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Research Article

The Impact of Essential Oils Blend on Experimental Colisepticemia in Broiler Chickens

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ABSTRACT

Day-old male Arbor Acres plus broiler chickens (n=288) were divided into 4 equal groups (72 each with 3 replicates). Duration of the study extended from one day of age up to slaughter (42 days). At day 22 of age; birds of groups 2 (T1), 3 (T2) and 4 (PC) were subcutaneously infected with *E. coli* (serogroup O78) in a dose of 10^9 CFU/bird while those of group 1 served as NC group. Groups T1 and T3 were orally treated with essential volatile oils blend (EVOB) that consist of eucalyptus, thyme and oregano in drinking water at day 22 of age or on appearance of first clinical signs of colisepticaemia respectively. Dead as well as survived birds were sacrificed and subjected to post mortem examination for lesion scoring. Other parameters including productive performance and carcass characteristics assay, HA and HI immune assay against SRBC'S and New Castle disease vaccine, oxidative stability and lipid markers assay, blood oxygen saturation percentage as well as histopathology were measured. Challenged EVOB treatment significantly improved productive performance parameters and carcass quality of colisepticaemic broiler chickens, reduced disease picture clinically and pathologicaly (macroscopic and microscopic lesions as well), prevented hypoxia associated with *E. coli* infection, improved oxidative stability, lipid markers and immune responsivness ($P<0.05$).

Key words: Essential Oils, Colisepticemia, Broiler chickens performance, Oxidative stability and Lipid markers test.

INTRODUCTION

There is an increasing interest for developing alternative disease control strategy to enhance chicken health and to reduce the use of antimicrobials. One promising new possibility to achieve this goal is the use of natural herbal products to enhance feed efficiency, gut health, and innate immunity (Lillehoj *et al.,* 2010). Recently aromatic plants and their extracts were introduced to the animal feeding (Awaad *et al.,* 2014). Most studies investigated blends of various active compounds and reported their effects on production performance rather than the physiological impacts (Lee *et al.,* 2013). The defense and immune mechanisms of the body are currently usable for stimulating the non-specific immune responsiveness in both the human and veterinary medical practice (Awaad *et al.,* 1999). In terms of decreased animal welfare and production economy; *E. coli* infections in poultry constitute a severe animal health issue worldwide (Zhuang *et al.,* 2014). Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *E. coli* (Jahantigh and Dizaji, 2015). Peppermint and eucalyptus essential oils blend proved to have an immunostimulant effect on the humoral and cell mediated immune response against Newcastle disease in chickens (Barbour and Danker, 2005). Their utility in modulating the immune response of immunocompromised birds after vaccination or infection with Infectious bursal disease virus as compared with untreated control groups was also evident (Awaad *et al.,* 2004, Awaad *et al*., 2009, Barbour *et al*., 2013).

This study is dedicated to determine the possible effect of usage of eucalyptus, thyme and oregano oils blend on productive performance, carcass characteristics, immune responsiveness, oxidative stability, histopathology and blood oxygen saturation % in experimentally infected broiler chickens with *E. coli* serogroup O78.

MATERIALS AND METHODS

Essential volatile oils blend (EVOB)

Eucalyptus, thyme and oregano oils blend produced commercially under the trade name "Broclear®" by Newtrix Co., Belgium was used in a dose of 0.5 ml/L for 5 consecutive days.

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Experimental birds

Day-old male Arbor Acres plus broiler chickens (n=288) were used in this study. Duration of the trial extended from one day of age up to slaughter (42 days). The birds allotted into 4 equal groups; *Test1 (T1), Test2 (T2), Positive control (PC), and Negative control (NC)* consisting of 72 each. Chickens of all groups were assigned into 3 equal replicates (24 each), floor reared in separate pens. The birds were vaccinated against different diseases according to the vaccination programs usually adopted in Egyptian chicken broiler farms.

