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The effect of anise and mint as essential oils supplementtion on performance and leukogram in broiler chickens

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Abstract
This study was conducted to investigate the effect of essential oils (Anise, mint) as feed additives on performance and leukogram of vaccinated and non- vaccinated broiler chicks. A total of one hundred and sixty, day-old chicks were distributed into 8 equal groups (A-H).Control groups (A and B), anise oil as 400mg /kg diet (C and D), mint oil as 300 mg /Kg diet (E and F), mixture of anise and mint oil with the same previous dose (G and H). At 12 day of age half of groups (B, D, F and H) were vaccinated against Newcastle disease (ND) with IB+ND vaccine as eye drop. The statistical findings of this experiment indicated that a significant increase in the body weight, body weight gain, and improvement in FCR in the groups treated with Anise oil + mint oil then in groups treated with anise oils and mint oils. Concerning leukogram results there was increase in TLC with characteristic heterophilia, lymphocytosis, and monocytosis in vaccinated and non- vaccinated broiler chicks at 3 and 6 weeks of age. The study concluded that combination of anise oil and mint oil has growth promoting effect in broilers.

Keywords: essential oils, Anise oil, Mint oil, broilers

Introduction
Poultry industry is the fastest growing sources of meat in the world. The modern production system can produce market ready broiler chicks in less than 6 week (Perez-Carbajal *et al*., 2010) [29]. Antibiotic growth promoters have been successfully used at sub therapeutic doses in poultry production to promote growth and protect health of the birds (Engberg *et al*., 2000) [13]. Recently, published data indicated that unsystematic use of antibiotics increases several strains resistance to antibiotics used for human health and enhances transferring the resistance to other bacteria (Moser *et al*., 2003) [25]. Aromatic plants and herbal products have been used worldwide as natural additives for medicinal purposes because they have been accepted by consumers as natural additives (Nanekarani *et al*., 2012) [26]. Among the alternatives, essential oils have a great potential. The essential oils are generally considered natural, less toxic, and free from residues when compared with antibiotics (Gong *et al*., 2014) [20]. Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin (Franz and Novak, 2010) [18]. Anise (*Pimpinella anisum L*.) is an aromatic annual herb widely grown in the Mediterranean region. The part used is the fruit or seeds. Anise seeds have a number of active compounds, particularly volatile oil (1- 4%), which consists of largely trans-anethol (70-90%) with estragole (methyl chavicol), anisealdehyde, bcaryophlline, anise ketone (methyloxy phenylacetone) and the polymers of anethole (Ciftci *et al*., 2005) [8]. Peppermint (*Mentha piperita*) or mint which is a member of the Labiatae family. It is widely used in herbal medicine and believed to be particularly beneficial in building of the immune system and fighting secondary infections (Nanekarani *et al*., 2012) [26]. This study aimed to investigate the effect of anise oil, mint oil and their combination on performance and leukogram of vaccinated and non-vaccinated broiler chickens.

Materials and methods
1. Experimental chicks
One hundred and sixty, one day old, Indian River chicks with an average body weight 45-50 grams were obtained from Ismailia/ Misr Poultry Company. Chicks were randomly assigned to eight treatment groups each of 20 birds and reared for 6 weeks, the end of experimental period.Feed and water were provided adlibitum**.** The diet was formulated to meet the nutritional requirements as suggested by the (NRC 1994) [27]. Essential oils was dissolved in vegetable oil and then gently mixed with the standard diets.

2. Vaccination
Chickens of all groups were vaccinated with live vaccine (IB+ND) by spray route at hatchner. Birds of groups B, D, F and H were vaccinated against Newcastle disease (ND) with IB+ND vaccine vaccine at 12 day of age as eye drop.

