

The protective Effects of Volatile Oils against Complicated Chronic Respiratory Disease (CCRD) in ChickensElbestawy A.R.¹, Eman Khalifa², Sadek K.M.³, Ahmed H.A.⁴ and Ellakany H.F.¹¹Dept. of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt.²Dept. of Microbiology, Faculty of Veterinary Medicine, Alexandria University – Matrouh branch, Egypt.³Dept. of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Egypt.⁴Dept. of Nutrition and Veterinary Clinical Nutrition, Faculty of Veterinary Medicine, Damanhour University, Egypt. ahmed.elbestawy@vetmed.dmu.edu.eg

Abstract: A product mixture of essential volatile oils was tested experimentally for evaluation of its effect on *Mycoplasma gallisepticum* (MG) and *Escherichiacoli* (*E.coli*) experimental infection, growth performance and NDV immune response of commercial broiler chickens, 160 birds were divided into 4 groups as follows: group 1 non infected non treated, fed with basal diet (control group); group 2 infected with *E.coli* and MG at 14th day of age; group 3 infected with MG and *E.coli* at 14th day of age and supplemented with a bronchodilator patent preparation as 0.5 ml /liter drinking water for 3 days and group 4 non infected treated with a bronchodilator patent preparation as 0.5 ml /liter drinking water for 3 days. The results indicated that the use of essential oils had a good effect on performance, weight gain, immune response and decreasing the lesion score of MG infected chickens.

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Key Words: Complicated Chronic Respiratory Disease, Volatile oils, Chickens.

1. Introduction

Complicated Chronic Respiratory Disease (CCRD) in chickens is a common disease in many poultry flocks around the world. Although the clinical manifestations are usually slow to develop, MG in combination with *E.coli* can cause severe airsacculitis. Beside feed and egg production reduction, these problems are of high economic significance since respiratory tract lesions can cause high morbidity, high mortality and significant carcass condemnation and downgrading. Also, the miss-use of antibiotics creates bacterial resistance rapidly and affects human health through its residues in poultry meat. Consequently, the efforts to limit the losses from these infections would be of primary importance to the poultry industry.

On the other hand, essential oils are very complex natural mixtures of compounds that have antibacterial and anti-inflammatory effects and their chemical compositions and concentrations are variable. For example, the concentrations of two predominant components of thyme essential oils, i.e. thymol and carvacrol have been reported to range from as low as 3-60% of total essential oils (Lawrence and Reynolds, 1984). *Eucalyptus* oil is one of essential oils that mainly composed of terpenes and terpene-derivatives in addition to some other non-terpene components (Edris, 2007). The principal constituent found in *Eucalyptus* is 1, 8-cineole (eucalyptol), however, other chemotypes such as α -phellandrene, ρ -cymene, γ -terpinene, ethanone, spathulenol, among others have been documented (Akin *et al.*, 2010).

The essential oils of *Eucalyptus* species possess important biological activities including antibacterial, anti-inflammatory, diaphoretic, antiseptic, analgesic effects (Cimanga *et al.*, 2002) and antioxidant properties (Lee and Shibamoto, 2001; Damjanović-Vratnica *et al.*, 2011). Antioxidant agents are compounds that have the potentials to scavenge reactive oxygen species or free radicals. These free radicals play an important roles in energy production, synthesis of some biomolecules, phagocytosis, and cell growth in living systems (Packer *et al.*, 2008). An imbalance in the rate of production of free radicals or removal by the antioxidant defense mechanisms leads to a phenomenon referred to as oxidative stress. A mixture of Oregano (carvacrol, cinnamaldehyde and capsicum oleoresin) beneficially affected the intestinal microflora, absorption, digestion, weight gain and also had antioxidant effect on chickens (Bassett, 2000). Also, Barbouret *et al.* (2011) examined the effect of Mentofin® (*Eucalyptus* and peppermint) essential oil mixture in the protection of the respiratory system of broilers against controlled challenges by MG and/or a mixture of respiratory viruses as avian influenza virus H9N2 and infectious bronchitis virus and they concluded that the Mentofin® treated group showed higher feed conversion, less mortality rate and lower in clinical signs and lesions than infected non treated groups.

