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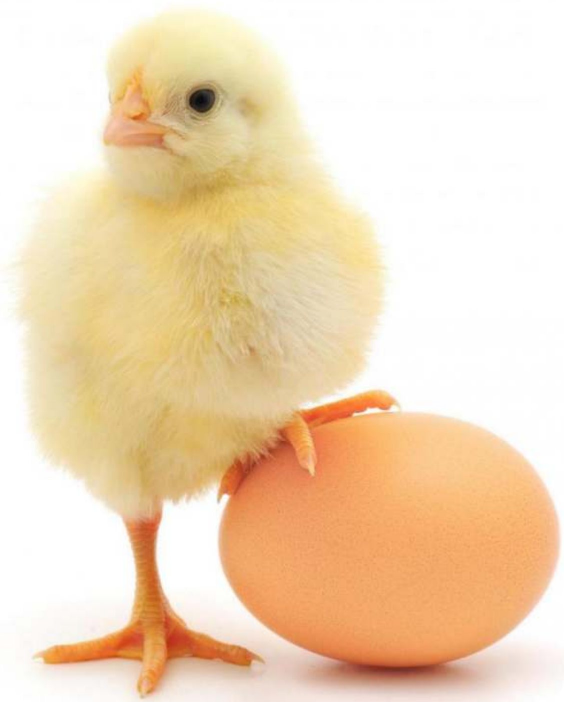

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# Research Article
# Effect of Dietary Supplementation with a Plant Extract Blend on the Growth Performance, Lipid Profile, Immune Response and Carcass Traits of Broiler Chickens

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## Abstract
**Objective:** This study was conducted to investigate the effect of supplementing broiler chicken diets with a blend of plant extracts on the growth performance, serum lipid profile, humoral immune response against Newcastle disease virus vaccines and carcass traits. **Methodology:** The plant extract blend was supplemented in the diets as a natural growth promoter at five different levels (100, 200, 500, 1000 and 2000 ppm). Broilers fed on the plant extract supplanted diets were compared to those fed on the un-supplemented (negative control) and antibiotic (oxytetracycline) supplemented (positive control) diets making seven dietary treatments. **Results:** Inclusion of the plant extract blend at levels of 200 and 1000 ppm resulted in a significant improvement in the overall performance parameters compared to the negative control. The best results were observed in broilers fed on the positive control diet. No significant differences were found between broilers fed on the positive control and those fed on 200 and 1000 ppm of the plant extract blend. Dietary intake of the plant extract blend, but not oxytetracycline resulted in a significant increase in the humoral immune response. No differences were observed among treatments in the measured serum lipid parameters or carcass traits ($p>0.05$). **Conclusion:** The tested extract blend can be utilized at a level of 200 ppm as an alternative to antibiotic growth promoters and to improve broiler performance and immune response.

Key words: Plant extracts, broilers, performance, lipids, immunity, carcass

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Feed antibiotics have played a crucial role in promoting chicken growth and lowering the incidence of enteric diseases. However, their use has been banned by the European Union since 2006 (Regulation 1831/2003/EC), with a potential ban in other parts of the world due to the development of multiple drug resistance and presence of residues in the slaughtered animal tissues[1,2]. Under this ban, scientists have searched for safe and efficient alternatives to maintain efficient poultry production. Enzymes, probiotics, prebiotics, acidifiers and phytogenics are several approaches that can replace feed antibiotics and simultaneously improve chicken performance[3,4]. Phytogenics, a category that involves herbs and their extracts, possess a number of biologically active compounds that are supposed to promote growth, enhance feed intake, activate digestive enzymes and stimulate immune function[5]. Among phytogenics, thyme, peppermint, green tea and licorice have been reported to possess several activities.

Thyme (*Thymus vulgaris* L.), a worldwide perennial plant of the Lamiaceae family, typically contains 1-3% essential oil. The sum of thymol and carvacrol constitutes approximately 64% of this oil[6]. Thyme and its active constituents have shown antimicrobial[7], immunostimulant[8] and hypocholesterolemic[9] activities. The growth promoting effect of thyme has been emphasized in broiler chickens by several researchers[10-12].

