The effects of different amounts of Mentha pulegium L. on immune system performance of broiler chickens

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ABSTRACT: The purpose of this study was to investigate the effects of different amounts of Mentha pulegium L. on immune system performance of broiler chickens. 300 one-day-old broilers (308 Ross strain) were used in a completely randomized design in 5 treatments and three replicates (20 birds per replicate) for 42 days. The treatment groups consisted of a control (Group 1) assigned to the basal diet and experimental groups 2, 3, 4 and 5 were basal diet supplemented with 0.5%, 1%, 1.5% and 2% Mentha pulegium L. of aerial parts powder in the feedstuff, respectively. B1 Newcastle vaccine was used in all groups of broilers at 10, 25 and 35 days of age. Two birds from each replicate were randomly chosen and blood sample was taken on the 24th and 34th days of experimental period and Hemagglutination Inhibition (HI) test was performed on sera and the heterophil: lymphocyte ratio was calculated.

Regarding HI test (Antibody levels against Newcastle virus vaccine), it didn’t show statistical significance in different treatment groups. Regarding heterophil: lymphocyte ratio, it didn’t show statistical significance in different treatment groups (P> 0.05). But, on the basis of results, group 3 (1% Mentha pulegium L.) showed the best performance in comparison with the other groups.

Key words: Broiler; Heterophil: Lymphocyte ratio; HI test; Immune system; Mentha pulegium

INTRODUCTION

There is considerable research interest in the possible use of natural products, such as essential oils and extracts of edible and medicinal plants, herbs and spices, for the development of new additives in animal feeding (Lavinia et al, 2009). Herbs, spices, and various plant extracts have received increased attention as possible antibiotic growth promoter alternatives (Mehmet et al, 2005). These extracts are specifically known for their antiseptic properties and beneficial effects on digestion (Fritz et al, 1993). The digestive stimulant action of spices has been well recognized for a long time, and a few of them find pharmacological application against digestive disorders (Platel and Srinivasan, 2001). Spices may exert their digestive stimulant action possibly through a beneficial stimulation of activity of the enzymes responsible for digestion, and/or secretion of bile which plays an important role in facilitating digestion and absorption of dietary fat (Platel and Srinivasan, 2001). Mentha, the genus in Labiatae family, includes 20 species that can be found all over the world. Mentha pulegium L. is one of the Mentha species commonly known as pennyroyal. It is native to Europe, North Africa, Minor Asia and the near East. The flowering aerial parts of Mentha pulegium L. have been traditionally used for its antimicrobial properties in the treatment of cold, sinusitis, cholera, food poisonings, bronchitis and tuberculosis (Zargari, 1990). In addition, it is also used as an antiflatulent, carminative, expectorant, diuretic, antitussive and menstruation agent (Nobakht et al, 2011). Some pharmacological effects of Mentha pulegium L. oils such as antimicrobial (Mahboubi and Haghi, 2008) and abortifacient effects in rat myometrium (Nobakht et al, 2011), cytotoxic activity against different human cell lines (Shirazi et al, 2004) and its antioxidant effect (El-Ghorab, 2006) have also been confirmed. Studies showed three chemotypes of Mentha pulegium L. with the following major oil components: (1) pulegone, (2) pipiritenone and/or pipiritone and (3) isomenthone/
neoisomenthol (Cook et al., 2007). In an experiment, the antimicrobial effect of Mentha pulegium L. was demonstrated (Jazani et al., 2009). Mentha pulegium L. also exhibit antioxidant activity (Nobakht et al., 2011). It was demonstrated that Mentha pulegium had significant effects on performance, egg quality, blood and immunity parameters of laying hens (p<0.05). In this experiment, the highest egg production percent, egg mass, better feed conversion, haugh unit and egg yolk color index were observed with using 2% Mentha pulegium L. (Nobakht and Mehmawnavaz, 2010). The antioxidants can prevent nutrients oxidation and the antimicrobial component can decrease the harmful bacterial populations in the gastrointestinal tract of broilers. The presence of harmful bacterial populations in the gastrointestinal tract may cause the breakdown of amino acids and thereby reduce their absorption (Lee et al., 2003). Therefore, the antimicrobial properties of Mentha pulegium L. (Nobakht et al., 2011) can reduce the harmful bacterial populations in the gastrointestinal tract and improve the levels of absorbed amino acids. The mechanism of action of herbal products has not been very clearly defined yet but there are suggestions that they alter the permeability of cell membranes and cause the destruction of pathogenic bacteria (Skandamis and Nychas, 2001). The immune system generally benefits from the herbs and spices rich in flavonoids, vitamin C and carotenoids (Frankic et al., 2009). The plants contain flavonoid and trepenic compounds by improving activity of vitamin C and their antibacterial effects cause improve immune system performance in animals (Samman and Cook, 1996). The aim of the present study was the investigate of the effects of different amounts of Mentha pulegium L. on immune system performance of broiler chickens.

