



## Effect of pennyroyal (*Mentha pulegium* L.) extract on performance, blood constitutes, immunity parameters and intestinal microflora in broiler chickens

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Received: 2 January 2020; Accepted: 23 October 2020

### ABSTRACT

This study aimed to test the hypothesis that lower levels of pennyroyal (*Mentha pulegium* L.) extract can improve growth traits, blood constitutes, immunity and microflora in broilers up to 42-days production cycle. A total of 300 broiler chicks (Ross 308) were randomly distributed into five dietary treatments, with three replicates per treatment (20 birds per replicate) as follows: control group received a basal diet with no pennyroyal supplementation, whereas the treatment-groups fed the basal diet including different pennyroyal levels (100, 200, 300 and 400 ppm, respectively) in drinking water. Feeding of pennyroyal during the whole rearing period partly affected body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broilers. Pennyroyal extract at different levels significantly lowered blood total cholesterol and triglycerides of birds. Results also showed significantly higher high-density lipoprotein (HDL)-cholesterol and lower low-density lipoprotein (LDL)-cholesterol levels in pennyroyal-supplemented group. Further, a significant increase of antibody production to total sheep red blood cells (SRBC) and IgM at 42 days of age was observed. Broilers receiving 100 ppm pennyroyal extract had the lowest count of *E. coli* bacteria in ileum. Overall, based on our findings, supplementing pennyroyal affected positively broiler growth traits, blood parameters and reduced harmful intestinal bacteria.

**Keywords:** Broiler, Microbiota, Pennyroyal, Performance

The genus *Mentha* belongs to the family Lamiaceae including about 25 species native from Europe, North Africa, the Middle East including Asia Minor and near East (Mahboubi and Haghi 2008). *Mentha pulegium* L. (pennyroyal) is an aromatic perennial herb distributed around the world (Díaz-Maroto *et al.* 2007). Pennyroyal leaves have a strong mint-like odour and have been traditionally used in folk medicine for gastrointestinal disorders, as astringent, antibacterial and also for culinary preparations (Agnihotri *et al.* 2005, Mahboubi and Haghi 2008). The bioactive constituents of pennyroyal have shown variations depending on the cultivation region and environmental conditions (Mahboubi and Haghi 2008, Erhan *et al.* 2012). According to Iscan *et al.* (2002), the principal component of pennyroyal is menthol (28–42%) and menthone (18–28%). In another study, the major components were piperitone (38%), piperitenone (33%),

$\alpha$ -terpineol (4.7%) and 1,8-cineole (4%) (Mahboubi and Haghi 2008). Some authors have reported positive effects of *Mentha piperita* L. on broiler growth performance, blood biochemical and immunity parameters (Nobakht *et al.* 2011, Fallah *et al.* 2013, Goodarzi and Nanekarani 2014), while others assessed no influence on performance, humoral immunity, internal organ weights and carcass characteristics in broilers (Abdulkarimi *et al.* 2012, Ghalamkari *et al.* 2012, Mahdavi *et al.* 2013).

Therefore, the purpose of the present investigation was to test the hypothesis that at low levels, pennyroyal extract in drinking water can improve productivity, blood constitutes, immunity and microflora in the gut of broilers up to 42-days.

### MATERIALS AND METHODS

This study was conducted in a commercial poultry farm at Rasht, Iran. The experiment was approved by the Ethics Committee of the Islamic Azad University with respect to the International Guidelines for research involving animals (Directive 2010/63/EU), and care was taken to minimize the number of animals used.

**Animals and experimental design:** A total of 300 one-day-old commercial male chicken (Ross 308) were randomly distributed into five treatments, with three

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replicates per treatment, in a total of 20 birds per replicate. Chicks were assigned into treatment groups with similar mean body weight and reared until the age of 42 days, covering three periods: starter (1–14 days), grower (15–28 days) and finisher (29–42 days). The control group received a basal diet with no pennyroyal supplementation, whereas the treatment-groups fed the basal diet including pennyroyal extract at different levels (100, 200, 300 and 400 ppm, respectively) in drinking water. Pennyroyal was harvested and sun-dried and extract was prepared as described by Bombik *et al.* (2012). Briefly, dried pennyroyal was infused with boiling water at 100°C (1 L water: 200 g dry herb) for 10 min, then cooled at room temperature and strained to obtain plant extract. Birds were housed in land cages, with 1.0×2.0 m. Thermo-neutral conditions were maintained according to usual brooding practices (Aviagen 2014). A constant lighting program was provided for 24 h on day 1, and thereafter for 23 h/day, following one hour of darkness per day.