Peppermint (*Mentha piperita* L.), a perennial aromatic herb of the same family, contains 1-3% essential oil, of which menthol constitutes 30-55% of its composition[6]. The polyphenolic constituents found in peppermint leaves include rosmarinic acid, eriocitrin, luteolin-7-o-rutinoside and hesperidin are known to be potent antioxidants and free radical scavengers both *in vitro* and *in vivo*[13-15]. In addition to its antimicrobial[16], antiviral[17], immunostimulant[18] and hypolipidemic[19] activities, peppermint has the capacity to lower respiratory tract distress, relieve digestive tract discomfort and promote poultry growth[20-21].

Green tea (*Camellia sinensis*) and their major active constituents (polyphenolic catechins) can be utilized as a powerful antioxidant[22-23], antiviral[24], immunostimulant[23,25] and hypocholesterolemic[26] agent. Furthermore, green tea (extract) has been reported to improve the growth performance, meat yield and gut microflora of poultry[27-29].

Extracts from the roots of the *Glycyrrhiza glabra* plant (Licorice) have been used as a medicinal product and as a sweetener since ancient times[30]. Glycyrrhizinic acid (Glycyrrhizin) is considered the main active constituent of licorice[31]. The dried aqueous extracts of Glycyrrhiza glabra contain 4-25% glycyrrhizinic acid[32]. Glycyrrhizin possess several biological activities such as anti-oxidant[33] and immunomodulat[34]. Additionally, incorporation of licorice extract in the feed has been reported to induce positive effects on bird health and performance[35-36].

This experiment hypothesized that dietary intake of various dosages of a plant extract blend based on thyme, peppermint, green tea and licorice could enhance the growth performance, serum lipid profile, immune response and carcass characteristics of broiler chickens and could therefore be used as an alternative to antibiotic growth promoters. Oxytetracycline, the broad-spectrum antibiotic of the tetracycline family that utilized to control poultry enteric and respiratory bacterial diseases, was included in one of the study treatments as a positive control.

## MATERIALS AND METHODS

**Tested products:** The plant extract blend used in this study was water extracted, spray-dried blend; each kg of the blend contained 400 g thyme extract, 300 g peppermint extract, 200 g green tea extract and 100 g licorice extract. Its major active constituents are 1.4% essential oils (0.4% thyme oil/0.9% peppermint oil), 25% polyphenols, 15% catechins and 0.5% glyzzhirizinic acid. It was obtained from plant extract GmbH and Co. KG (Germany). Terramycin® (oxytetracycline, 40% purity) was purchased from Delta Vet Center Company, the sole agent of Phibro Animal Health in Egypt.

**Chicks and management:** This experiment was conducted at the Animal Research House of Nutrition and Clinical Nutrition Dept., Faculty of Veterinary Medicine, Zagazig University, Egypt. It was extended from 1-42 days of age and followed the animal experimentation guidelines of the Local Veterinary Ethics Committee, Zagazig University. In a completely randomized design, 245 day-old broiler chicks (Cobb 500 strain), were obtained from a commercial hatchery, weighed and distributed into 7 dietary treatments. Each treatment included 35 chicks (7 chicks/pen and 5 pens/treatment). The environmental temperature was 33°C at the trial start and then lowered approximately 2°C each week. The experimental house was open-sided and was ventilated using electric fans. The lighting program was continuous. The chicks were raised on bedding composed of wood shaving and were daily observed for mortality. Experimental chicks were vaccinated against Newcastle disease virus using Hitchner B1 strain at day 7 (eye drop), Inactivated oil adjuvant Clone 30 strain at day 14 (s/c injection) and LaSota strain at day 16 (eye drop). They were also vaccinated against Gumboro disease virus using D78 stain at 12 and 22 days of age (drinking water), Infectious

Bronchitis virus using H120 strain at day 7 (eye drop) and Avian Influenza disease virus using Reassortant inactivated H5N1 Subtype, Re-1 Strain at day 9 (s/c injection).

**Diets:** The chicks were fed on corn-soy-gluten-sunflower meal based diets. Birds of treatment 1 were fed on the basal un-supplemented diet and kept as negative control, while birds of treatment 2 were fed on the basal diet supplemented by 200 ppm of the oxytetracycline antibiotic (OTC) and kept as a positive control. Birds of treatment 3-7 were fed on basal diets supplemented by 100, 200, 500, 1000 and 2000 ppm of the plant extract blend, respectively. The OTC dosage was chosen according to the recommendation of the manufacturing company. The diets were formulated to meet or exceed broiler nutrient requirements set by the National Research Council[37], but adjusted for age and energy. There were three feeding periods [starter (0-21 days of age), grower (22-35 days of age) and finisher (36-42 days of age]. The diets did not contain coccidiostats and were provided in mash form. Feed and water were provided *ad libitum* throughout the experiment. Utilized feed ingredients and diets were chemically analyzed for dry matter (method 967.03), crude protein (method 984.13), ether extract (method 920.29) and ash (method 942.05) according to the standard procedures of the AOAC[38]; the analyzed values showed close agreement with the calculated values (Table 1).

