A comparative evaluation of the two commercial essential oils of oregano in broiler diets on growth performance and immune responses was the purpose of this experiment. A total of 200-d-old Ross 308 chicks were used in a completely randomized design with 4 treatments and 5 replicates, with 10 birds in each. Dietary treatments included: (1) control (without phytogenic), (2) commercial blend of phytogenics (CBP; 150 ppm), (3) oregano essential oil (OEO; 300 ppm), and (4) OEO (500 ppm). During the entire period, ADFI was lower (P < .05) in CBP-fed birds than control, but was not different among CBP and either level of OEO (P > .05). European production efficiency factor was greater (P < .05) for broilers fed 300 ppm OEO than those fed control diet or CBP. Broilers fed 300 ppm OEO produced higher secondary total antibody titre against sheep red blood cell (P < .01) and their Immunoglobulin G titre was higher (P < .05) than those fed control or CBP. Serum heterophil counts (P < .01) and heterophils to lymphocytes ratio (P < .05) were lower in birds fed 300 ppm OEO or CBP than the control. Supplementation of OEO at the rate of 300 ppm in diet led to beneficial effects on performance and immune response of broilers.

1. Introduction

Phytogens elicited great interest in animal researches and their application is currently common in the feed industry. Essential oils are usually mixtures of several components that can be included individually or in combination in animal feed. Phytogenics showed beneficial effects on poultry performance and immune-enhancing activity, and anti-inflammatory potential (Acamovic and Brooker 2005). Amad et al. (2011) observed that feed conversion ratio (FCR) improved linearly by the administration of phytogenic feed additive containing essential oils of thyme and star anise in broiler diet. In contrast, Botsoglou et al. (2002) indicated that dietary oregano oil exerted no growth-promoting effect on broilers when added at 50 or 100 ppm to the feed. Although, Cross et al. (2007) reported that dietary thyme had different effects when used as a herb or as an oil on weight gain and body mass, which may primarily relate to differences in the terpene composition. The use of combinations of essential oils and their isolated components is a new approach to increase the efficacy of essential oil and taking advantage of their synergistic and additive effects (Bassole and Juliani 2012).

The immune status of the host plays an important role in resistance to various infections and essential oils may enhance cellular and humoral responses of broilers (Acamovic and Brooker 2005). Hashemipour et al. (2013) reported that feed supplementation with thymol and carvacrol improved the immune response of broilers. However, there is a considerable body of literature providing in vitro evidence of the antibacterial, antifungal, and antiviral activity of plant extracts, and there are fewer in vivo studies that confirm growth-promoting effects (Khattak et al. 2014). Moreover, researches on the comparison of individual and combined essential oils are limited; it seems that their effects may be different. The use of individual instead of combined oregano essential oils may have superior impacts on broiler performance. Therefore, the current experiment was designed to assess the effect of a combined phytogenic feed additive containing essential oils from oregano, anise, and citrus peel in comparison to the other phytogenic containing only essential oils of oregano on growth performance and immune response of broiler chickens.

2. Materials and methods

2.1. Birds, diets, and management

All the experimental procedures used in this research were approved by the Animal Ethics Committee of the University of Guilan. Two hundred-d-old straight-run Ross 308 broiler chicks, with average body weight of 46.1 ± 0.7 g, were used in this study. The chicks were feather sexed and distributed into 20 homogeneous groups of floor pens (1 m × 1 m) according to their sex and initial weight. Pens were randomly assigned to 4 treatments each having 5 replicates of 10 birds. Dietary treatments included: (1) control (without phytogenic), (2) commercial blend of phytogenics (CBP; 150 ppm), (3) oregano oil; performance; immune response; oregano; essential oil; phytogenics; broilers; comparison; growth; immunity; poultry; performance; essential oils; phytogenics; comparison; experimental; feed; diet; management; essential oil; in vitro; antibacterial; antifungal; antiviral; activity; plant; extracts; in vivo; studies; growth; effects; individual; combined; oregano; essential oils; superior; impacts; broiler; performance; experiment; designed; effect; combined; phytogenic; feed; additive; containing; essential; oils; oregano; anise; citrus peel; comparison; only essential; oils; oregano; growth; performance; immune; response; broiler; chickens.
essential oil (OEO; 300 ppm), and (4) OEO (500 ppm). CBP (Digestarom® Biomin GmbH, Herzogenburg, Austria) containing a blend of essential oils from oregano, anise, and citrus peel at 150 ppm was administered. Based on SPME-GC analysis (Mountzouris et al. 2011), carvacrol, the main active compound of the essential oil blend, was 102 g of the chemical component per kg of CBP. The OEO was tested at two rates of 300 and 500 ppm, was in the form of a powder called Orego-Stim® (Meriden Animal Health Ltd., Luton, UK) that contains 5% essential oil of Origanum vulgare subsp. Hirtum plants and 95% natural feed grade inert carrier (Giannenas et al. 2003). Diets were provided in mash form and formulated according to Ross 308 recommendations (Ross Broilers Manual 2012). Feed and fresh water were offered ad libitum throughout the experiment. Table 1 shows ingredients and nutrient contents of the basal diet. Chicks were kept on wood shavings at 32°C during the first 7 d of age, and then the temperature was stepped down to 23°C by 21 d of age. There was a continuous light regimen during the first 2 d, and then 23 h lighting was applied up to 42 d of age. Light intensity was 20 lux in the first 2 d; then it was decreased to 5 lux for the remaining period (3–42 d). All the chicks were vaccinated based on a routine programme.

