

Full Length Research Paper

The effects of different amounts of *Mentha pulegium* L. (pennyroyal) on performance, carcass traits, hematological and blood biochemical parameters of broilers

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Accepted 1 May, 2011

This experiment was conducted to evaluate the effects of different levels of powdered *Mentha pulegium* L. (pennyroyal; medicinal plant) obtained from its dried aerial part on performance, carcass traits, hematological and blood biochemical parameters of broilers. 300 broiler chicks (Ross 308) were used in a completely randomized design in five treatments and three replicates (20 birds per replicate) for 42 days. The treatment groups consisted of a control group (1) with no *Mentha pulegium* L. supplementation, and 2, 3, 4 and 5 experimental groups with different levels of *Mentha pulegium* L. in 0.5, 1, 1.5 and 2% in the feedstuff, respectively. There were significant differences between treatments on performance, carcass traits and blood biochemical parameters of broilers ($p < 0.05$). The lowest amount of daily weight gain (38.82 g) and the highest feed conversion (1.95) were observed in the control group, whereas the highest amount of daily weight gain (46.24 g), the lowest feed conversion (1.71), and the highest breast percent (33.29) were observed in Group 2. The blood samples of Group 5 showed the lowest amount of glucose (157.12 mg/dl) but there was no significant difference between Groups 2 and 5 in the blood glucose levels. The overall results showed that the use of 0.5% of *M. pulegium* L. medicinal plant in the diets of broilers has positive effects on their performance and carcass traits.

Key words: Antibiotic alternatives, blood metabolites, medicinal plant, *Mentha pulegium* L.

INTRODUCTION

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 (Foster and Duke, 1999). Researches on the use of herbal mixtures in poultry diets have produced inconsistent results (Fritz et al., 1993). In an experiment, broilers fed with 0.5% of peppermint performed better than those fed with 1.5% peppermint as regards weekly body weight gain; there was no significant effect with the addition of the peppermint to the diet on blood traits (PCV, RBC, Hb and WBC), however, liver weight significantly decreased whereas the H/L ratio

significantly increased in treatments compared with control (Al-kassie, 2010).

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 (Chalchat et al., 2000). 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 antifatulent, carminative, expectorant, diuretic, antitussive and menstruation agent (Newall, 1996). Some pharmacological effects of *M. pulegium* L. essential oils such as antimicrobial (Mahboubi and Haghi, 2008) and abortifacient effects in rat myometrium (Soares et al., 2005), cytotoxic activity against different human cell lines (Shirazi et al., 2004) and its antioxidant effect

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(El-Ghorab, 2006) have also been confirmed.

The ingredients in *M. pulegium L.* oil have been subjected to a number of studies which have shown differences in its constituents depending on the region of cultivation.

Studies showed three chemotypes of *M. pulegium L.* with the following major oil components: (1) pulegone, (2) piperitenone and/or piperitone and (3) isomenthone/neoisomenthol (Topalov and Dimitrov, 1969; Cook et al., 2007). In an experiment, the antimicrobial effect of *M. pulegium L.* was demonstrated by Jazani et al. (2009). *Mentha pulegium L.* also exhibit antioxidant activity (Souri et al., 2004).

In an experiment investigating the effects of *Thymus vulgaris L.*, *Lamiaceae menthapiperita L.* and *M. pulegium L.* (all medicinal plants), it was demonstrated that *M. 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% *M. pulegium L.* (Nobakht and Mehmannaavaz, 2010).

Based on the reports of Geran et al. (2010), of 0.1, 0.2 and 0.3% supplementation of *M. pulegium L.* essential oils did not significantly affect feed intake, weight gain and feed conversion in broilers. Another study in broilers showed that using *M. pulegium L.* in blend with other medicinal plants had positive effects on performance, carcass traits, blood biochemical and immunity parameters. Modiry et al. (2010) reported that the use of 1.5% of different mixtures of *Urtica dioica*, *M. pulegium* and *T. vulgaris* medicinal plants in broiler diets improved their performance and carcass quality. An application of 0.75% of mixtures of *Urtica dioica*, *M. pulagum* and *T. vulgaris* medicinal plants in the growing period had positive effects on performance and carcass traits of broilers (Nobakht et al., 2010). By application of the leaf extracts of *M. pulegium L.* in rats, the total protein and albumin of their blood significantly decreased (Mokhtari et al., 2008). Hardari et al. (2010) reported that using 1.5% of *M. pulegium L.* decreases the amount of blood cholesterol ($p < 0.05$). From where few studies on the use of medicinal plants especially *M. pulegium L.* in broilers are present, this study was conducted to evaluate the effects of using different levels of *M. pulegium L.* on performance, carcass traits, blood biochemical and immunity parameters of broilers.

