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The Effect of *Echinacea purpurea* Extract on Performance, and Interleukin-2 Gene Expression in Broilers

Research Article

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ABSTRACT

The effects of various concentrations of *Echinacea purpurea* extract were evaluated on performance, antioxidant activity, intestinal morphology, immune system, and interleukin-2 (IL-2) gene expression in broiler chickens. To conduct the experiment, 240 one-day-old male chickens of Ross 308 strain were used in a completely randomized design with 4 treatments, and 5 replicates with 12 chickens in each replicate. The experimental treatments included a control diet void of *Echinacea purpurea*, and diets supplemented with either 10 mg/kg, 20 mg/kg, or 30 mg/kg *Echinacea purpurea* extract. Addition of *Echinacea purpurea* extract at 20 and 30 mg/kg in the diet significantly increased the live weight and weight gain of the entire period and decreased the conversion ratio of the entire period ($P<0.01$). Antioxidant activity was significantly improved only in the treatment group supplemented with 30 mg/kg compared with the control ($P<0.05$). The villus height and the ratio of villus height to crypt depth in the treatments with 20 and 30 mg/kg extract in feeds were significantly increased compared with the control ($P<0.01$). Consumption of 20 and 30 mg/kg *Echinacea purpurea* extract enhanced total antibody production against SRBC and Newcastle vaccine (NDV) in the entire period ($P<0.01$). Chickens fed diets supplemented t with 30 mg/kg extract had a enhanced relative IL-2 gene expression compared with other treatments ($P<0.05$). In general, the results of this experiment showed that the consumption of 30 mg/kg extract in the feed of broiler chickens had a positive effect on growth performance, antioxidant activity, and immune system compared with other negative control diet.

KEY WORDS *Echinacea purpurea*, extract, IL-2, immune system, performance.

INTRODUCTION

In the broiler breeding and production industry, the highest cost is related to the feeding sector. For this reason, extensive research has been conducted to determine the optimal use of feed to reduce feeding costs (Irawan *et al*. 2020). An effective means of optimizing feeding efficiency by poultry is to use supplements. Previous research has demonstrated that supplements can be beneficial for providing nutrients, increasing feed efficiency, and poultry health. One of these supplements is antibiotics, but their use has been banned in many countries (Poorghasemi *et al*. 2017). Due to the importance of economic efficiency in the production of broiler chickens and taking into account the fact that the prohibition of antibiotics reduces the production and efficiency of feed, many studies have been conducted regarding the use of antibiotic alternatives in feed in recent years (Kostadinović and Lević, 2018). Medicinal plants and their extracts are among the most prominent compounds that have been used as alternatives to growth-promoting antibiotics. The response of poultry to specific plant materials have included beneficial effects of these additives on the

growth performance of broiler chickens, digestive performance, antioxidant activities, and immunity have been reported (Duskaev *et al*. 2021). Previous studies have shown that plant additives can increase the digestibility of nutrients, reduce competition for nutrients, reduce microbial toxins, and ultimately increase bird performance by improving the microbial population of the digestive system and changing the type of digestive secretions (Liao *et al*. 2021). One of the most important biological activities of medicinal plants is related to antioxidant activities, which are related to their bioactive molecules such as Carvacrol, Thymol, Cinol, Allysine, Capsaicin, and Piperine (Forte *et al*. 2017; Mirsalehi *et al*. 2024). Preventing the multiplication of the disease agent or the lethality of the disease agent is the antibacterial strength of medicinal plants. Creating the appropriate environment in the intestine to prevent the penetration and growth of harmful bacterial agents and improve the function of the digestive system against pathogenic agents is the specific property of some medicinal plants (Wan *et al*. 2016).

The use of medicinal plants with substances such as phenols, alkynes, and alkenes leads to an increase in blood lymphocytes, which can help improve the broiler immune system (Attia *et al*. 2017). Increased interleukin-2 gene expression has been reported when using herbal compounds in broiler chickens (Nobakht and Muhaghegh Dolatabady, 2019).

Echinacea purpurea is a medicinal plant of North America whose use has increased all over the world. Different structures of this plant (root and aerial part) have antioxidant properties and stimulate the immune system (Manayi *et al*. 2015). *Echinacea purpurea* contains effective substances such as caffeic acid, alkamide, flavonoids, essential oils, and polyacetylenes, which, in addition to anti-inflammatory and antimicrobial effects, can stimulate the phagocytic activity of macrophages and increase the number of lymphocytes and improve cellular antioxidant activity (Woelkert and Bauer, 2007). Researchers reported the use of *Echinacea purpurea* had a positive effect on the daily weight gain of broilers (Nosrati *et al*. 2017).