### Table 1. Composition and calculated nutrient composition of the basal diets (g/kg, as fed basis)

| Item                      | Starter (d 0–10) | Grower (d 11–24) | Finisher (d 25–42) |
|---------------------------|------------------|------------------|-------------------|
| Ingredient                |                  |                  |                   |
| Corn                      | 533.10           | 561.50           | 596.10            |
| Soybean meal, 44% CP      | 392.60           | 357.20           | 319.00            |
| Soybean oil               | 28.30            | 41.60            | 47.30             |
| Dicalcium phosphate       | 20.00            | 17.80            | 16.80             |
| Calcium carbonate         | 11.80            | 9.50             | 9.30              |
| Common salt               | 3.60             | 2.90             | 3.00              |
| L-Lysine HCl, 7%          | 1.80             | 0.80             | 0.40              |
| dl-Methionine, 98%        | 3.00             | 2.40             | 2.00              |
| L-Threonine, 99%          | 0.80             | 0.30             | 0.10              |
| Vitamin premixa           | 2.50             | 2.50             | 2.50              |
| Mineral premixb           | 2.50             | 2.50             | 2.50              |
| Sodium bicarbonate        | 0.00             | 1.00             | 1.00              |
| Total                     | 1000             | 1000             | 1000              |
| Calculated composition    |                  |                  |                   |
| Metabolizable energy, kcal/kg | 2870            | 3000             | 3080              |
| Crude protein, g/kg       | 218.20           | 204.80           | 190.60            |
| Digestible lysine, g/kg   | 12.00            | 10.50            | 9.30              |
| Digestible methionine, g/kg | 6.00           | 5.20             | 4.70              |
| Digestible methionine + cysteine, g/kg | 8.90      | 8.00             | 7.30              |
| Digestible threonine, g/kg | 7.90           | 7.00             | 6.30              |
| Calcium, g/kg             | 10.00            | 8.60             | 8.20              |
| Available phosphorus, g/kg | 4.70            | 4.30             | 4.00              |
| Total phosphorus, g/kg    | 7.50             | 7.00             | 6.70              |
| Sodium, g/kg              | 1.60             | 1.60             | 1.60              |
| Potassium, g/kg           | 9.10             | 8.50             | 7.90              |
| Chloride, g/kg            | 2.90             | 2.30             | 2.30              |
| DEB, meq/kg               | 221              | 222              | 207               |

Vitamin premixa provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 5000 IU; vitamin E (dl-a-tocopherol acetate), 50 IU; vitamin K₃ (bisulphate menadione complex), 3 mg; thiamine (thiamine mononitrate), 3 mg; riboflavin, 9 mg; nicotinic acid, 50 mg; pantothenic acid (o-calcium pantothenate), 15 mg; vitamin B₆, 4 mg; d-biotin, 0.1 mg; folic acid, 2 mg; vitamin B₁₂ (cyanocobalamin), 0.02 mg and choline (choline chloride), 1000 mg.

Mineral premixb provided the following per kilogram of diet: iron (FeSO₄·7H₂O), 55 mg; iodine (Ca(IO₃)₂), 1.3 mg; manganese (MnSO₄·H₂O), 120 mg; zinc (ZnO), 100 mg; copper (CuSO₄·5H₂O), 16 mg; selenium (Na₂SeO₃), 0.3 mg.

DEB = dietary electrolyte balance; Na + K + Cl.

