Influences of an essential oil mixture supplementation to corn versus wheat-based practical diets on growth, organ size, intestinal morphology and immune response of male and female broilers

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Abstract

The aim of this study was to investigate the effect of diet type, supplementation diet with an essential oil mixture (EOM), and gender on the growth performance, carcass yield, internal organ weight, immune response, and small intestine histology of broiler chickens. To do this, a 2×2 factorial arrangement was designed. The variables used were: two diet types (based on either wheat or corn), 2 feed additives (with or without EOM), and gender (male or female). EOM supplementation in the diet decreased body weight in corn-fed male birds at Days 21 and 42, but not in those fed the wheat-based diet, signifying a diet × EOM × gender interaction. Cumulative feed intake was not influenced by either the diet type or EOM. The feed conversion ratio was not affected by diet type, whereas EOM improved feed conversion ratio over the 42-day growth period. Feeding birds on wheat decreased the carcass yield while it increased relative small intestine and large intestine weight. Relative weights of liver, bursa fabricius and serum infectious bursal disease (IBD) and Newcastle disease (ND) titers were not affected by any of the variables studied. EOM supplementation and feeding birds on corn increased jejunal villus height at both 21 and 42 days of age, while bird gender showed no effect. In general, EOM positively influenced body weight gain and efficiency of feed conversion in broiler chickens. Birds receiving the corn-based diet were more efficient in converting feed to body mass as compared to those fed on the wheat-based diet.

Introduction

Antibiotic growth promoters (AGP) have been used since the 1950s via prophylactic inclusion in poultry feed, particularly in broiler feeds, to reduce the impact of pathogenic microorganisms and thus improve zootechnical performance and profitability (Engberg et al., 2000). However, the effects of phased out AGP from poultry diets in the EU, and recent moves toward reduction or removal of these compounds in other parts of the world, have put substantial pressure on the poultry industry to look for viable alternatives. Due to increased consumer resistance, regulatory scrutiny, and demands for safe food, scientists have prompted initial research programs for alternative growth promoters to use in poultry (Bach Knudsen, 2001).

Supplementation poultry diets with essential oils of some herbs have been one available approach that has aroused great interest on the part of the poultry industry (Brenes and Roura, 2010; Wallace et al., 2010). Essential oils are volatile oils obtained from plants, normally by steam and/or water distillation (Grathead, 2003). Herbal essential oils are naturally safe and free from toxic and residual effects. They can, therefore, be considered an important and sustainable solution to AGPs (Langhout, 2000). Essential oils derived mainly from aromatic herbs and species may possess various biological properties, such as acting as antimicrobials (Cowen, 1999; Ulte et al., 2002), antioxidans (Basmacoglu et al., 2004), antifungals (Shin and Lim, 2004), coccidiosstats (Allen et al., 1997), and stimulating the digestive enzyme activity (Jamroz et al., 2005; Basmacoglu et al., 2010).

The results of various field trials and experimental studies conducted under different management conditions over the last decade have shown that incorporation of essential oils in broiler diets can ensure improved weight gain and feed efficiency, and reduced mortality, in addition to inhibiting common pathogenic bacteria growth (Jamroz et al., 2003; Halle et al., 2004; Mitsch et al., 2004; Çabuk et al., 2006). However, the bio-response (in terms of feed conversion ratio or body weight gain) to essential oil administration has not always been consistent (Windsch et al., 2008; Brenes and Roura, 2010) because the bioeffects of essential oils appeared to be influenced by environmental factors, including house hygiene, stock density, nutritional status of the feed, and general health status of the bird (Hernandez et al., 2004; Botsoglou et al., 2004; Jamroz et al., 2005).

To our knowledge, two common diet types are currently used worldwide in broiler feeding, either practical corn-based diets or wheat-based diets; the former have generally been used in the USA and Brazil, while the latter have been used in the EU and Russia. Undoubtedly, both contribute in different ways to broiler feeding, such as nutrient digestibility, gut microbial balance, intestinal viscosity, water intake, litter condition, and also pellet quality (Choct and Annison, 1999; Jamroz et al., 2006). However, there are hardly any scientific articles assessing the interaction between essential oil supplementation and ingredient composition of the broiler diet. Furthermore, the gender response to feed additives including essential oils as a performance enhancer has rarely been discussed in relation to nutrition for today’s modern broiler hybrids.

