Performance, Blood Metabolites and Immunocompetence of Broiler Chicks Fed Diets Included Essential Oils of Medicinal Herbs

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Abstract: This study was undertaken to investigate growth performance, blood metabolites and humoral immune response of broiler chicks fed diets supplemented with essential oils of thyme, cinnamon and clove. Two hundred and sixty four days old Ross-308 chicks were weighed and randomly allocated between 24 battery pens. Four iso-nitrogenous and iso-caloric diets including: basal diet (control) and basal diet included 200 mg kg$^{-1}$ essential oils of Thyme (THY), Cinnamon (CIN) and Clove (CLO) were formulated to meet bird requirements and fed chicks in starting (1-21 days), growing (22-42 days) and finishing (43-49 days) periods. Each diet was fed to birds in six pens (replicate) with 11 chicks per pen. At 17 and 26 days of age, all 360 chicks were vaccinated against NDV. Blood samples were withdrawn from the wing vein at days 17, 26, 34, 41 and 47 of age. The non-heparinized blood samples (1.5 mL/chicken-one bird per pen) were placed at 37°C for 2 h, centrifuged (3000×g for 15 min) to separate sera and stored at 20°C until analysis. The sera were applied to HI test to determine Antibody (Ab) to NDV expressed as reciprocal log 2 values for the highest dilution that displayed HI. The data was analyzed based on completely randomized design by GLM procedure of SAS. Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) were not affected by dietary treatment, except for BWG and FCR during the 22-42 days of age. Chicks fed on THY included diets showed improved FCR and BWG comparing to chicks fed other experimental dietary groups during the growing period. Plasma total cholesterol, triglyceride and HDL were not affected by dietary essential oil inclusion (p>0.05). The highest and lowest levels of RBC and HCT were observed in chicks fed on CLO and CIN-included diets, respectively (p<0.05). Primary antibody response of CIN chicks was higher than THY and CIN chicks (p<0.05). Secondary antibody response were not affected by dietary inclusion of essential oil (p>0.05).

Key words: Essential oils, performance, immunity, broilers, Iran, blood metabolism

INTRODUCTION

A number of feed additives such as antibiotics have been widely used in the poultry industry for several decades. After the use of most antibiotic growth promoters as feed additives has been banned by the European Union due to cross-resistance against pathogens and residues in tissues, scientists have searched for alternatives to antibiotics. In this view, aromatic plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects and the stimulating effect on animal digestive systems. Aromatic plants have been used traditionally in the therapy of many diseases for a long time in the world. Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable. There are many records in literature working on including herbal plant powders or essential oils extracted from medicinal plants in animal diets (Lopez-Bote et al., 1998; Zheng and Wang, 2001; Botsoglou et al., 2002; Miura et al., 2002; Burt and Reinders, 2003; Jamroz et al., 2005).

Thymol, a major component of thyme-essential oils has been widely studied for its antimicrobial properties (Dorman and Deans, 2000). Carvacrol, an isomer of thymol is found in essential oils isolated from oregano and thyme. Like thymol, carvacrol also displays antimicrobial activity (Helander et al., 1998). Given their antimicrobial activity, it would be expected that thymol and carvacrol could have positive effects on growth performance in broilers.

Cinnamaldehyde, a main principle of cinnamon essential oil, amounts to approximately 60-75% of the total oil. Lee and Ahn (1998) found that cinnamaldehyde, derived from the cinnamon essential oil, strongly inhibits Clostridium perfringens and Bacteroides fragilis and...
moderately inhibits *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from human feces. The selective inhibition by cinnamaldehyde of pathogenic, intestinal bacteria may have a pharmacological role in balancing the intestinal microbiota. The wide range of *in vitro* anti-microbial activities of essential oils derived from cinnamon and thyme have been published (Paster et al., 1990; Cozzentino et al., 1999; Dorman and Deans, 2000), supporting their possible use as anti-microbial agents.

Similarly, Clove (*Syzygium aromaticum L.*) has been used as an antiseptic (Robenost, 1996) and antimicrobial (Dorman and Deans, 2000; Ouattara et al., 1997; Teissedre and Waterhouse, 2000; Valero and Salmeroj, 2003). In addition, it has appetite and stimulating effect of digestion, antifungal (Velluti et al., 2003), antiparasitic (Kim et al., 2004) and antioxidant (Gülçin et al., 2004; Lee and Shiyamoto, 2002).

There are very little information in literature evaluating effects of dietary inclusion of essential oils of thyme, cinnamon and clove on performance and immunity of growing broiler chicks at the single experiment, so the objective of the present study was to examine the effect of adding essential oils of these herbal plants on growth performance, blood metabolites and humoral immune response of broilers.