MATERIALS AND METHODS

300 one-day-old broilers (308 Ross strain) were used in a completely randomized design in 5 treatments and three replicates (20 birds per replicate) for 42 days. The treatment groups consisted of a control (Group 1) assigned to the basal diet and experimental groups 2, 3, 4 and 5 were basal diet supplemented with 0.5%, 1%, 1.5% and 2% Mentha pulegium L. of aerial parts powder in the feedstuff, respectively. B1 Newcastle vaccine was used in all groups of broilers by muscular injection (IM) and drinking water route at 10 days of age. Indeed, this vaccine was used in all groups of broilers by drinking water route at 25 and 35 days of age. In order to investigate the effect of above mentioned medicinal plants on humoral immunity performance in broilers, two birds from each replicate were randomly chosen and about 1 cc blood sample was taken from brachial vein in each bird on the 24th and 34th days of experimental period. Then, blood samples were taken into laboratory in every two experiments and Hemagglutination Inhibition (HI) test was performed on sera and humoral immunity was assessed as antibody production to Newcastle disease virus. Indeed, 1 ml of collected blood sample was taken and transferred to tubes with EDTA for determination of heterophil and lymphocyte blood cell counts. 100 leukocytes per sample was counted by heterophil to lymphocyte separation under an optical microscope. The heterophil: lymphocyte ratio was calculated and recorded (Gross and Sigel, 1983).

Statistical analysis

This study was performed in a completely randomized design in 5 treatments and three replicates with totally 15 experimental units. Comparing between groups was done by Duncan’s multiple rang test and 0.05 was used as the significance level.

RESULTS

Humoral immune level

Humoral immune level in the first record (HI1) and (the) second record (HI2) and the difference between them (dHI) did not show statistical significance in different treatment groups. But the highest increase and the lowest increase of immune level in two records distance numerically were associated with 1% and 1.5% Mentha pulegium L., respectively.

Heterophil

Regarding heterophil, the highest and the lowest amounts were associated with 2% and 1.5% Mentha pulegium L., respectively. All of the groups didn’t show statistical significance in comparison with each other (P>0.05).

Lymphocyte
Regarding the number of lymphocytes, statistical significance was not seen among different experimental groups, but the highest number was associated with 0.5% Mentha pulegium L. and the lowest number was associated with the 2% Mentha pulegium L.

**Heterophil: Lymphocyte ratio**

The highest ratio (Heterophil: Lymphocyte ratio) was shown in 2% Mentha pulegium L. and the lowest ratio was shown in 1.5% Mentha pulegium L. All of the groups didn’t show statistical significance in comparison with each other (Table 1).

<table>
<thead>
<tr>
<th>Mentha pulegium</th>
<th>H</th>
<th>L</th>
<th>H/L</th>
<th>H1</th>
<th>H2</th>
<th>dH1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>23.667</td>
<td>76.333</td>
<td>0.317</td>
<td>2.833</td>
<td>6.833</td>
<td>4.000</td>
</tr>
<tr>
<td>0.5%</td>
<td>19.500</td>
<td>81.167</td>
<td>0.251</td>
<td>2.167</td>
<td>6.500</td>
<td>4.333</td>
</tr>
<tr>
<td>1%</td>
<td>20.500</td>
<td>76.333</td>
<td>0.274</td>
<td>1.833</td>
<td>7.167</td>
<td>5.332</td>
</tr>
<tr>
<td>1.5%</td>
<td>18.833</td>
<td>79.500</td>
<td>0.240</td>
<td>4.000</td>
<td>6.333</td>
<td>2.333</td>
</tr>
<tr>
<td>2%</td>
<td>24.667</td>
<td>73.167</td>
<td>0.343</td>
<td>3.333</td>
<td>7.167</td>
<td>3.833</td>
</tr>
<tr>
<td>p-value</td>
<td>0.481</td>
<td>0.214</td>
<td>0.174</td>
<td>0.285</td>
<td>0.896</td>
<td>0.580</td>
</tr>
<tr>
<td>SEM</td>
<td>2.735</td>
<td>2.487</td>
<td>0.043</td>
<td>0.756</td>
<td>0.736</td>
<td>1.267</td>
</tr>
</tbody>
</table>

H=Heterophil, L=Lymphocyte, H/L= Heterophil: Lymphocyte ratio

**DISCUSSION**

According to the results of the present study, the effect of different amounts (0.5%, 1%, 1.5% and 2%) of Mentha pulegium L. on humoral immunity (antibody titer against Newcastle virus) of broiler chickens didn’t show statistical significance in comparison with the control group (P>0.05). Regarding heterophils: lymphocyte ratio, none of the experimental groups didn’t show statistical significance. These results are in agreement with the experimental results of Nobakht et al.(2011) and Deschpper et al.(2003) who reported that using the different amounts of Mentha pulegium didn’t show statistical significance in heterophil: lymphocyte ratio (Deschpper et al.,2003; Nobakht et al.,2011). Also, this results are in agreement with Lavinia et al.(2009) who reported that using aromatic plants (Satureja hortensis, Mentha piperita and Hippophae rhamnoides) didn’t show significant differences in the leucocytes formula (Lavinia et al.,2009). Because of improving the activity of lymphocytes, macrophages and natural killer cells (NK), medicinal plants cause increasing the phagocytosis and stimulating the production of interferons (IFN). This leads to improvement of immune system performance. In present study, group 2 (0.5% Mentha pulegium L.) showed the highest increase of lymphocytes production. Probably, because of preventing stress and increasing the activity of lymphocytes and stimulating the immune system, Mentha pulegium L. Causes increasing the number of lymphocytes (Craig, 1999). In this study, it was showed that using 1.5% Mentha pulegium L. Caused improvement of heterophil: lymphocyte ratio. While, Nobakht et al.(2011) reported 0.5% of Mentha pulegium L. Caused improvement of heterophil: lymphocyte ratio (Nobakht et al.,2011).

**REFERENCES**


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