The selection of six essential oils constituting the mixture was based on in vitro scientific evidence demonstrating marked antimicrobial and antioxidant properties for each essential oil (Baratta et al., 1998; Cowan, 1999). Furthermore, those herbal plants grown either wild or cultivated in Turkey are already commercial commodities that provide a sustainable, voluntary and economically feasible supply of corresponding essential oils. The essential oil mixture used herein showed remark-
able benefit in terms of performance enhancer feed additive in broiler nutrition (Alçiçek et al., 2003, 2004; Bozkurt et al., 2012; Küçükyılmaz et al., 2012). However, the possible interactions between essential oil and diet type and gender have not yet been examined.

Therefore, we assessed dietary supplemental effect of an essential oil blend on performance traits, carcass yield, intestinal histology, and some serum antibody titers of male and female broilers reared up to 42 days of age fed on wheat- or corn-based diets.

Materials and methods

Birds and housing

One-day old male (n=800) and female (n=800) broiler chicks of a commercial strain (Ross 308) were included in a feeding experiment. In a 2 x 2 factorial arrangement, broiler chicks (males or females) were fed two different types of diet (wheat-based or corn-based) with or without the supplemental essential oil mixture (EOM) (0 or 48 mg/kg diet). Birds were randomly assigned to eight dietary treatments replicated four times with 50 birds per replicate. Birds were reared in an environmentally controlled grower house with automated heating and ventilation systems. The birds were kept in 32 wire pens (2.4 x 1.6 m) with wood shavings as the litter material. Each pen was equipped with two hanging feeders and one bell-type drinker. Bird density was 13 chicks per square meter. The lighting cycle was maintained at 23 h/day. Ambient temperature was maintained at 32°C during the first three days and was gradually decreased from 32°C on Day 4 to 22°C on Day 21. Chicks were vaccinated against infectious bursal disease (D-78, Nobilis®) and Newcastle disease (Hitchner, Intervet®) at Days 10 and 16, respectively, via drinking water.

Feed supplements and diets

The birds were fed a starter diet from Days 1 to 21 and a grower diet from Days 22 to 42. The ingredients and chemical compositions of the diets are presented in Table 1. Experimental diets were isoenergetic and isonitrogenous. An EOM (Herbronmix, Herba Ltd. Co., Iznir, Turkey), including carvacrol, thymol, 1,8-cineole, p-cymene, and limonene as active components, and were composed of six totally different essential oils, i.e., oregano oil (Origanum sp.), laurel leaf oil (Laurus nobilis), sage leaf oil (Salvia triloba), myrtle leaf oil (Myrtus communis), fennel seeds oil (Foeniculum vulgare), and citrus peel oil (Citrus sp.). These major components comprise about 80% of the total oil ranging from as high as 60% to as low as 3% of total essential oil mixture, respectively. Thus, each kg of feed added with EOM provided 28.8 mg carvacrol as the main plant bioactive. The pre-mixed essential oil (i.e. EOM) used 952 g of zeolite as a carrier for 48 g of essential oil. Hydrodistillation was used to isolate the essential oils. The EOM premix was added as a supplement to the basal diet (i.e. 1 kg of supplement per 1000 kg feed was added in place of the sawdust conventionally found in the feed mix). Thus, each kg of the experimental diet without or with EOM included 0 or 48 mg of EOM premix, respectively. The EOM premix was added into the diet together (1 g/kg) with the micronutrients. All of the experimental diets met or exceeded NRC (1994) nutrient recommendations for broilers. The experimental diets in mash form and the drinking water were prepared ad libitum.

