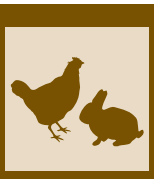
Effect of peppermint (*Mentha piperita L.*) in broiler chicken diet on production parameters, slaughter characteristics and gut microbial composition



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SUMMARY

This study was conducted to evaluate the productive performance, carcass quality and cecal microbial composition of broiler chickens fed diets supplemented with different concentrations of peppermint powder. Peppermint (*Mentha piperita L.*) is characterized by strongly scented leaves. It is used as a remedy in herbal medicine, and consists of up to 4% essential oil (with 35-45% menthol). The study included 960 one-day mixed-sex Ross 308 broilers. The chickens were assigned to 4 treatments and were housed in 24 boxes, with 6 replicates per treatment. Broilers received three diets (starter, grower and finisher) differing in the amount of supplemental peppermint powder, and were fed *ad libitum*. The diets contained different levels of supplemental peppermint powder: control group (C) - without peppermint, 02P - 0.2% peppermint, 04P - 0.4% peppermint, and 06P - 0.6% peppermint.

The body weight of chickens was measured when changing their feed (days 10 and 24) and at the end of the experiment (day 42). Average feed intake, average daily gain, mortality, feed conversion, and the European Production Efficiency Factor (EPEF) were determined. At the end of the trial, 12 broilers of both sexes were randomly selected from each group and slaughtered to measure their carcass traits. Positive effects of peppermint supplementation were identified. The 04P chickens had significantly higher ($p<0.01$) values of average daily gain, feed conversion and EPEF compared with the C broilers. There were no significant differences in slaughter results (dressing percentages, the proportions of breast, drumsticks, thighs, wings, abdominal fat, heart, liver and stomach). The total numbers of aerobic bacteria and *Lactobacilli* were not affected by the peppermint supplementation into broiler diet. The *Escherichia coli* count in the 04P and 06P birds was lower than that in the C broilers ($p<0.05$). The results showed that the supplementation of 0.6% peppermint powder to broiler diet had positive effects on weight gain, feed conversion and cecal microbial composition.

KEY WORDS

Broiler chickens, cecal microflora, nutrition, peppermint, productive performance.

INTRODUCTION

There has been an increasing use of antibiotics for growth promotion in animal nutrition worldwide although they are banned in some countries. Since antimicrobial agents cause bacterial resistance, they can be replaced with alternative growth promoters, such as organic acids, probiotics, prebiotics or phytobiotics. In contrast to antibiotics, phytobiotics pose no risk of bacterial resistance. Phytogenic feed additives are plant components used as whole plant parts (spices) or plant extracts and essential oils to improve animal health. Phytobiotics have a positive effect on the health and gut microbial status of animals (by inhibiting pathogen growth and increasing the total number of beneficial bacteria), thus improving their resistance to digestive tract diseases and, hence, their performance[20].

Peppermint (*Mentha piperita L.*) belongs to the family *Lamiaceae*, and is characterized by strongly scented leaves. It is used as a remedy in herbal medicine, and consists of up to 4% essential oil (with 35-45% menthol). Pattnaik et al. [16] compared the essential oils of various plants, and found significant antimicrobial and antibacterial efficacy of menthol. In addition to essential oil, the leaf of peppermint contains flavonoids, 6-12% tannins, triterpenes, bitter substances and other useful ingredients, which contribute to the beneficial effect of this plant. A number of authors have studied the effect of peppermint as a feed supplement in broiler diet. Ocak et al. [15], Al-Kassie [2], and Gurbuz and Ismael [8] evaluated the effects of various levels of dietary dried peppermint on the performance and carcass traits of chickens. Al-Kassie and Witwit [3] and Narimani-Rad et al. [14] used several medicinal plants, including peppermint. Nanekarani et al. [13] added different concentrations of the ethanolic extract of peppermint to drinking water. Hernandez et al. [9] found that the chemical composition of the phytobiotic used in diet and the essential oil extraction method employed can affect the test results. Cross et al. [6] reported differences in body weights between chickens fed diets supplemented with essen-

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tial oils (rosemary and Breckland thyme (*Thymus serpyllum*). The hypothesis tested in this research was that peppermint contains a mixture of complex bioactive components that can improve the productive performance, health and gut microbial status of broilers, and that the use of certain concentrations of peppermint powder in broiler diet, as a simpler and more accessible method compared with the use of peppermint extract, can produce the expected effects. The aim of the study was to assess the effect of the dietary inclusion of different concentrations of dried peppermint leaf on the productive performance, carcass quality and intestinal microflora in broilers.

