Thyme oil (*Thyme vulgaris* L.) as a natural growth promoter for broiler chickens reared under hot climate

Youssef A. Attia, Ahmed A. Bakhashwain and Nehal K. Bertub

Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia; Animal Production Administration, Agriculture Directorate - El Beheira, Ministry of Agriculture and Land Reclamation, El-Beheira, Damanhour, Egypt

**ABSTRACT**

This study aims to utilise thyme oil (TO) as a natural growth promoter in comparison to mannanoligosaccharides (MOS) for broilers raised under hot climates from 1 to 28 days of age. Thus, a total of 180, day-old broilers chicks were divided into five groups (36 broilers/group in 6 replicates of 6 broilers/replicate). The chickens were fed the same corn-soybean meal basal diet and were submitted to one of the following five dietary treatments: (a) positive control group, fed the basal diet supplemented with MOS at 1g/kg feed; (b) negative control group, fed the basal diet without supplementation; (c) thyme oil 1.0 (TO_1.0) group fed TO at 1.0 g/kg feed, (d) thyme oil 1.5 (TO_1.5) group fed TO at 1.5 g/kg feed and (e) thyme oil 2.0 (TO_2.0) group fed TO at 2.0 g/kg feed. The TO_1.0 displayed a better feed conversion ratio (FCR) than did the other treatments (*p* < .01). The MOS, TO_1.5 and TO_2.0 groups had higher (*p* < .01) plasma total protein than the control, and TO_1.5 also increased plasma globulin (*p* < .01) compared to the control, but decreased plasma albumin/globulin ratio. Moreover, the TO groups significantly decreased the plasma AST. Groups on the MOS and TO_1.0 diets showed higher (*p* < .01) white blood cells (WBCs) than the other groups. In addition, the MOS and TO_2.0 groups exhibited a greater (*p* < .01) antibody titre to infectious bursa disease (IBD) than the control group. In conclusion, TO at 1.0 g/kg diet may be used as a potential growth enhancer for broilers in hot region during 1–28 days.

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**Introduction**

Worldwide, high environmental temperatures have resulted in negative influences on broiler production. Thus, nutritional management, such as the use of natural growth promoters, can increase growth and immune responses while decreasing economic losses from deaths, culling and morbidity.

Among the 49 phytogenic plants, thyme (*Thyme vulgaris* L.) is a medicinal plant that is used for the medical and 50 spices purposes worldwide. This vegetation is spread throughout the Mediterranean area and is well adopted to hot and dry summer weather. Thyme volatile essential oils are present in its countless glandular hairs in different forms, evaporate when the glandular hairs are damaged and produce concentrated essences that surround the plant. It is probably due to thyme’s strong scent that humans have always been attracted to this plant and have exploited its essential volatile oils for popular and industrial utilisation (Stahl-Biskup & Saez 2002).

Plant extracts can have a beneficial impact on animal growth, health status and welfare under high temperature environments (Attia et al. 2016; Sethiya 2016). Feeding strategies are the most practical ways to alleviate the negative effects of high environmental temperatures on animal performance, health status and immunity (Attia et al. 2009; Bovera et al. 2013; Akbarian et al. 2014).

The ban on the use of antibiotic growth promoters (AGP) in animal diets, per European Union Reg. no. 1831/2003/EC, has increased many health problems and losses (Attia et al. 2016). Thus alternatives to antibiotic growth promoter such as probiotics, prebiotics, medical herbs and essential oils have become essential (Bozkurt et al. 2012; Mašek et al. 2014; Hady et al. 2016).

Plant extracts, such as essential or volatile oils, are usually utilised in animal feeding and are considered growth and immune enhancers due to their antioxidant, antimicrobial and digestion properties (Abdulkarimi et al. 2011; Assiri et al. 2016;
Fallah & Mirzaei (2016). The main functions of volatile essential oils are the control of pathogenic bacteria, the stimulation of endogenous digestive enzyme activity, increasing absorbed nitrogen and the control of excreta odour and ammonia content (Bölükbäşi et al. 2006; Sethiya 2016). The antimicrobial action of volatile oils consists of a change in cell membrane permeability, cations’ permeability for K+ and H+ (Alcicek et al. 2004).

Thymol and carvacrol are the major components of herbal oil, which form 20–55% of thyme oil extract, and showed considerable antimicrobial properties with no negative effects such as residues in animal meat and bacterial cross resistance (Cross et al. 2003; Sengul et al. 2008). It was demonstrated that thymol and carvacrol concentrations from 100 to 1000 ppm displayed a positive effect on broiler production (Hosseini et al., 2013; Pourmahmoud et al. 2013) and on blood metabolites and immune responses (Fallah & Mirzaet 2016). The difference in results cited in literature regarding the effect of thyme on animal performance may be due to the different thyme products used in the trials (oil, powder or different extracts), emphasising the need for continued research.

