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Bioactive Effect of Dietary Supplementation with Essential Oils Blend of Oregano, Thyme and Garlic oils on Performance of Broilers Infected with Eimeria species

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Abstract: A detailed understanding of the bioactive effect of feeding plant by-products or its extract to reduce the incidence of coccidial infection in birds remains a challenge. Essential oils (EO) from Oregano *(Origanum vulgare L),* Thyme *(Thymus vulgaris L.)* and Garlic *(Allium sativum)* as natural alternative feed additives were evaluated on the growth performance and health state of broiler chickens` infected with Eimeria species (spp).Seven groups of broiler chickens were conducted in the present study. G1 is the control negative group fed on basal diet (BD). G2 is a group fed on basal diet and medicated with Amporolium sulfate 20% (2gm/litter) as a reference drug. Groups G3 and G4 were fed on basal diet supplemented with EO blend of Oregano (10 mg/kg diet), thyme (10 mg/kg diet) and Garlic (5mg/kg diet) up to 35 day of age and 15 day of age, respectively. G5 and G6 were fed on basal diet supplemented with EO blend of Oregano (20 mg/kg diet), thyme (20 mg/kg diet) and Garlic (10mg/kg diet) up to 35 days and 15 day of age respectively. Group G7 fed on basal diet and act as control positive. At 15 day of age all groups were infected with Eimeria spp oocyst (20000 sporulated oocyst/ bird) except control negative group (G1). Results showed improvement in body weight, relative growth rate and feed conversion ratios of EO` supplemented groups specially G3 and G4. The lowest growth performance was for the control positive group, which infected with Eimeria without medication or essential oils supplementation. There were gradually decrease in oocysts count that shed from infected supplemented groups (G5 and G6) comparing with Amprolium sulfate treated group. No significant changes were found in serum total protein, albumin and globulin while significant increases in serum glucose, total cholesterol and significant decrease in triglycerides levels in all groups compared with the control groups. Belonging to carcass traits, EO supplementation showed no significant improvement in carcass traits. It was concluded that essential oils blend of Oregano, Thyme and Garlic improved broiler`s performance and could be used as anticoccidial phytobiotic.

Key words: Broilers · Garlic · Oregano · Thyme · Coccidia · Eimeria

INTRODUCTION

Phytogenic feed additives or phytobiotics are plant derived natural bioactive products used to improve the performance of animals and poultry affecting their growth and health, positively. This class of feed additives has recently gained increasing interest, especially for their use in poultry nutrition. Phytobiotics have the ability to positively modulate microflora, improve broiler performance and control pathogen, especially those with zoonotic implications [1]. One category of phytobiotics is essential oils (EO) and their blend, which can exert a variety of production enhancements including the potential for selective antimicrobial effects [2] and enhance digestion [3]. Coccidiosis is a parasitic disease that influences farm animals and poultry leading to real economic losses. In poultry, this occurs by affecting different parts of intestine therefore impairs feed utilization, decrease broilers growth and egg production of laying hens causing death of birds and expending a lot of money for vaccination and inclusion of anticoccidial drugs into the diet for long period [36, 37]. Anticoccidial

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drugs have been reasonably effective on preventing serious outbreaks of coccidiosis among birds reared for broiler market. However, the life of most of these drugs is limited due to the emergence of resistant strains of Eimeria [38]. Therefore, a pressing need for an alternative method of control is required. Hence necessity for cheap and safe alternatives rises up. Essential oils from Oregano (*Origanum vulgare L*), Thyme (*Thymus vulgaris L.*) and Garlic (*Allium sativum*) supplements were evaluated as natural alternatives bioactive compounds in broiler` diet.

The essential oil obtained from *O. vulgare* comprises more than 20 ingredients acting synergitical, most of which are phenolic antioxidants [4]. Major components are carvacrol and thymol that constitute about 78 to 82% of the total oil [5]. Thyme (*Thymus vulgaris*) is a member of lamiaceae family with the main components of phenols, thymol 40% and carvacrol 15% [6]. The beneficial value of thyme in poultry industry has been reported [7]. Garlic (*Allium sativum L.*) has antimicrobial, antioxidative, hypocholesterolemic and antihypertensive properties [8,9] and have been known from ancient times. Scientific research has shown that these effects can be attributed to its bioactive components, the most important among which are sulphuric compounds, allin, diallylsulphide, allyldisulphide and allicin defined by Kumar and Berwal [10]. Moreover, garlic is very rich in aromatic oils which enhance digestion and positively influenced respiratory system. A detailed understanding of the bioactive effect of feeding plant products and phytochemical blends to reduce the incidence of coccidial infection in birds remains a challenge. So the aim of the present study is to evaluate the effect of dietary supplementation of commercially available essential oils blend of Oregano, Thyme and Garlic on the growth performance and health state of broilers infected or non infected with Eimeria species.