Broiler chicks received feeds and water *ad lib.* throughout the trial. Ingredients and chemical composition of the basal diet are presented in Table 1. Routine vaccination was designed by the veterinarian and coped with regional veterinary authority: vaccination against infectious bronchitis (Infectious Bronchitis Virus (IBV) (H120); Razi Co, Iran) at days 1 and 8; Gumboro vaccination (Gumboro–IBD071IR; Razi Co, Iran) at days

16 and 32; vaccination against Newcastle disease was performed at days 1 and 8, and Influenza at day 1.

**Growth traits and blood metabolite:** Performance parameters including body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of chickens were weekly measured. The economic index was calculated based on conventional protocols as described by Nosrati *et al.* (2017). At the end of the trial, five birds from each replicate were randomly selected for blood sampling. Prior to blood collection and slaughter, the feed was withdrawn from all birds for a period of 4 h (to stabilize plasma constituents). The sampling was done in the morning to avoid the variability of the blood parameters. Care was taken to choose the most representative male birds, based on the average BW of group. Blood samples (~5 mL/bird) were collected from the wing vein using tubes without EDTA for further analysis. Plasma was harvested after centrifugation (3,000 g × 10 min at room temperature) and stored at –20°C until analysed. Blood parameters assessed in the study included glucose (GI), uric acid (UAc), total cholesterol (Chol), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and HDL/LDL ratios. Blood parameters were analysed by Roche Cobas Integra autoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany), based on standard protocols using commercial kits from Pars Azmoon (Pars Azmoon Co., Tehran, Iran) as described by Shabani *et al.* (2015).

**Immune competency:** The antigenic challenge with total sheep red blood cells (SRBC) was performed twice (13 and 24 days of age), and blood sampling was performed at days 22 and 38 for assessment of total antibody titer, IgG and IgM production. A 0.5 ml of 10% suspension of SRBC in sterile PBS (phosphate buffered saline solution; v/v) was inoculated under the skin of the breast of two birds per replicate. For the assessment of the immune parameters, blood samples (2 ml) were collected from the wing vein on the pre-scheduled days. The samples were centrifuged at 1,500 rpm for 10 min and the serum was harvested and stored at –20°C until analysis. Response to the Newcastle lentogenic vaccine was assessed in blood samples twice, at days 15 and 26; commercial lyophilized vaccines (Razi Co, Iran), was prepared with the strains Hitchner B1, LaSota and Clon30, were administered on days 1, 35 and 42 respectively. Haemagglutination inhibition (HI) assays were used to determine the vaccine titers of Newcastle disease (ND) following the procedure described in previous works (Seidavi *et al.* 2014, Ebrahimi *et al.* 2015). Three birds from each treatment were chosen at random on day 42 to collect blood samples from the brachial vein. Sera were separated by centrifugation (3000 × g for 15 min) to measure antibody titers against infectious bursal disease (IBD) and infectious bronchitis virus (IBV) using commercially available ELISA kits (Bio-check BV, Gouda, Holland). The absorbance of all samples was measured at 405 nm using an ELISA reader (Bio-Tek Instruments Inc. ELX 800; Winooski, VT, USA).

**Measurements of ileal microflora:** At day 42, three birds per replicate were selected for sample collection. Agar plates