MATERIALS AND METHODS

Animals and dietary treatments

300 broiler chicks (Ross 308) were used in a completely randomized design in five treatments and three replicates (20 birds per replicate) for 42 days. The treatment groups consisted of a control (Group 1) with no *M. pulegium L.* supplementation, and experimental groups 2, 3, 4 and 5 receiving varying amounts (0.5,

1, 1.5 and 2%, respectively) of *Mentha pulegium L.* The diets were formulated (Tables 1 and 2) to meet the requirements of broilers as established by the NRC (1994).

Dried aerial part of *M. pulegium L.* was supplied from the local market and the compositions of it was determined according to AOAC (1994). After fine milling, the grounder aerial parts were mixed with other ingredients. The diet and water were provided *ad libitum*. The lighting program during the experimental period consisted of a period of 23 h light and 1 h of darkness. Environmental temperature was gradually decreased from 33 to 25°C on day 21 and then kept constant.

Performance parameters

Body weight, feed intake and feed conversion were determined weekly on a per bird basis. Mortality was also recorded.

Blood biochemical and immunity parameters

At 42 days of age, two birds from each replicate (male and female) were randomly chosen for blood collection and approximate 5 ml blood samples were collected from the brachial vein. 1 ml of collected blood was 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 Siegel., 1983). The remaining 4 ml blood was centrifuged to obtain serum for the determination of blood biochemical parameters including: glucose, cholesterol, triglyceride, albumin, total protein and uric acid. t Kit package (Pars Azmoon Company; Tehran, Iran) was used for the determination blood biochemical parameters using Anision-300 auto-analyzer system.

Carcass components

At 42 days of age, two birds per replicate were randomly chosen, slaughtered and carcass percent to total weight and percents of carcass parts to carcass weight were calculated.

Statistical analysis

Data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of the SAS Institute (2005). Means were compared using the Duncan multiple range test. Statements of statistical significance were based on $P < 0.05$.

RESULTS AND DISCUSSION

Performance and carcass traits

The effects of different levels of *M. pulegium L.* in feeds on the performance of broilers are summarized in Table 3. Using different levels of *M. pulegium L.* showed significant effects on weight gain and feed conversion in broilers ($p > 0.05$). The lowest amount of daily weight gain (38.82 g) and the worst feed conversion (1.95) were observed in the control group, whereas the highest amount of daily weight gain and the best feed conversion

Table 1. The ingredients and nutrients composition of starter diets of broilers (1-21 days)

| Diet | Control group | 0.5% <i>M. pulegium</i> | 1% <i>M. pulegium</i> | 1.5% <i>M. pulegium</i> | 2% <i>M. pulegium</i> |
|---------------------------------|---------------|----------------------------|--------------------------|----------------------------|--------------------------|
| Ingredient | | | | | |
| Yellow corn | 58.51 | 57.81 | 57.12 | 56.43 | 55.74 |
| Soybean meal | 32.72 | 32.74 | 32.77 | 32.79 | 32.82 |
| Fish meal | 3 | 3 | 3 | 3 | 3 |
| <i>M. pulegium</i> (Pennyroyal) | 0 | 0.5 | 1 | 1.5 | 2 |
| Vegetable oil | 2.53 | 2.71 | 2.89 | 3.06 | 3.24 |
| Dicalcium phosphate | 1.06 | 1.07 | 1.07 | 1.07 | 1.07 |
| Oyster shell | 1.30 | 1.29 | 1.27 | 1.26 | 1.24 |
| Salt | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| Vitamin premix ¹ | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral premix ² | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| DL- Methionine | 0.15 | 0.15 | 0.15 | 0.16 | 0.16 |
| Calculated composition | | | | | |
| Metabolisable energy (Kcal/kg) | 3000 | 3000 | 3000 | 3000 | 3000 |
| Crude protein (%) | 21.56 | 21.56 | 21.56 | 21.56 | 21.56 |
| Calcium (%) | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 |
| Available phosphorous (%) | 0.42 | 0.42 | 0.42 | 0.42 | 0.42 |
| Sodium (%) | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Laysin (%) | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Methionine + Cysteine (%) | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 |
| Threonine (%) | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| Thryptophan (%) | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 |

¹Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; Antioxidant 100 mg. ²Mineral premix per kg of diet: Fe (FeSO₄.7H₂O, 20.09% Fe), 50 mg; Mn (MnSO₄.H₂O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO₄.5H₂O), 10 mg; I (KI, 58% I), 1mg; Se (NaSeO₃, 45.56% Se) , 0.2 mg.

