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Tackling of Experimental Colisepticaemia in Broiler Chickens Using Phytobiotic Essential Oils and Antibiotic Alone or in Combination

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Abstract: This study was designed to compare between the efficacy of a phytobiotic containing a mixture of essential oils of *Oreganum aetheroleum* and an antibiotic containing ciprofloxacin as an active principle for the treatment of experimental (*E. coli*) infection in broiler chickens. Two hundred, day old broiler chickens were divided into 5 equal groups. The 1st group was neither challenged nor treated but groups 2, 3, 4 and 5 were challenged with *E. coli*. The 2nd group was challenged only while the 3rd, 4th and 5th group were treated with phytobiotic, ciprofloxacin and phytobiotic and ciprofloxacin combination, respectively. Results confirmed significant ($p<0.05$) improvement of productive performance parameters, reduction in signs, mortalities, post mortem lesions and bacterial re-isolation, significant ($p<0.05$) enhancement in cell mediated and humoral immune responses, significant ($p<0.05$) reduction in levels of liver and kidney function tests as well as significant ($p<0.05$) increase in the total protein and globulin levels in chickens challenged with *E. coli* and treated with either essential oils or ciprofloxacin compared with challenged non treated chickens. Moreover, the best significant ($p<0.05$) results in all measured parameters were detected in group treated with combination in comparison with those treated with single treatment. In conclusion, a mixture of essential oils of *Oreganum aetheroleum* is effective for the treatment of *E. coli* infection in broiler chickens when compared with ciprofloxacin. However, combined treatment could be superior in controlling such infection of broiler chickens.

Key words: Poultry, colibacillosis, phytobiotics, ciprofloxacin, treatment, phytobiotic

INTRODUCTION

Colibacillosis is a disease in poultry caused by (*E. coli*) that induces an acute fatal septicaemia or sub-acute infection that extends to the serosal surfaces, joints and other organs (Rawivet and Chansiripornchai, 2009). Serotype O78 is one of the most common pathogenic one for poultry (Chansiripornchai and Sasipreeyajan, 2002). Ciprofloxacin is the 2nd generation of fluoroquinolones group; a broad-spectrum antimicrobial agent that is effective in the treatment of *E. coli* infection in poultry (Medders *et al*., 1998). Unfortunately, frequent use of this group may result in prevalence of fluoroquinolone-resistant *E. coli* in poultry ecosystem and consequently play a role in the treatment of humans' *E. coli* infections (Kola *et al*., 2005). So, alternative strategies for controlling of avian *E. coli* infection are being studied, among which using of natural additives (Nouzarian *et al*., 2011). Recently, emphasis for using phytogenic or herbal plants containing essential oils in poultry farms have been developed all over the world with successful results (Hashemi and Davoodi, 2010). Among theses phytobiotics are species of *Origanum* genus which are aromatic plants of the family Lamiaceae. The volatile oils of *Origanum vulgare* species have been used traditionally for respiratory disorders, indigestion, dental caries, rheumatoid arthritis and urinary tract disorders (Ertas *et al*., 2005). Some studies have demonstrated potent antibacterial (Saeed and Tariq, 2009), antiviral (Meschino, 2005), antifungal (Soylu *et al*., 2007), antiparasitic (Toulah *et al*., 2012), antioxidant as well as antimutagenic (Martinez-Rocha *et al*., 2008) and recently disinfecting (Debes and Basyony, 2011) properties of oregano.

For this, the current study was designed to compare between the effect of a phytobiotic product containing a mixture of essential oils of *Oreganum aetheroleum* and an antibiotic with active principle ciprofloxacin for the treatment of experimental infection with *E. coli* in broiler chickens.

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MATERIALS AND METHODS

The used phytobiotic: A commercial phytobiotic natural water additive supplement based on oregano (Orego-stim®) produced by the Meriden Animal Health Company, UK was tested. The main active ingredient of the product is *Oreganum aetheroleum* that is produced from the plant *Origanum vulgare* as it contains many phenolic substances such as carvacrol (81.89%) and thymol (2.12%) as well as the monoterpene hydrocarbons γ-terpinene (5.1%) and p-cymene (3.76%). It was used at a dose of 0.3 mL L^{-1} of drinking water for 5 consecutive days.

