

EFFECT OF *ECHINACEA PURPUREA* AND GARLIC ON GROWTH PERFORMANCE, IMMUNE RESPONSE, BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN BROILER CHICKS

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ABSTRACT

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This study was conducted to investigate the effect of *Echinacea purpurea* and garlic on growth performance, immune response, biochemical and hematological parameters in broiler chick. A total of (180) chickens were divided into 3 main groups A,B and C. First group as control group received only commercial basal diet, second group was fed on commercial basal diet plus *Echinacea purpurea* as 5 mg/kg feed and the third group was fed on commercial basal diet plus garlic powder as 1 g/kg feed. All the other conditions were the same for all the groups. At 21th days of age, each group was subdivided into two groups as group A: subgroups G1 and G2, group B: subgroups G3 and G4 and group C: subgroups G5 and G6. Groups G2, G4 and G6 were challenged with 0.5 ml *E. coli* O78 (4×10^6 CFU) by intraperitoneal route. Performance, immunity system and some blood parameters were measured. As well as clinical signs, postmortem and mortality were recorded. Experimentally infected and treated chickens with *Echinacea purpurea* and garlic powder displayed less symptoms with the mortality rate of 16.66 % (5/30) in comparison to infected non treated group with mortality rate of 40% (12/30). The postmortem lesions were reduced to greater extent as compared to infected untreated birds. There were significant differences in *Echinacea purpurea* and garlic treated groups with control in body weight gain, feed conversion ratio from performance, heterophils/lymphocytes ratio, lymphocyte stimulation index, albumin and protein differentiation from immune system, and triglyceride from biochemical parameters. Cholesterol level was lower in garlic and *Echinacea purpurea* groups. According to the results, *Echinacea purpurea* and garlic can be used as a good alternative for commercial antibiotics, growth promoter and immune stimulant feed additives.

Key words: *Echinacea purpurea*, Garlic, Immune response, Broiler chicks.

INTRODUCTION

For the past several decades, different strategies have been applied to improve poultry productivity and profitability. The most important of them were always directed towards maintaining health, reducing disease outbreak and improving general immunity. Antibiotics growth promoter feed additives have been successfully used at sub therapeutic doses in poultry production to protect health and maximize the efficiency of production, product quality, to control diseases and the genetic potential of poultry (Landy *et al.*, 2011).

However, their use of Antibiotics as growth promoters has been completely banned throughout Europe and alternative strategies are now needed. In this context, there has been increased interest in the

use of biological products, including enzymes, probiotics, prebiotics, synbiotics, organic acids and plant extracts (phytobiotics), as alternatives to antibiotic feed additives in diets for monogastric animals (Bedford, 2000 and Wenk, 2003).

Phytobiotics are plant-derived natural bioactive compounds that can be incorporated into diets in order to enhance the performance and well-being of animals. One of the most important Phytobiotic and popular medical herb is *Echinacea purpurea* [coneflower] (Barrett, 2003). This herbal medicine has been used from long time ago for a variety of purposes including treatment, growth enhancement and immunostimulation (Percival, 2000).

The positive effects of herbal plants on broilers have been reported by many studies (Tekeli *et al.*, 2006).

Their anti-biotoxic potential, hypocholesterolemic effects, growth promoting and availability are the most beneficial part of herbs, which have drawn the scientists' attention. Garlic (*Allium sativum*), has been approved scientifically for use as anti-atherosclerosis, anti-microbial, hypolipidemic, anti-thrombosis, anti-hypertension, anti-diabetes. Garlic has ajoene, S-allyl cysteine, di allyl (di/tri) sulphide and the most active one allicine (Rahmatnejad *et al.*, 2009). Allicine possibly reduces LDL, triglyceride and cholesterol in serum (Alder and Holub, 1997), hence been used for cardiovascular diseases (Tanamai *et al.*, 2004).

The medicinal herb *Echinacea purpurea* L. (E.P) is commonly known as an immune stimulating substance which is most widely used as herbal medicines throughout Europe and North America for the prevention or treatment of infectious diseases (Akhtar *et al.*, 2003 and Nasir and Grashorn, 2006). *Echinacea* and its different derivatives contain a variety of active substances like alkaloids, glycoproteins, phenolic compounds, cinnamic acid, essential oil and flavonoids (Barrett, 2003). These substances are effective in treatment of various ailments and proved to be beneficial in improving immunity (Bauer, 1999). However, there have been few reports on the effect of the herb in chickens.

So, the present study aimed to: investigate the supplementation of garlic and *Echinacea purpurea* in diet on broiler chickens to study their possible effect

on performance, immune system and some biochemical blood parameters.

MATERIALS and METHODS

1-Animals and dietary treatment: 180, one day old chicks were purchased from Ismailia/Misr poultry farm, individually weighted, wing banded and randomly divided into 3 main groups.

