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Efficacy of concurrent use of phytogenic and synbiotic in *Escherichia coli* experimentally infected broilers

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Abstract

Feed additives like synbiotics and phytogenics consider one of the successful strategies that applied in the recent years to improve growth performance as well as to combat bacterial infection in broilers especially after mounting of crisis of bacterial resistance to antibiotics. Although the individual use of synbiotics and phytogenics in broilers was studied in many of previous scientific studies, the combined use of both synbiotics and phytogenics is still a lacking scientific point of interest, therefore the present study was designed to investigate and cover this point. Here, a total of one hundred Hubbard broilers were randomly allocated in 5 groups (20 chicks per group). Except group no. (1), all groups were infected with *E. coli* O125 via oral route and groups no. (3), (4) and (5) were dietary treated with synbiotics, phytogenics and combination of synbiotics and phytogenics, respectively. Results showed that birds in the group (5) that dietary supplemented with combination of synbiotics and phytogenics were showed higher body weights, best improvement in Feed conversion ratio (FCR), lowest re-isolation percentages of *E. coli* O125 from examined organs and significant increase ($p<0.05$) in glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity and significant decrease in malondialdehyde (MDA) at the end of experiment (35 day) compared with other groups. In conclusion the combined use of synbiotics and phytogenics reflected positively in a synergy that provide better improvement of growth performance and antioxidant parameters as well as expand the selective antibacterial effect of both synbiotic and phytogenics against infection of E. coli O125.

Keywords: *Broilers, Escherichia coli, Phytogenics, Synbiotic*

Introduction

The poultry industry is one of the essential pillars of achieving food security in Egypt and providing a cheap source of animal protein. Poultry diseases represent one of the important threats to the industry and its investments. *Escherichia coli* was one of the normally inhabitant microflora in the poultry intestine, but certain strains of pathogenic *E. coli* (APEC) is able to cause a systemic fatal disease, colibacillosis through its invasive to various organs causing different lesions including typically pericarditis, perihepatitis, peritonitis, airsacculitis and other extra intestinal Lesions **(Oh *et al.*, 2011).** Avian colibacillosis is still continuing a major significant problem to poultry industry worldwide **(Dziva and Stevens, 2008).** In the past years sensitivity to antibiotics were used as successful strategy to combat the disease but recently *E. coli* strains that caused colibacillosis were shown to be resistant to almost antimicrobial agents **(Makhol *et al.*, 2010 and Zhang *et al.*, 2012)**.

The global problem, antimicrobial resistance was developed as a result of miss and excessive-use of antibiotics growth promoters

(AGP) in livestock production during last decade. Since 2006, the European Union took a decision to ban the use of antibiotics growth promoters (AGP) in animal production. There are an active and continuous search from scientific community for combating this problem by applied alternative strategies to substitute the use of antibiotic in animal feed with an effective natural compounds (**Megaache *et al.*, 2018**). Several forms of growth promoters including probiotics, prebiotics, antioxidants and phytogenic additives could be successfully used as alternative for antibiotics in broilers production **(Perić *et al.*, 2009).**

Synbiotics were among the natural compounds that have the advantages of prebiotics and probiotics in a form of single synergism product **(Marrero *et al.*, 2013; Sarangi *et al.*, 2016 and Fornazier *et al.*, 2019).** Probiotics are a viable microorganisms have a beneficial effects in broilers through enhancement microbial balance of the indigenous microflora **(Hossain *et al.*, 2015).** The prebiotics refer to non-digestible feed ingredients including glucose, fructose, galactose, and mannose that affect the host through stimulating the growth and activity of beneficial bacteria in intestine **(Hume, 2011).** The synergistic effect of synbiotic compound provide specific substrate for fermentation that needed to improve the survival of the probiotic organism **(Mehdi *et al.*, 2018).** The combination of prebiotics and probiotics create the optimum condition for growth and colonization of beneficial bacteria in the intestine **(Gibson and Roberfroid, 1995 and Bomba *et al.*, 2002).** Several studies clarified the advantage of combination of probiotics and prebiotics in improving the growth of beneficial indigenous bacteria as well as enhancing the immune fuction **(Mookiah *et al.*, 2014).** More importantly, synbiotic was found to play an important role in improving the growth performance of broilers by combat the excess of oxidative free radicals that responsible for cell damage **(Li *et al.*, 2012).** Moreover, dietary supplementation of synbiotic have been found to improve haematological and intestinal histological aspects in broilers that reflected positively on the productive performance **(Beski and Al-Sardary, 2015).**

Phytogenic additives consider a safe natural substances of plant origin (herbs, spices and essential oils) that provide positive effects on animal health and production performances as well as quality of products **(Steiner *et al.*, 2008, Windisch *et al.*, 2008, Hashemi and Davoodi, 2010 and Banerjee *et al.*, 2013).** Phytogenics have gained the scientist's attention as a promising option alternatives for AGP in poultry diets **(Toghyani *et al.*, 2011; Cherian *et al*. 2013 and Ghasemi *et al.*, 2014)** and it could be aid in solving of the problem of antibacterial resistance that occurs as a results of excessive miss-using of antibiotics as growth promoters **(Silva Cardoso *et al.*, 2012)**. Some research articles showed the positive effects of phytogenics on production performances via its direct effect on caecal microflora **(Roofchaee *et al.*, 2011)** and digestive enzyme activity **(Basmacioğlu Malayoğlu *et al.*, 2010).** Moreover, some literature referred to the positive effects of phytogenic additives to reduce the harmful microorganisms in the intestine without adverse effect on intestinal microflora **(Penalver *et al.*, 2005)** and it could successfully act as antimicrobial, antiviral, antioxidative and stimulate immune system. **(B_lükbaşi and Erhan, 2007, Brenes and Rthea, 2010 and Attia *et al.*, 2017).**

