Effect of AROMAX® on performance, local and humoral immunity against vaccination of Newcastle disease in the low management level in broiler chicken

Faleh Thajel and Amjed H Ulaiwi

Abstract
The present study was conducted to evaluate the adding of the commercial product (AROMAX®) (Eucalyptus oil, Mint oil, L-Menthol, Thyme oil) essential oils as a protective product against respiratory diseases and respiratory problems due to low management level in the broiler during the period from December – November 2016. Also, to determine its effect on some parameters such as: local and humoral immunity, body performance and levels of liver enzymes. A total of 210 one-day- old broilers Ross 308 mixed sex were divided into seven equal groups (30 chicks each group). The results of the final body weight showed that the differences were not significant among groups. The weight gain showed that G2 (576.60 gm) was significantly higher (P<0.05) as compared with other groups. Also the results of lesion score of air sac recorded the G7 was significantly differed (P<0.05) as compared with other groups. The results of local immunity (IgA) indicated that the groups G2, G3, G4 showed significant differences at (P<0.05) compared with other groups in all periods. The result of liver enzymes (ALT and AST) showed that the means in the G3, G4 ( ALT- 4.20, 4.22) (AST- 214.4 212.6) were significantly different (P<0.05) compared with other groups. In conclusion: The AROMAX® product reduced the respiratory signs resulted from the low management, and improved the local and humoral immune response in broiler.

Keywords: AROMAX®, respiratory signs, local and humoral immunity, body performance, liver enzymes

1. Introduction
The essential oils (EOs) represent one of the important modern improvements in poultry hygiene and food production techniques and have many improvements properties like bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, medicinal and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural and food industries. The essential oils can be extracted mostly by distillation from aromatic plants. It contains many of volatile molecules like terpenes and terpenoids, phenol-derived aromatic components. Some studies discovered the role of EOs like thyme oil on body health by improving the body weight and decreasing the feed consumption, this suggested that may be due to the strong taste of thyme oil that could be unpalatable for the young chicks. The EOs act on the avian digestive system as a positive effect by restoring the balance of microflora and increase the nutrient absorption, which may chiefly be attributed to terpenoid compounds. Also the EOs act as antiviral effect as different studies on Newcastle disease (ND) in the chicks reported that the adding of Eos to the diet could reduce the clinical signs, improving the immune response as well as decreasing the mortality and severity of disease. The adding of EOs to the broilers diet can increase the levels of immunoglobulin (IgA) lead to improving the local immunity in respiratory and GIT systems. Mainly aromatic essential oils contain compounds that possess strong anti-inflammatory properties, which suppress the metabolism of inflammatory prostaglandins. The other EOs have properties like anti-inflammatory, pain-relieving, or reducing edema, as well as reducing respiratory signs, such as eucalyptus oil, mint oils and menthol oils. The antioxidant effect of EOs linked with the chemical structure due to the presence of phenolic OH groups which act as hydrogen donors to the peroxyl radicals created during the first step in lipid oxidation, also EOs has potential health benefits activities through act on liver enzymes to a protective effect on foods from lipid oxidation.
2. Materials and methods

2.1 Chickens
The experiment contains 210 one day old mixed sex chicks (Ross -308 / Turnkey source) experiment was conducted in two separated poultry field in December 2016 Kut -Iraq. All Birds were exposed to lower management level (Crowding, wide variation of ventilations, and Variations of temperature, bad humidity) and pounded by food and water (ad libitum) in all ages.

2.2 Experimental design
The experiment was divided randomly into 7 equal groups all groups were kept in separate cages as follows:

G1: Chicks (30) were given orally (0.2 ml /L) Aromax® in every day of experiment with oral route of vaccination of NDv.
G2: Chicks (30) were given a spray (2 ml /L) Aromax® twice weekly with spraying rout of vaccination of NDv.
G3: Chicks (30) were given a spray (2 ml /L) Aromax® twice weekly + give orally (0.2 ml /L) Aromax® in every day of experiment with oral route of vaccination of NDv.
G4: Chicks (30) were given a spray (2 ml /L) Aromax® twice weekly + give orally (0.2 ml /L) Aromax® in every day of the experiment with spraying rout of vaccination of NDv.
G5: Chicks (30) were given orally (0.2 ml /L) Aromax® in every day of the experiment without vaccination of NDv.
G6: Chicks (30) were given a spray (2 ml /L) Aromax® twice weekly without vaccination of NDv.
G7: Chicks (30) vaccinated orally of NDv. Only.