Group A: was used as a control group and fed commercial basal diet.

Group B: was fed commercial basal diet plus (5 mg/kg feed) *Echinacea purpurea* (Immunvita-EMA pharm pharmaceutical co.) (Landy *et al.*, 2011).

Group C: was fed commercial basal diet plus (1 g/kg feed) garlic powder (Kamal and Kassab, 2009) At day 21, each group was subdivided into two groups as:

group A: group1(G1) and group2(G2), group B: group3(G3) and group4(G4) and group C: group5(G5) and group6(G6). Group of 2,4 and 6 were challenged with 0.5 ml 24 hrs broth culture of field strain of *E. coli* O78 serotype containing 4×10^6 colony-forming units by intraperitoneal route. The experiment design was illustrated in table (1):

Table 1: Showing design of experiment

Day1 N=180	Group A Commercial ration alone		Group B Commercial ration 5mg/kg+ Echinacea purpurea		Group C Commercial ration + garlic powder 1 g/kg	
Number of chickens	60		60		60	
Commercial ration	+		+		+	
Echinacea purpurea 5mg/kg	-		+		-	
garlic powder 1 g/kg	-		-		+	
Day 21	Group1	Group2	Group3	Group4	Group5	Group6
Number of chickens	30	30	30	30	30	30
<i>E. coli</i> inoculation 4×10^6 colony-forming	-	+	-	+	-	+

* Five chickens from each group were subjected for postmortem examination at 2days post infection.

The environmental conditions were equal for every group. Vaccination schedule were carried out against Newcastle disease, Avian influenza, Infectious Bronchitis and Infectious Bursal Disease at recommended age and dose.

2- Live body Performance:

Body weight was measured in 14th, 28th and 42th day of experiment. Feed consumption, feed conversion

ratio and mortality rate were determined and calculated.

3- Blood samples:

Blood was collected from wing vein from 10 birds of each group at 7 and 14 days post infection and divided as follow:

EDTA blood: Blood was collected in clean dry bottle containing dipotassium salt of EDTA as

anticoagulant in concentration of 2mg/1ml of blood (Jain, 2000) for hematological studies.

Serum: blood was collected in plain clean well-dried centrifuge tube and used for separation of serum to be used in estimation of biochemical parameters.

4- Hematological analysis:

Total leucocytic (WBCs $10^3/\mu\text{l}$) and differential leucocytic counts were determined according to routine hematological examination and standard blood smear (Jain, 2000). After that, estimation of Heterophil/Lymphocyte ratio (H/L) by follow formula: H/L= Percent of Hetrophil in blood film /Percent of Lymphocyte in blood film.

5- Serum biochemical analysis:

The collected sera were assayed for serum biochemistry. The level of alanine aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel (1957), creatininewas determined according to Henry (1979), uric acid (Caraway, 1963), total serum protein (Henry, 1964), albumin (Doumas, 1971), cholesterol and triglyceride were determined by using ready-made kits from (BioMeriux –France) by Auto analyzer Hitachi 912.

6- Immunologicalstudies:

a- Weight of lymphoid organs index: Five birds were randomly selected from each subgroup at 42 days of age; slaughtered and lymphoid organs (thymus, spleen and Bursa of Fabricius) were dissected out, weighed and calculated according to the formula of (Halouzka and Jurajda, 1991).

b- Protein electrophoresis: was done using SDS-Polyacrylamide gel electrophoresis according to Laemmli (1970).

c- Lymphocyte proliferation assay: Lymphocyte blastogenesis was applied to estimate the effect of *Echinacea purpurea* and garlic alone or in combination with *E. coli* infection on chicken lymphocyte proliferation. Briefly, the heparinized blood was layered carefully on the surface of lymphocyte separation medium, Ficollhpaque (1:1) in a 5 ml sterile centrifuge tube, then centrifuged for 40 min at 2400 r.p.m. at 4 °C and the separated Buffy coat was washed with RPMI-1640 medium three times each for 10 min at 2400, 2000 and 1500 r.p.m., respectively. The sediment washed lymphocytes were re-suspended in one ml of RPMI-1640 medium containing 10 % fetal calf serum. Then to 100 μl of lymphocytes suspension, 100 μl of 0.4% trypan blue were added. The number of viable lymphocytes were adjusted at a final concentration of 2×10^6 cells/ml according to (Hanks and Wallace, 1985 and Husdan and Hay, 1980) and suspended in RPMT medium containing 10 % fetal calf serum. The

phytohaemagglutinin (PHA) was reconstituted in RPMI-medium and the required concentration could be made to 10 $\mu\text{g}/\text{ml}$, then filtered through millipore filters (0.22 μm pore size, Millipore, USA) and store at - 20 °C. Flat bottom sterile tissue culture plates with 96 wells were used as follows: 3 wells containing 100 μl of suspended lymphocytes (2×10^6 cells) in 50 μl growth media (RPMI + 10 % fetal calf serum) served as cell control, 3 wells containing 100 μl of suspended lymphocyte + 50 μl of PHA mitogen (15 $\mu\text{l}/\text{ml}$) and 3 wells containing 150 μl of RPMT-1640 medium (medium control), then the plates were incubated at 37°C in a humid CO₂ atmosphere (5-10% CO₂), then blastogenesis was assayed after 48-72 hrs using residual glucose in the supernatant of tissue culture medium according to Decock *et al.* (1980).