MATERIALS AND METHODS

Birds and Treatments: The experiment was carried out under the protocol approved by the faculty of veterinary medicine Sadat City University, Egypt. A total of 210 one-day old chicks "$cobb_{500}$" were randomly allotted to 6 floor pens with 30 birds each. Broilers were raised to 35 days and fed corn- soya bean based diet (1-15 day of age) and grower-finisher diet (15-35 day of age) which were formulated to guarantee or exceed recommended nutrients requirements [11]. One basal diet was mixed for each dietary period and the additives were blended in according to treatment distribution at the later time (Table 1). The chicks were allocated in groups as follow:

G1 = Fed on basal diet with no supplementation (Control negative)

G2 = Fed on basal diet and medicated with amprolium sulfate 20% at dose 2g/liter

G3= Fed on basal diet supplemented with EO blend of Oregano (10 mg/kg diet), thyme (10 mg/kg diet) and Garlic (5mg/kg diet) till 35 day age

G4 = Fed on basal diet supplemented with EO blend of Oregano (10 mg/kg diet), thyme (10 mg/kg diet) and Garlic (5mg/kg diet) till 15 day age

G5 = Fed on basal diet supplemented with EO blend of Oregano (20 mg/kg diet), thyme (20 mg/kg diet) and Garlic (10mg/kg diet) till 35 day age.

G6 = Fed on basal diet supplemented with EO blend of Oregano (20 mg/kg diet), thyme (20 mg/kg diet) and Garlic (10mg/kg diet) till 15 day age.

G7 = Fed on basal diet without supplementation (Control positive)

Table 1: Ingredients and nutrients composition (% DM) of broiler's basal diets

Ingredients	Starter	Grower-Finisher
Yellow corn %	56.5	58.6
Soybean meal (44%)	32.0	30.6
Corn gluten (60%)	6.0	3.6
Vegetable oil	1.0	3.0
Common salt	0.3	0.3
Essential oil blend[1]	0.1	0.1 or 0.2
Dicalcium phosphate[2]	1.7	1.7
Vit. [3] and min. mixture[4]	0.3	0.3
Limestone	1.6	1.44
DL-Methionine[5]	0.13	0.12
L- Lysine-HCL[6]	0.17	0.14
Sodium bicarbonate	0.20	0.10
Chemical composition		
Crude protein	21.80	19.90
ME Kcal/Kg	2900	3015
C/P ratio	133	151
Calcium	1.10	1.0
Available Phosphorous	0.45	0.42
Methionine	0.47	0.45
Lysine	1.2	1.0

[1]Essential oil blend: 1 Kg contains 10 g of Oregano, 10 g of Thyme and 5 g of Garlic), which is patent product (Mg2Mix, France, Intermedicavet Co., VMD, sole agent, Egypt). The recommended dose is one Kg per ton feed (G3 and G4), which is duplicated in group G5 and G6 (2Kg/ton). [2]Dicalcium phosphate, 18% granular phosphate and 23 % calcium. [3]Each 1.5 kg contain: vitamin A 12000000 IU, vitamin D3 3000000 IU, vitamin E 40000 mg, vitamin K3 3000 mg, vitamin B1 2000 mg, vitamin B2 6000 mg, vitamin B6 5000 mg, vitamin B12 20 mg, niacin 45000 mg, biotin 75 mg, folic acid 2000 mg, pantothenic acid 12000 mg. [4]Each 1.5 Kg contain: manganese 100000 mg, zinc 600000 mg, iron 30000 mg, copper 10000 mg, iodine 1000 mg, selenium 200 mg and cobalt 100 mg. [5]DL-Methionine, Met AMINO® (DL-2-amino-4-(methyl-thio)-butane acid, DL-methionine, α-amino-Y-methyl-oily acid) by Feed Grade 99% (EU). [6]L-Lysine HCL 99% (Feed Grade) L-Lysine: 78.0% Min (Indonesia)

Management and Housing: Top-dressed litter with 2 inch of fresh wood shavings was used as bedding. The temperature was set at 30 to 33°C during the first week and was reduced by 2 °C per week until 20 °C was reached. Relative humidity was about 60 to 80%. The lighting program was 23L:1D. Access to feed and water was provided on an *ad libitum* basis.

Coccidial Strain and Infection: A native strain of sporulated *Eimeria spp* oocysts were obtained from Department of Parasitology, Faculty of Veterinary Medicine, University of Sadat City. The strain is a pooled colony of the oocysts *(E. tenella* 30%, *E. maxima* 20%, *E. acervulina* 20%, *E. necatrix* 15% and *E. mitis* 15%). At 15 days old, all groups were infected orally except the control negative (G1) with 20.000 sporulated oocysts/birds. Mean oocyst count/gm of dropping was performed at days 5, 6, 7, 8 post infection using the McMaster counting technique according to the method described by Long and Joyner [12].

Performance Measurements: Body weight (BW) and feed intake were monitored on a pen basis weekly; while weight gain (BWG), relative growth rate RGR [39] and feed conversion ratio FCR [40] values were consequently calculated. Mortality was also recorded on a daily basis in each pen. Chickens were killed by cervical dislocation at the end of the trial. Six birds per treatment group were randomly selected for determining of carcass yield and traits. They were defeathered, eviscerated and dressed. Carcass parts such as liver, gizzard, heart, spleen, bursa of Fabricius and breast muscle were collected by removing skin, fat and connective tissue.