Plant extracts have the ability to increase the absorption of micronutrients that needed for digestion and secretion of digestive enzymes **(Usha *et al.*, 2010)** and to exhibit antibacterial, antiviral and antioxidant activities **(Brenes and Rthea, 2010).** The most important antioxidant enzymes are GPx and SOD which have an important role in maintaining a balance between oxidants and antioxidants through eliminating free radicals **(Aguilar *et al.*, 2007)**. More importantly, MDA content has considered main parameter that detect the lipid peroxidation level and also has indirect reflection of the extent of cell damage through fragmentation of deoxyribonucleic acid (DNA), destruction of cell membrane structure and accelerate apoptosis (**Puvaĉa *et al.*, 2015 and Wang *et al.*, 2011).**

The present study aimed to evaluate the synergistic use of synbiotic and phytogenics on growth performance, re-isolation percentages, antioxidant status and biochemical status of broilers challenged with

Escherichia coli.

Materials and Methods

Dietary additives

Synbiotic :

Synbiotic additives used in the present study were probiotics, Lacteol Fort® (active component: bacillus subtilis 2×10^8 cfu/gm) and prebiotic, Lactulose® (active component: lactose 99.8%).

Phytogenics:

Phytogenics used in the present study was Promozen -plus® (active component: Artemisia capillaris 5.3%, Diospyrus 7%, Organic acid 20% and pure water up to 100%).

Experimental protocol

Birds

A total of one hundred broiler chicks (Hubbard) were obtained from a commercial hatchery located in Qalyoubia governorate. The chicks were randomly allocated into five groups of 20 birds per group. Chicks were confirmed for the absence of *E. coli.* Feed was provided freely for birds, the basal diet was formulated according to National Research Council **(NRC, 1994)** recommendations for all other nutrients to meet the nutritional requirements of growing broilers **(table 1).**

Table (1). Composition of the experimental diets.

	Starter ration (Kg)	Grower and Finisher ration (Kg)
Ground yellow corn	55.9	60.0
Soybean meal (44% CP1)	31.5	29.0
Fish meal (72.3% CP)	3	0
Meat and bone meal (50.4% CP)	3	4
Dicalcium phosphate	1.3	1.4
Limestone	1.1	1.3
Common salt	0.30	0.30
Premix2	0.30	0.30
DL-Methionine	0.1	0.1
Chemical composition		
ME4 K cal/Kg diet	3047.2	3043.1
Crude protein %	22.29	19.88
Crude fat %	2.96	3.16
Crude fiber %	3.5	3.5
Calcium	1.19	1.21
Available phosphorus %	0.47	0.48
Methionine %	0.51	0.47
Methionine + Cystine	0.88	0.86
Lysine %	1.125	1.125

Basal diet was formulated according to National Research Council (NRC) (1994) recommendations.

Challenged inoculums
All groups except the control group no.1 were challenged by *E. coli* O125 on day 5 of age at rate of 1ml of 3×10^8 CFU/ml via oral route. The isolate was kindly supplied from bacteriology department Animal Health Research Institute, Benha branch, Egypt.

Dietary treatment
The dietary treatments of the present study were conducted as following: birds in groups1 were non infected with *E. coli* (control negative) and birds in group2 were infected with E. coli O125 (control positive), the birds in both control groups were fed on basal diet without phytogenic additives nor synbiotic supplementation. Birds in group 3 infected with *E. coli* O125 and supplemented with synbiotic at dose1 ml/liter (each 1 ml of synbiotic contain 2×10^8 cfu of bacillus subtilis + 998 µl lactose) from day 1 until the end of experiment 35 day. Birds in group 4 infected with *E. coli* O125 and supplemented with phytogenic additives (Promozen- plus®) at dose 1ml/liter (1-14 days old), 3ml/liter (15-28 days old) and 5ml/liter (29-35 days old) **(Abeer Abd EL-Alim, 2017)**. Birds in group 5 infected with *E. coli* O125 and supplemented with a combination of synbiotic and phytogenic additives from day one till the end of the experiment at 35 days old (as described in groups 3 and 4, respectively).

Growth Performance parameters
Body weights and feed consumption per group were recorded weekly and the obtained values were used to calculate weight gain, feed intake and Feed conversion ratio for each group expressed according to **Wagner *et al.*, (1983)** as follows

FCR= Average feed intake (g) bird/week
Average body weight gain (g) bird/week

Sampling
Blood samples of six birds from each group were collected at days 21 and days 35 of age for estimating serum biochemical and antioxidant parameters. The samples were immediately transferred into sterile test tubes and serum was harvested after centrifuged at 3000 rpm for 10 min. The serum was stored at −20°C for later analysis of antioxidant and biochemical parameters.

