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COMPARISON OF EFFICACIES OF FOSFOMYCIN, THIAMPHENICOL, *NIGELLA SATIVA* OIL AND ITS COMBINATION AGAINST *ESCHERICHIA COLI* INFECTION IN CHICKENS

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ABSTRACT

The efficacies of fosfomycin, thiampheicol and *Nigella sativa* oil were compared against experimentally infected chickens with *E.coli* O_{111}. One hundred and twenty six 12-day old broiler chickens were divided into seven groups:1st group was control non infected non medicated , 2nd group was infected with *E.coli* and non medicated, the 3rd group was infected with *E.coli* and treated with disodium fosfomycin (40mg/kg b.wt), the 4th group was infected with *E.coli* and treated with thiamphenicol (30mg/kg b.wt), the 5th group was infected with *E.coli* and orally administrated with *Nigella sativa* oil (0.025 ml/bird), the 6th group was infected with *E.coli* and treated with disodium fosfomycin (40mg/kg b.wt), in addition to orally administration with *Nigella sativa* oil (0.025 ml/bird) and the 7th group was infected with *E.coli* and treated with thiamphenicol (30mg/kg b.wt) in addition to *Nigella sativa* oil (0.025 ml/bird). The performance, clinical signs, *E. coli* reisolation, antibacterial activity and the effect on immune response of chickens were compared. The treated groups showed fewer symptoms and gross lesions than those of infected group. The body weight and average daily gain were highly improved in the 6th, 7th and 5th group respectively. The combination of *Nigella sativa* oil with fosfomycin and thiamphenicol increase their antibacterial activity. The specific antibody titers against the *E.coli* O_{111} were significantly increased in the groups which received *Nigella sativa* oil. The results indicate that addition of *Nigella sativa* oil with fosfomycin or thiamphenicol increases their efficacy, antibacterial activity and immune response against *E. coli* O_{111}.

Key words: *E. coli* O_{111}, fosfomycin, thiamphenicol, *Nigella sativa* oil, ELISA.

INTRODUCTION

Escherichia coli is one of the most important and frequently encountered bacterial avian pathogens causing a wide variety of disease syndrome in birds causing up to 30% of poultry mortality (Georgnaras *et al.*, 2001), also it is responsible for severe economic losses for the world's poultry industries (Barnes *et al.*, 2008).

Avian pathogenic *Escherichia coli* induces different syndromes in poultry including systemic and localized infections such as respiratory colibacillosis, acute colisepticemia with characteristics fibrinous lesions (air saculitis, perihepatitis, and pericarditis), salpengitis, yolk sac infection, and swollen-head syndrome (Dho-Moulin and Fairbrother, 1999). The infection is generally initiated or enhanced by predisposing agents, such as mycoplasmal or viral infections, and environmental factors. Chickens of all ages are susceptible to collibacillosis (Barnes *et al.*, 2003).

Fosfomycin is a broad-spectrum bactericidal agent that attacks bacteria by inhibiting cell wall synthesis (Dàmaso, 1990). This antibiotic posses high activity in vitro against a wide range of Gram- positive bacteria such as *Streptococcus sp., S. aureus* and some Gram- negative bacteria such as *Pseudomonas aerugenosa* and *E.coli* (Tessier& Quentin, 1997; Grif *et al.*, 2001). It is used in chickens for the treatment of infection caused by *E.coli* and *Salmonella sp.* (Prescott, 2000). Pharmacokinetics studies in broiler chickens have shown that fosfomycin sodium salt is a product very soluble in water, with low protein binding, and low molecular weight, which hinders its diffusion within tissues such as muscle or fat, reaching a high concentration in the kidney (Aramayona *et al.*, 1997).

Thiamphenicol is a broad-spectrum bacteriostatic antibiotic, inhibiting the bacterial protein synthesis at the ribosome (Cannon *et al.*, 1990). It is a structural analogues of chloramphenicol with lower toxicity, in

addition to it has a greater *in vivo* activity against pathogenic bacteria than other structural analogues and it's also active against some bacteria that are resistant to chloramphenicol. It has been approved in European Union for use in cattle, sheep, pigs and chickens (Giorgi *et al.*, 2000).

The antimicrobial agents of plant origin such as essential oils, plants extracts and complete plant substances, have gathered significant consciousness as alternatives to the traditional antibacterial feed additives. Extracts of *Nigella sativa* oil have shown promising effects against bacteria, fungi, viruses, parasitics and worms. Seed extracts of *Nigella sativa* were found to inhibit the growth of *Escherichia coli*, *Bacillus subtilis* and *streptococcus faecalis* (Hala *et al.,* 2006).

Supplementing broiler diets with *Nigella sativa* seed, oil or meal improve growth performance, biochemical and hematological response and mortality rate (Nofal *et al*., 2006). Feeding different forms and levels of NS has been reported to possess anti stress activity (Tolba and Hassan, 2003), antimicrobial activity (Nasir and Grashorn, 2006) and has a stimulatory effect on the immune system (Tolba *et al*., 2005).

