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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

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ABSTRACT

Objective: The trial involved 240 Ross 308 broiler chicks in order to investigate the effects of *Mentha pulegium* L. (pennyroyal) on performance, carcass traits and immune responses in broiler chickens. **Methods:** Birds were assigned to 4 treatments: control feed, antibiotic group receiving 4.5 mg/kg flavophospholipol, and 5 and 10 g/kg pennyroyal powder added to the basal diet. Body weights of broilers were determined at d 1, 14, 28 and 42, feed intake was determined at the same periods, and feed conversion ratio was calculated accordingly. At day 42, two birds per replicate were slaughtered for the determination of carcass traits. Antibody titers against newcastle, influenza viruses and sheep red blood cell (SRBC) were determined. **Results:** Performance, Internal organ weights and carcass characteristics were not significantly influenced by the dietary treatments at day 42. Humoral immune responses were not affected by dietary treatments. **Conclusions:** In conclusion, the results of this study showed that addition of pennyroyal powder seem not to have a positive influence on growth performance of broiler chicks.

1. Introduction

Use of antibiotics as growth promoters is no longer acceptable and therefore it is forbidden in European Union countries. Because of that it was necessary to offer other feed additives as an alternative such as enzymes, organic or inorganic acids, Phytogenic additives, immunostimulators, microelements, probiotics and prebiotics [1]. Phytogenic feed additives have received increasing attention as possible antibiotics alternatives. The plants have been used traditionally in the therapy of some diseases for a long time in the world, and they have a significant role in maintaining human health. Also, they have been used in research of broiler nutrition to see their effects on their performance and other indices such as immunity and serum biochemical profiles [2].

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 [3]. 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 [4]. Most of the plant parts contain compounds with antiflatulent, carminative, expectorant, diuretic, antitussive and menstruation agent [5]. *Mentha pulegium L.* and other species of the genus *Mentha* possess antimicrobial [6], antioxidant [7], cytotoxic [8] and abortifacient properties [9].

Furthermore, Nobakht and Mehmannavaz[10] observed the beneficial influence of pennyroyal on performance, egg quality, blood and immunity parameters of laying hens. Also, Modiry *et al*[11] 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. Geran *et al*[12] reported that, supplementation of pennyroyal essential oils did not significantly affect feed intake, weight gain and feed conversion in broilers. The present study was designed to compare the efficacy of different levels of dried aerial part powder of pennyroyal an antibiotic growth promoter on growth performance, carcass characteristics and immune responses in broiler chickens when used as supplements in the diet.

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2. Materials and methods

2.1. Animals and dietary treatments

A total of 240 day-old Ross 308 broilers were assigned to 4 treatments with 4 replicates. Each replicate consisted of 15 as-hatched birds per pen. Chicks were raised on floor pens (120 × 120 × 80 cm) for 6 wk and had free access to feed and water throughout the entire experimental period. . The ambient temperature in experimental house was maintained at 32℃ during the first week and gradually decreased by 3 ℃ in the second and third weeks, and finally fixed at 22 ℃ thereafter. Feed was composed according to nutrient requirements of broilers provided by Ross Broiler Manual [13] and it was the same for all groups, except added additives (Table 1). The dried aerial parts were added to experimental diets of broilers after carefully grinding. The dietary treatments consisted of the basal diet as control, antibiotic group receiving 4.5 mg/kg flavophospholipol, 5 and 10 g/kg pennyroyal powder added to the basal diet in inclusion of corn. The birds were fed a starter diet from d 0 to 14, a grower diet from d 14 to 28, and finisher diet from d 28 to 42.

2.2. Data collection

Body weights of broilers were determined at d 1, 14, 28 and 42 of age. Feed intake and weight gain were recorded in different periods and feed conversion ratio (FCR) was calculated. Mortality was recorded as it occurred and was used to adjust the total number of birds to determine the total feed intake per bird and FCR. At d 42, two male broilers per replicate randomly selected, based on the average weight of the group and sacrificed. Carcass yield was calculated by dividing eviscerated weight by live weight. Abdominal fat, gizzard, proventriculus, small intestine, cecum, were collected, weighed and calculated as a percentage of live body weight.