The used antibiotic: Ciprofloxacin (CIPRO 20%®) is a synthetic antibacterial agent from the fluoroquinolone group produced by ArabcoMed Company, Egypt. Each gram contains 200 mg ciprofloxacin. It was given as 5 mg kg^{-1} body weight/day (1 g/4 L of the drinking water) for 5 consecutive days.

The bacterial culture: Avian field strains of *E. coli* serotype (O78) was obtained from the Animal Health Research Institute, Dokki, Giza, Egypt. The bacterial culture was propagated on MacConkey's agar and incubated at 37°C for 24 h. For experimental challenge of chickens, a nutrient broth culture of *E. coli* (O78) containing 10^8 colony forming unit CFU/mL was prepared (Fernandez *et al*., 2002).

Antibiotic sensitivity test: The *in vitro* antibiotic sensitivity test using the challenge organism *E. coli* serotype (O78) and ciprofloxacin antibiotic disc as well as other antibiotics like enrofloxacin, norfloxacin oxytetracycline and neomycin discs (Oxoid) were done according to standard disc diffusion technique (Prasad *et al*., 1997).

Experimental design: Day old, two hundred and twenty, Hubbard breed of broiler chicks was obtained from Cairo Poultry Company. Upon arrival, all chicks were kept in cleaned and disinfected houses and fed on a balanced commercial ration without any antimicrobial agents or feed additives. The vaccination schedule for the birds were the following; vaccination against Newcastle disease at 5 and 21 days old and against Gumboro disease at 12 days old using the eye drop inoculation method while vaccination against avian influenza was given at 7 days old through intramuscular inoculation. Just before the challenge, twenty chicks were selected randomly and sacrificed to be examined for insurance of absence of *E. coli* infection. At 14 days old, two hundred birds were divided into 5 equal groups, 40 each; the 1st group was not inoculated and left as a blank control negative. From the 2nd till the 5th group, each bird was inoculated intramuscularly with 0.5 mL of the nutrient broth culture containing 10^8 CFU *E. coli* O78/mL. The 2nd group was challenged but not treated and kept as control positive. The 3rd group was challenged and treated with the phytobiotic (essential oils mixture of *Oreganum aetheroleum*) at the recommended dose of the manufacturer (0.3 mL L^{-1} of drinking water for 5 consecutive days). The 4th group was challenged and treated with the antibiotic (ciprofloxacin) at the recommended dose of the manufacturer 5 mg kg^{-1} body weight/day (1 g/4 L of the drinking water) for 5 consecutive days. The 5th group was challenged and treated with the phytobiotic and ciprofloxacin at doses and duration as mentioned in the 3rd and 4th groups, respectively. The experiment was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee (IAEC).

Blood samples collection: Blood samples were collected by heart puncture from ten birds/group. Each sample was divided into 2 parts; the 1st was citrated for measurement of cell mediated immune response while the 2nd one was kept to clot and centrifuged to separate serum and then stored at -20°C till using for assessment of the humoral immune response and clinical serum chemistry (liver and kidney function tests and total protein and albumin).

Evaluation parameters

Productive performance: Average Body Weight (ABW), Feed Conversion Rate (FCR) and European Production Efficiency Factor (EPEF) of the chickens in each group were determined weekly till the end of the experiment (6 weeks old) according to Sainsbury (1984).

Morbidity and mortality rates: Each bird in the challenged group was observed daily after challenge for any symptoms and deaths. Moreover, dead birds were examined for the specific *E. coli* lesions.

Bacteriological investigation: Under complete aseptic condition, samples from the lung and liver were collected from 10 sacrificed birds in each group at the 1st, 2nd, 3rd and 4th weeks after challenge. The samples were inoculated on MacConkey agar and then incubated at 37°C for 24 h, then identified biochemically and serologically (Sambrook *et al*., 1989).