Sadek *et al.* (2014) revealed that Digestaron® 1317 supplementation increased significantly the body weight and the levels of antioxidant enzymes and significantly decreased protein nitrosylation and non-

significantly increased the level of liver-function enzyme. The addition of phytogetic additives ameliorated the intestinal microflora via the reduction of *Coliforms* at the ages of 14 and 28 days and via the fortification of beneficial gut flora, such as lactobacilli. Due to characteristic adverse effects of synthetic antioxidants (Zheng and Wang, 2001 and Peschel *et al.*, 2006), there is need to explore phytotherapies to develop viable alternatives and the impetus has shifted to look for plant derived products as food preservatives and antioxidants. Earlier, studies have reported the radical scavenging ability of oils from *Eucalyptus* species (Kaur *et al.*, 2010 and Marzoug *et al.*, 2011). Our primary objective in the present study is to assess *in vivo* the possible antioxidant, hepatoprotective, renoprotective and growth performance of essential oils of *Eucalyptus* on *M. Gand* *E. coli* experimentally infected chickens. The antioxidant activity was determined in terms of antioxidant enzymatic activities.

2. Materials and Methods

Birds and treatments

This work was carried out at Faculty of Veterinary Medicine, Damanhour University to investigate the possible effects of Ventoline[®] on *M. Gand* *E. coli* (Serotype O₂₇) infection, growth performance and NDV immune response in commercial broiler chickens. Briefly, 160 one-day-old chicks (avian 48) obtained from a local broiler chicken hatchery were divided into 4 groups 40 birds each, the experiment was conducted in accordance with animal welfare laws. All birds in each group were fed with basal diet and treated as follows: Group 1 non infected non treated (control group); Group 2, infected with *E. coli* and *MG* at 14th day of age; Group 3, infected with *E. coli* and *MG* at 14th day of age and supplemented with Ventoline[®] at 0.5 ml /liter drinking water for 3 days and Group 4 non infected treated with a bronchodilator patent preparation as 0.5 ml /liter drinking water for 3 days. Ventoline[®] contains essential oils of *Eucalyptus* 15%, carvacol 10%, thymol 15% in combination with menthol 5%. The initial brooding temperature of 32°C was reduced sequentially according to the age of the birds until reaching 26°C at 21 d. The chicks were kept on a 23-h light program, with free access to feed and water throughout the experiment. The birds were fed a starter diet until 21 d of age followed by a finishing diet from d 21 to 35.

Birds were vaccinated as follows:

7 days: Clone Ma5 (Eye drop)

8 days: Inactivated ND+H9 injection S/C

12 days: Gumboro intermediate plus (Bursin plus) via Drinking water

21 days: LaSota via Drinking water

Respiratory signs and mortality rate were recorded daily. *MG* lesion scoring was recorded weekly for 3 weeks PI through airsacculitis lesion scoring visually (Ellakany, *et al.*, 1997). Re-isolation of *MG* and *E. coli* were done also weekly for 3 weeks PI

Experimental infection:

Chickens were inoculated with 0.2 ml of Frey's broth containing *MG* at a concentration of 10⁸ CFU/ml of fresh culture into the right abdominal air sac at the age of 14 days (Kempf *et al.*, 1997). Also chickens were injected simultaneously into the air sac of the left side by 0.2 ml of MacFarland tube no.1 containing 10⁸ *E. coli* organisms /1ml saline suspension. The chickens of the non-infected control group were inoculated with 0.2 ml sterile saline. The chickens were observed daily for respiratory signs and/or mortalities up to 3 wks PI.

Culture and biochemical characterization:

MG re-isolation from air sacs and trachea was carried out on Frey's broth and agar medium (Frey *et al.*, 1968)

<i>Mycoplasma</i> agar base	35.0 g
Dextrose	3.00 g
Horse serum	120 ml
Phenol red (1%)	2.50ml
Thallium acetate	0.5g
PenicillinG-sodium	1000,000IU
DW up to	1000ml
pH adjusted at 7.8	

While *E. coli* re-isolation from heart, liver and air sac were carried out on MacConkey's agar media (OXOID, Basingstoke, UK) and incubated aerobically at 37°C for 18 to 24 h. The identification of *E. coli* was based on the results of colony morphology and biochemical tests as catalase, oxidase and IMViC tests (Someya *et al.*, 2007).