MATERIALS AND METHODS

A total number of 264 days old unsexed Ross 308 broiler chicks were distributed in a completely randomized experimental design in cage batteries with four treatments and six replications of eleven chicks each. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4 the lighting schedule was 24 h light. At days 5-49 the dark time was increased to 1 h. Birds were fed diets (starter: 1-21 days, grower: 22-42 days and finisher: 43-49 days). The composition of experimental diets is shown in Table 1. Diets prepared without additive as Control (CON) or with 200 mg kg⁻¹ essential oils of thyme, cinnamon and clove. Feed and water were available *ad libitum*. Data were collected for Body Weight (BW) at 21, 42 and 49 days of age and for Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) during periods of 0-21, 22-42 and 43-49.

One bird of each replicate was randomly selected, weighed after feed deprivation for 12 h and killed by cervical dislocation at 47 days of age. The relative weights of breast, drumstick and thigh were calculated as organ weight (g/100 g BW). The visceral were then opened and the thymus, spleen, bursa of Fabricius, liver, pancreas, gizzard, fat pad, heart and intestinal segments removed and weighed. Relative organ weights were calculated as organ weight (g/100 g BW).

Blood taking happened on the 34th day of age from on birds per each cage to determine HDL, TG and total cholesterol. Blood samples were centrifuged (15 min, 3000 rpm) and the obtained serum was preserved in -70°C to be analyzed latter. Serum cholesterol concentration, TG, and HDL were determined. To count WBC, a diluted sample of 1/100 was a sed on a counting chamber, thin blood slides were made and after staining with Geimsa, differential count glucosidases was performed.

Broiler vaccination against Newcastle virus happened in two turns as the following; the first on the 17th day of the experiment as an eye drop and the second or the booster on the 26th day as a drink. Blood samples were withdrawn from the wing vein on 17, 26, 34, 41 and 47th days of age. The non-heparinized blood samples (1.5 mL/
RESULTS AND DISCUSSION

According to the Table 2, BWG, FI and FCR were not affected by dietary treatment except for BWG and FCR during the 22–42 days of age. Chicks fed on THY-included diets showed improved FCR and BWG comparing to chicks fed other experimental dietary groups during the growing period.

Cross et al. (2007) who investigated the effect of the dietary inclusion of 5 culinary herbs (thyme, oregano, marjoram, rosemary or yarrow) or their essential oils on the growth, digestibility and intestinal microflora status in female broiler chicks reported that dietary thyme oil or yarrow herb inclusion had the positive effects on chick performance, while oregano herb and yarrow oil were the poorest supplements. Some other researchers (Botsoglou et al., 2003; Papageorgiou et al., 2003) reported that essential oils were not effective in improving animal performance. In addition, in vitro studies have shown that essential oils to have antibacterial properties against Listeria monocytogenes, Salmonellatyphimurium, Escherichia coli, Bacillus cereus and Staphylococcus aureus (Cosentino et al., 1999).

Some researchers have shown that essential oils of rosemary (Rosmarinus officinalis), sage (Salvia sclarea), thyme (Thymus vulgaris) were among the most active in this respect against strains of E. coli (Smith-Palmer et al., 1998; Hammer et al., 1999).

The results for visceral measurements are presented in Table 3. Among the visceral organs, only the relative weights of drumstick and pancreas were affected from dietary inclusion of essential oils. Chicks fed on CIN and THY included diets shown the highest and the lowest relative weight of drumstick, respectively. However, only the difference between these two groups was significant (p<0.05).

Chicks fed on THY and CIN included diets shown the highest and lowest relative weight of drumstick, respectively. However, only the difference between these two groups was statistically significant (p<0.05). The results for relative weight of intestinal segments and the viscosity of ileal digesta are presented in Table 4. Dietary addition of essential oils of medicinal herbs in this experiments did not affect the relative weight of intestinal segments and the viscosity of ileal digesta (p>0.05).

Plasma total cholesterol, triglyceride and HDL were not affected by dietary essential oil inclusion (Table 5). The highest and lowest levels of RBC and HCT were observed in chicks fed on CLO and CIN included diets.
Table 4: Relative weight (% body weight) of intestinal segments and the viscosity of ileal digesta in broilers fed the control diet (CON) and the diets containing 200 mg kg⁻¹ essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO) in 47 day of age.

