Effect of adding Ginkgo Biloba leaf powder to the diet of laying hens on microbial and histological characteristics of the intestine (jejunm)

Sudad Ayad Khaleel AL –Zubaidi1*, Ammar Taleb Dhiab Al-Tememy 2
Student at Diyala University - College of Agriculture - Department of Animal Production
2Dept. Animal production – College of Agriculture -University of Diyala –Iraq
*Corresponding author's email: sudad940@gmail.com.
Email addresses of coauthors ammaraltememy@uodiyala.edu.iq.

Abstract

This study was conducted at the poultry farm Department of Animal Production - College of Agriculture – University of Diyala for the period from 1/11/2023 until 22/2/2024, with the aim of finding out the effect of adding Ginkgo Leaf powder to the diet of Laying hens lohmann Brown. 120 laying hens at the age of 18 weeks were used, distributed to four treatments by three replicates for each treatment, on 12 pins by 10 hens per replicate, the first treatment)T1) fed a standard diet without addition (control), the second treatment)T2) A standard diet with 2.5 Kg /ton of ginkgo biloba leaf powder, the third treatment)T3) a standard diet with 5 Kg / ton of ginkgo biloba leaf powder, the fourth treatment)T4) a standard diet was fed with an addition of 7.5 Kg / ton of ginkgo leaf powder and, The results showed an improvement in the microbial balance and the jejunum small intestine environment, the numbers of total bacteria and coliform bacteria were significantly decreased and the numbers of lactic acid bacteria were significantly increased, in the hens fed ginkgo biloba leaf powder compared with the once in the control treatment (no additive). as for the histological characteristics, showed that the third treatment had a highly significant effect ($P \leq 0.01$) on the rest of the treatments in the length, thickness of the villi and the depth of the crypts. Thus, it concluded that adding Ginkgo biloba leaf powder has improved the number of beneficial bacteria and histological characteristics in the jejunum of the small intestine.

Keywords: Ginkgo biloba, microbial and histological count, Lohmann brown .

Introduction

After the use of antibiotics was banned by the World Health Organization, the global trend began to limit the use of antibiotics in the poultry industry. alternatives to antibiotics include organic acids, vitamins, amino acids, medicinal herbs and their extracts [1], Feed additives play a role in ensuring the integrity and development of the intestinal mucosa and thus improving the production of poultry depending on good animal performance [2], such as growth , digestion and absorption of of food, also to maintain effective and sustainable intestinal and digestive system health [3], It also affects immune balance and chronic diseases[4] Strengthening the functions of the immune barrier can increase egg production by enhancing the immunity of the intestinal tissues in laying hens, if the estrogen hormone stimulates the growth and differentiation of the mucous cells of the oviduct and the influx of immunocompetent

ISSN 2072-3857

cells such as T and B Lymphocyte [5],thus Ginkgo Biloba, is known as a medicinal plant that has medicinal therapeutic and antimicrobial properties, including nerve protection, anti- cancer, anti-inflammatory, free radicals , antioxidant and stimulates blood circulation. , [6], Therefore, this study was aimed to determine the effect of adding ginkgo biloba leaf powder to the diet of laying hens on microbial and histological characteristics in the jejunum of the small intestine.

Material and Methods

Thise experiment was conducted poultry farm department of Animal Production - College of Agriculture - University of Diyala for the period from 1/11/2023 to 22/2/2024, for a period of 12 weeks, using 120 laying hens distributed to four treatments with three replicates for each treatment, and 10 hens per replicate. The treatments were as the follow: first treatment (T1) feed without additives, The second treatment (T2) is a feed supplemented with 2.5 kg of ginkgo biloba leaf powder/ton of feed. Third treatment (T3) feed supplemented with 5 kg of ginkgo biloba leaf powder/ton of feed Fourth treatment(T4) feed supplemented with 7.5 kg of ginkgo biloba leaf powder/ton of feed.

Statistical analysis using SPSS Inc.2011.Statistical Package for social Sci version 20 for windows LEAD Technologies. Inc. USA .