Table 1. Ingredients and nutrient analysis of diets fed to broiler chickens

| Ingredient (%)                    | Starter<br>(1–14<br>DOA) | Grower<br>(15–28<br>DOA) | Finisher<br>(29–42<br>DOA) |
|-----------------------------------|--------------------------|--------------------------|----------------------------|
| Corn                              | 57.83                    | 58.59                    | 61.55                      |
| Soybean meal (44% CP)             | 36.71                    | 45.47                    | 31.66                      |
| Soybean oil                       | 1.60                     | 2.20                     | 3.17                       |
| Limestone                         | 1.26                     | 1.24                     | 1.09                       |
| Dicalcium phosphate               | 1.56                     | 1.30                     | 1.15                       |
| NaCl                              | 0.20                     | 0.25                     | 0.32                       |
| Mineral-vitamin premix*           | 0.50                     | 0.50                     | 0.50                       |
| DL-Methionine                     | 0.20                     | 0.25                     | 0.30                       |
| L-Lysine                          | 0.04                     | 0.05                     | 0.07                       |
| <i>Calculated analysis</i>        |                          |                          |                            |
| Metabolizable energy<br>(kcal/kg) | 2,900.00                 | 2,950.00                 | 3,000.00                   |
| Crude protein (%)                 | 21.00                    | 20.50                    | 18.75                      |
| Crude fat (%)                     | 2.42                     | 4.64                     | 2.86                       |
| Linoleic acid (%)                 | 2.81                     | 2.13                     | 1.46                       |
| Calcium (%)                       | 0.94                     | 0.87                     | 0.78                       |
| Available phosphorus (%)          | 0.42                     | 0.38                     | 0.35                       |
| Sodium (%)                        | 0.19                     | 0.17                     | 0.15                       |
| Lysine (%)                        | 1.30                     | 1.10                     | 1.10                       |

\*Calcium Pantothenate, 4 mg/g; Niacin, 15 mg/g; Vitamin B6, 13 mg/g; Cu, 3 mg/g; Zn, 15 mg/g; Mn, 20 mg/g; Fe, 10 mg/g; K, 0.3 mg/g; Vitamin A, 5000 IU/g; Vitamin D3, 500 IU/g; Vitamin E, 3 mg/g; Vitamin K3, 1.5 mg/g; Vitamin B2, 1 mg/g; DOA, days of age.

were streaked with ileal content and sent to the laboratory to determinate bacterial growth and colony counts. Tubes for collecting samples were weighted, wrapped, and autoclaved for 10 min. The media was prepared 24 h before sampling. The MRS agar (Man Rogosa Sharpe agar, 1.10660.500) was used to culture Lactobacilli, and eosin-metilen blue (EMB, 1.01347.0500) was used to culture *Escherichia coli*. The samples from gastrointestinal content were sent to the laboratory and again weighed. The amount of sample in each tube was calculated from the difference between these two values. After 30 min, the tubes were shaken, 1 ml was removed from the prepared suspension and added into 9 ml buffer phosphate saline (PBS) in another tube. Bacterial suspensions were prepared by repeated dilutions ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ , respectively) and 100  $\mu$ l was removed from  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  and poured into the petridishes previously prepared. Lactobacilli bacteria were incubated at 37°C in an aerobic conditions for 72 h. Counting bacteria in petri dishes were completed by colony counter. Logarithms of number of bacteria per 1 g were reported.

**Statistical analysis:** The Shapiro-Wilks's test confirmed the normal distribution of data, which was then analyzed using general linear model (GLM), using the following equation:

$$Y_{ij} = \mu + T_j + e_{ij},$$

where  $Y_{ij}$  is the dependent variable;  $\mu$  is the overall mean;  $T_j$  is the effect of the treatment; and  $e_{ij}$  is the residual error. The significance of the differences among group-means was analyzed using the ANOVA procedure, followed by a Tukey's post-hoc test, using IBM SPSS Statistics software for Windows® (SPSS, 1997). A P value of 0.05 was used to assess significance among means.

## RESULTS AND DISCUSSION

The effect of pennyroyal extract on broiler performance parameters are summarized in Table 2. Feeding of different levels of pennyroyal extract led to significant changes on BWG and FI ( $P < 0.05$ ) among groups. Furthermore, feed consumption was increasing gradually each week (data not shown) while FCR did not report differ during the entire feeding period. In general, the best FCR was observed at the first week of age, conversely the highest values were detected at the end of the trial (6th week of age).