Sampling: At the end of experiment, 4 birds from each group were randomly selected for blood analysis. Blood samples were obtained from wing vein and directly aliquoted into 2-mL sterile vials and allowed for one hour at room temperature (23°C) for complete coagulation and for 3h in refrigerator (4°C) for complete separation of serum before centrifugation at 1500 rpm, 6°C for 20 min. After centrifugation, the serum was aliquoted into 1-mL vials and stored at -20 °C for serum determination of glucose, total protein, albumin, cholesterol and triglycerides, using Spectrophotometer and commercially available kits (Biosystem S.A, Costa Brava, 30, Barcelona, Spain) according to manufacturer's instructions.

Statistical Analysis: Experimental data were analyzed as a randomized block design. All data were subjected to one-way ANOVA by the GLM procedure using the SPSS 18.00 statistical package (SPSS Ltd., Surrey, UK). Duncan's test was carried out to assess any significant differences at the probability level of $P< 0.05$ among the experimental treatments. Mortality data were not subjected to statistical analysis because just 4 chicks died throughout the experiment (One bird from G3, G4, G5, G6 each) and only after infection.

RESULTS

The mortality rate was 3.3% in groups G3, G4, G5 and G6 while in G7 was 28% and there was no mortality in G1and G2

Growth Performance: Body weight (BW), body weight gain (BWG), relative growth rate (RGR) and feed conversion ratio (FCR) are presented in Table 2 and showed that:

- Live BW, BWG and RGR increased significantly in birds of group G3 and G4 when compared with other groups all over the experimental period.
- FCR improved significantly in G3 and G4 when compared with other groups.
- The lowest values were for control positive group G7.

Serum Parameters: Table 3, presents the effects of experimental diets on some serum parameters of broiler chickens at 35 days of age, which showed:

- No significant differences were found among groups on concentration of serum total protein, albumin and globulin, except for G2 which showed significant decrease in serum total protein and albumin compared to control and other groups.
- Increased serum glucose levels in all treated infected groups, which were significant in groups G2, G3 and G5 while was insignificant in groups G4, G6 when compared with control positive and negative groups.
- Significant increase in serum cholesterol level in all groups compared to the control negative and positive groups while G2 showing significant decrease.
- Significant decrease in serum triglycerides level in all groups compared to the control groups.

Carcass Characteristics: Table 4 showed the effects of experimental diets on carcass characteristics showing that:

Table 2: Body weight (BW), body weight gain (BWG), relative growth rate (RGR) and feed conversion ratio (FCR)values of broiler chickens in response to diet and age