Sampling, measurements and analysis

During the 42-day experimental period, the growth performance of broiler chickens was evaluated by recording body weight (BW), feed intake (FI), feed conversion ratio (FCR) and mortality. Birds were weighed individually at 1, 21 and 42 days of age. FI and FCR was determined per pen according to the 1-21 and 1-42 day experimental periods. FCR was calculated as

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FCR = \frac{FI}{BW} / \text{days}
\]

where FI is feed intake in g, BW is body weight in g, and days is the number of days for each measurement.

Table 1. Ingredients and nutrient composition of the experimental basal diets, as fed.

| Ingredients, g/kg | Starter, 1-21 days | Grower, 22-42 days |
|------------------|--------------------|--------------------|
|                  | Wheat based        | Corn based         | Wheat based        | Corn based         |
| Wheat            | 649.47             | 547.27             | 581.80             |
| Soybean meal, 48% CP | 248.46             | 335.16             | 203.15             | 254.73             |
| Fullfat soybean  | 50.00              | 50.00              | 44.37              | 90.08              |
| Soybean oil      | 17.73              | 32.04              | 36.95              | 40.83              |
| Dicalcium phosphate | 15.14              | 19.17              | 15.38              | 16.05              |
| Ground limestone | 5.69               | 4.92               | 6.93               | 5.39               |
| NaCl             | 2.64               | 2.96               | 3.05               | 2.98               |
| Lysine HCL       | 1.58               | 0.22               | 2.01               | 0.36               |
| DL-Methionine, 99% | 2.99               | 2.76               | 2.53               | 2.28               |
| Threonine        | 0.80               | 0.80               |                 |                   |
| Vitamin premix$^*$ | 2.50               | 2.50               | 2.50               |                   |
| Mineral premix$^*$ | 1.80               | 1.00               | 1.00               |                   |
| NaHCO$_3$        | 0.50               | 0.50               | 0.50               | 0.50               |
| Anticoccidial$^*$ | 0.50               | 0.50               | 0.50               | 0.50               |
| Saw dust$^*$     | 1.00               | 1.00               | 1.00               | 1.00               |
| Chemical composition, % |                   |                     |                   |                   |
| Dry matter       | 89.95              | 89.08              | 90.18              | 88.79              |
| Crude protein, N x 6.25 | 22.12              | 22.23              | 19.86              | 19.78              |
| Ether extract    | 5.43               | 6.34               | 7.03               | 7.54               |
| Crude fibre      | 5.95               | 2.39               | 6.00               | 2.36               |
| Crude ash        | 6.62               | 5.23               | 6.81               | 4.82               |
| Starch           | 36.63              | 35.12              | 40.02              | 38.93              |
| Sucrose          | 5.12               | 5.09               | 4.78               | 4.98               |
| Calcium          | 1.01               | 0.98               | 0.91               | 0.87               |
| Total phosphorus | 0.67               | 0.70               | 0.62               | 0.64               |
| Calculated composition |
| Phosphorus, nonphytate, % | 0.46               | 0.46               | 0.39               | 0.39               |
| Lysine, %        | 1.21               | 1.21               | 1.10               | 1.10               |
| Methionine, %    | 0.60               | 0.60               | 0.53               | 0.53               |
| Methionine + cysteine, % | 0.98               | 0.86               | 0.86               | 0.86               |
| Threonine, %     | 0.86               | 0.86               | 0.77               | 0.76               |
| Metabolizable energy, kcal/kg | 3093               | 3112               | 3272               | 3276               |

CI: Crude protein. $^*$Provides per kg of diet: vitamin A (retinyl acetate), 12,000 U; cholecalciferol, 37.5 µg; vitamin E (DL-α-tocopheryl acetate, 68 U; vitamin K$_3$, 5 mg; vitamin B$_6$, 3 mg; vitamin B$_2$, 6 mg; vitamin B$_1$, 5 mg; vitamin B$_12$, 0.03 mg; nicotine amid, 40 mg; Ca-pantothenate, 10 mg; folic acid, 0.75 mg; biotin, 0.075 mg; choline chloride, 375 mg. $^*$Provides per kg of diet: Mn, 90 mg; Fe, 50 mg; Zn 60 mg; Cu, 5 mg; Co, 0.2 mg; Se, 0.15 mg; I, 0.18 mg. $^*$Provides per kg of diet: Narasin, 70 mg. $^*$Sawdust replaced by the EOM preparation.

