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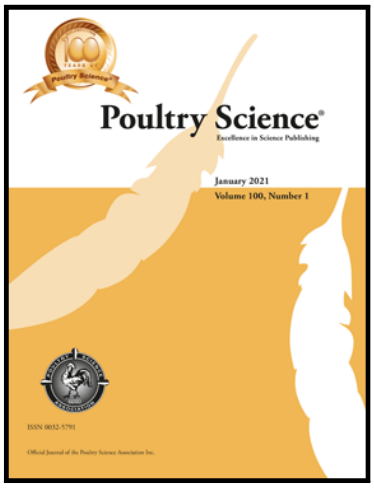

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Short title: *EFFECTS OF ECHINACEA EXTRACT ON BROILERS*

The effect of using Echinacea extract as an immune system stimulant and antioxidant on blood indicators, growth efficiency, and carcass characteristics in broiler chickens to produce a healthy product

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Abstract

This study aimed to discover how echinacea powder extract (EPE), an antioxidant, affects the growth rate, body composition, and blood parameters in broilers as an alternative to antibiotics. In a completely randomized design study, four experimental groups received 280 broiler chicks, 5 days old (Cobb 500). Each group was distributed into seven replicates, each containing ten unsexed chicks. Four groups were randomly selected from the chicks; the first group (the control group) was given drinking water only. Conversely, the birds in treatments 2, 3, and 4 were supplemented with EPA in their drinking water. They received 1.0, 2.0, and 3.0 cm^3 EPA/liter water every 3 days weekly until the end of the trial (30 days). Our findings found significant differences in avian mass between experimental treatments during growth phases. The control group weighed 887 g, while the 3.0 cm^3 EPE group weighed 850 g. The Duncan test showed a difference in FI and FCR among those favoring the EPE groups. Our results showed no significant changes in carcass features, except for spleen, gizzard, and abdominal fat, with the 3.0 cm^3 EPE group having weight-higher organs and lower abdominal fat. Also, the control had higher TP, albumin, and globulin levels, while the EPE diet increased ALT and AST and decreased TC, TG, LDL, and VLDL levels. Furthermore, EPE-containing diets resulted in higher IgG concentrations, lower MDA levels, and higher SOD activity compared to the control group. According to the ANOVA analysis, the water consumption rate did not significantly differ across the trial groups. It included adding EPE with 3.0 cm^3 enhanced growth performance, carcass traits, and antioxidant activity to obtain health outcomes for end users.

Keywords: Echinacea extract, broilers, growth, carcass, blood.

INTRODUCTION

Poultry is a popular global meat that is widely consumed. Its production in the European Union has increased worldwide, second only to pig (Thorp, 2021). Dietary additives are essential in the chicken sector, a significant source of animal protein. About 60–70% of all costs of producing chickens are connected to feeding (Abd El-Hack et al., 2024a; Mohamed et al., 2024). Several studies have demonstrated that using natural nutritional additives instead of artificial growth stimulants and antibiotics improves growth effectiveness, immunity, and carcass quality in birds and farm livestock (Kamal et al., 2023a, b; Abd El-Hack et al., 2024b; El-Ratel et al., 2024). The growing global population has increased chicken consumption, necessitating quality and safety assurance. The use of traditional antibiotics in livestock farming, particularly poultry, has developed resistance to antimicrobial drugs, a major public health issue (Abd El-Hack et al., 2023a, b; Abreu et al., 2023).

Herbal plant-based natural feed additives can enhance chicken growth, feed effectiveness, nutritional assimilation, and antioxidant capacity. Their consumption has increased in recent years due to their antioxidative properties, ability to lower cholesterol levels, and antibacterial activity (Li et al., 2018; Yong et al., 2020; Elmahallawy et al., 2021). Components derived from medicinal plants have been scientifically demonstrated to possess anti-parasitic (Gohel et al., 2019), antiviral (Alagbe et al., 2018), and anti-mycotic (Abed et al., 2021) properties.

Echinacea purpurea, also known as the purple coneflower, is an herbal remedy with immune system benefits (Weishaupt et al., 2020). Its leaves have higher concentrations of elements like Cu, Zn, Ni, and Mg than its flowers (Burlou-Nagy et al., 2022). Furthermore, it is noteworthy that research conducted on the effects of administering *Echinacea purpurea* to humans has shown that even at high dosages, the substance is either minimally toxic or non-toxic, even when supplied intravenously (Stanisavljević et al., 2009). A wide range of chemical substances, including volatile chemicals, alkaloids, polysaccharides, polyphenols, alkylamides, derivatives of caffeine, and several other structures, have been extracted from the roots or aerial sections of the plant (Yu et al., 2013; Erenler et al., 2015).

The popular medicinal herb *Echinacea purpurea* is recognized for its immunostimulatory and anti-inflammatory effects, which stimulate the immune response by producing T cells and phagocytosing them (Saeed et al., 2018). *Echinacea purpurea* (EP) possesses both antibacterial (Chiellini et al., 2017) and immunomodulatory properties (Seckin et al., 2018). Furthermore, Rady et al. (2023) found that adding *Echinacea purpurea* to the diets of broilers improves their growth, gene expression, immune system health, antioxidant status, and the structure of their intestines. Echinacea supplementation in broilers may increase the feed conversion ratio, alter blood cell counts, improve immune response, and modify total and differential leukocyte counts, as well as antibody titers against avian influenza and Newcastle disease (Dehkordi and Fallah, 2011).