Habibian Dehkordi (2011) showed that the use of *Echinacea purpurea* root powder at the level of 0.5% in the diet of broiler chickens increased the feed conversion ratio and immune response, but had no significant effect on the weight of the lymphoid organs of broiler chickens. Lee *et al*. (2013) reported that *Echinacea purpurea* powder in the diet of broiler chickens can increase the antioxidant capacity of broiler chickens. Landy *et al*. (2011) reported that the use of the powder of the aerial parts of *Echinacea purpurea* in the level of 5 grams per kilogram of feed caused a significant increase in the antibody titer against Newcastle virus and SRBC in broilers.

The results of another research showed that the addition of *Echinacea purpurea* medicinal plant can play an effective role in the morphology of the intestinal tissue and also more absorption due to the improvement of the growth environment of beneficial intestinal bacteria (Rahimi *et al*. 2011).

A common variable measured in studies of herbal medicines is their effects on the expression of genes related to the immune system and their relationship with growth performance. Interleukin-2 can be mentioned among the important genes in this field.

Based on the results of existing research, medicinal plants are able to have beneficial effects, especially on the growth performance and immune system of chickens, which are considered important goals of poultry breeding. But due to the lack of sufficient information about the benefits of using medicinal plants and how to use them correctly, you do not get good results. According to the limited reports that are available, the animal's response to the consumption of medicinal plants is affected by the method of consumption and its amount, so that if these types of plants are consumed in large quantities, they can even have harmful effects for the animal.

Considering the few researches about the effect of *Echinacea purpurea* on the expression of interleukin 2 gene and the dependence of the efficiency of this plant on the type and amount of the product used in the diet, the present research can be of great importance from the aspect of innovation. Therefore, the present experiment was conducted to determine the effect of *Echinacea purpurea* extract on performance, antioxidant activity, intestinal morphology, immune system and interleukin-2 gene expression in broiler chickens.

MATERIALS AND METHODS

Animal material

This experiment was conducted using 240 one-day-old male chickens of Ross 308 strain in a completely randomized design with 4 treatments, and 5 replicates with 12 chickens in each replicate. The experimental treatments included: 1- The control diet (C) (no extract), 2- C + 10 mg/kg *Echinacea purpurea* extract, 3- C + 20 mg/kg *Echinacea purpurea* extract, and 4- C + 30 mg/kg *Echinacea purpurea* extract. The basal diet was adjusted based on the nutrient supply recommended in the Ross commercial strain catalog and using the UFFDA software for the starter (1-10 days), grower (11-24 days) and finisher (25-42 days) periods (Table 1).

To prepare the extract of *Echinacea purpurea*, leaves were first dried in shadow. To prevent the destruction of the effective substances of the plant, drying was for 48 hours in an oven at a temperature of 40 ˚C.

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)	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 (%)	1.10	0.92	0.84
Ca (%)	0.99	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

[1] Mineral supplement per kilogram of diet: Manganese (manganese sulfate): 110 mg; Iron (ferrous sulfate): 50 mg; Zinc (zinc oxide) 100 mg; Iodine (calcium iodide): 1.2 mg; Selenium (sodium selenite): 0.3 mg and Copper (copper sulfate): 15 mg.
[2] Vitamin supplement per kilogram of diet: vitamin A: 12.500 IU; vitamin D_3: 3100 IU; vitamin E: 40 IU; vitamin K_3: 2 mg; vitamin B_1: 2 mg; vitamin B_2: 4.6 mg; vitamin B_6: 1.5 mg; B_{12}: 0.015 mg; B_3: 30 mg; B_5: 15 mg; Biotin: 10 mg; Choline: 30 mg; Folic acid: 50 mg and Antioxidants: 50 mg.

Then a 5% weight-volume suspension of the sample and solvent was prepared by placing it in a vibrating machine for 72 hours. The mixture was centrifuged at 3000 rpm for 10 minutes. Then, a laboratory sample of Rotary evaporator model HS-3001 (Hahnshin S&T Co., Ltd., Republic of Korea) was used to evaporate the solvent. In the end, the resulting extract was used in the required proportions in the diet.

Performance

During the experiment, the chickens had free access to water and feed. Chickens in each treatment group and amount of feed offered were weighed at the beginning and end of the rearing period, and then the feed consumption and weight gain of the period were calculated. Also, the feed conversion ratio was calculated from the ratio of feed intake to weight gain. Losses were weighed and recorded daily. Feed consumption and feed conversion ratio were also corrected for losses.

