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ORGINAL ARTICLE

The Effect of *Echinacea purpurea* Extract on Carcass Quality, Blood Biochemical Parameters, and Immune System in an Animal Model

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KEYWORDS

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ABSTRACT: This experiment was conducted to investigate the effect of adding different levels of *Echinacea purpurea* extract to the diet on carcass quality, blood biochemical parameters, and the immune system of broiler chickens. A total of 240 one-day-old male Ross 308 chicks were assigned in a completely randomized design to 4 experimental treatments with 5 replicates and 12 chicks per replicate. The experimental treatments included: 1) Control (without additive), 2) Control + 10 mg kg^{-1} *Echinacea* extract, 3) Control diet + 20 mg kg^{-1} *Echinacea* extract, and 4) Control + 30 mg kg^{-1} *Echinacea* extract. The results of the experiment showed that the carcass percentage in the treatment containing 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.01$). The amount of total protein and high-density lipoprotein (HDL) in the treatment containing 30 mg kg^{-1} extract had a significant difference compared to the control ($P<0.05$). Also, adding 20 and 30 mg kg^{-1} extract to the diet significantly increased the weight of the spleen and bursa of Fabricius compared to the control ($P<0.01$). Based on the results of this experiment, 30 mg kg^{-1} extract in the diet of broiler chickens had a better effect on carcass quality, blood lipid reduction, and the immune system.

INTRODUCTION

In the broiler chicken industry, optimal feed utilization for achieving desirable nutritional efficiency is of great importance. One of the useful strategies for better utilization of feed by poultry is the use of growth supplements. Previous research has proven that growth supplements can be beneficial for providing nutrients, improving feed efficiency, and maintaining poultry health [1]. One of these supplements is antibiotics. However, with the review of reports on antibiotic resistance, their use has been banned in many countries [2].

Over time, the use of antibiotics in poultry diets as growth promoters has been criticized due to the presence of antibiotic residues and the emergence of bacterial resistance as a result of the consumption of livestock and poultry products, and it has been recognized globally as a problem related to human health and the environment [3]. On the other hand, the ban on the use of antibiotic growth promoters may lead to an increase in disease incidence and a decrease in production in the poultry industry [4]. Therefore, finding a suitable alternative to antibiotics, such as organic acids, probiotics, prebiotics, and medicinal plants (essential oils, extracts, and dried powders), is essential.

In recent years, the tendency to use medicinal plants as natural growth-promoting additives in animal nutrition

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has gained attention [5]. Among the positive effects of medicinal plants in broiler chickens are growth stimulation, immune system stimulation, increased phagocytic activity in white blood cells, stimulation of digestive enzyme secretion, inhibition of pathogenic microbes, stimulation of lactobacillus activity, reduction of blood cholesterol and triglycerides, and antioxidant properties [6]. Echinacea purpurea is a medicinal plant native to North America, and its use has increased worldwide. Different parts of this plant (roots and aerial parts) have antioxidant and immune-stimulating properties [7]. Echinacea contains active compounds such as caffeic acid, alkamides, flavonoids, essential oils, and polyacetylenes, which, in addition to anti-inflammatory and antimicrobial effects, can stimulate macrophage phagocytic activity and lead to increased growth and strengthening of the immune system [8]. A group of researchers reported that herbal medicines such as Echinacea can significantly reduce serum lipid levels in broiler chickens due to their phenolic compounds, which is important for the quality of the produced products [9]. Given the global trend toward organic food and the increasing tendency to use medicinal plants due to their metabolic potential to improve growth, it is expected that the use of Echinacea purpurea in poultry farming will gain more attention. Therefore, the present study was conducted to investigate the effect of Echinacea extract on carcass characteristics, blood biochemical parameters, and the immune system of broiler chickens.

MATERIALS AND METHODS

This experiment was conducted using 240 one-day-old male Ross 308 chicks in a completely randomized design with 4 treatments, 5 replicates, and 12 chicks per replicate. The experimental treatments were: 1) Control (without additive), 2) Control + 10 mg kg^{-1} Echinacea extract, 3) Control + 20 mg kg^{-1} Echinacea extract, and 4) Control + 30 mg kg^{-1} Echinacea extract. The basal diet was formulated based on the nutrient requirements recommended in the Ross commercial strain catalog and using UFFDA software for the starter (1-10 days), grower (11-24 days), and finisher (25-42 days) periods (Table 1). To prepare the Echinacea extract, first, the leaves were shade-dried. To prevent the degradation of active compounds, they were dried in an oven at 40°C for 48 hours. A 5% weight/volume suspension of the sample and solvent was prepared by placing it in a shaker for 72 hours. The mixture was centrifuged at 3000 rpm for 10 minutes. Then, to evaporate the solvent from the laboratory sample, a Rotary evaporator model HS3001 (Hahnshin S&T Co., Ltd., Republic of Korea) was used. Finally, the resulting extract was added to the diet at the required ratios.