Thus, this study investigated the effects of EPE as an antioxidant and antibiotic substitute in the broiler feed on growth efficiency, carcass features, and blood indicators to produce healthier products.

MATERIALS AND METHODS

The study was conducted at the Poultry Research Farm, part of the Department of Poultry in the Faculty of Agriculture at Zagazig University in Egypt. The experimental techniques were conducted according to the guidelines set by the Institutional Animal Care and Use Committee.

The Ethics of the Institutional Committee of the Zagazig University, Zagazig, Egypt, authorized them.

Prepare the echinacea solution

The Echinacea was procured from a commercial enterprise known as 2 M Group, located in the 10th of Ramadan area in Sharqia, Egypt. We soaked 250 g of smashed ripe Echinacea in a liter of extraction solvent (Water - Ethanol) to prepare aqueous Echinacea solutions. We left the solutions at room temperature for at least 24 hours before filtering them. We utilized filter sheets labeled Whatman No. 42 (Hawach Scientific Co., Ltd., Xi'an, China). The administration of EPE in the drinking water commenced when the birds were 5 days old and persisted until they attained 35 days of age. During this period, we incorporated the solution into their water consumption.

Birds, diets, and design

Using a completely randomized design, we distributed 280 5-day-old chicks (Cobb 500) with similar initial body weights into four experimental groups. The original groupings yielded seven replicate groups, each containing 10 unsexed chicks. We randomly allocated the chicks to four treatments. We provided the first group with water to drink without additional substances, acting as the control group. Conversely, we added Echinacea supplements to the drinking water of groups 2, 3, and 4. The amount of Echinacea solution added to each liter of water was 1.0, 2.0, and 3.0 cm^3, respectively. This supplementation was administered every 3 days every week until the completion of the 35-day trial.

The chicks were raised at a house temperature of 29°C and a relative humidity of 70% until the experiment's conclusion, which occurred at the age of 35 days. Initially, the chicks were housed in an open house at a temperature of 33°C for three days. Following their arrival, the chicks underwent a continuous illumination program, which included a lighting schedule of 23 hours of light and 1 hour of darkness. The baseline diets were formulated to meet the nutritional requirements established by the NRC (1994). The chicks were fed pellet-form dieters from 5 to 35 days of age, as shown in Table 1. Two separate phases were used to administer the diets: the first stage (starter phase) ran from day 1 to day 21, and the second stage (finisher phase) ran from day 22 to day 35. Every chick was grown under the same environmental, managerial, and sanitary conditions. The birds were housed in conventional 1.0×1.0×50 cm^3 cages with free-flowing food and water.

Data collection

The daily water intake was measured for each experimental group, from 5 to 35 days of age, several times during the trial. The water-to-feed ratio was estimated throughout the various periods analyzed. The chicks were weighed individually at 5, 21, and 35 days of age to determine their live body weight (LBW). Additionally, their daily body weight gain (DBWG) was calculated for 5-21 days, 22-35 days, and 5-35 days of age. The feed conversion ratio (FCR) and average daily feed intake (FI) were calculated.

Carcass characteristics

Following the trial's end, we randomly selected seven birds from each group to perform carcass examinations when they reached 35 days of age. The birds were carefully weighed and slaughtered. The weight of the carcass and the total weight of all edible components were measured.

Blood biochemical parameters

Following a period of not eating for an entire night, we slaughtered seven birds from each group to collect blood samples. The blood was gathered into non-heparinized tubes and centrifuged the sample at 5000 revolutions per minute for 15 minutes at 4ºC. The resultant serum was preserved at a temperature of -20ºC until it was prepared for biochemical examination. Samples of serum were used to identify serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, as well as the measurement of total protein (TP), albumin, total cholesterol (TC), triglycerides (TG), LDL, and HDL levels. Additionally, measure levels of uric acid, creatinine, and immune response markers such as IgG and IgM using commercially available kits and following the manufacturer's instructions. The levels of malondialdehyde (MDA) and superoxide dismutase (SOD), the indices of oxidative status, were quantified using the methodologies outlined in our previous research (Abd El-Hack et al., 2017). PPE was utilized appropriately during the process of collecting and organizing the samples.

Statistical analysis

The data underwent ANOVA using a completely randomized design, employing the one-way ANOVA techniques of SPSS. The disparities among the averages were assessed utilizing the post-hoc Duncan test. The statistical value was evaluated by applying a threshold of $P<0.05$. Afterwards, we employed the subsequent statistical framework:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} = observed value of the concerned treatment, μ = observed mean for the concerned treatment, T_i treatment effect, and e_{ij} = the error related to individual observation.

RESULTS

Growth performance

Table 2 illustrates the impact of incorporating EPE into the diet on LBW. The five-day-old chicks' initial BW was evenly distributed among all treatment groups, with a mean of 82 ± 0.06 g. This implies that the trial groups received the birds randomly. The ANOVA indicated substantial differences among the experimental treatments during the early stages of development. When the birds were weighed on the 21st day of age, there were significant differences ($P \leq 0.05$) across the treatments. The control had an average bird weight of 887 g, whereas the 3 cm^3 treatment with EPE had an average weight of 850 g. On the 35th day, the ANOVA observed no statistically significant differences among the trial treatments. However, the Duncan test did demonstrate significant variations across the groups. The birds' mean weight was 1883 g for the 3 cm^3 EPE than 1840 g for the control.