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as feed consumed per unit of body weight gain, and was adjusted for weight of chicks at the first day and bird mortality. Mortality was recorded daily and was used to adjust the total number of birds to determine the total feed intake per bird. At the end of the experiment (at Day 42), 12 birds whose body weight was similar to the group average (±5%) were selected from each of the treatment groups (3 birds per replicate). Sampled birds were then electrically stunned and slaughtered by severing the jugular vein, and blood samples were taken. The slaughtered birds were then eviscerated to measure carcass yield, some internal organs, and bursa of fabricius. The intestinal organs, i.e., liver, spleen, small and large intestines, abdominal fat pad and bursa of fabricius, were weighed individually. Weight of these internal organs was expressed as a percentage of live body weight. The hot carcass yield was determined as a percentage of the pre-slaughter live body weight of broilers.

Serum was isolated and stored at -20°C. Individual serum samples were analyzed for antibody responses against infectious bursal disease virus (IBD) and Newcastle disease (ND) by Elisa technique using commercial kits (BioChek, Reeuwijk, The Netherlands), and the plates were read at 405 nm on an ELISA reader (Hitachi EL 3096 Microplate Reader).

At 21 and 42 days of age, 2 birds per replicate (8 birds per treatment) were randomly selected and killed by cervical dislocation for the collection of tissue samples from the jejunum. The contents of the duodenum and jejunum were carefully hand-stripped. The jejunum was defined as the portion of intestine extending from the bile duct entrance to Meckel’s diverticulum. A 2-cm segment of the jejunum (3 cm from the end of the duodenum) was excised, washed in physiological saline solution, and fixed in 10% buffered formalin. Then, the tissue samples were processed using routine histological methods and later embedded in paraffin wax blocks. Then 6 µm thick sections were cut and stained with Masson’s trichrome stain (Culling et al., 1985). All specimens were examined under a light microscope (Nikon Eclipse E-400) equipped with a digital camera head (DS-5M) and a camera control unit (DS-L1, Nikon). The villus height was measured using an image analysis system (BS200 PRO 2005, BAB Ltd. Comp., Istanbul, Turkey). Villus height was measured from the top of the villus to the top of the lamina propria. Six measurements were taken per bird and the average of these values was recorded.

The standard techniques of proximate analysis were used to determine the nutrient concentrations in the diets (Naumann and Bassler, 1993). The experimental diets were also analyzed for starch, sugar, total calcium and phosphorus according to the chemical analytical methods for feedstuffs of the Association of German Agricultural Analysis and Research Institutes (VDLUFA) (Naumann and Bassler, 1993).

### Statistical analysis

The experiment was conducted as a completely randomized design with 8 treatments arranged factorially, and main effects (diet type, EOM, bird gender) and their interactions were analyzed using the GLM procedure of SAS (SAS, 2001). Arc-sin transformation was applied to the percentage values before testing for differences. Significant differences between treatment means were separated using Duncan’s multiple range test with a 5% probability. All statements of significance are based on probability of less than 0.05.

### Results and discussion

#### Performance

Body weight at 1, 21 and 42 days of age and mortality rate for the two periods of 1-21 and 1-42 days are presented in Table 2. The supplementation of a wheat-based diet with EOM increased BW in both males and females at 21 and 42 days of age. Nonetheless, EOM depressed the BW of corn-fed males at both 21 and 42 days of age. As such, the obtained increases in female BW with respect to EOM supplementation to the corn-based diet were inferior to that of the wheat-based diet. These indicate significant 3-way interactions on both 21- (P=0.08) and 42-day body weight (P<0.01).

