

Evaluation of *Echinacea* Immunomodulatory Effect on the Immune Response of Broiler Chickens

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Abstract

Immunomodulators provide an excellent approach as they mainly have fewer side effects than chemical drugs and have no affinity for creating microbial resistance. In order to investigate the *Echinacea* immunomodulatory effect on the immune response of broiler chickens, one hundred twenty (1-day-old) chicks of both sexes were randomly allocated into 2 dietary groups on floor pen (60 chicks/group), group1: Chicks were offered basal diets and kept as control group, group2: Chicks were offered basal diets supplemented with *Echinacea purpurea* (5gm/kg diet for 6 weeks). Evaluating the effects of *Echinacea purpurea* on growth performance, Serum Protein profile, total and differential leucocytic count, humeral immunity which was carried out by measuring antibody titer against Newcastle (ND) and (AIV) Avian Influenza vaccine, and on innate immunity which was carried by measuring some function of peripheral blood monocyte (PBM) as phagocytic activity, fungicidal activity, nitric oxide NO production and reactive oxygen species ROS production. Challenge test was carried out using virulent *E. coli* strain. The results revealed that, *Echinacea* improve growth performance, significantly increase antibody titer against ND and AIV vaccine as well as enhance the innate immunity by increasing the phagocytic activities, fungicidal activities, ROS production and nitric oxide production of monocytes. In challenge test, *Echinacea* reduce *E. coli* lesions, morbidity and mortality rate. Based on the results of this study, it could be concluded that growth performance and both humeral and innate immune responses of broiler chicks were improved by of dietary supplementation of *Echinacea purpurea* (5 g/kg diet), also it could protect broiler chickens from virulent *E. coli* infection.

Key words: *Echinacea*, broiler chickens, *E. coli*, humeral immunity, innate immunity challenge test.

Introduction

The use of antibiotic in treatment of various bacterial diseases has an important concern in human health and animal production. The use of dietary antibiotics in feed to promote growth of poultry and other livestock has been implicated in the increased microbial resistance and the rise in number of human patients infected with these pathogens (*Threlfall et al., 2000 and Teuber, 2001*).

The social pressure to reduce and completely eliminate the use of antibiotics in poultry feed has increased; food safety standards have also been heightened recently (*USDA FSIS, 2010*). Dietary immunomodulation is an important method used for decreasing the prevalence of food borne pathogens in poultry. One of the most popular natural immunomodulator is *Echinacea purpurea*. This herbal immunomodulator has been used from long period of time for many purposes including immunostimulation, therapy and growth promotion (*Barrett, 2003; Porchezian and Punniamurthy, 2006*).

Echinacea purpurea is mainly used globally for stimulation of immune system. It is a natural drug used in treatment of respiratory manifestations, cancer and inflammatory lesions. Many of the active ingredients of *Echinacea* have been recognized including; polysaccharides, alkylamides, chicoric acid, arabinogalactan and

glycoproteins. The percentage of these constituents varies depending on which part of *Echinacea* was analyzed (*Percival, 2000; Redondo, 2000*). *Echinacea* could be used in increasing the immune response, especially of cells such as neutrophils, monocytes and lymphocytes (*O'Neill et al., 2002; Cundell et al., 2003*).

Echinacea extracts have the ability to increase the antibody titer against antigens, such as keyhole hemocyanin and sheep RBCs, in sera of the tested animals. The root extracts enhance the production of cytokines, such as IL-1, IL-10 and TNF- α by human macrophages (*Currier and Miller, 2002; Mishima et al., 2004*).

This work was planned to evaluate the effect of dietary supplementation of *Echinacea* on innate and humeral immunity of broiler chickens, as well as the antibacterial effect of *Echinacea* against *E. coli* (in vivo) by challenge test.

Materials and Methods

1. Experimental design:

One hundred twenty (1-day-old) chicks (Hubbard breed) of both sexes were randomly allocated into 2 dietary groups on floor pen (60 chicks/group). **Group1 (G1):** broiler chicks were offered basal diets and kept as a control group. **Group2 (G2):** broiler chicks in this group were offered basal diets supplemented with *Echinacea*

(5gm/kg diet for 6 weeks) (*Landy et al., 2011*).