Intestine morphology

To investigate the intestinal morphological traits, three birds from each replicate were killed at the age of 42 days. In the next step, the small intestine along with the gizzard and a part of the large intestine were removed from the bird's abdominal area. Then, a two-centimeter-long part was excised from the middle part of the jejunum of the small intestine. The samples were transferred to 10% buffered formalin and the formalin was changed after 24 hours. After one week for the stabilization phase, the samples entered the usual histological steps and five to six-micrometer sections were prepared. For morphometric studies, the prepared sections were stained with hematoxylin-eosin stain. Morphological indicators such as villus height, villus width, and crypt depth were measured by use of an AM7025X digital camera (manufactured by Dino-Lite company) and Dino-capture 2 software, and finally, the ratio of villus height to crypt depth was calculated (Poorghasemi *et al.* 2017).

Antioxidant activity

To investigate the antioxidant activity at the end of the experimental period (day 42), 3 birds were selected from each experimental unit and 2.5 ml of blood was taken through the wing vein and transferred to heparin tubes, and the plasma was collected after centrifugation at 3000 rpm for 15 minutes.

The activities of glutathione peroxidase and superoxide dismutase enzymes were measured using Biorex commercial kits and ELISA devices (PowerWave XS2, Biolek, USA). Malondialdehyde (MDA), reaction with thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were measured by spectrophotometric absorption measurement, and comparison of absorption with the standard curve (Hosseini-Vashan *et al*. 2012).

Immunity system

To investigate the status of the immune system of the birds, changes in antibody titer were investigated by injecting 2% sheep red blood cell (SRBC) suspension as a non-pathogenic antigen, on two occasions. 0.5 milliliters of 2% SRBC suspension was injected into the wing vein of 3 chickens from each replicate at 27 days of age. Blood was drawn from the chickens 6 days after the primary injection at 33 days of age and the second injection of SRBC was then administered. Six days after the second injection at 39 days of age, blood was taken again to check the secondary response. Finally, the antibody titer was determined by the serial dilution method (microtiter hemagglutination test) (Cheema *et al*. 2003).

Blood samples were also taken from 3 chickens in each replicate through the wing vein to measure anti-Newcastle and influenza titers on the 35th and 42nd days of rearing. An agglutination inhibition test measured anti-Newcastle and influenza titers (Bagherzadeh *et al*. 2012).

***IL-2* gene expression**

To investigate the relative interleukin-2 (IL-2) gene expression, thymus tissue was separated from slaughtered chickens (three birds from each replicate), transferred to a nitrogen tank (-196 ˚C) and sent to the laboratory. RNA extraction from tissue samples was performed by the RNA extraction kit according to the manufacturer (Cinagen). After evaluating the quantity and quality of extracted RNA by agarose gel electrophoresis and spectrophotometer, Bionier lyophilized Mastermix (Takapu Zist) was used for cDNA synthesis. After cDNA synthesis, its quality was evaluated using 1% agarose gel.

The design of primers was performed to investigate the expression of the IL-2 gene and reference gene using AllellD and Olgo 7 software (Table 2). Q-RT-PCR Mastermix (Cinnagen Company) was used for Real-Time PCR reaction. The thermal program used in the Real-Time PCR reaction for genes included 95˚C for 10 minutes and 60˚C for 1 minute. Finally, the relative amount of IL-2 gene expression was analyzed based on Ct values (the number of cycles required to reach a threshold level) obtained from Real-Time PCR, by REST software, 2009, V2.0.13.

Statistical analysis

The data obtained from the experiment were analyzed with the help of SAS software (SAS, 2005) and the averages were compared with Duncan's test. The statistical model of the design is $Y_{ij} = \mu + T_i + E_{ij}$, where Y_{ij} is the numerical value of each observation of the experiment, μ is the population mean, T_i is the effect of diet, and E_{ij} is the effect of the error of the experiment.

RESULTS AND DISCUSSION

The use of *Echinacea purpurea* in the diet of broiler chickens during the starter period caused a significant increase in feed intake and weight gain compared to the control ($P<0.005$) (Table 3). In the same period, the addition of *Echinacea purpurea* extract to diets had no significant effect on the live weight and conversion ratio of broiler chickens compared to the control ($P>0.01$).

The use of 20 and 30 mg *Echinacea purpurea* extract in diets during the grower period had a significant improvement compared with the control ($P<0.01$) on live weight, weight gain, and conversion ratio of broilers. The feed intake was significantly increased with the addition of 30 mg extract in the diet ($P<0.01$). In the finisher period, the weight of the chickens that had been fed 20 or 30 mg *Echinacea purpurea* were greater compared with the control diet birds ($P<0.01$). However, the only the increase in live weight in chickens fed 30 mg extract had a significant increase compared with the controls ($P<0.01$). The feed intake in chickens fed all levels of the extract had a significant increase compared with the controls ($P<0.01$). The conversion ratio was not significantly different among treatment groups ($P>0.01$). Addition of *Echinacea purpurea* extract at 20 and 30 mg/kg diet significantly increased the live weight and weight gain and decreased the conversion ratio of the experimental period ($P<0.01$). The feed intake in chickens fed with all three levels of *Echinacea purpurea* extract in the diet had a significant increase compared with the control group ($P<0.01$).