Table 1: Composition and nutrient content of the basal diet (g kg$^{-1}$ as fed)

| Feed ingredients | Starter | Grower | Finisher |
|---|---|---|---|
| Corn, ground yellow | 515.0 | 562.8 | 605.5 |
| Soybean meal, 460 CP | 320.5 | 270.0 | 220.6 |
| Corn gluten meal, 600 CP | 35.0 | 35.0 | 35.0 |
| Sunflower meal, 360 CP | 50.0 | 50.0 | 50.0 |
| Limestone | 10.4 | 9.4 | 9.1 |
| Dicalcium phosphate | 20.4 | 18.0 | 16.8 |
| Premix[1] | 3.0 | 3.0 | 3.0 |
| Sodium chloride | 3.1 | 2.6 | 2.6 |
| Sodium bicarbonate | 2.0 | 2.0 | 2.0 |
| DL. Methionine | 2.6 | 2.2 | 1.8 |
| L. Lysine HCl | 3.2 | 3.0 | 2.7 |
| L. Threonine | 0.4 | 0.4 | 0.3 |
| Vegetable oil | 33.3 | 40.6 | 49.5 |
| **Nutrient composition** | | | |
| Crude protein | 230.0 | 210.0 | 190.0 |
| AME[2], kcal kg$^{-1}$ diet | 3000.0 | 3100.0 | 3200.0 |
| Calcium | 10.0 | 9.0 | 8.5 |
| Available phosphorus | 5.0 | 4.5 | 4.2 |
| Lysine | 14.0 | 12.5 | 11.0 |
| Methionine | 6.7 | 6.0 | 5.4 |
| Methionine+cystine | 10.4 | 9.5 | 8.6 |
| Sodium | 2.0 | 1.8 | 1.8 |

[1]Supplied the following per kg of diet: *trans*-retinol 4.128 mg, cholecalciferol 0.075 mg, DL-α-tocopheryl acetate 10 mg, menadione 1 mg, thiamine 1 mg, riboflavin 5 mg, pyridoxine 1.5 mg, calcium pantothenate 10 mg, cyanocobalamin 0.01 mg, niacin 30 mg, folic acid 1 mg, biotin 0.05 mg, Zn 60 mg, Mn 60 mg, Fe 30 mg, Cu 4 mg, I 0.3 mg, Co 0.1 mg and Se 0.1 mg. [2]Apparent metabolizable energy

### Measurements

**Growth performance parameters:** Bird weights and feed consumption were measured at 21, 35 and 42 days of age to determine the average body weight, average daily gain, average daily feed intake and feed conversion ratio Corrected For Mortality (FCR) for each feeding period. Mortality was recorded daily to estimate the overall percent mortality for each treatment.

**Serum lipids:** At 42 days of age, blood samples were collected from the wing veins of 10 birds/treatment (2 birds/pen) into test tubes without anti-coagulant. The collected blood samples were centrifuged at 3500 rpm for 15 min to separate the serum. Serum samples were stored in vials at -20°C until analysis. The following lipid parameters were measured using commercial kits: Total lipid[39], triglycerides[40], total cholesterol[41] and High-Density Lipoprotein (HDL)[41]. Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were determined according to equations[42]:

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

$$\text{LDL} = \text{Total cholestrol-(HDL+VLDL)}$$

**Humoral immune response against Newcastle disease virus vaccines:** Blood samples were collected from the wing veins of 10 birds/treatment (2 birds/pen) at 21, 28 and 35 days of age. The serum was then separated and stored at -20°C for further analysis. Specific antibody titer against Newcastle disease virus vaccines was measured in the serum samples by an Enzyme Linked Immuno Sorbent Assay (ELISA) using a commercial kit (BioChek B. V., Reeuwijjk, The Netherlands) according to the instructions provided by the manufacturer. Optical density values were read at 405 nm using an ELISA reader.