	Groups						
Age	G1	G2	G3	G4	G5	G6	G7
7 days							
BW, g	157.8 ± 5.9	169.4 ± 4.9	162.5 ± 4.7	162.5 ± 4.7	160.9 ± 3.9	160.9 ± 3.9	163.8 ± 6.79
BWG,g	117.8 ± 5.9	129.4 ± 4.9	122.5 ± 4.7	122.5 ± 4.7	120.9 ± 3.9	120.9 ± 3.9	122.8 ± 8.8
RGR, g	1.18 ± 2.5	1.22 ± 1.9	1.194 ± 1.9	1.194 ± 1.9	1.193 ± 1.6	1.193 ± 1.6	1.12 ± 4.5
FCR	1.66^{b} ± 9.0	1.50^{a} ± 6.2	1.93^{b} ± 8.10	1.93^{b} ± 8.10	1.92^{b} ± 6.8	1.92^{b} ± 6.8	1.92^{b} ± 13.3
14 days							
BW, g	345.0^{b} ± 9.1	345.7^{b} ± 12.5	393.1^{a} ± 9.9	393.1^{a} ± 9.9	366.0^{ab} ± 8.4	366.0^{ab} ± 8.4	390.0^{a} ± 15.1
BWG, g	185.3^{b} ± 10.2	175.7^{b} ± 14.9	231.5^{a} ± 10.8	231.5 ^{a}± 10.8	205.0^{ab} ± 9.1	205.0^{ab} ± 9.1	226.3^{a} ± 16.2
RGR, g	0.738 ± 4.05	0.673 ± 5.08	0.831 ± 3.3	0.831 ± 3.3	0.773 ± 3.1	0.773 ± 3.1	0.817 ± 7.05
FCR	1.9 ± 0.19	2.1 ± 0.34	1.8 ± 0.12	1.8 ± 0.12	2.09 ± 0.2	2.09 ± 0.2	1.84 ± 0.38
21 days							
BW, g	644.7^{a} ± 20.6	608.0 ^{a} ± 21.1	651.1^{a} ± 27	662.0^{a} ± 24	619.2^{a} ± 18	611.5 ^{a} ± 27	510.7^{b} ± 40.6
BWG, g	322.0 ±16.5	267.5 ± 29.2	252.1 ± 30.4	251.2 ± 28.0	231.4 ± 19.6	222.6 ± 29.1	120.0 ± 43.5
RGR, g	0.635^{a} ± 3.1	0.566^{ab} ± 6.3	0.465^{b} ± 4.8	0.463^{b} ± 4.9	0.455^{b} ± 3.2	0.566^{ab} ± 5.8	0.268^{a} ± 11.1
FCR	2.05 ± 0.1	2.5 ± 0.29	2.4 ± 1.5	3.0 ± 0.5	2.8 ± 0.26	1.7 ± 0.87	2.05 ± 4.0
28 days							
BW, g	1008^{bc} ± 26.5	915.0^{c} ± 31.4	1210 ^{a} ± 45.2	1152.0^{a} ± 28.3	1034.2^{b} ± 18.9	1039.6^{b} ± 18.9	678^{d} ± 42.5
BWG, g	343.6^{cd} ± 34.8	305.7^{d} ± 36.4	576.0^{a} ± 55.0	481.1^{ab} ± 32.2	413.4^{bcd} ± 20.1	428.0^{bc} ± 32.6	167.3^{c} ± 66.8
RGR, g	0.412^{b} ± 3.8	0.400^{b} ± 4.6	0.620^{a} ± 5.2	0.529^{ab} ± 3.5	0.525^{ab} ± 2.7	0.525^{ab} ± 4.9	0.281^{c} ± 9.1
FCR	2.7^{a} ± 0.28	3.0^{a} ± 0.41	1.3^{c} ± 0.12	1.6^{bc} ± 0.11	1.9^{b} ± 8.3	1.9^{b} ± 0.2	2.39^{d} ± 1.4
35 days							
BW, g	1667^{b} ± 29.7	1577^{b} ± 59.5	1836^{a} ± 58.5	1840^{a} ± 47.4	1606^{b} ± 37.8	1676^{b} ± 56.4	967^{c} ± 80.7
BWG, g	705.0^{a} ± 48.3	662.0^{a} ± 82.0	626 ^{a} 4 ±76.0	692.7^{a} ± 49.6	584.0 ^{a} ± 40.7	645.6 ^{a} ± 61.9	289.0^{b} ± 110.3
RGR, g	0.520 ^{a} ± 3.3	0.527 ^{a} ± 5.8	0.410 ^{a} ± 4.6	0.462 ^{a} ± 3.0	0.442 ^{a} ± 2.7	0.473 ^{a} ± 3.8	0.350^{b} ± 6.3
FCR	1.4 ^{a} ± 0.13	1.6 ^{a} ± 0.18	1.8 ^{a} ± 0.2	1.5 ^{a} ± 0.12	1.7 ^{a} ± 0.12	1.6 ^{a} ± 0.15	3.11 ^{b} ± 0.32
0-35days							
BWG, g	1627^{b}±29.7	1537^{b} ± 59.9	1796.4^{a} ±58.5	1800^{a} ± 47.48	1566^{b} ± 37.8	1636.2^{b} ± 56.4	967^{c} ± 75.7
RGR, g	1.910^{b} ± 1.6	1.900^{b} ± 3.5	1.913^{a} ± 2.6	1.914^{a} ± 2.1	1.902^{b} ± 2.1	1.906^{b} ± 2.9	0.93^{c} ± 5.6
FCR	1.79^{bc} ± 3.5	1.95^{a} ± 7.3	1.61^{d} ± 5.1	1.66^{cd} ± 4.4	1.90^{ab} ± 4.4	1.82^{ab} ± 6.1	2.58^{d} ± 12.5

^{abcd}Values in the same row with a different superscript differ significantly at P □ 0.05

Table 3: Effect of experimental diets on some serum parameters of broiler chickens at 35 days of age

	Groups						
Parameters	G1	G2	G3	G4	G5	G6	G7
Total protein (g/dl)	3.5^{a}±0.02	2.7^{b}±0.02	3.6^{a}±0.07	3.2^{a}±0.17	3.4^{a} ±.011	3.3^{a}±0.2	3.3^{a} ± 0.34
Albumin (g/dl)	1.5^{a}±0.016	1.03^{b}±0.03	1.3^{a}±0.01	1.2^{a}±0.17	1.2^{ab} ±0.17	1.4^{a}±0.01	1.4^{a} ± 0.09
Globulin (g/dl)	2.0±0.18	1.6±0.00	2.3±0.08	2.05±0.34	2.1±0.09	1.8±0.20	1.8. ± 0.61
Glucose (g/dl)	208.75^{c}±2.01	244.68^{a}±0.75	217.95^{b}±4.6	213.52^{bc}±1.15	249.39^{a}±1.15	210.68^{bc}±1.8	206.43^{c}±5.01
Cholesterol (mg/dl)	95.75^{f}±2.8	71.42^{e}±3.6	143.43^{b}±1.2	155.28^{a}±1.7	125.04^{c}±1.1	85.14^{d}±1.7	98.35^{f} ± 4.8
Triglyceride (mg/dl)	69.48^{a}±0.74	43.28^{d}±0.50	67.46^{bc}±1.0	60.28^{c}±1.7	58.71^{c}±1.7	61.19^{c}±5.6	71.33^{a} ± 1.45

^{abcd}Values in the same row with a different superscript differ significantly at P □ 0.05

Table 4: Effect of experimental diets on the carcass traits and relative weights of organs (% BW) of broilers at 35 days age