2.3 Sample collection
Blood samples (2.5 ml) were collected from the jugular vein randomly from 10 chicks for estimation of maternal immunity against ND at 1,15,25,35 day old by using 5 ml disposable clean syringes. Gel and clot activator tube glass were used for blood collection. Serum was separated by centrifuge (1500 / RPM for 15 minutes) then stored at -20 °C for later analysis.

2.4 ELISA Test
The procedure used in this test was performed according to the manufacturer's instructions listed in the ProFLOK ELISA Kit (Symbiotics–USA), which is a rapid serologic test for the detection of antibody in chicken serum samples.

2.5 Vaccination
All Live attenuated vaccines (1000 dose) were vaccinated on (9, 18, 27 day -old) NDv. (LaSota) the vaccine was administered to each bird of the group (1,3,7) using orally method a, and group (2,4)using spray method.

2.6 Aromax® oil treatment
(Eucalyptus oil, Mint oil, L-Menthol, and Thyme oil. Essential volatile oils) in the commercial product, under the trade name “AROMAX®” by Germany was used. The route of administration in the groups (1,3,4,5) was in the drink water (0.2 ml/Liter) every day and was given to the birds of group (2,3,4,6) a spray (2ml/L /twice weekly).

2.7 Body Weight and Weight Gain
The mean of ten samples from each group was weighed randomly for live body weight weekly for seven-week of the experiment.
Body weight gain = B.W at the end of the week - B.W at the beginning of the week.

2.8 Score lesion
Gross lesion scores of air sac was taken from 0 to 4 units ascendingly according to the degree of turbidity in the ages (15,25,35 days old) of chicken.

2.9 Detection local immunity
Detection local immunity at (25,35) days of the experiment were measured by chicken immunoglobulin-A (IgA) ELISA kit SHANGHAI YEHUA Biological Technology Co., Ltd., China.

2.10 Detection of humoral immunity
Detection (AB) The blood serums at 1,15, 25, and 35 days of age were used for the humoral immunity test. Antibody titers against Newcastle Disease Virus (NDV) were measured by ProFLOK® NDV ELISA Kit with ELISA Reader.

2.11 Liver enzymes
Five blood samples were collected randomly from each group at (35 days old) for measuring the concentration of liver enzymes Aspartate transaminase (AST) and Alanine transaminase (ALT), after serum collection and analyzed by (Automatic Biochemical Analyzer System).

2.12 Statistical analysis
All data submitted for analysis by SAS (2012). One and Two way ANOVA was performed and Least significant difference –LSD test was used to assess the differences among means [11].

3. Results and Discussion

3.1 Body weight and weight gain
The results of body weight are shown in (Table 1). The G3 and G4 showed significant difference (P<0.05) as compared with other groups. The results of weight gain (Table 2) showed the G2 was significantly different (P<0.05) compared with other groups. The results are consistent with the findings of Awaad et al. [12] who found that essential oils of eucalyptus and mint were improved the performance parameters of the body weight as compared with the control birds. Also, the results agreed with the results obtained by El-Ghosein and Al-Beitawi [13] who reported that the using of the thyme oils showed significantly increasing in the body weight and weight gain in addition to improving the feed conversion ratio. The main action of essential oil acts as a digestibility enhancer, balancing the GIT microflora and stimulate the secretion of endogenous digestive enzymes, and thus increase the body performance in poultry [14-16]. On the other hand, the results of the present study were disagreed with results reported by Rehman[17] and Toghyani[18].