(46.24 g and 1.71, respectively) were observed in Group 2. Using more than 0.5% of *Mentha pulegium L.* did not have any significant effects on feed intake and feed conversion. Antimicrobial, antioxidant and phenolic substances in *M. pulegium L.* (Jazani et al., 2009) and (Souri et al., 2004) may be the main cause of improvements in daily weight gain and feed conversion. 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 *M. pulegium L.* (Gülcin et al., 2003) 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). Decrease in performance with increasing

levels of *M. pulegium L.* may be related with the accumulation of antimicrobial substances in the gastrointestinal tract. This may negatively affect the symbiotic bacterial populations and finally decrease animal performance. Our findings on performance in this experiment are in agreement with study results of Modiry et al. (2010). Nobakht et al. (2010) reported that the presence of *M. pulegium L.* blended with other medicinal herbs significantly improved the performance of broilers. Whereas it is not in agreement with the findings of Geran et al., (2010) who did not find significant effects in using 0.1, 0.2 and 0.3% of *M. pulegium L.* essential oils on performance of broilers.

Application of different levels of *M. pulegium L.* significantly affected carcass traits ($P < 0.05$). The effects of different levels of *M. pulegium L.* on carcass traits of broilers are presented in Table 4. The highest percent of breast (33.16%) were observed in Group 2 whereas the highest percent of thigh (27.24%) was recorded in Group 5, but in the percent of thigh, there was no significant difference observed between the control group and Group 5. However, the lowest percent of abdominal fat

Table 2. The ingredients and nutrients composition of grower diets of broilers (22-42 days) of broilers.

| Diet | Control group | 0.5% | 1% | 1.5% | 2% |
|---------------------------------|---------------|--------------------|--------------------|--------------------|--------------------|
| Ingredient | | <i>M. pulegium</i> | <i>M. pulegium</i> | <i>M. pulegium</i> | <i>M. pulegium</i> |
| Yellow corn | 65.61 | 64.94 | 64.27 | 63.6 | 62.93 |
| Soybean meal | 29.43 | 29.43 | 29.43 | 29.43 | 29.43 |
| Vegetable oil | 1.6 | 1.78 | 1.96 | 2.13 | 2.31 |
| <i>M. pulegium</i> (Pennyroyal) | 0 | 0.5 | 1 | 1.5 | 2 |
| Dicalcium phosphate | 1.25 | 1.24 | 1.24 | 1.23 | 1.23 |
| Oyster shell | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 |
| Salt | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 |
| Vitamin premix ¹ | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Mineral premix ² | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| DL- Methionine | 0.05 | 0.05 | 0.04 | 0.04 | 0.04 |
| Calculated composition | | | | | |
| Metabolisable energy (Kcal/Kg) | 3000 | 3000 | 3000 | 3000 | 3000 |
| Crude protein (%) | 18.75 | 18.75 | 18.75 | 18.75 | 18.75 |
| Calcium (%) | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Available phosphorous (%) | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| Sodium (%) | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Lysine (%) | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 |
| Methionine + Cysteine (%) | 0.67 | 0.67 | 0.67 | 0.67 | 0.67 |
| Threonine (%) | 0.79 | 0.79 | 0.79 | 0.79 | 0.79 |
| Thryptophan (%) | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |

¹Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; Antioxidant 100 mg.²Mineral premix per kg of diet: Fe (FeSO₄.7H₂O, 20.09% Fe), 50 mg; Mn (MnSO₄.H₂O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO₄.5H₂O), 10 mg; I (KI, 58% I), 1 mg; Se (NaSeO₃, 45.56% Se), 0.2 mg.

Table 3. The effects of different levels of experimental diets on performance of broilers (0 to 42 days).

| Treatment | 1 | 2 | 3 | 4 | 5 | SEM |
|-----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| Parameter | | | | | | |
| Feed intake (g) | 75.54 | 79.24 | 76.84 | 80.06 | 75.67 | 1.63 |
| Weight gain (g) | 38.82 ^b | 46.24 ^a | 43.74 ^a | 45.53 ^a | 43.77 ^a | 1.09 |
| Feed conversion (g:g) | 1.95 ^a | 1.71 ^b | 1.76 ^b | 1.76 ^b | 1.73 ^b | 0.02 |

Values in the same row not sharing a common superscript differ significantly (p<0.05). SEM = Standard error of mean.