The effect of different levels of pennyroyal extract on blood biochemical and immunity parameters of broilers are presented in Table 3. The serum glucose level in broilers varied from 131.5 to 157.7 mg/dl and it was found to be statistically non-significant ( $P > 0.05$ ). Pennyroyal extract supplementation affected significantly the total cholesterol, plasma triglycerides, HDL, and LDL. Total cholesterol decreased ( $P < 0.05$ ) as pennyroyal extract increased up to 300 ppm in drinking water. The blood level of triglycerides was considerably reduced in the pennyroyal extract-supplemented groups ( $P < 0.05$ ). The effect of the different levels of the extract on uric acid was not significant

Table 2. Performance traits of broilers fed different levels of *Mentha pulegium* L. extract

| Concentration                    | BWG<br>(g/chick)    | FI<br>(g/chick)      | FCR<br>(g/g) | Economic<br>index   |
|----------------------------------|---------------------|----------------------|--------------|---------------------|
| <i>Starter period (1-14 DOA)</i> |                     |                      |              |                     |
| Control (0 ppm)                  | 316.40 <sup>a</sup> | 374.51 <sup>a</sup>  | 1.180        | 190.86 <sup>a</sup> |
| 100 ppm                          | 292.00 <sup>b</sup> | 351.42 <sup>ab</sup> | 1.200        | 173.33 <sup>b</sup> |
| 200 ppm                          | 275.00 <sup>b</sup> | 327.05 <sup>b</sup>  | 1.190        | 165.29 <sup>b</sup> |
| 300 ppm                          | 274.60 <sup>b</sup> | 326.18 <sup>b</sup>  | 1.190        | 165.27 <sup>b</sup> |
| 400 ppm                          | 285.70 <sup>b</sup> | 336.48 <sup>b</sup>  | 1.180        | 173.24 <sup>b</sup> |
| SEM                              | 4.92                | 6.08                 | 0.002        | 3.04                |
| <i>Grower (15-28 DOA)</i>        |                     |                      |              |                     |
| Control (0 ppm)                  | 885.70              | 1443.80              | 1.630        | 388.54              |
| 100 ppm                          | 913.20              | 1481.60              | 1.620        | 402.55              |
| 200 ppm                          | 908.30              | 1412.80              | 1.560        | 417.41              |
| 300 ppm                          | 916.40              | 1473.00              | 1.610        | 407.60              |
| 400 ppm                          | 882.30              | 1395.00              | 1.580        | 398.63              |
| SEM                              | 8.54                | 15.37                | 0.012        | 6.04                |
| <i>Finisher (29-42 DOA)</i>      |                     |                      |              |                     |
| Control (0 ppm)                  | 1100.00             | 2124.10              | 1.930        | 407.21              |
| 100 ppm                          | 1002.30             | 1972.20              | 1.970        | 364.73              |
| 200 ppm                          | 1028.00             | 1919.10              | 1.880        | 394.82              |
| 300 ppm                          | 1069.70             | 2025.30              | 1.900        | 404.79              |
| 400 ppm                          | 1065.00             | 2121.80              | 1.990        | 383.36              |
| SEM                              | 21.11               | 40.81                | 0.028        | 11.37               |
| <i>Total period (1-42 DOA)</i>   |                     |                      |              |                     |
| Control (0 ppm)                  | 2302.0              | 3942.40              | 1.710        | 320.26              |
| 100 ppm                          | 2207.6              | 3805.30              | 1.720        | 305.09              |
| 200 ppm                          | 2211.3              | 3659.00              | 1.650        | 318.47              |
| 300 ppm                          | 2260.6              | 3824.50              | 1.690        | 318.24              |
| 400 ppm                          | 2233.5              | 3853.30              | 1.720        | 308.58              |
| SEM                              | 21.6                | 49.18                | 0.013        | 3.77                |

<sup>a-b</sup>Means within each column of levels of *Mentha pulegium* extract with different superscripts, differ significantly at  $P < 0.05$ . DOA: days of age.

compared to control diet. The HDL/LDL ratio was lower in 200 ppm group (0.66) increasing constantly up to 0.87 in birds fed 400 ppm of pennyroyal extract.