This present study was designed to compare the efficacy of fosfomycin and thiamphenicol as antibacterial agents and their effects in combination with *Nigella sativa* oil for treatment of experimental infections with *E. coli* O_{111}. Also, the effect of *Nigella sativa* oil on the immune response was investigated.

MATERIALS and METHODS

Birds

A total of 126 Hubbard broiler chicks, one-day old were floor reared under suitable conditions of husbandry. Commercial diets (antibacterial free) and water were provided ad libitum. The chicks were allowed a 5 days acclimatization period prior to the study.

Bacteria

The *E. coli* strain O_{111} was kindly supplied by Dokki animal health research institute. The bacteria were grown in BHI broth (Oxoid) for 24 hours at 37˚C and then on BHI agar (Oxoid) for 24 hours at 37˚C. To prepare the inoculums, the colonies were suspended in sterile saline and compared with McFarland 0.5 to obtain a concentration of 1.8×10^8CFU/ml.

Drugs:

Disodium fosfomycin, Thiamphenicol and its solvent (dimethylacetamide) were provided as pure powdered from (Pharma Swede Company, Egypt) and *Nigella sativa* oil was obtained from (Royal herbs, ltd company, Egypt).

Experimental design:

The 126 one-day old chicks were divided into seven groups (18 chicks each). At the 5th day of age the birds in 5th, 6th, 7th groups were orally administered with *Nigella sativa* oil daily till the end of the experiment (35 day of age). At the 12th day of age the chicks were experimentally infected with *E. coli* O_{111} (1ml of 1.8×10^8 CFU/ bird) and the treatment by fosfomycin and thiamphenicol began after 6 hours from infection and lasted for 5 successive days (Rawiwet *et al.,* 2010) as the following.

The 1st group was left as control (non infected, non medicated), the 2nd group was infected with *E. coli* O_{111} and not medicated, the 3rd group was infected with *E. coli* O_{111} and treated with disodium fosfomycin in a dose of 40mg/kg b.wt (Gutierrez *et al.*, 2010) in drinking water, the 4th group was infected with *E. coli* O_{111} and treated with thiamphenicol in a dose of 30mg/kg b.wt (Switala *et al.*, 2007) in drinking water.

While the 5th group was infected with *E. coli* O_{111} and orally administrated with *Nigella sativa* oil in a dose of 0.025 ml/bird (by using plastic tube directly into the crop) (Hodžic *et al.,* 2012). The 6th group was infected with *E. coli* O_{111} and treated with disodium fosfomycin in a dose of 40mg/kg b.wt in drinking water and orally administered with *Nigella sativa* oil in a dose of 0.025 ml/bird. The 7th group was infected with *E. coli* O_{111} and treated with thiamphenicol in a dose of 30mg/kg b.wt in drinking water and orally treated with *Nigella sativa* oil in a dose of 0.025 ml/bird.

The chicks were examined daily for clinical signs of the disease and mortality. The body weight of all chicken were determined at the beginning of the experiment and daily for 5 days during administration of the tested drugs then weekly till the end of experiment (35 day), also feed and water consumption were determined during the 5 days of medication to determine the feed conversion rate (FCR=feed intake (g)/ average weight gain (g)) and also Average daily gain (ADG) was determined and calculated from the formula (ADG= weight gain (g)/ days).

Blood sample were taken from 5 birds from each group (from wing vein) in the first day of infection and medication and after 2 hour from administration of the drugs and/or oils and daily during the 5 days of medication for determination of the antibacterial activity of tested drugs as diameter zone of inhibition against *E coli* O_{111}. Also, the blood samples were collected from chicken of each group at 0, 7th, 15th and 21st day of infection for detection of the immune response against *E. coli* O_{111}. The blood samples allowed to clot at room temperature for 30min., after which the sample were centrifuged at 3000 rpm for

15 min. The separated serum was decanted and immediately frozen at -20˚C until use.

Efficacy criteria:
The air sac, pericardial and perihepatic lesions of colisepticemia in each of the three sacrificed birds from each group at the 2nd, 5th, 8th and 10th day of the infection were scored. According to Charleston *et al.*, 1998, the air sac lesions of colisepticemia were scored as follows: 0: no lesions, 1: cloudness of air sacs, 2: air sac membranes are thickened, 3: "meaty" appearance of membranes with large accumulation of cheesy exudates confined to one air sac and 4: lesions with the same score as score 3 but with lesion in two or more air sacs while the pericardial lesions were scored as follows: 0: no lesions, 1: excessive clear or cloudy fluid in the pericardium, 2: extensive fibrination in pericardial cavity. For perihepatic lesions, 0: no visible lesion, 1: definite fibrination on the surface of the liver, 2: extensive fibrination, adhesions, liver swelling and necrosis.

To re-isolate the challenge *E.coli* O_{111}, swabs from the liver, spleen and heart were cultured on MacConkey agar (Oxoid) at 37˚Cfor 24 hours.