Experimental design

Broiler Chickens were fed on a standard ration supplemented with semduramicin at a concentration of 25 ppm as a coccidiostat. No antibiotics were administrated in water or feed for the whole experimental period. Birds had free access to feed and water. At day 22 of age; birds of groups T1, T2 and PC were subcutaneously infected with *E. coli* (serogroup O78) in a dose of 10^9 CFU/bird (Morsy, 2015). Birds of groups NC and PC were kept without treatment and served as negative and positive controls respectively. Those of groups T1 and T2 were orally dosed with EVOB in drinking water as the following regime; T1 group treated at day 22 of age, while T2 group treated on appearance of first clinical signs of *E. coli* infection. Dead as well as survived birds at the end of the trail were sacrificed and subjected to post mortem examination for lesion scoring.

Feeding

From d 1-16 of age, the birds received a starter diet (23% crude protein; 3000 kcal/Kg ME), from d 17-28 of age a grower diet (21% crude protein; 3100 kcal/Kg ME) and from d 29-35 of age a finisher diet (19% crude protein; 3150 kcal/ Kg ME).

Productive performance and carcass characteristics assay

Chicken performance response variables were determined according to (Brady, 1968), (Sainsbury, 1984) and (North, 1984). Body weight (BW) measured on all birds [individual chick weights (to the nearest 1g) were measured at one day, and weekly for the entire period of the experiment (6 weeks)]. Body weight gain (BWG) was determined subtracting the initial weight from the final weight. Growth rate (GR) was calculated according to (Brady, 1945), for each bird at 2, 4 and 6 weeks of age, as follows: $(BW_2 – BW_1) / 0.5 * (BW_1 + BW_2) *100$ where BW_1: BW at the initial phase and BW_2: BW at the end phase. Feed consumption was calculated for each replicate on the same days of birds weighting. Feed conversion ratio (FCR) was calculated for each replicate, as follows: Feed consumed for the replicate (kg)/Total BW gained for the same replicate (kg). Production number [that equals (kilograms of growth per day * (100–mortality %)/FCR) * 100 after (Timmerman *et al.*, 2006). Daily mortalities were recorded for each replicate. For carcass characteristics; at days 42 of age randomly chosen 12 birds per each replicate were taken, slaughtered by slitting the throat, cutting the carotid arteries, jugular veins, esophagus and trachea without severing the head (Sams, 2001). After slaughtering each bird was hanged in bleeding funnel for 3 minutes. Birds were then immersed in 68ºC water bath for 30 seconds and plucked in a rotary drum. Viscera were manually removed and abdominal fat pad weighed. The shanks with feet, neck (with skin), and head were removed. The gizzard (without their contents), heart and liver were removed and weighed. Chilled carcasses were weighed to obtain the dressed weight. Dressing percentage was expressed as the percentage of dressed weight to live weight. Each carcass was then carefully cut into front and hind parts, weighed and carcass characteristics (dressing %, front part %, hind part %, breast meat %, thigh drumstick %, carcass meat %, heart wt. %, gizzard wt. %, liver wt.%, giblet wt.%, and intestinal length and diameter) measured on all bird groups.

Immune assay

Twelve birds, within treatment, were injected, at 21 days of age, intramuscularly, by 1 ml of 10 % Sheep red blood cells (SRBC'S) suspension prepared in 0.9 % physiological saline. At 3, 6 and 9 days post immunization, blood samples were collected from the wing vein to determine the primary antibody responses as a measure of humoral immunocompetence using Haemagglutination (HA) test. Antibody titer values were expressed as $\log_2$ of the highest serum dilution giving total agglutination. Additionally; blood samples were also collected from 12 randomly selected birds/group (4 birds/ replicate) at 21, 28 and 35 days of age for determining antibody titers against Newcastle disease (ND) vaccination. Collected sera were subjected to Hemagglutination inhibition (HI) test employing 8 HA units as described by (Susan, 2016).

Oxidative stability and lipid markers assay

At day 42 of age, blood samples were collected from 12 randomly chosen birds out of each group, immediately placed on ice in heparinized tubes, centrifuged at 1000 rpm for 20 minutes and plasma stored at -20°C for further analysis. Plasma samples were analyzed and assayed for oxidative parameters; Total antioxidant capacity (TAC) and Malnodialdehyde (MDA) (Diamond Biodiagnostic, Egypt). Lipid markers including Triglycerides (TG), Total lipids (TL), Low density lipid (LDL) and High density lipid (HDL) were calorimetrically determined using commercial kits (Diamond Diagnostics, Egypt).