Table 3 presents the impact of EPE feed addition on BWG. The ANOVA results showed no statistically notable variations between the groups during the various trial periods. Additionally, Table 4 demonstrates the impact of EPE on FI in the diet. The ANOVA exhibited no statistically significant variations between the test groups in the trial. Nevertheless, the Duncan test did exhibit differences among the groups. The control group of birds had an average FI of 98 g, while the treatment groups that got 3 cm^3 EPE on the 35th day had an average FI of 93 g.

Table 5 displays the effects of incorporating EPE into the diet on FCR. The ANOVA showed no statistically substantial alterations in FCR between all groups for the whole trial duration. However, the Duncan test did demonstrate variations among the groups. The EPE groups demonstrated a superior FCR compared to the control group.

Carcass traits

Table 6 displays data regarding the influence of EPE on the weights of different components in chickens, encompassing carcass, giblets, liver, heart, spleen, gizzard, and abdominal fat. Furthermore, it offers specific data on the hens' physical traits, including their percentage of carcass weight. The ANOVA analysis revealed no significant changes in carcass features across the groups, save for the percentage of spleen, gizzard, and abdominal fat. The group with a volume of 3.0 cm^3 of EPE had a greater proportion of these organs than the other groups. In addition, the group that received 3.0 cm^3 of EPE had a lower percentage of abdominal fat than the control group.

Biological indicators of blood

Table 7 provides a concise overview of the effects of EPE in the diet of broiler chickens on blood parameters. The effects were evaluated through the analysis of the blood. The ANOVA analysis demonstrated significant differences across the TP, albumin, and globulin treatments. The control exhibited higher TP, albumin, and globulin levels than the other treatments. In birds, the EPE diet raised ALT and AST levels. In addition, including 1.0, 2.0, and 3.0 cm^3 of EPE in the basil diet resulted in lower levels of TC, TG, LDL, and VLDL than the control treatment.

Immunological and antioxidant characteristics

A summary of the effects of including EPE in the feeding of broiler chicks is shown in Table 8 on their immune responses and the evaluation of antioxidants. The examination of statistics demonstrated substantial variation among the treatments for IgG, MDA, and SOD. The diet, including EPE, led to elevated levels of IgG, with concentrations of 482 and 408 ng/ml for 1.0 and 3.0 cm^3 EPE, respectively. In addition, the level of MDA was greater in the control group than in the other groups. Furthermore, SOD activity was higher in diets containing 3.0 cm^3 of EPE than in the other groups.

Water consumption and feed ratio

Table 9 illustrates the effects of including EPE in broiler chicks' diets on their water intake and water feed ratio. The ANOVA analysis revealed no significant variations in water consumption rate between the experimental treatments throughout the trial. Nevertheless, the Duncan test did exhibit differences among the groups. The birds in the experimental group had a higher water consumption rate than the other groups.

DISCUSSION

Our study's results revealed significant variations in avian mass among the different experimental treatments throughout the growth phases. The control group weighed an average of 887 g, whereas the group treated with 3.0 cm^3 with EPE weighed 850 g. In addition, our findings indicated that there were no noteworthy disparities between the groups in terms of BWG during the trial periods. However, the Duncan test revealed distinctions, as the control group exhibited an average FI of 98 g, while the treatment groups had an average FI of 93 g. Also, the Duncan test revealed significant disparities across the EPE groups, with the EPE group demonstrating a higher level of FCR than the control group.

According to our study, Nosrati et al. (2017) suggested that incorporating Echinacea into the drinking water of broiler chicks can enhance their efficacy as growth enhancers. Additionally, Seifi et al. (2018), adding EPE at a concentration of 0.5 mL L-1 to quails' drinking water improved their FCR. Also, Roth-Maier et al. (2005) discovered that adding Echinacea cob, which contains similar chemicals as caffeic acid derivatives, reduced body weight. Hassan et al. (2004) observed that adding herbal supplements to the diet enhanced FCR. Growth-promoting feed additives improve livestock health by controlling gut pathogens and regulating feed hygiene. Herbal derivatives, like photobiotic, stimulate digestive enzymes and intestinal mucous, thereby stabilizing microbial balance (Wang et al., 2024).

Shen et al. (2020) say that 200 mg of EPE can improve the growth and quality of broiler meat by increasing redness, lowering feed-to-gain ratios, and increasing average daily gain and evisceration yield rates. Rady et al. (2023) also found that supplementing broiler meals with

1.0% *Echinacea purpurea* positively impacted broiler chicken growth efficiency and gene expression. Furthermore, it appeared to improve the birds' gastrointestinal histomorphology. Additionally, Hashem et al. (2020) and Bagno et al. (2021) discovered that including *Echinacea purpurea* in the meal of broilers resulted in enhanced BWG. In contrast, Lee et al. (2012) and Nosrati et al. (2017) discovered that adding Echinacea did not influence growth performance measures. The improved growth performance associated with Echinacea powder extract may be due to herbal derivatives, such as probiotics, which activate digestive enzymes and intestinal mucus, thus stabilizing microbial equilibrium. Moreover, it seems to enhance the shape of the avian digestive system.

Our findings showed no significant changes in carcass features, except for spleen, gizzard, and abdominal fat, with the 3.0 cm^3 EPE group having a higher percentage of organs and lower abdominal fat. The data on carcass attributes corroborated the results obtained by Pourasghar et al. (2021) and Rady et al. (2023), who found that EPE had no impact on the percentage of carcass consumption or the weight distribution of certain internal parts. Shen et al. (2020) discovered that adding EPE had a beneficial impact on the performance of slaughtered animals. On the other hand, Shen et al. (2020) discovered that whereas 100 mg of *E. purpurea* had negligible positive impacts, 200 mg dramatically enhanced broiler slaughter performance. 600 mg reduced both the half and destruction percentages. According to Awad et al. (2020), adding 2.5 g/kg of dietary EP can maximize and enhance the growth, carcass characteristics, and economic effectiveness of Sudani ducklings, particularly males, during the growing period in Egyptian summer settings. According to Atay (2023), using medicinal and aromatic plant extract powders in broiler chicken diets improves FI, BWG, and FCR. These plants also significantly impact carcass yield, thigh, wings, heart, spleen, bursa Fabricius, breast, and thigh colors, positively affecting performance and carcass parameters.

