Original Article

The effect of different dietary levels of Pennyroyal (Mentha Pulegium L.), probiotic and antibiotic on performance, carcass characteristics and, selected nutrients digestibility in broiler chickens

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Abstract

Background: The aim of this study was to investigate the effects of different dietary levels of Pennyroyal (Mentha Pulegium L.) essential oil (PEO), probiotic (Bioplus 2B) and antibiotic (Flavophospholipol) on performance, carcass characteristics and nutrients digestibility in broiler chickens in a completely randomize design (CRD). **Materials and Methods:** The treatments included: A corn-wheat-soybean meal basal diet without any additives as control group and adding three levels of Flavophospholipol (0.015, 0.03 and 0.05 % of diet), three levels of BioPlus-B2 (0.1, 0.2 and 0.3 % of diet) and three levels of Mentha pulegium essential oil (0.03, 0.05 and 0.07 % of diet) to the basal diet. 5 replicates of 12 chicks were allocated to each experimental treatment. **Results:** The results showed that the treatments significantly affected body weight gain (WG) and feed conversion ratio (FCR) in the all experimental periods (P<0.05); but, they had no significant effect on feed intake (P>0.05). PEO at the level of 0.07% significantly decreased WG during 11-25 d (P<0.05) but observed no effect during 11-25 and 11-42 d in compared to control group (P>0.05). Breast and abdominal fat percentage were not significantly affected by treatments (P>0.05). Lowest crude protein (CP) digestibility was observed in 0.015% antibiotic treatment that was significantly lower than 0.1% probiotic treatment (P<0.05). **Conclusion:** More significantly, dietary supplements represented desirable performance in compared to antibiotics and control group. Hence, the possible usage of these components as antibiotics alternatives in poultry feeds should be outlined in future.

Keywords: Antibiotics, broilers, carcass, pennyroyal essential oil, probiotics

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Introduction

Antibiotics are a group of chemical compounds that are biologically produced by plants or certain microorganisms, usually fungi. Since the 1950s, antibiotics have been used in poultry production for improvement of growth performance and feed efficiency and also, reduction of mortality. Most of antibiotics used in poultry feeding are the same as those used in humans. Flavophospholipol is a phosphoglycolipid antimicrobial that is produced by various Streptomyces strains. It is active against harmful bacteria because of inhibition of bacterial wall synthesis. Flavophospholipol is used as a feed additive growth promoter in chickens and turkeys. This antibiotic has no withdrawal period, because it is not absorbed in the gastrointestinal tract and no measurable residues are found in edible tissues even with higher doses (9). With increasing concerns about antibiotic resistance, use of them was banned in sub-therapeutic levels in poultry production from 2006 in European Union. Therefore, there is an increasing interest in finding alternatives of antibiotics in poultry production (4). Possible alternatives to antibiotics in animal rearing are essential oils and probiotics, in addition to prebiotics and symbiotics.

Essential oils (EOs) are a mixture of odorant and volatile compounds that are usually found in plants. Several EOs, such as those extracted from garlic bulbs, onion and mint (Mentha spp.) have been reported to have anti-parasitic and anti-microbial properties in gastrointestinal tract; in addition to other beneficial effects of EOs including appetite stimulation, improvement of digestive enzymes secretion and immune responses stimulation. Recently, use of EOs in broilers has drawn attentions. It has been revealed that EOs can be used in poultry feeding, but there are still questions concerning their action and optimal dosage, which have to be investigated in detail (13).

One part of the healing properties of plants is related to secondary metabolites such as phenolic compounds, essential oils and saponins. EOs are irregularly scattered throughout the plants but they often found in some glands, tissues or epidermis of the plants. These materials are not homogeneous in terms of chemical composition, but in various combinations, these are usually belonging to the terpenes, sesquiterpene, alcohols, ethers, aldehydes, phenols, ethers or peroxides (7). Pennyroyal (Mentha Pulegium) is a member of Labiatae family. This family includes 20 species that are scattered around the world (20). The herbals, in addition to antimicrobial and high antioxidant and anti-fungal properties, stimulate appetite, increase nutrient digestibility and feed intake, improve the state of the gastrointestinal tract environment (2, 20). A lot of researches have shown that Pennyroyal improves digestibility of feed and food in animals and humans (3, 20) Some researchers also reported that live performance and carcass weight of chickens that fed with oregano oils were higher than other non-treated birds (1).

