Essential oils (EOs) consisting of 4.5 g cinnamaldehyde and 13.5 g thymol per 100 g of EOs as phytogenic growth promoters in broiler diet. A total of 216, one-day-old male Arbor Acres broiler chickens were randomly allotted into six groups, each with six replicates. The 1st group was fed a basal diet (Control) without supplementation; the 2nd group was fed the basal diet including zinc bacitracin (ZnB); the 3rd, 4th, 5th and 6th groups were fed the basal diet including EOs at 25 (EOs_25), 50 (EOs_50), 100 (EOs_100) and 150 (EOs_150) mg/kg diet, respectively. The group EOs_150 showed significantly increased (p < .05) body weight gain (BWG), enhanced feed conversion rate (FCR) and production efficiency index (PEI) compared to the other groups, except for EOs_100. Increasing concentrations of EOs above 25 mg/kg significantly (p < .05) increased protein, lipid and fibre digestibility; the addition of EOs also increased the amount of edible parts and dressing percentage of carcases. Diets EOs_100 and EOs_150 resulted in significantly increased plasma total protein and globulin levels, while EOs_150 led to higher plasma glucose concentrations. From the findings, supplementation with 100 mg/kg EOs in encapsulated, heat-stable forms could be used as an alternative growth promoter to ZnB in broiler chickens.

HIGHLIGHT

- Essential oils can replace antibiotic in broiler nutrition with no adverse effects on growth performance.
- Essential oils improved total edible parts and dressing percentage.
- Essential oils enhanced digestibility of protein, lipid and fibre. Thus essential oils contained of 4.5 g cinnamaldehyde and 13.5 g thymol per 100 g can be used at 100 mg/kg feed as alternative growth promoter for broilers chickens.

Introduction

The use of antibiotics growth promoters (AGPs) for livestock species has been banned in the EU since 2006 (Castanon 2007). Successively, the US Food and Drug Administration restricted the use of AGPs in animal nutrition in December 2016. Increasing mortality and culling are challenges faced by the poultry industry after the withdrawal of AGPs (Zhao et al. 2007). Thus, the cost of potential alternatives for AGPs is essential to ensure poultry farming sustainability (Valenzuela-Grijalva et al. 2017; Yang et al. 2018). In this regard, medium-chain fatty acids (Boyen et al. 2008) and essential oils (EOs) (Windisch et al. 2008; Attia et al. 2017a) were suggested as substitutes for AGPs.

Essential oils are oily, volatile or aromatic liquids obtained from flowers, seeds, herbs, leaves, fruits, roots and bark (Brenes and Roura 2010). As widely reported in the literature, EOs increase BWG (Falaki et al. 2016; Yang et al. 2018), feed intake (Fi; Zhang et al. 2005; Mukhtar et al. 2013; Valiollahi et al. 2014), feed conversion ratio (FCR; Yang et al. 2018) nutrient digestibility and absorption (Boyen et al. 2008), dressing percentage (Alcicek et al. 2004; Mahmoodi et al. 2014) and reduce serum cholesterol (Mukhtar et al. 2013) and abdominal fat (Rafiee et al. 2013; Valiollahi et al. 2014). The beneficial effects of EOs were attributed to their antioxidant (Placha et al. 2010; Silva et al. 2012), antimicrobial (Du et al. 2016) and immunological functions (Hosseini et al. 2016). In addition, EOs improve the ecological conditions of the gut and simulate the activity of the digestive enzymes (Cross et al. 2007; Jang et al. 2007). For example, thymol and cinnamaldehyde have shown promising...
effects on broiler performance, gut ecosystem and immunity (Lee et al. 2003; Attia et al. 2017a).

The progress in the feed additive industry and the production of encapsulated, heat-stable essential oils can protect them from oxidative damage and improve their overall quality (Attia et al. 2017a). Digestibility and metabolic profiles reflect the responses of animals to feed additives (Khan et al. 2012; Tufarelli et al. 2016; Mokhtari et al. 2018). In this context, we investigated the replacement of antibiotics by EOs as growth promoters in terms of growth performance and production efficiency index as well as biochemical, haematological and immune indices of broilers.