According to Przybylski et al. (2016), EPE could shield cells in the intestines, improve alkaline phosphatase action, enhance antioxidant activity, improve protein metabolism, and improve the conversion and absorption of dietary fiber. These factors may also improve broiler slaughter efficiency (Lee et al., 2013). To grasp the mechanism precisely, more research is necessary.

Our findings showed that the EPE diet had lower TP, albumin, and globulin levels and increased ALT, AST, and decreased TC, TG, LDL, and VLDL levels. In partially with our study, Rady et al. (2023) discovered that adding *Echinacea purpurea* to the meals of broilers led to a rise in TP. This outcome aligns with the discoveries made by Gilani et al. (2018), who observed a numerical rise in TP using probiotics. The lipid profile data we obtained were corroborated by the studies conducted by Rahimi et al. (2011) and Nosrati et al. (2017). These studies found that adding *Echinacea purpurea* led to an important rise in serum levels of HDL and a reduction in cholesterol and LDL. A possible cause of this could be the anti-atherosclerotic characteristics exhibited by probiotics, according to Sharifi et al. (2013). Bouchama et al. (2024) discovered that adding medicinal plant extract powder to chick feed significantly improved their growth performance, including weight gain, ADG, FCR, blood serum parameters, and inhibition of coliform proliferation. It enhanced hepatic function, intestinal microbiota, and growth metrics. Nazari et al. (2024) assert that incorporating *Echinacea purpurea* and *Thymbra spicata* into the diet of broiler hens at a concentration of 0.25% can enhance growth performance.

Additionally, giving *Echinacea purpurea* and *Thymbra spicata* as supplements can improve the structure and function of the intestines and the immune system in broilers infected with *C. jejuni*. This includes raising the levels of antibodies against NDV. Medicinal plants suppress cholesterol resynthesis by targeting 3-hydroxy-3-methylglutaryl-coenzyme A, a critical enzyme in cholesterol synthesis regulation (Eilam et al., 2022). This illustrates how EPE addition reduces cholesterol and triglycerides.

Additionally, our study found significant variation in IgG, MDA, and SOD treatments among diets. EPE-containing diets resulted in higher IgG concentrations, lower MDA levels, and higher SOD activity compared to control. Lee et al. (2012) discovered incorporating *Echinacea purpurea* led to an elevation of SOD levels, as evidenced by the enzymatic antioxidant activity results. Rady et al. (2023) reported a marked reduction in MDA levels in the groups that received echinacea and nucleotide supplementation. MDA is a result of the peroxidation of PUFA in cells. A rise in free radicals causes excessive formation of MDA (Gaweł et al., 2004). SOD and GPX are the primary antioxidant enzymes. The GPX enzyme is the main enzyme responsible for scavenging hydrogen peroxide in the cell. It converts hydrogen peroxide into water. On the other hand, the SOD enzyme is responsible for eliminating superoxide radicals and promoting the conversion of hydrogen peroxide and molecular oxygen into other substances (Guruprasad et al., 2012). Furthermore, EPE increased broilers' antioxidant capacity.

According to Lee et al. (2013), EP has promising antioxidant properties in chickens. Arbor Acres broilers can benefit from dried EP as a feed addition, as it enhances meat quality and improves the oxidative state. Ghalamkari et al. (2011) showed that adding EPE from the dried apical component of the meal at a level of 10 g/kg can enhance the overall antioxidant capacity in the blood of broiler chicks. As a result of adding EPE to poultry feed, the concentrations of IgG and SOD increased while MDA decreased, which is one of the results of fat oxidation. This, in turn, leads to improved poultry health and thus enhances their growth performance.

CONCLUSION

Despite being raised in open sheds at a temperature of 29°C and relative humidity of 70%, the study discovered substantial changes in bird weight throughout the final growth stage between experimental treatments, where the 3.0 cm^3 EPE group had the highest weight of 1883.33g. Additionally, EPE-rich diets had a good impact on blood lipid parameters. Furthermore, adding 3.0 cm^3 of EPE improved growth efficiency, some carcass characteristics, and antioxidant and immunity activities.

COMPETING INTERESTS

The authors declare no competing interests.

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REFERENCES

Abd El-Hack, M. E., E. A. Ashour, N. Aljahdali, N. M. Zabermawi, S. A. Baset, M. Kamal, and S. S. Bassiony. 2024a. Does the dietary supplementation of organic nano-zinc as a growth promoter impact broiler's growth, carcass and meat quality traits, blood metabolites, and cecal microbiota? Poult. Sci. 103: 103550.

Abd El-Hack, M. E., K. A. Majrashi, K. Fakiha, M. Roshdy, M. Kamal, R. M. Saleh, and M. Alagawany. 2024b. Effects of varying dietary microalgae levels on performance, egg quality, fertility, and blood biochemical parameters of laying Japanese quails (*Coturnix coturnix Japonica*). Poult. Sci. 103: 103454.