Antibacterial activity of the tested drugs on *E.coli* O_{111}:
In vitro antibacterial activity was determined by the agar well-diffusion method (Mukherjee *et al*., 1995). The *E. coli* O_{111} was cultured on Muller Hinton agar for 24h at 37˚C then the bacterial cells were harvested and re-suspended in saline to make a suspension of 10^5 CFU/ ml and used for assay. The bacterial suspension (10^5 CFU/ ml) was mixed with Muller Hinton agar medium then the media was transferred to sterile Petri-plates (25ml/plate) and allowed to solidify. About 100µl of serum sample (which collected from chicken after 2h from administration of drugs) was placed in the wells and allowed to diffuse, then the plates were incubated at 37˚C for 24h. The antibacterial activity of the tested drugs was determined by measuring the diameter of the inhibition zones. The assay was performed in triplicate.

Immune-responsiveness against E. coli O_{111}:
The antibody titers against *E. coli* serotype O_{111} were determined in serum samples collected from each group on 0, 7, 15 and 21 days post infection by using Enzyme linked Immnosorbent Assay (ELISA) according to Briggs and Skeeles (1984).

Statistical analysis
ANOVA, student –t test was used for statistical comparison of the body weight, food conversion rate and average daily gain. The lesion scores were analyzed by Mann-Whitney U-test. The ELISA data were analyzed by using the General Linear Models. SPSS for windows was used for statistical analysis.

RESULT

Efficacy of fosfomycin, thiamphenicol and *Nigella sativa* oil against *E. coli* O_{111} infection:
Clinical signs were absent from control (non infected non medicated) group. The chicken infected with *E. coli* O_{111} and not treated were depressed, ruffled feathers, showed signs of anorexia, diarrhea and respiratory signs without mortalities. The chicken infected with *E. coli* and treated with fosfomycin, thiamphenicol and *Nigella sativa* oil showed less signs than infected group.

The post mortem pathology of the sacrificed chickens revealed that the infected non treated group individuals had severe lesions airsacculitis of, perihepatitis, pericarditis, while the lesions were less severe in chicken of the treated groups. The results of the lesion scores were recorded in table (1), which showed that there was significant decrease ($p<0.05$) in lesion scores of treated group.

Table 1: Mean macroscopic lesion scores in sacrificed chicken in infected non- treated and treated groups.

Group	Liver		Heart		Air sacs	
	2nd day of infection	5th day of infection	2nd day of infection	5th day of infection	2nd day of infection	5th day of infection
1st group	0.0	0.0	0.0	0.0	0.0	0.0
2nd group	2±0.00***a	1±0.0***a	1.67±0.33***a	1±0.0***a	2±0.00***a	1±0.0***a
3rd group	1±0.58	0.0***b	0.33±0.33***a,b	0.0***b	0.33±0.33***b	0.0***b
4th group	0.67±0.33**a,b	0.33±0.33**b	0.67±0.33**a,b	0.33±0.33**a,b	0.67±0.33**a,b	0.33±0.33**a,b
5th group	0.33±0.33**a,b	0.33±0.33**b	0.0***b	1±0.0	1±0.58	0.67±0.33**a,b
6th group	0.67±0.33**a,b	0.0***b	0.0***b	0.0***b	0.33±0.33**a,b	0.0***b
7th group	0.67±0.33**a,b	0.0***b	0.33±0.33**a,b	0.0***b	0.67±0.33**a,b	0.0***b

1st group was Control group, 2nd group was Infected group, 3rd group was Fosfomycin treated group, 4th group was thiamphenicol treated group, 5th group was *Nigella sativa* oil treated group, 6th group was Fosfomycin + *Nigella sativa* oil treated group and 7th group was Thiamphenicol + *Nigella sativa* oil treated group
a Group that significantly different ($P < 0.05$) from the 1st group
b Group that significantly different ($P < 0.05$) from the 2nd group

The *E. coli* O_{111} was re-isolated in pure culture from sacrificed chicken of infected non treated group until the 10th day of infection while in the treated groups the re-isolation of the *E. coli* O_{111} was negative from the 8th day of infection.

Performance:

The mean body weight and feed conversion rate at the treatment period were determined in all groups (table, 2). Also the mean body weight and the average daily gain were determined at the end of experiment, and illustrated in (table, 3). The results showed that the groups infected with *E. coli* O_{111} and treated with fosfomycin, thiamphenicol and/or *Nigella sativa* oil showed improvement in body weight than infected non treated group, also chickens infected with *E. coli* O_{111} and non medicated showed a decrease in food consumption and feed conversion ratio (FCR) when compared with control group, while infected chicken medicated with fosfomycin, thiamphenicol and/or *Nigella sativa* oil showed significant increase in FCR when compared with infected group.

The ADG were significantly increased in all infected treated groups, especially groups treated with fosfomycin and thiamphenicol in combination with *Nigella sativa* oil than infected non treated group.

Table 2: The mean body weight (MBW; g/chick) and feed conversion rate (FCR) at the treatment period in all groups.