Studied traits:

Microbial characteristics

Samples were taken from the intestinal contents of the carcasses from the jejunum a nd placed in sterile, tightly sealed plastic bottles. 1 g of the sample was placed in normal saline solution, and after shaking, decimal dilutions were made by transferring 1 ml from the first bottle to 9 ml of the vial containing normal saline, and so on several times until a dilution of 10-5 is reached to estimate the numbers of the following bacteria:

.1Preparation of total aerobic bacteria

.2Preparation of Coliform Bacteria

.3Preparation of lactic acid bacteria

The numbers of these bacteria were calculated using the spread plating method according to the method recorded by [7]. This was done by using solidified food media: nutriet agar to estimate the total number of bacteria, MacCeonkey agar to estimate the number of algal bacteria, and MRS to estimate the number of lactic acid bacteria. This was done by transferring 0.1 ml of each decimal dilution using a sterile pipette (Micropipet) to each of two Petri dishes prepared in advance from the special culture medium for each one, and spreading them on the surface of the solidified medium using a sterile curved glass rod resembling the letter L, and then incubating the special dishes. With total aerobic bacteria and coliform bacteria, they were incubated upside down at a temperature of 37°C for 24 hours. As for special dishes for medium lactic acid bacteria (MRS Agar), they were incubated upside down and isolated from the air in a glass jar (Anaerobic Jar) at atemperature of 37°C for 48 hours, and the colonies were counted. Development in each of the three media by testing the good agricultural plate resulted in the growth of colonies from each of the two plates for each decimal dilution, and according to the number of Points of Colony Forming Units and multiplied by the reciprocal to obtain the

ISSN 2072-3857

number of bacterial novae per gram of sample, the concentrations of these bacteria were converted into numbers Logarithmic to base (10) and expressed as log 10.

Histological characteristics:

Asample was taken from the jejunum area of the intestine by cutting 2 cm of it, which was the area between the vestigial scar connecting the yolk sac and the duodenum. Then it washed with normal saline solution, and placed in tubes containing 10% formalin to preserve it until the histological sectioning process was carried out and tissue slides were prepared for it. and the measurements were taken.

Results and Discussion

Table 1 showed that adding ginkgo biloba leaf powder was not significantly different from the control in the total number of bacteria. While the number of coliform bacteria was significantly decreased (P≤0.01) in the ginkgo biloba leaf powder treatments compare to the control. However, the number of lactic acid bacteria was linearly increased (223, 235, and 243.83 logs cfu/gm) for T2, T3, and T4 respectively by increasing the level of ginkgo biloba leaf powder compare to the control

Table 1. The effect of adding different levels of ginkgo biloba leaf powder to the diet on the numbers of total bacteria, coliform bacteria, and lactic acid bacteria inside the jejunum of laying hens (average ±SE.(

Treatment	Total bacteria(cfu/gm 10^5)	coliform bacteria(cfu/gm10^5 **)**	Lactic acid bacteria(cfu/gm 10^5)
T1	383.50± 5.97	199.33±[a] 5.88	180.67[b] ± 6.46
T2	370.33± 8.35	135.33±[b] 4.89	223.00±[a] 9.02
T3	377.50± 9.98	139.67±[b] 6.03	235.00±[a] 7.19
T4	375.50± 8.38	128.00±[b] 4.00	243.83[a] ± 9.22
Significance level in the analysis of variance table	0.73	0.01E-6	0.01E-2

ISSN 2072-3857

Different letters within one Column indicate the presence of significant differences between the means at a level level of P≤0.05 according to Duncan's multiple range test.

Ginkgo leaf extract contains 6% terpenoids (3.1% are ginkgolides A, B, and C, although 2.9% is biloba), and 24% flavonoid glycosides (containing quercetin, kaempferol, isor hamnetin, etc.) and 5-10% organic acids [[8 . As for flavonoids and terpenoids, they are the pharmacologically active components9] . [ginkgo have flavonoids that contain antioxidants and scavenge free radicals while ginkgolides protect the central nervous system, traumatic brain injury, cerebrovascular disease[10 .[
the medicinal activity of ginkgo is derived from flavonoids and ginkgolides [11] The flavonoids present in Ginkgo biloba affect the intestinal flora in broiler chickens [12], reduce colon bacteria and increase the Bifidobacteria and Lactobacillus communities in the cecum, thus regulating the intestinal environment [13]. in addition flavonoids inhibited the growth of

Staphylococcus aureus and Escherichia coli, and promoted the growth of Bifidobacteria to maintain intestinal health in the cecum of broiler chickens [14], as well as it alleviating metabolic disorders by reducing The fat level [15], and the intestinal flora and internal environment of the jejunm improved as shown in the results we obtained in Table (1,2), thus producing a number of digestive enzymes and vitamins and improving the digestion and absorption process and the readiness of nutrients, especially calcium and phosphorus. It has been proven that the sugars found in ginkgo are soluble in water, enhancing intestinal function and improving the productive and immune performance of bird.[16]This is shown in Table1.