3 Anise oil
Anise oil obtained from Jiangxi Yisenyuan Plant Spices Co., Ltd (China) with more than 85% anethole.

4. Mint oil
Mint oil obtained from Jiangxi Yisenyuan Plant Spices Co., Ltd (China) with more than 50% menthol.

5. Experimental design
One hundred and sixty, one day old, apparent healthy chicks

were classified into 8 equal groups; each group was fed for 6 weeks. Group (A): Control without any treatment and non-vaccinated. Group (B): Vaccinated and not received any treatment. Group (C) were received anise oil as 400mg / Kg diet throughout the experiment and non-vaccinated. Groups (D) were received anise oil with previous dose and vaccinated. Group (E) were received mint oil as 300mg /Kg diet throughout the experiment and non-vaccinated. Group (F) were Received mint oil with previous dose and vaccinated. Group (G) were received anise oil and mint oil as 400mg/Kg diet, 300mg/Kg diet respectively and throughout the experiment and non-vaccinated. Group (H) was received anise oil and mint oil with previous dose and vaccinated.

6. Growth Performance parameters

The chicks were weighed individually at the end of each week and feed consumption was recorded weekly. Four hours prior to bird weighing, the diets were removed and feed consumption was determined. Feed conversion ratio (FCR) was calculated weekly (Brady, 1968) [5].

7. Sampling

Five birds from each group were randomly selected at 3 and 6 weeks, blood samples were collected from wing vein from each bird on sodium salt of EDTA as anticoagulant and used for leukogram studies.

8. Leukogram studies

Leukocytic counts were performed using an improved Neubaur Hemocytometer and Natt&Herick solution. Total white blood cells and differential leukocyte count were calculated according to standard technique described by (Jain, 1986) [23]. For differential leukocytic count, blood films were made on clean slides, dried on air, fixed with absolute methyl alcohol and stained with Giemsa stain, the percentage and absolute value for each type of white cells were calculated according to (Feldman *et al*., 2000) [16].

9. Statistical analysis

Data collected from treated groups of chicks were statically analyzed in compare to control group for the mean and standard error using SPSS 16 (Coakes *et al*., 2009) [9]. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests according to Snedecor and William (1989) [33].

Results and Discussion

Medicinal plants can be used as natural growth promoters due to their antimicrobial and nutrient digestion improving properties (Hernandez *et al*., 2004) [21].

In the present study, anise and mint oils as a natural alternative growth promoter to antibiotics, significantly increase ($P<0.05$) final body weight and total body weight gain in chickens than control non-supplemented. Moreover, it significantly decreases ($P<0.05$) total FCR than control. The improvement of weight gain and FCR could be attributed due to the positive role of these medicinal plants on keeping a balanced microbial ecosystem in the digestive tract and stimulating digestive enzymes secretions (Ramakrisna *et al*., 2003) [31], which directly increased nutrient digestion and improved body weight gain. The favorable effects of adding anise oil and peppermint oil mixture to the basal diet could be attributed to the active and valuable components of such plants particularly essential oils. Cabuk *et al*., (2003) [6] mentioned that, the increase in live BW, BWG and the improvement in FCR may be due to the different active ingredients, particularly anethole and eugenol in anise which have digestive stimulating effects particularly protein, fats and cellulose digestion. In the same context, Giannenas *et al*. (2003) [19] and Ciftci *et al*. (2005) [8] reported that *Pimpinella anisum* oil significantly improves daily live BWG and FCR of male broilers. However, it also seems that, the improvement effect of *Pimpinella anisum* seeds might be due to the improvement of apparent whole tract and ileal digestibility of the nutrients (Hernandez *et al*., 2004) [21] and increases the effect of pancreatic lipase and amylase (Ramakrisna *et al*., 2003) [31].

Bioactive constituents, menthol, anethol and eugenol are currently being researched for their effects in broiler chickens. All of these components have been shown to promote growth performance (Erhan *et al*., 2012) [14]; (Fallah *et al*., 2013) [15] and (Hong *et al*., 2012) [22] and nutrient digestibility (Amad *et al*., 2011) [2] and (Emami *et al*., 2012) [12]. These results agree with Ciftci *et al*. (2005) [8] and Charal (2014) [7] who stated that, addition of 400 mg anise oil/kg diet improved ADG by approximately 6.5% and FCR by approximately 6% in broilers. This is likely attributed to different concentrations of the active substances and the methods used to isolate the effective compounds. The efficacy of star anise and its extracts also could be affected by other factors such as the diet type, animal age, hygiene, environmental factors, and so on (Amad *et al*., 2011) [2]. Similar findings have been reported by El Deek *et al*. (2003) [11] who investigated that, the higher body weight was found in birds fed with aniseed. Similarly, Simsek *et al*. (2007) [32] stated that, the improved body weight in the diet supplemented with 400 ppm of anise oil could be due to positive effects of anise oil on digestive system.