MATERIALS AND METHODS

Experimental design and nutrition

The study included 960 one-day mixed-sex Ross 308 broilers. The chickens were assigned to 4 treatments and were housed in 24 boxes, with 6 replicates per treatment. Twenty male and 20 female broilers were kept in 2 × 2 m cages (0.1 m^2 per bird). Ambient temperature was 33 °C at placement, and decreased gradually from day 24 onwards to achieve 24 °C. In the first week, the lighting regime was set to 23 hours of light (L) and 1 hour of darkness (D); thereafter, it was below 18L:6D. During the experiment, broilers were fed *ad libitum*, and received complete corn and soya based mash diets (starter, grower and finisher) formulated according to hybrid producer's recommendations and analyzed for their proximate composition in an accredited laboratory (Table 1). The diets contained different levels of supplemental peppermint powder: control group (C) - without peppermint, 02P - 0.2% peppermint, 04P - 0.4% peppermint, and 06P - 0.6% peppermint.

Peppermint plants were grown and processed (dried and ground) by a local producer and were safe for human use. The particle diameter of peppermint powder was 1 mm. Standard proximal chemical analysis showed that peppermint powder contained 91.1% dry matter, 13.7% crude protein, 5.4% crude fat, 16.9% crude fiber and 9.1% ash.

Table 1 - Composition of basal diets.

Ingredient, g/kg	Starter 0-10 d	Grower 11-24 d	Finisher 25-42 d
Corn	521.9	572.9	624.9
Soybean meal	320	260	207
Full fat soybean (extruded)	100	100	100
Vegetable oil	20	30	30
Limestone	14	14	14
Monocalcium phosphate	12	12	12
Salt	2	2	2
Vitamin + mineral premix[1]	10	10	10
DL-methionine	0.1	0.1	0.1
Nutrients (analyzed) and energy level (calculated)			
ME, MJ/kg	12.7	13.1	13.4
Crude protein, %	22.2	20.3	18.1
Crude fat, %	6.25	7.36	7.52
Crude fiber, %	3.35	3.11	2.92
Lysine, %	1.25	1.01	0.96
Methionine, %	0.49	0.48	0.45
Ca, %	1.01	0.98	0.96
P, %	0.62	0.59	0.58

[1]Contents per kilogram: vitamin A, 12000 IJ; vitamin D3, 5000 IJ; vitamin E, 50 mg; vitamin K3, 6 mg; thiamine, 3 mg; riboflavin, 9.4 mg; niacin, 5 mg; pantothenic acid, 10 mg; pyridoxine, 4 mg; folic acid, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.02 mg; choline, 400 mg; Mn, 100 mg; Fe, 30 mg; Zn, 100 mg; Cu, 8 mg; I, 0.5 mg; Se, 0.2 mg.

Production indicators

The body weights and feed consumption of chickens were measured when changing feed (days 10 and 24) and at the end of the study (day 42). Average feed intake, average daily gain, mortality and feed conversion were determined. The European Production Efficiency Factor (EPEF) was calculated using the average body weight, vitality, feed conversion and fattening duration, according to the formula: EPEF = (Live weight, *kg* x Livability, *%* / Age of depletion, *days* x Feed Conversion Ratio, *kg feed/kg gain*) x 100.

Slaughter indicators

At the end of the experiment, 1 male and 1 female chicken (12 chickens per treatment), whose body weight corresponded to the average body weight of the group, were selected from each box and slaughtered after 4 hours of starvation.

Chickens were processed by hand, and cooled to 4 °C for 24 hours. After cooling, carcass weight and abdominal fat content were determined, and carcasses were dissected into major parts (breasts, thighs, drumsticks, wings) to obtain their weights. The liver, heart and stomach were weighed during carcass dressing.

Microbiological analysis of cecal contents

The cecal contents of slaughtered broilers were placed in sterile bottles and taken to the laboratory as fast as possible. Proper dilutions of cecal contents were made in nutrient broth and within 1 hour plated under aseptic conditions on selected media. Total aerobic bacterial count was determined on plate count agar (PCA) (Torlak) after incubation at 30 °C for 72 hours under aerobic conditions. Total *Escherichia coli* count was determined on the modified UTI agar (UTI agar, Himedia) after incubation at 37 °C for 24 hours in an aerobic environment. MRS agar (Becton Dickinson) was used to determine the presence and total count of *Lactobacilli* at 37 °C for 72 hours under anaerobic conditions.

Statistical analysis

The results obtained were analyzed using the GLMM (Generalized Linear Mixed Model) of the statistical software SAS (version 9.3 - SAS Inst. Inc., Cary, NC, USA). The significance of differences was assessed at the probability level $p<0.05$. Prior to the statistical processing of the data for the microbiological analysis of the cecum, their transformation was performed using the logarithmic function log10(x) and tested for normal distribution.