7- Statisticalanalysis: After obtaining the data, they were analyzed by variance method (ANOVA) considering $P < 0.05$ using SPSS 18.0 software (2009). The significant differences were taken to Duncan multiple range tests to compare the means.

RESULT

Experimentally infected non treated chickens of group (G2) showed clinical signs of restlessness, ruffled feathers, loss of appetite, mouth breathing and sneezing 24hrs post infection. At autopsy of diseased chicks after 48 hour post infection, showed several gross pathological lesions such as airsacculitis, severe lung congestion, pericarditis, perihepatitis with kidney and liver congestion and general signs of septicemia with mortality 40% (12/30). The birds experimentally infected and treated with *Echinacea purpurea* and garlic powder displayed less clinical symptoms with the mortality rate 16.66 % (5/30). The postmortem lesions were reduced to greater extent as compared to infected untreated birds. The severity of signs was reduced in all infected and treated groups than infected untreated ones.

Table (2) shows the effect of different dietary supplements on performance of broiler chickens. The result showed that all treatments have better final result in comparison with control treatment. Live body weight was significantly different among all experimental groups at different age periods. At 42days of age the heaviest body weight ($P \leq 0.05$) was recorded in group 3 and group 5. The mean body weight of group 2 was significantly decreased ($P \leq 0.05$) from all other groups. The highest amount of FI and WG was seen in the group 3 in comparison with the infected group 2 and infected treated groups 4 & 6. The results of live body weight and feed intake are normally a reflection to FCR, therefore, at 1- 42 days of age, *Echinacea purpurea* and garlic powder recorded the best FCR (1.19) among all groups.

Table (3), showed that results of immune organs weight. In respect to Thymus Weight/body Weight (TW/BW), Spleen Weight/Body Weight (SW/BW) and Bursa Weight/Body Weight (BW/BW), the significant differences were found only in SW/BW and BW/BW. The means of spleen weight showed significant differences between the groups. The mean weight ratio of group1, group3, group4, group5 and group6 were highly significant in comparison withgroup2. In respect to bursal weight, the highest ratio was recorded in group3, group4, group5 and group6in comparison with group1 and group2.

Table (4), showed the results of hematological findings, at 1st week (leucocytosis, heterophilia, monocytosis, eosinophilia, significant increase in H/L ratio and lymphocyte stimulation index) in infected non treated and infected treated groups while birds which fed on *Echinacea purpurea* and garlicpowder showed a significant increase in lymphocytic count at 7 day. At 14 days post infection revealed significant increase in lymphocytic count in group2, group3and group5 and significant increase in lymphocyte stimulation index in infected treated groups and non-infected treated groups, while in group 2 significant increase in total leucocytic count all over the observation period.

In table (5) there were significant increases in transaminase ALT and AST enzymes in group2, group4 and group6 as well as hypercholesterolemia from first week were reported. While hypocholesterolemia was observed in *Echinacea purpurea* and garlicpowdertreated groups. The *E. coli* infected non treated birds displayed a significant increase in serum uric acid and creatinine levels all over the experiment. The chicks fed on *Echinacea purpurea* and garlicpowder (group 3 and group 5) induced significant decrease in serum uric acid and creatinine levels at 7th days post infection as compared with infected non treated group (group2) and infected treated groups (group4 and group6). On the other had founded non-significant changes in uric acid and creatinine in all treated groups (group3, group4, group5, and group6) at 14th days post infection in comparison to infected non treated group (group2). Table (6) Protienogram examination revealed hypoproteinemia, hypoalbuminemia, hyperglobulinemia (hyperalphanaglobulinemia, hyperbetaglobuliemia and hypergammaglo-buliemia) during the observation period in group 2 at 1st week post infection as well as non-significant changes in total protein, albumin and globulin at 2nd week after infection in all groupsbut significant increase in gamma globulin in group 3 and group 5 which treated by *Echinacea purpurea* and garlicpowder.

Table 2: The effect of dietary supplements of *Echinacea purpurea* and garlic powder on body weight, weight gain, feed intake, feed conversation ratio and mortality rates of broilers supplemented.