The positive effect of peppermint on improving growth performance caused by its role in strengthening the digestive system, improving feed efficiency and decreasing the gastrointestinal disorders. The active compounds such as essential oil that existence in the peppermint were stimulate appetite and improve the digestion and mineral absorption and increase feed efficiency in broilers (Asadi *et al*., 2017) [3]. Moreover, the antiseptic property of peppermint prevents harmful bacterial growth in the digestive system that, led to better digestion and absorption. The antiseptic property of peppermint results from the presence of menthol. It seems that, the presence of active compounds such as essential oil in the plant stimulate appetite and improve the digestion and mineral absorption and increase feed efficiency in broilers. Peppermint has also been shown to cure indigestion and gastroparesis and indigestion in humans (Ocak *et al*., 2008) [28].

In contrast, Botsoglou *et al*. (2004) [4] reported that, the supplementation of essential oils to a diet had no beneficial effect on body weight. Similar result was observed by Jamroz *et al*. (2005) [24] which noted that, a plant extract included in a broiler diet did not improve the body weight.

Avian leukocytes act as the first line of defense against invading microorganism (Powell, 1987) [30], the results of leukogram study showed leukocytosis in anise oil + mint oil treated group with vaccination followed by anise oil treated group with vaccination followed by other treated groups in

comparing with control groups at 3rd and 6th weeks of age with characteristic heterophilia, lymphocytosis, and monocytosis.

The WBCs known to be useful guide to the severity of disease fight infection and defend the body, it is well documented that, lymphocytes and monocytes perform a specific function against viral infections and several diseases (Fischbach and Dunning, 2004) [17]. The favorable effect obtained in total WBCs or in differential counts (hetrerophils, lymphocytes and monocytes) might be attributed to the main components of such medicinal plants, particularly essential oils.

This result agreed with Al-Beitawi *et al*. (2010) [1] who stated that, The increase in total WBCs and heterophils counts of vaccinated or non-vaccinated male broilers fed basal diet supplemented by 2.0% crushed Pimpinella anisum, Nigella sativa seeds and Thymus vulgaris mixture noticed at 21 days of age may be due to the active components of these medicinal plants.

The results of these study regarding increases in lymphocyte cell number are in accordance with previous reports (Eisa and El-Hamied, 2003) [10]; (Ziaran *et al*., 2005) [35] and (Kong *et al*., 2006). Similarly, Soltan *et al*. (2008) [34] reported that, anise 0.25, 0.5, 0.75 and 1 gm/kg diet significantly improved lymphocyte compared to control birds. Contrarily, Al-Beitawi *et al*., (2010) [1] found that, vaccinated male broiler chicks fed herbal mixture including anise had significantly lowered lymphocyte at 42th day compared to vaccinated non-supplemented birds.

Table 1: Effect of anise oil and mint oil on live body weight (g) of chickens with and without vaccination

