Egypt. J. Chem. **Vol. 65**, No. 6 pp. 269 - 282 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Prophylactic Effect of Oregano in Chickens Experimentally Infected with Avian Pathogenic *Escherichia coli* O27 with Special Reference to Hematology, Serum Biochemistry, and Histopathology of Vital Organs

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Abstract

This work was carried out to evaluate the efficacy of oregano powder 10% used as a prophylactic treatment in broiler chickens experimentally infected with avian pathogenic *E. coli* O27 (APEC O27) strain -local isolate-.Hematological and serum biochemical parameters, as well as histopathology of the vital organs, were investigated. A total of 200 chickens -aged 19 days old- were randomly allocated into 4 equal groups; Normal control (G1), Infected (G2), Oregano control (G3), and Oregano-Infected (G4). Chickens of (G3) and (G4) were supplemented with oregano powder that contains 10% oregano essential oil in the diet (250 g /ton diet) from the 1st day old till the end of the experiment.

At the age of the 19th, 20th, and 21st day,chickens of (G2), and (G4) were infected with 0.2 ml of APEC O27 (1×10^7 CFU/chick) via the intratracheal route. On the 3rd, 6th, 10th, and 21st day post-infection, blood and tissue samples from all groups were collected for laboratory examinations of the hemogram, serum biochemistry, and histopathology of the vital organs. Chickens of (G2) showed gasping, dullness, loss of appetite, and ruffled feathers. Birds of (G3) showed nosymptoms, while those of G4 showed mildsymptoms.
The mortality rates in chickens of (G2), (G3), and (G4) were recorded 30%, 0%, and 4%, respectively. Birds of (G2) revealed macrocytic hypochromic anemia, leukocytosis accompanied by heterophilia, and lymphopenia. The activities of AST and ALT enzymes and the values of uric acid and creatinine were increased while those of protein profile were decreased. In oregano supplemented groups, no significant alterations were detected. Birds of (G2) and (G4) revealed histopathological alterations in the lungs, trachea, liver, spleen, and kidneys.

Finally, APEC O27 caused changes in blood picture, liver and kidney functions as well as histopathology of the internal organs. Oregano powder (at a dose of 10% in diet) could be used as prophylactic treatment for APEC O27 in chickens from the 1st day of age.

Keywords: Avian pathogenic*E. coli* O27; oregano powder 10%; blood picture; serum biochemistry; pathology; chickens.

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Receive Date: 24 October 2021, Revise Date: 27 November 2021, Accept Date: 30 November 2021DOI: 10.21608/EJCHEM.2021.102510.4754

1. Introduction

Mortality due to infectious bacterial diseases always occurs in the poultry sector [1]. Avian colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) is one of the most significant bacterial diseases responsible for great losses for the poultry industry in Egypt and worldwide due to mortality and loss of production. The appearance of multidrug-resistant bacteria is due to excessive usage of antibacterial drugs [2]. Commercial antibiotics' non-therapeutic usage could cause tolerance or resistance in humans and animals. Accordingly, all over the world, there is a limitation on the usage of antibiotics to avoid emergence of antibiotic-resistant bacterial traits in poultry [3,4].

Some plants contain phytochemical components with antimicrobial activity that can be used as novel alternative drugs for the treatment of microbial diseases [5]. Essential oils derived from plants of the family "Lamiaceae" including oregano and thyme have antimicrobial properties [6]. Carvacrol and thymol are the major components of the essential oils [7, 9]. Thymol alone or in combination with carvacrol has antibacterial effects [9]. These compounds can inhibit the growth of Gram-negative and Gram-positive bacteria [10]. Supplementation of broiler diet with oregano essential oil significantly improved feed conversion ratio compared with the control diet [11]. It has been reported that the essential oil of oregano revealed an antibacterial effect against avian strains of *E. coli* [12]. Higher body weight gain, lower feed conversion ratio and lower percentage of abdominal fat were observed in broiler chickens fed with oregano oil compared to control one [13]. Furthermore, increasing levels of oregano supplementation to broiler diets improved the gut health and nutrient digestibility of the birds[14]. The phenolic compounds (carvacrol and thymol) found in the essential oil from oregano have a good antioxidant effect [15], and antimicrobial activities against pathogenic bacteria as *Salmonella* typhimurium, *E. coli*, *Staphylococcus aureus*,and *Staphylococcus epidermidis*[16,17]. It has been reported that chicken infected with *E.* coli exhibitedelevation of the activities of serum AST andALT and values of serum uric acid, creatinine, and globulin with a decrease of serum total protein and albumin [18, 19].

Therefore, the present experiment was carried out to evaluate the efficacy of oregano powder supplementation in diet (contains 10% oregano essential oil) as a prophylactic treatment for broiler chickens experimentally infected with APEC O27 strain "local isolate from lungs of diseased broiler chickens". Hematological and serum biochemical parameters as well as histopathology of the vital organs (lungs, trachea, liver, spleen, and kidneys), were also examined for assessment.

2. Material and methods

2.1. Ethics statement

This experiment was performed regarding the guidelines of animal experimentation and the protocol approved by the Institutional Animal Care and Use Committee, National Research Centre, Giza, Egypt. The protocol approval number was 1276072021.

2.2. Avian Pathogenic Escherichia coli O27 (APEC O27)

Avian Pathogenic Escherichia coli O27 local isolate from the lungs of broiler chickens was used in the experimental infection. The APEC O27 was identified at Animal Health Research Institute, Dokki, Giza, Egypt.

2.3. Oregano powder 10%

Oregano powder (Ropadiar®) containing 10% oregano essential oil is derived from Oregano (*Origanumvulgare* L.), family Lamiaceae. It was purchased from ROPA Pharm International Co., Netherlands.It contains more than 60 active components. The major components are the phenolic terpenoids; carvacrol (62 – 68%), thymol (1 – 3 %) and p-cymene (~8%). According to the manufacturer's instructions, oregano 10% powder was added to the diet at a dose of 250 g per ton diet.

2.4. Chicks

Two hundred –one–day–old– chicks **(**Cobb 500**)** purchased from El-Wattania Co. were used in this

experiment. Before the beginning of the experiment, five chicks were sacrificed randomly, and samples from lungs, trachea liver, spleen, and kidney were examined bacteriologically for pathogenic *E. coli*. All the results were *E. coli* negative. Chicks were raised under the required hygienic conditions, fed a balanced commercial diet according to NRC [20], and supplied with clean water in sufficient quantities. The experimental chicks were subjected to a traditional vaccine program for different viral diseases (Avian influenza, Newcastle, Infectious bronchitis, and Infectious bursal disease) until the end of the experimental period.