Cellular immune response: To investigate the possible effect of each treatment on the cell mediated immunity;

measurement of phagocytic activity and index of peripheral blood monocytes using *Candida albicans* was adopted at 3 and 5 weeks of age (Chu and Dietert, 1989).

Humoral immune response: At 21 days of age, all the birds belonged to each group were inoculated with 0.5 mL/bird of 5% suspension of washed Sheep Red Blood Cells (SRBC) in Phosphate Buffer Saline (PBS) intramuscularly. At week interval (4, 5 and 6 weeks of age), the total Haemagglutinating Inhibiting (HI) antibody titers produced in response to SRBC were determined by micro-agglutinating technique (Wegmann and Smithies, 1966). All titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination.

Serum biochemical metabolites: At the end of the study (6 weeks of age), the levels of serum biochemical metabolite parameters including Aspertate aminotransferase (AST) and alanine Aminotransferase (ALT) (liver function tests) also uric acid and creatinine (kidney function tests) were determined according to Santurio *et al.* (1999) and Sachan *et al.* (2002) using commercially available diagnostic kits according to manufacturer's instructions. Serum samples were also analyzed for total protein and albumin where globulin concentration was detected by subtracting albumin concentration of proteins and consequently albumin to globulin ratio was calculated (Doumas, 1971).

Statistical analysis: Data were presented as Mean±Standard deviation for numerical variables and as number (percentage) for qualitative variables. One way ANOVA was used to assess the statistical significance of mean difference between groups. Bonferroni post hoc test was used when ANOVA is significant. Pearson χ^2-test was performed to show the significance of association between categorical variables. The significance level was set at $p \leq 0.05$. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS©) Version 16.0.

RESULTS

Results of antibiotic sensitivity pattern *in vitro* showed that *E. coli* challenge strain was highly sensitive to ciprofloxacin compared with enrofloxacin, norfloxacin oxytetracycline and neomycin.

Table 1 shows the results of the productive performance (ABW, FCR and EPEF) of all groups from the first till the 6 weeks of age. Significant ($p<0.05$) improvement in overall productive parameters was observed in the blank control negative birds and other challenged treated groups when compared with control positive birds during the whole experimental period. The best significant ($p<0.05$) performance parameters were demonstrated in group treated concomitantly with oregano essential oils and ciprofloxacin rather than groups treated with each compound separately.

The clinical signs of challenged birds appeared 2 days post *E. coli* challenge. Signs of depression, ruffling, offood, thirst, nasal and ocular discharge, coughing and rals were observed in all challenged chickens. The severest signs (90% morbidity rate) were seen in control positive challenged group; however blank non challenged one showed no signs. The severity of signs decreased 10 days after treatment with oregano essential oils and ciprofloxacin in separate manner whereas the response of chickens to combined treatment with oregano essential oils and ciprofloxacin was more pronounced as signs continued to improve post-treatment more rapidly and few affected birds showed mild signs of respiratory distress at 5 days following the end of treatment.

No deaths were detected in non challenged group while challenged non treated chickens showed cumulative mortality rate of 40% at the 2nd day post challenge. This percentage decreased in the treated chickens to reach 6, 5 and 3%, respectively in groups 3, 4 and 5. Mortalities were reduced by the 5th day of combined treatment with essential oils and ciprofloxacin and completely disappeared by the 7th day of treatment.

The lesions observed during post-mortem examination of dead birds were varying degrees of serous membranes inflammation (serous to fibrinous pericarditis, perihepatitis and airsacculitis) associated with tracheitis and pneumonia. The mildest (serous) gross pathological lesions were recorded in groups challenged and treated with phytobiotic essential oils and ciprofloxacin alone or in combination than that challenged and not treated. Complete absence of the lesions were seen a week after combined treatment.