MOS, a yeast cell wall component, along with chitin, mannan and glucans are known as immunostimulants and have been used as a growth promoter in animal nutrition, as reviewed by Rodriguez et al. (2003), Hooge (2004) and Bovera et al. (2016). Prebiotics such as MOS are able to improve growth performance and the immune status of different animal species (Attia et al. 2014a,b). Besides, it is the well-known effects as immune stimulants, MOS was found to enhance nutrient digestibility, and improve gastrointestinal beneficial microbiota and the intestinal morphology as also reviewed by Hooge (2004), Rosen (2007) and Hooge and Connolly (2011).

The present study aims to utilise thyme oil as a safe and environmental friendly growth promoter in comparison to MOS on the growth performance, biochemical, haematological and immune indices of broilers raised under hot climate conditions from 1 to 28 days of age.

Materials and methods

A total of 180, one day-old Arbor Acres broilers were wing banded and randomly distributed among five treatment groups, keeping their initial body weight (BW) similar. Thus, each group consisted of 36 broilers, six replicates of six broilers per replicate.

During 1–28 days of age, the groups were fed the same corn-soybean meal basal mash diet. During the same period, they were submitted to the following dietary treatments: (a) positive control group, fed the basal diet supplemented with mannanoligosaccharides (MOS, Alltech Inc., Nicholasville, KY) group fed MOS at 1g/kg feed; (b) negative control group, fed the basal diet without supplementation; (c) thyme oil (TO) 1.0 (TO_1.0, steam distilled product obtained from the local market in Jeddah city, SA) group fed TO at 1.0 g/kg feed, (d) thyme oil 1.5 (TO_1.5) group fed TO at 1.5 g/kg feed and (e) thyme oil 2.0 (TO_2.0) group fed TO at 2.0 g/kg feed.

The feeds were mixed weekly with the additives and kept in tied, double layered plastic bags in a well ventilated room at 25 °C. The levels of TO oil were chosen based on previous results reported by Cross et al. (2003), Bölükbäşi et al. (2006) and Feizi et al. (2013). The basal diet was formulated according to NRC (1994) to satisfy the broilers’ requirements. Samples of the feeds were collected and their chemical-nutritional characteristics were determined according to AOAC (2004) or calculated according to NRC (1994), (Table 1). The antioxidant activity and fatty

Table 1. Composition and calculated and measured analyses of the starter diet fed from 1 to 6 days of age and the basal experimental diet fed from 1 to 28 days of age.

| Ingredients                                      | Starter, 1–15 days of age | Grower 16–28 days of age |
|--------------------------------------------------|---------------------------|--------------------------|
| **Dry matter, g/kg**                             |                           |                          |
| Maize                                            | 511.0                     | 518.5                    |
| Ryde                                             | 0.0                       | 50.0                     |
| Soybean meal (44% CP)                            | 328.2                     | 244.2                    |
| Vegetable oil                                    | 22.5                      | 20.0                     |
| Full fat soybean meal                            | 100.0                     | 130.0                    |
| Dicalcium phosphate                              | 18.0                      | 16.0                     |
| Limestone                                        | 10.0                      | 10.0                     |
| L-Lysine                                         | 1.0                       | 1.5                      |
| α-Methionine                                     | 1.5                       | 2.0                      |
| Vitamin and minerals premixα                     | 4.5                       | 3.0                      |
| NaCl                                             | 3.0                       | 4.5                      |
| Washed building sand                             | 0.3                       | 0.3                      |
| **Total**                                        | 1000.0                    | 1000.0                   |
| **Determined and calculated analyses**           |                           |                          |
| Dry matter, g/kg                                | 866.8                     | 875.5                    |
| ME, MJ/kg                                       | 12.72                     | 12.98                    |
| CP, g/kg                                        | 228.0                     | 211.2                    |
| Lysine, g/kg                                    | 13.3                      | 12.3                     |
| Methionine, g/kg                                 | 5.0                       | 5.2                      |
| Calcium, g/kg                                   | 9.1                       | 8.5                      |
| Available P, g/kg                                | 4.6                       | 4.1                      |
| Crude fat, g/kg                                  | 60.9                      | 64.5                     |
| Crude fibre, g/kg                                | 35.5                      | 35.1                     |
| Ash, g/kg                                       | 52.2                      | 54.8                     |
| NFE, g/kg                                       | 623.4                     | 634.4                    |

*Provided per kilogram of the diet: vitamin A (beta-carotene), 7200 g; vitamin E (α-tocopheryl acetate), 20 mg; menadione, 2.3 mg; vitamin D₃, 55 g; riboflavin, 5.5 mg; calcium pantothenate, 12 mg; nicotinic acid, 50 mg; choline, 250 mg; vitamin B₁₂, 10 g; vitamin B₆, 3 mg; thiamine, 3 mg; folic acid, 1 mg; and α-biotin, 0.05 mg. Trace mineral (mg/kg) of diet: Mn, 80; Zn, 60; Fe, 35; Cu, 8; and Se, 0.1 mg.