Table 4 lists the mean antibody titers ( $\log_{10}$ ) against Newcastle disease (ND), total sheep red blood cells (SRBC), IgG, IgM, infection bursal disease (IBD) and infectious bronchitis virus (IBV). The experimental treatments failed to induce marked effect on antibody titers against ND and IBV. At day 42 of age, fed pennyroyal extract had a significant effect on antibody titers against SRBC compared to control group. Furthermore, broilers receiving pennyroyal extract diets at day 42 had significantly higher titers of IgM antibodies than those in control diet. Broilers supplemented with 200 ppm extract showed highest antibody titers against IBD and tended to decrease with increasing concentrations of pennyroyal extract in drinking water. Broilers fed the diet containing pennyroyal extract had reduced *E. coli* counts in the ileum (Table 5), whereas the control group showed the highest concentration. Results also showed that broilers which received 100 ppm had the lowest counts of *E. coli*. Supplementing pennyroyal extract

Table 3. Blood parameters at 42 DOA in broilers fed different levels of *Mentha pulegium* L. extract

| Concentration   | Glucose<br>(mg/dl) | Uric acid<br>(mg/dl) | Total cholesterol<br>(mg/dl) | Triglycerides<br>(mg/dl) | HDL<br>(mg/dl)      | LDL<br>(mg/dl)      | HDL/LDL |
|-----------------|--------------------|----------------------|------------------------------|--------------------------|---------------------|---------------------|---------|
| Control (0 ppm) | 145.1              | 2.87                 | 192.31 <sup>a</sup>          | 155.90 <sup>a</sup>      | 53.67 <sup>b</sup>  | 107.46 <sup>a</sup> | 0.81    |
| 100 ppm         | 144.59             | 1.23                 | 169.28 <sup>ab</sup>         | 119.50 <sup>b</sup>      | 67.72 <sup>ab</sup> | 77.66 <sup>b</sup>  | 0.71    |
| 200 ppm         | 157.77             | 1.7                  | 153.70 <sup>bc</sup>         | 102.09 <sup>b</sup>      | 75.26 <sup>a</sup>  | 58.02 <sup>bc</sup> | 0.66    |
| 300 ppm         | 144.5              | 1.1                  | 135.40 <sup>c</sup>          | 112.57 <sup>b</sup>      | 71.63 <sup>a</sup>  | 41.25 <sup>c</sup>  | 0.83    |
| 400 ppm         | 131.53             | 1.19                 | 136.11 <sup>c</sup>          | 116.90 <sup>b</sup>      | 69.90 <sup>a</sup>  | 42.83 <sup>c</sup>  | 0.87    |
| SEM             | 4.509              | 0.197                | 6.875                        | 5.785                    | 2.667               | 7.456               | 0.031   |

<sup>a-c</sup>Means within each column of levels of *Mentha pulegium* extract with different superscripts differ significantly at P<0.05.

Table 4. Immunity parameters (log10) in broilers fed different levels of *Mentha pulegium* L. extract.

| Concentration   | ND<br>at 1<br>DOA | ND<br>at 35<br>DOA | ND<br>at 42<br>DOA | TSRBC<br>at 28<br>DOA | TSRBC<br>at 42<br>DOA | IgG<br>at 28<br>DOA | IgG<br>at 42<br>DOA | IgM<br>at 28<br>DOA | IgM<br>at 42<br>DOA | IBD<br>at 42<br>DOA  | IBV<br>at 42<br>DOA |
|-----------------|-------------------|--------------------|--------------------|-----------------------|-----------------------|---------------------|---------------------|---------------------|---------------------|----------------------|---------------------|
| Control (0 ppm) | 4.67              | 3.00 <sup>b</sup>  | 4.67               | 3                     | 5.00 <sup>b</sup>     | 1                   | 2.67                | 2                   | 2.33 <sup>b</sup>   | 7008.0 <sup>ab</sup> | 72.33               |
| 100 ppm         | 4.67              | 4.33 <sup>a</sup>  | 5.67               | 3.33                  | 6.67 <sup>a</sup>     | 1.67                | 4                   | 1.67                | 4.00 <sup>a</sup>   | 8866.3 <sup>a</sup>  | 114                 |
| 200 ppm         | 5.67              | 4.33 <sup>a</sup>  | 5.33               | 3.67                  | 6.67 <sup>a</sup>     | 2.33                | 3                   | 1.33                | 3.67 <sup>a</sup>   | 6997.3 <sup>ab</sup> | 68                  |
| 300 ppm         | 4.67              | 4.33 <sup>a</sup>  | 4.67               | 2.67                  | 5.67 <sup>ab</sup>    | 2                   | 3                   | 1.33                | 3.67 <sup>a</sup>   | 5572.0 <sup>ab</sup> | 84.67               |
| 400 ppm         | 4.67              | 5.00 <sup>a</sup>  | 5.67               | 2.33                  | 5.67 <sup>ab</sup>    | 1                   | 2.33                | 1.33                | 3.33 <sup>ab</sup>  | 4741.0 <sup>b</sup>  | 193.67              |
| SEM             | 0.165             | 0.223              | 0.262              | 0.309                 | 0.3                   | 0.254               | 0.258               | 0.165               | 0.214               | 582.019              | 21.482              |