Biochemical parameters analysis:
Activities of alanine aminotransferase(ALT), aspartate aminotransferase (AST) enzymes, total protein, albumin, uric acid, creatinine, total cholesterol in tested sera were determined using the spectrophotometric method (RAL, Barcelona, Spain.) and Bioanalytica test kits (Bioanalitika doo, Beograd, Serbia) as described by **(Rej and Hoder, 1983)**. Serum globulin (G) was calculated as follows: G = total protein – albumin.

Determination of antioxidant indices:
The superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and Malondialdehyde (MDA) levels were determined by ELISA kits produced by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to **Dalton *et al.*, (2000)**.

Re-isolation and colony count of inoculated *E. coli* O125 from examined organs of experimentally infected birds.
E. coli O125 was re-isolated from examined organs including, liver, heart, lung and kidney from 3 birds from each experimental group at 3rd, 6th and 10th day post infection. Re-isolation percentages of *E. coli* O125 were calculated from all aforementioned organs while *E. coli* O125 colony count was done only from liver and lung tissue and carried out according to **ISO 16649-2 (2001).**

Statistical analysis:
The data were analyzed by determine the normality via Shapiro-Willk test. The differences between groups were analyzed by One-Way ANOVA followed by Duncan's multiple comparison Post Hoc tests **(Duncan, 1955)**. The Statistical Package for Social Science **(SPSS Inc. Released, 2009)** (version 20.0; SPSS Inc., Chicago IL, USA) was used for performing the statistical analyses to determine difference between groups. Significance between mean values was set a statistically at $P<0.05$.

Results
Clinical signs, morbidity, mortality and post -mortem lesions in the experimentally infected broilers:
Clinical signs, morbidity and mortality:
Clinical signs were appeared in experimentally infected broiler chicks with *E. coli* O125 in groups (2) at 3rd day post infection in form of

ruffled feathers, closed eyes, lethargy, rapid respiration, loss of body weights and white diarrhea was commonly seen at 6th day post infection. Morbidity in the control positive group (2) was 80% while mortality was 10%.

The experimentally infected chicks in the other dietary treated groups (3), (4) and (5) showed no clinical signs and registered no mortalities post infection till the end of the experiment (35 day).

Post-mortem lesions:

The most post mortem lesions were seen in the experimentally infected group (2) were in the form of congested lung, congested liver, congested intestine, slight congestion of kidneys and slight pericarditis at 3rd day post infection while conciliated lung, pneumonia and Pale liver were commonly seen at 6th day post infection. There is no post mortem lesions were recorded at 10 day post infection.

On the other hand, there is no PM lesions recorded in the other infected treated groups unless congested lung and congested liver were seen in the infected groups 3 and 4 that treated with synbiotics and phytogenics, respectively at 3rd day post infection.

Effect of Synbiotic, Phytogenics and combination of them on body weights in experimentally infected broiler chicks with *E. coli* O125.

As shown in table (2), significant higher ($P<0.05$) body weights of broiler chicks were recorded in groups that dietary treated with phytogenics and combination of both synbiotic and phytogenics in groups 4 and 5, respectively while lowest body weights were recorded in infected not treated group (2) compared with control negative group (1).

Considering the comparison of the effect of synbiotic and phytogenics on body weights in broiler chicks in the experiment, we found that body weights of broilers chicks in phytogenics treated group (4) were significantly better ($P<0.05$) than that of synbiotic treated group (3) compared with groups (1) and (2).

Body weights of broiler chicks in group 5 that treated with the combination of synbiotic and phytogenics were significantly higher ($P<0.05$) than that of group 4 that treated with phytogenics starting from 3rd week till the end of the experiment (5th week of age).

Effect of Synbiotic, Phytogenics and combination of them on Feed conversion ratio (FCR) in experimentally infected broiler chicks with *E. coli* O125.

Data of Feed conversion ratio (FCR) that calculated weekly during the experiment period (35 day) as shown in table (3), revealed that, the best significant ($P<0.05$) FCR was recorded in group (4) that treated with phytogenics and group (5) that treated with combination of synbiotic and phytogenics. Regarding to group (5), the birds in this group were showed significantly higher ($P<0.05$) than that of group (4) from 4th week of age till the end of the experiment (5th week of age) compared with control groups (1) and (2).

FCR of group 4 (treated with phytogenics) was significantly ($P<0.05$) better than that recorded in group 3 (treated with synbiotic) from 2nd week of age till the end of the experiment (5th week of age) compared with control groups (1) and (2).

The lowest significant FCR was recorded in the infected non treated group (2) at weekly interval compared with either control negative group (not treated and not infected) or other dietary treated groups (3), (4) and (5).

Re-isolation percentages at different three interval isolation periods (3rd, 6th and 10th post infection) from different organs in the experimentally infected broiler chicks.

Re-isolation percentages of *E. coli* O125 from different examined organs (liver, lung, heart and kidney) from birds in the experimental groups were registered in the table (4). The results clarified that, the higher percentages of re -isolation were recorded in group (2) that infected and not treated at 3rd, 6th and 10th day post infection compared with other experimentally infected groups (3),(4) and (5) that treated with synbiotic, phytogenics and combination of synbiotic and phytogenics, respectively.