Materials and methods

Animals and supplements

All the procedures were approved by the department committee of Arid Land Agriculture under proposal number G 20-155-39H, that recommends animal rights, welfare and minimal stress and did not cause any harm or suffering to animals according to the Royal Decree number M59 in 14/9/1431H.

A total of 216, one-day-old male chickens (Arbor Acres) were randomly allotted into six groups, each with six replicates and six animals per replicate. All experimental groups were fed the same basal diet and submitted to the following treatments: the 1st group (control) was fed the basal diet without supplementation, the 2nd group (ZnB) was fed a diet with added 0.5 g/kg diet zinc bacitracin (Pucheng Lifecome Biochemistry Co. Ltd, Pucheng, P. R. China). The 3rd, 4th, 5th and 6th groups were fed the control diet supplemented with a heat-stable encapsulated essential oils (Enviva™ EOs 101 G, a product of Danisco Animal Nutrition, www.animalnutrition.dupont.com at 25, 50, 100 and 150 mg/kg diet (EOs_25, EOs_50, EOs_100 and EOs_150, respectively). The total level of EOs compounds in the commercial product was 18 g/100 g of product consisting of 4.5 g cinnamaldehyde and 13.5 g thymol. The product was encapsulated by forcing essential oil molecules into the maltodextrin matrix to provide protection. Diets were formulated based on recommendations tables (NRC 1994) to meet the animals’ nutrient requirements. The ingredients and chemical profiles of the basal diets (% as fed basis) are shown in Table 1. The additive was added at the top of the diet and mixed carefully to assure proper distribution; this preparation was carried out weekly.

Housing and husbandry

Chickens were housed in wire cages (60 cm length × 50 cm depth × 40 cm height) provided with galvanised feeders and automatic nipple drinkers in a semi-open room equipped with two exhaust fans to ensure ventilation. Chickens were fed the experimental diets in mash form ad libitum and given free access to water; they were maintained under similar management, environmental and hygienic conditions.

Responses criteria

Broilers were weighed according to a replicate basis at 1, 14, 28 and 36 days of age, and the BWG was calculated. Feed consumption was assessed for each replicate, and mortality was determined. The obtained data were used for the calculation of the FCR and live-ability (100 - mortality rate) during 1–14, 1–28 and 1–36 d of age.

The apparent digestibility of crude protein (CP), ether extract (EE), crude fibre (CF) and ash were analysed using six replicates of three animals housed in metabolic cages during 36–40 days of age (Attia et al. 2012). Nitrogen, EE, CF and ash content in the excrements, as well as those in the feed, were assessed (AOAC 2004). The production efficiency index (PEI) was calculated as cited by Hubbard (1999).

Six animals were taken randomly from each treatment group to represent all treatment replications and slaughtered at 36 days of age according to the Islamic method after being fasted overnight. The carcass and inner organs were separated, weighed and expressed as relative to live BWG as cited by (Attia et al. 2012).

At 36 days of age, 12 blood samples were collected from each treatment group (two samples per replication) for blood analysis. The samples were divided into two parts: the 1st part was collected in heparinised tubes, while the 2nd part was collected in non-heparinised tubes to obtain serum after coagulation. Plasma and serum were separated by blood centrifugation (1512 xg (3000 rpm) for 20 min) and stored at −20°C for later analysis. Biochemical indices total protein, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose, total lipids, triglycerides and cholesterol were determined by using commercially available kits (Diamond diagnostics, Cairo, Egypt).

Haematological traits, including haemoglobin (Hgb), red blood cells (RBCs), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration
Effect of different levels of essential oils and zinc bacitracin supplementation on growth performance traits of broiler (n = 6 per treatment).