The results related to the effect of experimental treatments on the antioxidant activity of broiler chickens showed that the addition of 30 mg *Echinacea purpurea* extract in the diet caused a significant increase in the GPX concentration and a significant decrease in the MDA concentration compared with the controls ($P<0.05$; Table 4).

Table 2 List of primers used

Gene	Primer sequence	Product length (bp)
IL-2	F:5′-TCTGTGAGCCAATTGTAGTCACC-3′ R:5′-TCCAGCACATAGAAGTATCATTACG-3′	135
β-actin	F:5′-GCAATCCTCACCGTAAGGCTGA-3′ R:5′-CTGTCGGTGTCTCTATCTCGT-3′	121

Table 3 The effect of experimental treatments on performance of broiler chickens

Treatment	Starter (1-10 days)			
	BW (g)	FI (g chick)	BWG (g chick)	FCR
Control	196.28±0.65[a]	204.80±2.07[b]	146.68±0.70[b]	1.04±0.01[a]
10 mg EPE/kg of diet	198.08±0.43[a]	214.54±1.16[a]	150.60±0.71[a]	1.08±0.007[a]
20 mg EPE/kg of diet	197.10±0.64[a]	216.26±4.16[a]	150.30±0.97[a]	1.09±0.02[a]
30 mg EPE/kg of diet	202.64±3.12[a]	221.14±2.55[a]	153.16±1.60[a]	1.09±0.02[a]
SEM[3]	1.64	2.72	1.06	0.01
P-value	0.06	0.00	0.00	0.22
Treatments	Grower (11-24 days)			
	BW (g)	FI (g chick)	BWG (g chick)	FCR
Control	1116.74±0.91[c]	1236.26±1.03[b]	920.46±1.11[c]	1.34±0.00[a]
10 mg EPE/kg of diet	1116.60±0.92[c]	1238.84±0.66[b]	918.52±1.11[c]	1.34±0.002[a]
20 mg EPE/kg of diet	1222.82±6.48[b]	1245.30±3.56[b]	1025.72±7.04[b]	1.21±0.00[b]
30 mg EPE/kg of diet	1335.40±9.37[a]	1276.84±6.13[a]	1132.76±11.81[a]	1.12±0.008[c]
SEM	5.73	3.59	6.92	0.00
P-value	0.00	0.00	0.00	0.00
Treatments	Finisher (25-42 days)			
	BW (g)	FI (g chick)	BWG (g chick)	FCR
Control	1977.00±2.72[c]	2242.00±24.93[c]	860.26±2.81[b]	2.60±0.02[a]
10 mg EPE/kg of diet	1982.20±1.15[c]	2297.30±0.97[b]	865.60±1.96[b]	2.65±0.00[a]
20 mg EPE/kg of diet	2131.80±19.96[b]	2338.76±18.03[b]	908.98±19.49[b]	2.57±0.06[a]
30 mg EPE/kg of diet	2350.30±26.08[a]	2441.08±12.99[a]	1014.90±34.58[a]	2.41±0.09[a]
SEM	16.49	16.70	19.92	0.05
P-value	0.00	0.00	0.00	0.06
Treatments	Total period (1-42 days)			
	BW (g)	FI (g chick)	BWG (g chick)	FCR
Control	1977.00±2.72[c]	3683.06±23.29[c]	1929.88±2.60[c]	1.87±0.00[a]
10 mg EPE/kg of diet	1982.20±1.15[c]	3750.68±1.33[b]	1935.90±1.10[c]	1.88±0.00[a]
20 mg EPE/kg of diet	2131.80±19.96[b]	3800.32±21.88[b]	2085.16±20.18[b]	1.79±0.02[b]
30 mg EPE/kg of diet	2350.30±26.08[a]	3939.06±17.42[a]	2302.30±26.11[a]	1.66±0.02[c]
SEM	16.49	18.21	16.56	0.01
P-value	0.00	0.00	0.00	0.00

BW: body weight; FI: feed intake; BWG: body weight gain and FCR: feed conversion ratio.
EPE: *Echinacea purpurea* extract.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).
SEM: standard error of the means.