The higher re-isolation percentages of *E. coli* O125 were from liver and lungs followed by heart and kidney (table4) with regarding to that the birds in group (4) that treated with phytogenics showed higher re-isolation rate from liver compared with that of lung at 3rd and 6th day post infection as shown in table (4).

Effect of different dietary treatments on *E. Coli* count from liver and lung in experimentally infected broiler chicks.

As shown in figure (1) significant highest *E. coli* count from liver were from birds in group 2 (infected and not treated) while the lowest E. coli count were from birds in group 5 (treated with combination of synbiotic and phytogenics) at 3rd, 6th and 10th day post infection compared with other experimental groups. Regarding to birds in group 3(treated with synbiotic), they showed significant lower *E. coli* count from liver compared with that of group 4 (treated with phytogenics) at 3rd and 6th day post infection.

In figure (2), the results showed that, the birds in both groups (4) and (5) that treated with phytogenics and combination of synbiotic and phytogenics, respectively were showed significant lowest *E. coli* count from lung at 3rd, 6th and 10th day post infection compared with other experimental groups while the significant highest E. coli count were from birds in group 2 (infected not treated). With regarding to birds in group 4 (treated with phytogenics), they showed significant lower *E. coli* count from lung compared with that of group 3 (treated with synbiotic) at 3rd post infection.

Table (2). Effect of different dietary treatments with Synbiotic, Phytogenics and combination of them on body weights in experimentally infected broiler chicks with *E. coli* O125 .

Group number	Infection with *E. coli* O125	Dietary treatment	Body weights (g) /chick (mean± SE)					
			0 day	1st week	2nd week	3rd week	4t week	5th week
1	Not infected	Non treated	45.00± 1.22 a	146± 2.11 b	336.42 ±5.59 b	659.84 ±7.49c	1092.60 ±4.78c	1527.66± 12.82 e
2	Infected	Non treated	46.20± 0.86 a	130.10 ±1.77 c	304.28 ±8.77 c	597.50 ±5.15 d	975.52± 8.10 d	1389.96± 12.24 d
3	Infected	Treated with synbiotic	44.80± 1.24 a	144.70 ±1.55 b	346.28 ±7.27b	672.38 ±7.95 c	1097.52 ±6.28 c	1555.56± 9.07 c
4	Infected	Treated with phytogenics	45.60± 1.17 a	154.60 ±2.31 a	387.98 ±4.52 a	708.20 ±5.29 b	1124.12 ±5.43 b	1586.92± 4.01 b
5	Infected	Treated with combination of synbiotic and phytogenics	46.20± 1.24 a	159.58 ±1.83 a	399.12 ±8.14 a	738.60 ±5.29 a	1147.54 ±10.07 a	1617.70± 3.12 a

a, b, c, mean values with different superscripts in a column are statistically different at ($p< 0.05$)

Table (3). Effect of different dietary treatments with Synbiotic, Phytogenics and combination of them on Feed conversion ratio (FCR) in experimentally broiler chicks infected with *E. coli* O125 .

Group number	Infection with *E. coli* O125	Dietary treatment	Feed conversion ratio (FCR) (mean± SE)				
			1st week	2nd week	3rd week	4th week	5th week
1	Not infected	Non treated	1.16±0.01 c	1.42±0.04 b	1.69±0.03 c	2.16±0.03 b	2.44±0.08 b
2	Infected	Non treated	1.46±0.02 a	1.89±0.11 a	2.22±0.07 a	2.50±0.05 a	2.67±0.11 a
3	Infected	Treated with synbiotic	1.23±0.02 b	1.37±0.05 b	1.92±0.04 b	2.11±0.02 b	2.35±0.04 bc
4	Infected	Treated with phytogenics	1.10±0.03 ab	1.20±0.03 c	1.60±0.02 cd	1.93±0.01 c	2.17±0.03 c
5	Infected	Treated with combination of synbiotic and phytogenics	1.08±0.03 a	1.19±0.04 c	1.48±0.04 d	1.78±0.02 d	1.97±0.03 d

a, b, cd, mean values with different superscripts in a column are statistically different at ($p < 0.05$)

Table (4). Re-isolation percentages from different organs in the experimentally infected broiler chicks at 3rd, 6th and 10th day post infection.

Group No.	Infection	Treatment	Re-isolation											
			Time	3rd day post infection				6th day post infection				10th day post infection			
			Organ	Liver	heart	lung	kidney	Liver	heart	lung	kidney	Liver	heart	lung	kidney
1	Non infected with *E. coli* O125 (Control negative)	Not treated	No. of positive birds	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
			Percentage %	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	Infected with *E. coli* O125 (Control positive)	Not treated	No. of positive birds	3/3	2/3	3/3	3/3	2/3	1/3	1/3	2/3	1/3	0/3	1/3	1/3
			Percentage %	100%	67%	100%	100%	67%	33%	33%	67%	33%	0%	33%	33%
3	Infected with *E. coli* O125	Treated with synbiotic	No. of positive birds	2/3	1/3	2/3	1/3	1/3	0/3	1/3	1/3	0/3	0/3	0/3	0/3
			Percentage %	67%	33%	67%	33%	33%	0%	33%	33%	0%	0%	0%	0%
4	Infected with *E. coli* O125	Treated with phytogenics	No. of positive birds	2/3	2/3	1/3	0/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
			Percentage %	67%	67%	33%	0%	67%	0%	0%	0%	0%	0%	0%	0%
5	Infected with *E. coli* O125	Treated with combination of synbiotic and phytogenics	No. of positive birds	2/3	1/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
			Percentage %	67%	33%	33%	33%	0%	0%	0%	0%	0%	0%	0%	0%