Probiotics are living and health-promoting bacteria that habituate in gastrointestinal tract of humans and animals. They prevent the establishment of pathogens, by 1) competition with pathogen bacteria for nutrients, 2) production of antimicrobial compounds (production of short-chain fatty acids and bacteriocins and lowering the lumen pH) 3) competition for attaching to absorption sites in intestinal epithelium and 4) stimulation of the immune system of the animals (12, 19). The major probiotic strains used in poultry nutrition are Lactobacillus, Saccharomyces, Bacillus and Streptococcus. Positive effects of probiotics on animals can be resulted from their both direct effect as bio-regulators of intestinal micro flora and augment the host natural defenses. There have been numerous studies in broilers on the ability of probiotics to improve growth performance and nutrients digestibility.

This study aimed to investigate the effects of different dietary levels of Pennyroyal essential oil and probiotics as a possible alternative to antibiotics on broiler chickens performance, carcass characteristics and, selected nutrients digestibility.

Methods

Ethical approval. The experimental procedure was approved by the Animal care and use committee of the Ferdowsi University of Mashhad, Iran.

Essential Oil Composition. Main components of the Pennyroyal essential oil were determined using a GC/MS system (GCMS-QP5050, Shimadzu, Japan); column (DB-1, 60 mm × 0.25mm fused silica capillary column, film thickness 0.25 μm). The column temperature was increased from 40 ºC to 220 ºC at a rate of 4 ºC/min; injector temperature, 260ºC; with helium as the carrier gas (Table 2).

Birds and Management. A total of 600 day-old male broiler chickens (Ross 308 strain) were obtained from a local commercial hatchery with average initial body weight of 40 g. Chicks were transferred from incubation center to the research field. The chicks were randomly assigned to 10 dietary treatments. Each treatment consisted of 5 replicates (floor pen; 1.5×1×0.8 m) of 12 birds each. Temperature was set at 32 ºC on day one and gradually decreased in a linear manner by 0.5◦C per day to reach 22 ºC on day 20 and this temperature was maintained during the experiment. Lighting program consisted of 23L: 1D from 1 to 42 d. The pens were equipped with a hanging feeder and a bell drinker.

Diets and Treatments. Corn, wheat and soybean meal basal diets were prepared for Starter (1 to 10 d), grower (11 to 24 d), and finisher (25 to 42 d) periods. The diets (Table 1) were formulated by UFFDA1 software to meet or exceed the nutrient requirements according to Ross-308 guideline (5). BioPlus 2B components were Calcium carbonate, dried Bacillus Licheniformis fermentation product, dried Bacillus Subtilis fermentation product, and Sodium silico aluminate. This product was prepared from Biochem Co., Germany. Its total Bacillus colony forming unit (CFU) was 2.21×109 g-1.

Different dietary levels of Pennyroyal essential oil, probiotic (Bioplus 2B) and antibiotic, Flavophospholipol (Teif Azmoon Pars, Co., Tehran, Iran) were supplemented to the basal diets. So, the experimental treatments included: 1) a corn, wheat, soybean meal basal diet without any additives (control), 2) Basal diet with 0.015% antibiotic, 3) Basal diet with 0.03% antibiotic, 4) Basal diet with 0.05% antibiotic, 5) Basal diet with 0.1% probiotic, 6) Basal diet with 0.2% probiotic, 7) Basal diet with 0.3% probiotic, 8) Basal diet with 0.03% Pennyroyal EO, 9) Basal diet with 0.05% Pennyroyal EO, 10) Basal diet with 0.07% Pennyroyal EO. Pennyroyal essential oil was bought from Barij Essence Company2. Dietary treatments were offered to the chicks from 11 to 42 days of age and the birds were fed ad libitum throughout the experiment (1-42 days). The essence was primarily dried by anhydrous sodium sulphate and then the containers tightly-closed in dark chambers and kept at 4°C until use. Prior to use, the essential oil, was dissolved in vegetable oil of the diets and uniformed by stirring and then was mixed with the experimental diets. The diets, during the experiment, were prepared weekly and were kept in containers tightly-closed (in order to prevent the infiltration of air into the containers and also to prevent the outflow of the essence).