Histopathology

At the end of the trial (42 days) samples of trachea (Cilia) and the primary immune organs [*Bursa of Fabricus* (BF), spleen and thymus glands] were collected from sacrificed 3 chickens per group (one bird/replicate) fixed in 15% buffered formalin, paraffin-embedded sections stained with hematoxylin and eosin (Bancroft *et al*., 1996) and scored for histopathological lesions according to the method described by (Rosales *et al*. 1989). BF lesions were subjectively scored as 1 = no lesions, 2 = focal mild cell necrosis or depletion, 3 = multifocal, 1/3 to ½ of the follicles showing atrophy and 4 = diffuse atrophy of all the follicles.

Blood oxygen saturation percentage

Blood samples were collected from wing veins of randomly chosen 10 birds at 3, 6, 9, 12 and 15 days post

infection (PI) for determination of oxygen level in blood using blood gas analyzer.

Statistical analysis

Data were analyzed using the SAS statistical package (SAS Institute, 2004). General liner model procedure with a one way ANOVA model using essential oils blend as main effect. Mean values were compared using multiple rang test (Duncan, 1955). The significant level was set at 5%.

RESULTS AND DISCUSSION

Significant increase in all studied productive performance and carcass characteristics parameters in T1 and T2 groups vs. PC group were obtained except in growth rate, proventriculus and gizzard weight % where there was numerical increase vs. PC group ($P<0.05$) (Tables 1 and 2). The proposed mode of action of herbal products might be attributed to their antimicrobial properties (Lee *et al*., 2013), oxidative-resistant activity (Dundar *et al*., 2008) or anti-inflammatory effect (Yanhong, 2011).

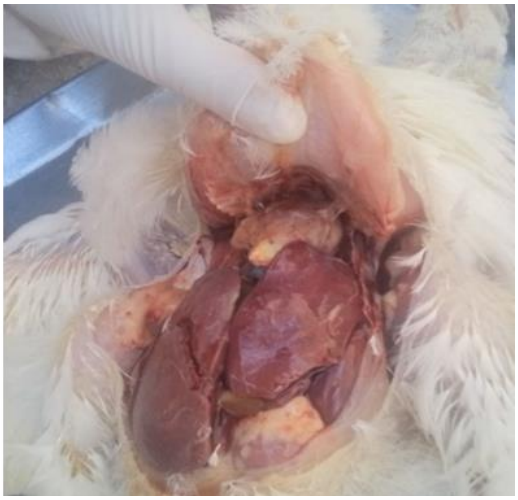

Fig. 1: Post-mortem examination of broiler chickens showing pericarditis and perihepatitis 2 weeks after *E. coli* serogroup O78 infection.

A numerical response to supplementation was observed for TAC and MDA activities in the present investigation. Numerical reduction in MDA (which is an end product of lipid peroxidation) with numeric increase in TAC were obtained in T1 and T2 vs. PC group (Table 3). MDA is a secondary dicarbonyl product of lipid oxidation that can interact with amine groups in proteins, generating protein-bound carbonyls (Hidalgo *et al*., 1998). Antioxidative properties are well described for herbs and spices (Wei and Shibamoto, 2007). Thyme and oregano are labiatae species with significant antioxidative properties, which contain large amounts of the monoterpenes thymol and carvacrol (Cuppett and Hall, 1998). Our results are on line with those described by (Ryzner *et al.,* 2013) who reported on similar results of MDA concentration in the broiler's plasma that found to be significantly decreased on study the effect of dietary Salvia officinalis essential oil and sodium selenite supplementation on antioxidative status in broiler chickens.