Table 4 The effect of experimental treatments on antioxidant enzymes and malondialdehyde (MDA) of broiler chickens

Treatments	GPX (mg/dL)	SOD (mg/dL)	MDA (mg/dL)
Control	1001.10±791.18[b]	9.78±1.32[a]	19.91±13.58[a]
10 mg EPE/kg of diet	1028.10±787.09[b]	9.88±1.39[a]	19.83±13.27[a]
20 mg EPE/kg of diet	1457.40±399.11[ab]	9.96±1.44[a]	18.79±8.98[a]
30 mg EPE/kg of diet	1998.89±401.2[a]	11.07±8.06[a]	11.01±8.01[b]
SEM	1002.01	6.95	7.97
P-value	0.03	0.22	0.01

GPX: Glutathione peroxidase and SOD: Superoxide dismutase.
EPE: *Echinacea purpurea* extract.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).
SEM: standard error of the means.

None of the levels of *Echinacea purpurea* extract had a significant effect on SOD concentration (P>0.05).

The results of experimental treatments on the morphology of the intestine showed that the birds that had been fed the diets containing 20or 30 mg/kg *Echinacea purpurea* extract had a significant increase in villus height and the ratio of villus height to crypt depth compared with the control birds (P<0.01) (Table 5). Also, chickens fed either 20 or 30 mg/kg extract had a significant decrease in crypt depth compared to the control chickens (P<0.01). The villus width was not significantly different from among treatments (P>0.05).

The consumption of *Echinacea purpurea* extract had no significant effect on total anti-SRBC, IgG, and IgM titers in 35 days for any of the experimental treatments (P>0.05) (Table 6). On the 42nd day, *Echinacea purpurea* extract at the levels of 20 or 30 mg/kg diet caused a significant increase in IgG (P<0.05), IgM, and total (P<0.01) anti-SRBC titers compared with the control diet.

The addition of different levels of *Echinacea purpurea* extract to the diet of broiler chickens had no significant effect on the antibody produced against the Newcastle vaccine at the age of 35 days (P>0.05), but there was a significant increase compared to the control at 42 days of age for the treatments containing 20 or 30 mg extract (P<0.01) (Table 7). *Echinacea purpurea* extract had no significant effect on the antibody produced against influenza vaccine (P>0.05).

The relative expression of the IL-2 gene of the chickens that received the treatment containing 30 mg/kg extract had a significant increase compared with the control chickens (Figure 1; P<0.05). Relative expressions of IL-2 in birds fed either 10 or 20 mg/kg *Echinacea purpurea* extract were not significantly different from the control birds (P>0.05).

In the present study, a significant improvement in live weight and weight gain in the grower period and the entire rearing period was observed by feeding either 20 or 30 mg/kg *Echinacea purpurea* extract. Also, with the consumption of different concentration of *Echinacea purpurea* extract, the feed intake increased significantly in the starter, finisher, and the entire period. In the grower period and the entire experimental period, a significant decrease in the feed conversion ratio was observed using 20 or 30 mg *Echinacea purpurea* extract, but it was not significant in the starter and finisher periods of the experiment.

Dora *et al*. (2008) observed that the body weight was significantly lower compared to the control group using a dose of 2.4% of *E. purpurea* extract in the diet, which was contrary to the results of the present experiment. Consistent with the results of the present experiment, Lee *et al*. (2012) showed the use of 0.5% aerial part of *Echinacea purpurea* in the diet of broiler chickens resulted in higher weight gain and feed efficiency in the entire rearing period compared with the control. Landy *et al*. (2011) observed that the body weight, daily weight gain, and feed intake of the birds that continuously consumed 0.5% *Echinacea purpurea* were higher than other treatments, and the weakest performance was related to the birds that received 1% *Echinacea purpurea*. The intermittent use of *Echinacea purpurea* extract or fermented extract of *Echinacea purpurea* improves poultry performance, which means better digestion and absorption of feed (Ghafouri *et al*. 2021).

In previous studies, it has been determined that herbal extracts can play an important role in improving the conversion and growth ratio (Platel and Srinivasan, 2004). Starch and triglycerides are important macromolecules in the diet, which are hydrolyzed by pancreatic amylase and lipase, respectively. Active herbal ingredients improve digestion by acting on salivary glands and secretions of the stomach, pancreas, and bile and intestinal mucus enzymes (Ali *et al*. 2019).

The phenolic components in plants can prevent the loss of nutrients by reducing the number of intestinal pathogenic microbes, thus improving performance and increasing protein in body tissues (Amiri *et al*. 2024). Nasir and Grashorn (2009) stated that when broilers consumed plant extracts, significant activity in intestinal lipase was observed. The improvement in poultry performance in the present experiment may be due to the increase in feed digestion and the use of nutrients that as a result, the phenolic compounds of *Echinacea purpurea* had been able to create better intestinal health and improve the microbial population by stimulating digestive enzymes (Abudabos *et al*. 2021).