Group	The mean body weight at the treatment period (g/chick)					FCR %
	1st day	2nd day	3rd day	4th day	5th day	
1st group	269.75±13.7	298.75 ± 6.4	336±12.35	386±6.87	412±6.11	1.976±0.079
2nd group	253±4.43	280.0±3.26	313±8.88	346.66±8.11	390.66±8.74	2.227±0.0905
3rd group	249.0±5.0	277±5.97	341.3±11.18	379.3±9.68	421.33±12.7	1.617±0.076***
4th group	248±12.0	281±4.43	324.91±1.74	362.66±12.79	396±8.326	1.826±0.074*
5th group	255±11.59	253.3±6.67	308±2.38	348.66±7.33	393.3±10.41	1.762±000**
6th group	260.25±4.6	296.82±7.3	358.66±7.42	406.66±9.61	458.66±14.8	1.666±0.071***
7th group	261.32±6.9	287.5±8.81	338.66±18.6	376±8.33	426±12.05	1.934±0.22

Groups that significantly different from the 2nd group, *p <0.05 ** p <0.01 ***p <0.001

Table 3: The mean body weight and the average daily gain at the start of experiment and at the end of experiment in all groups.

Group	MBW(g) at the start of experiment (12 day of old)	MBW(g) at the end of experiment (35 day of old)	ADG
1st group	216.84±7.76	1445±24.4	56.76±0.99
2nd group	207.5±5.93	1417±41.9	54.65±1.72
3rd group	206.5±8.9	1542±31.50	59.66±1.33
4th group	220.5±5.54	1442±32.9	54.43±1.43
5th group	181.66±6.919	1580±24.4	62.61±1.07*
6th group	196.66±5.72	1692±38.6	69.60±1.47**
7th group	201.1±6.75	1651±40.6	66.39±0.76**

Groups that significantly different from the 2nd group, *p <0.05 ** p <0.01

Antibacterial activity of the tested drugs against *E coli* O_{111}:

Antibacterial activity of fosfomycin, thiamphenicol and *Nigella sativa* oil against *E coli* O_{111} measured as diameter zone of inhibition (mm) and illustrated in table (4). The result showed that the administration of *Nigella sativa* oil with fosfomycin or thiamphenicol increase their antimicrobial activity, the serum samples of the group treated with fosfomycin and *Nigella sativa* oil showed the highest zone of inhibition than other groups.

Table 4: Antibacterial activity of fosfomycin, thiamphenicol and *Nigella sativa* oil against *E. coli* O_{111}.

Group	Zone of inhibition (mm)				
	Days of medication				
	1st day	2nd day	3rd day	4th day	5th day
3rd group	10.8±0.116	11±0.001	10.5±0.166	10.8±0.16	11.16±0.33
4th group	10.5±0.577	10.66±0.44	9.8±0.288	10.33±0.166	9.66±0.166
5th group	10±0.57	10±0.28	9.5±0.28	10±0.288	9.83±0.44
6th group	11.83±0.60	11.5±0.288	11.33±0.166	11.5±0.166	11.33±0.33
7th group	11.25±0.5	11±0.726	10.63±0.33	11.00±0.166	10.33±0.33

Immune-responsiveness against *E. coli* O_{111}:
The data showed significantly increase in antibody titer ($p < 0.05$) for *E. coli* O_{111} after two weeks of infection in all chicken groups which infected or infected and received antibacterials alone or in combination with *Nigella Sativa* oil than those of the control group (Table, 5).

The 3rd, 5th group, 6th group and 7th group revealed significant increase ($P < 0.05$) in antibody titer against *E. coli* O_{111} in comparison with infected group after two weeks of infection. The important comparison for antibody titer in groups which received *Nigella Sativa* oil either with fosfomycin or thiamphenicol showed that the level of antibody production significantly elevated after 15 days and continued after the 21 days of infection.

Table 5: Mean log_{10} antibody titer against *E. coli* serotype O_{111} in all groups at 0, 1st, 2nd and 3rd week post infection.

Group	Mean log_{10} antibody titer X ± SE			
	0	1st week	2nd week	3rd week
1st group	3.86 ± 0.042	3.87±0.05	4.05±0.089	3.99±0.040
2nd group	3.93 ± 0.15	4.15±0.049	4.30±0.072 [a]	4.43±0.090 [a]
3rd group	3.91 ± 0.042	4.23±0.041[a]	4.48±0.049 [ab]	4.51±0.040 [ab]
4th group	3.99 ± 0.030	4.06±0.035	4.29±0.064 [a]	4.17±0.045 [a]
5th group	3.97 ±0.14	4.05±0.030	4.45±0.07 [ab]	4.39±0.047 [a]
6th group	3.91 ±0.032	4.12±0.018	4.59±0.056 [ab]	4.63±0.089 [ab]
7th group	3. 83 ± 0.040	3.89 ± 0.014	4.43±0.075 [ab]	4.31±0.034 [ab]

a Group that significantly different ($p < 0.05$) from the 1st group
b Group that significantly different ($p < 0.05$) from the 2nd group