Growth Performance. Weight gain, feed consumption and feed conversion ratio were measured periodically (11-24 and 25-42 days of age). The chicks weighed on day 11 and then at the end of each feeding phase (25 and 42 days). To calculate the averages of daily weight gain and daily feed intake, the hen-day method was used to consider died chicks during the experiment. To calculate the feed intake, amount of feed remained at the end of the period subtracted from the amount of feed offered to each pen during the period.

Carcass characteristics. At the end of the experiment (42 d) two birds per pen closest to the mean body weight of the pen, slaughtered after 8 h feed withdrawal by cutting off the head between the head and the first cervical vertebra. The carcasses were peeled and their parts including breast, thighs, back and neck and abdominal fat were weighed, separately. The carcass yields were reported as percentage of pre-slaughter live body weight of the broilers (6).

Nutrients digestibility. On day 18, chromic oxide (Cr2O3) was mixed (3 g/kg diet) to all experimental diets and then one bird per pen with closest weight to the average body weight of the pen was selected and slaughtered by cervical dislocation at day 21. Digesta were collected from the between Meckel's diverticulum and 2 cm prior to ileocecal junction. The digesta were then frozen at -20◦C until analysis. The digesta samples were oven-dried at 65 ◦C to reach a constant weight, and then grounded. The diets and dried digesta samples were analyzed in duplicate, for crude protein (CP) and ether extract (EE) according to AOAC procedures (1990). The content of Cr2O3 in the diets and digesta was determined using a flame atomic absorption spectrophotometer (Shimadzu, AA 670, Tokyo, Japan) according to Fenton and Fenton method (10).

Then, nutrient digestibility was calculated by the following formula (23): Nutrient digestibility (%) = 100 – (% chromic oxide in feed/% chromic oxide in digesta) × (% nutrient in digesta /% nutrient in feed).

Apparent ileal digestibility of CP and EE of the diets were calculated using the following formula: Digestibility (%) = 100 – 100 × [(Cr2O3diet × CP (and or EE) digesta)/(Cr2O3digesta × CP diet)] in which, Cr2O3diet and Cr2O3digesta represent

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concentrations of Cr2O3 in the diet and digesta samples (g/kg), respectively, and CP diet and CP digesta stand for the concentrations of CP in the diet and digesta samples (g/kg), respectively.

Statistical analysis. Data were subjected to ANOVA appropriate for a completely randomized design; using the General Linear Models (GLM) procedure of SAS (22). Treatments means were separated using Duncan's new multiple range test. Pens served as the experimental unit for all data analyzed, and means were considered significant at P<0.05.

Results

Retention time and chemical composition percentages obtained in the analysis of Pennyroyal essential oil are shown in table 2. In this investigation, Pennyroyal essential oil was bought from Barij essence Co, Kashan, Iran. Eleven components were identified in the EO by our analysis; representing 100 % of the total compounds. Cyclopropane was the major group of the constituents (63.18%), followed by β- Myrcene (8.26%) and Dl- Limoene cyclohexene (6.22%). Other components present in remarkable contents included: Cyclohexanone, 5-Methyl-2 (5.53%), Sabinene (3.68%), 7-Methyl-3-Methyleneoctadiene (3.66%), Limoene (2.97%), 1, 8- Cineole (2.08%), β-Phellandrene (1.98%), Eucalyptol (1.34%) and Bicyclo (1.1%).

The effect of experimental treatments on broiler chickens performance is shown in Table 3. Results indicated that the treatments did not significantly affect feed intake (P>0.05). Probiotic increased body weight gain more than other treatments. The highest increase in body weight gain belonged to probiotic 0.2% which was significantly more than Pennyroyal 0.07% (P<0.01). Pennyroyal at the level of 0.07% deteriorated FCR in compare to all other experimental groups (Table 3).

Table 1. Ingredients and chemical composition of the basal diets.