Table 2 showeds that there wasasignificant superiority in the 3rd treatment compared to the control, 2nd and 4rth treatments in the length of the villi, Thickness of the villi

adding Ginkgo Biloba increased the thickness of the villi compared to the control treatment,and, the third treatment was superior to the rest of the treatments in the depth of the crypts

ISSN 2072-3857

Table 2. The effect of adding different levels of ginkgo biloba leaf powder on the length of the villi, the thickness of the villi, and the depth of the crypts within the intestinal jejunum (micrometers) (mean ± SE.(

Treatments	Length of villi	Thickness of villi	depth of the crypts
T1	176.70^{c} ± 5.57	41.83^{b} ± 1.61	$188.13\pm^{d}$ 16.61
T2	329.35^{b} ± 9.22	76.52^{a} ± 5.32	$331.95\pm^{b}$ 9.77
T3	430.35^{a} ± 20.54	$87.94\pm^{a}$ 5.21	$372.58\pm^{a}$ 10.23
T4	7.82 ± 321.54^{b}	$89.83\pm^{a}$ 9.51	274.91^{c} ± 6.78
Significance level in the analysis of variance table	0.04E-4	0.02E-1	0.02E-3

Different letters within one column indicate the presence of significant differences between the means at a level level of P≤0.05 according to Duncan's multiple range test

Show the researcher results [17] a significant effect of adding ginkgo leaf powder to the diet of laying hens on the length of the villi, the thickness of the villi, and the depth of the crypts within the intestinal jejunum, indicates shape as a healthy intestinal better growth performance, effective absorption of nutrients, and a strong defense against disease-causing bacteria. The reason for the increase in the length and thickness of the villi and the depth of the crypts may be due to the phenolic compounds that enter the colon unchanged and interact with the colon bacteria. polyphenols play vital roles in modifying the intestinal microbiota and not only change the bacterial composition of the intestine, but also They also improve the bioavailability of polyphenols by metabolizing them into absorbable metabolites. Altered microbial composition and bacteria-derived polyphenol metabolites influence intestinal development and can improve the health and productivity of chickens The health-promoting properties of these phenolic compounds was due to their effect on the gut microbiome, and the interactions between the gut microbiota and polyphenols are a two-way process in which the gut microbes convert the polyphenols into their active ingredient metabolites, leading to improved bioavailability and health effects, while the polyphenols and their metabolites are derived from Gut microorganisms can support the growth of beneficial bacteria and inhibit pathogens [18.[

The results of this study[19] indicate that phenolic compounds derived from Ginkgo biloba sarcotestas, because of their strong inhibitory characteristics towards food

ISSN 2072-3857

pathogens, can be considered ideal candidates for possible application in food microbiology due to their natural origins

Conclusion

Adding ginkgo biloba leaf powder played an important role in improving microbial characteristics during the production period 18- 29 weeks for laying hens, as it increases the number of beneficial

.2 bacteria and inhibits or minimize harmful bacteria, which leads to the development of the digestive tract and improves the process of digestion and absorption, and this reflects positively on enhancing the productive performance and egg quality characteristics of laying hens .

.2The addition of ginkgo biloba leaf powder was important for improving the histological characteristics of the intestine through the improvement that achieved in the length and thickness of the villi and the depth of the crypts for the treated treatments compared to the control .

.3Adding ginkgo biloba leaf powder at a level of 5 kg/ton gave the best results for textural characteristics.

Acknowledgment: To the professors of the Animal ProductionDepartment/College ofAgriculture /Universitykl of Diyala their instructions and supports are really appreciated.

References

[11]Boateng, I. D., and Yang, X. M. 2022. Ginkgo biloba L. seed; A comprehensive review of bioactives, toxicants, and processing effects. Industrial Crops and Products, 176, 114281.

[17]Carraturo, A., Raieta, K., Kim, J., and Russo, G. L. (2013). Antibacterial activity of phenolic compounds derived from Ginkgo biloba sarcotestas against food-borne pathogens. British microbiology research journal, 4(1), 18-27.

[19] Chee, S.H.; Iji, P.; Choct, M.; Mikkelsen, L.L.; Kocher. 2010. A. Characterisation and response of intestinal microflora and mucins to manno-oligosaccharide and antibiotic supplementation in broiler chickens. Br. Poult. Sci. , 51, 368–380.