3.2 Score lesion
The results of lesion score of air sacs revealed that the differences were significant (P<0.05) as the G7 showed a higher scores 2, 3, and 4 for the periods 15, 25, and 35 day respectively (Table 3). The our results agreed with the findings reported by Elbestawy [19] who observed that adding of Ventoline® contains(Eucalyptus 15%, carvacrol 10%, thymol 15% in combination with menthol 5% reducing the lesion score of air sac in infected chickens when compared with airsacculitis lesion in chickens of control groups. Also, the thyme oil has main active ingredients which lead to increase of (IgA) concentration in blood [20]. As well as the EOs could increase the IgA concentration leading to better local and intestinal immunocompetence in young chicks [7].
3.3 Detection local immunity
The results of local immunity- immunoglobulin (IgA) (Table 4) showed that the groups G2, G3, G4 recorded a higher significant difference (P<0.05) compared with other groups. These results agreed with Popovic et al., [21] who showed the addition of some EOs like thyme to broiler diets significantly increased (P<0.05) the IgA level as compared with the control group. As well as Barbour et al., [22] revealed that the addition of Thyme Vulgaris and some EOs on diets of broilers increased the IgA levels and stimulated phagocytic activity in blood.

3.4 Detection humoral immunity
The result of Antibody titer against NDv illustrated in the Table (5). The titer of the G3 differed significantly (P<0.05) compared with other groups. This result agreed with the results obtained by Barbour EK, Danker [23] who showed that the addition of eucalyptus and mint oils improved the humoral immune responses. Also, the titers of NDv were higher increased in groups that treated with mint and eucalyptus essential oil when compared with the untreated group [22]. As well as, the using of mint oil support the humoral immune response through increasing antibody production against NDv antigens [24]. While the current study disagreed with some studies that showed the using of thyme in the broiler diet and drinking water have no significant effect on anti bodies titers against NDv [25-27].

3.5 Liver enzymes
Table (6) showed the result of levels liver enzymes. The mean of the ALT enzyme was significantly high (P<0.05) in the G7 as compared with G1, G2, G3, G4. Similar trend was found in the AST as the means of the G5, G6, G7 were significantly higher (P<0.05) as compared with other groups. The results of liver enzymes agreed with the study reported that the mint extract supplement significantly decreased the levels of liver enzymes, including ALT and AST [28]. Another study found that the adding of Thyme extracts was significantly decreased (P< 0.05) the liver enzymes (ALT, AST) with concentrate of 250 mg/kg and 500 mg/kg when compared with untreated groups [29]. As well as the using of (3g) eucalyptus leaves powder significantly reduced (P< 0.05) the liver enzymes (AST and ALT) compared with the control group [30]. On the other hand, some studies confirmed that that adding of the thyme in drinking water [31], the adding of Mint oils [32] and the Eucalyptus oils [33] did not effect on liver enzyme activity like (ALT and AST)

Table 1: Effect of groups on the body weight (gm/day)

<table>
<thead>
<tr>
<th>The group</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>165.00 A</td>
<td>430.00 AB</td>
<td>842.78 B</td>
<td>1374.40 B</td>
<td>1900.00 A</td>
</tr>
<tr>
<td>G2</td>
<td>165.50 A</td>
<td>431.00 AB</td>
<td>850.00 B</td>
<td>1400.60 AB</td>
<td>1977.20 A</td>
</tr>
<tr>
<td>G3</td>
<td>171.00 A</td>
<td>462.22 A</td>
<td>937.22 A</td>
<td>1486.10 A</td>
<td>2026.70 A</td>
</tr>
<tr>
<td>G4</td>
<td>164.50 A</td>
<td>458.33 AB</td>
<td>918.89 A</td>
<td>1474.40 A</td>
<td>2002.20 A</td>
</tr>
<tr>
<td>G5</td>
<td>148.50 A</td>
<td>415.00 BC</td>
<td>836.67 B</td>
<td>1362.80 B</td>
<td>1833.30 A</td>
</tr>
<tr>
<td>G6</td>
<td>149.00 A</td>
<td>395.56 C</td>
<td>835.56 B</td>
<td>1330.60 B</td>
<td>1837.80 A</td>
</tr>
<tr>
<td>G7</td>
<td>147.00 A</td>
<td>400.00 BC</td>
<td>831.67 B</td>
<td>1329.40 B</td>
<td>1844.40 A</td>
</tr>
<tr>
<td>LSD value</td>
<td>27.86 NS</td>
<td>53.91 *</td>
<td>66.74 *</td>
<td>102.62 *</td>
<td>165.75 NS</td>
</tr>
</tbody>
</table>