Ingredients (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	50.12	39.58	35.43
Wheat	-	10	20
Soybean meal (44%)	40.56	40.39	35.62
Sunflower oil	4.33	6.16	5.35
Dicalcium Phosphate	2.00	1.69	1.53
Limestone	1.30	1.08	1.06
DL-Methionine	0.6	0.26	0.21
Common salt	0.30	0.30	0.30
L-Lysine HCl	0.29	0.04	0.00
Vitamin Premix *	0.25	0.25	0.25
Mineral Premix **	0.25	0.25	0.25
Calculated composition (%)			
Metabolizable Energy (kcal/kg)	3000	3100	3100
Crude protein	22	21.67	20.39
Calcium	1.05	0.9	0.85
Available Phosphorous	0.5	0.45	0.42
Lysine	1.43	1.24	1.10
Methionine	0.94	0.60	0.52
Methionine + Cystine	1.28	0.95	0.86
Threonine	0.89	0.88	0.81

*Supplied per kilogram of diet: Vitamin A (retinol), 22,500 IU; Vitamin D3, 5,000 IU; Vitamin E (DL-α-tocopheryl acetate), 45 IU; Vitamin K3 (menadione), 5 mg; vitamin B1, 4.375 mg; vitamin B2 (riboflavin), 16.5 mg; vitamin B3 (niacin), 24.5 mg; vitamin B5 (pantothenic acid), 74.25 mg; vitamin B6 (pyridoxine), 7.35 mg; vitamin B9 (folic acid), 2.5 mg; vitamin B12 (cyanocobalamin), 0.0375 mg; vitamin H2 (biotin), 0.25 mg; Choline chloride, 625 mg.

** Supplied per kilogram of diet: Mn (manganese sulfate), 248 mg; Fe (iron sulfate), 125 mg; Zn (zinc sulfate), 211.75 mg; Cu (copper sulfate), 25 mg; I (calcium iodate), 2.475 mg; Se (Sodium Selenite), 0.5 mg.

Table 2. The main constituents of the essential oil of Pennyroyal (Mentha pulegium).

Compounds	Retention time (min)	%
Cyclopropane	11.474	63.18
β- Myrcene	17.16	8.26
Dl- Limonene cyclohexene	9.088	6.22
Cyclohexanone, 5-Methyl-2-(1-methylethyl)	11.186	5.53
Sabinene	7.495	3.68
7- Methyl-3-Methyleneoctadiene	8.942	3.66
Limonene	17.683	2.97
1,8- Cineole	9.561	2.08
β- Phellandrene	11.842	1.98
Eucalyptol	18.289	1.34
Bicyclogermacrene	16.861	1.1

Table 3. Effect of different dietary levels of Pennyroyal essential oil, antibiotic and probiotic on growth performance of broiler chickens.

Treatment (% of diet)		Feed intake (g/day)			Body weight gain (g/day)			Feed conversion ratio (g:g)		
		(11-24 d)	(25-42 d)	(11-42 d)	(11-24 d)	(25-42 d)	(11-42 d)	(11-24 d)	(25-42 d)	(11-42 d)
Control		75.14	147.46	118.56	55.84ab	87.12ab	71.87ab	1.53c	1.69ab	1.65b
Antibiotic	0.015	90.84	152.48	125.52	55.23ab	90.91ab	72.80a	1.64bc	1.69ab	1.72b
	0.03	87.91	147.95	121.68	54.73ab	94.16a	76.90a	1.61bc	1.57b	1.58b
	0.05	87.95	150.45	122.48	57.66ab	92.06ab	77.01a	1.52c	1.63b	1.59b
Probiotic	0.1	93.09	149.98	125.12	59.36a	95.02a	77.16a	1.57c	1.57b	1.59b
	0.2	91.74	150.55	124.78	54.02abc	94.04ab	77.42a	1.69bc	1.60b	1.62b
	0.3	90.31	149.67	121.82	54.66abc	94.49a	75.19a	1.65bc	1.58b	1.61b
Pennyroyal EO	0.03	88.54	139.01	116.93	48.73bcd	85.72ab	69.53ab	1.82ab	1.62b	1.62b
	0.05	91.97	133.76	115.48	45.59cd	82.04ab	66.1ab	2.02a	1.70ab	1.78b
	0.07	89.14	144.25	120.12	44.87d	72.54b	60.31b	2.00a	2.00a	1.99a
P. value		0.15	0.09	0.16	0.0001	0.0001	0.0005	0.0001	0.0001	0.04
SEM		2.82	4.42	2.79	1.92	4.56	2.59	0.049	0.06	0.0001

Means within a column without any common superscript are significantly different (P<0.05).
SEM= Standard error of means.