	Groups							
Parameters	G1	G2	G3	G4	G5	G6	G7	P
Dressing	$0.67^{c} \pm 0.002$	$0.69^{b} \pm 0.007$	$0.72^{a} \pm 0.001$	$0.66^{cd} \pm 0.004$	$0.67^{c} \pm 0.007$	$0.65^{d} \pm 0.001$	$0.62^{c} \pm 0.02$	
Liver	$2.7^{a} \pm 0.008$	$2.2^{a} \pm 0.001$	$2.5^{a} \pm 0.005$	$2.7^{a} \pm 0.006$	$2.6^{a} \pm 0.006$	$2.4^{a} \pm 0.001$	2.2 ± 0.019	
Gizzard	2.3 ± 0.005	2.5 ± 0.001	2.2 ± 0.005	2.6 ± 0.001	2.4 ± 0.001	2.2 ± 0.001	2.2 ± 0.08	NS
Heart	$5.07^{a} \pm 0.006$	$6.06^{a} \pm 0.002$	$5.7^{a} \pm 0.006$	$4.6^{a} \pm 0.001$	$6.1^{a} \pm 0.001$	$5.2^{a} \pm 0.006$	5.2 ± 0.018	
Spleen	$1.02^{c} \pm 0.002$	$1.3^{b} \pm 0.002$	$1.2^{c} \pm 0.006$	$1.9^{b} \pm 0.005$	$1.7^{c} \pm 0.006$	$2.4^{a} \pm 0.005$	$1.01^{c} \pm 0.02$	
Bursa of fabricius	2.05 ± 0.001	1.8 ± 0.001	2.2 ± 0.003	2.1 ± 0.001	2.1 ± 0.001	2.2 ± 0.004	1.9 ± 0.06	NS
Breast meat %	$0.17^{a} \pm 0.003$	$0.14^{c} \pm 0.001$	$0.167^{b} \pm 0.003$	$0.164^{b} \pm 0.002$	$0.178^{a} \pm 0.009$	$0.143^{c} \pm 0.001$	$0.16^{a} \pm 0.08$	

[abcd]Values in the same row with a different superscript differ significantly at P □ 0.05

Table 5: Effect of experimental diets on Eimeria oocyst counts shed from infected birds

	Groups						
Day	G1	G2	G3	G4	G5	G6	G7
5^{th} Dpi	0	$13233.3^{b} \pm 272$	$10633.3^{c} \pm 650$	$13666.6^{b} \pm 440$	$8166.66^{d} \pm 881$	$9300.0^{d} \pm 145$	$40100.0^{a} \pm 1005$
6^{th} Dpi	0	$13233.3^{d} \pm 333$	$14166.6^{c} \pm 166$	$21666.6^{b} \pm 145$	$11033.3^{e} \pm 333$	$9833.33^{f} \pm 145$	$38033.33^{a} \pm 950$
7^{th} Dpi	0	$20033.3^{c} \pm 366$	$17733.3^{d} \pm 721$	$35366.6^{b} \pm 251$	$15500.0^{c} \pm 368$	$15266.6^{c} \pm 120$	$45266.6^{a} \pm 890$
8^{th} Dpi	0	$20533.3^{c} \pm 305$	$17400.0^{d} \pm 333$	$23666.6^{b} \pm 200$	$12200.0^{f} \pm 333$	$13666.6^{e} \pm 290$	$49626.6^{a} \pm 1100$
9^{th} Dpi	0	$14866.6^{c} \pm 100$	$11300.0^{d} \pm 296$	$16666.6^{b} \pm 173$	$8300.0^{e} \pm 666$	$10433.3^{d} \pm 185$	$44333.3^{a} \pm 1012$

[abcd]Values in the same row with a different superscript differ significantly at P □ 0.05

- No significance differences were found in the relative weights of liver, gizzard, heart and bursa.
- Significant increase in weights of spleens in groups G6, G4 and G5 compared to control groups and other groups.
- Significant increase in dressing weights of groups G2 and G3 when compared to control and other groups.
- Significant decrease in breast meat percentages in all groups when compared with control groups except G5 which showed no significant differences.

Oocyst Count: Table 5, showed the effect of EO blend supplementation on oocystic count shed from infected broilers from 5^{th} to 9^{th} days post infection, the data revealed significant decrease in oocyte count in groups G5 and G6 compared to amporolium treated group and other groups.

DISCUSSION

Higher values of final BW, BWG and RGR were observed in broiler chickens of groups G3 and G4 compared with the control and other groups. Dietary inclusion of EO blend of Oregano (10 mg/kg diet), Thyme (10 mg/kg diet) and Garlic (5mg/kg diet) improved the cumulative FCR in these two groups compared to the control group groups. Higher BW was recorded by broiler chickens in groups G3 and G4 in the second and 5^{th} week of age. The lowest values were for the control positive groups as a result of infection. These results indicate that dietary supplementation of broiler's basal diet with EO blend of Oregano (10 mg/kg diet), thyme (10 mg/kg diet) and Garlic (5mg/kg diet) either only till the end of starter period (G4) or throughout the experiment (G3), was effective in improving growth performance of broiler chickens. This effect was higher than the control negative group or even the groups supplemented with double dose of EO blend of Oregano (20 mg/kg diet), Thyme (20 mg/kg diet) and Garlic (10 mg/kg diet) or amprolium sulfate.