<sup>a-b</sup>Means within each column of levels of *Mentha pulegium* extract with different superscripts differ significantly at P<0.05; ND, Newcastle disease; TSRBC, total sheep red blood cells; IgG, immunoglobulin G; IgM, immunoglobulin M; IBD, infectious bursal disease; IBV, infectious bronchitis virus; DOA, days of age.

Table 5. *E. coli* and Lactobacillus concentrations (CFU/g) at 42 DOA in broilers fed different levels of *Mentha pulegium* L. extract \*

| Concentration   | <i>E. coli</i>     | Lactobacillus |
|-----------------|--------------------|---------------|
| Control (0 ppm) | 8.47 <sup>a</sup>  | 7.52          |
| 100 ppm         | 5.99 <sup>c</sup>  | 7.54          |
| 200 ppm         | 8.11 <sup>ab</sup> | 7.44          |
| 300 ppm         | 8.11 <sup>ab</sup> | 8.41          |
| 400 ppm         | 7.51 <sup>b</sup>  | 7.64          |
| SEM             | 0.251              | 0.218         |

<sup>a-c</sup>Means within each column of levels of *Mentha pulegium* extract with different superscripts differ significantly at P<0.05.

to broilers did not significantly affect the Lactobacillus count, and this findings are in agreement with those stated by Dhama *et al.* (2015).

In general, the growth performance of broilers were not directly affected by the consumption of different levels of pennyroyal extract. This is in accordance with previous results by Geran *et al.* (2010) who found that using 0.1, 0.2 and 0.3% of *M. pulegium* L. essential oils did not affect significantly broiler performance. According to previous research, comparing the BWG by using either antibiotic or pennyroyal extract in diet, it was found that the extract can affect feed intake by modulating the flavor (Goodarzi and Nanekarani 2014). Abdulkarimi *et al.* (2012) reported that the supplementation of *Mentha* extract in drinking water increased only the breast weight compared to the other carcass components.

Serum glucose level did not change among groups. However, *Mentha* extract at different levels significantly lowered total cholesterol at 300 and 400 ppm. Moreover, the consumption of extracts reduced triglycerides at all levels of supplementation compared to control group. Aghazadeh *et al.* (2011) reported similar triglycerides and cholesterol depressing effects due to mentha supplementation in broilers chicken. Also, Fallah *et al.* (2013) observed lowest HDL and LDL concentration by adding of *Cynara Scolymus* L. leaves and *Mentha piperita* extracts to broilers. Our observations confirm the reduction of low-density lipoprotein (LDL) and increase of high-density lipoprotein (HDL) by the addition of pennyroyal extract at any inclusion level. Conversely, Khursheed *et al.* (2017) reported no differences in serum cholesterol levels after evaluating the effect of different diets including *Mentha piperita* L. and enzyme supplementation. Other previous studies reported that active components of *Mentha* extracts, including menthol and menthon, inhibit hepatic 3-hydroxy-3-methylglutaryl (HMG) CoA-reductase activity, and therefore reducing the total serum cholesterol (Crowell 1999, Abdulkarimi *et al.* 2012). Based on the concern of human health, several researchers have shown that low HDL and high LDL levels are associated with increased risk of atherosclerosis, and that elevated serum HDL levels have a protective effect (Ozdogan and Aksit, 2003).