**Carcass traits:** At the end of the experiment, ten birds/treatment were randomly selected (2 birds/pen, with an approximate average weight of the pen) and restricted from feed for 8 h (with no limitation of water access). Next, they were individually weighed and euthanized by severing the jugular veins, followed by exsanguination, plucking and evisceration for carcass trait evaluation. Carcass, liver, gizzard, heart, spleen and abdominal fat pad weights were recorded. The percentages of the carcass (dressing) and organs relative to live body weight were calculated.

**Statistical analysis:** The statistical analysis was performed using Statistix®9[43]. Pen was served as the experimental unit for the growth performance parameters. For other measurements, individual birds were considered the experimental unit. One way analysis of variance (ANOVA) was conducted to determine the effect of the different treatments and LSD was used to determine if significant differences exists among treatments at p<0.05.

## RESULTS

Obtained data revealed significant differences among treatments in the average body weight at 35 and 42 days of age (p<0.01), but not at 21 days of age (p>0.05). Addition of the plant extract blend at levels of 200 and 1000 ppm significantly increased the final body weight of broiler chickens by 5.2 and 5.5%, respectively when compared to those fed on the negative control diet. The best final body weight was observed in broilers fed on the positive control diet, which was 8.8% heavier than those fed on the negative control diet. No significant difference was observed in the final body weight between broilers fed on the positive control diet and those fed on the 200 and 1000 ppm of the plant extract blend. The lowest average body weights were observed in broilers fed on diets contained 2000 and 100 ppm of the plant extract blends beside the negative control (Table 2).

A significant difference in the average daily gain was observed among treatments during the age periods of 22-35 and 0-42 days (p<0.01). The overall average daily gain of broilers fed on diets supplemented with 200 and 1000 ppm of the plant extract blend were significantly higher than those fed on the negative control diet by 5.5%. The overall average daily gain of broilers fed on the positive control diet was significantly higher than those fed on the negative control diet by 8.9%, but this become non-significant when broilers fed on the positive control diet were compared to those fed on diets contained 200 and 1000 ppm of the plant extract blend (Table 2).

The average daily feed intake was significantly differed among treatments at 22-35 (p<0.05) and 0-42 (p<0.05) days of age. Compared to negative control, the overall average daily feed intake was not significantly differed due to inclusion of the plant extract blend. Inclusion of the OTC resulted in a significant increase (5.3%) in the overall average daily feed intake compared to the negative control, but this effect was not significant when OTC was compared to 200-1000 ppm of the plant extract blend (Table 3).

Table 2: Effect of feeding plant extract blend on the average body weight and daily gain of broiler chickens

| | Control | | Plant extract blend (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | Negative | Positive | 100 | 200 | 500 | 1000 | 2000 | Pooled SEM | p-value |
| **Average body weight (g)** | | | | | | | | | |
| 1 day of age | 42.44 | 42.28 | 42.34 | 42.53 | 42.58 | 42.61 | 42.42 | 0.29 | - |
| 21 day of age | 859 | 910 | 868 | 883 | 880 | 902 | 859 | 43.2 | - |
| 35 days of age | 1777$^{c}$ | 1992$^{a}$ | 1825$^{bc}$ | 1879$^{b}$ | 1856$^{b}$ | 1888$^{b}$ | 1755$^{c}$ | 66.4 | 0.01 |
| 42 days of age | 2322$^{cd}$ | 2526$^{a}$ | 2354$^{bcd}$ | 2442$^{ab}$ | 2399$^{bc}$ | 2449$^{ab}$ | 2266$^{d}$ | 91.2 | 0.01 |
| **Average daily gain (g)** | | | | | | | | | |
| 0-21 days of age | 38.88$^{c}$ | 41.36$^{a}$ | 39.34$^{bc}$ | 40.06$^{abc}$ | 39.88$^{abc}$ | 40.96$^{ab}$ | 38.90$^{c}$ | 1.63 | - |
| 22-35 days of age | 65.56$^{cd}$ | 77.20$^{a}$ | 68.38$^{bc}$ | 71.12$^{b}$ | 69.72$^{b}$ | 70.42$^{b}$ | 64.02$^{d}$ | 2.85 | 0.01 |
| 36-42 days of age | 77.94$^{ab}$ | 76.44$^{ab}$ | 75.54$^{ab}$ | 80.32$^{a}$ | 77.58$^{ab}$ | 80.18$^{a}$ | 72.94$^{b}$ | 4.72 | - |
| 0-42 days of age | 54.29$^{cd}$ | 59.14$^{a}$ | 55.06$^{bcd}$ | 57.14$^{ab}$ | 56.12$^{bc}$ | 57.30$^{ab}$ | 52.94$^{d}$ | 2.17 | 0.01 |