The commercially available oil-adjuvant injectable emulsion against Newcastle Disease virus (NDV) and Avian Influenza virus (AIV) were used (H9N2 subtype) for vaccinating broiler chicks, and they were injected subcutaneously with 0.2 mL per chick at 9 d of age. Also, chicks were orally vaccinated against Newcastle Disease (Lasota) at 21 d of age. Antibody titers against NDV, AIV, and sheep red blood cells (SRBC) were measured as immune responses. At 25 d of the experiment, 2 birds per pen were inoculated via the brachial vein with 1 mL of 1% SRBC suspension. At d 6 post-SRBC, blood samples were taken and plasma was collected. Antibody titers against SRBC were measured by the microtiter procedure described by Wegmann and Smithies [14]. Titers were expressed as the log2 of the reciprocal of the highest dilution giving visible hemagglutination. At 28 d of age, 2 male broilers from each pen were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titers against NDV and AIV. Serum antibody titers against NDV and AIV were measured by the hemagglutination inhibition test (HI), and HI antibodies were then converted to log2.

2.3. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Means were compared using Tukey test. Statements of statistical significance are based on $P < 0.05$.

3. Results

3.1. Performance and carcass traits

Table 1
The ingredient and chemical composition of basal starter, grower and finisher diets.

Ingredients (g/kg)	Starter	Grower	Finisher
Corn	559.4	567.8	593.3
Soybean meal	379.0	371.4	346.1
Soybean Oil	16.7	24.4	26.7
DCP	19.0	16.2	14.7
Caco3	12.0	9.7	9.5
NaCl	3.2	3.2	3.0
Mineral-Premix1	2.5	2.5	2.5
Vitamin-Premix2	2.5	2.5	2.5
DL-Methionine	3.6	2.3	1.5
L-Lysine	2.1	–	–
Calculated composition			
M.energy (kcal/kg)	2870	2950	3000
Crude protein (g/kg)	208.7	206.0	196.8
Calcium (g/kg)	9.96	8.40	7.96
Av.phosphorus (g/kg)	4.7	4.2	3.9
Meth.+cysteine (g/kg)	10.1	8.8	7.7
Lysine (g/kg)	13.5	11.6	11.0
Sodium (g/kg)	1.5	1.5	1.5

1.To provide the following per kg of diet: Vit A 10,000 IU, vitamin D3 2000 IU, vitamin E 5 IU, vitamin K 2mg, riboflavin 4. 20 mg; vitamin B12 0.01 mg; pantothenic acid 5 mg; nicotinic acid 20 mg; folic acid, 0.5 mg. 2. To provide the following per kg of diet: ; choline 3 mg; Mg 56 mg; Fe 20 mg; Cu, 10 mg; Zn 50 mg; Co 125 mg; Iodine 0.8 mg.

Table2
Effect of experimental diets on performance indices of broilers at different ages.

Variables	Dietary treatments				
	Control	Flavophospholipol	5 g pennyroyal/kg	10 g pennyroyal/kg	SEM4
DFI1					
0-14 d	26.25	26.50	25.62	26.10	0.78
14-28 d	44.55	47.52	49.05	47.17	2.83
28-42 d	157.30	159.45	154.80	161.30	7.24
0-42 d	66.42	65.45	67.70	68.10	2.93
FCR2					
0-14 d	1.67	1.71	1.73	1.77	0.05
14-28 d	1.01	1.04	1.09	1.09	0.04
28-42 d	1.91	1.98	1.89	2.00	0.12
0-42 d	1.36	1.36	1.33	1.43	0.07
BW3(g)					
14 d	243.75	241.00	232.50	231.25	7.61
28 d	860.00	876.75	861.25	836.00	23.60
42 d	2008.00	1987.75	2086.50	1962.50	63.85

1. Daily Feed Intake (g per bird per day).2. Feed Conversation Ratio (g/g).3. Body Weight.4. Standard error of mean.

Table 3
Effect of experimental diets on carcass yield and internal relative organ weight of broilers at 42 d.