### 2.2. Collection of samples and measurements

Body weight of the birds and feed consumption were recorded weekly by replicate, and mortality was recorded and weighed at occurrence. From these data, average daily body weight gain (BWG), average daily feed intake (ADFI), and FCR were calculated by week and for the entire experimental period. At the end of the experiment (d 42), the European production efficiency factor (EPEF) was calculated using the following formula: BW (kg) × % liveability × 100/FCR × trial duration (d) (Huff et al. 2013).

### 2.3. Immune measurements

In order to assay the primary and secondary immune responses, at d 28 and 35 two chicks per pen were injected intravenously (brachial vein) with 0.1 ml/kg BW of 0.5% suspension of sheep red blood cell (SRBC) in phosphate buffer saline. On seventh day after each injection (d 35; primary immune response and d 42; secondary immune response), blood was collected from the brachial vein of the two same selected bird and serum was obtained by centrifugation at 1500 × g for 15 min at 15°C, and stored at −20°C for further analysis. Total antibody titres against SRBC were determined by agglutination, according to Wegmann and Smithies (1966). To determine IgG antibody titre (β-mercaptoethanol, 2-ME resistant), 25 µl of serum were incubated with 25 µl of 0.2 M 2-ME in the first column of a 96-well U-bottom microplate at 37°C for 1 h and then haemagglutination test was performed as for total antibody titre. To determine IgM antibody titre, IgG titre was subtracted from total antibody titre. All serum samples were tested in duplicate. Antibody titres measured against SRBC were expressed as the log₂ of the reciprocal of the highest serum dilution giving complete agglutination.

### 2.4. Hematological parameters

At d 42, two birds per replicate were selected and their blood samples were collected into tubes with EDTA to avoid blood clot formation for hematological analysis. The proportions of the individual leukocyte types (in 200 cells) were determined by classical histological methods using a light microscope with an immersion lens after staining blood smears with May-Grünwald and Giemsa-Romanowski solutions. Blood samples were prepared on slides and painted by the Giemsa method. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope, and then heterophils to lymphocytes ratio (H/L) was measured. The white blood cell (WBC) counts were determined by an improved Neubauer hemocytometer method (Jain 1986). The hematocrit values were measured by microhematocrit methods (Baker and Sliverton 1985).

### 2.5. Statistical analyses

Normal distribution of residuals and variance homogeneity of the data were tested by UNIVARIATE procedure and the Levene’s test, respectively. The experimental unit was the selected chicks for blood hematological parameters and
antibody production, constituting two observations in each replicate. For all the remaining studied traits including average daily BWG, ADFI, FCR, and EPEF, the pen was used as the experimental unit. Data were analysed as a completely randomized design using GLM procedure of SAS (SAS Institute 2002) and differences among the individual means were compared by the Tukey test ($P < .05$).

### 3. Result

#### 3.1. Performance

Mortality was <1% and was not related to the treatments. During the entire period, except from d 22 to 28 and d 22 to 42, all groups indicated similar average daily BWG (Tables 2–4). From d 22 to 28, average daily BWG of the control group or broilers fed 300 ppm OEO was higher ($P < .05$) than those fed 500 ppm OEO in the diet. From d 22 to 42, supplementation of 300 ppm OEO in the diet increased on average daily by BWG ($P < .05$) compared with the birds in the other groups.

During the entire period, except from d 22 to 28, all groups indicated similar ADFI (Tables 2–4). From d 22 to 28, broilers fed diets with 300 ppm OEO had higher ADFI ($P < .05$) than those fed the other supplements, whereas none of them were different from the control group ($P > .05$). From d 1 to 21, supplementation of 300 ppm OEO to the diet decreased ADFI when compared to the control diet (53.8 vs. 58.5 g; $P < .05$). Broilers fed CBP had lower ADFI compared to those fed control (165.8 vs. 175.0 g; $P < .05$) from d 22 to 42. For the entire experimental period, birds fed diet supplemented with CBP exhibited lower ADFI compared to the control group (109.2 vs. 115.3 g; $P < .05$), but no effect ($P > .05$) was observed from either level of OEO. From d 29 to 35, dietary supplementation of any of the tested phytogenics improved FCR ($P < .05$; Table 3) than

### Table 2. Effects of dietary oregano essential oil and the phytogenic blend on average daily body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) from 1 to 21 d of age.