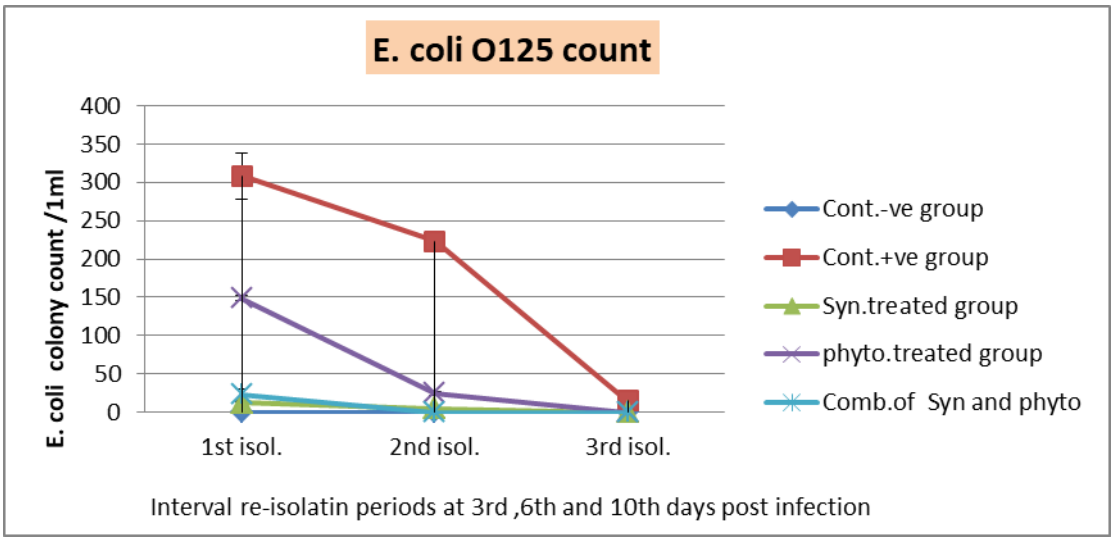

Figure (1). Effect of Synbiotic, Phytogenics and combination of them on the E. coli O_{125} count in liver of the experimentally infected groups.

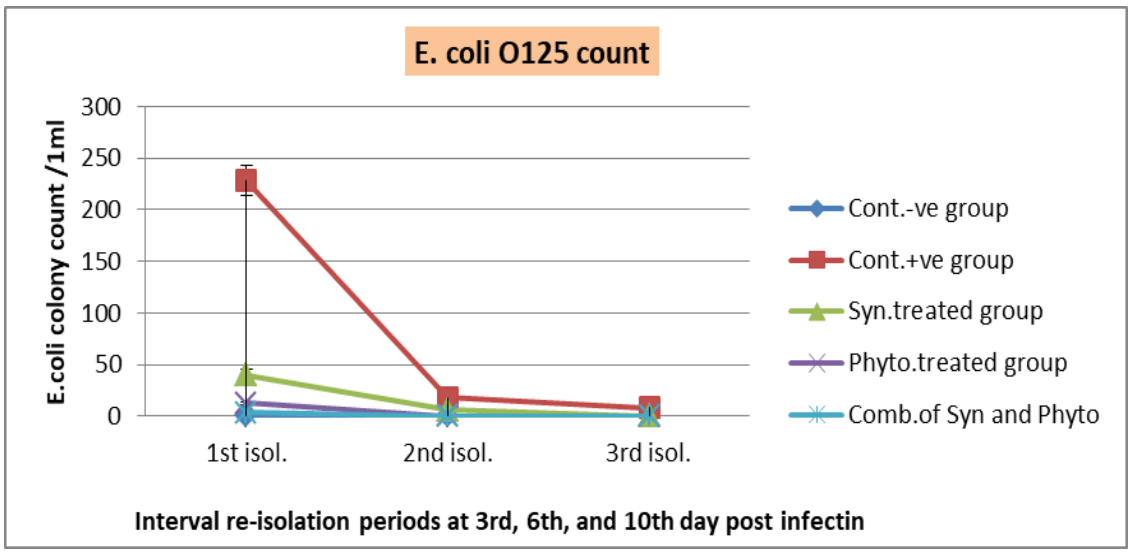

Figure (2). Effect of Synbiotic, Phytogenics and combination of them on the *E. coli* O_{125} count in lung of the experimentally infected groups.

Effect of Synbiotic, Phytogenics and combination of them on antioxidant enzymes status in experimentally infected broiler chicks with *E. coli* O125 at 21 and 35 day of age.

The effect of the dietary treatments on serum GSH-Px, MDA and SOD is shown in Table (5). At 21 and 35 day of age, There were significantly higher in the GSH-Px and SOD levels in broiler chicks treated with phytogenics (group 4) and in broiler chicks treated with synbiotic and phytogenics (group 5) than those in other treatments (synbiotic, infected and control). On the other hand, at 21 day of age there were no significant differences among dietary treatments in the MDA levels compared to that of the control. Furthermore, at 35 day of age the concentration of serum MDA levels decreased ($P < 0.05$) in chicks treated with phytogenics (group 4) and in chicks treated with synbiotic and phytogenics (group 5) compared to all groups.