*Analysed values (AOAC 2004).

*Calculated values.
acids profiles were determined in TO according to the method of Benzie and Strain (1998) and Radwan (1978), respectively.

The broilers were housed in battery brooders (35cm ×25cm ×30cm) in semi-opened housing with feed and water available ad libitum. The light programme was as follows: until 7 days of age, 23h light, followed by 20h of light from 8 to 28 days of age. The average outdoor minimum and maximum temperature and relative humidity during the experimental period were 28.6 and 35.2 °C (33.6 ± 3.1 °C), and 53.2 and 64.5% (55.8 ± 4.9%), respectively. The brooding temperatures (indoors) were 33.2, 28.3 and 26.7 °C during 1–7, 8–18 and 15–20 days of age, respectively. The chicks were vaccinated with Clone 30 at day 8, with dead H5N2 Avian Flu and Newcastle disease virus (NDV) at days 10, and with Clone 30 and Gumboro at day 21.

The broilers were weighed at 1 and 28 days of age, and the BWG (g/bird) per replicate was calculated. Feed intake was recorded for each replicate (g/bird) to calculate the feed conversion ratio (FCR, g feed/g gain). The survival rate (SR, 100− mortality rate) during 1–28 days of age was recorded. The European production efficiency index (EPEI) was calculated, as cited by Attia et al. (2012).

At 28 days of age, six blood samples per treatment (one per replicate) were collected from the wing vein. The blood samples were put into both non-heparinised and heparinised tubes. Blood serum and plasma were separated by centrifugation at 1500 g for 10 min at 4 °C and stored at −18 °C until the analyses were performed.

All biochemical traits of the blood plasma (total protein, albumin, alanine aminotransferase activity [ALT] and aspartate aminotransferase activity [AST]) were determined using the commercial diagnostic kits (Diamond Diagnostics Company, Egypt), as reported by Attia et al. (2011a,b). Globulin concentration was calculated as the difference between total protein and albumin.

Red blood cells (RBC), white blood cells (WBC), different types of leukocytes, the haemoglobin concentration, packed cell volume (PCV), average volume size of RBC (MCV), average weight of the haemoglobin in RBC (MHC) and the average concentration of haemoglobin in the RBC (MCHC) were measured, as reported by Attia et al. (2013).

Plasma malondialdehyde (MDA) was determined according to the method illustrated by Richard et al. (1992). The serum antibody titre for NDV was done using the Haemagglutination inhibition test (HI) according to the method described by Takatsy (1956), and that for IBD by enzyme-linked immunosorbent assay, performed according to the method described by Snyder et al. (1984).

Statistical analyses were performed using the GLM procedure of the Statistical Analysis Software (SAS® 2003) using a one-way ANOVA according to the following model:

\[ y_{ij} = \mu + \tau_i + \epsilon_{ij} \]

where \( y \) = the dependent variables; \( \mu \) = general mean; \( \tau \) = supplement effect and \( \epsilon \) = random error.

The mean differences at \( p < .05 \) were tested using the Student–Newman–Keuls test. The survival rate was analysed as percentage using chi-square analyses. The experimental unit was the replicate.

Results

The antioxidant activity, % inhibition of TO found herein is 69.5% (Table 2). The major fatty acids content in TO were linoleic acid, oleic acid, palmitic acid and stearic in descending order. The effect of different supplementations on growth performance from 1 to 28 days of age of broilers raised in a hot climate is found in Table 3. The dietary treatments had no effects on BWG.

Feed intake was reduced in the group supplemented with TO at 1 g/kg in comparison to the other groups (\( p < .01 \)). Broilers that were fed diets supplemented with TO at 1.0 g/kg had a more favourable FCR than the other TO groups (\( p < .01 \)), but they were not different from the control and MOS groups. EPEI was significantly greater for the MOS and TO_1 groups than for the other groups. Broilers chickens fed MOS

| Fatty acids profile and antioxidant activity of thyme powder and thyme oil. |
|-----------------------------------------------|
| Fatty acid | Thyme oil |
| Caprylic acid, C8:0 | 0.52 |
| Capric acid, C10:0 | 0.15 |
| Lauric acid, C12:0 | 0.18 |
| Triolein acid, C13:0 | 0.08 |
| Myristic acid, C14:0 | 1.07 |
| Pentadecanoic acid, C15:0 | 1.23 |
| Palmitoleic acid, C16:1 | 2.30 |
| Palmitic acid, C16:0 | 12.07 |
| Linolenic acid, C18:3c | 0.61 |
| Linoleic acid, C18:2c | 41.73 |
| Oleic acid, C18:1c | 33.04 |
| Stearic acid, C18:0 | 6.15 |
| SFA | 21.45 |
| MUFA | 35.34 |
| PUFA | 42.34 |
| UFA | 77.68 |
| SFA/UFA ratio | 27.61 |
| PUFA/MUFA | 119.7 |

Antioxidant activity, % inhibition of TO

69.5

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids.
and TO at 1.5 and 2.0 g/kg had higher plasma total protein than the control (p < .01). The group fed TO at 1.5 g/kg had a higher value of globulin in comparison to the control group (Table 4). The albumin to globulin ratio was lower in the TO_1.5 group (p < .05) than in the control group. The blood levels of AST observed in the control and MOS groups were higher than those measured in the TO groups.