Lactobacillus count was done using Rogosa Agar as a selective medium used for the isolation of *lactobacilli* and the typical colonies appeared after 48 hours of incubation at 37°C in 5% CO₂.

For 1 liter of Rogosa Agar medium (Rogosa *et al.*, 1951):

- Tryptone 10.0 g
- Yeast extract 5.0 g
- Glucose 20.0 g
- Sodium acetate 15.0 g
- Ammonium citrate 2.0 g
- Potassium dihydrogen phosphate 6.0 g
- Magnesium sulfate 575.0 mg
- Manganese sulfate 120.0 mg
- Ferrous sulfate 34.0 mg
- Tween 80 1.0 g
- Bacteriological agar 15.0 g

Approximately 100 mg of intestinal digesta were collected 3 times after the end of essential oil treatment at 3, 7 and 14 days and mixed each time with 900 μ L of sterile saline solution (0.9% NaCl) and homogenized 3 min using a homogenizer. Each digesta homogenate was serially diluted from initial 10^{-1} to 10^{-9} and subsequently plated on selective agar media (Rogosa Agar) and incubated anaerobically at 37°C for 48h for *Lactobacillus* count;

Haemagglutination inhibition test (HI): Twenty serum samples from chickens of each group were taken at 35 days of age for HI testing according to Allan *et al.* (1978).

Biochemical analysis:

Also, 20 serum samples from chickens of each group were taken at 35 days of age for all biochemical parameters which were analyzed using commercially available kit methods. UNICO 2100 UV-Spectrophotometers, ELx800 Absorbance Microplate Reader and other laboratory equipment aids were used for biochemical analysis. Moreover, each parameter was performed according to the instructions of its kit.

Final Body Weight and FCR: All birds were weighted at 35 days of age and the body weight gain and feed conversion rate were calculated of each group.

3. Results and Discussions

There was insignificant difference in initial body weight between different experimental groups. Chickens of group 4 which received Ventoline® 0.5 ml/liter drinking water for 3 days showed the highest final weight and weight gain, when compared with chickens of control non infected non treated group and showed 109.73 % weight gain relative to control non infected non treated group followed by chickens in group 3 which were infected and treated with Ventoline® 0.5 ml/liter drinking water for 3 days showed lower feed conversion ratio and higher final weight and weight gain, when compared with chickens of control non infected non treated group and showed 104.64 % weight gain relative to control non infected non treated group (Table 1).

The observed data indicated the better weight gain and FCR in chickens received the essential oils as it caused a significant increase in *Lactobacillus* count inducing better feed digestion, absorption, increased digestive enzymes as well as reducing the bad effect of harmful bacteria in the intestinal tract. Also, Zeng *et al.* (2015) indicated the positive effect of essential oils on the production of digestive secretions and nutrient absorption, reduce pathogenic stress in the gut, exert antioxidant properties and reinforce the animal's immune status, which help to explain the enhanced performance in poultry.

This growth promoting effect of essential oils through growth enhancing effects on the intestinal microflora agreed with those of Sadek *et al.* (2014) who found that the addition of essential oil mixture, showed a significant reduction in the counts of total bacterial, *E.coli* and *Coliform* count and an increase in the beneficial *lactobacillus* and lactic acid bacteria compared with the control group.

Although, Helander *et al.* (1998) indicated in vitro that carvacrol and thymol but not cinnamaldehyde, exert an antibacterial effects on *E.coli* O₁₅₇ through lipophilic effect by disintegrating the membrane of bacteria, leading to the release of membrane-associated material from the cells to the external medium. Our in vivo study of essential oil supplementation did not clarify their antibacterial effect because re-isolation of the bacteria still high along the 3 weeks of sampling.