2.5. Experimental design

Experimental chicks were assigned into 4 equal groups, (G1- G4); Normal control (G1), Infected (G2), Oregano control (G3), and Oregano-Infected (G4). At the age of the 19th, 20th, and 21st day, chickens of G2, and G4 were infected with 0.2 ml of bacterial suspension in saline of APEC O27 (1×10^7 colony-forming unit (CFU) per Chick) via the intratracheal route according to the method of Rosenberger *et al.* [21]. Chickens of G3 (Oregano control) and G4 (Oregano-Infected) were fed a diet with oregano at a dose of 250 mg/kg diet from the 1st day of age till the end of the experiment. Clinical signs and mortality rate of birds of all groups were recorded during the period of the experiment. On the 3^{rd}, 6^{th}, 10^{th}, and 21^{st} day post-infection (dpi), blood and tissue samples were collected from 5 chickens in each group and reisolation of APEC O27 was performed.

2.6. Reisolation of APEC O27 serotype

Samples from the lungs,trachea, liver, spleen, and kidney were taken under aseptic conditions and incubated overnight at 37°C in nutrient broth.Loopfulls from inoculated broth were then streaked onto MacConkey's agar medium and Eosin Methylene Blue agar (Oxoid, UK). The inoculated media were incubated at 37°C for 24–48hrs. *E. coli* colonies appeared pink colonies on MacConkey's agar and shiny metallic green colonies on Eosin Methylene Blue agar [22].

2.7. Blood and tissue sampling

Blood samples were collected from the wing vein of each bird and were divided into two portions. The first portion was collected into clean tubes containing EDTA anticoagulant (Ethylenediaminetetraacetic acid) and used for evaluation of the hemogram. The second portion was placed into a plain tube, left to clot then centrifuged at 3000 rpm for 15 min. for separation of serum. Sera were stored at -20°C until used for biochemical analyses. Tissue specimens were collected from the lungs, trachea, liver, spleen, and kidneys of each bird for histopathological examination.

2.7.1. Hematological examination

Complete blood picture of chickens of all groups were investigated according to the method of Weiss and Wardrop [23].

2.7.2. Serum biochemical analysis

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and levels of uric acid, creatinine, total serum proteins, and albumin were determined according to the manufacturer' instructionsof commercial Test kits supplied from Biodiagnostic Co., Egypt. A spectrophotometer (T80 UV/VIS PG instrument Ltd, UK) was used.Total globulins were calculated by subtracting the obtained value of albumin from total proteins.

2.7.3. Histopathological examination

Tissue specimens collected from the lungs, trachea, liver, spleen, and kidneys were immediately fixed in neutral buffered formalin 10%, washed, dehydrated, cleared, and embedded in paraffin. Paraffin blocks were sectioned at 4-5 μm thickness and stained with hematoxylin and eosin (H&E) according to Suvarna*et al.* [24]. Slides were examined under a light microscope (Olympus B x50, Japan).

2.8. Statistical analysis

All data were subjected to statistical analysis including the calculation of the mean ±standard error. Differences between the normalcontrol and the treated groups were tested for significance using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.The differences were considered significant at $P<0.05$ level [25] using

SPSSsoftware version 20 computer program (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Clinical signs and mortality rate

Clinical signs that appeared on infected birds (G2) were dullness, ruffled feather, gasping, loss of appetite, and depression. Chickens of G3 showed no clinical signs, while birds of G4 showed mildsymptoms.

Mortality rates among chickens of different experimental groups were 0%, 30%, 0%, and 4%, for G1, G2, G3, and G4, respectively (Table 1).

Table 1 Mortality rate of broiler chickens supplemented with oregano in diet and infected with avian pathogenic *E. coli* O27 during the different experimental periods.

Groups	Chickens Number	Mortalities at different days post infection					Mortality rate
		1-3	4-6	7-10	11-21	Total	
Normal control (G1)	50	0	0	0	0	0	0%
Infected (G2)	50	3	6	4	2	15	30%
Oregano (G3)	50	0	0	0	0	0	0%
Oregano-Infected (G4)	50	1	1	0	0	2	4%

3.2. Reisolation of APEC O27

At the 3rd dpi, APEC O27 was isolated from the lungs of G4 chickens, while it was isolated from chickens of G2 at the 3rd, 6th, and 10th dpi.

3.3. Hematological findings

Red blood cell counts (RBCs) and hemoglobin (Hb) concentration revealed significant (P<0.05) decrease in chickens of G2 at the 6th, 10th, and 21st dpi compared to chickens of (G1) and (G3). Chickens of (G3) showed marked increase in RBCs and Hb values at the 6th, 10th, and 21st dpi compared to values of (G1). Values of packed cell volume (PCV) of (G2) chickens exhibited gradual significant (P<0.05) decrease from the 6th dpi till the 21st dpi compared to the other groups. At the 6th, 10th, and 21st dpi, mean corpuscular volume (MCV) indicated a significant (P<0.05) increase, while mean corpuscular hemoglobin concentration (MCHC) revealed a marked (P<0.05) decrease in (G2) compared to the other groups. Mean corpuscular hemoglobin (MCH) generally showed no significant changes in all groups during the experiment. Oregano-Infected group (G4) showed no significant changes in erythrogram all over the experimental period. Evaluation of red cell indices revealed presence of macrocytic hypochromic anemia in (G2) chickens (Table 2).

Total white blood cell counts (WBCs) and heterophilswere significantly (P<0.05) increased all over the experimental period in (G2) and at the 6th and 10th dpi in the Oregano-Infected (G4) compared to birds of(G1) and (G3). Chickens of G2 showed marked lymphopenia from the 3rd dpi till the end of the experiment, while those of G4 showed lymphopenia at the 6th & 10th dpi in comparison with the other groups. Chickens of (G2) revealed marked (P<0.05) increase in monocytes at all periods after infection. Eosinophils count was significantly (P<0.05) increased at the 6th and 21st dpi and basophils count showed significant (P<0.05) increase starting from the 3rd till the 21st dpi compared to other groups (Table 3).