These findings are supported by the results of Roofchaee *et al*. [13] who found that inclusion of 600 mg/kg of EO in the grower diet of broilers increased significantly the body weight gain and improved FCR compared with control group. Also the findings are in agreement with those of Marcincak *et al*. [14] who suggested that the effect of herbal essential oils on the growth performance may be due to the greater efficiency in the utilization of feed resulted in enhanced growth. Similarly, Prasad *et al.* [15] found that some bioactive components of essential oils especially carvacrol and

thymol, improved FCR in broiler chickens related to increased efficiency of feed utilization. These results also are in consistent with Lewis *et al.* [16] who reported that garlic extract increased BWG and improved FCR in broilers between the 7th to the 27th day of age. Alcicek *et al.* [17] indicated that broilers which received dietary blend of essential oils had higher weights and feed intakes with lower FCR than control group. There is evidence to suggest that herbs, spices, various plant extracts especially herbal essential oils have appetite and digestion stimulating properties [18]. Windisch *et al.* [19] indicated that phytogenic compounds may specially enhance activities of digestive enzymes and nutrient absorption. Also, the improvement in bird' performance was greater with the diets contained *oregano* herb than in the control group in the study of Florou-Paneri *et al.* [20]. On contrary to these results, Cross *et al.* [7] reported that dietary inclusion of 1g/kg diet of oregano essential oil in broiler diet could not affect the growth performance parameters and Demir *et al.* [21] concluded that addition of Thyme and garlic powder to broiler's diet didn't affect growth and FCR during the experiment.

As shown in table 3, the total serum protein, albumin and globulin levels have not changed significantly in all treated groups compared with control groups. These results are in agreement with that of Jawad Ala Al Deen [22] who found no significant effect in serum total protein, albumin and glucose levels in broiler chickens fed diets supplemented with 10 and 15% of garlic. Similarly, Fadlalla *et al.* [23] found no significant effect in serum total protein, albumin and globulin of broiler chicken fed diet supplemented with 0.15, 0.45, 0.3 and 0.6 % of garlic extract. Concerning serum glucose level, it has been shown that all of the treated groups show increased serum glucose level, which was significant in groups G2, G3 and G5 while was insignificant in groups G4, G6 compared with the control groups. This means that the etheric oil supplementation either with the lower or higher level of EO blend along the study or onle till the end of starting period, increased serum glucose level. Actually many researchers found that garlic and thyme has hypoglycemic effect and proposed that garlic can act as anti-diabetic agent by increasing either pancreatic secretion of insulin from beta cells or its release from bound insulin [24].The principal active ingredients are believed to be allyl propyl disulphide and diallyl disulphide oxide (Allicin). Although, other constituents such as flavonoid may play a role as well. Some experimental evidence suggests that allyl propyl disulphide lower glucose levels by competing with insulin for insulin-inactivating sites in the liver. This led to an increase in free insulin [25]. However, another investigator noticed that garlic could not decrease the level of glucose in blood in non diabetic normal animals [26]. Grodsky *et al.* [27] found that substances that cause antagonist between garlic and natural insulin, such action may occur in this experiment as there was slight elevation in the glucose level in bird fed diets contained garlic EO.

The results also indicated higher total cholesterol in all groups when compared with the control groups, which is contradicting with the finding of Mansoub and Myandoab [28] who found decrease in cholesterol in chickens supplemented with thyme. Also the results are in contrary with that of Jawad Ala Al Deen [22] who found reduction of cholesterol levels in blood of chicken supplemented with garlic. It is clear in many studies that garlic extract and EO blend have been shown to lower plasma lipid and cholesterol which accompanied by depressed activities of lipogenic and cholestologenic enzymes and inhibition of hepatic cholesterol synthesis. In study on dogs [29] it was stated that dietary supplementation of garlic elevated the cholesterol level in blood then after 4-5 months decreased. They have attributed this finding to the effect of garlic on gradual releasing of cholesterol from its storage leading to elevation of cholesterol level in blood, then decreased when the storage is exhausted, which might be occurred in the present study. The results revealed significant decrease in triglycerides level in all groups compared to the control groups, this is supported by the finding of Mansoub and Myandoab [28] who found decreased blood triglycerides in chickens supplemented with thyme.

The results of carcass traits of birds showed that no significance differences were found in the relative weights of liver, gizzard, heart and bursa. There was no significant effect for EO supplementation on the relative weights of organs, which were in agreement with those of Ocak *et al.* [30] who found no significant effect on carcass traits in broiler chickens supplemented with thyme. Also, our findings agreed with those of Fadlalla *et al.* [23] who found no significant effect on the carcass weights and organs of broiler chickens fed diet supplemented with 0.15, 0.45, 0.3 and 0.6 % of garlic extract. Our results of dressing weight and breast meat yield weren't agree with some studies [28, 31] who found higher carcass and breast meat yield of broilers fed diet supplemented with garlic and thyme. The lower weight of carcass or lack of significant effect of etheric oil supplementation of broilers in the present study on relative weights of organs may support the principle of enhancing effect for essential oils of herbs on digestibility as a result of its stimulating effect

on endogenous digestive enzymes production [32].The second reason for lack of the effect of EO supplementation might be related to the stress of Eimeria oocyst infection.