Lipid markers revealed significant increase in HDL in T1 vs. PC group ($P\leq0.05$) with numerical increase in T2 vs. PC group. On the other hand, there were numerical decrease in TL, LDL and TG in T1 and T2 groups vs. PC group. TL and LDL showed numerical decrease in NC group vs. PC group which might be attributed to the effect of *E. coli* infection on the level of total cholesterol. Amelioration of this increase was shown in treated groups with EVOB. The improvement in HDL together with the reduction of TL, LDL and TG in treated infected groups vs. PC group might be attributed to the positive effect of the used EVOB. Regarding results of vitamin C illustrated in Table 3 showed a numerical increase in T1 and T2 groups vs. PC group provided that the increase was in T1 more prominent. Ascorbic acid is the most important water-soluble antioxidant provided with feed and synthesized within the animal/chicken body (Chakraborthy *et al.,* 2014).

HI antibody titers against ND vaccines and HA against SRBCs revealed significant increase in NC group ($P<0.05$) and numerical increase in T1 and T2 groups vs. PC group. Antibody titers against ND vaccination at d 3 and d 6 post inoculation of SRBCs revealed significant increase in NC group and T1 and T2 groups ($P<0.05$). These results indicate the immunostimulant effect of volatile oils in colisepticaemic broiler chickens (Table 3). EVOB proved to have an immunostimulant effect on the humoral and cell mediated immune response against Newcastle disease in chickens (Barbour and Danker, 2005). Enhancement of the immune system by herbal products and oils has been reported by other investigators (Lee *et al.,* 2010 and Lillehoj *et al.,* 2010). Moreover, immunocompromised birds by IBDV vaccination or infection has been modulated by supplementation of EVOB (Awaad *et al.,* 2010).

PC group showed significant reduction in blood oxygen saturation % at all studied intervals vs. NC group. This hypoxia might be attributed to septicaemia associated with *E. coli* infection that proportionately underdeveloped cardio pulmonary system that fails to fulfill required oxygen demand and consequently resulted in blood oxygen saturation reduction. Treatment of colisepticaemia in broiler chickens with EVOB resulted in numerical increase in blood oxygen saturation % vs. PC group at d 3 and d 6 PI, with significant increase in treated groups vs. PC group at d 9, 12 and 15 PI ($P<0.05$) (Table 2). The proportionately underdeveloped cardio-respiratory system of modern broilers fails to fulfill the required oxygen demand, resulting in hypoxemia (Aftab and Khan, 2005). In broilers raised at high altitudes, heart contraction rate increases to cope with high oxygen deficiency and high venous blood transformation leading to fatigue, depression and heart failure in animals (Tekeli, 2014). Hypoxia occurs as a result of diminished partial pressure of oxygen, such as occurs with increasing altitude, or reduced oxygen percentage in the air capillaries of the lung (Druyan, 2012). Although some investigators reviewed that blood oxygen saturation is a potential indicator trait for resistance to ascites in chickens (Navarro *et al*. 2006), no data are available to correlate between blood oxygen saturation and chicken infectious diseases which need further and comprehensive investigation.

Table 1: Productive performance and carcass characteristics of broiler chickens experimentally infected with *E. coli*.