Carcass and internal organ characteristics

To evaluate carcass and internal organ characteristics at the end of the period (42 days), 3 birds per replicate were randomly selected, weighed, and slaughtered by cutting the jugular vein. After de feathering, removing the head and feet, and evisceration, the weight of the empty carcass and its parts (breast and thigh weights), internal organs (liver, pancreas, heart, and gizzard), and abdominal fat were measured. The weight of different parts of the intestine (duodenum, jejunum, and ileum) was also calculated. The percentage of each part was determined by dividing its weight by the live weight before slaughtering.

Blood biochemical parameters

At 42 days of age, 2 birds from each experimental unit were selected, and 2 mL of blood was collected from the wing vein and transferred to tubes containing anticoagulants. The serum was separated from the blood samples after centrifugation at 2000 rpm for 15 minutes. The concentrations of glucose, triglycerides, cholesterol, total protein, albumin, HDL, LDL, total protein, albumin, and uric acid in serum samples were measured using Pars Azmun laboratory kits and a Ce1010 spectrophotometer (made in England).

Table 1. Dietary composition of different periods of rearing.

Ingredients (%)	Starter (1-10 Days Old)	Grower (11-24 Days Old)	Finisher (25-42 Days Old)
Corn	54.21	58.23	62.36
Soybean Meal	39.52	36.46	30.89
Soybean Oil	2.10	1.40	3.21
Dicalcium Phosphate	1.71	1.55	1.35
$CaCO_3$	1.26	1.19	1.07
NaCl	0.35	0.31	0.30
Mineral Premix[1]	0.35	0.25	0.25
Vitamin Premix[2]	0.25	0.25	0.25
L-Lysine-HCl	0.16	0.20	0.21
DL-Methionine	0.19	0.16	0.11
Total	100	100	100
Chemical analyses			
Metabolizable Energy (kcal kg^{-1})	2900	2910	3010
Crude Protein(%)	22	20.50	20
Lysine (%)	1.39	1.29	1.18
Methionine (%)	0.59	0.51	0.45
Methionine + Cysteine (%)	0.10	0.92	0.84
Ca (%)	1.00	0.90	0.81
Na (%)	0.19	0.17	0.16
Available Phosphorus (%)	0.50	0.46	0.45
Cl (%)	0.28	0.24	0.23
Threonine (%)	0.92	0.84	0.79
Arginine (%)	1.52	1.42	1.27

* antioxidants.Mineral supplement per kilogram of diet: 110 mg of manganese (manganese sulfate), 50 mg of iron (ferrous sulfate), 100 mg of zinc (zinc oxide), 1.2 mg of iodine (calcium iodide), 0.3 mg of selenium (sodium selenite), 15 mg of copper (copper sulfate).

** Vitamin supplement per kilogram of diet: 12.500 IU vitamin A, 3100 IU vitamin D_3, 40 IU vitamin E, 2 mg vitamin K_3, 2 mg vitamin B_1, 4.6 mg vitamin B_2, 1.5 mg vitamin B_6, 0.015 mg B_{12}, 30 mg B_3, 15 mg B_5, 10 mg biotin, 30 mg choline, 50 mg folic acid and 50 mg

Immune system

The immune system was evaluated by measuring the weight of lymphoid organs (spleen, thymus, and bursa of Fabricius) in slaughtered birds.

Statistical analysis

The data were analyzed using SAS (2005) software, and the means were compared using Duncan's test. The statistical model of the design was $Y_{ij} = \mu + T_i + E_{ij}$, where Y_{ij} is the numerical value of each observation, μ is the population mean, T_i is the effect of dietary treatment, and E_{ij} is the experimental error.

RESULTS AND DISCUSSION

Carcass and internal organ characteristics

The results related to the effect of experimental treatments on carcass characteristics are presented in Table 2. The carcass percentage in treatments containing 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.01$). The breast percentage of broilers fed with all three levels of extract significantly increased compared to the control ($P<0.01$). The thigh percentage in treatments with 20 and 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.01$).

Table 2. Effect of different levels of *Echinacea* extract in the diet on the mean (± standard error) of carcass characteristics of broiler chickens at 42 days of age

Treatment	Empty Carcass (%)	Breast (%)	Thighs (%)
Control	65.347±1.153[b]	22.754±0.627[c]	21.600±1.227[c]
10 mg kg^{-1} *Echinacea*	67.204±0.127[b]	25.804±0.238[b]	23.568±0.381[bc]
20 mg kg^{-1} *Echinacea*	66.340±0.446[b]	32.300±0.880[a]	24.165±0.144[b]
30 mg kg^{-1} *Echinacea*	69.814±0.902[a]	31.106±0.411[a]	29.672±0.567[a]
SEM	0.7668	0.590	0.706
P-value	0.005	0.000	0.000

In each column, means with at least one common letter do not have a significant statistical difference at the 5% level.