Groups Time	Group A		Group B		Group C		SEM
	G1	G2	G3	G4	G5	G6	
Body Weight.(BW)							
14 d	290.4 ± 3.3b	290.4 ± 3.3b	300± 2.4a	300± 2.4a	301.8 ±3.5a	301.8 ±3.5a	5.99
28d	990.5± 4.5a	940.5± 4.2d	994.4± 5.2a	960 ± 7.2b	990.0± 3.5a	965 ± 6.2b	17.4
42	1928.0± 3.5b	1890 ± 6.5d	2086± 5.7a	1930± 5.9bc	2074.5± 6.8a	1935 ± 7.1b	34.7
Weight Gain (g per bird per day).(DWG)							
14d	17.76± 0.28a	17.76± 0.28a	19.5±0.45a	19.5±0.45a	20.84±0.58a	20.84±0.58a	2.5
28d	69.04±0.38a	58.4±0.5b	69.64±0.61a	60.1±2.9b	69.88± 0.53a	60.3±7.2b	0.12
42d	91.1± 2.1b	89.1± 2.3b	99± 3.5a	90.2± 3.5b	94.3± 5.3a	92.2± 4.3b	1.5
feed intake(g/ bird/day)(DFI);;							
14d	29.2± 3.2b	29.2± 3.2b	36.8± 3.4a	36.8± 3.2a	34.9± 4.2a	34.9± 4.2a	2.2
28d	148± 4.6b	125± 6.2d	168± 5.1a	135± 4.7c	165± 6.2a	136± 6.4c	3.0
42d	109±5.6a	95 ± 3.2b	117.6±2.4a	110.1±4.6a	115.0±1.5a	109.5±6.4a	4.3
Feed Conversation Ratio (g/g)(FCR)							
14d	1.64± 1.5b	1.64± 1.5b	1.88 ± 2.2a	1.88± 2.2a	1.67± 2.5b	1.67± 2.5b	0.13
28d	2.14± 2.4c	2.14± 1.8c	2.41± 2.4a	2.25± 1.5b	2.36± 2.7a	2.26± 3.0b	0.10
42d	1.2 ± 2.1a	1.07± 1.7b	1.19± 1.6a	1.22± 1.5a	1.19± 2.0a	1.19± 1.4a	0.08
Percent of Mortality							
1-42d	2%	40%	2%	16.66%	2%	16.66%	

Means (± S.E) with different superscript (a,b,c,d) within the same column are significantly different at p<0.05

Table 3: Effects of *Echinacea purpurea* and garlic powder supplemented in diet on immune organs weight index

Groups	TW/BW	SW/BW	BW /BW
G1	0.22±0.01 a	0.13±0.01b	0.19±0.02b
G 2	0.22±0.01 a	0.12±0.01c	0.16±0.01c
G3	0.22±0.02a	0.14±0.01a	0.22±0.01a
G4	0.22±0.01 a	0.14±0.01 a	0.22±0.02a
G5	0.22±0.02a	0.14±0.01a	0.21±0.02a
G 6	0.22±0.02 a	0.14±0.01 a	0.21±0.01a
L.S.D 0.05	0.2	0.1	0.2

TW/BW; Thymus weight/Body weight, SW/BW; Spleen weight/Body weight, BW/BW; Bursa weight/Body weight. a, b and c different manuscripts indicated significant differences (PB 0.05)

Table 4: Leucogram & lymphocyte stimulation index in infected groups by *E. coli* and treated groups by *Echinacea purpurea* and garlic powder after one and two weeks. (n=10)

Parameters Time	Parameters groups	Total leucocytic count x 10 ³ /μl	Lymphocyte x 10 ³ /μl	Heterophils x 10 ³ /μl	Monocytes x 10 ³ /μl	Eosinophils x 10 ³ /μl	H/L ratio	Lymphocyte stimulation index
1 st week	G1	21.15± 1.6c	10.40±1.13b	9.70±1.16b	0.45± 0.3b	0.60±0.2b	0.9± 1.2b	1.14±0.1b
	G2	32.35± 1.5a	11.46± 2.4a	18.87±1.14a	1.32±0.4a	0.70±0.5a	1.6± 1.1a	1.14±0.13b
	G3	28.3±1.6b	18.18±1.2b	9.12±1.01b	0.35±0.4b	0.63±0.3b	0.5± 1.3b	1.65±0.2a
	G4	30.36 ±1.4a	10.5±2.3a	17.8± 3.2a	1.4±0.7a	0.76±0.4a	1.7±0.9a	1.18±0.4b
	G5	26.12±1.3b	17.1±1.5b	8.0±1.2b	0.4±0.5b	0.62±0.2b	0.5±0.8b	1.67±0.3a
	G6	30.68±1.2a	11.25±1.3a	17.7±1.9a	1.05± 0.5a	0.78±0.3a	1.6 ±1.0a	1.18±0.23b
	L.S.D 0.05	3.5	2.5	3.3	0.48	0.05	0.5	0.45
2 nd week	G1	12.26± 0.8b	9.30±1.2c	2.55± 1.3a	0.25± 0.3a	0.16±0.2a	0.27±0.1a	1.20±0.1b
	G2	18.60± 0.5a	15.2±1.3a	2.9± 1.01a	0.27±0.4a	0.23±0.5a	0.19±0.05a	1.14±0.13b
	G3	12.90±1.3b	10.1±1.01b	2.50±0.9a	0.25±0.5a	0.15±0.3a	0.25±0.3a	1.35±0.2a
	G4	12.67±1.7b	9.20± 3.2c	2.60±2.3a	0.35±0.7a	0.22±0.4a	0.28±0.04a	1.34±0.4a
	G5	12.66±1.1b	10.20±1.6b	2.40±1.5a	0.30±0.5a	0.16±0.2a	0.24±0.2a	1.37±0.3a
	G6	12.05±1.2b	9.12 ±1.9c	2.35±1.3a	0.35± 0.5a	0.23±0.3a	0.26±0.1a	1.34±0.23a
	L.S.D 0.05	1.5	1.5	1.4	0.48	0.32	0.12	0.11

