# EVAPORATION TECHNIQUE OF ESSENTIAL OILS FOR IMPROVING OF IMMUNE RESPONSE OF BROILER CHICKENS

**Waleed H. F.** **A. H. Ulaiwi**
**Researcher** **Assist. Prof.**
**College of Veterinary Medicine – University of Baghdad**
**E-mail: amjed.h@covmuobaghdad.edu.iq**

**ABSTRACT**

**This study aimed to investigate a new evaporation technique of essential oils; EOs (oregano, Lemongrass and bergamot essential oils) are in product called Fumite®Aroma. The study included 3 immunological parameters namely immunoglobulin A (IgA), interferon gamma (INF-γ), and newcastle disease (ND) antibody titer (Ab) as well as to compare between aerosol and ocular routes for ND vaccine. The study was designed as follow : {G1: is positive control given EOs(6g) only; G2: given Eos (6g) with ND vaccine by aerosol route; G3: given EOs (6g) with ND vaccine by ocular route; G4: given ND vaccine by aerosol route only; G5: given ND vaccine by ocular route only; G6: is negative control with no evaporation or vaccination}. Level of IgA at 16 days old there was a significant difference in G1 and G6 as compared with another groups. At 25 days old, G2, G3 and G5 revealed the highest levels as compared with another groups. At 34 days old, G3 was highest when compared to another groups. The results of IFN-γ showed at 16 days old showed a higher level in G3 as compared to another groups. the same in age 25 and 34. Levels of ND titer at 16 and 25 days old showed that G3 were significantly highest as compared to another groups. At age 34 days, G3 and G2 showed the highest levels as compared to another groups. In conclusion EOs as evaporation. It can increase level of IgA, INF-γ and it improve immune response against ND.**

**Key words: fumite®aroma , humoral and cellular immunity , newcastle disease antibody titer.**



## تقنية تبخير الزيوت الأساسية العطرية لتحسين الاستجابة المناعية في الدجاج اللاحم

وليد حامد فرحان امجد حسين عليوي
باحث أستاذ مساعد
كلية الطب البيطري– جامعة بغداد

**المستخلص**

**هدف البحث تقنية جديدة لتبخير الزيوت الأساسية العطرية ; EOs (زيت الأوريجانو الأساسي و وزيت الليمون العطري وزيت البرغموت العطري مجموعة بمركب يسمى Fumite®Aroma ) وشمل البحث على 3 معايير مناعية هي الغلوبولين المناعي A (IgA) و انترفيرون جاما (INF–γ) وعيار الأجسام المضادة (Ab) لمرض نيوكاسل (ND) كم تضمنت مقارنة طريقة اللقاح بالرش واللقاح عن طريق العين لل ND. كالتالي: {G1: هو السيطره الايجابي اعطيت(6g) EOs فقط ؛ G2: اعطيت Eos(6g) مع لقاح ND عن طريق الرش ؛ G3: اعطيت EOs(6g) مع لقاح ND عن طريق العين ؛ G4: اعطيت لقاح ND عن طريق الرش ؛ G5: اعطيت لقاح ND عن طريق العين فقط ؛ G6: سلبي السيطرة لم يبخر ولم يتم تحصينه} . اعطيت نتائج مستوى IgA بعمر 16 يومًا كان هناك فرق كبير بين G1 و G6 مقارنة بالمجموعات الأخرى. في عمر 25 يومًا ، كشفت G2 و G3 و G5 عن أعلى المستويات مقارنة بالمجموعات الأخرى. في عمر 34 يومًا ، كان G3 أعلى عند مقارنته بمجموعات أخرى. أظهرت نتائج IFNγ في عمر 16 يومًا G3 اعلى مستوى مقارنة بالمجموعات أخرى. نفس الشئ في عمر 25 و 34 . أظهرت مستويات عيار ND في عمر 16 و 25 يومًا أن G3 كانت أعلى بشكل ملحوظ مقارنة بالمجموعات الأخرى. في سن 34 يومًا ، أظهر G3 و G2 أعلى المستويات مقارنة بالمجموعات الأخرى. ونستنتج ان تبخير الزيوت الاساسية العطرية يزيد من مستوى IgA و ويزيد من مستويات INF–γ ويحسن الاستجابة المناعية ضد ND.**