2. Determination of growth Performance Parameters:

Birds were weighed at first day of age as one day-old weight and then final body weights (gm) were recorded at 42 days of age.

3. Blood sampling:

Blood samples were collected from the 2 groups, and divided into 3 portions; the first portion was collected in plain tubes, for separation of serum and kept frozen at -20°C till use. The 2nd portion was collected in EDTA tubes for leucocytic count. The 3rd portion was collected in heparinized tubes and was used for separation of peripheral blood monocyte cells (PBM) according to (*Grievink et al., 2016*).

4. Determination of total and differential leucocytic count:

The total and differential leucocytic counts were determined at the 1st, 2nd and 4th week according to (*Stoskopf, 1993*).

5. Determination of protein profile:

5.1. Determination of serum total protein:

Serum total protein concentration was determined at 1st, 2nd and 4th week as described by (*Bakerman, 1984*).

5.2. Determination of serum albumin:

Serum albumin was determined at 1st, 2nd and 4th week, according to the method of (*Young, 1997*).

5. 3.Calculation of serum globulin:

Serum globulin was estimated at 1st, 2nd and 4th week, by subtracting the concentration of albumin from the value of estimated total protein.

5.4. A/G ratio:

Albumin/ globulin were estimated at 1st, 2nd and 4th week by dividing value of albumin over globulin.

6. Evaluation of humeral immune response of chicks:

Humeral immune response was evaluated at 1st, 2nd, 3rd and 4th week by detecting antibody titer against ND and AI virus vaccine by haemagglutination inhibition test according to (*Miller, 2013*).

7. Estimation of Innate immune response:

7.1. Determination of phagocytic activity:

The phagocytic activity of monocyte cells was assessed according to *Elmowalid (2012)*, at 1st, 2nd, 4th and 6th week of age. The results are expressed as phagocytic percentage (number of phagocytic monocytes /total number of monocytes) and phagocytic index (the main of engulfed *Candida* spores per monocyte).

7.2. Fungicidal activity (Intracellular survival assay):

An intracellular survival assay was used to measure the fungicidal activity of monocytes at 2nd, 4th and 6th week of age, according to (*Peck, 1985*).

7.3. Nitric oxide assay:

Nitric oxide production was measured in PBM culture media

using Griess reagent at 1st, 2nd, 4th and 6th week as described by (*Blond et al., 2000*).

7.4. Reactive oxygen species production:

The reactive oxygen species production of PBM were determined using Nitro blue tetrazolium reduction test at 2nd, 3rd and 6th week of age, as described by (*Anderson and Siwicki, 1995*).

8. Challenge Test:

Laboratory multidrug resistant virulent *E. coli* strain was used at the end of the experiment for challenge test. The clinical symptoms were recorded since the first day of chicken inoculation for calculation of morbidity and mortality rate, the survived chickens were slaughtered and the internal organs were harvested and prepared to determine total CFU count in each organ of each chicken in each experimental group.

9. Statistical analysis:

The data were given as means \pm S.E. The statistical analysis was done using the One Way ANOVA. The significant (**P*) values were taken as $P \leq 0.05$ (*McCredie et al., 2006*).

Results

1-Effect of dietary supplementation with *Echinacea* on body weight gain in broiler chickens:

The results showed in Table (1) revealed that chickens in G2 fed *Echinacea* supplemented diet for 42 days showed a significant increase in final body weight and body

weight gain compared to control group.

2- Effect of dietary supplementation with *Echinacea* on total and differential leucocytic count in broiler chickens:

The obtained results in Table (2) revealed that there was a significant increase in total leucocytic count in chickens which fed on a ration containing *Echinacea* at all intervals compared with the control group.

The heterophilic count of chickens which fed a ration containing *Echinacea* at 1st, 2nd and 4th week in Table (2) revealed that there was a significant increase in the heterophilic count in chickens which fed on a ration containing *Echinacea* at all the experimental period when compared with the control group.

The lymphocytic count of chickens which fed a ration containing *Echinacea* in Table (2) revealed that there was significant increase in the lymphocytic count in chickens which fed on a ration containing *Echinacea* at all the experimental period when compared with the control group.