Group	Final BWt.(g)	Cumulative feed intake (g/bird)	Final FCR	Wt. gain (d 1-42) (g)	Mortality %	Growth rate	Dressing%	Breast Meat%	Carcass meat%	Intestinal lenghth (Cm)	Intestinal diameter (Cm)	Prove-ntriculus (%)	Gizzard%	Thigh drumstick meat %	Hind parts %	Front parts %
Blank ctrl	2718.6±35.74^{a}	5015.57±19.46^{a}	1.843±0.012^{a}	2678.1±29.25^{a}	7.00±0.65^{c}	18.1±0.97^{a}	71.10±0.52^{a*}	22.05±0.19^{a}	38.76±0.30^{a}	204.33±2.68^{a*}	0.875±0.025^{a}	0.503±0.056^{a}	1.878±0.138^{a*}	15.93±0.17^{a}	32.34±0.25^{a}	38.76±0.30^{a}
T1	1892.4±143.82^{b}	3022.64±99.54^{bc}	1.590±0.051^{b}	1851.0±143.73^{b}	36.00±6.11^{b}	17.6±4.19^{a}	66.68±0.29^{b}	19.27±0.14^{c}	36.21±0.31^{b}	188.83±3.34^{b}	0.708±0.034^{bc}	0.411±0.030^{ab}	1.611±0.053^{ab}	14.85±0.11^{b}	30.48±0.08^{bc}	36.21±0.31^{b}
T2	2041.0±168.61^{b}	3201.27±83.88^{b}	1.582±0.097^{b}	2000.3±168.36^{b}	34.67±3.80^{b}	15.1±4.40^{a}	67.72±0.65^{b}	20.29±0.35^{b}	37.13±0.43^{b}	195.83±2.74^{b}	0.758±0.029^{b}	0.424±0.036^{ab}	1.666±0.065^{ab}	14.72±0.24^{b}	30.59±0.28^{b}	37.13±0.43^{b}
Positive ctrl	1516.9±119.62^{c}	2840.18±111.18^{c}	1.859±0.105^{a}	1475.8±118.73^{c}	61.00±11.49^{a}	24.7±6.83^{a}	62.96±0.66^{c}	17.64±0.27^{d}	33.23±0.32^{c}	178.17±2.40^{c}	0.667±0.033^{c}	0.332±0.034^{b}	1.458±0.076^{b}	13.63±0.21^{c}	29.72±0.38^{c}	33.23±0.32^{c}
Probability	0.0001	0.0001	0.0108	0.0001	0.0001	0.3830	0.0001	0.0001	0.0001	0.0001	0.0001	0.0376	0.0167	0.0001	0.0001	0.0001

Means with different, lower case, superscripts, within age, are significantly different (P≤0.05).

Table 2: Results of European production efficiency factor, coefficient of variation and blood oxygen saturation % of broiler chickens experimentally infected with *E. coli*.

Trait	EPEF**	Coefficient of	Maximum weight (g)	Minimum weight (g)	Blood oxygen saturation (%)				
Treatment		variation at 45 ds	at 45 ds	at 45 ds	At 3 ds PI	At 6 ds PI	At 9 ds PI	At 12 ds PI	At 15 ds PI
Negative control	305.56 ±6.25^{a*}	9.57	3100.00	1845.00	86.80 ±1.12^{a*}	86.33±1.25^{a}	86.93±1.49^{a}	86.13±1.03^{a}	86.60±1.38^{a}
T1	168.15±8.04^{b}	34.83	2900.00	825.00	77.38±2.51^{b}	79.67±2.13^{b}	81.53±1.99^{b}	83.40±1.69^{a}	84.60±1.00^{a}
T2	192.45±19.85^{b}	36.94	2900.00	830.00	75.00±1.32^{b}	79.93±2.07^{b}	82.27±1.62^{ab}	85.80±2.24^{a}	86.53±1.01^{a}
Positive control	64.96±13.74^{c}	28.43	1980.00	800.00	74.93±2.28^{b}	75.13±2.85^{b}	75.20±1.63^{c}	77.27±1.11^{b}	78.67±1.55^{b}
Probability	0.0001	--	--	--	0.0001	0.0061	0.0001	0.0007	0.0001

*Means with different, superscripts, within trait, are significantly different (P≤0.05). **EPEF= European production efficiency factor.

Table 3: Results of antibody titers against ND vaccines and Sheep red blood cells, vitamins (C), oxidative stability biomarkers and lipid markers of broiler chickens experimentally infected with *E. coli.*