$^{a,b,c,d}$Means within the same row with different superscripts are significantly different at p<0.05, -: No significant effect (p>0.05)

Table 3: Effect of feeding plant extract blend on the average daily feed intake and feed conversion ratio of broiler chickens

| | Control | | Plant extract blend (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | Negative | Positive | 100 | 200 | 500 | 1000 | 2000 | Pooled SEM | p-value |
| **Average daily feed intake (g)** | | | | | | | | | |
| 0-21 days of age | 49.82$^{bc}$ | 52.14$^{a}$ | 50.0$^{abc}$ | 50.56$^{abc}$ | 50.66$^{abc}$ | 51.74$^{ab}$ | 49.14$^{c}$ | 0.79 | - |
| 22-35 days of age | 121.4$^{cd}$ | 136.0$^{a}$ | 124$^{bcd}$ | 128.2$^{b}$ | 125.5$^{bc}$ | 126.8$^{b}$ | 119.6$^{d}$ | 1.78 | 0.01 |
| 36-42 days of age | 166.9$^{ab}$ | 160.5$^{ab}$ | 163.1$^{ab}$ | 169.9$^{a}$ | 165.6$^{ab}$ | 167.5$^{ab}$ | 157.3$^{b}$ | 3.64 | - |
| 0-42 days of age | 93.20$^{bc}$ | 98.16$^{a}$ | 93.64$^{bc}$ | 96.30$^{ab}$ | 94.78$^{ab}$ | 96.10$^{ab}$ | 90.68$^{c}$ | 1.35 | 0.05 |
| **Feed conversion ratio** | | | | | | | | | |
| 0-21 days of age | 1.28 | 1.26 | 1.27 | 1.26 | 1.27 | 1.26 | 1.26 | 0.01 | - |
| 22-35 days of age | 1.85$^{ab}$ | 1.76$^{d}$ | 1.82$^{bc}$ | 1.80$^{cd}$ | 1.80$^{cd}$ | 1.80$^{cd}$ | 1.87$^{a}$ | 0.02 | 0.01 |
| 36-42 days of age | 2.14$^{ab}$ | 2.10$^{c}$ | 2.16$^{a}$ | 2.11$^{bc}$ | 2.14$^{ab}$ | 2.09$^{c}$ | 2.16$^{a}$ | 0.01 | 0.01 |
| 0-42 days of age | 1.72$^{a}$ | 1.66$^{d}$ | 1.70$^{abc}$ | 1.69$^{bcd}$ | 1.69$^{bcd}$ | 1.68$^{cd}$ | 1.71$^{ab}$ | 0.01 | 0.01 |

$^{a,b,c,d}$Means within the same row with different superscripts are significantly different at p<0.05, -: No significant effect (p>0.05)

Table 4: Effect of feeding plant extract blend on the serum lipid profile of 42 days old broiler chickens

| | Control | | Plant extract blend (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | Negative | Positive | 100 | 200 | 500 | 1000 | 2000 | Pooled SEM | p-value |
| Total lipid | 302.6 | 294.3 | 323.1 | 318.0 | 332.1 | 292.1 | 337.7 | 14.19 | - |
| Triglycerides | 84.30 | 78.33 | 83.37 | 86.25 | 82.55 | 79.60 | 89.00 | 5.44 | - |
| Total cholesterol | 187.5 | 162.6 | 178.4 | 178.0 | 164.6 | 154.4 | 176.2 | 8.94 | - |
| HDL | 69.1 | 65.77 | 60.1 | 63.25 | 61.33 | 62.70 | 67.66 | 4.27 | - |
| LDL | 101.3 | 81.22 | 101.2 | 97.25 | 86.55 | 75.6 | 90.55 | 8.57 | - |
| VLDL | 16.80 | 15.66 | 16.62 | 17.25 | 16.44 | 15.8 | 17.77 | 1.08 | - |

HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, -: No significant effect (p>0.05)