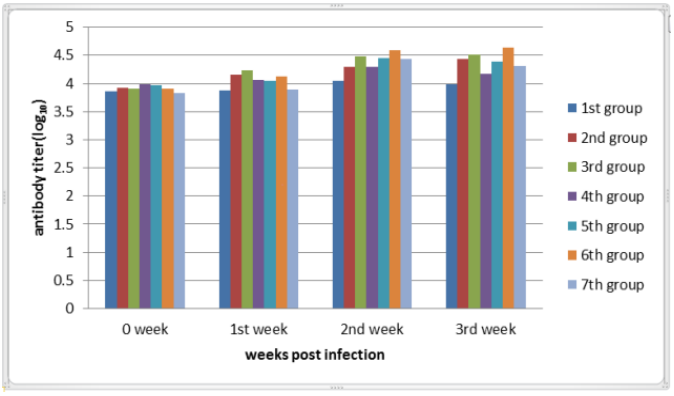

Figure 1: Mean log_{10} antibody titer against *E.coli* serotype O_{111} in all groups at 0, 1st, 2nd and 3rd week post infection.

DISCUSSION

A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which lead to insufficiency of antimicrobial treatment (Schelz *et al.,* 2006), so combination of different antimicrobials may result in a predictable therapeutic result as to increase the therapeutic efficacy or decrease the toxicity among the administrated drugs (Hurry *et al.*, 1998). Therefore the aim of this work is to compare the efficacy of fosfomycin, thiamphenicol alone or in combination with *Nigella Sativa* oil against experimental infection of broiler chickens with *E. coli* O_{111} and study their effect on immune response.

In the present study, the experimental infection of broiler chickens with *E. coli* O_{111} cause severe clinical signs (depression, decrease food intake, respiratory sings and diarrhea) with gross lesions (airsacculitis and pericarditis) in non medicated group, this was similar to those reported by (Nakamura *et al.,* 1992; Mogenet *et al.,* 1997). The clinical signs and lesion scores were less in chicken treated with fosfomycin, thiamphenicol alone or in combination with *Nigella sativa* oil, these results were similar to those reported by (Fernandez *et al.,* 2002) who proved that the treatment with calcium fosfomycin in the drinking water controlled the adverse effects of experimental colibacillosis., also agreed with (Kowalski, 2007) who reported that thiamphenicol has the potential to become available antibiotic in the treatment and control of a wide range of respiratory and alimentary tract infection of bacterial origin in live stock of mammals, poultry and fish, and (Erener *et al.,* 2010) who reported that *Nigella sativa* seed was effective against total coliform count in the intestine of broilers.

The mean body weight, Feed conversion rate (FCR) and Average daily gain were significantly improved in chicken treated with fosfomycin, thiamphenicol and *Nigella sativa* oil when compared with infected non treated group, the best significant performance parameters were demonstrated in groups treated with fosfomicin and thiamphenicol in combination of *Nigella sativa* oil rather than groups treated with each compound separately, our result was similar to findings reported in chicken treated with fosfomycin (Fernandez *et al.,* 1998, 2002), also (Halle *et al.,* 1999; Erener *et al.,* 2010; Shewita and Taha, 2011) showed that dietary black cumin (*Nigella sativa*) and its oil extract improved feed intake, body weight and FCR in the broilers even at low dose.

Treatment of *E. coli* O_{111} infected chickens by fosfomycin, thiamphenicol alone or in combination with *Nigella sativa* oil reduce *E.coli* reisolation rate from liver, heart and air sac when compared with those data recorded in infected non treated chickens. These findings were supported by previous report (Shaheen and El-Far, 2013) who recorded that combination of the therapeutic doses of pefloxacin and florfenicol reduce *E.coli* reisolation rate from liver and heart. Also agreed with the result obtained by (Fernandez *et al.,* 1998, 2002) who demonstrated that fosfomycin reduced the numbers of birds from which bacteria were reisolated and it was effective for treatment of *E.coli* infection in chickens.

The result of antibacterial activity of fosfomycin, thiamphenicol and *Nigella sativa* oil against *E coli* O_{111} which measured as diameter of zone of inhibition (mm) showed that the administration of *Nigella sativa* oil with fosfomycin or thiamphenicol increase their antimicrobial activity. Our result agreed with that obtained by (Hanafy and Hatem, 1991) who recorded that the extract of *Nigella sativa* showed antibacterial synergism with streptomycin and gentamycin and showed additive antibacterial action with some antibiotics such as spectinomycin, erythromycin, tobramycin and chloramphenicol.

The effect of *Nigella Sativa* on the immune- response has been investigated by several researches. Many studies have reported that the oil of *N.Sativa* and its component produce an increase in the T- helper cells and enhance natural killer cell activity, also they had a stimulatory effect on macrophages (El- Kadi and Kandil, 1986 and Haq *et al*., 1995). The ability of the most abundant component of *Nigella Sativa oil*; Thymoquinone (TQ) to modulate cytokines and enhance the immune system has been implicated as the main reason for its protective effect against schistosoma egg infection in the liver (Mahmoud *et al.,* 2002). Dorucu *et al*. (2009) proved that serum proteins and total immunoglobulin levels were significantly increased in rainbow trout fish.