There were no effects for different supplementations on the RBC characteristics (Table 5). The effect of dietary supplementations on WBC counts and different WBCs constituents of 28-day-old broilers under hot climate conditions are found in Table 6. The groups on the MOS supplemented diets showed a higher (p < .01) value of WBC than the other groups. In addition, TO_1 group showed higher WBC than the other TO groups. The monocyte, basophil, eosinophil and heterophil percentages observed in the blood of the broilers did not differ among the experimental groups.

The serum antibody titre for IBD was higher (p < .01) in the MOS and TO_2.0 groups than in the control group (Table 7). No significant differences were shown in serum MDA, the heterophil/lymphocyte (H/L) ratio and the survival rate of the different groups.

### Discussion

In general, TO_1 had comparable FCR and EPEI to MOS and showed more desirable FCR (−7.5%) and EPEI (+10.6) than the control group. The present results agree with the findings of Cross et al. (2003), El-Ghousin and Al-Betawi (2009), Najafi and Torki (2010) and Maşek et al. (2014). They reported that thymol had an enhancing effect on the growth performance of broilers. In addition, Feizi et al. (2013) showed that TO improved growth performance and reduced the mortality of broilers. On the other hand, Hosseini et al. (2013) and Fallah and Mirzaei (2016) concluded that thyme powder at 1, 1.5 and 5 g/kg diet did not affect the growth performance of broilers, and the hot water extracted thyme administered at 5g/l did not affect the growth performance of broilers during 1–21

### Table 3. Effect of different dietary thyme oil concentrations and MOS on growth performance of broilers under hot climate during 1–28 days of age.

| Treatment | Body weight gain, g/chick | Feed intake, g/chick | FCR, kg feed/kg BWG | European production efficiency index |
|-----------|--------------------------|----------------------|---------------------|-------------------------------------|
| Control + MOS | 1145 | 1929<sup>a</sup> | 1.689<sup>ab</sup> | 243.3<sup>a</sup> |
| Control | 1093 | 1859<sup>a</sup> | 1.706<sup>ab</sup> | 217.3<sup>b</sup> |
| Control +1 g TO/kg diet | 1088 | 1713<sup>b</sup> | 1.578<sup>b</sup> | 240.4<sup>a</sup> |
| Control +1.5 g TO/kg diet | 1028 | 1833<sup>a</sup> | 1.751<sup>a</sup> | 205.3<sup>b</sup> |
| Control +2 g TO/kg diet | 1061 | 1867<sup>a</sup> | 1.783<sup>a</sup> | 207.2<sup>a</sup> |
| CV | 6.4 | 5.1 | 6.1 | 11.2 |
| p Value | .059 | .003 | .001 | .001 |

<sup>a,b</sup>Means within the same column with different superscript letters are significantly different p < .05.

MOS: mannanoligosaccharides; TO: thyme oil; BWG: body weight gain; CV: coefficient of variation.

### Table 4. Effect of different dietary thyme oil concentrations and MOS on plasma proteins and liver function indices of broilers under hot climate at 28 days of age.

| Treatment | Total protein, mg/dl | Albumin, mg/dl | Globulin, mg/dl | Albumin/globulin ratio | AST, U/dl | ALT, U/dl | AST/ALT ratio |
|-----------|----------------------|----------------|-----------------|-----------------------|-----------|-----------|---------------|
| Control + MOS | 3.06<sup>a</sup> | 1.48 | 1.58<sup>ab</sup> | 0.945<sup>ab</sup> | 7.00<sup>a</sup> | 19.85 | 0.343<sup>a</sup> |
| Control | 2.79<sup>b</sup> | 1.38 | 1.41<sup>b</sup> | 0.996<sup>b</sup> | 6.62 | 21.50 | 0.336<sup>b</sup> |
| Control +1 g TO/kg diet | 2.86<sup>ab</sup> | 1.38 | 1.48<sup>b</sup> | 0.942<sup>b</sup> | 4.67<sup>b</sup> | 16.20 | 0.299<sup>b</sup> |
| Control +1.5 g TO/kg diet | 3.02<sup>a</sup> | 1.32 | 1.70<sup>a</sup> | 0.786<sup>a</sup> | 4.50<sup>b</sup> | 18.98 | 0.229<sup>b</sup> |
| Control +2 g TO/kg diet | 2.97<sup>a</sup> | 1.32 | 1.65<sup>ab</sup> | 0.804<sup>ab</sup> | 4.00<sup>b</sup> | 17.47 | 0.227<sup>b</sup> |
| CV | 7.0 | 10.3 | 10.2 | 13.8 |
| p Value | .008 | .264 | .26 | .022 |

<sup>a,b</sup>Means within the same column with different superscript letters are significantly different p < .05.