The results of the present research showed that the extract of *E. purpurea* significantly reduces the feed conversion ratio of broiler chickens, and the lowest conversion ratio was related to the treatment containing 30 mg/kg of the extract, which is consistent with the results of Hedia *et al*. (2008). The feed conversion ratio depends on the parameters of daily weight gain and daily feed consumption, and since *E. purpurea* extract improved the weight gain, as a result, the improvement of the conversion ratio in this treatment was predictable.

The performance of animals is significantly influenced by the state of health and safety of the animal. A weakened or stressed immune system when dealing with an infectious disease causes weight loss. The *Echinacea purpurea* plant has the property of stimulating the immune system, therefore, the use of immune system stimulants can increase performance by improving the immune status (Priya *et al*. 2022). In the present study, supplementing *Echinacea purpurea* extract from 10 mg to 30 mg in the diet resulted in the increase concentration of GPX and and decreased malendialdehyde compared with the control diet.

Table 5 The effect of experimental treatments on intestinal morphology of broiler chickens

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/Crypt depth
Control	481.66±35.64^{c}	86.64±8.31^{a}	110.14±3.65^{a}	4.42±0.44^{b}
10 mg EPE/kg of diet	553.85±21.50^{bc}	98.74±7.04^{a}	108.67±13.60^{a}	5.09±0.36^{b}
20 mg EPE/kg of diet	600.85±26.52^{ab}	110.60±9.25^{a}	85.15±9.30^{b}	7.28±0.60^{a}
30 mg EPE/kg of diet	644.86±17.85^{a}	107.92±10.29^{a}	84.80±4.63^{b}	7.70±0.53^{a}
SEM	26.24	8.81	8.75	0.49
P-value	0.00	0.25	0.00	0.00

EPE: *Echinacea purpurea* extract.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).
SEM: standard error of the means.

Table 6 The effect of experimental treatments on antibody titer against SRBC of broiler chickens

Treatments	35 days			42 days		
	IgG	IgM	Total	IgG	IgM	Total
Control	1.00±0.00^{a}	1.00±0.00^{a}	2.00±0.00^{a}	1.40±0.24^{b}	1.40±0.24^{b}	2.80±0.37^{b}
10 mg EPE/kg of diet	1.40±0.24^{a}	1.60±0.24^{a}	3.00±0.44^{a}	1.40±0.24^{b}	1.60±0.24^{b}	3.00±0.44^{b}
20 mg EPE/kg of diet	1.40±0.24^{a}	1.40±0.24^{a}	2.80±0.49^{a}	2.60±0.24^{a}	2.40±0.40^{a}	5.00±0.63^{a}
30 mg EPE/kg of diet	1.40±0.24^{a}	1.60±0.24^{a}	3.40±0.60^{a}	2.80±0.24^{a}	3.00±0.00^{a}	5.80±0.63^{a}
SEM	0.21	0.21	0.44	0.36	0.26	0.46
P-value	0.46	0.19	0.20	0.02	0.00	0.00

EPE: *Echinacea purpurea* extract.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).
SEM: standard error of the means.

Table 7 The effect of experimental treatments on antibodies titer against Newcastle Disease Virus (NDV) and Avian Influenza Virus (AIV) of broiler chickens

Treatments	35 days		42 days	
	NDV (Log2)	AIV (Log2)	NDV (Log2)	AIV (Log2)
Control	4.00±0.31^{a}	3.00±0.00^{a}	4.00±0.31^{c}	3.20±0.20^{a}
10 mg EPE/kg of diet	3.80±0.37^{a}	3.20±0.20^{a}	4.00±0.44^{c}	3.40±0.24^{a}
20 mg EPE/kg of diet	4.00±0.44^{a}	3.00±0.31^{a}	5.60±0.24^{b}	3.60±0.24^{a}
30 mg EPE/kg of diet	4.00±0.31^{a}	3.20±0.20^{a}	6.60±0.24^{a}	3.60±0.24^{a}
SEM	0.36	0.21	0.32	0.23
P-value	0.97	0.82	0.00	0.58

EPE: *Echinacea purpurea* extract.
The means within the same column with at least one common letter, do not have significant difference (P<0.05).
SEM: standard error of the means.