Chickens of (G2) revealed significant decrease in thrombocytes count starting from the 6th dpi till the end of the experiment, while in (G4),thrombocytes count was slightly decreased at the 6th and 10th dpi (Table 3).

Table 2 Erythrogram of broiler chickens supplemented with oregano 10% in diet and infected with avian pathogenic *E. coli* O27 during the different experimental periods. (Mean ± SE, N=5)

Parameters	Periods (dpi)	Groups			
		Normal Control (G1)	Infected (G2)	Oregano control (10%) (G3)	Oregano-Infected (G4)
Red blood cell count ($\times 10^6$/µl)	3	2.03±0.09^{b}	1.93±0.05^{b}	2.18±0.03^{a}	2.16±0.01^{a}
	6	2.11±0.05^{b}	1.88±0.01^{c}	2.22±0.09^{a}	2.08±0.01^{b}
	10	2.18±0.08^{a}	1.84±0.03^{b}	2.27±0.07^{a}	1.97±0.04^{a}
	21	2.34±0.05^{b}	2.03±0.03^{c}	2.35±0.02^{a}	2.25±0.02^{b}
Packed cell volume (%)	3	28.08±0.36^{ab}	27.60±0.39^{b}	29.00±0.32^{a}	28.10±0.12^{ab}
	6	28.44±0.09^{b}	27.28±0.12^{c}	29.60±0.18^{a}	28.10±0.10^{b}
	10	29.04±0.20^{a}	27.04±0.42^{b}	30.24±0.32^{a}	29.30±0.37^{a}
	21	29.30±0.33^{b}	27.20±0.22^{c}	30.90±0.29^{a}	30.00±0.35^{b}
Hemoglobin (g/dl)	3	9.20±0.17^{b}	8.72±0.11^{c}	9.74±0.10^{a}	9.50±0.07^{b}
	6	9.10±0.14^{b}	7.60±0.14^{c}	9.72±0.09^{a}	9.08±0.25^{b}
	10	9.08±0.14^{a}	7.08±0.05^{b}	9.68±0.10^{a}	9.02±0.57^{a}
	21	9.04±0.13^{b}	7.28±0.09^{c}	9.82±0.13^{a}	9.14±0.08^{b}
Mean corpuscle volume (fl)	3	139.24±6.95^{a}	143.54±3.96^{a}	132.71±3.61^{a}	130.34±0.99^{a}
	6	135.16±2.77^{b}	145.13±0.66^{a}	133.45±3.22^{b}	135.80±1.14^{b}
	10	133.61±3.87^{b}	147.09±2.27^{a}	134.22±1.87^{b}	135.68±1.13^{b}
	21	131.42±3.22^{a}	134.16±1.53^{a}	131.95±1.52^{a}	133.24±1.76^{a}
Mean corpuscle hemoglobin (pg)	3	45.63±2.39^{a}	45.39±1.53^{a}	44.55±0.77^{a}	43.92±0.70^{a}
	6	43.26±1.22^{a}	40.25±0.77^{a}	43.74±2.06^{a}	43.33±1.13^{a}
	10	41.45±1.61^{a}	38.59±0.92^{a}	42.96±0.51^{a}	41.76±2.59^{a}
	21	40.52±0.82^{a}	35.92±0.52^{b}	41.58±0.87^{a}	40.60±0.52^{a}
Mean corpuscle hemoglobin concentration (g/dl)	3	32.75±0.20^{a}	31.60±0.28^{b}	33.61±0.47^{a}	33.67±0.29^{a}
	6	31.00±0.50^{a}	27.11±0.42^{b}	32.83±0.31^{a}	32.32±0.99^{b}
	10	31.71±0.49^{a}	26.13±0.03^{b}	32.13±0.46^{a}	30.76±0.71^{b}
	21	30.89±0.63^{a}	26.78±0.54^{b}	31.80±0.41^{a}	29.99±0.52^{b}

Means with different superscripts (a, b, c) in the same row are significantly different at $P<0.05$.

Table 3 Leukogram of broiler chickens supplemented with oregano 10% in diet and infected with avian pathogenic *E. coli* O27 during the different experimental periods. (Mean ± SE, N=5)

Parameters	Periods (dpi)	Groups			
		Normal Control (G1)	Infected (G2)	Oregano control (10%) (G3)	Oregano- Infected (G4)
Total white blood cell count ($\times10^3/\mu l$)	3	19.68±0.21^{b}	24.10±0.50^{a}	19.89±0.08^{b}	19.72±0.16^{b}
	6	20.11±0.25^{c}	25.11±0.52^{a}	19.81±0.17^{c}	21.94±0.26^{b}
	10	20.63±0.25^{c}	24.62±0.52^{a}	20.18±0.26^{c}	21.54±0.45^{b}
	21	20.30±0.23^{b}	22.09±0.24^{a}	19.67±0.24^{b}	19.80±0.16^{b}
Heterophils ($\times10^3/\mu l$)	3	7.63±0.14^{b}	12.89±0.12^{a}	7.77±0.0 7^{b}	7.73±0.10^{b}
	6	7.53±0.31^{c}	13.27±0.27^{a}	7.64±0.18^{c}	11.44±0.23^{b}
	10	8.30±0.20^{c}	13.15±0.42^{a}	8.08±0.12^{c}	10.27±0.35^{b}
	21	7.88±0.13^{b}	12.11±0.16^{a}	7.83±0.05^{b}	7.73±0.11^{b}
Lymphocytes ($\times10^3/\mu l$)	3	10.11±0.11^{a}	8.95±0.47^{b}	10.28±0.06^{a}	10.04±0.10^{a}
	6	10.69±0.18^{a}	8.24±0.39^{b}	10.46±0.14^{a}	8.51±0.20^{b}
	10	10.36±0.18^{a}	8.90±0.17^{b}	10.23±0.05^{a}	8.97±0.17^{b}
	21	10.56±0.16^{a}	7.02±0.17^{b}	10.24±0.21^{a}	10.33±0.09^{a}
Monocytes ($\times10^3/\mu l$)	3	0.99±0.08^{a}	1.00±0.09^{a}	0.80±0.07^{a}	1.01±0.06^{a}
	6	0.89±0.05^{b}	1.91±0.07^{a}	0.76±0.08^{c}	1.21±0.07^{b}
	10	1.00±0.08^{b}	1.28±0.07^{a}	0.85±0.08^{c}	1.10±0.04^{b}
	21	1.05±0.11^{b}	1.66±0.12^{a}	0.93±0.05^{b}	0.85±0.08^{b}
Eosinophils ($\times10^3/\mu l$)	3	0.44±0.04^{a}	0.48±0.01^{a}	0.47±0.04^{a}	0.47±0.05^{a}
	6	0.44±0.04^{b}	0.74±0.10^{a}	0.47±0.05^{b}	0.49±0.04^{b}
	10	0.41±0.05^{a}	0.59±0.05^{a}	0.47±0.05^{a}	0.55±0.05^{a}
	21	0.45±0.01^{a}	0.53±0.05^{a}	0.44±0.04^{a}	0.49±0.05^{a}
Basophils ($\times10^3/\mu l$)	3	0.51±0.05^{b}	0.77±0.10^{a}	0.56±0.04^{b}	0.47±0.05^{b}
	6	0.44±0.07^{b}	0.95±0.09^{a}	0.48±0.05^{b}	0.49±0.05^{b}
	10	0.54±0.05^{b}	0.84±0.07^{a}	0.53±0.05^{b}	0.64±0.05^{b}
	21	0.28±0.05^{b}	0.75±0.11^{a}	0.32±0.05^{b}	0.40±0.06^{b}
Platelets ($\times10^3/\mu l$)	3	5.81±0.10^{ab}	5.35±0.15^{b}	5.99±0.22^{a}	5.61±0.22^{ab}
	6	6.85±0.27^{a}	5.66±0.11^{b}	6.11±0.27^{b}	6.08±0.09^{b}
	10	7.05±0.10^{a}	5.95±0.09^{c}	6.75±0.31^{b}	6.20±0.24^{bc}
	21	6.21±0.06^{b}	5.39±0.18^{c}	6.40±0.18^{a}	5.86±0.21^{bc}