The results of bacterial re-isolation are seen in Table 2. It was observed that during 4 weeks after challenge, *E. coli* was not re-isolated from blank control group. The organism was not recovered from challenged chickens that treated with ciprofloxacin or phytobiotic essential oils and ciprofloxacin combination at the 3rd and 4th week post challenge. Group treated with phytobiotic essential oils revealed absence of *E. coli* recovery only at the 4th week after challenge. Challenged non-treated group had a higher significant ($p<0.05$) frequency of *E. coli* re-isolation that ranged from 90-50% along all the observation period.

Table 1: Effect of oregano essential oils and antibiotic treatments on productive performance of non challenged and *E. coli* challenged and treated broiler chickens

		Average body weight/g							
		Age/week							
Group number	Treatments	1	2	3	4	5	6	FCR	EPEF
1	Non challenged+non treated	140.82±3.40^{a}	304.0±5.32^{ab}	598.30±21.80^{a}	940.2±51.200^{a}	1340.20±35.7^{a}	1678.0±42.30^{a}	1.93	210.03
2	Challenged+non treated	134.98±5.55^{a}	256.4±8.65^{c}	501.10±19.20^{b}	702.91±54.10^{b}	930.12±32.4^{c}	1250.1±14.30^{c}	2.40	145.21
3	Challenged+oregano essential oils	135.74±6.18^{a}	298.1±7.55^{ab}	563.41±23.86^{a}	876.45±71.80^{a}	1119.60±67.2^{b}	1479.1±33.20^{b}	2.00	181.41
4	Challenged+ciprofloxacin	133.96±6.72^{a}	285.9±8.40^{b}	556.31±1.530^{a}	824.99±0.890^{ab}	1089.51±3.20^{b}	1401.9±34.51^{b}	2.20	179.56
5	Challenged+oregano essential oils+ciprofloxacin	140.24±2.37^{a}	307.9±5.30^{a}	584.9±20.110^{a}	906.90±32.09^{a}	1198.00±68.2^{b}	1596.4±39.44^{a}	1.90	205.44

FCR = Feed Conversion Rate; EPEF = European Production Efficiency Factor; means with different letters (a-c) within the same column are significantly different at $p \leq 0.05$

Table 2: Effect of oregano essential oils and antibiotic treatments on *E. coli* re-isolation rate of non challenged and *E. coli* challenged and treated broiler chickens

			Weeks after challenge			
Group number	Treatments	Number of examined chickens	1st	2nd	3rd	4th
1	Non challenged+non treated	10	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2	Challenged+non treated	10	9/10 (90%)	7/10 (70%)	5/10 (50%)	5/10 (50%)
3	Challenged+oregano essential oils	10	3/10 (30%)	2/10 (20%)	2/10 (20%)	0/10 (0%)
4	Challenged+ciprofloxacin	10	3/10 (30%)	1/10 (10%)	0/10 (0%)	0/10 (0%)
5	Challenged+oregano essential oils+ciprofloxacin	10	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
p-value			<0.001	<0.001	0.003	0.001

Table 3: Effect of oregano essential oils and antibiotic treatments on cell mediated immunity (phagocytic activity and index) of non challenged and *E. coli* challenged and treated broiler chickens

		3 weeks of age		5 weeks of age	
Group number	Treatments	Phagocytic activity	Phagocytic index	Phagocytic activity	Phagocytic index
1	Non challenged+non treated	54.11±2.83^{bc}	0.5167±1.21^{a}	56.69±3.78^{c}	0.5293±0.22^{b}
2	Challenged+non treated	40.62±1.86^{d}	0.2001±0.12^{a}	48.11±3.20^{d}	0.3010±0.10^{b}
3	Challenged+oregano essential oils	61.31±4.72^{b}	0.5322±0.10^{a}	65.17±2.79^{b}	0.5460±0.10^{b}
4	Challenged+ciprofloxacin	49.78±5.60^{cd}	0.4140±0.31^{a}	51.97±0.22^{cd}	0.4789±0.10^{b}
5	Challenged+oregano essential oils+ciprofloxacin	72.03±0.29^{a}	0.7450±0.02^{a}	75.99±0.89^{a}	0.9889±0.18^{a}