Effect of Synbiotic, Phytogenics and combination of them on blood biochemical parameters in experimentally infected broiler chicks with *E. coli* O125 at 21 and 35 day of age.

Results of biochemical blood parameters were summarized in Tables (6, 7) showed that at 21 day of age there were no significant differences observed in serum ALT, AST, Total protein, Albumin and uric acid compared to that of the control. Also, creatinine level was decreased in chicks treated with phytogenics (group 4) and in chicks treated with synbiotic and phytogenics (group 5) compared to synbiotic group. Furthermore, the concentration of total cholesterol was lower ($P < 0.05$) in chicks treated with phytogenics (group 4) than those in other treatments. On the other hand, At 35 day of age, no significant differences among treatments in ALT, AST, Total protein, Albumin and Creatinine compared to that of the control. In addition, chicks treated with phytogenics (group 4) and in chicks treated with synbiotic and phytogenics (group 5) showed the lowest ($P < 0.05$) level of uric acid compared to synbiotic treatment group while total cholesterol was lower ($P < 0.05$) in chicks treated with phytogenics (group 4) than those in other treatments.

Table (5). Effect of Synbiotic and Phytogenics in *Escherichia coli* experimentally infected broilers on glutathione peroxidase (GPx), malondialdehyde (MDA) and superoxide dismutase (SOD) activity at 21 and 35 day of age (Mean*±SE).

Groups	Antioxidant parameters					
	GPX (ng/ml)		MDA (nmol/ml)		SOD (U/L)	
	21 day	35 day	21 day	35 day	21 day	35 day
Control	0.161± 0.015c	0.215± 0.016bc	0.347± 0.023ab	0.359± 0.019ab	0.207± 0.026b	0.216± 0.025c
Infected group (no treatment)	0.142± 0.014c	0.192± 0.011c	0.380± 0.015a	0.408± 0.023a	0.179± 0.020b	0.167± 0.016c
Infected group (treatment with synbiotic)	0.212± 0.007b	0.262± 0.016b	0.301± 0.027b	0.307± 0.026b	0.209± 0.011b	0.296± 0.021b
Infected group treatment with phytogenics)	0.294± 0.016a	0.398± 0.025a	0.281± 0.026b	0.228± 0.015c	0.344± 0.030a	0.422± 0.035a
Infected group (treatment with synbiotic and phytogenics)	0.280± 0.017a	0.377± 0.022a	0.295± 0.024b	0.243± 0.021c	0.288± 0.028a	0.416± 0.036a

a, b, c mean values with different superscripts in a column are statistically different at ($p \leq 0.05$) ; *mean of 6 birds.

Table (6). Effect of Synbiotic, Phytogenics and combination of them on blood biochemical parameters in experimentally infected broiler chicks with *E. coli* O125 at 21day of age (Mean*±SE).

Groups	Blood biochemical parameters at 21 day							
	ALT (U/L)	AST(U/L)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total Cholesterol (mg/dl)
Control	23.29± 1.12b	184.31± 2.18b	4.13± 0.21b	2.14± 0.13b	1.95± 0.27a	5.42± 0.27b	0.34± 0.008c	193.21± 2.24ab
Infected group (no treatment)	27.51± 1.31a	197.15± 1.54a	6.13± 0.31a	3.59± 0.31a	2.73± 0.25a	7.68± 0.28a	0.40± 0.011a	197.38± 3.19a
Infected group (treatment with synbiotic)	24.46± 1.14ab	182.60± 2.12b	4.47± 0.28b	2.31± 0.15b	2.18± 0.34a	5.57± 0.39b	0.37± 0.007b	188.78± 2.84b
Infected group treatment with phytogenics)	23.87± 0.67b	180.10± 2.59b	4.35± 0.17b	2.43± 0.21b	1.96± 0.14a	6.31± 0.31b	0.32± 0.010c	161.01± 1.60d
Infected group treatment with synbiotic and phytogenics)	23.53± 0.88b	181.97± 3.43b	4.29± 0.19b	2.08± 0.17b	2.16± 0.29a	5.56± 0.25b	0.33± 0.014c	173.86± 2.38c

a, b, c, mean values with different superscripts in a column are statistically different at ($p \leq 0.05$) ; *mean of 6 birds.

Table (7). Effect of Synbiotic, Phytogenics and combination of them on blood biochemical parameters in experimentally infected broiler chicks with *E. coli* O125 at 35 day of age (Mean*±SE).