Concerning the mortality rate, it was observed that group 1 and 4 showed 2% and chickens of group 3 showed 4% mortality rate respectively all over experiment period. While group 2 showed 20% mortality rate due to infection with *MG+E.coli* without any supplementation.

Respiratory signs (as coughing, sneezing and head swelling) started 3 days Pin group 2 and 5 days PI in chickens of group 3 and were milder than the infected control group 2 along the experiment. The cumulative *MG* lesion scoring was 0 in chickens of group 1 and 4, and the chickens of group 2 showed 4 lesion score and 2 in chickens of group 3 (Fig.1,2 and 3). The re-isolation of *E.coli* and *MG* (Fig. 4 and 5) were 0% in chickens of group 1 and 4, 100% in chickens of group 2 and 66.6% in chickens of group 3 while HI titers for NDV were 2^{6.6} in chickens of group 1, 2^{5.6} in chickens of group 2, 2⁷ in chickens of group 3 and 2^{7.3} in chickens of group 4.

These results showed that the immunostimulation effect of essential oils was prominent in the NDV antibody titers of the chickens in group 3 and 4 as it was higher than the control group and this agreed with Awaad *et al.* (2010), who recorded that the administration of volatile oils had a potent immunomodulatory effect and evoked the chicken immune response and also confirmed the results obtained by Barbour and Danker (2005), who reported that essential oils of *Eucalyptus* and peppermint improved the homogeneity of immune responses and performance in *MG/H9N2*-infected broilers. However, Rehman *et al.* (2013) stated that Mentofin® (a herbal product containing 10% *Eucalyptus*oil, 10% menthol, 33% liquid builders and 47% saponins) treated broilers showed higher consistent antibody titer against NDV as compared to untreated broilers but did not show any effect on weight gain and feed conversion ratio of the treated

broilers. Moreover, fecal droppings from Mentofin[®] treated birds showed no urease producing bacteria (*Proteus vulgaris*) while 100% of droppings of the untreated birds showed the presence of this bacteria.

The biochemical analysis showed that MG+E.coli infected group 2 resulted in significant increase in MDA level (a marker of lipid peroxidation) and significant decrease in all antioxidant enzymes as well as the level of GSH in different tissues (Tables, 3, 4 and 5). On the other hand, Ventoline[®] in the infected treated group 3 was able to ameliorate the disturbances in oxidative status induced by infection. Regarding the effect of experimental infection on liver and kidney functions, the results presented in tables (6 and 7) revealed that, there were an adverse effects reflected in significant decrease in total protein and significant increase in ALT and AST as well as serum urea, uric acid and creatinine while the administration of Ventoline[®] in chickens of group 3 mitigate such hazard effects and a better results were recorded in chickens of group 4 which received only Ventoline[®] treatment indicating the good efficacy of these essential oils on the oxidative stress, liver and kidney functions as well as uric acid in the birds.

Concerning the effect of Ventoline[®] on oxidative status, our results went paralleled with the result of Olayinka *et al.* (2012) who showed that the *Eucalyptus* oil exerted a concentration dependent radical scavenging activity. Some authors stated that, the strong antioxidant capacity of essential oils has been attributed to their phenolics constituents and synergistic effect such as tannins, rutin, thymol and carvacrol, and probably 1, 8-cineole with moderate DPPH radical scavenging activity reported by (Edris, 2007; El-Moein *et al.*, 2012 and Kaur *et al.*, 2011). The minimum 1, 8-cineole (*Eucalyptol*) content of pharmaceutical-grade *Eucalyptus* essential oil as defined in most standards is 70% (Singab *et al.*, 2011). Extractions of phenolic compounds as antioxidants from *Eucalyptus* bark were done by Vázquez *et al.* (2012) who demonstrated the potential of *Eucalyptus* bark as a source of antioxidant compounds. In that study they showed that *Eucalyptus* had ferric reducing

antioxidant power in the ranges 0.91–2.58 g gallic acid equivalent (GAE)/100 g oven-dried bark and 4.70–11.96 mmol ascorbic acid equivalent (AAE)/100 g oven-dried bark, respectively. Inside cell, essential oils serve as powerful scavenger preventing mutations and oxidants in cells (Bakkali *et al.*, 2008). Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* essential oils was reported by (Shahwar *et al.*, 2012). Also, Superoxide dismutase (SOD)-like activity for different compounds and fractions isolated from wood extracts was achieved by (Eyles *et al.*, 2004).