Table 2. Effect of different levels of essential oils and zinc bacitracin supplementation on growth performance traits of broiler (n = 6 per treatment).

| Treatment | Initial body weight, g | Body weight gain, g/d | Feed intake, g/d | Feed conversion ratio, kg feed/kg gain |
|-----------|------------------------|-----------------------|------------------|---------------------------------------|
|            | 1–14 | 1–28 | 1–36 |            | 1–14 | 1–28 | 1–36 |            |
| Control    | 49.90 | 314b | 113b | 1792c | 254d | 0.276 | .0005 | .0001 | .0004 | .0003 |
| ZnB, 500 mg/kg | 48.70 | 320d | 1141c | 1825c | 259d | 1.180b | 1.610b | 1.760b |
| EOs, 25 mg/kg | 48.70 | 364c | 1235c | 1873c | 274d | 1.280b | 1.720b | 1.900a |
| EOs, 50 mg/kg | 48.20 | 381b | 1260b | 1886b | 285c | 1.440a | 1.820ab | 1.950a |
| EOs, 100 mg/kg | 49.30 | 392c | 1269c | 1972b | 306 | 1.480 | 31 | 62.300 | 94 | 9.410 |
| Eos, 150 mg/kg | 49.80 | 396c | 1300a | 1999a | 323a | 1.800 | 3 | 67.00 | 9 | 10.00 |
| SEM        | 1.480 | 31 | 62.300 | 94 | 9.410 |

 SEM: Standard error of mean; ZnB: Zinc bacitracin; EOs: essential oils.

Table 3. Effect of different levels of essential oils and zinc bacitracin supplementation on feed intake and feed conversion ratio of broiler (n = 6 per treatment).

| Treatment | Feed intake, g/d | Feed conversion ratio, kg feed/kg gain |
|-----------|------------------|---------------------------------------|
|            | 1–14 | 1–28 | 1–36 |            | 1–14 | 1–28 | 1–36 |            |
| Control    | 456  | 2094 | 3531 | 1.460a | 1.850a | 1.970a |
| ZnB, 500 mg/kg | 460 | 2083 | 3558 | 1.440a | 1.820ab | 1.950a |
| EOs, 25 mg/kg | 462 | 2103 | 3543 | 1.280b | 1.720b | 1.900c |
| EOs, 50 mg/kg | 462 | 2107 | 3551 | 1.220b | 1.680b | 1.880c |
| EOs, 100 mg/kg | 459 | 2080 | 3510 | 1.190b | 1.640b | 1.780b |
| Eos, 150 mg/kg | 464 | 2095 | 3525 | 1.180b | 1.610b | 1.760b |
| SEM        | 8.840 | 19.300 | 19.900 | 0.057 | 0.042 | 0.035 |

SEM: Standard error of mean; ZnB: Zinc bacitracin; EOs: essential oils.

Statistical analyses

Prior to the analyses, percentages were transformed to the logarithmic values to normalise data distribution. One-way ANOVA of the GLM procedure was used, with the experimental unit as the replicates (SAS 2002). Mean differences were compared using the Student-Newman-Keuls test at p ≤ 0.05.

Results

The effects of ZnB and EOs on growth traits of broilers are shown in Table 2. During the different experimental periods, BWG, FCR and PEI were significantly (p < 0.05) influenced by dietary additives. Broilers on diets supplemented with EOs greater than 25 mg/kg showed similarly (p < 0.05) increased BWG and improved FCR than those fed ZnB and the control diet during 1–14 and 1–28 days of age. During 1–36 days of age, chickens fed the diets Eos_50, Eos_100 and Eos_150 mg/kg showed significantly (p < 0.05) improved protein, lipid and fibre digestibility compared with the control, ZnB and Eos_25 groups (Table 4). No differences were detected for dry matter and ash digestibility among the different treatments (p > 0.05).

Carcass traits and inner body organs are presented in Table 5. Dressing percentage significantly (p < 0.05)
increased in ZnB and EOs groups compared to the control; conversely, there were no effects of the EOs treatments on the inner body organs.

Total protein, globulin and glucose were affected ($p < .05$) by dietary additives (Table 6). The diets EOs_100 and EOs_150 resulted in significantly increased blood plasma total protein and globulin compared to the control, ZnB and EOs_25 and EOs_50 diets. Broilers fed the EOs_150 diet showed higher ($p < .05$) blood glucose levels than those in the other groups, with the exception of the ZnB diet. No effects of dietary additives on albumin, albumin/globulin ratio, total cholesterol and triglycerides were observed (Table 6).