Means with different superscripts (a, b, c) in the same row are significantly different at $P<0.05$**.**

3.4. Biochemical findings

Chickens of (G2) exhibited significant ($P<0.05$) increase in the activity of AST and ALT compared to (G1) starting from the 3rd dpi till the end of the experiment. In chickens of G3, serum enzymes activities were significantly ($P<0.05$) decreased all over the experimental period.While, there were no significant changes in the activities of ALT and AST in (G4)compared to normal control(Table 4).

Serum creatinine and uric acid levels markedly increased in chickens of (G2) from the 3rd till the 21st dpi, while serum creatinine increased at the 6th and 10th dpi in (G4) compared to chickens of (G1). Results of G3 revealed significant decrease of creatinine levels. Chickens of (G4) showed no changes in uric acid levels all over the experimental period (Table 4).

The values of total serum proteins, albumin, total globulins and A/G ratio were significantly decreased in (G2) compared to (G1)starting from the 3rd dpi till the end of the experiment.Chickens of (G3) exhibitedmarked increase in total serum proteins and albumin concentrations, while chickens of (G4) showed no significant changes during the experimental period (Table 5).

Table 4 Activity of serum enzymes (ALT and AST) and levels of creatinine and uric acid of broiler chickens supplemented with oregano 10% in diet and infected with avian pathogenic *E. coli* O27 during the different experimental periods. (Mean ± SE, N=5)

Parameters	Periods (dpi)	Groups			
		Normal Control (G1)	Infected (G2)	Oregano control (10%) (G3)	Oregano-Infected (G4)
Alanine aminotransferase (IU/l)	3	32.32±2.20^{b}	40.64±2.18^{a}	22.40±0.68^{c}	32.00±1.41^{b}
	6	30.68±2.26^{b}	41.58±2.34^{a}	22.00±0.95^{c}	30.60±1.08^{b}
	10	30.98±1.86^{b}	41.98±2.00^{a}	23.20±1.02^{c}	30.80±1.71^{b}
	21	29.24±1.94^{b}	38.02±2.12^{a}	21.60±1.07^{c}	28.42±0.58^{b}
Aspartate aminotransferase (IU/l)	3	101.26±4.19^{b}	121.70±4.06^{a}	89.80±0.73^{c}	105.40±2.20^{b}
	6	101.08±3.67^{b}	126.70±4.49^{a}	96.80±1.96^{c}	115.60±1.50^{b}
	10	100.38±3.73^{c}	130.70±4.49^{a}	91.80±1.39^{d}	106.20±1.88^{b}
	21	103.36±3.77^{b}	125.70±4.49^{a}	86.80±2.22^{c}	101.20±1.07^{b}
Creatinine (mg/dl)	3	0.77±0.05^{b}	0.93±0.04^{a}	0.68±0.02^{c}	0.82±0.03^{b}
	6	0.80±0.04^{b}	1.00±0.04^{a}	0.79±0.03^{b}	0.95±0.01^{a}
	10	0.91±0.03^{c}	1.25±0.04^{a}	0.79±0.02^{d}	1.00±0.01^{b}
	21	0.91±0.04^{b}	1.30±0.02^{a}	0.83±0.02^{c}	0.85±0.01^{b}
Uric acid (mg/dl)	3	4.19±0.17^{b}	4.74±0.06^{a}	4.16±0.10^{b}	4.16±0.13^{b}
	6	4.16±0.07^{b}	5.42±0.13^{a}	4.22±0.12^{b}	4.10±0.07^{b}
	10	4.20±0.13^{b}	6.00±0.09^{a}	4.18±0.06^{b}	4.20±0.10^{b}
	21	4.20±0.02^{b}	6.22±0.09^{a}	4.18±0.06^{b}	4.16±0.10^{b}

Means with different superscripts (a, b, c) in the same row are significantly different at $P<0.05$**.**

Table 5 Serum protein profile of broiler chickens supplemented with oregano 10% in diet and infected with avian pathogenic *E. coli* O27 during the different experimental periods. (Mean ±SE, N=5)