Abd El-Hack, M. E., M. Kamal, A. H. Alqhtani, R. M. Alreemi, R. S. Alazragi, H. Khojah, and S. Świątkiewicz. 2023a. Detoxification Impacts of Dietary Probiotic and Prebiotic

Supplements Against Aflatoxins: An Updated Knowledge–A Review. Ann. Anim. Sci. 23: 1049-1060.

Abd El-Hack, M. E., S. A. Abdelnour, M. Kamal, A. F. Khafaga, A. M. Shakoori, R. M. Bagadood, and S. Świątkiewicz. 2023b. Lactoferrin: Antimicrobial impacts, genomic guardian, therapeutic uses and clinical significance for humans and animals. Biomed. Pharmacother. 164: 114967.

Abd El-Hack, M. E., S. A. Mahgoub, M. Alagawany, and E. A. Ashour. 2017. Improving productive performance and mitigating harmful emissions from laying hen excreta via feeding on graded levels of corn DDGS with or without Bacillus subtilis probiotic. J. Anim. Physiol. Anim. Nutr. 101: 904-913.

Abed, A. H., I. A. Radwan, M. M. A. El-Aziz, and A. Ali. 2021. Antifungal activity of natural essential oils against molds and yeasts associated with respiratory problems in broiler chickens. Adv. Anim. Vet. Sci. 9: 348-355.

Abreu, R., T. Semedo-Lemsaddek, E. Cunha, L. Tavares, and M. Oliveira. 2023. Antimicrobial drug resistance in poultry production: current status and innovative strategies for bacterial control. Microorganisms. 11: 953.

Alagbe, J. O., J. O. Alagbe, and T. D. Tijani. 2018. Effects of dried *Centella asiatica* leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of broiler chicken. Pac. Int. J. 1: 172-180.

Atay, A. 2023. The effect medicinal plants on performance, carcass parameters and meat quality in broiler chickens. JIST. 13: 1418-1428.

Awad, A.L., H.N. Fahim, and A.M. El-Shhat. 2020. Response of Sudani Ducklings to Dietary *Echinacea purpurea* Addition on Growth Performance and Economic Efficiency. J. Anim. Poult. Prod. 11: 175-182.

Bagno, O. A., S. A. Shevchenko, A. I. Shevchenko, O. N. Prokhorov, and A. V. Shentseva. 2021. Efficiency of *Echinacea purpurea* extract in growing broiler chickens. Achievements of Science and Technology in Agro-Industrial Complex.

Bouchama, C., L. K. Yousfi, S. EL Akhdari, N. Chadli, K. F. Samouh, S. M. Raoui, and F. Errachidi. 2024. Effect of feed supplementation with Curcuma longa and Zingiber officinale spices on the zootechnical, biochemical, and microbiological parameters of broiler chickens. Not. Sci. Biol. 16: 11948-11948.

Burlou-Nagy, C., F. Bănică, T. Jurca, L. G. Vicaş, E. Marian, M. E. Muresan, and A. Pallag. 2022. *Echinacea purpurea* (L.) Moench: Biological and pharmacological properties. A review. Plants. 11: 1244.

Chiellini, C., I. Maida, V. Maggini, E. Bosi, S. Mocali, G. Emiliani, and R. Fani. 2017. Preliminary data on antibacterial activity of *Echinacea purpurea*-associated bacterial communities against Burkholderia cepacia complex strains, opportunistic pathogens of Cystic Fibrosis patients. Microbiol. Res. 196: 34-43.

Eilam, Y., N. Pintel, H. Khattib, N. Shagug, R. Taha, and D. Avni. 2022. Regulation of cholesterol metabolism by phytochemicals derived from algae and edible mushrooms in non-alcoholic fatty liver disease. Int. J. Mol. Sci. 23: 13667.

Elmahallawy, E. K., A. Fehaid, D. M. El-Shewehy, A. M. Ramez, A. A. Alkhaldi, R. Mady, and W. Abdo. 2022. S-methylcysteine ameliorates the intestinal damage induced by Eimeria tenella infection via targeting oxidative stress and inflammatory modulators. Front. Vet. Sci., 8: 754991.

El-Ratel, I. T., M. M. Amara, M. M. Beshara, M. F. El Basuini, S.F. Fouda, K.H. El-Kholy, and A. Mekawy. 2024. Effects of supplemental vitamin A on reproduction and antioxidative status of aged laying hens, and their offspring's growth, blood indices and immunity. Poult. Sci. 103: 103453.

Erenler, R., I. Telci, M. Ulutas, I. Demirtas, F. Gul, M. Elmastas, and O. Kayir. 2015. Chemical Constituents, Quantitative Analysis and Antioxidant Activities of *Echinacea purpurea* (L.) M oench and *Echinacea pallida* (N utt.) N utt. Food Biochem. 39: 622-630.

Gaweł, S., M. Wardas, E. Niedworok, and P. Wardas. 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie. 57: 453-455.

Ghalamkari, G., N. Landy, M. Toghiani, M. Modaresi, and Z. Ghalamkari. 2011. Efficiency of *Echinacea purpurea* on total antioxidant activity in serum of broiler chicks. J. Herb. Drug. 1: 7-14.

Gilani, S. M. H., S. Zehra, S. Galani, and A. Ashraf. 2018. Effect of natural growth promoters on immunity, and biochemical and haematological parameters of broiler chickens. Trop. J. Pharm. Res. 17: 627-633.