Our results showed that the antioxidant activity of GPX and GPT were elevated in Ventoline[®] treated group and this can be attributed to the possible synergistic effects of the components of this oil mixture (Basak and Candan, 2010 and Hasegawa *et al.*, 2008). The inhibitory effects of *Eucalyptus* towards malonaldehyde formation from lipid by oxidation with Fenton's reagent was measured (Lee and Shibamoto, 2001), they found that it inhibited malonaldehyde (MA) formation in cod liver oil. Gallic and ellagic acid in the ethanolic extract and flavones in supercritical carbon dioxide fluid extraction of *Eucalyptus* were found to be the prevailing *in vitro* antioxidant activity in comparison with that of butylated hydroxyanisole, which was used as a standard for the antioxidant activity measurement (El-Ghorab *et al.*, 2003). Also, Sadek *et al.* (2014) revealed the antioxidant effect of volatile oils in chickens. On the contrary, the oxidant effects of *Eucalyptus* oil were investigated in the erythrocytes of the koala especially effect on haemolysis and changes in the intracellular levels of glutathione (Agar *et al.*, 1998), the results indicated that koala erythrocytes are susceptible to *Eucalyptus* oil-induced oxidative damage to the intracellular constituents and increased degree of haemolysis. Regarding the hepatorenal protective effects of Ventoline[®], this might attributed to its antioxidant potential and subsequently protect cell membrane of this tissues from damage induced by free radicals associated with infections. Our results that revealed the antioxidant properties of Ventoline[®] confirm and support such hypothesis.

Table 1. Effect of Ventoline[®] supplementation on broiler performance:

Parameters	(1) Control-ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
Initial BW in grams	45.88	45.84	45.76	45.78
Final BW in grams	1280.10 ±43.06 ^{ab}	1228.30 ±48.39 ^b	1337.20 ±43.06 ^a	1400.10 ±40.06 ^{ab}
Weight gain in grams	1234.22±42.95 ^{ab}	1182.46±48.27 ^a	1291.44±42.95 ^a	1354.32±40.95 ^{ab}
Gain relative to control	100	95.81	104.64	109.73
Feed Intake	2144.00±0.00 ^a	2090.00±0.00 ^c	2099.00±0.00 ^b	2125.00±0.00 ^a
FCR	1.67±0.07 ^a	1.70±0.08 ^a	1.57±0.07 ^a	1.51±0.07 ^a
Mortality rate%	4	20	4	2

Means within the same column under the same category carry different superscripts are significantly different.

Table 2. Effect of Ventoline® supplementation on *Lactobacillus* count:

Groups <i>Lactobacillus</i> count	(1) Control-ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
3 dpt	6×10^4	4.12×10^4	8.5×10^4	9.1×10^4
7 dpt	3.65×10^5	0.7×10^5	4.48×10^5	7.3×10^5
14 dpt	1.8×10^5	0.3×10^5	1.65×10^5	3.14×10^5

Table 3. HI titers of NDV, MG lesion scores and the re-isolation of MG&*E. coli* of experimental chicken groups:

Parameters	(1) Control-ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
HI titers of ND at 35 days	$2^{6.6}$	$2^{5.6}$	2^7	$2^{7.3}$
MG lesion scoring average (weekly for 3 weeks PI)	0	4	2	0
MG re-isolation (%)	0	100	66.6	0
<i>E. coli</i> re-isolation (%)	0	100	66.6	0

Table 4. Effect of Ventoline® supplementation on GP_x Activity and GST Activity of broiler chickens:

Enzymes	Groups	(1) Control -ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
Glutathione Peroxidase (GP _x) Activity IU/g Wet tissue	Liver	53.61 ± 4.49^b	39.83 ± 3.57^c	67.30 ± 4.73^a	58.73 ± 1.55^b
	RBCs hemolysate	31.43 ± 3.36^b	29.37 ± 2.89^b	50.64 ± 3.57^a	34.22 ± 2.32^b
	Kidneys	44.38 ± 4.67^b	27.91 ± 3.62^c	61.32 ± 4.58^a	47.77 ± 2.69^b
Glutathione S Transferase (GST) Activity mol CDNB/min/g Wet tissue	Liver	537.3 ± 6.52^b	471.6 ± 5.47^c	603.7 ± 7.62^a	570.1 ± 4.11^b
	RBCs hemolysate	268.9 ± 4.53^a	231.2 ± 5.72^b	270.5 ± 3.62^a	275.9 ± 2.44^a
	Kidneys	487.8 ± 9.61^a	485.1 ± 8.90^a	489.3 ± 7.73^a	488.6 ± 4.55^a

Means within the same column under the same category carry different superscripts are significantly different.

Table 5. Effect of Ventoline® supplementation on SOD and CAT of broiler chickens:

Enzymes	Groups	(1) Control -ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
Superoxide dismutase SOD (U/mg protein)	Liver	129.82 ± 6.71^a	87.26 ± 4.46^b	131.23 ± 5.49^a	133.27 ± 3.41^a
	RBCs hemolysate	64.12 ± 3.19^b	62.31 ± 4.73^b	78.52 ± 3.42^a	66.33 ± 1.28^b
	Kidneys	81.27 ± 7.62^b	62.72 ± 5.752^c	99.41 ± 6.81^a	84.33 ± 3.33^b
Catalase CAT (K/Sec/mg protein)	Liver	79.16 ± 5.67^a	68.71 ± 5.19^{bc}	73.92 ± 6.83^{ab}	80.54 ± 2.77^a
	RBCs hemolysate	61.79 ± 5.18^a	62.79 ± 5.43^a	60.12 ± 6.68^a	62.11 ± 2.33^a
	Kidneys	56.64 ± 3.52^b	54.29 ± 3.92^b	82.71 ± 7.39^a	57.44 ± 2.11^b

Means within the same column under the same category carry different superscripts are significantly different.

Table 6. Effect of Ventoline® supplementation on SOD and CAT of broiler chickens:

Enzymes	Groups	(1) Control-ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
MDA (nmol /g wet tissue)	Liver	94.65 ± 3.42^{bc}	132.47 ± 4.64^a	100.26 ± 3.29^b	95.33 ± 1.22^{bc}
	RBCs hemolysate	76.28 ± 6.31^b	119.18 ± 5.53^a	74.64 ± 6.81^b	76.36 ± 3.25^b
	Kidneys	59.24 ± 3.32^b	87.68 ± 7.24^a	36.89 ± 6.56^c	57.33 ± 2.44^b
GSH (μ mol/g wet tissue)	Liver	107.62 ± 8.53^a	72.38 ± 5.41^c	89.25 ± 4.68^b	109.35 ± 4.53^a
	RBCs hemolysate	59.68 ± 7.87^b	57.25 ± 7.42^b	78.28 ± 3.69^a	61.87 ± 5.43^b
	Kidneys	63.32 ± 6.24^b	42.56 ± 7.61^c	81.19 ± 5.92^a	66.54 ± 4.32^b

Means within the same column under the same category carry different superscripts are significantly different.

Table 7. Effect of Ventoline[®] supplementation on total protein, GPT and GOT in serum of broiler chickens:

Enzymes	(1) Control-ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
Total protein(g/dl)	8.49±0.24 ^a	8.37±0.16 ^{ab}	8.52±0.27 ^a	8.78± 0.32 ^a
GPT(U/L)	43.22±4.16 ^{bc}	67.84±5.62 ^a	49.23±4.75 ^b	40.31± 5.34 ^{bc}
GOT(U/L)	51.73±5.45 ^a	50.38±3.59 ^a	51.17±3.26 ^a	48.98± 5.56 ^a

Means within the same column under the same category carry different superscripts are significantly different

Table 8. Effect of Ventoline[®] supplementation on Urea, Uric acid and Creatinine of broiler chickens:

Enzymes	(1) Control-ve	(2) Control infected	(3) Infected and Ventoline treated (0.5 ml /liter)	(4) Non infected and Ventoline treated (0.5 ml /liter)
Urea (mg/dl)	52.57±3.27 ^b	76.76±4.32 ^a	39.41±4.67 ^c	45.66± 2.34 ^b
Uric acid(mg/dl)	3.97±1.25 ^c	9.59±0.83 ^b	8.13±0.77 ^{ab}	3.01±0.12 ^c
Creatinine(mg/dl)	0.93±0.11 ^a	0.96±0.23 ^a	0.63±0.18 ^b	0.59±0.41 ^a

Means within the same column under the same category carry different superscripts are significantly different.

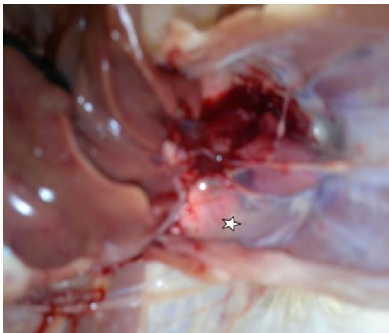


Fig I. 0 Score airsacculitis in chickens of control group 1 (star)

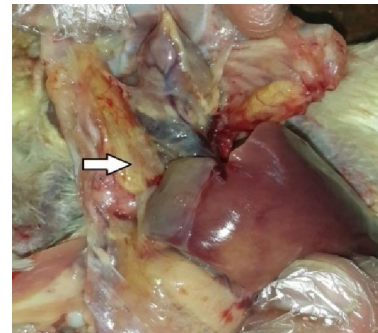


Fig II. Score 4 airsacculitis in infected chickens of control group 2 (Arrow)

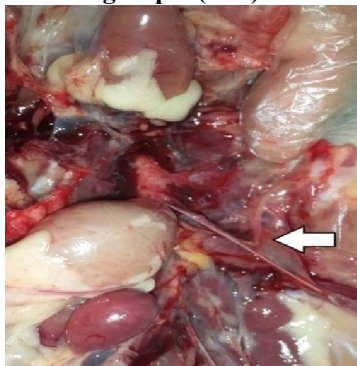


Fig III. Score 2 airsacculitis in chickens of group 3 (Arrow)



Fig IV. *E. coli* colonies on MacConkey's agar

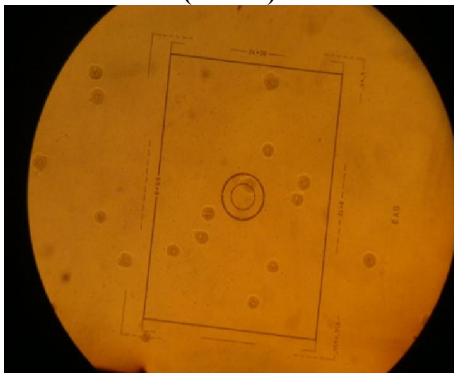


Fig V. Fried egg appeared colonies of *MG*

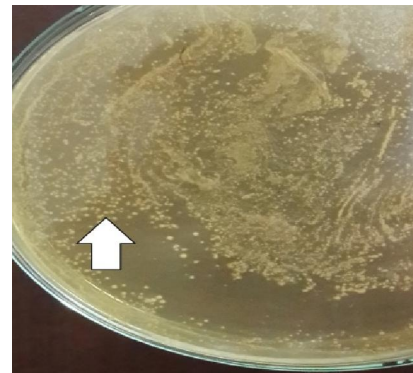


Fig VI. Typical small whitish colonies of *Lactobacillus* on Rogosa Agar

4. Conclusions

From the previously recorded data, we concluded that the use of essential volatile oils improved the weight gain; feed conversion rate, humoral immune response to NDV vaccines. It has also, antioxidant effect and such effect can protect the liver and kidney from injuries and damage associated with infection and reduced the lesions of CCRD but incompletely removed *MG* and *E.coli* infection so it is not recommended to use these products alone in controlling the bacterial infection and must be used in combination with antibiotics to get a better results in reducing the bacterial infection. Also, further studies should be done to detect the effect of these products on cell mediated immunity.

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