MOS: mannanoligosaccharides; TO: thyme oil; AST: aspartate amino transferase; ALT: alanine amino transferase; CV: coefficient of variation.

### Table 5. Effect of different dietary thyme oil concentrations and MOS on red blood cells parameters of broilers under hot climate at 28 days of age.

| Treatment | RBC, 10<sup>6</sup>/mm<sup>3</sup> | Haemoglobin, g/dL | PCV, % | MCV, µm<sup>3</sup>/RBC | MCH, pg | MCHC, % |
|-----------|-------------------------|-----------------|-------|------------------------|--------|--------|
| Control + MOS | 2.025 | 7.55 | 24.2 | 119.3 | 37.3 | 31.2 |
| Control | 2.028 | 7.87 | 26.0 | 128.0 | 38.8 | 30.4 |
| Control +1 g TO/kg diet | 2.031 | 7.62 | 24.5 | 120.7 | 37.5 | 31.1 |
| Control +1.5 g TO/kg diet | 2.016 | 8.00 | 25.2 | 125.1 | 39.7 | 31.8 |
| Control +2 g TO/kg diet | 2.028 | 7.72 | 24.9 | 122.8 | 38.0 | 31.2 |
| CV | 0.664 | 3.88 | 7.8 | 7.9 | 4.03 | 6.23 |
| p Value | .385 | .094 | .569 | .538 | .075 | .823 |

MOS: mannanoligosaccharides; TO: thyme oil; RBC: red blood cell; PCV: packed cell volume; MCV: mean cell volume; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; CV: coefficient of variation.

n = 6 with one blood sample/replicate.
days of age (Sadeghi et al. 2012). Another study with broilers indicated that, thyme extract added at 0.2, 0.4 and 0.6% to the broiler diet did not affect BWG, feed intake and FCR during 1–42 days of age (Pourmahmoud et al. 2013; Hady et al. 2016). Thus, these contradictions could be attributed to the thyme product, e.g. powder, extract or oil, the composition of the basal diet, and the environmental, hygienic conditions and animal age. In general, growth promoters had less favourable effects when animals were fed highly digestible diets or were raised in optimal environmental conditions (Bovera et al. 2012; Pourmahmoud et al. 2013; Mašek et al. 2014; Attia et al. 2016).

Hot climates deteriorated feed intake, the digestibility of nutrients and raised pathogens and oxidative markers (Attia et al. 2011a; Akbarian et al. 2014). There are three possible methods for the action of thymol: the first is a reduction of harmful microbiota; the second is a boosting of the antioxidant system due to polyphenol constituents (Abdulkarimi et al. 2011); the third may be due to increasing feed utilisation and enhancing immunity (Ghazalah & Ali 2008). Thus, the improved feed utilisation of the TO_1 group in the present study may be due to the decrease in the pathogen microflora and thus improved gut ecology and/or increased digestibility of the nutrients.

The increase in plasma total protein of the groups on the TO_1.5 and TO_2 g/kg diets, the globulin of the TO_1.5 group, the decrease in the AST of all the TO groups, and the AST/ALT ratios of the TO_1.5 and TO_2 groups suggest an improvement in general health status and a boosting in liver functions due to TO, which was found to be a good source of PUFA (42.34%) and antioxidants (69.5).

The antioxidant activity and the fatty acids profile are similar to those reported by (Assiri et al. 2016). The present results are in line with those reported by Abdulkarimi et al. (2011), who found that thyme decreased liver weight due to bile acid deconjugation, and thus reduced fat absorption (Pourmahmoud et al. 2013).

In addition, Zhu et al. (2014) suggested that thyme essential oil markedly increased serum total proteins and globulins on day 21, significantly decreased ALT, and the albumin to globulin ratio and serum urea on days 21 and 42, while increasing high-density lipoproteins on days 21 and 42. Further evidence for improved health status due to TO can be seen from the significant increase in antibody titres to IBD, particularly of the group on the TO_2 diet. In addition, a higher globulin level and a lower A/G ratio in TO_1.5 group when compared to the control, is suggestive for a better disease resistance and immune response of

### Table 6. Effect of different dietary thyme oil concentrations and MOS on white blood cells count and its fraction, %, of broilers under hot climate at 28 days of age.

| Treatment                  | WBCs, 10^3/mm^3 | Lymphocyte,% | Monocyte,% | Basophil, % | Eosinophil, % | Heterophil,% |
|----------------------------|-----------------|--------------|------------|-------------|---------------|--------------|
| Control + MOS              | 23.6^a          | 58.2         | 6.8^a      | 0.500       | 0.167         | 23.3         |
| Control                    | 22.4^bc         | 60.2         | 5.83^ab    | 0.500       | 0.167         | 23.2         |
| Control +1 g TO/kg diet    | 23.1^ab         | 58.0         | 5.63^ab    | 0.500       | 0.167         | 22.2         |
| Control +1.5 g TO/kg diet  | 20.0^d          | 59.2         | 5.17^d     | 0.333       | 0.333         | 22.8         |
| Control + 2 g TO/kg diet   | 21.7^c          | 58.3         | 5.50^ab    | 0.167       | 0.500         | 22.7         |
| CV                         | 3.7             | 3.7          | 17.4       | 29.1        | 73.2          | 4.84         |

^a–dMeans within the same column with different superscript letters are significantly different p < .05.