The positive effects of EOM on body weight gain in the present study (excluding the males fed on the corn-based diet) confirm the findings of previous research (Windsch et al.,

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**Table 2. The effects of diet type, gender and essential oil mixture on body weight and mortality of broilers.**

| Diet type | EOM | Body weight, g | Mortality rate, % |
|-----------|-----|----------------|------------------|
|           | 1 d | 21 d | 42 d | 1-21 days | 1-42 days |
| Male      |     |      |      |            |           |
| Wheat     | -   | 45.90| 722d | 2617c      | 0.50      | 1.50      |
| Corn      | +   | 45.82| 744c | 2708b      | 1.00      | 1.50      |
| Corn      | -   | 45.83| 822a | 2773a      | 0.50      | 1.00      |
| Corn      | +   | 45.79| 792b | 2693b      | 1.00      | 1.50      |
| Female    |     |      |      |            |           |
| Wheat     | -   | 45.66| 657f | 2333e      | 0.50      | 1.00      |
| Wheat     | +   | 45.70| 687c | 2393d      | 1.00      | 1.00      |
| Corn      | -   | 45.77| 738d | 2376b      | 2.00      | 2.00      |
| Corn      | +   | 45.63| 752c | 2406d      | 0.00      | 0.50      |

### Table 2. EOM, essential oil mixture. * Means within columns, within main effects, with different superscript differ at P<0.05. **Data are means of 4 replicate pens with SEM for each treatment; *data were analyzed as a 2×2×2 arrangement.**
2008). Specifically, dietary provision of the same essential oil mixture (Herbromix) at 36 or 48 mg/kg increased body weight and decreased feed conversion ratio in Ross-308 broiler chickens reared up to 42 days of age (Alicic et al., 2003, 2004; Bozkurt et al., 2012; Kucukylmaz et al., 2012). The available scientific evidence described in detail above underlines the mechanism of action of supplemental essential oils in promoting growth and efficiency of feed conversion in broilers. Since any signs of disease or toxicological symptoms were not diagnosed, we failed to explain the exact mechanism that retards growth in male birds fed the corn-based diet with added EOM (48 mg/kg diet).

In fact, the experimental wheat-based diets were seen to be differ completely from the corn-based diets in terms of digestibility (Choc and Annison, 1990; Engberg et al., 2004). It is well documented that the non-starch polysaccharides present in cereals such as wheat and barley lead to low growth performance by raising the viscosity of ileal contents and so subsequently decreasing the digestibility of the macronutrients, and also of some micronutrients (Steenfeldt et al., 1998). Considering that wheat contains a higher amount of anti-nutritional substances than corn, the lower BW in wheat-fed broilers at 21 and 42 days of age is consistent with the general expectations (Table 2).

None of the variables had any effect on mortality throughout the experimental period (Table 2). Although substantially proven in in vitro (Cowan, 1999; Lambert et al., 2001) and in vivo (Jamroz et al., 2003, 2005; Mitsch et al., 2004) antimicrobial action, essential oils did not improve the livability of broiler chickens in the majority of the earlier studies (Basmacoglu et al., 2004, 2010; Cabuk et al., 2006; Isabel and Santos, 2009; Bozkurt et al., 2012) and this was also the case in the present study when added as a supplement to broiler diets as in-feed antimicrobial agents.

Feed intake and FCR of male and female broilers given either corn-based or wheat-based diets with and without EOM over 1-21 day and 1-42 day periods are presented in Table 3. Diet type and, naturally, gender had a significant effect on FI of broiler chickens. Birds fed the corn-based diet received more food than those on the wheat-based diet over a 1-21 day period (P<0.01). There was no difference in overall consumption (1-42 days) between the two diet type applications (P>0.05). On the other hand, an EOM did not influence FI for any time period either on corn or wheat diets. However, study findings were inconsistent; some authors reported EOM to be a stimulator (Alicic et al., 2004; Bozkurt, et al., 2009) while others reported them to have no effect (Hernandez et al., 2004; Basmacoglu et al., 2004; Botsoglou et al., 2004) on FI in broiler chickens. The discrepancies between the results appear to be due to nutritional variables, including ingredient composition, variety and, in particular, amount of essential oils added.