Parameters	Periods (dpi)	Groups			
		Normal Control (G1)	Infected (G2)	Oregano control (10%) (G3)	Oregano-Infected (G4)
Total Proteins (g/dl)	3	3.50±0.09^{ab}	2.51±0. 09^{c}	3.66±0.02^{a}	3.42±0.08^{b}
	6	3.60±0.08^{b}	2.21±0.05^{c}	3.78±0.03^{a}	3.54±0.05^{b}
	10	3.63±0.10^{b}	2.15±0.09^{c}	3.94±0.02^{a}	3.63±0.04^{b}
	21	3.77±0.10^{b}	2.06±0.11^{c}	4.10±0.04^{a}	3.70±0.07^{b}
Albumin (g/dl)	3	2.15±0.08^{b}	1.52±0.07^{c}	2.45±0.05^{a}	2.27±0.05^{b}
	6	2.37±0.06^{b}	1.40±0.05^{c}	2.54±0.05^{a}	2.31±0.02^{b}
	10	2.35±0.07^{b}	1.20±0.04^{c}	2.63±0.02^{a}	2.34±0.03^{b}
	21	2.42±0.07^{b}	1.09±0.06^{c}	2.67±0.01^{a}	2.38±0.06^{b}
Total Globulins (g/dl)	3	1.35±0.03^{a}	0.98±0.03^{c}	1.22±0.07^{ab}	1.15±0.08^{bc}
	6	1.23±0.05^{a}	0.81±0.06^{b}	1.24±0.04^{a}	1.23±0.05^{a}
	10	1.29±0.06^{a}	0.95±0.06^{b}	1.31±0.03^{a}	1.29±0.05^{a}
	21	1.36±0.05^{a}	0.97±0.06^{b}	1.43±0.05^{a}	1.32±0.03^{a}
A/G Ratio	3	1.59±0.05^{b}	1.55±0.05^{b}	2.04±0.14^{a}	2.02±0.14^{a}
	6	1.94±0.08^{a}	1.77±0.18^{a}	2.06±0.10^{a}	1.89±0.07^{a}
	10	1.84±1.45^{a}	1.27±0.08^{b}	2.01±0.05^{a}	1.82±0.08^{a}
	21	1. 97±0.07^{a}	1.13±0.04^{b}	1.88±0.08^{a}	1.81±0.05^{a}

Means with different superscripts (a, b, c) in the same row are significantly different at $P<0.05$**.**

3.5. Pathological findings

3.5.1. Post-mortem findings

No gross lesions were observed in chickens of (G1) and (G3) all over the experimental period. Chickens of (G2) and (G4) showed slight congestion at the 3rd and 6th dpi in the lungs, livers, and kidneys. At the 10th and 21st dpi, severe congestion of lungs, liver, spleen, and kidney was seen in chickens of (G2).

3.5.2. Microscopic findings

No microscopic alterations were observed in the lung, trachea, liver, spleen, and kidney of birds of (G1) during different experimental periods (**Figs. 1A-5A).**

Lungs: Lung of (G2)showed congestion of the peri air blood capillariesat the 3rd and 6th dpi **(Fig. 1B).** At the 10th and 21st dpi, the lung showed congestion of the peri air blood capillaries and interstitial blood vessels**.** Lungs of (G3) showed normal histological architecture all over the different periods **(Fig. 1C).** Lungs of (G4)showed congestion of the peri air blood capillaries at the 3rd and 6th dpi **(Fig. 1D).** By the 10th dpi till the end of the experiment, the lung demonstrated a normal histological picture**.**

Trachea: Trachea of (G2) chickens showedhyperplasia in the mucosal tracheal epithelium and edema of the submucosa at the 3rd and 6th dpi**.**Proprial mononuclear cells' infiltration with congestion of the blood vessels was observed also in trachea at the 10th and 21st dpi, **(Fig. 2B)**. No pathological changes were detected in the trachea of (G3) groupin all periods **(Fig. 2C).** In (G4),the trachea showed activation of mucous secreting goblet cells at the 3rd dpi. andnecrosis in the mucosal lining epithelium with mononuclear cells' infiltration in the lamina propriaat the 6th and 10th dpi, **(Fig. 2D).**

Liver: Liver of (G2) showed congestion of hepatic sinusoids, central veins, and portal BVs at the 3rd dpi**.** Some livers revealed congestion in the central veinat the 6th dpi andby the 10th dpi, the liver showed aggregation of mononuclear cells' infiltrations forming minute foci with multiple areas of diffuse hemorrhages **(Fig. 3B)**. All over the experimental periods, the liver of (G3) showed normal hepatic parenchyma with normal hepatic architecture **(Fig. 3C).** Liver of (G4)showed focal peri-central mononuclear cells' infiltration at the 3rd dpi, **(Fig. 3D).** After that, the histological picture of livers appeared normal.

Spleen: Spleen of (G2) revealed congestion of splenic red pulp at the 3rd and 6th dpi. Also, hyperplasia of ellipsoidal reticular cells in the sub capsular sinuses and around capillaries was observed at the 10th dpi. and some spleens showed a thickened muscular layer of blood vessels **(Fig. 4B)**. The spleen of (G3) displayed a normal histological appearance **(Fig. 4C).** The spleen of (G4) demonstrated minute hemorrhage at the 3rd and 10th dpi **(Fig. 4D)** and by the end of the experiment histological picture of spleens appeared normal.

Kidneys: kidney of (G2) revealed congestion of interstitial blood vessels at the 3rd dpi. and degenerative changes in the renal epithelium. Slight degeneration of some renal tubules was noticed, as well as hemorrhage in the interstitial tissue of the kidney was detected at the 6th and 10th dpi**(Fig. 5B).** Kidneys of (G3) demonstrated normal renal histological architecture **(Fig. 5C)**. Kidneys of (G4) displayed some focal hemorrhage and necrosis at the 6th dpi **(Fig. 5D),** while the histological picture appeared normal at the end of the experiment.