Means (± S.E) with different superscript (a,b,c,d) within the same column are significantly different at p<0.05

Table 5: Some biochemical parameters in infected groups by *E. coli* and treated groups by *Echinacea purpurea* and garlic powder.

Parameters	Parameters	ALT	AST	Uric acid	Creatinine	Cholesterol	Triglyceride
		u/l	u/l	Mg/l	Mg/l	Mg/l	Mg/l
Time	Groups						
1 st week	G1	5.0± 1.5b	99.2±21.3b	7.9±0.4b	0.52±0.05b	122.5± 2.3b	114.5±13.2a
	G2	8.4± 0.8a	119.0± 23.3a	14.2±1.3a	0.71±0.05a	154±0.4a	115.3±0.5a
	G3	4.07±1.3b	101.4±20.1b	8.1±1.01b	0.54±0.3b	115±0.3b	110.2±0.3b
	G4	8.2 ±0.7a	116.0±21.3a	13.5± 3.2a	0.75±0.04a	148±0.7a	110.5±0.4b
	G5	4.1±0.3b	100±22.1b	8.5±1.6b	0.52±0.2b	112.3±0.5b	109.2±0.2b
	G6	8.3 ±1.2a	115.5±21.1a	13.6 ±1.9a	0.70±0.1a	147± 0.5a	109.5±0.3b
	L.S.D	0.05	2.3	9	1.2	0.12	17
2 nd week	G1	15.0± 1.9a	115.2±6.3b	4.4±0.3b	0.42±0.2b	115.5± 3.2b	71.8±2.1a
	G2	19.9± 0.7b	140.0± 8.6a	7.1±0.2a	0.55±0.2a	135.8±5.4a	76±5.0a
	G3	15.4±0.6a	117.4±1.1b	4.1±1.01b	0.44±0.1b	96.5±1.2c	61.2±3.4b
	G4	16.2 ±0.7a	126.0±1.3b	4.3± 1.2b	0.45±0.2b	96.0±0.7c	63.2±5.5b
	G5	14.1±0.3a	116.7±2.1b	4.5±1.6b	0.42±0.1b	92.2±0.5c	60.2±6.2b
	G6	16.3 ±1.2a	125.1±1.1b	4.7 ±1.9b	0.45±0.2b	97.0± 0.5c	65±3.5b
	L.S.D	0.05	3.5	11	1.8	0.05	7

Means (± S.E) with different superscript (a,b,c,d) within the same column are significantly different at p<0.05

Table 6: Protienogram on broiler chicks experimentally infected with *E. coli* and infected treated groups