Fortification diet with EOM provided benefit in terms of FCR when compared to unsupplemented treatment during the 1-21 (P=0.06) and 1-42 (P<0.05) day periods (Table 3). Besides this, numerical improvements were reported when corn was used instead of wheat throughout the experiment. The change in characteristics of food substrates due to the stimulated endogenous enzyme concentration and well-balanced intestinal microbial flora were seen to lead to improved feed absorption and utilization, and hence enhancement in feed efficiency (Bedford and Morgan, 1996). Jamroz et al. (2003) demonstrated that a supplemented broiler diet with phytogenetic extracts including oregano oil and thyme oil slightly increased pancreatic enzyme secretion in association with better feed efficiency in both wheat-based and corn-based diets as compared to unsupplemented controls. Increased chymotrypsin and amylase activity in response to oregano oil supplementation to a wheat-based diet was also recently reported (Basmacoglu et al., 2010). It appears that essential oil supplementation to a wheat-based diet may partly compensate for some nutritional shortcomings (Simon, 1998; Steenfeldt et al., 1998), thereby stimulating secretion of endogenous enzymes, as shown in the above-mentioned studies.

### Table 3. Feed intake and feed conversion ratio of broilers fed on corn vs wheat based diets with or without essential oil mixture.

| EOM | Feed intake, g | Feed conversion ratio, g feed:g gain |
|-----|----------------|-------------------------------------|
|     | 1-21 days | 1-42 days | 1-21 days | 1-42 days |
| Diet type | Male | | | |
| Wheat | - | 1165 | 4945 | 1.731 | 1.923 |
| Wheat + | 1154 | 4936 | 1.653 | 1.854 |
| Corn | - | 1208 | 4986 | 1.633 | 1.828 |
| Corn + | 1232 | 4882 | 1.651 | 1.844 |
| Female | - | 1079 | 4461 | 1.764 | 1.950 |
| Wheat | 1064 | 4435 | 1.659 | 1.889 |
| Corn | - | 1210 | 4535 | 1.748 | 1.946 |
| Corn + | 1162 | 4480 | 1.645 | 1.888 |
| SEM | 22.73 | 59.98 | 0.02 | 0.02 |
| Main effects | | | | |
| Wheat | 1116 | 4694 | 1.701 | 1.903 |
| Corn | 1218 | 4717 | 1.669 | 1.875 |
| EOM | - | 1180 | 4731 | 1.719 | 1.909 |
| EOM + | 1153 | 4680 | 1.651 | 1.864 |
| Male | - | 1204 | 4937 | 1.667 | 1.862 |
| Female | 1128 | 4477 | 1.704 | 1.920 |
| Probabilities | | | | |
| Diet type | 0.0001 | 0.5866 | 0.0638 | 0.1211 |
| EOM | 0.0957 | 0.5062 | 0.0321 | 0.0487 |
| Gender | 0.0001 | 0.0001 | 0.0377 | 0.0012 |
| Diet type x EOM | 0.3684 | 0.4290 | 0.4639 | 0.1622 |
| Diet type x gender | 0.4559 | 0.4862 | 0.5100 | 0.1143 |
| EOM x gender | 0.7986 | 0.9818 | 0.1929 | 0.3570 |
| Diet x EOM x gender | 0.9031 | 0.7574 | 0.4540 | 0.2454 |

EOM, essential oil mixture. * Means within columns, within main effects, with different superscript differ at P<0.05. ** Data are means of 4 replicate pens with SEM for each treatment; ‘ data were analyzed as a 2x2x2 arrangement.
Table 4. Carcass yield and relative weights (in percentage) of internal organs of broiler chicks fed on different dietary regimens.