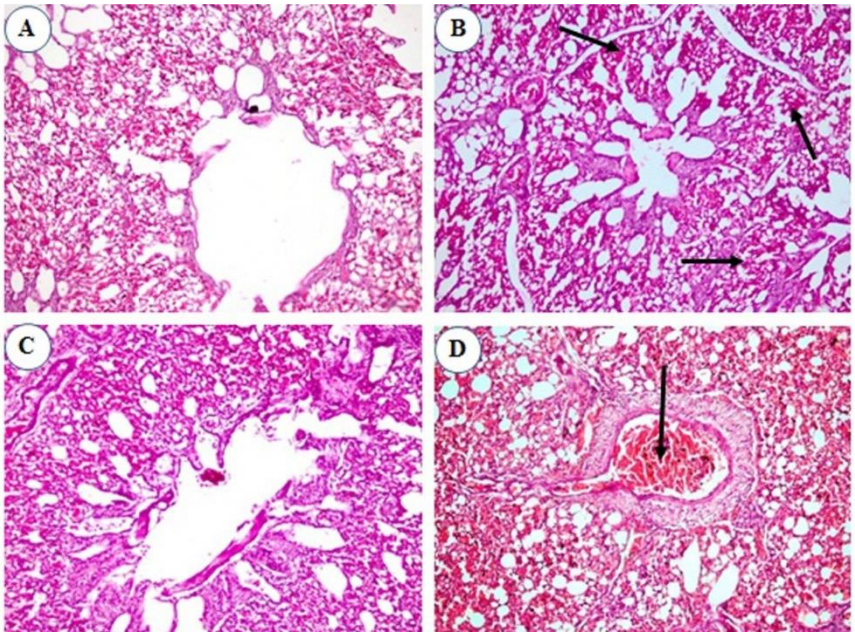

Fig. 1. Lung of broiler chickens: (A) Normal control group (G1); showing normal parenchyma of parabronchus and air capillaries, (B) Infected group (G2); showing congestion of the peri air blood capillaries (arrows) at the 3rd day post-infection (dpi), (C)Oregano control group (G3);showing normal parenchyma of parabronchus and air capillaries, and (D) Oregano- Infected group (G4); showing congestion of the peri air blood capillaries (arrow) at the 3rd day dpi. (H&E, x200)

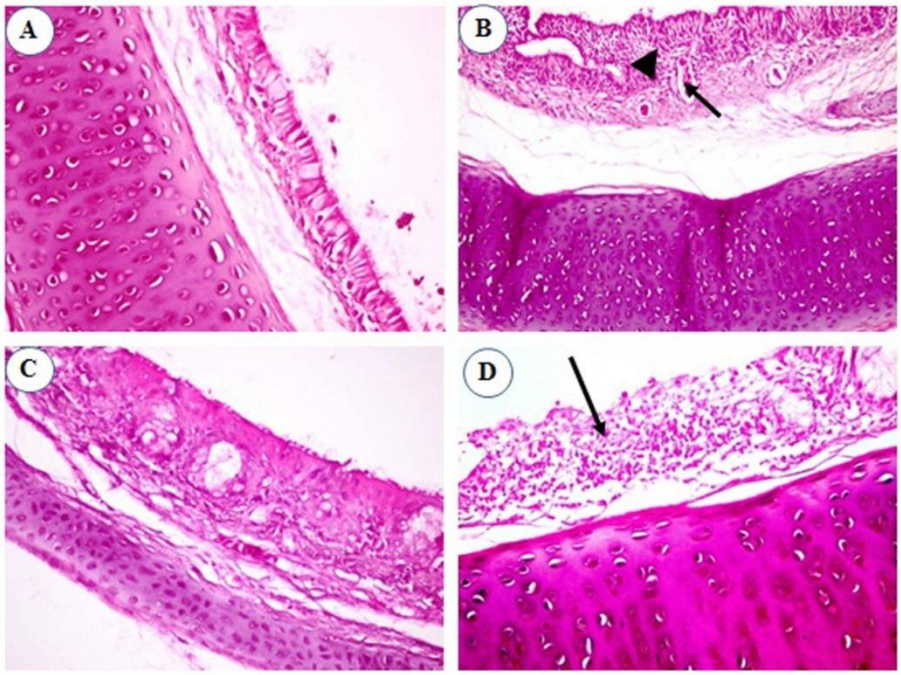

Fig. 2. Trachea of broiler chickens: (A) Normal control group (G1); showing normal mucosa (H&E, x200), (B) Infected group (G2); showing proprial mononuclear cells' infiltration (arrowhead) with congested blood vessel (arrow) at the 6th dpi (H&E, x200), (C)Oregano control group (G3); showing normal mucosal epithelium, (D) Oregano- Infected group (G4); showing necrosis of the mucosal layer with mononuclear cells' infiltration, (arrow). (H&E, x400)

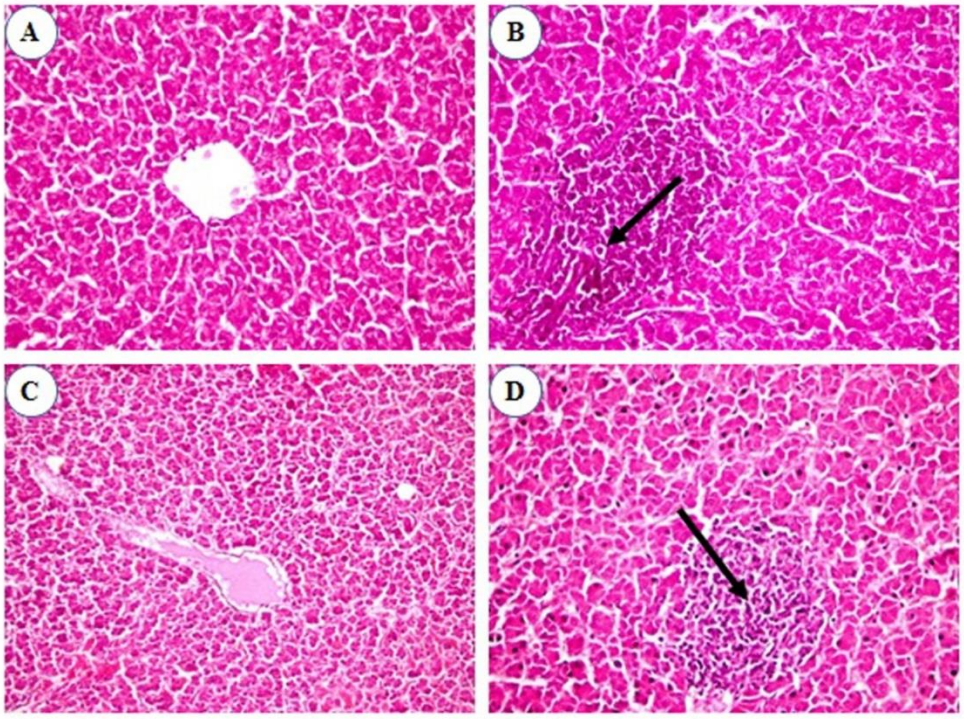

Fig. 3. Liver of broiler chickens: (A) Normal control group (G1); showing healthy hepatic parenchyma, central vein, and blood sinusoids, (B) Infected group (G2); showing a large focal area of mononuclear cells' infiltration (arrow) at the 10th dpi, (C)Oregano control group (G3); showing normal parenchyma of hepatocytes, central vein, and blood sinusoids, and(D) Oregano-Infected group (G4); showing focal aggregation of mononuclear cells (arrow). (H&E, x400)