Relative organ weight	Dietary treatments				
	Control	Flavophospholipol	5 g pennyroyal/kg	10 g pennyroyal/kg	SEM1
Carcass (%)	70.62	69.92	71.16	72.30	1.80
Abdominal fat (%)	1.53	1.62	1.41	1.65	0.16
Gizzard (%)	2.22	2.32	2.09	2.56	0.28
Proventriculus (%)	0.45	0.53	0.41	0.47	0.08
Small intestine (%)	3.6	3.8	4.07	4.00	0.23
Cecum (%)	0.63	0.58	0.68	0.65	0.04

1 Standard error of mean.

Table 4
Effect of experimental diets on antibody titers against Newcastle and influenza viruses at day 30 and SRBS at day 31.

Variables	Dietary treatments				
	Control	Flavophospholipol	5 g pennyroyal/kg	10 g pennyroyal/kg	SEM1
Influenza (log2)	3.37	2.87	2.75	2.87	0.35
New castle (log2)	2.50	1.87	1.87	2.00	0.4
SRBC (log2)	6.25	4.87	5.80	6.37	0.79

1. Standard error of mean.

Data on performance indices are summarized in Table 2. The body weight increased in broilers fed diet containing 5 g pennyroyal/kg diet at 42 d of age compared to other groups, but it was not significant ($P>0.05$). Treatments failed to induce any marked effect on daily feed intake and FCR at different period of trial. Table 3 shows relative weight means (as a percentage of live weight at slaughter) of digestive and non-digestive organs as a function of treatments. Carcass yield, abdominal fat, gizzard, proventriculus, cecum and small intestine weights were not markedly affected by dietary treatments.

3.2. Immune responses

The results for serum antibody titers against Newcastle and influenza viruses at day 30 and SRBC at day 31 in broilers are presented in Table 4. Treatments failed to induce any marked effect on antibody titers against Newcastle and influenza viruses. The treatments had not any significant effect on antibody titers against SRBC antigen, though it tended to decrease in broilers fed on 4.5 mg antibiotic/kg diets ($P>0.05$).

4. Discussion

Performance, Internal organ weights and carcass characteristics were not significantly influenced by the dietary treatments at day 42. In accord with our findings Geran *et al*[12] reported that, using 0.1, 0.2 and 0.3% of *M. pulegium* L. essential oils had not any significant effect on performance of broilers. Nobakht *et al*[15] reported that the presence of *M. pulegium* L. blended with other medicinal herbs significantly improved the performance of broilers. Modiry *et al*[11] 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. Also, Nobakht *et al*[15] reported positive effect of *M. pulegium* L. on performance indices of broiler chicks. In this experiment flavophospholipol had not any

effect on performance criteria, whereas it is not in agreement with the findings of Landy *et al*[16] who find significant effects in using flavophospholipol on performance of broilers. The study conducted by Coates *et al*[17] showed that antibiotics did not promote the growth of broilers raised in a germ-free environment as compared to those raised in a conventional environment, leading the authors to conclude that antibiotics suppressed the growth of some microorganisms responsible for growth depression, thus it is possible in present trial the treatments had not any effects on performance indices due to hygienic status of trial.

In accord with our results Nobakht *et al*[15] reported that, use of pennyroyal had not any significant effect on relative weight of carcass yield, abdominal fat pad, liver and gizzard. Also, Hernández *et al*[18] reported that, use of antibiotic or mixtures of plant extracts had not any significant effect on carcass traits of broilers. These results are consistent with those observed by Landy *et al*[19] who did not find any differences among the control treatment and those containing antibiotic or Echinacea purpurea L. for organ weight of 42-day-old broilers.

Result of this trial showed that, humoral immune responses were not affected by dietary treatments. As pennyroyal has been reported to have antimicrobial [6] and antioxidant activities [7], an increase in immune responses of chicks was anticipated. In this trial use of pennyroyal had not any significant effect on antibody titers against Newcastle, influenza viruses and SRBC antigen, though it tended to increase in broilers fed diet containing 10 g pennyroyal/kg (P>0.05). Nobakht *et al*[15] reported no effect of dietary pennyroyal on Heterophil/Lymphocyte ratio of broiler chicks. Consistently, Hardari *et al*[20] reported that, dietary supplemented with pennyroyal had not any marked effect on Heterophil/Lymphocyte ratio of broiler chicks. Unfortunately, no other reports are available on the effects of pennyroyal on bird immune responses.

In conclusion, the results of this study showed that addition of pennyroyal powder seem not to have a positive influence on growth performance of broiler chicks.

Conflict of interest statement

We declare that we have no conflict of interest.

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