RESULTS AND DISCUSSION

The effects of the peppermint supplementation of broiler diet on the productive performance of broilers are shown in Table 2. During the starter and grower periods, there were no significant differences in feed intake, average daily gain and feed

conversion. In the third experimental period (25-42 days), the increase in dietary peppermint level gave higher values for average daily gain and feed conversion. The 06P chickens had a significantly ($p<0.05$) higher average daily gain compared with the C birds. Feed conversion was significantly ($p<0.01$) better in the 06P chickens than in the C and 02P broilers. Over the whole experimental period, increasing supplemental peppermint levels led to an increase in the average daily gain. This parameter was higher ($p<0.01$) in the 06P chickens than in the C ones. Feed conversion in 06P broilers was better ($p<0.01$) than in the C and 02P birds.

Across groups, broilers showed no significant differences in mortality. As feed conversion and average daily gain were higher in the 06P and 04P chickens, significantly higher ($p<0.01$) EPEF values were determined compared with C broilers. The increase in peppermint levels in broiler diet increased the EPEF values. The research focused on the practical application of ground peppermint, as a simpler and cheaper way of using this plant compared with the purchase of its essential oil. The obtained results revealed beneficial effects of peppermint in broiler diet, as manifested by higher daily gains and better feed conversion. As the concentration of active ingredients in broiler feed increased, higher daily gains and better feed conversion were obtained. Narimani-Rad et al. [14] reported improvement in the performance and carcass quality of broilers receiving 0.5% peppermint in their diet containing a mixture of medicinal herbs. Antiseptic properties come from menthol, which prevents the growth of pathogenic bacteria in the digestive system while improving digestion and nutrient absorption[16]. The positive effects of peppermint supplementation on broiler performance were also recorded by Al Ankari et al.[1], Arab Ameri et al. [4], and Asadi et al. [5]. In a study by Nanekarani et al. [13], the addition of 0.3% of ethanolic extract to drinking water improved the production characteristics of broilers and decreased the proportion of abdominal fat. In contrast, Hernandez et al. [9] found no significant differences in feed intake and feed conversion between broilers after dietary treatment with two herbal extracts. Also, Toghyani et al. [19] obtained no significant differences in the final body weight between broilers fed diets supplemented with 4% peppermint and control birds. In our study, mortality rate was higher in control chickens than in the other groups of broilers, but without significant differences. Al-Kassie [2] reported a significantly lower mortality rate in chickens receiving 0.5% peppermint in their diet compared with the control, which may be associated with the favorable effect of active ingredients of essential oils on the intestinal microbiota and the resulting stimulation of endogenous enzyme secretion and vitality of chickens.

Table 2 - Production performance of broilers receiving peppermint powder supplemented diets.

	C	02P	04P	06P	SEM	p
Starter period (1-10 d)						
FI, g/d	21.37	21.30	21.53	21.97	0.132	0.284
ADG, g/d	13.83	13.84	14.00	14.81	0.167	0.108
FCR, g/g	1.55	1.54	1.54	1.48	0.012	0.382
Grower period (11-24 d)						
FI, g/d	74.82	76.06	74.67	76.76	0.391	0.175
ADG, g/d	44.44	45.65	44.81	45.85	0.397	0.573
FCR, g/g	1.68	1.67	1.67	1.68	0.011	0.959
Finisher period (25-42 d)						
FI, g/d	144.53	143.64	144.18	140.36	0.760	0.192
ADG, g/d	68.60^{b}	69.34^{ab}	71.99^{ab}	72.90^{a}	0.622	0.028
FCR, g/g	2.10^{a}	2.07^{a}	2.01^{ab}	1.93^{b}	0.019	0.001
Whole period (1-42 d)						
FI, g/d	91.80	91.90	91.19	91.01	0.295	0.696
ADG, g/d	47.51^{c}	48.23^{bc}	49.12^{ab}	50.06^{a}	0.246	0.000
FCR, g/g	1.93^{a}	1.90^{a}	1.87^{ab}	1.82^{b}	0.012	0.002
Mortality, %	2.08	1.25	0.83	0.83	0.369	0.619
EPEF	244.27^{c}	255.66^{bc}	266.27^{ab}	278.93^{a}	3.223	0.001

C - without peppermint, 02P - 0.2% peppermint, 04P - 0.4% peppermint, and 06P - 0.6% peppermint.
SEM - Standard error of the means; FI - Feed intake; ADG - Average daily gain; FCR - Feed conversion rate;
$^{a, b, c}$ In a row, the least squares means with different superscripts differ significantly ($p<0.05$)