The results related to the effect of experimental treatments on the relative weight of internal organs and abdominal fat are presented in Table 3. The percentage of abdominal fat in treatments with 20 and 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.01$). No significant differences were observed in the percentage of liver, pancreas, heart, and gizzard in any of the experimental treatments compared to the control ($P>0.05$).

Table 3. Effect of different levels of *Echinacea* extract in the diet on the mean (± standard error) of relative weight of internal organs and abdominal fat of broiler chickens at 42 days of age

Treatment	Abdominal Fat (%)	Liver (%)	Pancreas (%)	Heart (%)	Gizzard(%)
Control	1.746±0.034[a]	2.106±0.037	0.306±0.007	0.508±0.007	1.010±0.074
10 mg kg^{-1} *Echinacea*	1.690±0.007[a]	2.002±0.009	0.310±0.020	0.504±0.002	1.062±0.080
20 mg kg^{-1} *Echinacea*	1.328±0.099[b]	1.850±0.210	0.300±0.005	0.500±0.007	1.064±0.056
30 mg kg^{-1} *Echinacea*	1.196±0.080[b]	1.966±0.060	0.280±0.007	0.500±0.004	1.118±0.026
SEM	0.066	0.111	0.012	0.005	0.063
P-value	0.000	0.458	0.302	0.695	0.690

* In each column, means with at least one common letter do not have a significant statistical difference at the 5% level.

According to the results, the group receiving 30 mg kg^{-1} extract had the highest significant percentage of carcass, breast, and thighs, as well as the lowest percentage of abdominal fat. Consistent with the present results, Hoseinyan Bilandi et al. [10] reported in their experiments that active compounds of herbal medicines can increase the carcass yield of broiler chickens. One of the disadvantages of harmful microbes in the gastrointestinal tract is the increased breakdown of proteins and amino acids by microbes. Since medicinal plants reduce the microbial population in the gastrointestinal tract, they can reduce the rate of breakdown of proteins and amino acids, leading to greater absorption and storage in the body, which ultimately reduces fat accumulation and improves carcass percentage.

The results related to the effect of experimental treatments on the relative weight of different parts of the small intestine are presented in Table 4. The duodenum percentage in the treatment with 20 mg kg^{-1} extract and the ileum percentage in the treatment with 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.05$). No significant difference was observed in the jejunum percentage in any of the experimental treatments compared to the control ($P>0.05$).

Table 4: Effect of different levels of *Echinacea* extract in the diet on the mean (± standard error) of relative weight of different parts of the small intestine of broiler chickens at 42 days of age

Treatment	Duodenum (%)	Jejunum (%)	Ileum (%)
Control	38.800±0.800[b]	53.700±7.826	30.800±0.970[b]
10 mg kg^{-1} *Echinacea*	40.800±2.131[ab]	54.000±4.868	31.700±1.530[b]
20 mg $^{-1}$*Echinacea*	45.400±1.939[a]	54.800±7.479	32.200±0.735[b]
30 mg kg^{-1} *Echinacea*	43.200±1.114[ab]	56.600±8.641	36.000±0.707[a]
SEM	1.595	3.557	1.039
P-value	0.049	0.473	0.014

* In each column, means with at least one common letter do not have a significant statistical difference at the 5% level.

A few other studies, consistent with the present results, have shown that the consumption of thyme and *Echinacea* extracts increases the weight of the small intestine in broiler chickens [11]. Researchers have stated that the consumption of medicinal plants can influence the growth and development of gastrointestinal tissues and organs [12]. In general, any factor that increases the efficiency of nutrient absorption from the gastrointestinal tract will lead to increased weight and length of digestive organs. Extracts of medicinal plants enhance metabolism and strengthen the immune system, which improves digestion and absorption efficiency and the weight of digestive organs [13]. Also, plant extracts reduce the secretion of the enzyme urease, preventing protein breakdown in the gastrointestinal tract, which can lead to better protein absorption and growth of digestive organs [14].

Blood biochemical parameters

The results related to the effect of experimental treatments on blood biochemical parameters are presented in Table 5. The concentration of cholesterol and low-density lipoprotein (LDL) in the treatment with 30 mg kg^{-1} extract significantly decreased compared to the control ($P<0.05$). Also, the concentration of high-density lipoprotein (HDL) and total protein in the treatment with 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.05$). No significant differences were observed in glucose, triglycerides, albumin, and uric acid concentrations in any of the treatments compared to the control ($P>0.05$).