Means with different letters (a-d) within the same column are significantly different at $p \leq 0.05$

Table 4: Effect of oregano essential oils and antibiotic treatments on Humoral Immunity (HI) titers of non challenged and *E. coli* challenged and treated broiler chickens

		Weeks after inoculation of sheep red blood cells		
Group number	Treatments	1st	2nd	3rd
1	Non challenged+non treated	5.5±0.310^{a}	6.1±0.230^{a}	5.0±0.210^{a}
2	Challenged+non treated	2.91±0.11^{c}	3.83±0.30^{b}	3.22±0.19^{b}
3	Challenged+oregano essential oils	4.90±0.36^{ab}	5.4±0.280^{a}	5.1±0.360^{a}
4	Challenged+ciprofloxacin	4.1±0.400^{b}	5.2±0.370^{a}	4.9±0.360^{a}
5	Challenged+oregano essential oils+ciprofloxacin	5.36±0.56^{a}	5.78±0.40^{a}	5.1±0.460^{a}

Means with different letters (a-c) are significantly different at $p \leq 0.05$

The effect of different treatments on cell mediated immune response parameters (phagocytic activity and index) is illustrated in Table 3. It was observed that at the 3rd and 5th weeks of age, challenged non treated control positive birds produced the lowest significant ($p<0.05$) mean values of both phagocytic activity and index in comparison with other experimental groups. Added to that, blank non challenged control negative birds didn't show any significant ($p<0.05$) differences in its phagocytic activity and index at the examined dates. At 3 weeks of age, treated birds either by phytobiotic essential oils or by ciprofloxacin reveled significant ($p<0.05$) higher mean phagocytic activity and index compared with challenged non treated chickens and this difference remain significant ($p<0.05$) at 5th week of age. The measured cell mediated immune parameters at both sampling times of chickens treated by combined phytobiotic essential oils along with ciprofloxacin showed the best significant ($p<0.05$) mean values than other non treated or treated birds.

Data presented in Table 4 reveals the results of Humoral Immunity (HI) antibody titers against SRBC in

Table 5: Effect of oregano essential oils and antibiotic treatments on liver and kidney functions of non challenged and *E. coli* challenged and treated broiler chickens

		Liver function tests		Kidney function tests	
Group number	Treatments	AST (IU/L)	ALT (IU/L)	Uric acid (mg dL^{-1})	Creatinine (mg dL^{-1})
1	Non challenged+non treated	201.7±5.830^{c}	61.9±4.62^{d}	6.92±0.46^{d}	0.42±0.990^{a}
2	Challenged+non treated	259.4±3.270^{a}	90.3±1.25^{a}	15.11±0.23^{a}	0.60±0.123^{a}
3	Challenged+oregano essential oils	230.3±21.31^{abc}	77.1±1.23^{bc}	10.99±0.20^{b}	0.39±0.100^{a}
4	Challenged+ciprofloxacin	236.5±8.250^{ab}	82.3±1.52^{b}	11.53±0.81^{b}	0.50±0.130^{a}
5	Challenged+oregano essential oils+ciprofloxacin	220.1±9.310^{bc}	71.2±2.90^{c}	9.45±0.41^{c}	0.43±0.121^{a}

AST = Aspertate aminotransferase; ALT = Alanine aminotransferase; means with different letters (a-d) are significantly different at $p \leq 0.05$

Table 6: Effect of oregano essential oils and antibiotic treatments on albumin and globulin levels of non challenged and *E. coli* challenged and treated broiler chickens