The monocytic count of chickens which fed a ration containing *Echinacea*. The obtained results in Table (2) revealed that there was non-significant increase in monocytic count in chicks fed on ration containing *Echinacea* when compared with the control group.

The eosinophilic count of chickens which fed supplemented diet with Echinacea in Table (2) revealed that there was non-significant alterations in the eosinophilic count in chicks fed supplemented diet Echinacea when compared with the control group.

3- Effect of dietary supplementation with Echinacea on total protein (gm/dl), albumin (gm/dl) and globulin (gm/dl) in broiler chickens:

The mean values of the proteinogram of chicken's fed supplemented diets with Echinacea were tabulated in Table (3). There were no significant changes in the total proteins and albumin at 1st and 4th week from dietary supplementation in the 2 groups. There were insignificant changes in globulins at 1st week in all groups, while there was significant increase at 4th week in the chickens fed supplemented diet with Echinacea when compared with the control group. A/G ratio at 1st and 4th week there were significant decrease at chickens fed supplemented diet with Echinacea when compared with the control group.

4- Effect of dietary supplementation with Echinacea on the antibodies titers of Newcastle and AI viruses' vaccine of broiler chickens:

Results shown in Table (4) revealed that chickens fed on *Echinacea* supplemented diet showed significant increase in antibody titer of ND and AI vaccines at 3rd and 4th

week post vaccination compared to the control group.

3-Effect of dietary supplementation with Echinacea on the innate immunity of broiler chickens:

5.1- phagocytic activity of peripheral blood monocyte:

The phagocytic activity of monocyte cells of chickens fed *Echinacea* supplemented diet Table (5) and Photo(1), showed significant increase in the phagocytic % at 4th and 6th week, and significant increase in phagocytic index at all over the experimental duration compared to the control group.

5.2- Fungicidal activity of the peripheral blood monocyte:

Fungicidal % of the PBM showed a significant increase in G2, *Echinacea* treated group, compared to control group at 2nd, 4th and 6th weeks (Table 6).

5.3- Nitric oxide production (μmol/ml) of the peripheral blood monocytes:

The data recorded in Table (6) revealed that, chickens fed diet supplemented by Echinacea (2) showed a significant increase in NO at 4th and 6th weeks compared with the control group.

5.4- Reactive oxygen species production (OD) of the peripheral blood monocytes:

By using nitro blue tetrazolium reduction test, ROS production of PBM of chickens fed *Echinacea* supplemented diet showed a significant increase on ROS

production at 6th weeks compared to control group (Table 6 and photo2).

6-Effect of dietary supplementation of broiler chickens with *Echinacea* on susceptibility to infections (challenge test):

Clinical signs of *E.coli* infection were observed on both challenged groups within 2-3 days post infection, including; fever, ruffling feather, weight loss, and diarrhea, which were more severe in control group than that fed *Echinacea* supplemented diet, morbidity rate was 80% in control group, while it

was (46.7%) in *Echinacea* in supplemented group. Mortality rate was (60%) in the control group (9/15 were died within 10 days post challenge), while it was (26.7%) chickens died in the *Echinacea* dietary supplemented group (4/15 were died after 10 days) as shown in Table (7).The results shown in Table (8) revealed that there was a significant reduction in *E. coli* count in the spleen, liver and intestine of chickens fed on supplemented ration with *Echinacea* when compared with the control group.

Table (1): Effect of dietary supplementation with *Echinacea* on growth performance of broiler chicks:

Group	Initial body weight (g)	Final body weight (g)	body weight gain(g)
Control	46.00± 0.67 ^a	1779.40±5.89 ^b	1731.79±5.05 ^b
<i>Echinacea</i>	46.91± 0.38 ^a	2220.97±46.87 ^a	2173.79±14.58 ^a

*Data are presented as (means ± S.E) *Means with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (2): Effect of dietary supplementation with *Echinacea* on total and differential leucocytic count in broiler chickens:

Group	TLC			Heterophils			Lymphocytes			Monocytes			Eosinophils		
	1week	2week	4week	1 week	2 week	4 week	1 week	2 week	4 week	1 week	2 week	4 week	1week	2week	4week
Control	23.87 ±0.85 ^b	24.46 ±0.85 ^b	24.78 ±1.17 ^b	820.00 ±31.75 ^b	820.00 ±57.16 ^b	775.3 ±21.62 ^b	1379 ±26.20 ^b	1445 ±44.10 ^b	1499 ±33.80 ^b	175.00 ±13.28 ^b	175.00 ±8.66 ^b	189.00 ±12.70 ^b	14.00 ±1.16 ^b	14.00 ±1.15 ^b	14.00 ±1.16 ^b
<i>Echinacea</i>	31.70 ±1.28 ^a	31.19 ±2.36 ^a	32.60 ±1.03 ^a	1106.00 ±44.46 ^a	1048.00 ±50.81 ^a	1178.3 ±57.16 ^a	1842 ±33.30 ^a	1866 ±86.20 ^a	1875 ±86.20 ^a	201.00 ±12.70 ^a	189.00 ±14.44 ^a	192.00 ±12.71 ^a	12.00 ±1.70 ^a	14.00 ±1.20 ^a	12.00 ±2.31 ^a

*Data are presented as (means ± S.E) *Mean with different superscript letters in the same column is significantly different at ($P \leq 0.05$).

Table (3): Effect of dietary supplementation with Echinacea on proteinogram of broiler chickens:

Parameters Group	Total protein (gm/dl) 1week	Total protein (gm/dl) 4 week	Albumin (gm/dl) 1 week	Albumin (gm/dl) 4 week	Globulin (gm/dl) 1 week	Globulin (gm/dl) 4week	A/G ratio 1 week	A/G ratio 4 week
Control	3.68 ± 0.30 ^a	4.54 ± 0.21 ^a	1.99 ± 0.12 ^a	2.08 ± 0.17 ^a	1.69 ± 0.06 ^a	2.46 ± 0.05 ^a	1.17 ± 0.05 ^a	0.85 ± 0.02 ^a
Echinacea	3.96 ± 0.16 ^a	4.91 ± 0.17 ^a	1.94 ± 0.12 ^a	2.00 ± 0.12 ^a	2.02 ± 0.07 ^a	2.91 ± 0.05 ^b	0.96 ± 0.03 ^b	0.69 ± 0.03 ^b

*Data are presented as (means ± S.E) *Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (4): Effect of dietary supplementation with Echinacea on the antibody titer (log₂ HI titer) against Newcastle and Avian influenza viruses at different ages:

Type	ND antibody				AIV antibody			
Group Age	1 week	2week	3week	4Week	1 week	2 week	3 week	4week
Control	4.26± 0.21 ^a	4.26±0.10 ^b	5.89±0.17 ^b	6.47±0.23 ^b	5.19± 0.23 ^a	2.26± 0.07 ^b	3.77± 0.32 ^b	4.81±0.21 ^b
Echinacea	4.06± 0.13 ^a	5.18± 0.07 ^a	6.77± 0.19 ^a	7.12± 0.16 ^a	5.73± 0.21 ^a	2.74±0.17 ^a	4.45± 0.16 ^a	6.18± 0.25 ^a

*Data are presented as (means ± S.E) *Mean with different superscript letters in the same column is significantly different at ($P \leq 0.05$).

Table (5): Effect of dietary supplementation of Echinacea on phagocytic %and phagocytic index of the peripheral blood monocytes of broiler chickens:

Type	Phagocytic %				Phagocytic index			
Age Group	1week	2week	4week	6week	1 week	2 week	4week	6 week
Control	55.00± 2.85 ^a	53.00± 2.66 ^a	57.00± 2.19 ^b	56.00± 1.54 ^b	3.10± 0.17 ^b	3.40± 0.17 ^b	2.90± 0.10 ^b	2.80± 0.09 ^b
Echinacea	54.00± 3.21 ^a	58.00± 2.07 ^a	68.00± 2.19 ^a	68.00± 2.29 ^a	4.50± 0.25 ^a	4.80± 0.21 ^a	4.60± 0.19 ^a	5.10± 0.21 ^a