Parameters	Parameters	Total protein	albumin	Globulin	Beta globulin	Alpha globulin	Gamma globulin	A/G ratio
		gm/dl	gm/dl	gm/dl	gm/dl	gm/dl	gm/dl	
Time	Groups							
1 st week	G1	2.69 ±0.03a	2.1±0.04a	0.59±0.02b	0.18±0.05b	0.16± 0.03b	0.25±0.03c	3.56±0.01a
	G2	2.15±0.1b	1.38±0.02b	0.87±0.01a	0.23±0.02a	0.27±0.04a	0.37±0.02b	1.58±0.03b
	G3	2.73 ±0.03a	2.1±0.01a	0.75±0.01a	0.16±0.01b	0.15±0.05b	0.44±0.02a	3.33±0.2a
	G4	2.34±0.1b	1.55±0.05b	0.79±0.02a	0.18±0.05b	0.26±0.03a	0.35±0.01b	1.96±0.4b
	G5	2.96 ±0.03a	2.2±0.01a	0.76±0.03a	0.17±0.03b	0.15±0.02b	0.44±0.01a	3.49±0.3a
	G6	2.34±0.02b	1.57±0.05b	0.77 ±0.04a	0.16±0.05b	0.25±0.05a	0.36±0.02b	2.04±0.23b
	L.S.D	0.05	0.3	0.5	0.12	0.3	0.8	0.5
2 nd week	G1	3.91± 0.13a	2.13± 0.05a	1.78±0.2a	0.64±0.1a	0.47± 0.03a	0.67±0.02b	1.14±0.01a
	G2	3.80± 0.15a	1.94± 0.04a	1.86±0.3a	0.67±0.05a	0.49±0.04a	0.70±0.05b	0.88±0.03a
	G3	3.94±0.3a	2.1 ±0.09a	1.84±0.01a	0.55±0.3a	0.41±0.01a	0.88±0.03a	1.04±0.2a
	G4	3.86±0.33a	2.04±2.3a	1.82± 0.2a	0.59±0.04a	0.46±0.04a	0.80±0.04b	0.96±0.4a
	G5	3.96±0.3a	2.3±0.04a	1.86±0.6a	0.56±0.2a	0.43±0.05a	0.87±0.02a	0.82±0.3a
	G6	3.80±0.16a	2.2±13a	1.80 ±0.2a	0.58±0.01a	0.42± 0.05a	0.80±0.03b	0.96±0.23a
	L.S.D	0.05	0.43	0.31	0.3	0.15	0.16	0.07

Means (± S.E) with different superscript (a,b,c,d) within the same column are significantly different at p<0.05

DISCUSSION

Phytogenic feed additives (PFA) (often also called as phytobiotics or botanicals) are plant-derived products, used in animal feeding to improve performance of agricultural livestock through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (Windisch *et al.*, 2008). Echinacea is popular herbal immune-stimulator in North America and Europe, while for their beneficial effects on immune system and as natural remedy in many diseases (Nasir and Grashorn, 2009).

Concerning, the effect of supplementation of *Echinacea purpurea* and garlic in diet on body performance, the results showed a significant improvement ($P \leq 0.05$) in live body weight, weight gain and feed intake and feed conversion rate through 5 weeks in *Echinacea purpurea* and garlic powder feeding groups. This improvement of feed conversion ratio with feeding *Echinacea purpurea* is in agreement with the findings of Maass *et al.* (2005) who reported that *Echinacea purpurea* botanicals (herbs and/or spices), supplementation as feed additive improved feed conversion. Gbenga *et al.* (2009) found that supplementation of chicken diets with garlic marginally improved weight gain and it was better at high level of supplementation (5g/kg diet). Meanwhile, there are some controversies on the effect of *Echinacea purpurea* on feed conversion where, Ma *et al.* (2009) reported that *Echinacea purpurea* extract significantly lowered the feed conversion efficiency in broilers.

Birds fed *Echinacea purpurea* and garlic in diet and experimentally challenged with *E. coli* developed less response in the form of clinical signs, morbidity and mortalities in comparison to infected non treated group, this may be due to inhibitory effect of garlic and *Echinacea purpurea* in colonization of pathogenic *E. coli* which agreed with Rahimi *et al.* (2011) who found that feeding garlic and *Echinacea purpurea* in diet reduce the intestinal bacterial populations in broiler chickens and the colony forming units of *Escherichia coli* in digesta of ileo-cecum. In the same context, the fighting ability of chickens against infection may be regarded to immunostimulatory effects of garlic and *Echinacea purpurea* which confirmed in immunological investigations in this study. Antibacterial activity of garlic is due to the inhibition of succinic dehydrogenase via the inactivation of thiol group (Jonkers *et al.*, 1999). Also Allen (2003) and Mass *et al.* (2005) showed that dried extract of *Echinacea purpurea* can stimulate the immune system and increase the immune response.

Birds infected and non-treated developed decrease in growth rate, body performance and feed conversion

in addition to mortality 40% and morbidity in the form of airsacculitis, severe lung congestion, pericarditis, perihepatitis with kidney and liver congestion and general signs of septicemia. These results agreed with Russell (2003) and Ask *et al.* (2006) reported that colibacillosis had adverse effects on growth and health, growth retardation being the main problem, reduced eating behavior leading to reduce feed intake.

Echinacea purpurea and garlic powder groups showed an elevated ratio of the organs weight/ body weight in comparison to the infected non treated group. This indicates that these two herbs had an immune-stimulating effect against *E. coli* infection when comparing the results of pathological lesions with the organ weights. It may be suggested that *Echinacea purpurea* and garlic were antibacterial and immunogenic agent (Nasir and Grashorn, 2006 and Nasir and Grashorn, 2008). Also, Mahmood (2006) reported that the highest ratio of SW/BW and BW/BW was recorded in groups containing 1% garlic powder in the feed of broiler chickens.