**لكلمات المفتاحية: فومايت العطريه ، المناعة الخلطية والخلوية ، عيار الأجسام المضادة لمرض نيوكاسل**

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## INTRODUCTION

Newcastle disease virus has gained an interest of being used as anti-cancer virus in many researches concerning human (19). Feed additives has gained many interest recently in Iraq to be added in poultry feed (2). It is a major threat viral disease of poultry as it so using EOs can lessen signs and improve immunity (5). Vaccination alone is not enough, therefore EOs can act as immunomodulatory and immunostimulatory at the same time (11) The process of essential oil inhalation as vapor is a quick, practical, safe, easy and comfortable technique (16). Aromatherapy can be used as an immunomodulatory and immunostimulatory effect in some aromatic compounds (25). The mixture for feed additives proved to be reliable in broiler feed as reliable and supportive to immune system (26). Oregano (*Origanum vulgare*), has been used for a long time and a reliable source of useful plant-based medicines as phytotherapy, it is worldwide in distribution (6). Bergamot is part of The Rutaceae family includes the aromatic plant bergamot *(Citrus bergamia)* The fruit is grown in Italy, france, Ivory Coast, Mauritius, Brazil, Turkey, Morroco and several other Mediterranean nations (15). Lemongrass is related to *Cymbopogon* spp. are grown is for their essential oils. from the Poaceae family of grasses. Around 180 species of the genus lemongrass exist (24). The goal of the study is to evaluate EOs for improvement of vaccine immune response against ND in spray and eye drop method also investigation of evaporation technique of EOs on cellular and humoral immunity.

## MATERIALS AND METHODS

### Study design

A total of three hundred of one day broiler chicks for the (Ross 308 strain) one day old, were adopted. They were purchased from a licensed hatchery and divided into six equal groups housed in a separated room. The experiment lasted for 35 days as follows:

**G1**: Fifty chicks( Control+) exposure to evaporation of EOs at the recommended dose at 9,18 and 27 days only.

**G2:** Fifty chicks exposure to evaporation of EOs at 9,18 and 27 days before 1hr of (NDV)vaccination (spray method).

**G3:** Fifty chicks exposure to evaporation of essential oils at 9,18 and 27 days old before 1hr of (NDV)vaccination (eye drop method).

**G4:** Fifty chicks vaccination of (NDV) (spray method) at 9,18 and 27 days old only.

**G5:** Fifty chicks vaccination of (NDV) (eye drop method) at 9,18 and 27 days old only.

**G6:** Fifty chicks (control –ve) no evaporation and no vaccinations.

### Experimental location

This experiment was carried out at poultry Farm in Veterinary Medicine College in Al-Fallujah, the rooms was 2×3×3 for each separated group. It was exactly 35 days in the interval of 12 of December up to 14 of January 2021). The dose was 6g for each group according to company recommendation, 1hr before vaccination

### Vaccination

Live attenuated vaccine were given in (9, 18, 27 day -old) (ND B1 BIO-VAC B1®,ND Clone 30 CLONE®, ND LaSota AVI ND LaSota® respectively.

### Serum sampling

At the age of 3 day old 10 birds were sacrificed to measure the material immunity in order to decide the vaccination program, Blood sampling of this experiment was collected at age of 16, 25 and 34 days of age for INF-γ ,IgA and polyclonal antibody for ND (4). ELISA was used to detect the parameters.

### evaporation Technique of essential oil

The process of evaporation was done on the basis of company instruction which is based on the reaction between a special igniter and the powder of essential oils to produce an evaporation that can be inhaled from chickens for about half an hour fans and lights should be turn off, the doors should be closed.

### Statistical analysis

The Statistical analysis system software has been done to determine the influence of variables on the study parameters. Significant comparisons among means were performed using the LSD test (22). (Analysis of Variance-ANOVA).

## RESULTS AND DISCUSSION

The results of immunoglobulin A revealed that there was no significant difference among G2, G3, G4 and G5 at 16 days old. On the other hand, G1 and G6 exhibited lower (P≤0.01) levels (31.538 and 30.407 respectively) when compared to the results of other groups as in Table (1). At 25 days old, G2, G3 and G5 revealed the highest (P≤0.01) levels followed by G4 (116.471). Additionally, G1 (43.145) was higher than G6 (10.145). At 34 days old, the G3 (250.835) was the highest (P≤0.01) followed by G2 (223.717) and G5 (224.886), and then followed by G4 (196.992). Moreover, G1 (2.615) was higher than G6 (42.971). While When comparing the results of the same group at 16, 25 and 34 days of age there was no significant difference when comparing the results of IgA in G1. On the other hand, there was an increase when compared to those of G2, G3, G4 and G5 at 16 days old with results of the same groups at 25 days old. Results of these groups at 34 days old, showed increase (P≤0.01) when compared with those at 25 days old. Results of G6 showed decrease between the levels at 16 days old and those at 25 days old and 34 days old. These results agreed with