Dietary treatment	Blood biochemical parameters at 35 day							
	ALT (U/L)	AST (U/L)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total Cholesterol (mg/dl)
Control	25.20± 0.83b	200.03± 1.25b	4.16± 0.27b	2.13± 0.13b	1.88± 0.23a	4.27± 0.24c	0.35± 0.016b	212.79± 3.44a
Infected group (no treatment)	28.55± 0.57a	236.75± 1.64a	5.91± 0.28a	3.59± 0.31a	2.32± 0.47a	7.40± 0.21a	0.40± 0.012a	215.90± 3.97a
Infected group (treatment with synbiotic)	25.05± 0.92b	198.98± 2.24bc	4.47± 0.28b	2.31± 0.15b	2.16± 0.34a	6.52± 0.18a	0.37± 0.013ab	210.95± 2.49a
Infected group treatment with phytogenics)	24.68± 0.82b	193.74± 1.97c	4.07± 0.25b	2.43± 0.20b	1.64± 0.31a	5.36± 0.46b	0.35± 0.014b	168.55± 2.67c
Infected group treatment with synbiotic and phytogenics)	25.27± 0.63b	196.19± 2.18bc	4.06± 0.16b	2.08± 0.17b	1.98± 0.29a	4.99± 0.44bc	0.35± 0.016b	186.13± 3.42b

a, b, c, mean values with different superscripts in a column are statistically different at ($p \leq 0.05$) ; *mean of 6 birds.

Discussion

With the increase of fierce attacks of antibiotic resistant bacteria, especially after prevention of the use of antibiotics as growth promoters in animal feed. There was a priority to use feed additives as synbiotic and phytogenics in poultry diets instead of antibiotic growth promoters (AGP) to take advantages of their promising effects in enhancing growth performances **(Al-Sultan *et al.,* 2016).**

Focusing on the obtained results in the present study regarding with the effect of synbiotic and phytogenics either solely or in a combination on the performances of the experimentally infected birds with *E. coli* O125, the results showed that body weights and FCR was significantly improved in the dietary treated infected birds in groups 3, 4 and 5 compared with infected birds in control diet group 2. These results were in agreement with the previous studies that carried on synbiotic (**Ateya *et al.,* 2019)** and phytogenics **(Abudabos *et al.,* 2016; Alaeldein *et al.,* 2018)** that showed the positive effects of these additives on the improvement of performances of infected broilers with different type of pathogenic bacteria including *E. coli*.

One of the substantive points in the present results was the superior effect of phytogenics than that of synbiotic on broilers performances compared with the uninfected control diet group (1). These results were in accordance with **peric *et al.* (2010)** who reported that probiotic and phytogenic treatments had a significant positive effect on body weight of broilers with consideration of the best result was achieved in phytogenic group. Moreover, the results were agree with other scientific studies that showed the positive effects of phytogenics on production performances **(Awad *et al.*, 2009; Abdel-Raheem *et al.,* 2012 and Sara kamal *et al.*, 2016)** via its direct effect on caecal microflora **(Roofchaee *et al.*, 2011)** and digestive enzyme activity **(Basmacioğlu Malayoğlu *et al.,* 2010).** On contrary the results disagree with other studies that reported the lack of effect of phytogenics on broilers production performances **(Kirkpinar *et al.,* 2011)** and caecal microflora **(Cross *et al.,* 2007).** These differences may attributed to several factors related to phytogenics that have been used as feed additives like species of plant, time of picking, technology of production **(Yang *et al.,* 2009**).

At the end of the experiment 35 days, the birds in group 5 that supplemented with combination of synbiotic and phytogenic were showed the highest body weights and FCR compared with other groups. These results provide a good opportunity to take advantages of synergy combination between synbiotic and phytogenic to maximize their effects on broilers growth performance .

One of the main objectives in the present study is to evaluate the antibacterial effect of both synbiotics and phytogenics, either alone or in a combination on the experimentally infected broilers with *E. coli* O125. Here, birds that dietary treated with synbiotic alone (group 3) showed significant ($P<0.05$) reduction in re-isolation percentages of *E. coli* from different examined organs including liver, heart, lung and kidney at interval re-isolation periods compared with control diet infected group (2). These results were agree with previous studies that showed the inhibitory effect of B. subtilis on *E. coli* in broilers **(Hooge, 2008, Gao *et al.,* 2017**) and reported that B. subtilis could significant reduce ($P < 0.01$) the population E. coli in cecum of challenged birds **(Manafi *et al.,* 2017).** Concerning with phytogenics, the group 4 that dietary treated with Artemisia was showed a significant lower ($P<0.05$) re-isolation percentages of E. coli from examined organs including liver, heart, lung and kidney at interval re-isolation periods compared with the untreated infected group no.(2). These results agree with previous studies that proven the highly antimicrobial activity of Artemisia species against *Escherichia coli* **(Hakimi *et al.,* 2003; Erel *et al.,* 2012 and Habibi *et al., 2013*)** and its significant role in the reduction of *E. coli* population and amelioration of lactobacilli population in the intestine of broiler chicks **(Siragusa *et al.,* 2008; McReynolds *et al.*, 2009; Khalaji *et al.,* 2011; Murugesan *et al.,* 2015; Wati *et al.,* 2015; Manafi, *et al.,* 2016).** On the contrary, the obtained results were disagreed with other scientific studies that showed the lower efficacy of Artemisia species against *E. coli* **(Hasanshahian and Khosravi, 2015).** Furthermore, other studies showed that Artemisia couldn't reduce *E. coli* population in the intestine of broilers **(Vukic-**

Vrajnes *et al.*, 2013;Mountztheis *et al.*, 2014; and Ahsan *et al.*, 2018).