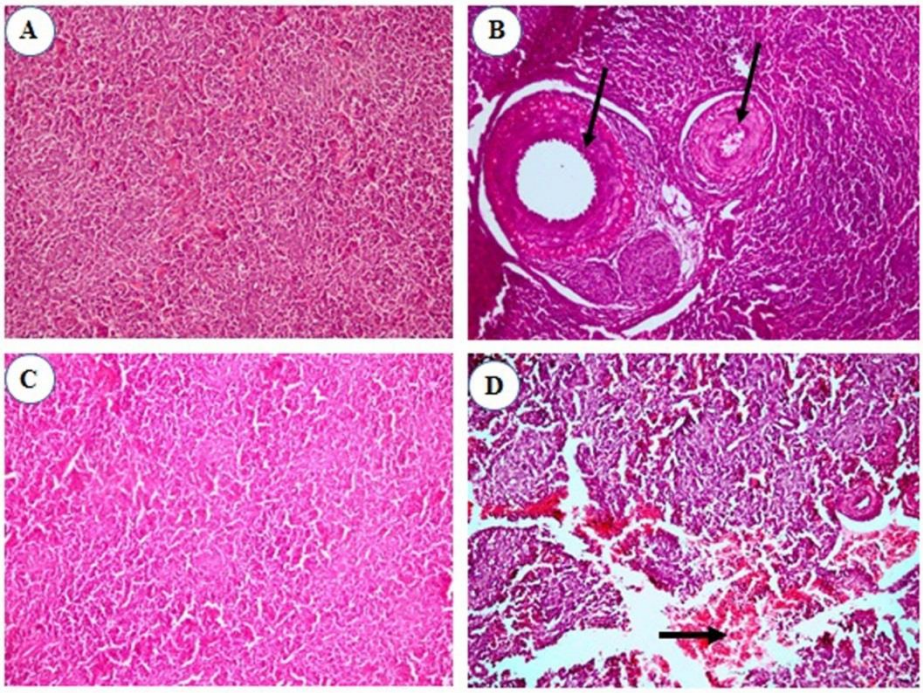

Fig. 4. Spleen of broiler chickens: (A) Normal control group (G1); showing normal follicles, white and red pulps, (H&E, x200), (B) Infected group (G2); showing thickened muscular layer of blood vessels (arrows) at the 10th dpi (H&E, x400), (C)Oregano control group (G3); showing normal follicles, white pulp, and red pulp (H&E, x200), and(D)Oregano-Infected group (G4): showing focal areas of hemorrhage (arrow) (H&E, x400).

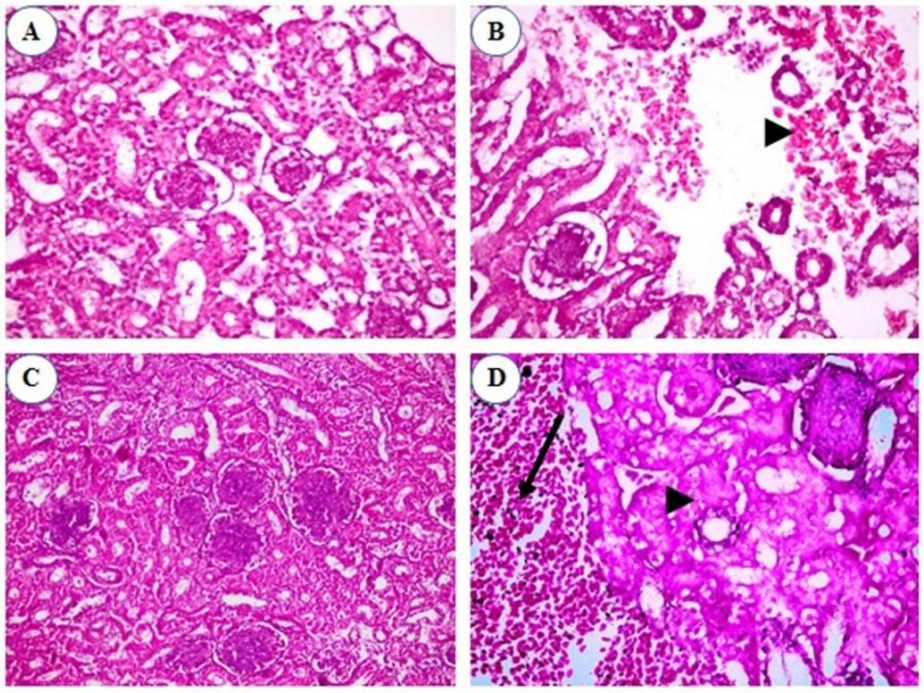

Fig. 5. Kidney of broiler chickens: (A) Normal control group (G1); showing normal parenchyma of renal glomeruli and renal tubules (H&E, x400), (B) Infected group (G2); showing hemorrhages in the interstitial tissue (arrowhead) at the 6th dpi (H&E x400), (C)Oregano normal group (G3); showing normal parenchyma (H&E, x400), and (D) Oregano-Infected group (G4); showing necrosis of renal tubules (arrowhead) and focal area of hemorrhage (arrow), (H&E, x200).

4. Discussion

This study was conducted to assess the efficacy of using oregano powder 10% supplementation in the diet as a prophylactic treatment in broiler chickens experimentally infected with APEC O27 strain -local isolate-. Hematological and serum biochemical parameters, as well as pathological changes of the vital organs were evaluated during the experimental period.

The APEC is one of the most common bacterial diseases causing economic losses in poultry all over the world [26]. In the present experiment, the infected group showed clinical signs that began at the 3rd dpi in the form of gasping, huddling together, dullness, ruffled feather, loss of appetite,and depression. The mortality rate among the infected group, oregano control and oregano-infected were 30%, 0%, and 4%, respectively. Similar results were previously observed by De Carli*et al.* [27].