MOS: mannanoligosaccharides; TO: thyme oil; WBCs: while blood cells; CV: coefficient of variation.

n = 6 with one blood sample/replicate.

### Table 7. Effect of different dietary thyme oil concentrations and MOS on lipid peroxidation biomarker (MDA), lymphocyte/heterophil ratio and serum antibody titre to NDV and infection bursa disease and survival rate of broilers under hot climate at 28 days of age.

| Treatment                  | MDA, μmol/l | Heterophil/lymphocyte ratio | Antibody titre log₂ NDV IBD | Survival rate during 1–28 days of age, % |
|----------------------------|-------------|----------------------------|-----------------------------|----------------------------------------|
| Control + MOS              | 1.333       | 0.404                      | 1.66                        | 4.33^a                                  | 100                                     |
| Control                    | 1.533       | 0.386                      | 2.16                        | 3.33^b                                  | 94.4                                    |
| Control +1 g TO/kg diet    | 1.250       | 0.402                      | 1.83                        | 3.50^ab                                 | 97.2                                    |
| Control +1.5 g TO/kg diet  | 1.317       | 0.387                      | 1.83                        | 3.50^ab                                 | 97.2                                    |
| Control + 2 g TO/kg diet   | 1.350       | 0.389                      | 2.00                        | 4.33^a                                  | 97.2                                    |
| CV                         | 16.1        | 7.55                       | 39.2                        | 13.9                                    | 5.92                                    |

^a,bMeans within the same column with different superscript letters are significantly different p < .05.

MOS: mannanoligosaccharides; TO: thyme oil, MDA: malondialdehyde; NDV: Newcastle disease virus; IBD: infections bursa disease; CV: coefficient of variation.

n = 6 replicates per treatment.
birds as indicated by Bovera et al. (2015). The enhancements in the general health status of the broilers fed different TO supplementations could be attributed, as previously mentioned, to the increase in digestion and absorption of feeds and to the antimicrobial, antioxidants effects and fatty acid profile (Bozkurt et al. 2012; Hosseini et al. 2013; Mašek et al. 2014; Hady et al. 2016).

In the literature, thymol, carvacrol and linalool are the major components in the TO, which are found to have antioxidant, antimicrobial and digestion-enhancing effects (Cross et al. 2003; Bozkurt et al. 2012; Sethiya 2016). Thyme extract, and particularly the phenolic and terpenic compounds of TO, protect against DNA damage (Sengul et al. 2008), suggesting that these compounds may act as free radical scavengers (Akbarian et al. 2014).

In addition, Böyükbaşı et al. (2006) and Mansoub and Nezhady (2011) found that thyme decreased the peroxidation biomarkers and increased antioxidant abilities more than that did by vitamin E. Similarly, the present results showed that TO groups showed 14.8% lower MDA than the control group, showing an antioxidant effect. The antioxidant activity % inhibition of TO found herein is 69.5%.

In general, MOS and TO showed a similar effect on growth performance (1.689 vs. 1.704), blood profile and the immune titre (4.33 vs. 3.78), indicating that TO also displayed prebiotic-like effects. In the literature, MOS was reported to improve the performance and immunity of animals (Rosen 2007; Bovera et al. 2010; Attia et al. 2014a,b). The manner of action of MOS could be attributed to enhancing the nutrient digestibility, and improving gastrointestinal beneficial microbiota and the intestinal morphology (Hooge 2004; Hooge & Connolly 2011). On the other hand, some studies indicated a lack of significant influence of MOS on the growth performance of broilers (Yalçınkaya et al. 2008).

Conclusions
Thyme oil at 1g/kg diet may be used as an alternative growth promoter with positive effects on economic performance (FCR and EPEI) and the immune responses (IBD) during 1–28 days of age of broiler chickens raised under hot climates, and showed a prebiotic-like effect, similar to that of MOS.