| Item | 1–7 d | 8–14 d | 15–21 d |
|------|-------|--------|---------|
|      | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g |
| Control | 18.9 | 27.0 | 1.43 | 34.9 | 56.7 | 1.62 | 54.7 | 91.8 | 1.68 |
| CBP | 18.4 | 26.1 | 1.42 | 32.0 | 52.6 | 1.65 | 52.7 | 87.9 | 1.68 |
| OEOd 300 | 18.0 | 26.0 | 1.45 | 32.2 | 50.3 | 1.56 | 53.0 | 85.1 | 1.61 |
| OEO 500 | 18.3 | 26.4 | 1.44 | 33.8 | 52.5 | 1.55 | 55.8 | 89.0 | 1.60 |
| SEM, ($n = 5$) | 0.47 | 0.57 | 0.018 | 1.23 | 1.62 | 0.034 | 1.71 | 1.95 | 0.033 |
| $P$-value | 0.610 | 0.615 | 0.606 | 0.293 | 0.078 | 0.154 | 0.573 | 0.148 | 0.188 |

aCommercial blend of phytogenic.

bOregano essential oil.

cStandard error of mean.

### Table 3. Effects of dietary oregano essential oil and the phytogenic blend on average daily body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) from 22 to 42 d of age.

| Item | 22–28 d | 29–35 d | 36–42 d |
|------|--------|--------|--------|
|      | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g |
| Control | 74.4a | 129.5ab | 1.74 | 94.6 | 183.1 | 1.94a | 100.7 | 219.7 | 2.18 |
| CBP | 69.2ab | 122.8b | 1.78 | 94.3 | 169.0 | 1.79b | 103.9 | 212.2 | 2.06 |
| OEOd 300 | 74.4a | 131.9a | 1.77 | 99.9 | 180.1 | 1.80a | 111.9 | 226.1 | 2.02 |
| OEO 500 | 67.7b | 122.1b | 1.80 | 95.9 | 174.9 | 1.82b | 106.8 | 219.7 | 2.06 |
| SEM, ($n = 5$) | 1.76 | 2.50 | 0.029 | 1.48 | 3.61 | 0.035 | 3.28 | 4.38 | 0.049 |
| $P$-value | .029 | .032 | .546 | .058 | .065 | .043 | .140 | .205 | .156 |

aMeans within a column with different superscripts are significantly different ($P < .05$).

bCommercial blend of phytogenic.

cOregano essential oil.

dStandard error of mean.

### Table 4. Effects of dietary oregano essential oil and the phytogenic blend on average daily body weight gain (BWG), average daily feed intake (ADFI), feed conversion ratio (FCR), and European production efficiency factor (EPEF) from 1 to 42 d of age.

| Item | 1–21 d | 22–42 d | 1–42 d | EPEF |
|------|--------|--------|--------|------|
|      | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g | BWG, g/d | ADFI, g/d | FCR, g/g | EPEF |
| Control | 36.2 | 58.3a | 1.62 | 89.4a | 175.0a | 1.96 | 62.1 | 115.3a | 1.86 | 362.2a |
| CBP | 34.3 | 55.6ab | 1.62 | 88.4a | 165.8b | 1.88 | 60.7 | 109.2b | 1.81 | 370.3b |
| OEOd 300 | 34.4 | 53.8b | 1.57 | 94.6a | 177.1a | 1.87 | 63.8 | 113.9b | 1.79 | 398.0b |
| OEO 500 | 36.0 | 56.0ab | 1.56 | 89.2a | 169.9ab | 1.90 | 62.0 | 111.5b | 1.80 | 377.9ab |
| SEM, ($n = 5$) | 0.66 | 0.98 | 0.019 | 1.28 | 2.44 | 0.027 | 0.76 | 1.46 | 0.019 | 7.73 |
| $P$-value | .125 | .029 | .548 | .014 | .020 | .120 | .079 | .048 | .080 | .022 |

aMeans within a column with different superscripts are significantly different ($P < .05$).

bCommercial blend of phytogenic.

cOregano essential oil.

dStandard error of mean.
the control group. However, no significant effect from any treatment was observed on FCR (P > .05) in the other periods. Broilers fed either 300 or 500 ppm of OEO were equal in EPEF (P > .05) but were higher (P < .05; Table 4) in comparison to broilers fed the control diet or CBP.

### 3.2. Immune response

Regarding primary antibody response (at 35 d of age), no difference was observed between treatments for antibody titres against SRBC (P > .05; Table 5). Broilers fed 300 ppm OEO in diet had higher titres for IgG (P < .05) and total antibodies (P < .01) in secondary response (at 42 d of age) compared with those fed control diet or those fed CBP, but no significant effect (P > .05) were detected among either supplementation rate of OEO.