Gohel, B. C., D. D. Garg, S. S. Patil, H. H. Savsani, S.P. Trivedi, and S.J. Kadam. 2019. Efficacy of *Ocimum sanctum* (Tulsi) and *Aloe vera* leaves powder as phytogenic growth promoter in diet of broiler chickens. J. Entomol. Zool. Stud. 7: 379-383.

Gurudath, S., R. M. Naik, K. S. Ganapathy, Y. Guruprasad, D. Sujatha, and A. Pai. 2012. Superoxide dismutase and glutathione peroxidase in oral submucous fibrosis, oral leukoplakia, and oral cancer: A comparative study. J. Orofac. Sci. 4: 114-119.

Hashem, M. A., A. N. Neamat-Allah, H. E. Hammza, and H. M. Abou-Elnaga. 2020. Impact of dietary supplementation with *Echinacea purpurea* on growth performance, immunological, biochemical, and pathological findings in broiler chickens infected by pathogenic E. coli. Trop. Anim. Health Prod. 52: 1599-1607.

Hassan, I. I., A. A. Askar, and G. A. El-Shourbagy. 2004. Influence of some medicinal plants on performance; physiological and meat quality traits of broiler chicks. Egy. Poult. Sci. 24: 247-266.

Kamal, M., N. Aljahdali, F. A. Jaber, K. A. Majrashi, W. H. Kishk, H. A. Khalil, A. M. Abdel-Khalek, M. A. Ayoub, and M. E. Abd El-Hack. 2023a. Influence of dietary chitosan supplementation on ovarian development and reproductive performance of New Zealand White rabbit. Ann. Anim. Sci. 23: 757-764.

Kamal, M., W. H. Kishk, H. A. Khalil, A. M. Abdel-Khalek, M. A. Ayoub, A. A. Swelum, A. H. Alqhtani, H. A. Ba-Awadh, and M. E. Abd El-Hack. 2023b. Effect of dietary chitosan supplementation on growing New Zealand white rabbits' productive and physiological performance parameters. Int. J. Biol. Macromol. 230: 123166.

Lee, T. T., C. L. Chen, C. C. Wang, and B. Yu. 2012. Growth performance and antioxidant capacity of broilers supplemented with *Echinacea purpurea* L. in the diet. J. Appl. Poult. Res. 21: 484-491.

Lee, T. T., J.Y. Ciou, C.L. Chen, and B. Yu. 2013. Effect of *Echinacea purpurea* L. on oxidative status and meat quality in Arbor Acres broilers. J. Sci Food Agric. 93: 166-172.

Li, B., J. Q. Zhang, X. G. Han, Z. L. Wang, Y. Y. Xu, and J. F. Miao. 2018. *Macleaya cordata* helps improve the growth-promoting effect of chlortetracycline on broiler chickens. J. Zhejiang Univ. Sci. B. 19: 776-784.

Mohamed, L. A., W. M. Dosoky, M. Kamal, G. Alshehry, E. H. Algarni, N. M. Aldekhail, and S. A. Farag. 2024. Growth performance, carcass traits, and meat physical characteristics of growing Japanese quail-fed ginger powder and frankincense oil as feed additives. Poult. Sci. 103: 103771.

Nazari, Z., H. Shirzadi, K. Taherpour, E. Rahmatnejad, and A. Khatibjoo. 2024. Effect of dietary medicinal plants on physiological responses of broiler chickens challenged with Campylobacter jejuni. Vet. Med. Sci. 10: e70028.

Nosrati, M., F. Javandel, L. M. Camacho, A. M. E. R. Khusro, M. Cipriano, A. Seidavi, and A. Z. M. Salem. 2017. The effects of antibiotic, probiotic, organic acid, vitamin C, and *Echinacea purpurea* extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. J. Appl. Poult. Res. 26: 295-306.

Pourasghar, F., H. R. Aliakbarpour, and G. Maliji. 2021. Effects *Echinacea purpurea* (L.) Moench) extract on the yield and some immunological and blood biochemical parameters in broilers. Iran. J. Med. Arom. Plant. Res. 36: 975-984.

Przybylski, W., B. Sionek, D. Jaworska, V. Santé-Lhoutellier. 2016. The application of biosensors for drip loss analysis and glycolytic potential evaluation. Meat Sci. 117: 7-11.

Rady, W. F., A. B. N. Sayed, and H. A. Abdel-Raheem. 2023. Effect of dietary supplementation of echinacea and nucleotides on productive performance, intestinal histomorphology and gene expression of broiler chickens. Assiut Vet. Med. J. 69: 141-155.

Rahimi, S., Z. Teymori Zadeh, K. Torshizi, R. Omidbaigi, and H. Rokni. 2011. Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. J. Agric. Sci. Technol. 13: 527-539.

Roth-Maier, D. A., B. M. Böhmer, N. Maaß, K. Damme, and B. R. Paulicks. 2005. Efficiency of *Echinacea purpurea* on performance of broilers and layers. Europ. Poult. Sci. 69: 123-127.

Saeed, M., D. Babazadeh, M. A. Arain, M. Naveed, Q. Shah, A. A. Kamboh, and S. Chao. 2018. The use of chicoric acid from *Echinacea purpurea* as a feed additive in poultry nutrition. World's Poult. Sci. J. 74: 69-78.

Seckin, C., G. Alpun Kalayci, N. Turan, A. Yilmaz, U. Y. Cizmecigil, O. Aydin, and H. Yilmaz. 2018. Immunomodulatory effects of Echinacea and Pelargonium on the innate and adoptive immunity in calves. Food Agric. Immunol. 29: 744-761.