Trait	Antibody Titer Against NDV (Log_2)			Antibody Titer Against Sheep Red Blood Cells (Log_2)			Vitamin C (mg/L)	Oxidative biomarkers		Lipid Markers			
treatment	21 days	28 days	35 days	3-ds PI	6-ds PI	9-ds PI		Total antioxidant capacity (TAC) (mM/L)	Malnodialdehyde (MDA) (nmol//mL)	TG ml/dl	TL ml/dl	LDL ml/dl	HDL ml/dl
Negative control	4.67±0.28	4.42±0.26^{a*}	5.43±0.30^{a}	7.17±0.21^{a*}	3.92±0.48^{a}	1.58±0.26	22.87±2.16	1.418±0.128	6.32±0.12^{b*}	187.5±8.67	133.80±10.69	101.06±11.5	32.74±1.66^{b*}
T1	4.58±0.31	3.00±0.49^{b}	3.10±0.46^{b}	6.17±0.24^{b}	2.50±0.23^{bc}	0.83±0.21	23.01±1.90	1.380±0.079	7.79±0.48^{ab}	180.79±9.09	126.75±5.49	91.83±6.50	34.92±2.40^{ab}
T2	4.50±0.40	3.11±0.31^{b}	4.08±0.78^{ab}	5.75±0.52^{bc}	3.08±0.36^{ab}	1.33±0.28	20.54±1.66	1.325±0.075	7.65±0.35^{ab}	192.26±7.41	135.65±6.55	94.47±7.73	41.18±2.80^{a}
Positive control	4.91±0.21	2.92±0.42^{b}	3.08±0.23^{b}	4.83±0.27^{c}	1.75±0.37^{c}	0.83±0.11	18.21±1.78	1.318±0.021	8.77±0.81^{a}	194.22±5.31	146.71±10.94	112.25±10.47	34.46±1.88^{b}
Probability	0.8200	0.0120	0.0272	0.0002	0.0015	0.0534	0.2395	0.8104	0.0222	0.6236	0.4527	0.4204	0.0446

PI=PLDH= Lactate Dehydro-genase. TG=Triglycerides. TL=Total cholestrol. LDL=Low density lipid. HDL=High density lipid. * Means with different, superscripts, within trait, are significantly different (P≤0.05).

Table 4: Microscopic lesion scoring of broiler chickens experimentally infected with *E. coli.*

Trait	Organs										
Treatment	Trachea						BF	Spleen	Caecal tonsils	Thymus glands	
	Deciliation	Hyperplasia of mucosal epi.	Necrosis of mucosal epi.	Inflammatory reaction of sbmucosa	Submucosal Hg	Goblet cell hyperplasia	Lymphoid depletion	Lymphoid depletion	Lymphoid depletion	Lymphoid depletion	Medullary Hg
T1	1.333±0.333^{b*}	0.889±0.200	0.778±0.278^{b}	1.444±0.176^{b}	0.667±0.236^{ab}	1.444±0.176	1.263±0.214^{b}	1.000±0.169^{b}	1.000±0.169^{b}	1.091±0.211^{ab*}	0.364±0.203^{b}
T2	0.889±0.200^{b}	1.000±0.000	0.333±0.167^{b}	1.000±0.000^{b}	0.111±0.111^{b}	1.000±0.236	1.235±0.106^{b}	0.615±0.140^{b}	0.714±0.125^{b}	0.600±0.163^{b}	0.400±0.163^{b}
Positve Ctrl	2.222±0.147^{a}	1.333±0.287	1.556±0.176^{a}	2.111±0.200^{a}	1.125±0.441^{a}	1.222±0.324	2.529±0.125^{a}	1.769±0.201^{a}	1.688±0.176^{a}	1.455±0.282^{a}	1.636±0.310^{a}
Probability	0.0022	0.2910	0.0017	0.0001	0.0493	0.4723	0.0001	0.0002	0.0003	0.0445	0.0007

* Means with different, superscripts, within trait, are significantly different (P≤0.05).