Table 5: Effect of feeding plant extract blend on the antibody titer against Newcastle disease virus vaccines (titer range)

| | Control | | Plant extract blend (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | Negative | Positive | 100 | 200 | 500 | 1000 | 2000 | Pooled SEM | p-value |
| 21 day of age | $2067^{bc}$ | $2302^{bc}$ | $1938^{c}$ | $2564^{ab}$ | $3041^{a}$ | $2647^{ab}$ | $2125^{bc}$ | 196 | 0.01 |
| 28 day of age | $3074^{cd}$ | $3115^{bcd}$ | $2946^{d}$ | $3745^{ab}$ | $3916^{a}$ | $3675^{abc}$ | $3224^{bcd}$ | 210 | 0.05 |
| 35 day of age | $4524^{c}$ | $4820^{bc}$ | $4662^{c}$ | $5281^{ab}$ | $5516^{a}$ | $5044^{abc}$ | $4892^{bc}$ | 191 | 0.05 |

$^{a,b,c,d}$Means within the same row with different superscripts are significantly different at p<0.05, -: No significant effect (p>0.05)

Table 6: Effect of feeding plant extract blend on the carcass characteristics of 42-day-old broiler chickens

| | Control | | Plant extract blend (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Trait (%) | Negative | Positive | 100 | 200 | 500 | 1000 | 2000 | Pooled SEM | p-value |
| Carcass dressing | 71.4 | 69.8 | 71.5 | 69.8 | 71.4 | 69.2 | 70.7 | 0.87 | - |
| Liver | 2.15 | 2.00 | 2.20 | 2.27 | 2.20 | 2.34 | 2.33 | 0.13 | - |
| Gizzard | 1.99 | 1.90 | 1.85 | 1.95 | 1.93 | 1.97 | 2.08 | 0.09 | - |
| Heart | 0.40 | 0.43 | 0.40 | 0.41 | 0.64 | 0.43 | 0.42 | 0.03 | - |
| Spleen | 0.36 | 0.39 | 0.38 | 0.40 | 0.43 | 0.40 | 0.41 | 0.02 | - |
| Abdominal fat pad | 1.38 | 1.40 | 1.47 | 1.36 | 1.44 | 1.43 | 1.43 | 0.04 | - |

-: No significant effect (p>0.05)

Except for 0-21 days of age (p>0.05), a significant difference was observed among treatments in the FCR during different age periods (p<0.01). The overall FCR of broilers fed on the plant extract blend at levels ranged from 200-1000 ppm was significantly improved by 2.3% compared to those fed on the negative control diet. The best overall FCR was observed in broilers fed on the OTC supplemented diet, which was significantly improved by 3.5% when compared to those fed on the negative control diet. No significant difference was observed in the overall FCR between broilers feed on the positive control diet and those fed on 200-1000 ppm of the plant extract blend. The worst overall FCR was observed in broilers fed on the negative control diet and 2000 ppm plant extract diet (Table 3).

The overall percent mortality was 4.1%, which is in the normal range of mortality in broiler farms. The mortality percentage was 11.4, 0, 5.7, 2.9, 2.9, 0 and 5.7% in broilers fed on the negative control, positive control, 100, 200, 500, 1000 and 2000 ppm of the plant extract blend, respectively. This indicates that the percent mortality was decreased by 50% in broilers fed on 100 and 2000 ppm of the plant extract blend and by 75% in broilers fed on diets supplemented with 200 and 500 ppm of the plant extract blend when compared to those fed on the negative control diet. No mortality was found in broilers fed on the positive control diet or diet supplemented with 1000 ppm of the plant extract blend.

As shown in Table 4, data analysis revealed no significant differences in the measured serum lipid parameters of 42 day old broiler chickens fed on different test diets (p>0.05).

A significant difference was observed among treatments at 21 (p<001), 28 and 35 (p<0.05) days of age. The best serum antibody titer against Newcastle disease virus vaccines was observed in broilers fed on a diet supplemented with 500 ppm of the plant extract blend, which was significantly higher than those fed on the negative control, positive control, 100 and 2000 ppm of the plant extract blend. No significant difference was observed between broilers fed on diets supplemented by 200, 500 and 1000 ppm of the plant extract blend (Table 5).

No significant differences (p<0.05) were observed in the measured carcass characteristics due to inclusion of the plant extract blend or OTC (Table 6).

## DISCUSSION

The blend tested in this trial was a plant extracts mix derived from thyme, peppermint, green tea and licorice. Most of the tested products in the published literatures composed only of essential oils, but the plant extract blend tested in the current study contained several active constituents beside essential oil such as polyphenols, catechins and glycyrrhizinic acid, which are supposed to work synergistically in improving broiler growth performance and immune response. The chemical structures of some of the phytochemical constituents found in the plant extract blend are shown in Fig. 1.