Seifi, S., R. Khoshbakht, H. Hajati, and A. Gilani. 2018. Appraisal of purple coneflower (*Echinacea purpurea*) extract on production performance, internal organs, and gut microflora of Japanese quail. *Acta Scientiarum*. Anim. Sci. 40: e37230.

Sharifi, S. D., S. H. Khorsandi, A. A. Khadem, A. Salehi, and H. Moslehi. 2013. The effect of four medicinal plants on the performance, blood biochemical traits and ileal microflora of broiler chicks. Vet. Arh. 83: 69-80.

Shen, C., S. Li, Z. Cai, R. Man, and X. Wang. 2020. Effect of *Echinacea purpurea* extract given in drinking water on performance, slaughter variables, and meat quality of broilers. ESFAF. 2: 42-49.

Stanisavljević, I., S. Stojičević, D. Veličković, V. Veljković, and M. Lazić. 2009. Antioxidant and antimicrobial activities of Echinacea (*Echinacea purpurea* L.) extracts obtained by classical and ultrasound extraction. Chin. J. Chem. Eng. 17: 478-483.

Thorp, B. 2021. The poultry industry. Poultry health: a guide for professionals. CABI, Nosworthy Way, Wallingford, Oxfordshire OX10 8DE. 25.

Wang, J., L. Deng, M. Chen, Y. Che, L. Li, L. Zhu, and T. Feng. 2024. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. Anim. Nutr. Anim. Nutr. 17: 244-264.

Weishaupt, R., A. Bächler, S. Feldhaus, G. Lang, P. Klein, and R. Schoop. 2020. Safety and dose-dependent effects of echinacea for the treatment of acute cold episodes in children: a multicenter, randomized, open-label clinical trial. Children. 7: 292.

Yong, T., M. Chen, Y. Li, X. Song, Y. Huang, Y. Chen, Z. Yin. 2020. Anticoccidial effect of Fructus Meliae toosendan extract against *Eimeria tenella*. Pharm. Biol. 58: 636-645.

Yu, D., Y. Yuan, L. Jiang, Y. Tai, X. Yang, F. Hu, and Z. Xie. 2013. Anti-inflammatory effects of essential oil in *Echinacea purpurea* L. Pak. J. Pharm. Sci. 26: 403-408.

Table 1. Composition and quantitative examination of the foundational diet.

Items	Starter (1 – 21 days)	Finisher (22 – 35 days)
Ingredients (%)		
Soybean meal (44%)	34.50	29.5
Yellow corn	54.10	58.7
Corn germ (60%)	5.50	5.50
Dicalcium phosphate	2.00	1.75
Limestone	1.08	0.95
Premix[1]	0.30	0.30
L-lysine	0.29	0.24
Salt	0.30	0.30
DL-methionine	0.20	0.18
Soybean oil	1.80	2.30
Calculated analysis[2]		
CP %	23.12	21.10
ME (Kcal/kg)	3001	3180
Calcium %	0.99	0.89
Available phosphorus %	0.51	0.46
Potassium %	0.54	0.52

[1]Minerals and vitamins premix manufactured by Multi Vita Animal Nutrition® (Tenth of Ramadan City, Sharkia Governorate, Egypt) provides vitamin A 12000 IU, vitamin D3 2500 IU, vitamin E 20 mg, vitamin K3 2 mg, vitamin B1 2 mg, vitamin B2 5 mg, vitamin B6 2 mg, vitamin B12 0.05 ug, niacin 30 mg, biotin 0.05 ug, folic acid 1 mg, pantothenic acid 10 mg, manganese 60 mg, zinc 50 mg, iron 40 mg, copper 10 mg, iodine 0.6 mg, selenium 0.3 mg per 1 kg diet. DL-methionine (manufactured by Evonik Industries, Essen, Germany) contains 99 % methionine. Lysine = lysine hydrochloride (Evonik Industries) and contains 70 % Lysine. CP, crude protein; ME, Metabolizable energy.

[2]Calculated according to NRC (1994).

Table 2. The effect of broilers' diets adding varying amounts of echinacea on LBW.

Items	LBW (g)		
	5 D	21 D	35 D
Echinacea (Cm^3/ liter drinking water)			
0.0	82.30	887.50[a]	1840.00[ab]
1.0	82.20	792.50[b]	1810.00[ab]
2.0	82.25	815.00[b]	1661.66[b]
3.0	82.45	850.00[ab]	1883.33[a]
SEM	0.06	13.36	37.02
P value	0.635	0.028	0.037

D: days; LBW: live body weight; SEM: standard error mean.
[a,b] different letters within one column are significantly different ($P < 0.05$).

Table 3. The impact of broiler diets incorporating different quantities of echinacea on BWG.

Items	BWG (g/day)		
	5-21 D	22-35 D	5-35 D
Echinacea (Cm^3/ liter drinking water)			
0.0	50.32[a]	68.03	58.59
1.0	44.39[b]	72.68	57.59
2.0	45.80[b]	60.47	53.26
3.0	47.97[ab]	73.81	60.02
SEM	0.83	2.31	1.14
P value	0.027	0. 148	0.174

D: days; BWG: body weight gain; SEM: standard error mean.

Table 4. The effect on FI of broiler diets with varying amounts of echinacea.

Items	FI (g/day)		
	5-21 D	22-35 D	5-35 D
Echinacea (Cm^3/ liter drinking water)			
0.0	66.51	128.33	98.76[a]
1.0	63.08	112.35	87.71[b]
2.0	63.10	119.94	91.52[ab]
3.0	66.45	120.26	93.35[ab]
SEM	0.87	2.92	1.67
P value	0.319	0.321	0.108

D: days; FI: feed intake; SEM: standard error mean.
[a,b] different letters within one column are significantly different (P < 0.05).