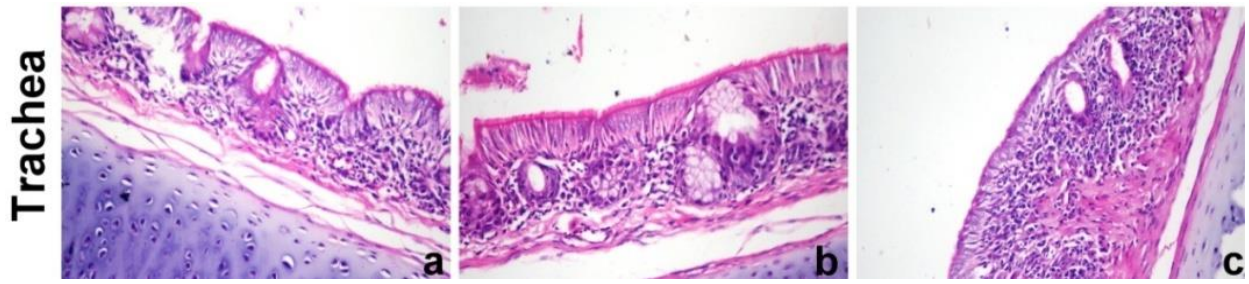

Fig. 2: Histopathological sections of Trachea. a) T1 group is showing focal deciliation, goblet cell hyperplasia & submucosal mononuclear cell infiltration. b) T2 group is showing minimal histopathological alterations. Note the intact normal cilia with mild mononuclear cell infiltartion. c) PC group is showing deciliation of tracheal epithelium with intense submucosal mononuclear cell infiltration (H&E,X400).

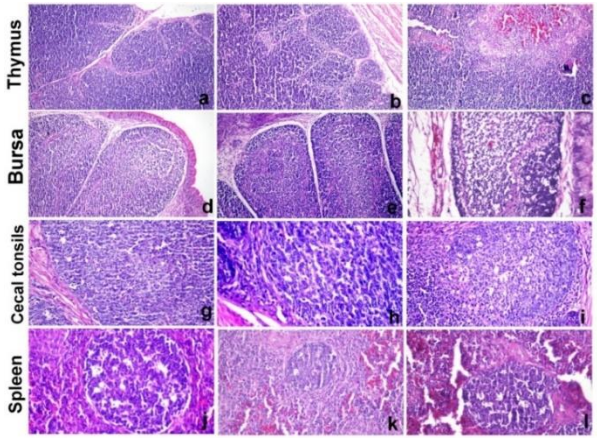

Fig. 3: Histopathological sections of Thymus gands, BF, Cecal tonsils and Spleen. Thymus glands: a) T1 group is showing mild lymphocytic depletion comprising the cortex. b) T2 group is showing minimal lymphocytic depletion of lymphoid elements. c) PC group is showing moderate depletion with medullary hemorrhages (H&E, X200). BF: d) T1 group showing mild medullary lymphocytic depletion with distinct corticomedullary junction. e) T2 group showing minimal lymphocytic cortical depletion(X200). f) PC group is showing severe medullary lymphocytic depletion with obvious cortcomedullary zone (X400). Cecal tonsils: g) T1 group is showing lymphoid depletion with appearance of mitotic figures. h) T2 group is showing minimal lymphoid depletion with increases mitosis. i) PC group is showing moderate lymphoid depletion and lymphocytolysis (X400). Spleen: k) T1 group is showing lymphocytolysis of lymphoid elements(X400). k) T2 group is showing minimal lymphocytolysis of lymphoid elements with mitosis (X200). l) PC group is showing lymphocytolysis comprising the lymphoid follicle(X400) (H&E).

Histopathological assay revealed significant reduction in the lesion scoring and the inflammatory reaction of tracheal submucosa and the lymphoid depletion of cecal tonsils, BF, spleen and thymus glands of EVOB treated groups (T1 and T2) vs. PC group ($P<0.05$). (Table 4 and Figs. 2, 3).

Microscopically, all dead birds did not reveal homogenous severity of lesions in all organs in the present study corresponded with the findings of others (Islam *et al*., 2003 and Ghosh *et al*., 2006). The treated groups showed mitosis of lymphoid elements comprising the cecal tonsils (Fig.3g-i). Severe lymphoid depletion of lymphoid follicles of spleen has been observed in PC group which was less in T1 and T2 groups that also showed mitotic figures (Fig.3 J-l).

Conclusions

EVOB improved productive performance parameters and carcass quality of experimentally infected colisepticaemic broiler chickens. Its usage resulted in reduction of the disease picture clinically and pathologicaly, prevented hypoxia associated with *E. coli* infection, and improved oxidative stability, lipid markers and immune responsivness.

Authors contributions

MA Elmenawey: Productive performance assay, carcass characteristics assay and statistical analysis. Faten A Mohamed: Histopathology assay. Eman A Morsy: Bacteriological study and *E. coli* challenge. GA Abdel-Alim: Immune assay, oxidative stability and lipid markers assay. MHH Awaad: Research Plan, experimental design and writing of the manuscript.

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