### 3.3. Hematological parameters

Compared with the control group, broilers fed 300 ppm OEO or CBP in diet indicated lower counts of heterophil (P < .01; Table 6) and heterophils to lymphocytes ratio (P < .05); however, there was no difference (P > .05) between the two levels of OEO. The other determined parameters, including hematocrit percentage, WBC count, lymphocytes and monocytes count were not influenced (P > .05) by any of the phytogenics.

### 4. Discussion

Improved FCR from d 28 to 35 by phytogenic supplementation to the diets could be explained by higher secretions of endogenous digestive enzymes, which enhance nutrient digestion and gut passage rate in broilers (Lee et al. 2003, 2004). Amad et al. (2011) reported that phytogenic feed additives improved apparent ileal digestibility of nutrients. However, other researchers did not observe any positive impacts of phytochemical compounds on broiler performance (Botsoglou et al. 2002; Celikbilek et al. 2014). In the current trial, the inclusion of 300 ppm OEO in broiler diets increased average daily BWG from d 22 to 42. This finding is consistent with the results of Khattak et al. (2014) who reported that dietary supplementation of 300 ppm OEO, with thymol and carvacrol as its major component, increased BWG of broilers from d 22 to 42. Hashemipour et al. (2013) reported that supplementation of thymol and carvacrol at a level of 200 ppm in broiler diets enhanced BWG, and decreased ADFI from d 25 to 42. Moreover, Alp et al. (2012) reported that broilers fed the OEO in diets consumed less feed and had better FCR from d 21 to 42 and from d 1 to 42 than those fed control diet. However, the present results contradict the findings of Amad et al. (2011) who reported no difference in BWG and ADFI (in grower phase and total period) in broilers fed diets supplemented with thyme and star anise. Hernandez et al. (2004) reported that the inclusion of 5000 ppm of phytochemical compounds containing sage, thyme, and rosemary oils increased growth rate compared with the inclusion of 200 ppm phytogenic compounds containing oregano, cinnamon, and pepper in broilers from d 14 to 21. A probable reason for improved growth performance parameters with OEO in the present study could be related to the digestion-stimulating effect of essential oils, as reported by Langhout (2000) and Williams and Losa (2001).

In the current study, dietary supplementation of CBP reduced ADFI and FCR from d 1 to 42 and from d 28 to 35, respectively. Mountzounis et al. (2011) reported that overall feed intake decreased quadratically when the CBP levels (80, 125, and 250 ppm) increased in broiler diet. They also had indicated that feeding CBP to broilers improved linearly FCR from d 29 to 35 and d 1 to 42. However, the current results contrast with the findings of Reisinger et al. (2011) who reported no difference in feed intake or FCR of broilers fed diets supplemented with 125 ppm the CBP. A potential reason for inconsistent results of previous experiments that studied the growth performance of broiler fed essential oil may be the content of active substances in essential oil samples. In the current study, dietary supplementation of 300 ppm OEO resulted in better growth performance compared to CBP-fed broilers. A similar finding was reported by Peric et al.

### Table 5. Effects of dietary oregano essential oil and the phytogenic blend on antibody production (log).

| Item | Primary (35 d of age) | Secondary (42 d of age) |
|------|----------------------|------------------------|
|      | Total antibody | IgG | IgM | Total antibody | IgG | IgM |
| Control | 3.21 | 1.84 | 1.43 | 3.42<sup>c</sup> | 2.01<sup>b</sup> | 1.42 |
| CBP<sup>a</sup> | 2.83 | 1.82 | 1.01 | 3.63<sup>b</sup> | 2.41<sup>b</sup> | 1.22 |
| OEO<sup>c</sup> 300 | 4.01 | 2.43 | 1.62 | 5.03<sup>b</sup> | 3.63<sup>b</sup> | 1.41 |
| OEO 500 | 3.42 | 2.01 | 1.43 | 4.42<sup>a,b</sup> | 2.82<sup>a,b</sup> | 1.63 |
| SEM<sup>e</sup>, (n = 10) | 0.631 | 0.424 | 0.282 | 0.312 | 0.374 | 0.232 |
| P-value | .179 | .442 | .277 | .007 | .046 | .698 |

<sup>a,b</sup>Means within a column with different superscripts are significantly different (P < .05).
<sup>c</sup>Commercial blend of phytogenic.
<sup>e</sup>Oregano essential oil.
<sup>f</sup>Standard error of mean.