Table 4. The effects of different levels of experimental diets on carcass traits of broilers.

| Treatment | 1 | 2 | 3 | 4 | 5 | SEM |
|--------------------|---------------------|---------------------|--------------------|---------------------|--------------------|------|
| Carcass traits (%) | | | | | | |
| Carcass | 70.56 | 70.82 | 72.04 | 70.79 | 72.32 | 0.84 |
| Abdominal fat | 3.65 | 2.89 | 3.74 | 3.25 | 2.96 | 0.28 |
| Gizzard | 3.92 | 3.34 | 3.29 | 3.88 | 3.79 | 0.19 |
| Breast | 29.04 ^b | 33.16 ^a | 33.29 ^a | 31.74 ^a | 31.61 ^a | 0.68 |
| Thigh | 26.54 ^{ab} | 25.89 ^{bc} | 25.43 ^c | 26.96 ^{ab} | 27.24 ^a | 0.34 |
| Liver | 3.74 | 3.14 | 2.93 | 3.28 | 3.42 | 0.21 |

Values in the same row not sharing a common superscript differ significantly (P<0.05). SEM = Standard error of mean.

Table 5. The effects of different levels of experimental diets on blood parameters of broilers.

| Treatment | 1 | 2 | 3 | 4 | 5 | SEM |
|------------------------|----------------------|----------------------|---------------------|---------------------|---------------------|-------|
| Blood parameter | | | | | | |
| Glucose (mg/dl) | 194.28 ^{ab} | 178.52 ^{ab} | 212.48 ^a | 205.36 ^a | 157.12 ^b | 11.57 |
| Cholesterol (mg/dl) | 130.60 | 118.45 | 128.12 | 131.35 | 121.35 | 13.66 |
| Triglyceride (mg/dl) | 51.65 | 45.18 | 45.56 | 43.59 | 46.47 | 3.01 |
| Albumin (mg/dl) | 1.97 | 1.76 | 1.92 | 1.97 | 2 | 0.1 |
| Total Protein (mg/dl) | 4.17 | 3.69 | 3.94 | 4.1 | 4.5 | 0.25 |
| Uric Acid (mg/dl) | 3.26 | 3.68 | 2.73 | 2.61 | 4.57 | 0.55 |
| Heterophil (%) | 23.67 | 19.50 | 20.50 | 18.84 | 24.67 | 3.24 |
| Lymphocyte (%) | 76.34 | 81.17 | 76.44 | 79.50 | 73.17 | 2.73 |
| Heterophil/Lymphocyte | 0.312 | 0.247 | 0.274 | 0.237 | 0.341 | 0.05 |

Values in the same row not sharing a common superscript differ significantly ($P < 0.05$). SEM = Standard error of mean.

was obtained in Group 2. Like performance, the antimicrobial substances of *M. pulegium L.* can reduce harmful bacteria populations in the gastrointestinal tract and improve the levels of absorbed nutrients especially amino acids. By increasing the amount of absorbed amino acids, highest levels can accumulate in tissues like the breast. Besides its antimicrobial effects, the antioxidants and phenolic substances in *M. pulegium L.* can prevent nutrient oxidation and increase the amounts absorbed thus resulting in an accumulation in broiler tissues (Lee et al., 2003). The improvement of carcass traits by using of *M. pulegium L.* is in agreement with the experimental results of Nobakht et al. (2010) who reported that a blend of *M. pulegium L.* with another medicinal herb significantly improved the carcass traits of broilers.

The effects of different levels of *M. pulegium L.* on blood biochemical and immunity parameters of broilers are presented in Table 5. The effect of different levels of *M. pulegium L.* on blood glucose was significant ($p < 0.05$). The lowest (157.12 mg/dl) and the highest (212.48 mg/dl) amounts of glucose were observed in Groups 5 and 3 but there are no significant differences with the Control group.

Except glucose, there were no other observed biochemical and immunity parameters between treatments. Our observation in this experiment on blood biochemical and immunity parameters is not in agreement with Hardari et al. (2010) report. They concluded that using 1.5% of *M. pulegium L.* had positive effects on blood cholesterol level.

Conclusion

It can be concluded that the use of *M. pulegium L.* medicinal plant in broiler diets have positive effects on there performance and carcass traits and only use 0.5% of it is sufficient and more than 0.5% not recommended.

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