Concerning the hematological results that there were clear at 1st week post infection, leucocytosis, heterophilia, monocytosis and eosinophilia, in infected non treated and infected treated groups, while birds which treated with *Echinacea purpurea* and garlic powder showed a significant increase in total leucocytic count, lymphocytic count and lymphocyte stimulation index. At 14 days post infection revealed significant increase in lymphocytic count in group 2, group 3 and group 5 and significant increase in lymphocyte stimulation index in infected treated groups (group 4 & group 6) and non-infected treated groups (group 3 & group 5). The significant increase in lymphocytes might indicate the specific and nonspecific immune stimulant role of *Echinacea purpurea* and garlic. This is in agreement with Cundell *et al.* (2003) who found a significant increase of lymphocytes in rats fed with dried *Echinacea* preparations. Bohmer *et al.* (2009) reported that ethanolic juice of *Echinacea purpurea* increased the number of lymphocytes and total leucocytes in hens and pigs. While in group 2 significant increase in total leucocytic count at 14 days after infection. This suggestion is confirmed by those found by Fraser *et al.* (1991) who stated that leucocytosis, lymphocytosis and monocytosis might be associated with infection.

The results of Hetrophil (H) / Lymphocyte (L) ratio showed that significant differences were evident between groups. Birds in groups (group 2, group 4 and group 6) had the highest ratio at 1st week after infection and the lowest H/L ratio was recorded in the *Echinacea purpurea* group (group 3) and Garlic group (group 5) which suggests that *Echinacea purpurea* and Garlic are good anti stress factors when

added. This result agreed to some extent with Kamal and Kassab (2009). Many workers reported that Hetrophil/Lymphocyte (H/L) ratio was an excellent indicator for stresses in chickens (Gross and Siegal, 1983; Kassab *et al.*, 2000; Mahmood, 2006 and Abuzeed, 2008). The stress may be caused by pathological, nutritional or environmental factors (Al-Murrani *et al.*, 1997 and Amedy, 2008).

The results of biochemical tests indicated that a significant increase in transaminase ALT and AST enzymes were noticed in infected non treated group (group2) all over the experimental period while in infected treated groups (group4 and group6) at 1st week after infection. This increased in serum AST level has been associated with hepatocellular damage in chickens, turkeys and ducks as described by Campbell and Coles (1986) and Thrall *et al.* (2004). The *E. coli* infected chicks treated either with *Echinacea purpurea* or garlic denoted a significant decrease in serum AST and ALT values as compared with infected untreated group (group2) at 2nd weeks after infection. These findings may be attributed to the antioxidant effect of *Echinacea purpurea* as stated by Zhai *et al.* (2007) and Nasir and Grashorn (2009) who reported that *Echinacea purpurea* is an important herb rich in phenolic compounds with antimicrobial and antioxidant activity. The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure which can play an important role in neutralizing free radicals, chelating transitional metals and quenching singlet and triplet oxygen by delocalization or decomposing peroxides. Also, Jackson *et al.* (2002) who reported the antimicrobial effect of garlic and the antioxidant activity in vivo and in vitro.

From the previously mentioned results which indicates that *Echinacea purpurea* do not have any negative effect on liver functions and activity of different enzymes (Nasir and Grashorn, 2008). Similarly, no significant treatment effect on liver enzymes was observed by Maass *et al.* (2005) by in-feed application of *Echinacea purpurea* preparations to pigs. This shows that *Echinacea purpurea* supplementation has obviously no harmful effect on health of broilers.

The *E. coli* infected non treated birds displayed a significant increase in serum uric acid and creatinine levels all over the experiment. These findings are close to those of Mellata *et al.* (2003) who stated that avian pathogenic *E. coli* belongs to extra intestinal pathogenic group of *E. coli* which causes septicemia in poultry. The chicks fed on *Echinacea purpurea* and garlic powder (group 3 and group 5) induced significant decrease in serum uric acid and creatinine levels at 7th days post infection as compared with infected non treated group (group2) and infected

treated groups (group4 and group6). On the other hand founded non-significant changes in uric acid and creatinine in all treated groups (group, group, group and group 6) at 14th days post infection in comparison to infected non treated group (group2). These results mean improvement in body health of chicks. These improvements may be owing to the kidney protective effect of *Echinacea purpurea* and garlic owing to its antioxidant effect of its phenolic compounds content.

The serum total cholesterol and Triglycerides concentration were significantly reduced by dietary with *Echinacea purpurea* and Garlic compared to the control group. Ologhobo *et al.* (2008); Metwally (2009) and Mansoub and Nezhady (2011) reported that Garlic has reducing effect on triglyceride and cholesterol level and the best result was obtained in 2 % of garlic in the commercial basal diet.