The uric acid concentration in serum was higher in the control group with no statistical significance among dietary treatments. In broilers, plasma uric acid can vary directly



with dietary protein level produced by nitrogen metabolism. Therefore, plasma urea viable variables determine amino acid requirements or the efficiency of amino acid utilization (Bowes *et al.* 1989). The finding of this trial showed that humoral immune responses were affected by dietary treatments causing an increase of antibody levels to SRBC and Infectious bursal diseases at 42 days of age. In a study, Ghalamkari *et al.* (2012) reported no effect using different levels of *Mentha pulegium* L. (pennyroyal) on antibody titers against Newcastle, influenza viruses and SRBC antigen. Our results suggest that pennyroyal extract may induce humoral immunity of birds by increasing IgM levels in the treated groups.

Supplementing pennyroyal extract to broilers in drinking water was effective in reducing counts of *E. coli* compared to the basal diet, whereas the lactobacillus count did not vary among treatments. The antibacterial activity, especially against different microorganisms known to be pathogenic to broiler chickens has been also reported by Iscan *et al.* (2002) and Saeed and Tarik (2005). According to Mahboubi and Haghi (2008), piperitone and piperitene were the main components of pennyroyal essential oil (71.1%) exhibiting bactericidal effect against *S. aureus*, *S. epidermidis*, *B. cereus* and *E. coli*. Therefore, pennyroyal antimicrobial bioactive compounds may inhibit intestinal pathogenic organisms, balancing gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes (Ocak *et al.* 2008).

In conclusion, the findings of this study suggested that addition of pennyroyal extract to drinking water can modulate growth performance of broiler chicks. Supplementing pennyroyal extract had few effects on immunity parameters mainly increasing the antibody production to SRBC, IgM and IBV at 42 days of age. However, adding pennyroyal extract from 100 to 400 ppm led to higher decrease of *E. coli* count in ileum. Thus, according to our results, the supplementation of pennyroyal extract in drinking water may represent a valuable natural feed extract to promote and support broilers growth and health status.

#### ACKNOWLEDGMENTS

Financial support by Rasht Branch, Islamic Azad University, Grant number 4.5830 is gratefully acknowledged.