Table 5. Influences of broilers' diets adding varying levels of Echinacea on FCR.

Items	FCR (g feed/ g gain)		
	5-21 D	22-35 D	5-35 D
Echinacea (Cm^3/ liter drinking water)			
0.0	1.32	1.90	1.66[a]
1.0	1.42	1.54	1.52[b]
2.0	1.37	2.01	1.65[a]
3.0	0.02	1.63	1.55[b]
SEM	0.28	0.08	0.02
P value	0.397	0.153	0.032

D: days; FCR: feed conversion ratio; SEM: standard error mean.
[a,b] different letters within one column are significantly different (P < 0.05).

Table 6. Carcass traits of broilers fed different levels of dietary echinacea supplementation.

Items	Relative to pre-slaughter weight, %							
	Carcass	Dressing	Giblets	Heart	Liver	Spleen	Gizzard	Abdominal fat
Echinacea (Cm^3/ liter drinking water)								
0.0	69.52	73.75	4.21[a]	0.38[b]	0.95	2.88[a]	0.06[b]	0.98[a]
1.0	65.88	69.12	3.23[b]	0.40[a]	0.95	1.87[b]	0.06[b]	0.61[b]
2.0	72.84	76.00	3.15[b]	0.37[ab]	0.98	1.79[b]	0.07[b]	0.50[c]
3.0	72.84	77.04	4.42[a]	0.35[c]	1.09	2.97[a]	0.10[a]	0.60[b]
SEM	1.44	1.42	0.22	0.008	0.05	0.19	0.005	0.06
P value	0.307	0.205	0.043	0.037	0.826	0.020	0.000	0.013

SEM: standard error mean.
[a,b] different letters within one column are significantly different (P < 0.05).

Table 7. Blood indices of broilers fed different levels of dietary echinacea addition.

Items	TP (g/dl)	ALB (g/dl)	GLOB (g/dl)	A/G ratio	ALT (U/L)	AST (U/L)	CREAT (mg/dl)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Echinacea (Cm^3/ liter drinking water)												
0.0	5.82[a]	3.50[a]	2.31	1.55[a]	5.21[b]	173.86[d]	0.74	135.63[a]	121.68[a]	45.71	64.99[a]	24.33[a]
1.	4.0	2.06[b]	2.02	1.02[b]	12.8	229.	0.71	121	105.	47.	52.4	21.13

0	8^{c}	^{c}			6^{a}	86^{a}		$.45^{b}$	66^{b}	83	8^{b}	^{b}
2.0	3.70^{d}	1.72^{c}	1.97	0.88^{c}	14.60^{a}	199.03^{c}	0.56	106.06^{c}	103.38^{b}	49.17	36.21^{c}	20.67^{b}
3.0	4.73^{b}	2.37^{b}	2.36	1.01^{b}	19.96^{a}	209.87^{b}	0.67	93.59^{d}	84.34^{c}	46.30	31.01^{c}	16.86^{c}
SEM	0.24	0.20	0.07	0.09	1.83	8.30	0.05	5.15	4.85	0.64	4.37	0.97
P value	0.000	0.000	0.185	0.035	0.009	0.048	0.365	0.001	0.023	0.230	0.001	0.023

TP: total protein; ALB: albumen; GLOB: globulin; ALT: alanine transaminase; AST: aspartate aminotransferase; CREAT: creatinine; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; SEM: standard error mean.
[a,b,c] different letters within one column differ significantly (P < 0.05).

Table 8. Immunity and antioxidative parameters of broilers fed various amounts of dietary echinacea supplementation.

Items	IgG (ng-ml)	IgM (ng-ml)	MDA (U-ml)	SOD (nmol-ml)
Echinacea (Cm^3/ liter drinking water)				
0.0	190.48^{b}	237.41	6.41^{a}	81.32^{c}
1.0	482.98^{a}	380.23	3.34^{b}	120.01^{bc}
2.0	347.04^{ab}	357.14	3.59^{b}	144.51^{b}
3.0	408.44^{a}	409.83	1.11^{c}	202.97^{a}
SEM	39.47	38.89	0.59	14.36
P value	0.023	0.471	0.000	0.001

IgG: Immunoglobulin G, IgM: Immunoglobulin A, SOD: Superoxide dismutase, MDA: Malondialdehyde, SEM: standard error mean.
[a,b,c] different letters within one column differ significantly (P < 0.05).

Table 9. The broilers' water consumption and water feed ratio fed different levels of dietary echinacea supplementation.

Items	Water consumption			Water/ feed ratio		
	5-21d	22-35d	5-35d	5-21d	22-35d	22-35d
Echinacea (Cm^3/ liter drinking water)						
0.0	171.80	355.21[a]	263.50[a]	2.57	2.76	2.7
1.0	155.11	297.30[c]	226.20[b]	2.46	2.64	2.58
2.0	161.64	332.01[b]	246.82[ab]	2.56	2.77	2.69
3.0	173.86	321.59[b]	247.72[ab]	2.61	2.67	2.65
SEM	3.79	8.35	5.72	0.04	0.02	0.02
P value	0.277	0.042	0.031	0.741	0.202	0.353

SEM: standard error mean. Water Con, water consumption; Water FR, water feed ratio.
[a,b] different letters within one column are significantly different ($P < 0.05$).

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.