Groups	1st WK	2nd WK	3rd WK	4th WK	5th WK	6th WK
A (Control without vaccine)	134.44 ±1.76^{d}	387.78 ± 8.04^{d}	716.11 ±8.81^{d}	1055.60±14.94^{d}	1504.40±10.91^{d}	1770.60 ±7.84^{d}
B (Control+ vaccine)	135.00 ±2.89^{d}	399.44 ± 3.27^{d}	731.67 ±4.56^{d}	1067. 80± 9.39^{d}	1512.80±11.82^{d}	1765.00 ±8.66^{d}
C (Anise oil without vaccine)	144.44 ±2.11^{bc}	432.22 ±6.83^{bc}	791.11 ±7.90^{c}	1208.90±14.69^{c}	1686.10±8.45^{c}	2050.60 ±13.03^{c}
D (Anise oil+ vaccine)	145.56 ±1.55^{bc}	431.1 ±7.30^{bc}	821.11 ±8.81^{b}	1315.00 ±15.72^{ab}	1804.40±10.19^{a}	2270.00 ±12.91^{a}
E (Mint oil without vaccine)	142.78±2.06^{c}	425.56 ±4.89^{c}	786.11 ±6.05^{c}	1176.70 ±13.54^{c}	1675.00±11.93^{c}	2021.70±7.86^{c}
F (Mint oil + vaccine)	141.67 ±2.20^{c}	427.22 ±8.54^{c}	806.11 ±8.15^{bc}	1280.00±15.32^{b}	1767.20±13.00^{b}	2106.10 ±19.77^{b}
G (Anise oil+ Mint oil without vaccine)	149.44 ±1.00^{ab}	448.33 ±3.91^{ab}	851.11 ±4.91^{a}	1343.30±15.57^{a}	1835.00±10.61^{a}	2295.00±6.61^{a}
H (Anise oil+ Mint oil+ vaccine)	152.22 ±1.21^{a}	454.44 ±4.75^{a}	861.11 ±9.71^{a}	1323.30±11.93^{a}	1815.60±9.88^{a}	2278.90±6.55^{a}

Values are expressed as mean (Mean± SE). Means with the same letter in the same column are non-significant at P<0.05.

Table 2: Effect of anise oil and mint oil on mean weight gain (g/bird) of chickens with and without vaccination

Age Groups	1st WK	2nd WK	3rd WK	4th WK	5th WK	6th WK
A (Control without vaccine)	88.33±1.56^{d}	253.33± 7.07^{b}	328.33±8.29^{d}	339.44±14.40^{c}	448.89±8.69^{b}	266.11±9.35^{c}
B (Control+ vaccine)	88.11±2.68^{d}	264.44± 4.52^{b}	332.22±2.51^{d}	336.11± 11.45^{c}	445.00±9.32^{b}	252.22 ±11.09^{c}
C (Anise oil without vaccine)	96.78±2.19^{bc}	287.78±7.32^{a}	358.89±9.16^{c}	417.78±11.00^{b}	509.44±16.40^{a}	311.11±11.23^{b}
D (Anise oil+ vaccine)	98.78±1.48^{bc}	285.56 ±8.39^{a}	390.00 ±7.95^{ab}	493.89 ±14.55^{a}	489.44±14.44^{a}	465.56 ±12.17^{a}
E (Mint oil without vaccine)	95.33±2.23^{c}	282.22 ±5.78^{a}	360.56±4.82^{c}	390.56 ±11.94^{b}	498.33±11.27^{a}	335.56±8.68^{b}
F (Mint oil + vaccine)	94.22 ±2.40^{c}	285.56±8.39^{a}	378.89±9.57^{bc}	473.89±12.98^{a}	487.22±12.28^{a}	338.89 ±12.49^{b}
G (Anise oil+ Mint oil without vaccine)	101.89 ±1.47^{ab}	298.89±3.89^{a}	402.78±6.72^{a}	492.22±13.77^{a}	491.67±14.33^{a}	460.00±13.23^{a}
H (Anise oil+ Mint oil+ vaccine)	105.00±0.93^{a}	302.22 ±4.94^{a}	406.67 ±9.79^{a}	462.22±7.73^{a}	492.22±14.32^{a}	463.33±9.13^{a}

Values are expressed as mean (Mean± SE). Means with the same letter in the same column are non-significant at P<0.05

Table 3: Effect of anise oil and mint oil on feed intake (g/bird) of chickens with and without vaccination