#### REFERENCES

- Abdulkarimi R, Mirza Aghazadeh A and Daneshyar M. 2012. Effect of *Mentha* extract (*Mentha piperita*) supplementation in drinking water on performance, plasma lipoproteins, carcass characteristic and liver color index or weight in broiler chickens. *Indian Journal of Animal Sciences* **82**(9): 1070.
- Aghazadeh A M, Abdolkarimi R and Ashkavand Z. 2011. Effect of dietary thyme (*Thymus vulgaris*) and mint (*Mentha piperita*) on some blood parameters of broiler chickens. *Journal of Agricultural Science and Technology A*, 1288.
- Agnihotri V K, Agarwal S G, Dhar P L, Thappa R K and Kapahi B K *et al.* 2005. Essential oil composition of *Mentha pulegium* L. growing wild in the north western Himalayas India. *Flavour and Fragrance Journal* **20**(6): 607–10.
- Aviagen. 2014. Ross Broiler Management Manual. Aviagen Ltd., Newbridge, UK.
- Bombik T, Bombik E, Frankowska A, Trawińska B and Saba L. 2012. Effect of herbal extracts on some haematological parameters of calves during rearing. *Bulletin of Veterinary Institute in Pulawy* **56**: 655–58.
- Bowes V A, Julian R J and Stirtzinger T. 1989. Comparison of serum biochemical profiles of male broilers with female broilers and White Leghorn chickens. *Canadian Journal of Veterinary Research* **53**(1): 7.
- Crowell P L. 1999. Prevention and therapy of cancer by dietary monoterpenes. *Journal of Nutrition* **129**(3): 775–78.
- Dhama K, Latheef S K, Saminathan M, Abdul Samad H, Karthik K, Tiwari R, Khan R U, Alagawany M, Farag M R, Alam G M, Laudadio V and Tufarelli V. 2015. Multiple beneficial applications and modes of action of herbs in poultry health and production—a review. *International Journal of Pharmacology* **11**: 152–76.
- Díaz-Maroto M C, Castillo N, Castro-Vázquez L, Ángel González Viñas M and Pérez-Coello M S. 2007. Volatile composition and olfactory profile of pennyroyal (*Mentha pulegium* L.) plants. *Flavour and Fragrance Journal* **22**(2): 114–18.
- Ebrahimi A, Santini A, Alise M, Pourhossein P and Miraalami N *et al.* 2015. Effect of dried citrus sinensis peel on gastrointestinal microbiota and immune system traits of broiler chickens. *Italian Journal of Animal Science* **14**(4): 712–17.
- Erhan M K, Bölükbaşı S C and Ürüsan H. 2012. Biological activities of pennyroyal (*Mentha pulegium* L.) in broilers. *Livestock Science* **146**(2): 189–92.
- Fallah R, Kiani A and Azarfar A. 2013. Effect of artichoke leaves meal and mentha extract on immune cells and blood biochemical parameters of broilers. *Global Veterinaria* **10**: 99–102.
- Geran M P, Irany M and Dehpourjoybari A. 2010. The effect of pennyroyal essential oil on performance of broilers. Proceedings of the 5th Congress of New Idea in Agriculture, pp. 1–3.
- Ghalamkari G, Toghyani M, Landy N and Tavalaeian E. 2012. Investigation the effects using different levels of *Mentha pulegium* L. (pennyroyal) in comparison with an antibiotic growth promoter on performance, carcass traits and immune responses in broiler chickens. *Asian Pacific Journal of Tropical Biomedicine* **2**(3): 1396–99.
- Goodarzi M and Nanekarani S. 2014. Effects of feeding *Mentha pulegium* L. as an alternative to antibiotics on performance of broilers. *APCBEE Procedia* **8**: 53–58.
- Iscan G, Kirimer N, Kürkcüoğlu M, Baser H C and Demirci F. 2002. Antimicrobial screening of *Mentha piperita* essential oils. *Journal of Agricultural and Food Chemistry* **50**(14): 3943–46.
- Khursheed A, Banday M T, Khan A A, Adil S and Ganai A M *et al.* 2017. Effect of mint leaves with or without enzyme supplementation on blood biochemistry, carcass characteristics and sensory attributes of broiler chicken. *Advances in Animal Veterinary Science* **5**(11): 449–55.
- Mahboubi M and Haghi G. 2008. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *Journal of Ethnopharmacology* **119**(2): 325–27.
- Mahdavi S, Mehmannaavaz Y, Nobakht A and Zakeri A. 2013. The effects of different amounts of *Mentha pulegium* L. on immune system performance of broiler chickens. *International*

- Research Journal of Applied and Basic Science* **4**: 381–84.
- Nobakht A, Norani J and Safamehr A. 2011. The effects of different amounts of *Mentha pulegium* L. (pennyroyal) on performance, carcass traits, hematological and blood biochemical parameters of broilers. *Journal of Medicinal Plants Research* **5**(16): 3763–68.
- Nosrati M, Javandel F, Camacho L M, Khusro A, Cipriano M, Seidavi A and Salem A Z M. 2017. The effects of antibiotic, probiotic, organic acid, vitamin C, and *Echinacea purpurea* extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *Journal of Applied Poultry Research* **26**(2): 295–306.
- Ocak N, Erener G, Ak B F, Sungu M and Altop A *et al.* 2008. Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves as growth promoter source. *Czech Journal of Animal Science* **53**(4): 169–75.
- Özdoğan M and Aksit M. 2003. Effects of feeds containing different fats on carcass and blood parameters of broilers. *Journal of Applied Poultry Research* **12**(3): 251–56.
- Saeed S and Tariq P. 2005. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pakistan Journal of Botany* **37**: 997–1001.
- Seidavi A R, Asadpour L, Dadashbeiki M and Payan-Carreira R. 2014. Effects of dietary fish oil and green tea powder supplementation on broiler chickens immunity. *Acta Scientiae Veterinariae* **42**(1205): 1–13.
- Shabani S, Seidavi A, Asadpour L and Corazzin M. 2015. Effects of physical form of diet and intensity and duration of feed restriction on the growth performance, blood variables, microbial flora, immunity, and carcass and organ characteristics of broiler chickens. *Livestock Science* **180**: 150–57.
- SPSS. 1997. SPSS Base 7.5 for Windows. SPSS, Chicago, IL.