Liver leakage indices (AST and ALT) were significantly influenced ($p < .05$) by dietary additives; however, the effects of the treatments on renal functions were not significant (Table 7). Groups fed ZnB and EOs_50 diets showed higher ($p < .05$) blood plasma AST than those fed the other treatments, except for group EOs_100. In addition, the EOs_50 diet resulted in a significantly higher blood ALT than the other diets, with the exception of the EOs_100 diet.

There were no effects on the characteristics of the RBCs and WBCs (Tables 8 and 9). The immune status of the broilers is shown in Table 10. Phagocyte activity, LTT and BA were affected ($p < .05$) by dietary additives. Broilers fed the diets EOs_25 and EOs_100

Table 4. Effect of different levels of essential oils and zinc bacitracin supplementation on nutrients digestibility of broiler during 36–40 days of age ($n = 6$ per treatment).

| Treatment     | Crude protein, % | Ether extract, % | Crude fibre, % | Ash, % | Dry matter, % |
|---------------|------------------|-----------------|----------------|--------|---------------|
| Control       | 68.900<sup>b</sup> | 73.300<sup>b</sup> | 15.200<sup>b</sup> | 37.000 | 73.200        |
| ZnB, 500 mg/kg| 72.400<sup>b</sup> | 75.100<sup>b</sup> | 14.900<sup>b</sup> | 38.000 | 73.500        |
| EOs_25 mg/kg  | 70.100<sup>b</sup> | 74.600<sup>b</sup> | 15.000<sup>b</sup> | 35.700 | 73.400        |
| EOs_50 mg/kg  | 78.500<sup>a</sup> | 83.500<sup>a</sup> | 20.400<sup>a</sup> | 38.000 | 76.700        |
| EOs_100 mg/kg | 78.900<sup>a</sup> | 83.900<sup>a</sup> | 20.600<sup>a</sup> | 35.200 | 76.100        |
| EOs_150 mg/kg | 81.300<sup>a</sup> | 86.500<sup>a</sup> | 24.100<sup>a</sup> | 38.400 | 75.200        |
| SEM           | 2.650             | 2.820            | 1.310           | 2.370  | 1.110         |

**<sup>a,b</sup>Differences among means within a column within each factor not sharing similar superscripts are significantly different ($p < .05$).**

SEM: Standard error of mean; ZnB: Zinc bacitracin; EOs: essential oils.

Table 5. Effect of different levels of essential oils and zinc bacitracin supplementation on carcass traits of broiler at 36 days of age ($n = 6$ per treatment).

| Treatment     | Dressing | Abdominal fat | Proventriculus | Gizzard | Intestinal | Pancreas | Liver | Heart |
|---------------|----------|---------------|----------------|---------|------------|----------|-------|-------|
| Control       | 67.900<sup>b</sup> | 0.968          | 0.388          | 1.200   | 3.950      | 0.245    | 2.110 | 0.471 |
| ZnB, 500 mg/kg| 69.700<sup>a</sup> | 1.023          | 0.358          | 1.240   | 2.960      | 0.235    | 2.170 | 0.483 |
| EOs_25 mg/kg  | 72.100<sup>a</sup> | 0.900          | 0.362          | 1.210   | 3.440      | 0.266    | 1.980 | 0.495 |
| EOs_50 mg/kg  | 73.300<sup>a</sup> | 0.862          | 0.395          | 1.180   | 2.780      | 0.248    | 1.770 | 0.424 |
| EOs_100 mg/kg | 74.900<sup>a</sup> | 0.663          | 0.354          | 1.120   | 2.890      | 0.241    | 2.020 | 0.489 |
| EOs_150 mg/kg | 75.100<sup>a</sup> | 0.617          | 0.429          | 1.190   | 2.740      | 0.276    | 2.150 | 0.492 |
| SEM           | 1.180    | 0.166         | 0.019          | 0.071   | 0.340      | 0.025    | 0.159 | 0.042 |

**<sup>a,b</sup>Differences among means within a column within each factor not sharing similar superscripts are significantly different ($p < .05$).**

SEM: Standard error of mean; ZnB: Zinc bacitracin; EOs: essential oils.