<table>
<thead>
<tr>
<th>Items</th>
<th>CON</th>
<th>THY</th>
<th>CIN</th>
<th>CLO</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum weight</td>
<td>0.48</td>
<td>0.50</td>
<td>0.46</td>
<td>0.50</td>
<td>0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Jejunum weight</td>
<td>1.24</td>
<td>1.17</td>
<td>1.01</td>
<td>1.16</td>
<td>0.010</td>
<td>0.06</td>
</tr>
<tr>
<td>Ileum weight</td>
<td>1.04</td>
<td>0.88</td>
<td>0.95</td>
<td>1.01</td>
<td>0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>2.21</td>
<td>2.21</td>
<td>2.67</td>
<td>1.82</td>
<td>0.582</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Mean values within a row with different superscript letters (a, b and c) were significantly different (p<0.05). CON (Control), THY (Thyme essential oil, 200 mg kg⁻¹ diet), CIN (Cinnamon essential oil, 200 mg kg⁻¹ diet) and CLO (Clove Essential Oil, 200 mg kg⁻¹ diet).

Table 5: Total cholesterol, triglyceride and high density lipoprotein of serum, red blood cell, Hematocrit (HCT) and white blood cell counts on day 34 of age in broilers fed the control diet (CON) and the diets containing 200 mg kg⁻¹ diet essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO).

<table>
<thead>
<tr>
<th>Items</th>
<th>CON</th>
<th>THY</th>
<th>CIN</th>
<th>CLO</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>130.17</td>
<td>128.67</td>
<td>134.83</td>
<td>135.83</td>
<td>13.943</td>
<td>0.84</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>115.50</td>
<td>120.83</td>
<td>113.00</td>
<td>113.50</td>
<td>27.585</td>
<td>0.95</td>
</tr>
<tr>
<td>HDL (mg dl⁻¹)</td>
<td>77.67</td>
<td>77.00</td>
<td>83.17</td>
<td>79.17</td>
<td>7.879</td>
<td>0.54</td>
</tr>
<tr>
<td>RBC</td>
<td>2.24₇</td>
<td>2.3₆₇</td>
<td>2.2₈₇</td>
<td>2.5₇₆</td>
<td>0.15₈</td>
<td>0.05</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>32.7₇₅</td>
<td>32.6₅₆</td>
<td>31.0₇₆</td>
<td>34.8₈₆</td>
<td>2.1₆₈</td>
<td>0.04</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>59.3₅₆</td>
<td>68.₃₅₆</td>
<td>73.₅₆₆</td>
<td>66.0₅₆</td>
<td>0.0₅₄</td>
<td>0.02</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>0.8₃₅</td>
<td>1.₅₃₅</td>
<td>1.₃₃₅</td>
<td>1.₈₃₅</td>
<td>0.0₈₈</td>
<td>0.8₇</td>
</tr>
<tr>
<td>Basophile (%)</td>
<td>0.₃₃₅</td>
<td>0.0₃₅</td>
<td>0.₀₃₅</td>
<td>0.₅₃₅</td>
<td>0.₄₇₈</td>
<td>0.4₁</td>
</tr>
<tr>
<td>Eosinophile (%)</td>
<td>0.₁₇₅</td>
<td>0.₀₁₅</td>
<td>0.₀₁₅</td>
<td>0.₀₂₀</td>
<td>0.₀₂₁</td>
<td>0.₄₁</td>
</tr>
</tbody>
</table>

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respectively so that the differences between these two dietary groups were significant (p<0.05) but no statistically significant differences was found with CON and THY (p>0.05). Among the white blood cell counts, only heterophils and lymphocytes were significantly affected from dietary treatments. Chicks fed on THY and CIN included diets had significantly lower number of heterophiles comparing to control group. In the case of lymphocyte numbers, chicks fed on THY and CIN included diets had significantly higher number of heterophiles comparing to control group.

As it was shown in Fig. 1, primary antibody response (Anti-NDV titers 9 days after first vaccination) of CON chicks was higher than THY and CIN chicks (p<0.05) but no statistically significant differences was found between CON and CLO chicks. Secondary antibody response (Anti-NDV titers 8, 15 and 21 days after second vaccination) were not affected by dietary essential oil inclusion (p>0.05). Diet inclusion of essential oils of THY caused an accelerating increase in secondary antibody response comparing to other dietary groups.

Fig 1: Humoral immune response against Newcastle disease virus (log 2) of broiler chickens fed the Control diet (CON) and the diets containing 200 mg kg⁻¹ diet essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO).

CONCLUSION

In this study, it can be concluded that adding essential oils of thyme to broiler diets may improve FCR with no adverse effect on other biochemical of immunological traits of birds.

REFERENCES


