, and thigh muscle fatty acid profile in broiler chickens fed with *Eucalyptus globulus* essential oil. Poultry Science, 100(3): 100922. https://doi.org/10.1016/j.psj.2020.12.020

29- **Mustafa, M.A. 2019.** Effect of Eucalyptus leaves and its supplementation with diet on broiler performance, microbial and physiological statues to alleviate cold stress. Iraqi Journal of Agricultural Sciences, 50(1):953-963. https://doi.org/10.36103/ijas.v50i1.302

30- **Nasr, A., Saleem Khan, T. and Zhu, G.P. 2019.** Phenolic compounds and antioxidants from *Eucalyptus camaldulensis* as affected by some extraction conditions, a preparative optimization for GC-MS analysis. Preparative Biochemistry & Biotechnology, 49(5): 464-476. https://doi.org/10.1080/10826068.2019.1575860

31- **Petrolli, T.G., Sutille, M. A., Petrolli, O. J., Stefani, L. M., Simionatto, A. T., Tavernari, F. D. C. and Girardini, L. K. 2019.** Eucalyptus oil to mitigate heat stress in broilers. Revista Brasileira de Zootecnia, 48. https://doi.org/10.1590/rbz4820160306

32- **Porres-Martínez, M., González-Burgos, E., Carretero, M.E. and Gómez-Serranillos, M.P. 2016.** In vitro neuroprotective potential of the monoterpenes α-pinene and 1, 8-cineole against H2O2-induced

oxidative stress in PC12 cells. Zeitschrift für Naturforschung C, 71(7-8): 191-199. https://doi.org/10.1515/znc-2014-4135

33- **Riberean-Gayon, P. 1972.** Plant phenolics Oliver and body. U.S.A. 254 pp.

34- **Seol, G. H. and Kim, K. Y. 2016.** Eucalyptol and its role in chronic diseases. Drug discovery from mother nature, 389-398. https://doi.org/10.1007/978-3-319-41342-6_18

35- **Singab, A.N., Ayoub, N., Al-Sayed, E., Martiskainen, O., Sinkkonen, J. and Pihlaja, K. 2011.** Phenolic constituents of *Eucalyptus camaldulensis* Dehnh, with potential antioxidant and cytotoxic activities. Records of Natural Products, *5*(4).

36- **Singh, H.P., Mittal, S., Kaur, S., Batish, D.R. and Kohli, R.K. 2009.** Characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*. Journal of Agricultural and Food Chemistry, 57(15): 6962-6966. https://doi.org/10.1021/jf9012407

37- **SPSS, Statistical Package for the Social Sciences 2018.** Quantitative Data Analysis with IBM SPSS version 24: A Guide for Social Scientists. New York: Routledge. ISBN, 978-0-415-57918-6. https://doi.org/10.4324/9780203180990

38- **Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. 2008.** Use of phytogenic products as feed additives for swine and poultry. Journal of animal science, 86(suppl_14): E140-E148. https://doi.org/10.2527/jas.2007-0459

39- **Yagi, K. 1998.** Simple assay for the level of total lipid peroxides in serum or plasma. Free Radical and Antioxidant Protocols,108: 101-106. https://doi.org/10.1385/0-89603-472-0:101

40- **Yang, B., Wang, J., Zhao, M., Liu, Y., Wang, W.and Jiang, Y. 2006.** Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities. Carbohydrate research, 341(5): 634-638. https://doi.org/10.1016/j.carres.2006.01.004