Table 4. Effect of different dietary levels of Pennyroyal essential oil, antibiotic, probiotic and on carcass traits of broiler chickens at 42 d (% of live body weight).

Treatment (% of diet)		Carcass	Thighs	Breast yield	Back and neck	Abdominal fat
Control		65.46a	19.22b	24.34	19.76ab	1.34
Antibiotic	0.015	65.28ab	17.75b	24.73	22.30a	1.28
	0.03	64.26ab	17.70b	23.73	19.58ab	0.84
	0.05	64.90ab	18.58b	24.75	21.46ab	0.89
Probiotic	0.1	61.73b	19.10b	21.80	20.30ab	0.96
	0.2	66.48a	18.86b	21.87	21.44ab	1.32
	0.3	66.36a	19.03b	25.30	21.09ab	0.93
Pennyroyal EO	0.03	66.73a	19.17b	24.67	21.23ab	0.88
	0.05	67.09a	22.45a	22.14	20.63ab	1.25
	0.07	66.56a	19.59b	24.26	18.78c	0.91
P. value		0.0001	0.0021	0.1101	0.0001	0.085
SEM		0.74	0.52	0.89	0.61	0.11

Means within a column without any common superscript are significantly different (P<0.05).
SEM= Standard error of means.

In grower (11-25 d), finisher (25-42 d) and whole experimental (11-42 d) periods, different levels of probiotics decreased feed conversion ratio that was significantly better than 0.07% Pennyroyal (P<0.05) with no significant differences in compare to other groups.

The effect of experimental treatments on average relative weights of broiler carcass components is shown in Table 4. Effect of treatments on carcass relative weight indicated that 5% Pennyroyal yielded highest relative weights of carcass and thigh (P<0.05). Different levels of Pennyroyal compared with probiotic numerically increased the relative weight of carcass and thigh percentages. Different treatments did not significantly affect weights of the breast and abdominal fat pad. The lowest weight of neck and back belonged to Pennyroyal 0.07% which had a significant difference compare to antibiotic 0.015% (P<0.05).

The crude protein and fat digestibility in various experimental treatments is presented in Table 5. The results of statistical analysis indicated that lowest CP digestibility was observed in 0.015% antibiotic treatment that was significantly lower than 0.1% probiotic treatment (P<0.05), but not with control group. Lowest fat digestibility among the treatments was observed in 0.3% probiotic, that was not significantly different with control group (P>0.05).

Table 5. Effect of different dietary levels of Pennyroyal essential oil, antibiotic and probiotic on nutrients digestibility (%).

Treatment (% of diet)		Crude protein	Fat
Control		66.62ab	80.82ab
Antibiotic	0.015	56.40b	79.45ab
	0.03	61.00ab	85.59a
	0.05	69.17ab	91.26a
Probiotic	0.1	79.53a	85.78a
	0.2	70.80ab	79.72ab
	0.3	74.74ab	72.48b
Pennyroyal EO	0.03	59.66ab	82.63ab
	0.05	64.91ab	83.52ab
	0.07	63.04ab	84.06ab
		0.001	0.001
		4.54	2.48

Means within a column without any common superscript are significantly different (P<0.05).
SEM= Standard error of means.

Discussion

In other studies (24), carvone (49.5 %) was also the major constituent, followed by menthone (21.9 %) and limonene (5.8 %) of the extractable essential oils from Mentha Pulegium. In contrast, in a study about Mentha spicata essential oil, menthone was absent (13).