Groups	1st WK	2nd WK	3rd WK	4th WK	5th WK	6th WK
A (Control without vaccine)	97.17±0.45^{a}	370.99± 1.43^{a}	440.76±2.84^{d}	818.49±2.42^{c}	978.28±6.55^{a}	980.24±7.64^{a}
B (Control+ vaccine)	95.42±0.59^{a}	375.75± 1.43^{a}	450.80±6.94^{bc}	822.31± 2.16^{c}	966.75±4.37^{a}	953.58 ±7.25^{b}
C (Anise oil without vaccine)	82.59±0.71^{d}	275.25±3.31^{d}	445.09±3.26^{d}	853.23±3.36^{b}	866.40±4.87^{b}	876.67±7.26^{d}
D (Anise oil+ vaccine)	82.84±0.74^{d}	349.09 ±2.21^{b}	454.03±2.99^{bc}	843.95 ±3.52^{b}	864.76±2.11^{b}	932.01 ±9.83^{c}
E (Mint oil without vaccine)	91.60±0.34^{bc}	266.46 ±5.47^{d}	464.55±4.67^{b}	785.50 ±3.04^{d}	817.31±7.26^{c}	928.55±7.46^{c}
F (Mint oil + vaccine)	92.34±0.40^{b}	345.58±2.86^{b}	485.12±6.49^{a}	785.62±5.21^{d}	806.64±3.51^{c}	870.28±5.77^{d}
G (Anise oil+ Mint oil without vaccine)	90.04±0.72^{c}	272.49±2.18^{d}	482.20±3.50^{a}	919.07±2.23^{a}	865.53±3.07^{b}	885.30±4.32^{d}
H (Anise oil+ Mint oil+ vaccine)	92.61±0.90^{b}	286.07 ±3.04^{c}	480.49±5.99^{a}	915.69±3.81^{a}	870.66±5.30^{b}	920.41±5.76^{c}

Values are expressed as mean (Mean± SE). Means with the same letter in the same column are non-significant at P<0.05

Table 4: Effect of anise oil and mint oil on FCR ((g food /g gain) of chickens with and without vaccination

Groups	1st WK	2nd WK	3rd WK	4th WK	5th WK	6th WK
A (Control without vaccine)	1.10±0.01^{a}	1.46± 0.01^{a}	1.34±0.02^{a}	2.41±0.03^{a}	2.18±0.03^{a}	3.69±0.05^{a}
B (Control+ vaccine)	1.08±0.01^{a}	1.42± 0.01^{a}	1.36±0.02^{a}	2.45± 0.03^{a}	2.17±0.01^{a}	3.80±0.08^{a}
C (Anise oil without vaccine)	0.85±0.01^{cd}	0.96±0.02^{c}	1.24±0.02^{bc}	2.04±0.02^{b}	1.63±0.01^{c}	2.82±0.04^{b}
D (Anise oil+ vaccine)	0.84±0.01^{d}	1.22±0.02^{b}	1.16±0.01^{d}	1.71±0.03^{d}	1.77±0.02^{b}	2.00±0.01^{d}
E (Mint oil without vaccine)	0.96±0.01^{b}	0.94±0.02^{c}	1.29±0.01^{b}	2.01±0.01^{b}	1.60±0.01^{c}	2.77±0.01^{b}
F (Mint oil + vaccine)	0.98±0.01^{b}	1.21±0.02^{b}	1.28±0.01^{b}	1.66±0.03^{d}	1.66±0.01^{c}	2.57±0.05^{c}
G (Anise oil+ Mint oil without vaccine)	0.88±0.01^{c}	0.91±0.01^{c}	1.20±0.01^{cd}	1.87±0.01^{c}	1.76±0.01^{b}	1.93±0.03^{d}
H (Anise oil+ Mint oil+ vaccine)	0.88±0.01^{c}	0.95±0.02^{c}	1.18±0.03^{d}	1.98±0.01^{b}	1.78±0.03^{b}	1.99±0.01^{d}

Values are expressed as mean (Mean± SE). Means with the same letter in the same column are non-significant at P<0.05

Table 5: Effect of anise oil and mint oil on leukogram parameters at 3weeks age of chickens with and without vaccination