The effect of dietary supplementation of broilers with EO blend on oocystic count shed from infected birds from the 5th to 9th day post infection revealed that gradual decrease in the number of oocysts count in group G5 and G6 compared with amprolium medicated group. The least number of oocysts is in group G5 which received BD supplemented with EO blend of Oregano (20 mg/kg diet), thyme (20 mg/kg diet) and Garlic (10mg/kg diet) till the end of the experiment while the highest oocysts numbers were for the control positive group (G7) as a result of Eimeria infection without medicaments. These results are in accordance with those of Giannenas *et al.* [33] who observed that oregano EO, mainly carvacrol and thymol, existed an anticoccidial effect against *E. tenella* and reduced oocyst count shed from infected birds. Similarly, Oviedo-Rondón [34] concluded that EO thymol, carvacrol and eugenol can control the proliferation of Clostridium perfringens in the intestines and could potentially reduced the effects of complications associated with coccidiosis such as necrotic enteritis. Also, the current results are concurrent with those of Kim *et al.* [35] who found that dietary supplementation of broilers with EO enhanced coccidiosis resistance as demonstrated by increased BW gains, reduced fecal oocysts shedding and decreased gut lesions compared with infected birds fed a non supplemented control diet.

CONCLUSION

It was concluded that, supplementation of broilers`diet with essential oil blend composed of either 10 mg/kg diet from Oregano, 10 mg/kg diet from thyme and 5mg/kg diet from Garlic oils or 20 mg/kg diet from Oregano, 20 mg/kg diet from thyme and 10mg/kg diet from Garlic oils up to 35 day of age protect the birds from Coccidiosis and improved their growth performance without significant effect on serum metabolites and carcass traits. However, the lower dietary levels were comparatively more effective than the higher levels.

REFERENCES

1. Yang, Y., P.A. Iji and M. Choct, 2009. Dietary modulation of gut microflora in broiler chickens: A review of therole of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J., 65: 97-114.
2. Kim, S.W., M.Z. Fan and T.J. Applegate, 2008. Non ruminant nutrition symposium on natural phytobiotics for health of young animals and poultry: Mechanisms and application. J. Anim. Sci., 86: E138-E139.
3. Brugalli, I., 2003. Alimentação alternativa: a utilização de fitoterαpicos ou nutracêuticos como moduladores da imunidade e desempenho animal. Anais do Simposio sobre Manejo e Nutrição de Aves e Suínos; Campinas, São Paulo. Brasil. Campinas: CBNA, pp: 167-182.
4. Vekiari, S.A., V. Oreopoulou, C. Tzia and C.D. Thomopoulos, 1993. Oregano flavonoids as lipid antioxidants. J. Am. Oil. Chem. Soc., 70: 483-487.
5. Adam, K., A. Sivropoulou, S. Kokkini, T. Lanaras and M. Arsenakis, 1998. Antifungal activities of *Origanum vulgare* ssp. Hirtum, Menthaspicata, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agric. Food Chem., 46: 1739-1745.
6. Mikaili, P., M.A. Mohammad Nezhady, J. Shayegh and M.H. Asghari, 2010. Study of anticonceptive effect of Thymus Vulgaris and Vulgare Foeniculum essential oil in Mouse. International J. of Academic Research, 2: 1-3.
7. Cross, D.E., R.M. McDevitt, K. Hillman and T. Acamovic, 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poult. Sci., 48: 496-506.
8. Sivam, G.P., 2001. Protection against Helicobacter pylori and other bacterial infections by garlic. J. Nutr., 131: 1106S-1108S.
9. Prasad, G. and V.D. Saharma, 1981. Antifugnal property of garlic (*Allium Sativum Linn*.) in poultry feed substrate, Poult. Sci., 60: 541-545.
10. Kumar, M. and J.S. Berwal, 1998. Sensitivity of food pathogenes to garlic (*Allium Sativum*). J. Appl. Microbiol., 84: 213-215.
11. NRC., 1994. Nutrient Requirements of Poultry. (9th rev.ed.). National Research Council. National Academy Press, Washington, D.C., USA.
12. Long, P.L. and L.P. Joyner, 1984. Problems in the identification of species of Eimeria. Journal of Protozoology, 31: 535-541.
13. Roofchaee, A., M. Irani1, M.A. Ebrahimzadeh and M.R. Akbari, 2011. Effect of dietary oregano essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens. African Journal of Biotech., 10: 6177-6183.