Table 6. Effect of different levels of essential oils and zinc bacitracin supplementation on blood biochemistry of broiler at 36 days of age ($n = 6$ per treatment).

| Treatment     | Total protein, mg/dL | Albumin, mg/dL | Globulin, mg/dL | Albumin/globulin ratio | Total cholesterol, mg/dL | Triglycerides, mg/dL | Glucose, mg/dL |
|---------------|----------------------|----------------|----------------|------------------------|-------------------------|---------------------|---------------|
| Control       | 3.700<sup>b</sup>   | 2.000          | 1.700<sup>b</sup> | 1.340                  | 168.300                 | 168.500             | 230<sup>b</sup> |
| ZnB, 500 mg/kg| 4.120<sup>b</sup>   | 2.120          | 2.000<sup>b</sup> | 1.060                  | 175.600                 | 178.000             | 242<sup>a</sup> |
| EOs_25 mg/kg  | 4.500<sup>b</sup>   | 2.450          | 2.050<sup>b</sup> | 1.310                  | 163.700                 | 166.500             | 232<sup>b</sup> |
| EOs_50 mg/kg  | 4.450<sup>b</sup>   | 2.470          | 1.980<sup>b</sup> | 1.410                  | 172.700                 | 174.800             | 228<sup>b</sup> |
| EOs_100 mg/kg | 6.130<sup>a</sup>   | 2.770          | 3.700<sup>a</sup> | 0.907                  | 176.200                 | 175.800             | 234<sup>a</sup> |
| EOs_150 mg/kg | 6.120<sup>a</sup>   | 2.320          | 3.800<sup>a</sup> | 0.643                  | 169.000                 | 167.300             | 248<sup>a</sup> |
| SEM           | 0.362                | 0.213          | 0.3620          | 0.231                  | 9.530                   | 5.500               | 4.6300        |

**<sup>a,b</sup>Differences among means within a column within each factor not sharing similar superscripts are significantly different ($p < .05$).**

SEM: Standard error of mean; ZnB: Zinc bacitracin; EOs: essential oils.
showed \( p < .05 \) higher PA than those on the EOS_50 diet. In addition, different concentrations of EOS resulted in higher blood LTT levels \( p < .05 \) than those found for the control and ZnB diets. In addition, the BA of various EO groups was significantly \( p < .05 \) higher than that of the control, while broilers fed ZnB did not differ from the control and the EOS_25 and EOS_50 groups.

There were no significant effects of different feed additives on the PI, the LA and on lymphoid organs.
(bursa, thymus, spleen), as well as on survival (Table 10).

Discussion

Several studies have focussed on the use of EOs as substitutes for antibiotics to promote the growth of livestock species, to improve the FCR and to reduce feed costs (Amal et al. 2013; Attia et al. 2017a; Mokhtari et al. 2018). However, the available results are contradictory as some studies have observed positive effects (Amal et al. 2013; Falaki et al. 2016; Tufarelli et al. 2016; Attia et al. 2017a; Mokhtari et al. 2018), while others did not show differences in comparison to the control (Lee et al. 2003; Jang et al. 2007; Cerisuelo et al. 2014). These differences can be explained by the type, dose and form of the different EOs. For this reason, we used encapsulated, heat-stable forms of EOs, which were protected from high temperatures, high pressure and oxidant factors during manufacturing as well as after manufacturing, thereby ensuring high quality.

It has been suggested that EOs can perform as antioxidants, immune enhancers and antimicrobials, consequently maintaining the quality of the feeds and enhancing the gut health of animals reared in an antibiotic-free environment (Khan et al. 2012; Attia et al. 2017a). In the present study, broilers fed EOs at 150 mg/kg of diet showed the best BWG, FCR and production index values, without differences between EOs levels of 100 and 150 mg/kg, suggesting that, considering the cost of supplementation, an EO level of 100 mg/kg of diet is adequate as growth promoter for broilers from 1–36 days of age. This improvement due to EOs may be attributed to the antimicrobial (Du et al. 2016) and antioxidant activities of EOs (Minute et al. 2000; Tufarelli et al. 2016), that may result in improve gut health and stimulate digestive enzymes (Falaki et al. 2016; Hosseini et al. 2016) and stimulating digestive enzymes. Hence, an increased digestion of dietary nutrients consequently enhances the availability of nutrients for growth (Attia et al. 2017a). Likewise, broilers fed diets including EOs in 150 mg/kg of the diet significantly boosted BWG compared to broilers fed the control diet (Amal et al. 2013; Falaki et al. 2016).