### Table 6. Effects of dietary oregano essential oil and the phytogenic blend on hematological parameters at 42 d of age.

| Item | Hematocrit | WBC<sup>c</sup> | Heterophil | Lymphocyte | Monocyte | Het/Lym |
|------|------------|----------------|------------|------------|----------|---------|
| Control | 32.9 | 41775 | 38.4<sup>c</sup> | 61.2 | 2.75 | 0.66<sup>c</sup> |
| CBP<sup>a</sup> | 31.1 | 45125 | 28.0<sup>c</sup> | 71.0 | 2.25 | 0.40<sup>b</sup> |
| OEO<sup>c</sup> 300 | 30.7 | 43875 | 28.0<sup>c</sup> | 69.1 | 2.60 | 0.41<sup>b</sup> |
| OEO 500 | 31.2 | 42625 | 33.4<sup>b</sup> | 62.0 | 1.80 | 0.54<sup>b</sup> |
| SEM<sup>e</sup>, (n = 10) | 1.07 | 2626.3 | 1.77 | 2.89 | 0.379 | 0.055 |
| P-value | .481 | .817 | .002 | .060 | .328 | .012 |

<sup>c</sup>WBC = white blood cell.
<sup>e</sup>Heterophil to lymphocyte ratio.
<sup*f</sup>Commercial blend of phytogenic.
<sup>e</sup>Oregano essential oil.
<sup>f</sup>Standard error of mean.
(2010) who observed no significant effect on EPEF by the CBP supplementation to broiler diet.

Moreover, in the current study broilers fed 300 ppm OEO in diet indicated higher IgG titres and total titre of antibodies in response to SRBC compared with those fed control diet or those fed CBP. Similar results have been reported by Hashemipour et al. (2013) who observed that the inclusion of 200 ppm thymol and carvacrol to broilers’ diets improved immune response by increasing IgG and total antibody titre. However, Alp et al. (2012) observed a slight increase in the serum IgG titre of broilers fed 300 ppm OEO in the diet. Hong et al. (2012) reported that IgG and total antibody titres were not affected by 125 ppm of CBP supplementation, which is similar to our finding by CBP supplementation. It is indicated that carvacrol inhibited the production of prostaglandin E₂, suggesting carvacrol has an anti-inflammatory effect (Luna et al. 2010). Acamovic and Brooker (2005) reported immunostimulating activity of polyphenol fraction of thymol and OEO with respect to the system of mononuclear phagocyte system, cellular, and humoral immunity.

The reliability of heterophils to lymphocytes ratio as a biological index of stress in birds is well documented. In the current study, supplementation of 300 ppm OEO or CBP to broiler diets reduced heterophils and the ratio of heterophils to lymphocytes. Stressors generally elevate the number of heterophils and depress the number of lymphocytes in birds (McFarlane and Curtis 1989). Inflammation, whether it results from either feed or disease, inversely effects the growth and health. Nuclear factor kappa B (NF-kB) acts as a master regulator of inflammatory process which up-regulates a battery of genes mediating inflammatory response (cytokines, chemokines, and adhesion molecules) and it is also stimulated by antigens such as bacteria, viruses, toxins, and heat stress. The CBP decreases the release of NF-kB, reduces mRNA level and reduces inflammatory proteins. The condition of inflammation is always dominated by heterophils in species condition and length of the inflammation. Lymphocytes and cell plasma increased in the chronic inflammation, when there is a virus infection. Few hours after infection, the number of heterophil cells in the blood increased. It is caused by inflammation products which enter the blood and push the heterophil cells to enter the blood circulation faster, and hence make a lot of heterophil cells in the inflamed tissues (Hall 2016). Thymol used alone exhibits some local anti-inflammatory properties, as indicated by a decrease in TNF-mRNA in the stomach of post-weaned pigs (Trevisi et al. 2010). Reports on the effect of OEO supplementation to poultry diet on blood hematological parameters are very rare. Al-Kassie (2008) reported that dietary supplementation of oil extract derived from thyme and cinnamon increased hematocrit and WBC of broilers.

5. Conclusion

It is concluded that there is a difference in broiler response to individual or combined phytogenics. Dietary inclusion of 300 ppm OEO improved performance which led to greater EPEF. Moreover, the titre of antibody against SRBC increased by 300 ppm OEO. Either individual usage of 300 ppm OEO or combined phytogenics decreased heterophil counts and heterophils to lymphocytes ratio, which is an index for stress status.

Disclosure statement

No potential conflict of interest was reported by the authors.

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