Data of the erythrogram in the infected group showed significant decrease in both RBCs and Hb concentration starting at the 3rd dpi and macrocytic hypochromic anemia was recorded. A similar result was previously mentioned by Huff *et al.* [28].The decrease in hematological parameters may be due to breakdown of erythrocytes by hemolytic enzymes found in endotoxins produced by *E. coli* [29] leading to decrease of RBCs count, PCV and hemoglobin concentration [23]. Erythrocyte indices revealed increase in the values of MCV and a decrease in MCHC which indicated that chickens were suffering from macrocytic hypochromic anemia. Saini [30]reported normocytic normochromic anemia in *E. coli* infection in broiler chickens. Oregano-fed chickens (G3) showed elevation of RBCs and Hb values at the 6th, 10th and 21st dpi.

Hematological parameters are good indicators of the physiological, pathological, and nutritional status of animals. Al-Kassie [31] showed that feeding broiler on diets supplemented with oregano significantly increased red and white blood cells, PCV, and hemoglobin values compared with the control group. On the other hand, Toghyani*et al.* [32] showed that red and white blood cell counts, hemoglobin concentration, and PCV% did not differ significantly among broiler chickens fed dietary thyme.

Regarding the leukogram, a marked increase was recorded in the total leukocytic count and heterophils together with lymphopenia in infected birds of (G2) throughout the experiment, and in G4 during the 6th and 10th dpi. In this respect, heterophils are part of natural immunity and cellular defense against microbial infections. Moreover, the observed changes of the leukogram may be due to increase in the level of corticosterone secretion due to stress of infection [33].

Thrombocytopenia was detected in infected groups (G2 and G4), which may be due to endotoxins produced by APEC [27]. This assumption is supported by the present histopathological findings of congestion and hemorrhage in the internal organs.

Biochemical studies revealed significant increase in the serum AST and ALT activity of the infected group. Similar results have been previously reported in *E. coli* -infected broiler chickens [34, 35] who worked on *E. coli* infection in broiler chickens. These increases could be attributed to hepatocellular damage. In the present study, the elevation of the activities of the hepatic enzymes was supported by the histopathologicalfindings of the liver. Chickens of G3 showed decrease of liver enzymes that suggest a hepatoprotective and cardioprotective effects of oregano supplementation due to its active materials such as carvacrol, and thymol that have antioxidant and antibacterial effects [10,11,13].

The present work showed significant increase in serum creatinine and uric acid levels in (G2) that may be attributed to kidney damage caused by the effect of APEC and its toxin onthe kidney tissue. This result was confirmed by observation of degenerative changes in the renal epithelium,slight degeneration of some renal tubules, and hemorrhage in the interstitial tissue of the infected group. Our results agree with Kumari*et al.* [34, 35].Creatinine returned to the normal level at the end of the experiment which may be due to an antioxidant and antibacterial effects of the oregano bioactive constituents[10, 11, 13]. Chickens of oregano control showed decrease of creatinine levels which could indicate that oregano has a nephroprotective effect.

Regarding serum protein profile, results revealed significant reduction in the concentrations of total proteins, albumin, total globulins, and A/G ratio in the serum of infected chickens which started after infection with APEC O27 till the end of the experiment. Similar results have been previously reported in chickens infected by APEC [34, 36]. Hypoproteinemia may be due to failure in plasma protein synthesis resulted from hepatocytes' damage, and renal affection which led to protein loss [37].

This is confirmed by histopathological alteration of the liver and kidneys. Our results disagree with Saini [30] who recorded hyperglobulinemia associated with liver cirrhosis, hepatitis, and Kupffer cells proliferation in broiler chickens infected withAPEC. Serum protein profile of oregano supplemented chickens exhibited increases from the 3rd dpi till the end of the experiment indicating a hepatoprotective effect of oregano supplementation.

Microscopically, lungs of chickens experimentally infected with APEC O27showed congestion and hemorrhage of pulmonary blood vessels and capillaries in most cases. Similar findings were recorded by Reese *et al.* [38]. Edema of the submucosa and hyperplasia in the mucosal tracheal epithelium were constant observations at the 3rd and 6th dpi in the infected group. Mononuclear cells infiltration in the lamina propria with blood vessels congestion was detected at the 6th till 21st dpi. Liver sections of experimentally infected chickens revealed inflammatory mononuclear cells infiltration and congestion of some portal blood vessels. These findings were in complete agreement with Antão*et al.* [39]. At the 3rd and 6th dpi, congestion of splenic red pulp was noticed in infected groups. At the 10th dpi, hyperplasia of ellipsoidal reticular cells in the subcapsular sinuses and around capillaries in the spleen was observed. In the spleen of the oregano group, no pathological alterations were detected. The present microscopic lesions were similar to the results of Koutsianos*et al.* [40]. Kidney sections of the infected group revealed congestion of parenchymatous blood vessels at the 3rd dpi. Also, degenerative alterations in the renal tubular epithelium were observed. At the 6th and 10th dpi, slight degeneration of some renal tubules and hemorrhage in the parenchymal tissue were noticed. These results were recorded by Rodriguez-Siek*et al.* [41], and Kumari*et al.* [34, 35]. Kidneys of the oregano-infected group showed necrosed renal tubules and focal areas of hemorrhage at the 3rd dpi which may be due to the effect of APEC infection and its toxin on the kidneys. Similar results were recorded byEwers *et al.* [42].

Chicks fed on a diet supplemented with oregano 10% from the 1st day till the end of the experimental period showed blood picture and serum biochemical parameters within the normal levels. Histopathological structures of the lung, trachea, liver, spleen, and kidneys were normal. It may be due to bioactive phenolic compounds of oregano essential oil such as carvacrol, thymol, and p-cymene that have anti-inflammatory, antibacterial [43], and antioxidant [10-11,13,44] activities. Such compounds decrease the activity of reactive oxygen species that are produced by macrophage cells stimulated by APEC lipopolysaccharide [45]

5. Conclusion

Experimental infection of chickens with APEC O27 caused macrocytic hypochromic anemia, leukocytosis, heterophilia, and lymphopenia, and alterations in the liver and kidney functions, as well as histopathological changes in the internal organs. Supplementation of diet with oregano could ameliorate the severity of the pathogenicity caused by APEC O27 and so, the pathological and clinicopathological alterations were diminished. Oregano could be used as a feed additive for controlling APEC O27 infection.

6. Formatting of funding sources

None.

7. Conflicts of interest

There are no conflicts to declare.

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