14. Marcincak, S., R. Cabadaj and P. Poplka, 2008. Antioxidative effect of oregano supplemented to broilers on oxidative stability of poultry meat. Slov. Vet. Res., 45: 61-66.
15. Lee, K.W., H. Everts, H.J. Kappert, K.H. Yeom and A.C. Beynen, 2003. Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. J. Appl. Poult. Res., 12: 394-399.
16. Lewis, M.R., S.P. Rose, A.M. Mackenzie and L.A. Tucker, 2003. Effects of dietary inclusion of plant extracts on the growth performance of male broiler. Br. Poult. Sci., 44: S43-S44.
17. Alcicek, A., M. Bozkurt and M. Cabuk, 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. S. Afr. J. Anim. Sci., 33: 89-92. Chem., 46: 1739-1745.
18. Hernandez, F., J. Madrid, V. Garcia, J. Orengo and M. Magias, 2004. Influence of two plant extracts on broilers performance, digestibility and digestive organ size. Poult. Sci., 83: 169-174.
19. Windisch, W., K. Shedle and A. Kroismayr, 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim Sci., 86: E140-E148.
20. Florou-Paneri, P., I. Giannenas, E. Christaki, A. Govaris and N.A. Botsoglou, 2006. Performance of chickens and oxidative stability of the produced meat as affected by feed supplementation with oregano, vitamin C, E and their combinations. Arch. Geflu, 70: 232-240.
21. Demir, E., S. Sarica, M.A. Ozcan and M. Suicmez, 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. Br. J. Poultry Sci, 44: S44-S45.
22. Jawad, H. Ala Al Deen, 2007. Some haematological and biochemical effect of garlic in broiler chickens. Bas. J. Vet. Res., 6(2): 56-63.
23. Fadlalla, L.M.T., B.H. Mohammed and A. Bakhiet, 2010. Effect of feeding garlic on the performance and immunity of broilers. Assian Journal of Poultry science, 4: 182-189.
24. Jain, R.C. and C.R. Vyas, 1975. Garlic in alloxan induced diabetic rabbits. Am. J. Clin. Nutr., 28: 684-685.
25. Banerjee, S.K. and S.K. Maulik, 2002. Effect of garlic on cardiovascular disorders: a review. Nutrition Journal, 1: 4-18.
26. Al-aummar, L.W., 1994. Effect of garlic on blood glucose and cholesterol level in normal and diabetic rabbits, Msc. Thesis, College of Veterinary Medicine, university of Baghdad.
27. Grodsky, G.M., J.H. Karam and P.H. Forsham, 1965. Serum insulin response to glucose in prediabitic subjects, 6: 1: (7380): 290-1.
28. Mansoub, N.H. and M.P. Myandoab, 2011. The effects of different levels of Thyme on performance, carcass traits, blood parameters of broilers. Annals of Biological Research, 2(4): 379-385.
29. Das, S.N., A.K. parmanik, S.K. Mitra and B.N. Mukherjee, 1982. Effect of garlic peak (Ranbaxy) on blood cholesterol level in normal dogs. Ind., Vet. J., 29: 936-938.
30. Ocak, N., G. Erenter, A.K.F. Burak, M. Sungue, A. Altop and A. Ozmen, 2008. Performance of broilers fed diets supplemented with dry peppermint (Mentha Piperita L.) or Thyme (Thymus Vulgaris L.) leaves as growth promoter source. Czech. J. Anim. Sci., 53: 169-175.
31. Raeesi, M., S.A. Hoseini-Aliabad, A. Roofchaee, A. Zare Shahneh and S. Pirali, 2010. Effect of Periodically Use of Garlic (*Allium sativum*) Powder on Performance and Carcass Characteristics in Broiler Chickens. World Academy of Sci., Engine. Techno, 68: 1213-1219.
32. Bampidis, V.A., V. Christodoulou, P. Florou-Paneri, E. Christaki, P.S. Chatzopoulou, T. Tsiligianni and A.B. Spais, 2005. Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. Brit. Poult. Sci., 46: 595-601.
33. Giannenas, I., P. Florou-Paneri, M. Papazaha-riadou, E. Christaki, N.A. Botsoglou and A.B. Spais, 2003. Effect of dietary supplementation with Oregano essential oil on performance of broilers after experimental infection with Eimeria Tenella. Arch. Anim. Nutr., 2: 99-196.
34. Oviedo-Rondón, E.O., S. Clemente-Hernαndez, F. Salvador, P. Williams and R. Losa, 2006. Essential oils on mixed coccidia vaccination and infection in broilers. International Journal of Poultry Science, 5: 723-730.
35. Kim, D.K., H.S. Lillehoj, S.H. Lee, S.I. Jang, E.P. Lillehoj and D. Bravo, 2013. Dietary Curcuma longa enhances resistance against *Eimeria maxima* and *Eimeria tenella* infections in chickens. Poult. Sci., 92: 2635-2643.
36. Lillehoi, H.S., C.H. Kim, C.L. Keeler Jr and S. Zhang, 2007. Immunogenomi approaches to study host immunity to enteric pathogen. Poult. Sci., 86: 1491-1500.

37. Lillehoi, H.S., W. Men and R.A. Dalloul, 2004. Recent progress on the cytokine regulation of intestinal immune response to Eimeria. Poult. Sci., 83: 611-623.
38. Lillehoi, H.S., D.K. Kim, D.M. Bravo and S.H. Lee, 2011. Effects of dietary plant-derived phytonutrients on the genome-wide profiles and Coccidiosis resistance in the broiler chickens. BMC Proc., 5 Suppl. 4: S34.
39. Brody S., 1945. Bioenergetics and growth. 1st Ed., Baltimore,USA., pp: 502-507.
40. Singh, K.S. and B. Panda, 1992. Feed efficiency. "Poultry Nutrition", 2 nd Ed. Kalyam Publishers, Rajinder Nagar, India, pp: 199.