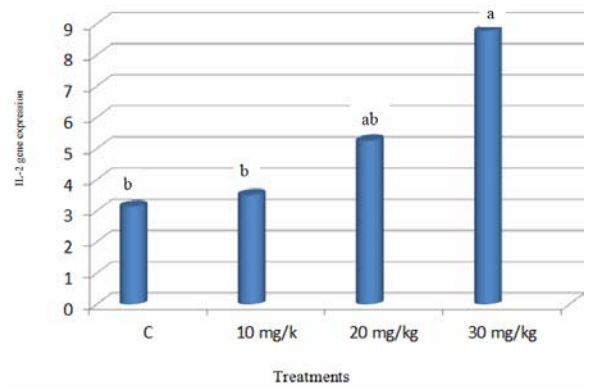

Figure 1 The effect of experimental diets on the relative expression of IL-2 gene in broilers

Ghalamkari *et al.* (2012) reported that adding 10 grams/kg powder of aerial part of *Echinacea purpurea* plant increased the antioxidant activity of total blood serum in broilers, which is consistent with the results of the present experiment.

The extract of *Echinacea purpurea* can have the same antioxidant effect as other antioxidants such as ascorbic acid (Tzu Tai *et al.* 2009). Plant extracts have the role of protecting body tissues against oxidative stress by having biologically active compounds and antioxidant properties (Wang *et al.* 2019). The active ingredients of medicinal plants are mainly secondary metabolites that include phenolic compounds with antioxidant properties (Su *et al.* 2020). These active ingredients inhibit free radicals produced by the body's metabolism. Inhibition of free radicals prohibits the use of antioxidant enzymes and high activity levels of these enzymes in the blood, and they reduce the production of malondialdehyde by preventing the peroxidation of lipids in the body (Chi *et al.* 2020). Plants containing phenolic and flavonoid compounds have a high capacity as powerful antioxidants to clear free radicals and eliminate oxidative reactions. These compounds can increase the activity level of antioxidant enzymes and give hydrogen ions to free radicals to destroy them, reduce peroxidation and also reduce the level of malendialdehyde (Hosseini and Meimandipour,

2018). Regarding the mechanism of the effect of *Echinacea purpurea* in increasing antioxidant enzymes as well as preventing and reducing the process of peroxidation, studies have shown that *Echinacea purpurea* is rich in antioxidant compounds (Tzu Tai *et al*. 2013). The phenolic compounds in *Echinacea purpurea* include echinacoside and caffeic acid, which can play an effective role in removing free radicals, including hydroxyl radicals and superoxide (Hu and Kitts, 2000). Thereforethe improvement of the antioxidant activity in the current experiment is probably related to the phenolic and antioxidant compounds mentioned in the extract of *Echinacea purpurea*.

In the current experiment, the use of *Echinacea purpurea* extracts greater than 10 mg/kg in the diet significantly increased the villus height in the jejunum and decreased the crypt depth, which is consistent with the results of the experiment of Rady *et al*. (2023). Gastrointestinal mucosa is the first tissue that comes into contact with nutritional compounds. The condition of the mucus and its microscopic structure is a good indicator of the intestinal response to the active substances in the feed and changes in intestinal morphology (Pascual *et al*. 2022). Deeper crypt depths indicate rapid tissue breakdown and regeneration and high demand for new tissue. The villus height depends on the destruction speed of the mucous cells at the surface of the villi (enterocytes) located at the top of the villi and on the speed of their replacement in the crypt. If the proliferation of crypt cells is faster than cell destruction, the length of the villi will increase. An increase in the height of the villi leads to an increase in the contact surface and, as a result, more nutrients are absorbed (Brus *et al*. 2018). The effective compounds present in plant extracts reduce the number of pathogenic bacteria in the wall of the small intestine, and by reducing the production of toxic compounds by the bacteria causes a change in the morphology of the intestinal wall of broiler chickens and thus prevent the destruction and damage of the mucosal cells of the intestinal wall. In fact, with the reduction of harmful microorganisms, intestinal inflammation is reduced, which can prevent protein from falling into the intestinal lumen and reducing the rate of regeneration (Farahat *et al*. 2021). Also, on the other hand, medicinal plants, due to their antioxidant compounds, neutralize free radicals and thus prevent their destructive effects on the intestinal villi, and the villi will grow better (Kheiri *et al*. 2018). Adedokun and Olojede (2019) also observed plant-derived supplements containing phenolic compounds had stimulating effects on the production and secretion of intestinal mucin, which may lead to the adhesion of pathogens and have positive effects on chicken intestinal tissue. Therefore, the effect of *Echinacea purpurea* extract on the significant improvement of the intestinal morphology of broiler chickens in the present experiment can probably be due to the presence of anti-microbes compounds that have been effective in creating normal conditions in the intestinal lining tissue.