In this study, the specific antibody titers against the *E. coli* O_{111} detected by *ELISA* in serum were significantly increased after two weeks of infection in 5^{th}, 6^{th} and 7^{th} groups which received the *Nigella Sativa* oil in comparison to other groups. These results agreed with those of Durrani *et al.,* 2007; Al-Beitawi *et al.,* 2009; Shewita and Taha (2011) and Al-Mufarrej, (2013) who reported significant improvement in antibody titer. Using *Nigella Sativa* as feed supplementation enhanced antibody production in laying hens (Yalçin *et al.,* 2012). Moreover, using *Nigella Sativa* oil significantly enhanced the immune system through increased lymphocyte production, and inhibited development of advanced dysplastic changes after topical application of DMBA (7,12- Dimethylbenz(a) anthracene) in hamsters to induce immune-suppression (Al-Jawfi *et al.,* 2008). The results of this study disagreed with the finding of (Jang, 2011) who recorded no significant effects on immunity parameters except in lymphocyte ratio. In conclusion, the use of *Nigella*

Sativa oil has beneficial effects on the performance, feed intake, body weight and immunological response in the broilers. Future researches should focus on the mechanisms by which the *Nigella Sativa* oil exerts their effects.

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REFERENCES

AL-Beitawi, N.A.; El-Ghousein, S.S. and Nofal, A.H. (2009): Replacing bacitracin methylene disalicylate by crushed *Nigella sativa* seeds in broiler rations and its effects on growth, blood constituents and immunity. Livest. Sci., 125, pp. 304–307.

AL-Jawfi, K.A.M.; Hassan, M.M.A. and El-Gohary, A.M. (2008): Effect of *Nigella sativa* oil on the Hamster lymphocytes secondary to DMBA-induced carcinogenesis. Suez Canal Univ. Med. J., 11 (1): pp. 75–80.

Al-Mufarrej, S.I. (2013): Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella Sativa* L.) powder. J. Saudi Soci. Agricult. Sci., 13 (1): 75–80.

Aramayona, J.; Bregante, M.; Solans, C.; Rueda, S.; Fraile, L.J. and Garcia, M.A. (1997): Pharmacokinetics of Fosfomycine in chickens after a single intravenous dose and tissue levels following chronic oral administration. Vet. Res., 28, 581-588.

Barnes, H.J.; Vaillancourt, J.P. and Gross, W.B. (2003): Diseases of poultry. Iowa State Univ. Press, Ames, IA. p. 631-652.

Barnes, J.; Nolan, L.K. and Vaillancourt, J.P. (2008): Colibacillosis. In: Diseases of poultry, 12th ed. Blackwell publishing, Ames, IA. pp. 691-732.

Briggs, D.J. and Skeeles J.K. (1984): An enzyme linked immunosorbent assay for detecting antibodies to *Pasturella multocida* in chickens. Avian Dis. 28 (1): 208 -215.

Cannon, M.; Horfard, S. and Davies, J. (1990): A comparative study on the inhibitory actions of Chloramphenicol, Thiamphenicol and some fluorinated analogs. J. Antimicrob. Chemoth. 26, 307-317.

Charleston, B.; Gate, J.J.; Aitken, I.A.; Stephan, B. and Froyman, R. (1998): Comparison of the efficacies of three fluoroquinolone antimicrobial agents, given as continuous or pulsed-water medication, against *Escherichia coli* infection in chickens. Antimicrob. Agents Chemother.; 42(1): 83-7.

Dàmaso, L.D. (1990): Fosfomicina en Antibacterianos, (Marketing pharm, S. A., Madrid), 435-452

Dho-Moulin, M. and Fairbrother, J.M. (1999): Avian pathogenic *Escherichia coli* (APEC). Vet. Res., 30, 299-316.

Dorucu, M.; Ozesen Colak, S.; Ispir, U.; Altinterim, B. and Celayir, Y. (2009): The effect of black cumin seeds, *Nigella sativa*, on the immune response of rainbow trout, *Oncorhynchus mykiss*. Mediterr. Aquacult. J., 2 (1), pp. 27–33.

Durrani, F.R.; Chand, N.; Zaka, K.; Sultan, A.; Khattak, F.M. and Durrani, Z. (2007): Effect of different levels of feed added black seed (*Nigella sativa* L) on the performance of broiler chicks. Pakistan J. Biol. Sci., 10, pp. 4164–4167.

Erener, G.; Altop, N.; Ocak, H.; Aksoy, S. and Ozturk, E. (2010): Influence of black cumin seed (*Nigella sativa* L) and seed extract on broilers performance and total coliform bacteria count. Asian J. Anim. Vet. Adv., 5, pp. 128–135.

El-Kadi, A. and Kandil, O. (1986): Effect of *Nigella sativa* (the black seed) on immunity, Proceeding of the 4th International Conference on Islamic Medicine, Kuwait. Bull Islamic Med., 4: 344–348.

Fernàndez, A.; Lara, C.; Puyuelo, R.; Gomez J.; Ramos J.J.; Loste, A.; Marca, M.C. and Verde M.T. (1998): Efficacy of phosphomycin in the control of *Escherichia coli* infection of broiler chickens. Res. Vet. Sci.; 65(3): 201-204.