*Data are presented as (means ± S.E) *Means with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (6): Effect of dietary supplementation with Echinacea on Fungicidal activity, Nitric oxide production and reactive oxygen species (ROS):

Test Age Group	Fungicidal %			NO($\mu\text{mol/ml}$)			ROS(OD)		
	2week	4week	6week	2week	4week	6week	2week	4week	6week
Control	43.00 $\pm 2.48^b$	53.00 $\pm 2.24^b$	52.00 $\pm 1.54^b$	23.90 $\pm 0.34^a$	32.10 $\pm 1.36^b$	29.50 $\pm 1.28^b$	0.034 $\pm 0.003^b$	0.038 $\pm 0.002^b$	0.037 $\pm 0.002^b$
Echinacea	76.00 $\pm 3.18^a$	81.00 $\pm 1.54^a$	89.00 $\pm 1.83^a$	20.40 $\pm 0.30^a$	36.00 $\pm 1.70^a$	36.90 $\pm 1.24^a$	0.057 $\pm 0.002^a$	0.049 $\pm 0.002^a$	0.061 $\pm 0.002^a$

*Data are presented as (means \pm S.E) *Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (7): Effect of dietary supplementation of Echinacea on mortality and morbidity rate of broiler chickens after challenge with E.coli:

Group Parameter	Control	Echinacea
Total No	15	15
Morbidity rate	12	7
Morbidity %	80%	46.7%
Mortality rate	9	4
Mortality %	60%	26.7%

Table (8): Effect of dietary supplementation of Echinacea on CFU of E.coli in involved organs of broiler chickens:

Group Organ	Spleen	Liver	Intestine
Control	$14 \times 10^6 \pm 577530^a$	$21 \times 10^3 \pm 11574^a$	$16 \times 10^6 \pm 1154700^a$
Echinacea	$6 \times 10^5 \pm 28867^c$	$7 \times 10^3 \pm 288.70^b$	$12 \times 10^3 \pm 577.35^b$

*Data are presented as (means \pm S.E) *Mean with different superscript letters in the same column is significantly different at ($P \leq 0.05$).

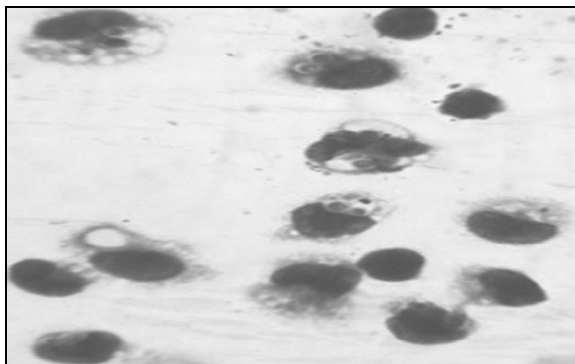


Figure (1): Effect of dietary supplementation of Echinacea on phagocytic activity :(Giemsa stain X 100).

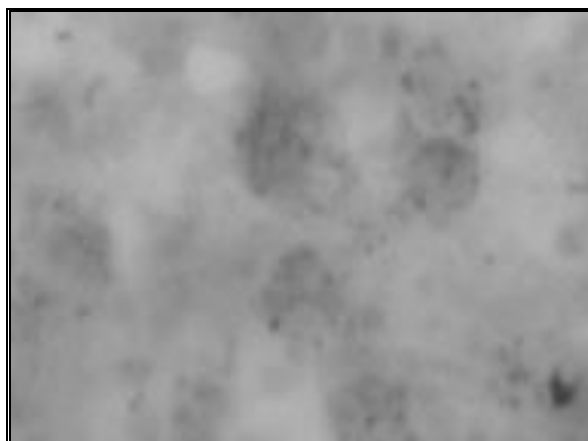


Figure (2): Peripheral blood monocytes cells of broiler chickens fed Echinacea supplemented diet have blue formazane deposit granules (Leishman's stain 100x).