Humoral immunity is a part of the body's acquired immunity that protects the body against pathogenic agents by B lymphocytes and large protein molecules, including secreted antibodies and antibacterial peptides (Pirgozliev *et al*. 2019). In the present experiment, , humoral immune responses in chickens responded on day 42, so that IgG, IgM, and total antibody titers against SRBC, as well as antibody titers against Newcastle vaccine in birds fed diets containing either 20 or 30 mg/kg extract were significantly enhanced compared with the control. Landy *et al*. (2011) reported that adding 5 grams from the powder of the aerial part of the *Echinacea purpurea* plant per kilogram of feed continuously caused a significant increase in antibodies against SRBC. Habibi and Firouzi (2017) showed that the addition of *Echinacea purpurea* extract to the drinking water of broiler chickens increased the function of the humoral immune system in broiler chickens. *Echinacea purpurea* caused a significant increase in antibody titers against Newcastle vaccine in laying hens and broilers, which is consistent with the results of the present research (Bohmer *et al*. 2009; Landy *et al*. 2011). The Bursa of Fabricius is the place of differentiation of immune B cells. Extract of *Echinacea purpurea* has caused an increase in the relative size of the bursa and the proliferation of B lymphocytes (Erenler *et al*. 2015). The positive effect of *Echinacea purpurea* extract in increasing the relative size of lymphatic organs (spleen and bursa) has been attributed to its stimulating and antioxidant properties (Erenler *et al*. 2015). Lymphatic organs are very active and the active oxidant compounds are formed during aerobic metabolism as well as the specific function of some cells such as phagocytosis. Immune cells are very sensitive to oxidative damage. These damages can be related to the unsaturated fatty acids of the membrane of immune cells or the DNA of immune cells and their constituent tissues (Cheng *et al*. 2021). Due to its antioxidant properties, *Echinacea purpurea* can protect tissues and lymph cells from these damages and improve the performance of the humoral immune system (Erenler *et al*. 2015). Studies have shown that a part of humoral immune responses depends on the function of helper T cells and antigen-presenting cells. It is expressed in CD4 helper T cells. The receptors of these cells are involved in the activation of killer T cells, B cells, and other immune cells (Pirgozliev *et al*. 2019). *Echinacea purpurea* contains compounds such as caffeic acid derivatives, alkylamides, flavonoids, essential oils, and polyacetylenes. The pharmacological properties of each of these substances have not been fully characterized (Erenler *et al*. 2015). However, the modulating property of immune system has been proven by

caffeic acid derivatives and alkalamides (Matthias *et al.* 2008). Also, herbal medicines and compounds derived from them have direct effects on macrophages, causing them to express pro-inflammatory mediators and antimicrobial agents (Ismail *et al.* 2021). In the present experiment, it is likely that the use of *E. purpurea* extract together with the Newcastle and influenza vaccine has caused inflammatory and specific responses against the Newcastle and influenza virus and has increased the effectiveness of the vaccine.

In the thymus tissue of broiler chickens that were fed with a diet containing 30 mg/kg extract, IL-2 gene expression was significantly increased compared with the control broiler chickens. Interleukin-2 is one of the known groups of interleukins that help the growth and rapid division of immune cells. Interleukin-2 is also called T-cell growth factor. Interleukin-2 causes the proliferation of type 1 helper T cells and NK cells due to its effect on them. Interleukin-2 affects B lymphocytes and has an effective role in the production of interferon-gamma. Also, interleukin-2 is necessary for the survival and function of regulatory T cells (Ibrahim *et al.* 2022). Studies have shown that the consumption of *Echinacea purpurea* extract in mice caused an increase in the levels of IL-2 and interferon-gamma (INT-γ) in response to concanavalin (Bodinet *et al.* 2002). Also, it has been determined that the consumption of *Echinacea purpurea* extract in mice increases the release of cytokines from macrophages in the blood, increases the production of helper T cells, INT-γ, CD4, CD8, increases the proliferation of lymphocytes and granulocytes (Bany *et al.* 2003). Some researchers reported that *Echinacea purpurea* extract has immune-stimulating factors such as glycoproteins and polysaccharides. They announced that the stimulation of cytokine production in *Echinacea purpurea* is caused by its glycoproteins, which can cause immune system stimulating effects with its mitogenic activities. On the other hand, fucogalactoxyloglucans and arabinogalactans are also part of the known polysaccharides in *Echinacea purpurea*, which have the effect of stimulating the immune system and respectively increase phagocytosis and stimulate macrophages, which can be effective in the production of cytokines (Hashem *et al.* 2020). Since *Echinacea purpurea* extract has a stimulating effect, increasing the consumption of *Echinacea purpurea* extract significantly improved IL-2 in broilers.

CONCLUSION

In general, in the present experiment, the results showed that the use of 30 mg/kg of *Echinacea purpurea* extract in the feed had better effects compared to other levels used, so that a significant improvement was seen for the growth performance, antioxidant activity and immune system.

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