| Diet type | EOM | Carcass yield | Liver | Small intestines | Large intestines | Spleen | Bursa of fabricius |
|-----------|-----|---------------|-------|------------------|-----------------|--------|-------------------|
| Male      |     |               |       |                  |                 |        |                   |
| Wheat     | -   | 77.12         | 2.29  | 2.63             | 0.208           | 0.141  | 0.181             |
| Wheat     | +   | 76.33         | 2.17  | 2.56             | 0.192           | 0.117   | 0.178             |
| Corn      | -   | 78.08         | 2.89  | 2.21             | 0.172           | 0.106   | 0.190             |
| Corn      | +   | 78.85         | 2.28  | 2.39             | 0.143           | 0.117   | 0.217             |
| Female    |     |               |       |                  |                 |        |                   |
| Wheat     | -   | 75.51         | 2.16  | 2.70             | 0.223           | 0.106   | 0.195             |
| Wheat     | +   | 77.16         | 2.26  | 2.63             | 0.196           | 0.105   | 0.181             |
| Corn      | -   | 77.47         | 2.05  | 2.31             | 0.161           | 0.108   | 0.177             |
| Corn      | +   | 77.75         | 2.26  | 2.39             | 0.157           | 0.137   | 0.182             |
| SEM\(\delta\) | & 0.481 | 0.087 & 0.137 & 0.010 & 0.010 & 0.014 & |

Main effects\(\delta\)
- Wheat \(\beta\)
  - 76.53\(\beta\)
  - 78.03\(\beta\)
  - 77.64\(\beta\)
  - 77.52\(\beta\)
  - 77.59\(\beta\)
  - 76.97\(\beta\)

Probabilities
- Diet type \(\delta\)
  - 0.0001
  - 0.1655
  - 0.0714
  - 0.8835
  - 0.5001
  - 0.1601
  - 0.0534

- EOM \(\delta\)
  - 0.023
  - 0.0238

- Gender \(\delta\)
  - 0.0714
  - 0.2192

- Diet type x EOM \(\delta\)
  - 0.8434

- Diet x gender \(\delta\)
  - 0.609

EOM, essential oil mixture. a,bMeans within columns, within main effects, with different superscript differ at P<0.05. °Data are means of 4 replicate pens with SEM for each treatment; #data were analyzed as a 2x2x2 arrangement.

Carcass yield and relative organ weights
Carcass yield and relative weights of some internal organs of broilers were unaffected by EOM and bird gender (P<0.05) (Table 4). However, carcass yield and small and large intestine weights are significantly influenced by diet type (P<0.01). The current data indicated a positive response to corn-based feeding on hot carcass yield by 1.96% as compared to the wheat-based program (P<0.01). This is probably because of the decreased nutrient digestibility due to the nutritional shortcomings mentioned above when birds were fed on a wheat-based diet. The significant increment in intestinal weight indicates a thickening of the intestinal wall in association with unfavorable conditions through the gut line that might result from the marked level of dietary wheat supplementation (65-68%) in this study. Notably, considering the present ingredient composition in Table 1, such a higher level of wheat inclusion rate has not really been seen in the recent scientific literature. Those measurable histological changes through the intestines in wheat-fed birds are in accordance with the lower body weight gain (Table 2), slightly inferior feed conversion ratio (Table 3) and,

Table 5. Jejunal villus height of broilers fed on two diet types, with or without essential oil mixture.

| Diet type | EOM | 21 days of age | 42 days of age |
|-----------|-----|----------------|---------------|
| Male      |     |               |               |
| Wheat, µ  | -   | 0.760         | 1.012         |
| Wheat, µ  | +   | 0.840         | 1.100         |
| Corn, µ   | -   | 0.950         | 1.213         |
| Corn, µ   | +   | 0.916         | 1.186         |
| Female    |     |               |               |
| Wheat, µ  | -   | 0.753         | 0.992         |
| Wheat, µ  | +   | 0.809         | 1.063         |
| Corn, µ   | -   | 0.890         | 1.160         |
| Corn, µ   | +   | 0.934         | 1.193         |
| SEM\(\delta\) | & 0.023 | & 0.028 & | |

Main effects\(\delta\)
- Wheat, µ \(\beta\)
  - 0.790\(\beta\)
  - 0.922\(\beta\)
  - 0.838\(\beta\)
  - 0.874\(\beta\)
  - 0.840\(\beta\)