Means with differences letter in the same column significantly different (P<0.05)

Table 2: Effect of groups on the gain weight (gm/day)

<table>
<thead>
<tr>
<th>The group</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>125.00 A</td>
<td>265.00 B</td>
<td>412.78 B</td>
<td>531.62 AB</td>
<td>525.60 AB</td>
</tr>
<tr>
<td>G2</td>
<td>125.50 A</td>
<td>265.50 B</td>
<td>419.00 B</td>
<td>550.60 A</td>
<td>576.60 A</td>
</tr>
<tr>
<td>G3</td>
<td>131.00 A</td>
<td>291.22 A</td>
<td>475.00 A</td>
<td>548.88 A</td>
<td>540.60 AB</td>
</tr>
<tr>
<td>G4</td>
<td>124.50 A</td>
<td>293.83 A</td>
<td>460.56 A</td>
<td>555.51 A</td>
<td>527.80 AB</td>
</tr>
<tr>
<td>G5</td>
<td>108.50 A</td>
<td>266.50 B</td>
<td>421.67 AB</td>
<td>526.13 A</td>
<td>520.50 B</td>
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<tr>
<td>G6</td>
<td>109.00 A</td>
<td>246.56 B</td>
<td>440.00 AB</td>
<td>495.04 B</td>
<td>527.20 AB</td>
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<tr>
<td>G7</td>
<td>107.00 A</td>
<td>253.00 B</td>
<td>431.67 AB</td>
<td>497.73 B</td>
<td>515.00 B</td>
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<tr>
<td>LSD value</td>
<td>30.58 NS</td>
<td>43.71 *</td>
<td>55.92 *</td>
<td>51.03 *</td>
<td>55.75 *</td>
</tr>
</tbody>
</table>

Means with differences letter in the same column significantly different (P<0.05)

Table 3: Lesion Scores

<table>
<thead>
<tr>
<th>The group</th>
<th>15 Day</th>
<th>25 Day</th>
<th>35 Day</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1 BA a</td>
<td>1 BC a</td>
<td>2 BC a</td>
<td>1.02 NS</td>
</tr>
<tr>
<td>G2</td>
<td>0 B a</td>
<td>0 C a</td>
<td>1 C a</td>
<td>1.00 NS</td>
</tr>
<tr>
<td>G3</td>
<td>0 B a</td>
<td>0 C a</td>
<td>1 C a</td>
<td>1.00 NS</td>
</tr>
<tr>
<td>G4</td>
<td>0 B a</td>
<td>0 C a</td>
<td>1 C a</td>
<td>1.00 NS</td>
</tr>
<tr>
<td>G5</td>
<td>1 AB a</td>
<td>2 AB a</td>
<td>3 AB a</td>
<td>1.25 *</td>
</tr>
<tr>
<td>G6</td>
<td>0 B a</td>
<td>0 C a</td>
<td>1 C a</td>
<td>1.00 NS</td>
</tr>
<tr>
<td>G7</td>
<td>2 A b</td>
<td>3 A b</td>
<td>4 A a</td>
<td>1.37 *</td>
</tr>
<tr>
<td>LSD value</td>
<td>1.25 *</td>
<td>1.33 *</td>
<td>1.28 *</td>
<td>---</td>
</tr>
</tbody>
</table>

Means with different small letters horizontally refer to the presence of significant (P<0.05).
Means with different capital letters vertical refer to the presence of significant value at (P<0.05).
The present study indicated that The AROMAX® product reduced the respiratory signs resulted from the low management, and improved the local and humoral immune response in broiler.

5. Acknowledgement

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6. References