Group number	Treatments	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	A/G ratio
1	Non challenged+non treated	2.90±0.230^{b}	1.41±0.020^{c}	1.49±0.05^{c}	0.946±0.03^{ab}
2	Challenged+non treated	3.92±0.084^{a}	1.97±0.050^{a}	1.95±0.04^{b}	1.011±0.03^{a}
3	Challenged+oregano essential oils	3.85±0.086^{a}	1.81±0.042^{b}	2.04±0.05^{ab}	0.887±0.02^{b}
4	Challenged+ciprofloxacin	3.72±0.109^{a}	1.80±0.043^{b}	1.92±0.08^{b}	0.937±0.04^{ab}
5	Challenged+oregano essential oils+ciprofloxacin	4.07±0.100^{a}	1.90±0.041^{ab}	2.17±0.07^{a}	0.875±0.02^{b}

A/G = Albumin/Globulin; means with different letters (a-c) are significantly different at $p \leq 0.05$

different experimental groups. These data demonstrated that chickens inoculated with *E. coli* had significant ($p<0.05$) decrease in antibody titers against SRBC at the 1st, 2nd and 3rd week post SRBC inoculation when compared with the non challenged blank control negative chickens. Antibody titers to SRBC were found to be significantly ($p<0.05$) the highest in broilers challenged with *E. coli* and treated with phytobiotic essential oils and ciprofloxacin combination and these titers were significantly ($p<0.05$) higher than that treated with phytobiotic essential oils and that treated with ciprofloxacin along the whole sample collection intervals.

The effect of oregano essential oils and ciprofloxacin treatment on serum biochemical metabolites parameters is collected in Table 5 and 6. The serum analysis of challenged non treated broiler chickens denoted a significant ($p<0.05$) increase in the activity of liver enzymes including AST and ALT as well as significant ($p<0.05$) increase in the kidney parameters considering uric acid and creatinine (Table 5), all of these findings indicating dysfunction of both liver and kidney. On the other hand, aforementioned parameters in challenged-treated broiler chickens decreased significantly ($p<0.05$) toward the values that recorded in blank control negative birds. It could be demonstrated that concurrent treatment of chickens with the phytobiotic essential oils and ciprofloxacin displayed the lowest significant ($p<0.05$) levels of liver and kidney function tests when compared with birds treated only with phytobiotic or with ciprofloxacin.

As shown in Table 6, treatment of *E. coli* challenged broilers with either phytobiotic essential oils or ciprofloxacin significantly ($p<0.05$) reduced serum albumin concentration however, application of combined treatment resulted in marked elevation of serum total protein and globulin level compared to the other treatments ($p<0.05$). Albumin to globulin ratio was lower in non challenged or treated birds than challenged non treated birds on the other hand, the this ratio showed the lowest values in treated birds by phytobiotic essential oils, ciprofloxacin and their combinations.

DISCUSSION

The results of *in vitro* antibiotic sensitivity pattern of the challenged *E. coli* strain agree with that of Blanco *et al.* (1997) who showed that avian *E. coli* strain were highly sensitive to ciprofloxacin *in vitro*.

The mechanism of phytobiotic essential oils in improving the productive performance of broilers may be due to improving of feed conversion ratio and increasing the efficiency of feed utilization (Marcincak *et al*., 2008; Mocar *et al*., 2010; Symeon *et al*., 2010), promoting better sedimentation of muscle proteins (Zheng *et al*., 2009), stimulation of appetite, digestive and absorption enzymes (Mountzouris *et al*., 2008; Windisch *et al*., 2009; Christaki *et al*., 2011) or stimulating effect on Lactobacillus proliferation (Roofchaee *et al*., 2011). Contrary results were obtained by Ocak *et al*. (2008) and Karimi *et al*. (2010) who recorded that performance of growing broilers was not affected by using an oregano-based supplement. This discrepancy is probably dependent on the ingredient composition and dosage of the used phytogenic compounds (Yang *et al*., 2009).

Development of performance parameters in ciprofloxacin treated birds could be attributed to the bactericidal effect of drug on *E. coli* and consequently improving of general health condition (Brown, 1996).