Probiotics have useful effects in poultry nutrition with variety ways such as decreasing Escherichia coli infection, increasing lactic acid producing bacteria and increasing phagocyte activity of leukocytes (15). Supplementation of probiotics to wheat based diets decreases intestine contents viscosity and population of the ceca coliforms bacteria, increases body weight gain and villus height and crypt depth. Also, probiotic supplementation decreases ammonium concentration in excreta and increases total volatile fatty acids and lactic acid of intestine contents. It has been reported that probiotics have positive effects on bird's health and improve body weight gain (21).

Essential oils of Pennyroyal may affect nutritional value of the feeds. These oils stimulate enzymes secretion such as amylase and other digestive enzymes. Also, they may change population of digestive tract microbia and act similar to antibiotic, which decrease population of harmful bacteria that break down amino acids, eventually this process ameliorates their absorption (8). In addition, antioxidant nature of Pennyroyal essential oil can prevent nutrients oxidation and improves nutrient efficiency (14). Other investigations, confirm our results which reported Pennyroyal supplementation significantly improves broiler chickens performance (25). In current experiment, Pennyroyal at the level of 0.07% decreased broiler chickens performance that may be due to its toxic effect in higher doses.

Pennyroyal may improve the levels of nutrients absorption, especially amino acids. Absorbed amino acids can accumulate in carcass parts, especially breast and thighs. Antioxidant effect of Pennyroyal essential oil due to phenolic substances, decreases oxidation of nutrients and increases amount of the nutrient absorption and substrate accumulation in broiler tissues (2, 17). Also, odorous materials of herbals stimulate the secretion of digestive enzymes especially pancreatic lipase, amylase and proteases and also brush border enzymes in the intestinal cells. Then, PEO not only affects enzyme activity but also affects bile production which stimulates growth performance and feed retention capacity (11). Our results, in current sturdy, are in agreement with other findings, which reported significant effect of Pennyroyal on carcass characteristics (20).

In general, the addition of antibiotics, probiotics and Puneic essential oils in corn-wheat based diets did not significantly improve protein and fat digestibility compared to the control group. Wheat contains high levels of non-starch polysaccharides (NSP) in the aleuron layer and endosperm wall. The NSP in wheat, by creating viscosity, prevents the effective movement of enzymes and nutrients and reduces their digestibility, as the increase in viscosity of the intestines greatly reduces the physical mixing of intradermal contents. It also reduces the passage of dissolved materials in the gastrointestinal tract, and limits the transfer of material within the intestine and reduces the digestibility of feed and nutrients (18). Antibiotic use in this test did not have a positive effect on digestion of protein and fat, too. This is probably due to the use of vegetable oil in the basal diet, which has high digestibility and also less interference with NSP in wheat. But in general, antibiotics increase digestion and absorption of carbohydrates and lipids (16). An increase in the population of microorganisms disrupts digestion and absorption of fats and, by causing problems in the biliary system of the hepatic-intestinal cycle, reduces the digestion and absorption of the fats (15). The use of probiotics, in current study, did not affect apparent digestibility of the nutrients. Researchers reported that activity of intestinal proteases in germ free animals was similar to that of ordinary animals (26). The use of probiotics increases the population of lactobacilli in the intestine, resulting in disturbances in the absorption of fat by the bird due to altering the biological changes in bile acids. In addition to bacteria such as bacterioede, bifidobacteria and clostridium, lactobacilli may also be effective in hydrolyzing bile salts that are critical for fat absorption. There are limited studies on the effects of phytobiotics and plant essential oil compounds on the function and activity of digestive enzymes and digestibility of nutrients. Some herbs and essential oils affect the digestion and secretion of digestive enzymes, which, by increasing digestive enzymes secretion, increase the digestibility of nutrients and improve the regulation and stability of the intestinal microbial flora. Phenolic compounds in broilers increase pancreatic enzymatic secretions such as amylase, lipase, trypsin and chymotrypsin due to the incomplete activity of digestive enzymes in young chicks (17). In conclusion more significantly, dietary supplements represent desirable performance compare to antibiotics and control group. Hence, the possible use of these components as antibiotics alternatives in future in poultry diets should be outlined.

Conflicts of Interest

There is no conflict of interest among authors.

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