Probabilities
- Diet type \(\delta\)
  - 0.0001

- EOM \(\delta\)
  - 0.0238

- Gender \(\delta\)
  - 0.2192

- Diet type x EOM \(\delta\)
  - 0.3811

- Diet x gender \(\delta\)
  - 0.6934

EOM, essential oil mixture. a,bMeans within columns, within main effects, with different superscript differ at P<0.05. °Data are means of 4 replicate pens with SEM for each treatment; #data were analyzed as a 2x2x2 arrangement.
ultimately, the lower meat yield (Table 4) as compared to birds on the corn diet.

In the present study, the administered diet with EOM did not modify intestinal weight or carcass yield (Table 4). In accordance with findings presented herein, some earlier studies had reported no change in carcass yield and intestinal weight in broiler chickens when fed diets with different essential oils (Basmacıoğlu et al., 2004; Jamroz et al., 2005; Çabuk et al., 2006). The results showed that the weight of the liver or bursa of fabricius was not affected by any of the variables tested (P<0.05), whereas the weight of the spleen yielded variable responses to different treatments with significant interactions (Table 4). However, there is a considerable and marked tendency towards overgrowth in organs associated with the immune system, i.e. spleen and bursa of fabricius, in both male and female birds fed corn diets with added EOM.

Villus height

The mean height of villus sampled from jejunum is shown in Table 5. EOM affected jejunal villus height at both 21 and 42 days of age (P<0.05). Birds given diets with EOM had longer (P<0.05) villus than those of untreated birds. Villus length was shorter (P<0.01) in birds fed wheat-based diets as compared to those on the corn-based diet. In poultry, intestinal villus plays a crucial role in digestion and absorption of nutrients, ensuring maximum absorption capacity with changes in intestinal morphology and increased villus surface area (Dibner and Richards, 2004; Sklan and Tucker, 2004). Villus morphology and histology are correlated with growth and feed efficiency in broiler chickens (Jamroz et al., 2006; Garcia et al., 2007). In agreement with this, birds with longer villus in groups fed the corn-based diet showed a better growth rate and meat yield than those receiving the wheat-based diet. Our findings also suggest villi-related protective properties of bioactive compounds of EOM including mainly carvacrol, thymol, and 1,8-cineole. The suggestion by Jamroz et al. (2006) that the oregano leaf extract and essential oil could accelerate the renewal rate of mature enterocytes at the surface of villi of the intestine appears to confirm the positive relationship between villus height and performance in EOM-fed birds in this study.

Immune response

In the present study, the nutritional factors (i.e. diet type and inclusion of essential oil to the diet) did not influence the specific immune response of broiler chickens measured as serum IBD and ND titers at 42 days of age (Table 6). Also, the generation of specific immune response was not influenced by bird gender. Indeed, the gut and resident microbiota, and also nutrition, play a pivotal role in shaping the immune system (Sklan et al., 1994; Noverr and Hufnagle, 2004). Therefore, it is appropriate to postulate that the EOM could increase the immune response due to its antimicrobial activity since various improved immune globulin concentrations and serum NDV titers have been observed with essential oil supplementation in monogastric farm animals (Cho et al., 2006; Basmacıoğlu et al., 2010). However, results of the current study showed no difference in terms of serum IBD and ND titers. It has also been suggested that the anti-oxidant properties of some plant bioactives may play a role in the development of immune response in birds by protecting cells from oxidative damage and enhancing the function and proliferation of these cells (Deying et al., 2005). However, the specific experimental verification of this in broiler chickens is rather limited. We are not, therefore, able to draw any conclusions on the effects of essential oils on specific immune response in this study.

Conclusions

Production efficiency of broiler chickens was not compromised when a specific blend of essential oils was added to a wheat-based diet. However, a noticeable reduction in growth in male birds fed a corn-based diet with EOM was seen. It is likely that the significance of the effects of the essential oils varies with diet type and bird gender, and further research is warranted in this area considering the diverse range of diet types and the inclusion dose responses.
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