The results obtained in this study revealed that dietary intake of this plant extract blend at a level of 100 ppm was not sufficient to promote broiler growth. Likewise, its inclusion in excess of 1000 ppm could adversely affect the measured growth performance parameters. The best growth promoting effect was observed when this mix was included at levels ranging from 200-1000 ppm. The poor growth performance observed in broilers fed on a diet supplemented with 2000 ppm compared to the lower dosages of the blend could be attributed in part to the high tannin or licorice components of the diet. A high content of tannins in the feed diet may decrease nutrient digestion and absorption[44]. Additionally, incorporation of high concentrations of licorice in broiler feed has been reported to decrease carcass abdominal fat[45].

Dietary inclusion of thyme oil, the main active component of thyme, has been reported to increase the growth performance parameters of broiler chickens when included at levels of 200 ppm[10] or 500 ppm[8]. Inclusion of thyme extract in the drinking water was also reported to improve bird performance, particularly at levels ranging from 500-2000 ppm[11,12]. Interestingly Feizi *et al*.[46] reported a 3% improvement in weight gain and 2.2% in the feed conversion ratio due to inclusion of thyme extract in the feed at a level of 200 ppm. Peppermint extract was included in the blend with the aim of improving growth performance by supporting digestive health. Ocak *et al*.[20] and Al-Kassie[21] observed positive effects on the growth performance due to inclusion of peppermint extract in broiler diets.

Green tea extract has been reported to improve broiler growth performance when utilized at a level of 200 ppm[29]. Licorice extract was included in the studied mix for the aim of immune stimulation. However, its inclusion level should be at a minimum level to avoid any growth depression. High levels of licorice extract has been reported to lower carcass fat and subsequently body weight[47]. In Japanese quails, licorice extract has been reported to increase feed intake and weight gain when included at a level of 200 ppm in combination with a probiotic bacteria[12]. The variation in response to inclusion of plant extracts in published studies could be attributed to the difference in the concentration and type of the active constituents contained in the tested products.

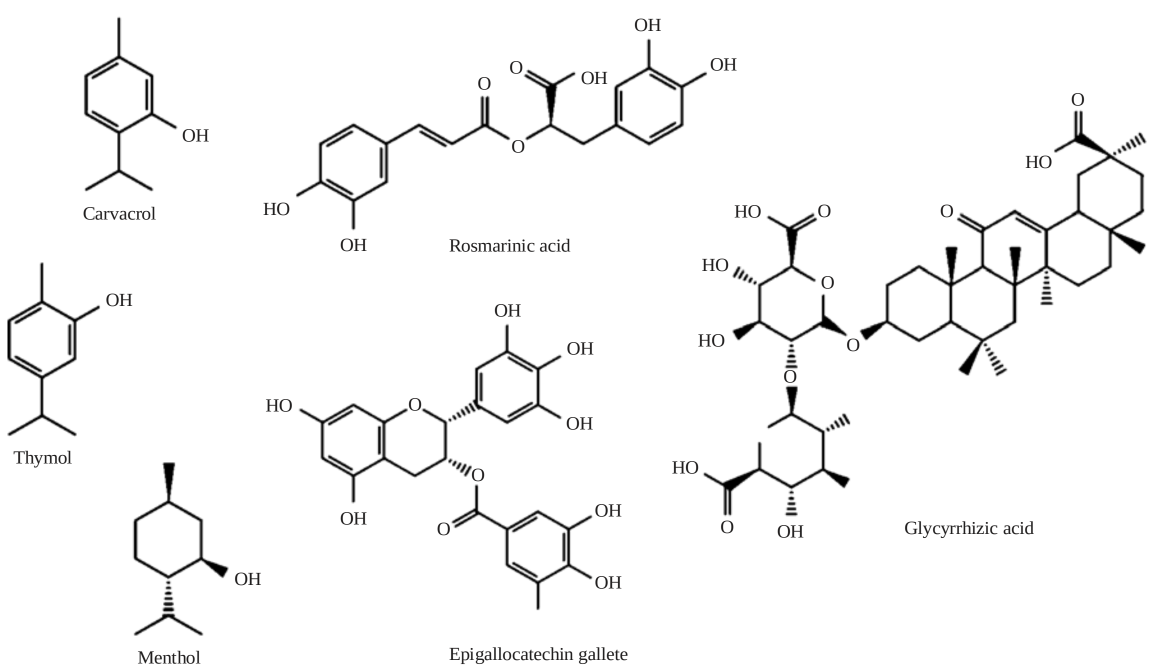


Fig. 1: Chemical structures of some of the main phytochemicals found in the plant extract blend used in this study