Fernàndez, A.; Lara, C.; Loste, A. and Marca, M.C. (2002): Efficacy of calcium fosfomycin for the treatment of experimental infection of broiler chickens with *Escherichia coli* O78:K80. Vet. Res. Commun., 26, 427-436.

Geornaras, I.; Hastings, J.W. and Von Holy, A. (2001): Genotypic analysis *E.coli* strains from poultry carcasses and their susceptibilities to antimicrobial agents. Appl. Environ. Microbial. 67: 1940-1944.

Giorgi, M.; Romani, M.; Bagliacca, M. and Mengozzi, G. (2000): Determination of thiamphenicol residues in albumin and yolk of hen eggs. J. Vet. Pharmacol. Therap. 23, 397-399.

Grif, K.; Dierich, M.; Pfaller, K.; Miglioli, P. and Allerberger, F. (2001): In vitro activity of fosfomycin in combinations with varies antistaphylococcal substances. J. Antimicrob. Chemoth., 48, 209-217.

Gutierrez, L.; Ocampo, L.; Rosario, C. and Sumano, H. (2010): Pharmacokinetics of disodium fosfomycin in broilers and dose strategies to comply with its pharmacodynamics versus

Escherichia coli. Poult Sci., 89(10): 2106-2115.

Hala G. Muhtasib; Nahed El. Najjar and Regine S. Stock (2006): The medicinal potential of black seed (*Nigella sativa*). Advances in phytomedicine, 2, 133-153.

Halle, I.; Thomann, R.; Flachowsky, G.; Schubert, R.; Flachowsky, G.; Bitsch, R. and Jahreis, G. (1999): Effect of ethereal (essential) oil and oil seed on the growth of broilers. Vitamin and Zusatzstoffe in der Ernaehrung, Von Mensch undTier: 7, symposium Jena, Thuringen, Germany.

Hanafy, M.S. and Hatem M.E. (1991): Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J Ethnopharmacol., 34: 278-280.

Haq, A.; Abdullatif, M.; Lobo, P.I.; Khabar, K.S.; Sheth, K.V. and Al-Sedairy, S.T. (1995): Nigella sativa: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. Immunopharmacol., 30(2): 147-55.

Hurry, W.; Lampiris, M.D. and Aniel, S.M. (1998): Clinical use of antimicrobial agents in, Basic and clinical pharmacol. 7th edn., Katzung, B.G.(ed) Stanford, Appleton and lange, New York, 812-826.

Hodžic, A.; Hrković, P.A.; Gagić, A.; Hamamdžić, M.; Kavazović, A.; Pašić Juhas, E.; Kustura, A. and Goletic, T. (2012): Dietary Black cumin (*Nigella sativa*) seed oil affects serum lipids in chicken broilers. Veterinaria, 61(1-2), 23-33.

Jang, J.P. (2011): The evaluation of different levels of *Nigella Sativa* seed on performance, and blood parameters of broilers. Ann. Biol. Res., 2 (5): pp. 567–572.

Kowalski, P. (2007): Capillary electrophoretic determination of thiamphenicol in turkeys' serum and its pharmacokinetic application. J. pharmaceut. Biomed. Analy. 43, 222-227.

Mahmoud, M.R.; El-Abhar, H.S. and Saleh, S. (2002): The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J. Ethnopharmacol., 79(1): 1-11.

Mogenet, L.; Bezille, P.; Guyonett, J. and Karembe, H. (1997): Comparison de la fluméquine (Flumisol) à l'amoxicilline (Vetrimoxin poudre orale) dans deux modes d'administration par voie orale, en traitement de la colibacillose du poulet: approche pharmacodynamique et Clinique. Revue Médecine Vétérinaire, 148, 793-804.

Mukherjee, P.K.; Balasubramanian, R.; Saha, K.; Saha, B.P. and Pal, M. (1995): Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizomes extract; Indian Drugs, 32, 274-276.

Nakamura, K.; Cook, J.K.A.; Frazier, J.A. and Narita, M. (1992): Escherichia coli multiplication and lesion in the respiratory tract of chickens inoculated with infectious bronchitis virus and/or *E.coli* .Avian Dis., 36, 881-890.

Nasir, Z. and Grashorn, M.A. (2006): Use of Black cumin (*Nigella sativa linn.)* as alternative to antibiotics in poultry diets. 9 Tagung. Schweine und Geflugelernahrung, Martin Luther Universitat Halle Wittenberg Halle, Germany, 28-30 November, 210-213.

Nofal, M.E.; Abo-Etta, E.M. and Salem, A.A. (2006): Some productive and physiological responses to dietary *Nigella Sativa* seeds supplementation of Mamourah laying hens. Egypt poult. Sci., 26: 455-476.

Prescott, J.F. (2000): Peptide antibiotics: polymixins, glycopeptides, streptogramins and bacitracin.in antimicrobial therapy in Veterinary Medicine, 3rd edn. Eds Prescott, J., Baggot, D. and Walter, R., pp.190. Iowa State University Press, Ames, IA.