The improvement in FCR indicated an increase in feed use and reflected the enhancement in the digestibility of ether extract, crude fibre and crude protein due to EOs. This corroborates the findings of several previous trials (Brenes and Roura 2010; Yang et al. 2018) reporting improvements in FCR with EO supplementation. In our study, the positive effects of EOs on the digestibility of CP, EE and CF agree with previous observations reporting that EOs improved protein digestibility (Ding et al. 2017). An EO blend similar to that used in the present study, containing thymol and cinnamaldehyde, resulted in improved nitrogen use and increased ileal energy digestibility (Cao et al. 2010). Some studies have reported that EOs supplementation might enhance the functions of the digestive tract by increasing trypsin, lipase and amylase activities of the pancreas and the gastric mucosa, thus increasing the levels of nutrients available for absorption and ultimately enhancing animal performance (Jang et al. 2007; Boyen et al. 2008). In addition, EOs may improve the gut ecosystem (Cross et al. 2007); they may also decrease crypt depth and increase the microvilli in the intestines, leading to enhanced animal growth (Boyen et al. 2008).

Maybe the encouraging effects of EOs on the PEI of broilers are in line with available findings demonstrating that the addition of garlic EO to broiler diets was economically profitable (El Tazi Safa et al. 2014), which was also observed for the addition of black cumin, lemon grass, clove oil, halfa-bar, thyme and spearmint EOs (Amal et al. 2013; Mukhtar et al. 2013; Attia et al. 2017a).

The present results show that the supplementation of EOs significantly increased dressing percentage, similarly, an improvement in dressing percentage with supplementation of EOs was reported by Alcicek et al. (2004; Dieumou et al. (2012) and Mahmoodi et al. (2014). Similar to the present findings, EOs did not influence the weight of gizzard, liver and pancreas (Ding et al. 2017). Likewise, dietary EOs did not affect the percentages of abdominal fat, gizzard, liver, lymphoid organs and heart (Falaki et al. 2016). The differences in the responses to EOs among various studies could be attributed to hygienic condition, dietary composition, environmental conditions as well as the type and dose of feed additives (Attia et al. 2017b).

In this research, EOs induced metabolic changes in total blood protein, globulin, glucose, AST, ALT, PA, LLT and BA, showing an enhanced immune response of broilers. However, EOs supplementation did not affect the survival rate of broilers, which is in line with previous observations (Amal et al. 2013; Mukhtar et al. 2013; El Tazi Safa et al. 2014). Studies conducted by Tekeli et al. (2011) and Attia et al. (2017a) indicated that broilers fed thyme oil at 1.5 and 2.0 g/kg showed higher plasma total protein levels than the control, whereas thyme oil at 1.5 g/kg increased globulin levels. In addition, thyme EO raises serum globulins and
total proteins at 21 days (Zhu et al. 2014). Moreover, urea and cholesterol levels were lower, with significant differences groups fed on halfa-bar oil compared to the control and antibiotic groups (Amal et al. 2013).

The present results indicate that EOs had no negative effects on biomarker indices of renal and liver functions, RBCs and WBCs. Likewise, (Dieumou et al. 2012; Amal et al. 2013; Mukhtar et al. 2013; Attia et al. 2017a) indicated that RBC characteristics as well as monocyte, basophil, eosinophil and heterophil percentages did not differ among different thyme EOs groups.

Conclusions

Essential oil supplementation consisting of 4.5 ginnamonaldehyde and 13.5 g thymol per 100 g of EOs in encapsulated, heat-stable forms at 100 mg/kg of the diet could substitute ZnB and resulted in enhanced growth performance, production efficiency index and immune responses of broilers. Further studies on alternative environmental friendly feed additives are essential to replace AGPs in animal nutrition to reduce hazards and promote health benefits throughout feed chain.

Disclosure statement

The authors declare that they have no conflict of interest.

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