Discussion

In the present work, *Echinacea* provided a significant improvement in the final body weight and body weight gain as shown in Table (1), these results are in agreement with the findings of (*Jahanian et al., 2014 and Maisa and Fatma, 2014*), other researchers (*Roth et al., 2005 and Böhmer et al., 2009*) reported that the using of *Echinacea* as a feed additive for broilers and layers is not beneficial for growth or layer

performance. The main causes for this discrepancy in recorded results are the plant origin, climate conditions, host species, the host health status and disease occurrence. (*Maass et al., 2005*). The mode of action of the herb mixtures on feed conversion is through the enhancement of the digestive functions (*Przybilla and Weiss, 1998*).

Concerning the leucogram of the broiler chickens, the results shown in

Table (2) revealed that the dietary supplementation of *Echinacea purpurea* resulted in a significant increase in total leucocytic counts as well as the neutrophils and lymphocytic count. These results are in agreement with those obtained by **Maisa et al. (2014)** who found a significant increase of lymphocytes in broiler chicks supplemented diet with *Echinacea purpurea* as 5 mg/kg feed and **Barbara et al. (2009)** who recorded significant increase in lymphocytes and total leucocytic count in groups receiving ethanolic juice of *Echinacea purpurea* for 5 days. *Echinacea purpurea* extract from root significantly increased in vivo the number of leucocytes and lymphocytes of rats (**Skaidickas et al., 2003**). Moreover, the various immune cells (macrophages, monocytes and natural killer cells) were stimulated in vitro by *Echinacea purpurea* extract (**O'Neill et al., 2002 and Cundell et al., 2003**).

Serum proteins were synthesized in liver so it used as indicators of metabolic and synthetic activity of liver. Enhancement of immunoglobulin production leads to increase in serum globulin contents, which represent status of immune health. Regarding the effect of *Echinacea* on the proteinogram of the treated boiler chicken as shown in Table (3) only treated group with *Echinacea* showed a numerical increase in serum total protein level which is attributed to a significant increase in serum globulin in the 4th

week. These findings are in agreement with those obtained by (**Nasir, 2009 and Miraghaee et al., 2011**) who reported that *Echinacea* application improved serum globulin concentration.

In order to evaluate the effect of dietary supplementation of *Echinacea purpurea* on humoral immune response of broiler chickens, antibody titers against ND and AI V-vaccines were measured. In the present work, broiler chicks fed supplemented diet with *Echinacea purpurea* showed significant increase in antibody titer against ND and AI as shown in Table (4). These findings are in accordance with that reported by (**Dehkordi et al., 2011; Landy et al., 2011**). Dried extract of *Echinacea purpurea* can stimulate the immune system and increase the immune response of Newcastle vaccination (**Feizi and Dadian, 2012**).

In the present study, as shown in (Table 5 and photo 1), there is a marked increase in the phagocytic % and index in experimental group compared to the control group. These results are in agreement with those obtained by **Allen (2003)**. The increase of phagocytic activity associated with *Echinacea* are exactly linked with its active ingredients including; polysaccharides, glycoproteins, and alkylamides of *E. purpurea* (**Barrett, 2003**).

In the present study (Table 6), the chickens in *Echinacea* treated group

showed a significant increase in the fungicidal % of PBM compared to control group. These results are in agreement with those obtained by **Hudson (2012)**. It was known that *Echinacea* extracts have a powerful antimicrobial activity (**Goel et al., 2002**).

Regarding to NO production (Table 6), chickens fed supplemented diet with *Echinacea* showed significant elevation in NO value at 4th and 6th weeks of age, This was agree with **Goel et al. (2002)** who reported that *Echinacea* stimulates macrophage function in the lung and spleen of normal rats, this is due to the effects of active components of *Echinacea* as cichoric acid, polysaccharides and alkylamides. Moreover, these finding are disagree with those obtained by **Tyler et al. (2012)** who reported that there were no significant effects following six weeks of *Echinacea* supplementation for nitrite ($\mu\text{mol}\cdot\text{L}^{-1}$).

In the present work, the nitro blue tetrazolium test (NBT) was used to investigate the effect of *Echinacea* on production of ROS by PBM, as showed in (Table 6), there is a marked increase in ROS at 2nd, 4th and 6th week compared to control group. These findings are agreed with those obtained by (**Zhai et al., 2007**), *Echinacea* Purified polysaccharides could enhance the production of oxygen radicals, proinflammatory cytokines and inflammatory mediators by macrophages and neutrophils.