The results of slaughter traits of broiler chickens fed diets supplemented with different levels of peppermint are shown in Table 3. Dressing percentages, and the proportions of breast, drumsticks, thighs, wings, abdominal fat, heart, liver and stomach were not affected by the examined factor ($p>0.05$). The increase in the dietary level of peppermint had no significant effect on carcass quality parameters. These results are supported by the findings of Ocak et al. [15], who found no differences in the dressing percentages of broilers receiving 0.2% peppermint in their diet; Toghyani et al. [19], who reported no significance for the effect of peppermint supplementation on the proportion of carcass parts and organs; Hernandez et al. [9], who determined no significant effect on a mixture of herbal extracts on the weight of internal organs; and Khursheed et al. [11], who found no significant differences in the dressing percentage and the proportions of heart, stomach and liver between chickens fed diets supplemented with 0%, 1% and 2% peppermint.

The effects of dietary peppermint powder on the microbiological composition of the cecum of broiler chickens are shown in Table 4. The total numbers of aerobic bacteria and *Lactobacilli* did not differ significantly between groups. Significant differences ($p<0.05$) were determined for the *Escherichia coli* count, which was higher in control birds than in the 04P and 06P broilers. The increase in peppermint level led to a gradual reduction in the number of *Escherichia coli*.

In this study, the use of peppermint in the nutrition of broiler chickens was found to improve the ratio of beneficial to harmful bacteria in the cecum. A favorable microbiological status of the digestive tract results in better health and better nutrient digestibility. These results are consistent with Jamroz et al. [10], who found a significant reduction in the number of *Escherichia coli* and an increase in the number of *Lactobacilli* in chickens fed diets supplemented with plant extracts. Giannenas et al. [7] reported an increase in lactic acid bacteria and a decrease in coliforms in the cecum of turkeys receiving a mixture of essential oils and thymols. The authors also determined a significant improvement in the antioxidant status of turkeys. Roofchaee et al. [17] obtained a decrease in the number of *Escherichia coli*, but no change in the count of *Lactobacilli* in the cecum of broilers fed broccoli-containing diets supplemented with oregano essential oil. Also, Kirkpinar et al. [12] recorded no differences in the number of *Lactobacilli* in chickens receiving a mixture of essential oils. Saki et al. [18] reported a significant decrease in *Escherichia coli* in broiler chickens given the essential oil of thyme in drinking water.

Table 3 - Slaughter traits of broilers receiving peppermint powder supplemented diets.

	C	02P	04P	06P	SEM	p
Body weight (BW), g	2013.3	2055.8	2045.0	2079.2	36.21	0.939
Carcass weight (CW), % BW	67.74	67.42	67.50	67.76	0.204	0.918
Breasts, % CW	28.65	29.01	29.30	29.51	0.170	0.313
Drumsticks, % CW	15.11	15.27	14.83	15.15	0.107	0.537
Thighs, % CW	17.00	17.28	16.94	17.16	0.097	0.602
Wings, % CW	11.27	11.38	11.18	11.40	0.082	0.770
Abdominal fat, % CW	0.90	0.87	0.88	0.75	0.027	0.218
Organ weights, % BW						
Heart	0.51	0.53	0.52	0.51	0.008	0.872
Liver	1.73	1.72	1.74	1.78	0.028	0.870
Gizzard	1.91	1.87	1.92	1.95	0.022	0.599

C - without peppermint, 02P - 0.2% peppermint, 04P - 0.4% peppermint, and 06P - 0.6% peppermint.
SEM - Standard error of the means.

Table 4 - Bacterial counts from the cecal content of broilers receiving peppermint powder supplemented diets.

	C	02P	04P	06P	SEM	p
The total number of aerobic bacteria	8.55	8.59	8.33	8.26	0.075	0.331
Escherichia coli	6.92^{a}	6.83^{a}	6.44^{ab}	6.23^{b}	0.091	0.019
Lactobacilli	7.33	7.50	7.46	7.60	0.047	0.244

C - without peppermint, 02P - 0.2% peppermint, 04P - 0.4% peppermint, and 06P - 0.6% peppermint.
SEM - Standard error of the means.
$^{a-b}$ In a row, the least squares means with different superscripts differ significantly ($p<0.05$)

CONCLUSIONS

The results of the present study indicated that the use of 0.6% of peppermint in broiler diet had a beneficial effect on average daily gain, feed conversion, EPEF value and cecal *E. coli* count, without affecting carcass quality.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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