Table 5. Effect of different levels of *Echinacea* extract in the diet on the mean (± standard error) of blood biochemical parameters of broiler chickens at 42 days of age

Treatment	Glucose (mg dL^{-1})	Triglycerides (mg dL^{-1})	Cholesterol (mg dL^{-1})	LDL (mg dL^{-1})	HDL (mg dL^{-1})	Total Protein (g dL^{-1})	Albumin (mg dL^{-1})	Uric Acid (mg dL^{-1})
Control	257.13±3.27	119.02±21.70	148.76±9.01[a]	37.97±12.79[a]	65.97±13.73[b]	3.25±1.02[b]	1.38±0.21	7.12±0.791
10 mg kg^{-1} *Echinacea*	254.0±3.46	110.32±19.13	128.20±7.63[ab]	26.13±10.35[a]	66.13±16.07[b]	3.27±1.13[b]	1.42±0.20	6.96±0.788
20 mg kg^{-1} *Echinacea*	261.98±3.35	108.87±33.20	119.18±7.51[ab]	25.03±9.05[a]	78.03±7.35[ab]	3.29±1.11[b]	1.49±0.39	6.11±0.785
30 mg kg^{-1} *Echinacea*	263.9±6.24	101.56±31.24	99.91±8.91[b]	19.83±9.62[b]	89.93±11.21[a]	3.81±1.28[a]	1.53±0.08	5.89±0.774
SEM	8.13	7.92	5.97	1.91	7.21	0.248	0.07	0.35
P-value	0.264	0.211	0.028	0.023	0.020	0.044	0.061	0.201

* In each column, means with at least one common letter do not have a significant statistical difference at the 5% level.

The highest level of Echinacea extract in the diet led to an increase in total protein and a decrease in cholesterol and low-density lipoprotein (LDL) in the serum of broiler chickens. The present results are consistent with the findings of Dauqan and Abdullah [15], who reported the effect of adding thyme to the diet on the serum parameters of broiler chickens. The antimicrobial and antioxidant properties of medicinal plants can reduce

harmful bacteria and prevent protein oxidation, increasing amino acid absorption and subsequently elevating total serum protein levels [16]. On the other hand, the reduction in serum lipid profiles of broilers fed with medicinal plants is related to their active compounds (e.g., flavonoids, alkaloids, and alkamides) [17]. A possible mechanism for this is the binding of these compounds to bile acids and increased excretion from the gastrointestinal tract [18]. Also, Pirmohamammadi et al. [19] stated that compounds such as flavonoids can reduce LDL levels in broiler serum by inhibiting enzymes involved in cholesterol synthesis.

Immune system

The results related to the effect of experimental treatments on the relative weight of lymphoid organs are presented in Table 6. The relative weight of the spleen in treatments with 20 and 30 mg kg^{-1} extract significantly increased compared to the control ($P<0.01$). Also, the relative weight of the bursa of Fabricius in all treatments containing the extract significantly increased compared to the control ($P<0.01$). No significant differences were observed in the relative weight of the thymus in any of the treatments containing the extract compared to the control ($P>0.05$).

Table 6. Effect of different levels of *Echinacea* extract in the diet on the mean (± standard error) relative weight of lymphoid organs of broiler chickens at 42 days of age

Treatment	Spleen (%)	Thymus (%)	Bursa of Fabricius (%)
Control	0.064±0.002^{c}	0.066±0.007	0.071±0.008^{d}
10 mg kg^{-1} *Echinacea*	0.076±0.003^{c}	0.329±0.208	0.144±0.011^{c}
20 mg kg^{-1} *Echinacea*	0.457±0.157^{b}	0.192±0.003	0.175±0.006^{b}
30 mg kg^{-1} *Echinacea*	0.742±0.022^{a}	0.232±0.011	0.215±0.009^{a}
SEM	0.079	0.104	0.009
P-value	0.000	0.379	0.000

In each column, means with at least one common letter do not have a significant statistical difference at the 5% level.

The results showed that the consumption of 10 to 30 mg kg^{-1} extract significantly increased the weight of the bursa of Fabricius in broiler chickens. The bursa of Fabricius is one of the main immune organs in poultry, where lymphocyte maturation occurs. Consistent with the present results, other studies have reported that phenolic compounds in plants can influence the weight of lymphoid organs as they inhibit prostaglandin synthesis by their antioxidant and anti-inflammatory properties, consequently, strengthening the immune system [20].

CONCLUSIONS

In this experiment, carcass characteristics and abdominal fat percentage in broiler chickens significantly improved with increasing levels of *Echinacea* extract. Increased total protein and decreased serum cholesterol were observed in broilers fed with higher levels of the extract. Also, the improvement in lymphoid organs was dependent on the increased amount of extract in the diet. Overall, the results showed that the use of 30 mg kg^{-1} *Echinacea* extract in the diet had better effects compared to other applied levels.

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Conflict of interests

There are no conflicts of interest to declare by the authors.

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary information

Not applicable.

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