Group	TLC x10^3/µl	Heterophils x10^3/µl	Lymphocyte x10^3/µl	Monocyte x10^3/µl	Eosinophils x10^3/µl	Basophils x10^3/µl
A(Control without vaccine)	23.60±1.72^{d}	11.58±1.28^{d}	9.54±0.55 ^{c}	1.77±0.36^{d}	1.30±0.41 ^{a}	0.00±0.00^{a}
B(Control+ vaccine)	24.60±1.72^{d}	11.90±1.18^{cd}	9.99±0.65^{c}	1.63±0.05^{d}	1.02±0.05 ^{a}	0.05±0.05^{a}
C(Anise oil without vaccine)	40.40±2.13^{bc}	20.27±2.21^{ab}	15.42±2.08^{ab}	2.68±0.45^{bcd}	2.03±0.19 ^{a}	0.00±0.00^{a}
D(Anise oil+ vaccine)	42.00±2.28^{ab}	20.31±1.35^{ab}	15.25±1.36^{ab}	3.25±0.43^{ab}	2.32±0.46 ^{a}	0.09±0.09^{a}
E (Mint oil without vaccine)	33.60±2.79^{c}	16.90±1.98^{bc}	13.05±1.37^{bc}	2.13±0.29^{cd}	1.39±0.45 ^{a}	0.12±0.08^{a}
F (Mint oil + vaccine)	37.60±3.65^{bc}	17.26±1.88^{ab}	15.48±2.07 ^{ab}	2.93±0.45^{ab}	1.93±0.61 ^{a}	0.00±0.00^{a}
G (Anise oil+ Mint oil without vaccine)	35.20±2.15^{bc}	16.70±1.40^{bc}	13.38±0.70 ^{bc}	2.74±0.17^{bcd}	2.39±0.59 ^{a}	0.00±0.00^{a}
H (Anise oil+ Mint oil+ vaccine)	48.00±1.41^{a}	22.36±1.82^{a}	18.70±0.89^{a}	4.67±0.38^{a}	2.28±0.48 ^{a}	0.10±0.10^{a}

Values are expressed as mean (Mean± SE). Means with the same letter in the same column are non-significant at P<0.05

Table 6: Effect of anise oil and mint oil on leukogram parameters at 6 weeks age of chickens with and without vaccination

Group	TLC x10^3/µl	Heterophils x10^3/µl	Lymphocyte x10^3/µl	Monocyte x10^3/µl	Eosinophils x10^3/µl	Basophils x10^3/µl
A (Control without vaccine)	18.00±.089^{c}	8.90±0.58^{b}	6.88±0.40 ^{c}	1.10±0.11^{a}	1.12±0.22 ^{a}	0.00±0.00^{a}
B(Control+ vaccine)	20.00±2.45^{c}	9.36±1.11^{b}	8.21±1.13^{bc}	1.26±0.09^{a}	1.11±0.28 ^{a}	0.05±0.05^{a}
C(Anise oil without vaccine)	25.40±0.75^{b}	12.72±0.77^{a}	9.38±0.72^{ab}	1.38±0.48^{a}	1.87±0.24 ^{a}	0.05±0.05^{a}
D(Anise oil+ vaccine)	26.20±1.62^{b}	12.09±0.91^{a}	9.60±0.75^{ab}	2.51±0.35^{a}	2.09±0.30 ^{a}	0.10±0.06^{a}
E(Mint oil without vaccine)	25.60±1.33^{b}	12.68±0.89^{a}	9.93±0.22^{ab}	1.81±0.43^{a}	1.18±0.31 ^{a}	0.00±0.00^{a}
F(Mint oil + vaccine)	27.20±6.10^{ab}	13.36±1.17^{a}	9.53±1.38 ^{ab}	2.38±0.44^{a}	1.89±0.23 ^{a}	0.11±0.07^{a}
G(Anise oil+ Mint oil without vaccine)	27.60±1.72^{ab}	13.28±1.02^{a}	9.90±0.85 ^{ab}	2.45±0.31^{a}	1.97±0.38 ^{a}	0.00±0.00^{a}
H(Anise oil+ Mint oil+ vaccine)	31.80±1.28^{a}	14.83±0.82^{a}	11.77±0.40^{a}	2.55±0.82^{a}	1.96±0.50 ^{a}	0.07±0.07^{a}

Values are expressed as mean (Mean± SE). Means with the same letter in the same column are non-significant at P<0.05

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