The *E. coli* infected non treated and infected treated groups provoked a significant decrease in serum total protein, albumin levels, as compared with control group. These results indicated hepatic damage because liver is responsible for the production of a great proportion of plasma protein (Coles, 1986). The infected and treated with *Echinacea purpurea* and Garlic elicited non-significant changes in total proteins, albumin and globulin at 2nd week post treatment as compared with group1; meanwhile these changes are non-significantly increased when compared with infected non medicated group. These results indicating improvements in hepatic functions because of hepatoprotective effect of *Echinacea purpurea* and Garlic owing to the antioxidant effect of its higher contents of phytochemicals as previously discussed. Also, there was significant increase in serum alpha, beta and gamma globulins of infected non treated group compared to the control group. While there was significant increase in serum alpha and beta globulins in infected treated groups with *Echinacea purpurea* and garlic compared to control group (group1) at 1st week post infection. Also, showed that significant increase in serum gamma globulins of treated (*Echinacea purpurea* group and garlic group) non infected group compared to the control group at 1st and 2nd weeks. This result may be due to effect of *Echinacea purpurea* group and garlic group on immunity. An elevation in the alpha and gamma globulins usually indicates activation of the immune system and is most often due to infection or inflammatory diseases (Butler, 1983). However, *Echinacea purpurea* fermented juice improve health and immunity of the birds by improving serum globulin contents and stabilizing serum creatine kinase activities (reducing the risk of sudden death syndrome) (Nasir and Grashorn, 2010). Albumin, which is synthesized only in the liver, is the main responsible for maintaining the oncotic blood pressure; it may occur hypoalbuminemia in

cases of hepatic insufficiency, malnutrition and gastrointestinal disturbances within other conditions (Meyer *et al.*, 1995).

In conclusion *Echinacea purpurea* and garlic powder feed has an antibacterial effect against *Escherichia coli*, reduces stress, immunostimulant and improve body performance in broiler chickens.

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تأثير حشيشة القنفذ الارجوانية والثوم على أداء النمو والاستجابة المناعية والقياسات البيوكيميائية والدموية في بداري التسمين

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أجريت هذه الدراسة لدراسة أثر حشيشة القنفذ الارجوانية (بوربوريا يشيناسيا) والثوم على مستوى الاداء فى النمو والاستجابة المناعية والقياسات البيوكيميائية والدموية في بداري التسمين. تم تقسيم عدد (180) ككتوت عمر يوم إلى 3 مجموعات رئيسية (أ) و (ب) و (ج). المجموعة الأولى اعطيت علف تجارى بدون اضافات والمجموعة الثانية علف تجارى مضافا اليه 5 ملجم / كجم يشيناسيا بوربوريا والمجموعة الثالثة اعطيت علف تجارى مضافا اليه 1 جم / كجم مسحوق الثوم. عند اليوم 21 من العمر، تم تقسيم كل مجموعة إلى مجموعتين فرعيتين حيث قسمت المجموعة (أ) : G1 و G2 ، المجموعة (ب) : G3 و G4 ، ومجموعة ج: G5 و G6 وتم اجراء العدوى الاصطناعية بالميكروب القولونى عترة 0.78 بمعدل 0.5 مل يحتوى على (4×10^6) وحدة ميكروبية وذلك عن طريق الحقن فى الغشاء البريتونى لكل من المجموعات (G2 ، G4 و G6). تم قياس مستوى الاداء للنمو ، والاستجابة المناعية و بعض مكونات الدم . وسجلت كذلك الاعراض المرضية ونسب الاصابة والنفوق لكل المجموعات. وقد اسفرت النتائج عن ان الطيور المصابة تجريبيا وغذيت على اعلاف مضافا اليها مسحوق بوربوريا يشيناسيا والثوم اظهرت اعراض اقل ومعدل نفوق اقل بلغ 16.66 ٪ (30/5) بالمقارنة مع المجموعة المصابة بدون اضافات والتي بلغت معدل نفوق 40 ٪ (30/ 12) . كما انخفضت الصفات الباثولوجى التشريحية بعد الوفاة إلى حد أكبر بالمقارنة مع الطيور المصابة دون علاج . ووضحت النتائج ان هناك فروق ذات دلالة إحصائية في اضافة البوربوريا يشيناسيا والثوم على معدل الزيادة في الوزن ، ونسبة التحويل الغذائى ، وكذلك نسبة هنيروفيلالى الخلايا اللمفية ، الزلال من الجهاز المناعي، والدهون الثلاثية في الدم. وكان مستوى الكوليسترول أقل في الثوم ويشيناسيا بوربوريا عن المجموعات الغير معالجة. وفقا لتلك النتائج ، ينصح ان بوربوريا يشيناسيا والثوم يمكن استخدامهما فى إضافات الأعلاف كبديل جيد وامن للمضادات الحيوية ، ومحفز النمو ومنشطا مناعيا.