This research proved that essential oils succeeded in elimination of the challenged *E. coli* organism. Similar records were obtained by Friedman *et al.* (2002), Mitsch *et al.* (2004), Horosova *et al.* (2006), Bendahou *et al.* (2008), Ouwehand *et al.* (2010), Stofan *et al.* (2010) and Rahimi *et al.* (2011) who found that essential oils extracted from the plant *Origanum vulgare* have broad spectrum antimicrobial activities against avian pathogens either *in vitro* or *in vivo*. High concentrations of essential oils lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander *et al.*, 1998) also theses oils increased the bacterial membrane permeability causing leakage of protons and potassium ions, decreased in pH gradient across the cytoplasm membrane, collapse of the membrane potential, inhibition of ATP synthesis and consequently induced cells death (Ultee *et al.*, 2002). Reduction of *E. coli* re-isolation in ciprofloxacin treated group was due to inhibition of DNA gyrase and Topoisomerase II enzymes which are needed for the transcription and replication of bacterial DNA (Brown, 1996).

In this study, the results of cell mediated and humoral immune response pointed out that using of phytobiotics essential oils in combination of ciprofloxacin induce an immune potentiating effect. The effect of oregano essential oils on stimulation of immune response in chickens is somewhat rare but thymol and carvacrol have been indicated to possess potent antioxidant properties and consequently elevated immune responses of chicks (Gabor *et al.*, 2010; Feizi and Nazeri, 2011). In other species, some plants contain steroidal saponins affecting human cytokine production, macrophage activation and lymphocyte activity (Tan and Vanitha, 2004) *Origanum vulgaris* modulated ovine neutrophils immune function (Farinacci *et al.*, 2008) thyme essential oil significantly inhibited the total mRNA IL-1B expression in the mice colon (Juhas *et al.*, 2008); essential oils were associated with an increased proportion of swine CD4+, CD8+ and double positive T cells in peripheral blood and mesenteric lymph nodes (Walter and Bilkei, 2004) and also thymol enhanced the total IgA and IgM serum levels and exhibited some local anti-inflammatory properties as indicated by a reduction in TNF-mRNA in the swine stomach (Trevisi *et al.*, 2007). Conversely results were obtained by Toghyani *et al.* (2010), Abdulkarimi (2011) and Mansoub and Myandoab (2011) who detected that the dietary treatments of broiler chickens with thymol did not induce any significant effect on HI antibodies humoral immune response to SRBC and ND virus. Although, the results showed that ciprofloxacin treatment stimulated either cellular or humoral immune responses but Punniamurthy and Porchezhian (2007) demonstrated that ciprofloxacin had an immunosuppression effect as it induced significant reduction in HI antibody titers against SRBC and didn't produce any significant effect against La Sota antigen.

It was suggested essential oils of oregano posse biological activities as antioxidants that enhanced liver and kidney functions (Hernandez *et al.*, 2004). The high antioxidant activity of thymol is due to the presence of phenolic OH groups which act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation thus retarding the hydroxy peroxide formation (Farag *et al.*, 1989). Besides, El-Boushy *et al.* (2006) investigated that ciprofloxacin had no hepato or nephro-toxicity in *E. coli* infected broilers. However, it is important to mention that plants with their essential oils could be used as hepato and nephro-tonics to counter act the side effects of some chemotherapeutic agents (Sylvestre *et al.*, 2006). Albumin to globulin ratio was the lowest in the treated groups than challenged control and this is a good indication of high level of globulins and consequently immunostimulation.

CONCLUSION

Therefore, it could be concluded that a mixture of essential oils of *Oreganum aetheroleum* is an effective phytobiotic for the treatment of *E. coli* infection for broiler chickens when compared with ciprofloxacin. Moreover, this mixture along with ciprofloxacin treatment could be superior in controlling *E. coli* infection of broilers as they soluble in water, easily applied at any time, improve the performance parameters, reduce signs and mortalities, reduce the bacterial count, immunomodulators and have hepatic and rental tonic effects. Other complementary comprehensive researches in this era are needed in the future.

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