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The authors certify that they have no affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria, educational grants, participation in speakers’ bureaus, membership, employment, consultancies, stock ownership or other equity interest, expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

ORCiD
Youssef A. Attia http://orcid.org/0000-0001-6505-3240

References
Abdulkarimi R, Daneshyar M, Aghazadeh A. 2011. Thyme (Thymus vulgaris) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. Ital J Anim Sci. 10:101–105.
Akbarian A, Golian A, Kermanshahi H, De Smet S, Michiels J. 2014. Antioxidant enzyme activities, plasma hormone levels and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and Curcuma xanthorrhiza essential oil. J Anim Physio Anim Nutr. 99:150–162.
Alcicek A, Bozkurt M, Cabuk M. 2004. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. South Africa J Anim Sci. 34:217–222.
AOAC. 2004. Official methods of analysis. 18th ed. Washington, DC: AOAC.
Assiri AMA, Elbanna K, Abuleeesh HH, Ramadan MF. 2016. Bioactive Compounds of Cold-pressed Thyme (Thymus vulgaris) oil with antioxidant and antimicrobial properties. J Oleo Sci. 65:629–640.
Attia YA, Abd Al-Hamid AE, Zeweil HS, Qota EM, Bovera F, Monastra G, Sahledom MD. 2013. Effect of dietary amounts of inorganic and organic zinc on productive and physiological traits of White Pekin ducks. Animal. 7:895–900.
Attia YA, Bovera F, Abd El-Hamid AE, Tag El-Din AE, Al-Harthi MA, El-Shafy AS. 2016. Effect of zinc bacitracin and phytase on growth performance, nutrient digestibility, carcass and meat traits of broilers. J Anim Physiol Anim Nutr (Berl). 100:485–491.
Attia YA, El-Tahawy WS, Abd El-Hamid AE, Hassan SS, Nizza A, El-Kelaway MI. 2012. Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital J Anim Sci. 11:303–308.
Attia YA, Hamed RS, Abd El-Hamid AE, Shahba HA, Bovera F. 2014b. Effect of inulin and mannan-oligosaccharides compared with zinc-bacitracin on growing performance,
nutrient digestibility and hematological profiles of growing rabbits. Anim Prod Sci. 55:80–86.

Attia YA, Hassan RA, Qota MA. 2009. Recovery from adverse effects of heat stress on slow-growing chicks in the tropics 1: effect of ascorbic acid and different levels of betaine. Trop Anim Health Prod. 41:807–818.

Attia YA, Hassan RA, Tag El-Din AE, Abou- Shehema BM. 2011a. Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress. J Anim Phys Anim Nut. 95:744–755.

Attia YA, Zeweih LS, Alsaaffar AA, El-Shafy AS. 2011b. Effect of non-antibiotic feed additives as an alternative to flavomycin on broiler chickens production. Arch Gefügelk. 75:40–48.

Attia YA, AbdAl-Hamid AE, Ibrahim MS, Al-Harthi MA, Bovera F, Elnaggar ASh. 2014a. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannanoligosaccharides continuously or intermittently. Livestock Sci. 164:87–95.

Benzie IF, Strain JJ. 1998. Ferric reducing antioxidant power assay, direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299:15–27.

Böülübaşı SC, Erhan MK, Özkan A. 2006. Dietary effect of thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. S Afr J Anim Sci. 36:189–196.

Bovera F, Lestangi A, Marono S, Iannaccone F, Nizza S, Mallardo K, de Martino L, Tateo A. 2012. Effect of dietary mannan-oligosaccharides on in vivo performance, nutrient digestibility and caecal content characteristics of growing rabbits. J Anim Phys Anim Nut. 96:130–136.

Bovera F, Lestangi A, Piccolo G, Iannaccone F, Attia YA, Tateo A. 2013. Effects of water restriction on growth performance, feed nutrient digestibility, carcass and meat traits of rabbits. Animal. 7:1600–1606.

Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi F, Gasco L, Nizza A. 2016. Use of Tenebrio molitor larvae meal as protein source in broiler diet: effect on growth performance, nutrient digestibility, and carcass and meat traits. J Anim Sci. 94:639–647.

Bovera F, Marono S, Di Meo C, Piccolo G, Iannaccone F, Nizza A. 2010. Effect of mannanoligosaccharides supplementation on caecal microbial activity of rabbits. Animal. 4:1522–1527.

Bovera F, Piccolo G, Gasco L, Marono S, Loponte R, Vassalotti G, Mastellone V, Lombardi P, Attia YA, Nizza A. 2015. Yellow mealworm larvae (Tenebrio molitor, L) as a possible alternative to soybean meal in broiler diets. Br Poult Sci. 56:569–575.

Bozkurt M, Kucukyilmaz K, Pamukcu M, Cabuk M, Alciek A, Cati AU. 2012. Long-term effects of dietary supplementation with an essential oil mixture on the growth and laying performance of two layer strains. Ital J Anim Sci. 11:23–28.

Cross DE, Sovboda K, McDevitt RM, Acamovic T. 2003. 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. Br Poult Sci. 48:496–506.

El-Ghosein SS, Al-Betawi NA. 2009. The effect of feeding crushed thyme Thymus valgaris L. on growth, blood constituents, gastrointestinal tract and carcass characteristics of broiler chickens. J Poult Sci. 46:100–104.

Fallah R, Mirzaei E. 2016. Effect of Dietary inclusion of turmeric and thyme powders on performance, blood parameters and immune system of broiler chickens. J Livestock Sci. 7:180–186.