### Results of immunoglobulin A

Ruan *et al*. (21) who proved that dietary supplementation of oregano essential oil plays an important role in immune-stimulation on humoral immunity (IgA) in chicken. Du *et al*. (9) proved that the increase in serum IgA is related to the active ingredients of the EOs of which is carvacrol and thymol these are present in oregano with other minor ingredients. On other hands Alagawany *et al*. (3) revealed that lemongrass essential oil caused a considerable increase in immunoglobulin levels, the elevation in serum immunoglobulin (IgA) in broiler chicken. Moreover Zeng *et al*. (27) returned this increase to the increased B-cell proliferation which in term increase their levels in serum. Mallick *et al*. (17) explained the difference between aerosol (G4) and ocular vaccination(G5) that the presence of plasma cells in the interstitial regions of Harderian gland which increase in numbers in older birds. Also the lachrymal gland which is small size has plasmacytic infiltration, but many IgA-producing cells were discovered in which is absent in respiratory system in aerosol.

**Table.1 Effects of group and days in titers of chicken immunoglobulin A in different ages**

| | Mean ± SE of IgA (µg/ml) | | | LSD value |
|---|---|---|---|---|
| Groups | 16 Days Old | 25 Days Old | 34 Days Old | |
| G1 | 31.538 | 43.145 | 42.971 | 12.56 NS |
| | B a | C a | D a | |
| G2 | 80.262 | 146.612 | 223.717 | 17.05 ** |
| | A c | A b | B a | |
| G3 | 89.942 | 161.565 | 250.835 | 17.47 ** |
| | A c | A b | A a | |
| G4 | 77.809 | 116.471 | 196.992 | 16.96 ** |
| | A c | B b | C a | |
| G5 | 84.742 | 155.942 | 224.886 | 21.04 ** |
| | A c | A b | B a | |
| G6 | 30.407 | 10.145 | 2.615 | 15.82 ** |
| | B a | D b | E b | |
| LSD value | 15.28 ** | 19.03 ** | 24.66 ** | --- |

**Means with different capital letters in the same column and small letters in the same row are significantly different. * (P≤0.05), ** (P≤0.01). G1: Control positive (Evaporation of Essential oil only). G2: Evaporation of Essential oil with aerosol vaccination. G3: Evaporation of Essential oil with ocular vaccination. G4: Aerosol vaccination only. G5: Ocular vaccination only. G6: Control negative.**

### Results of interferon gamma

The results of interferon gamma showed that G3 (219.407) were the highest (P≤0.01) followed by G2 (199.098) and then followed by G4 and G5 and then G1 and G6 at 16 days old as in Table (2). At 25 days old, levels in G3 (257.847) were the highest (P≤0.01) followed by G2 (199.654). The levels in G5 (191.978) was similar to the levels in G2. Results in G4 (174.942) was also close to the

levels in G5, but lower than G2 and higher (P≤0.01) than the levels in G1 and. At 34 days old, levels of G3 were the highest (P≤0.01) followed by G5. The levels of G2 was similar to those of G5. Results of G4 was also close to the levels of G2. When comparing the results of the same group at different age at sampling, G1, G2 and G6 showed no difference. G3 showed lower value at 16 days old compared to day 25 and 34, G4 and G5 increase gradually among times G4 has significant difference (P≤0.05). in G5 have highly significant difference (P≤0.01). The results agreed with Seyedabadi *et al*. (23) who revealed that feeding broiler chicken with oregano essential oil could increase expression of IFN-γ and it is dose dependent. El-Sheikh *et al*. (10) revealed that active ingredients of oregano essential oil (carvacol and thymol) could do up-regulation of gene expression of IFN-γ in chicken by increasing systemic lymphocyte. additionally Han *et al*. (14) concluded providing bergamot essential oil can enhance immunomdulatory effect on which can increase IFN-γ -induced protein 10 which is produced as a result of IFN-γ. Additionally, Ghanima *et al*. (13) concluded that lemongrass essential oil can increase cellular immunity and phagocytic activity and different serum parameters for broiler chickens reared in different stocking density. Furthermore, Rauw *et al*. (20) showed the difference between aerosol vaccination and ocular vaccination related to the route of vaccination in which ocular route have the head-associated lymphoid tissue (Harderian gland) and also going down to the respiratory system via naso-lacrimal duct can act both cellular and humoral immunity.