[13]Dong, Y., Lei, J., and Zhang, B. 2020. Effects of dietary quercetin on the antioxidative status and cecal microbiota in broiler chickens fed with oxidized oil. Poultry science, 99(10), 4892-4903.

[10] Dzah, C. S., Duan, Y., Zhang, H., Boateng, N. A. S., and Ma, H. 2020. Latest developments in polyphenol recovery and purification from plant by-products: A review. Trends in Food Science & Technology, 99, 375-388.

[4] Gao, J., Xu, K., Liu, H., Liu, G., Bai, M., Peng, C., Li.,T. & Yin, Y. 2018. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Frontiers in cellular and infection microbiology, 8, 13 .

[15] Hirata, B. K., Pedroso, A. P., Machado, M. M., Neto, N. I., Perestrelo, B. O., De Sá, R. D.,

Vale.MI.,Nogueira,F.N.,Oyama,L.M.,Ribeiro, E.B.,Tashima,A.k and Telles, M. M. 2019. Ginkgo biloba extract modulates the retroperitoneal fat depot proteome and reduces oxidative stress in diet-induced obese rats. Frontiers in Pharmacology, 10, 686.

[16] Hossain M.E, Kim G.M, Lee S.K, Yang CJ .2012. Growth performance, meat yield, oxidative stability, and fatty acid composition

of meat from broilers fed diets supplemented with a medicinal plant and probiotics. Asian-Austr J Anim Sci 25: 1159-1168.

[18] Iqbal, Y., Cottrell, J. J., Suleria, H. A., and Dunshea, F. R. 2020. Gut microbiota-polyphenol interactions in chicken: A review. Animals, 10(8), 1391 .

[2]Lemos, M.J.D., Calixto, L.F.L., Torres-Cordido, K.A.A. and Reis, T.L. 2016. so de aditivo alimentar equilibrador da flora intestinal em aves de corte de postura. Arq. Inst. Biol. 83

[1]Li L., Liu Z., Fang B., Xu J., Dong X., Yang L., Zhang Z, Guo S,and Ding B .2022. Effects of vitamin a and K-3 on immune function and intestinal antioxidant capacity of aged laying hens. Braz. J. Poult. Sci. 24:eRBCA-2021-1572.

[3] Miao, S., Zhou, W., Li, H., Zhu, M., Dong, X., and Zou, X. 2021. Effects of coated sodium butyrate on production performance, egg quality, serum biochemistry, digestive enzyme activity, and intestinal health of laying hens. Italian Journal of Animal Science, 20(1), 1452-1461.

[5]Nii, T. 2022. Relationship between mucosal barrier function of the oviduct and intestine in the productivity of laying hens. The Journal of Poultry Science, 59(2), 105-113.

[6] Ražná, K., Sawinska, Z., Ivanišová, E., Vukovic, N., Terentjeva, M., Stričík, M., Łukasz, K., Hlavačková, L., Rovná, Kand Kačániová, M. 2020. Properties of Ginkgo biloba L.: Antioxidant characterization, antimicrobial activities, and genomic microRNA basedmarkerfingerprints. International journal of molecular sciences, 21(9), 3087.

[7] Samanta, S, Haldar S, Ghosh TK. 2010. Comparative efficacy of an organicacid blend and bacitracin methylene disalicylate as growth promoters in broiler chickens: effects on performance, gut histology, and small intestinal milieu. Vet Med Int:645–650.

[9]Singh, S. K., Srivastav, S., Castellani, R. J., Plascencia-Villa, G., and Perry, G. 2019. Neuroprotective and antioxidant effect of Ginkgo biloba extract against AD and other neurologicaldisorders. Neurotherapeutics, 16(3), 666-674.

[12]Sun, L., Guo, L., Xu, G., Li, Z., Appiah, M. O., Yang, L., and Lu, W. 2022. Quercetin reduces inflammation and protects gut microbiota in broilers. Molecules, 27(10), 3269.

[8] Tomino, C., Ilari, S., Solfrizzi, V., Malafoglia, V., Zilio, G., Russo, P., Proietti, Marcolongo,F., Scapagnini,G., Muscoli,C. and Rossini, P. M. 2021. Mild cognitive impairment and mild dementia: the role of Ginkgo biloba (EGb761®). Pharmaceuticals,14(4),30.

[14]Wang, S., Yao, J., Zhou, B., Yang, J., Chaudry, M. T., Wang, M., Xiao,F., and Yin, W. 2018. Bacteriostatic effect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. Journal of Food Protection, 81(1), 68-78.

ISSN 2072-3857