Inclusion of oxytetracycline in one of the study treatments improved the overall growth performance in comparison to the negative control. This may be attributed to the antimicrobial and anti-inflammatory properties of antibiotic growth promoters[48]. Similar findings have been reported in the literature[48-50]. The growth promoting effect of the feed additives may be enhanced when chickens are fed on less digestible diets or reared under less hygienic conditions[51]. Sunflower meal was included in the diets of the current study at a level of 5% with the aim of lowering diet digestibility. Furthermore, the use of coccidiostats was omitted from the diets in an attempt to challenge the birds with less hygienic conditions.

According to Elson and Qureshi[52], plant extracts may lower the blood cholesterol level in broilers via inhibition of the controlling enzyme for cholesterol synthesis (3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. However, no significant differences were observed in the measured lipid components of the current study. Similarly, El-Deek and Al-Harthi[53] did not observe significant differences in the total lipid and cholesterol levels due to supplementation of the chicken diets with green tea powder. Nevertheless, other researchers reported significant decreases in the triglyceride and cholesterol concentrations due to inclusion of thyme extract or oil in broiler diets at levels ranging from 200-500 ppm[12]. Likewise, Aghazadeh *et al.*[19] reported a significant decrease in the triglyceride, cholesterol and LDL concentration due to inclusion of peppermint extract at levels ranging from 2000-6000 ppm. Furthermore, significant decreases in the blood cholesterol and/or LDL were observed due to supplementation of broiler chickens with licorice extract[36,45]. Dietary intake of OTC in the current study did not result in a significant difference in the serum lipid profile. Similar findings were reported by El-Deek *et al.*[54].

Inclusion of the plant extract blend in the broiler feed increased the antibody titer against Newcastle disease virus vaccines. This effect may be attributed to the immunostimulant and antioxidant properties of the plant extracts used in the blend. Polyphenolic substances found in the plant extracts have the capacity to scavenge free radicals and maintain the structural integrity of immune cells[18]. Rafiee *et al.*[12] and Manafi *et al.*[8] revealed that inclusion of thyme extract at a level of 500 ppm in the drinking water or feed respectively resulted in a significant increase in the antibody titer against Newcastle disease vaccine of broiler chickens. Green tea extract has been reported to decrease the incidence of poultry diseases such as avian influenza[25] and also increase the antibody titer against Newcastle disease virus vaccines when included at levels ranged from 125-500 ppm[23]. On the other hand, inclusion of thyme extracts in the drinking water did not result in significant immune stimulation against Newcastle disease vaccines in broiler chickens[11]. Supplementation of chicken diet with antibiotic growth promoter (OTC) did not significantly affect the humoral immune response against Newcastle disease virus vaccines. Similarly, Zulkifli *et al.*[49] and El-Deek *et al.*[54] reported no positive effect on the humoral immune response against Newcastle disease virus due to supplementation of the diet with OTC antibiotic.

Dietary intake of the plant extract mix did not improve the carcass characteristics of broiler chickens. Likewise, Hernandez *et al.*[55] revealed no significant differences in the chicken's liver and gizzard weights due to dietary inclusion of 5000 ppm of a product based on sage, thyme and rosemary extracts. Schulze *et al.*[56] did not observe a significant effect in the broiler carcass yield due to the dietary inclusion of essential oil blend based on thyme and cinnamon. Published studies have revealed that licorice extract has no significant effect in chicken carcass traits other than lowering abdominal fat level[45]. On the other hand, Khaksar *et al.*[9] revealed significant improvements in some carcass traits due to inclusion of thyme essential oils. In addition, Al-Kassie[21] recorded positive effects in the carcass and liver yields of broilers due to inclusion of dry peppermint powder at levels ranging from 2500-15000 ppm. In compliance with the observation of El-Deek *et al.*[53], dietary inclusion of OTC in the current study did not result in significant differences in the measured carcass traits.

## CONCLUSION

A blend of plant extracts containing thyme, peppermint, green tea and licorice at a level of 200 ppm could be included in broiler chicken diets to promote their growth performance and immune response. Furthermore, it can be utilized as an alternative to antibiotic growth promoters.

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