**Table 2. Effects of group and days in titers of chicken interferon gamma in different ages**

| | Mean ± SE of IFNγ (ng/L) | | | LSD value |
|---|---|---|---|---|
| **Groups** | **16 Days Old** | **25 Days Old** | **34 Days Old** | |
| **G1** | **127.489** | **128.463** | **122.548** | **14.37 NS** |
| | **D a** | **D a** | **D a** | |
| **G2** | **199.098** | **199.654** | **201.483** | **12.58 NS** |
| | **B a** | **B a** | **BC a** | |
| **G3** | **219.407** | **257.847** | **266.532** | **24.06 **** |
| | **A b** | **A a** | **A a** | |
| **G4** | **159.213** | **174.942** | **185.820** | **22.46 *** |
| | **C b** | **C ab** | **C a** | |
| **G5** | **173.988** | **191.978** | **211.792** | **24.93 **** |
| | **C b** | **BC ab** | **B a** | |
| **G6** | **115.866** | **117.311** | **121.801** | **16.48 NS** |
| | **D a** | **D a** | **D a** | |
| **LSD value** | **18.94 **** | **23.04 **** | **20.17 **** | **---** |

**Means with different capital letters in the same column and small letters in the same row are significantly different. * (P≤0.05), ** (P≤0.01) G1: Control positive (Evaporation of Essential oil only). G2: Evaporation of Essential oil with aerosol vaccination. G3: Evaporation of Essential oil with ocular vaccination. G4: Aerosol vaccination only. G5: Ocular vaccination only. G6: Control negative.**

## Results of Newcastle disease titer

The results of Newcastle disease titer showed that levels of G3 (1881) were the highest (P≤0.01) followed by G2, G5, G1 and G6 of 16 days old as seen in Table (3). At 25 days old, levels of G3 were the highest (P≤0.01) followed by G2, G4 and G5. There was no significant difference when comparing levels in G2, G4 and G5. Results of G1 were higher (P≤0.01) than levels in G6. At 34 days old, higher (P≤0.01) levels in G2) and G3 followed by G5 and then G4. The results in different ages in the same groups levels in (G2, G3, G4 and G5) at 34 days old were higher (P≤0.01) than those at 16 days old , in contrary, levels of G1 at 16 days old were higher (P≤0.05) than levels at 34 days old. levels of G6 similar to G1. These results agreed with Mohiti-Asli and Ghanaatparast-Rashti (18). who found there is an increase in Abs titer (serum IgY) generally mononuclear phagocyte system, cellular, and humoral immunity with the adding of oregano essential oil to the diet of broilers showed significant increase compared to control group and also EOs mixture**.** Bolukbasi *et al*. (7) revealed that adding of bergamot essential oil

in diet result in increase serum IgG in broiler chicken. similarly Abdulkadhim *et al*. (1) proved that the adding of oregano essential oil to broiler diets stimulated humoral immunity, cell mediated immunity and mononuclear phagocyte proliferation as thymol and carvacrol are added to the feed. Earlier study (5). has shown that broilers may exhibit possible humoral immunostimulant activity with thymol and carvacol by having higher IgG titers against ND, and other immune system markers. Galal *et al*. (11) expressed another explanation the INF-α induce antiviral property which leads to production of interferon regulatory factor 7, and virus restriction factors, such as myxovirus1 gene , in this research oregano essential oil act as immunomodulatory to enhance INF-α which increase Abs titer against ND. Furthermore, thymol and carvacol can enhance systemic lymphocyte activity thus increase immunity against ND (12). The comparison between routes the highest response (93%) comes through ocular administration, whereas only 53-60% of birds may develop protective antibodies in response to vaccination administrated by water or spray , it is clear in G3 was significantly higher than G2 (8).

**Table 3. Effect of group and days in titers Newcastle disease in different ages**

| | Mean ± SE of ND | | | LSD value |
|---|---|---|---|---|
| **Group** | **16 Days Old** | **25 Days Old** | **34 Days Old** | |
| **G1** | **881** | **471** | **239** | **281.52 **** |
| | **C a** | **C b** | **D b** | |
| **G2** | **1447** | **3129** | **4692** | **355.08 **** |
| | **B c** | **B b** | **A a** | |
| **G3** | **1881** | **3868** | **5016** | **346.13 **** |
| | **A c** | **A b** | **A a** | |
| **G4** | **1066** | **2756** | **3036** | **296.62 **** |
| | **C b** | **B a** | **C a** | |
| **G5** | **1116** | **2915** | **3868** | **306.59 **** |
| | **BC c** | **B b** | **Ba** | |
| **G6** | **139** | **47** | **0** | **102.77 *** |
| | **D a** | **D ab** | **D b** | |
| **LSD value** | **339.66 **** | **391.03 **** | **398.83 **** | **---** |

**Means with different capital letters in the same column and small letters in the same row are significantly different. * (P≤0.05), ** (P≤0.01). G1: Control positive (Evaporation of Essential oil only). G2: Evaporation of Essential oil with aerosol vaccination. G3: Evaporation of Essential oil with ocular vaccination. G4: Aerosol vaccination only. G5: Ocular vaccination only. G6: Control negative.**

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