Rawiwet, V.; Chansiripornchai, P. and Chansiripornchai, N. (2010): Comparison of the Efficacy of Enrofloxacin against *Escherichia coli* or *Pasteurella multocida* Infection in Chickens. Thai J. Vet. Med. 40(3): 297-301.

Schelz, Z.; Moniar, J. and Hohmann, J. (2006): Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 77; 279-285.

Shaheen, H.M. and El-Far, A.H. (2013): Evaluation of the therapeutic efficacy of pefloxacin and florfenicol combination in broilers experimentally challenged by *Escherichia coli*. Int. J. Pharm. Sci. Rev. Res., 23(2), 396-404.

Shewita, R.S. and Taha, A.E. (2011): Effect of dietary supplementation of different levels of black seed (*Nigella Sativa* L.) on growth, performance, immunological, hematological and carcass parameters of broiler chicks. World Acad. Sci. Eng. Technol., 77, pp. 788–794.

Switala, M.; Hrynyk, R.; Smutkiewicz. A.; Jaworski, K.; Pawlowski, P.; Okoniewski, P.; Grabowski, T. and Debowy, J. (2007): pharmacokinetics of florfenicol, thiamphenicol, and chloramphenicol in turkeys. J. Vet. Pharmacol. Therap. 30, 145-150.

Tessier, F. and Quentin, C. (1997): In vitro activity of fosfomycin combined with ceftazidine, imipem, amikacin and ciprofloxacin against *pseudomonas aerugenosa*. Clin. Microbiol. Infec. 16, 159-162.

Tolba, A.A.H. and Hassan, M.S.H. (2003): Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions. 2-Black cumin (*Nigella Sativa*) or garlic (*Allium sativum*). Egypt poult. Sci., 23: 327-340.

Tolba, A.A.H.; Abd El-Galyl, M.A. and Abd El-Samad, M.H. (2005): The effect of using some herbal additives on physiological and productive performance of two Egyptian chicken strains winter and summer seasons. Egypt poult. Sci., 25: 107-123.

Yalçin, S.; Yalçin, S.; Uzunoğlu, K.; Duyum, H.M. and Eltan, Ö. (2012): Effects of dietary yeast autolysate *(Saccharomyces cerevisiae)* and black cumin seed (*Nigella sativa* L.) on performance, egg traits, some blood characteristics and antibody production of laying hens. Livest. Sci., 145, (1-3): 13–20.

مقارنة كفاءة الفوسفومايسن، الثيامفينيكول وزيت حبة البركة وخليط مع كلاهما فى علاج الدجاج المصاب بميكروب القولون الإشريكى

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تهدف هذة الدراسة الى مقارنة كفاءة كلا من الفوسفومايسن، الثيامفينيكول وزيت حبة البركة فى علاج الدجاج المصاب بميكروب القولون الإشريكى (O_{111}). تمت الدراسة على ١٢٦ دجاجة عمر ١٢ يوم حيث تم تقسيمهم الى سبع مجموعات: المجموعة الاولى هى الضابطة والمجموعة الثانية تم اصابتها بميكروب القولون الإشريكى (O_{111}) ولم يتم علاجها أما المجموعة الثالثة تمت اصابتها بالميكروب وعولجت بالفوسفومايسن بجرعة ٤٠ مجم/كجم من وزن الجسم فى مياة الشرب والمجموعة الرابعة تمت اصابتها بالميكروب وعولجت بالثيامفينيكول بجرعة ٣٠ مجم/كجم من وزن الجسم فى مياة الشرب والمجموعة الخامسة تم اصابتها بالميكروب وتم تجريعها زيت حبة البركة بجرعة ٠.٠٢٥ ملى / كجم من وزن الجسم عن طريق الفم اما المجموعة السادسة تم اصابتها بالميكروب وعولجت بالفوسفومايسن بالاضافة الى زيت حبة البركة اما المجموعة السابعة تمت اصابتها بالميكروب وعولجت بالثيامفينيكول بالاضافة الى زيت حبة البركة بنفس الجرعات السابقة. ثم تم دراسة تاثير هذة الادوية على معدل التحويل الغذائى وملاحظة الاعراض المرضية التى تظهر على الدجاج وتم عزل الميكروب من الانسجة الداخلية ودراسة نشاطهما المضاد لهذا الميكروب وتاثيرهما على الجهاز المناعى. ومن هذة الدراسة تبين ان الاعراض المرضية والتشريحية ظهرت بصورة اقل فى الدجاج المعالج بهذة الادوية وكان هناك زيادة فى وزن الجسم والنشاط المضاد البكتيرى وأيضا زيادة فى الأجسام المناعية المتخصصة لميكروب القولون الإشريكى (O_{111}) خاصة فى المجموعات التى عولجت بالفوسفومايسن والثايمفينيكول بالاضافة الى زيت حبة البركة. ومن هذة الدراسة نستنتج ان اضافة زيت حبة البركة مع كلا من الفوسفومايسن والثيامفينيكول زاد من كفائتهما العلاجية.