Feizi A, Bijanzad P, Kaboli K. 2013. Effects of thyme volatile oils on performance of broiler chickens. Eur J Exp Bio. 3:250–254.

Ghazalah AA, Ali AM. 2008. Rosemary leaves as a dietary supplement for growth in broiler chickens. Int Poult Sci. 7:234–239.

Hady M M, Zaki MM, Abd EL-Ghany W, Korany Reda MS. 2016. Assessment of the broilers performance, gut healthiness and carcass characteristics in response to dietary inclusion of dried coriander, turmeric and thyme. Int J Environ & Agric Res. 2:153–159.

Hooge DM, Connolly A. 2011. Meta-analysis summary of broiler chicken trials with dietary Actigen® 2009–2011. Int J Poult Sci. 10:819–824.

Hooge DM. 2004. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide,1993–2003. Int J Poult Sci. 3:163–174.

Hosseini SA, Meimandipour A, Alami F, Mahdavi A, Mohiti-Asli M, Lotfollahian H, Cross D. 2013. Effects of ground thyme and probiotic supplements in diets on broiler performance, blood biochemistry and immunological response to sheep red blood cells. Ital J Anim Sci. 12:116–120.

Mansoub NH, Nezhady MAM. 2011. The effect of using Thyme, Garlic, Nettle on performance, carcass quality and blood parameters. Annals Bio Res. 2:315–320.

Masek T, Starcević K, Mikulec Z. 2014. The influence of the addition of thymol, tannic acid or gallic acid to broiler diet on growth performance, serum malondialdehyde value and cecal fermentation. Europ Poult Sci. 78:2014 DOI: 10.1399/eps.2014.64.

Najafi P, Torki M. 2010. Performance, blood metabolites and immune competence of broiler chicks fed diets included essential oils of medicinal herbs. J Anim Vet Adv. 9:1164–1168.

NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. Washington (DC): National Academic Press.

Pourmahmoud B, Aghazadeh AM, Sis NM. 2013. The effect of thyme extract on growth performance, digestive organ weights and serum lipoproteins of broilers fed wheat-based diets. Ital J Anim Sci. 12:337–341.

Radwan SS. 1978. Coupling of two-dimension thin layer chromatography with gas chromatography for the quantitative analysis of lipids classes and their constituent fatty acids. J Chromatographic Sci. 16:538–542.

Richard MJ, Portal B, Meo J, Coudray C, Hadjian A, Favier A. 1992. Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin Chem. 38:704–709.

Rodriguez A, Cuesta A, Ortuno J, Esteban MA, Messeguer J. 2003. Immunostimulant properties of a cell wall-modified...
whole *Saccharomyces cerevisiae* strain administered by diet to sea bream *Sparus aurata* L. Vet Immun Immunopath. 96:183–192.

Rosen GD. 2007. Holo-analysis of the efficacy of Bio-Mos in broiler nutrition. Br Poult Sci. 48:21–26.

Sadeghi GH, Karimi A, Padidar Jahromi SH, Aziz T, Daneshmand A. 2012. Effect of cinnamon, thyme and turmeric infusions on the performance and immune response in of 1- to 21-day-old male broilers. Braz J Poult Sci. 14:15–20.

SAS®. 2003 Statistical analyses software. The SAS system for windows, Release 9.1.3 Service pack 2, TS-level 01M3. Cary (NC): SAS Institute Inc.

Sengul T, Yurtseven S, Cetin M, Kocyigit A, Sogut B. 2008. Effect of thyme *T. vulgaris* extracts on fattening performance, some blood parameters, oxidative stress and DNA damage in Japanese quail. J Anim Feed Sci. 17:608–620.

Sethiya NK. 2016. Review on natural growth promoters available for improving gut health of poultry: an alternative to antibiotic growth promoters. Asian J Poult Sci. 10:1–29.

Snyder DB, Marquardt WW, Mallinson ET, Savage PK, Allen DC. 1984. Rapid serological profiling by enzyme-linked immunosorbent assay. III. Simultaneous measurements of antibody titers to infectious bronchitis, infectious bursal disease, and Newcastle disease viruses in a single serum dilution. Avian Dis. 28:12–24.

Stahl-Biskup E, Saez F. 2002. Thyme, the genus *Thymus*. 1st ed. London, UK: Taylor and Francis.

Takatsy GY. 1956. The use of spiral loops in serological and virological micromethods. Acta Micro Academic Sci Hungary. 3:197–200.

Yalcinkaya L, Gungor T, Basalan M, Erdem E. 2008. Mannan Oligosaccharides MOS from *Saccharomyces cerevisiae* in boilers: effects on performance and blood biochemistry. Turkey J Vet Anim Sci. 32:43–48.

Zhu X, Liu W, Yuan S, Chen H. 2014. The effect of different dietary levels of thyme essential oil on serum biochemical indices in Mahua broiler chickens. Ital J Anim Sci. 13:3238. 576-581.