In the present work, the effect of dietary supplementation of *Echinacea* on susceptibility to infections was investigated by challenge test as shown in Tables (7 and 8); the morbidity rate was (80.3%) in control group, while it was (46.4%) in *Echinacea* in supplemented group. The mortality rate was (60%) in the control, while it was (26.7%) chickens died in the *Echinacea* dietary supplemented group. The results revealed that there was a significant reduction in *E. coli* count in the spleen, liver and intestine of chickens fed on supplemented ration with *Echinacea* when compared with the control group. These findings are in accordance with that reported by (**Savage et al., 1996**) who reported that supplementation with oligosaccharides may have a prebiotic effect through an increase in production of lactic acid, thus increasing the proliferation of beneficial bacteria and reducing the presence of Gram-negative bacteria also **Sullivan et al. (2008)** found that oral administration with *Echinacea purpurea* reduces bacterial burden in the spleen of mice infected by *listeria monocytogenes* demonstrating its efficacy *in vivo*.

In conclusion, *Echinacea* might be used as growth promoters in broiler chickens as it improve body weight gain, stimulate leukocyte production, stimulate globulin production, stimulate humeral immunity by increasing the

antibody titer against ND and AIV vaccine as well as enhance the innate immunity by stimulating the PBM function as phagocytic activity, fungicidal activity and production of both of ROS and NO. *Echinacea* have the ability to protect broiler chickens from virulent as *E. coli*.

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تقييم تأثير نبات الاشنسيا كمعدل مناعي على الاستجابة المناعية فى دجاج التسمين محمد السيد عناني¹، عبدالعظيم محمد الجمال¹، رفيق توفيق سليمان²، أشجان فهمي السيسى³ وأسماء عبدالله حبيشي³

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معدلات المناعة الطبيعية توفر دور هام في رفع كفاءة الجهاز المناعي في الطيور بدون حدوث اثار جانبية كما يحدث في حاله استخدام المضادات الحيويه. من اجل تقييم تاثير نبات الاشنسيا كمعدل مناعي على الاستجابة المناعية فى دجاج التسمين , تم استخدام 120 ككتوت عمر يوم خلال مده التجربه 6 اسابيع وذلك لقياس تاثير مستخلص نبات الاشنسيا علي الجهاز المناعي في الدجاج وتم تقسيم هذه الطيور الي مجموعتان المجموعه الاولى المجموعه المرقيه تتغذي علي العليقه الاساسيه فقط , المجموعه الثانيه تم اضافته مستخلص نبات الاشنسيا الي العليقه. ثم بعد ذلك تم تقييم معدل النمو في نهايه التجربه و تم قياس عد الخلايا الدم البيضاء الكليه والنوعيه وتم قياس بروتينات الدم عن طريق قياس البروتين الكلى , الاليومين والجلوبيولين وتم قياس المناعه المكتسبه للطائر بقياس تركيز الاجسام المناعية بعد التحصين بلقاح النيوكاسل والانفلونزا . بعد ذلك تم قياس مناعة الجسم الطبيعيه للطائر وذلك بفصل خلايا الدم البيضاء وحيدته النواة ثم دراسته تاثير الاشنسيا علي قدره الالتهامية لها ضد فطر الكانديدا وقياس قدرة الخلايا علي انتاج المركبات الاكسجنية واكسيد النيتريك المستخدم في قتل الميكروبات. فى نهايه التجربه تم عمل اختبار التحدى عن طريق عمل عدوي معملية بالايشريشياكولاي لتقييم مقدره الطيور علي مقاومة الميكروب وتقييم معدل النفوق والاعتلال بين المجاميع. بعد انتهاء التجربه وجد ان مستخلص الاشنسيا عمل علي تحسين معدل نمو الطيور , كما ساعد علي رفع مناعة الطيور الطبيعية والمكتسبة وبعد عمل اختبار التحدى وجد ان مستخلص الاشنسيا ساعد في تقليل معدل الاصابة بالعدوى ونفوق الطيور.