We found that, Artemisia capillaris as a type of phytogenics that used in the present study was exerted superior selectivity ($P<0.05$) in reduction of of *E. coli* population that re-isolated from lung than that of synbiotic (bacillus subtilis+lactose). These results were agree with **Chang *et al.*, (2015)** who found that Artemisia capillaris essential oil was exerted a potent antibacterial effect against different bacterial strains causing respiratory infection including *E. coli.* On the other hand, synbiotic was exerted superior selectivity ($P<0.05$) than that of phytogenics in the reduction of *E. coli* count that re-isolated from liver. These results may be attributed to the competitive exclusion nature of Bacillus subtilis **(La Ragione and Woodward, 2003)** especially when added with prebiotic that improve its survival and implantation in gastrointestinal tract **(Cencic and Chingwaru 2010).**

Although there was available of immense scientific studies that showed the antimicrobial effect of phytogenics and synbiotics on different pathogenic microorganisms but the knowledge about the expected benefits about the concurrent combination of phytogenics and synbiotics is still lacking. Here, as showed in group 5, the concurrent use of synbiotic and phytogenics could be maximize the improvement of broilers performances as well as expand the selective antibacterial effect of both synbiotic and phytogenics against *E. coli* O125 in different organs of the experimentally infected broilers.

Nutrition plays an important role in maintaining the pro-oxidant/antioxidant balance. The GSH-Px and SOD are two main antioxidant enzymes and the MDA level is used to evaluate the level of lipid peroxidation. In the present study, The GSH-Px and SOD levels were increased. Several factors cause increase the activity of these antioxidant enzymes like colonization resistance, susceptibility to environmental pathogens and age of the broilers **(Aluwong *et al.*, 2013).**

The MDA was decreased in the serum of chicks treated with phytogenics (group 4) and in chicks treated with combined use of synbiotic and phytogenics (group 5). These results were agreement with (**Wan *et al*. 2016 and Abeer Abd El-Alim, 2017)** who indicated that Artemisia species can improve the antioxidant status of poultry through increasing the levels of GSH-Px and SOD and decreasing MDA level in the serum and liver. On the contrary, the present results disagreed with (**Erdogan *et al.* 2010**) who showed that the combined use of synbiotics and phytobiotics was significantly increased plasma malondialdehyde (MDA) levels ($p\leq 0.05$) while the SOD level did not differ ($p > 0.05$) among the groups in broilers.

Artemisia leaves contain flavonoids and phenolic compounds (**Gouveia and Castilho, 2013)**. The nutritional values of Artemisia were improved when combined with vitamin E and other phenolic compounds in poultry diet. So, it makes Artemisia a natural phytogenic feed additive with high antioxidant activity that should be added in poultry rations (**Cherian *et al.*, 2013).**

The results of the present study illustrated no significant differences in ALT, AST, Total protein and Albumin concentrations among dietary treatments compared to those of the control. The results in the present study were agreed with that obtained by **Khavari *et al.* (2019)** who showed non-significant response of dietary supplementation of phytogenics on glucose content, albumin, ALT and AST and these findings are also in line with (**Kanani *et al*., 2018)** who reported no significant differences in ALT and AST concentrations among dietary treatments of phytogenics and synbiotics compared to those of the control. Similar non-significant effect was reported on synbiotics supplementation **(Oliva Das *et al.*, 2016)** in broilers which improves the overall performance without altering the normal blood values of broilers. Cholesterol was decreased significantly ($P < 0.05$) in chicks treated with phytogenics (group 4). The results of cholesterol in the present study were in agreement with **Lutgen (2013)** who reported that several Artemisia species can enhance lipid metabolism and reduce blood cholesterol concentration. These results might be attributed to "the fiber content of Artemisia leaves may stimulate the binding of cholesterol with bile acids, and the inhibition of micelle formation combined with the effect of fermentation on short-chain fatty

acid production are mechanisms that have been suggest to describe the potential cholesterol-lowering effects" (**Baghban-Kanani *et al.*, 2018).** In contrary to the findings, **Ghasemi *et al.*, (2014)** and **Jamshidparvar *et al.*, (2017)** did not observe any effect of phytogenics on cholesterol, This might be attributed to the variation in the kind of phytogenics supplemented, their phytochemicals, and the doses tested. Also, creatinine level was decreased in chicks treated with phytogenics (group 4) and in chicks treated with combined use of synbiotic and phytogenics (group 5) compared to synbiotic group. In addition, chicks treated with phytogenics (group 4) and chicks treated with combined use of synbiotic and phytogenics (group 5) showed the lowest ($P < 0.05$) level of uric acid compared to synbiotic additives. These results were disagreement with **Jamshidparvar *et al.* (2017)** who stated that phytogenics elevated the level of uric acid. The greatly difference in the biochemical parameters in broilers in response to different feed additives may be due to several factors including differences in the genetic, nutrition, age and experimental designs of the studies (**Abudabos *et al.*, 2018).**

Conflict of interest statement

The authors declare no conflict of interest.

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Conclusion

It is concluded that the phytogenics and synbiotic as feed additives in a combination treatment has a significant higher growth performances, significant best reduction of re-isolated *E. coli* O125 from different examined organs and a significant enhancement in antioxidant parameters compared with single treatment of either synbiotic or phytogenics without any side effects as detected by normal biochemical blood profile. These findings encourage further scientific studies to investigate the effect of combination of synbiotic and phytogenics on growth performances and antioxidative status of broiler chickens.

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