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The effect of four medicinal plants on the performance, blood biochemical traits and ileal microflora of broiler chicks

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ABSTRACT

This study was conducted with broilers to evaluate the effects of dietary supplementation of four medicinal plants on the performance, blood lipids and microflora population in the ileum. Three hundred and thirty-six day-old Ross broiler chicks were used in a completely randomized study with 6 treatments and 4 replicates each. The diets were iso-caloric and iso-nitrogenous and contained 15, 3, 2 and 2 g/kg of dried cumin, peppermint, yarrow and poley herbs, respectively. Two dietary treatments were considered the negative (containing no medicinal plant or antibiotic) and positive (containing Flavomycin at 0.4 g/kg) control groups. Flavomycin and peppermint supplementation to the diet increased the FI and BWG of the broiler chickens compared to the control ($P<0.01$). Dietary Flavomycin significantly increased body weight gain (BWG) in contrast to the other dietary treatments ($P<0.05$). Peppermint and cumin supplementation to the diet increased the BWG of the broiler chickens, whereas dietary poley and yarrow significantly reduced the BWG and increased feed conversion ratio (FCR) when compared with broilers fed the negative control diet ($P<0.05$). Dietary Flavomycin and peppermint increased the concentration of triglycerides (TG), low density lipoprotein (LDL) and total cholesterol in serum ($P<0.05$). Addition of Flavomycin or peppermint to the diet significantly reduced the ileal *Bifidobacteria* and *Clostridia* ($P<0.05$). In conclusion, under the conditions of this study, peppermint improved growth performance and adding it to the diet could be an alternative to the use of antibiotics as growth promoters in poultry production.

Key words: broiler chicken, medicinal plants, performance, flavomycin, growth promoters

Introduction

Antibiotic growth promoters (AGP) have made a tremendous contribution to the profitability of the poultry industry. Recently, it has been reported that the use of antibiotics

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as a growth promoter in chicken has caused some unwanted results (BOTSOGLOU and FLETOURIS, 2001). Therefore, most antibacterial performance promoters have been banned due not only to cross-resistance but also to multiple resistances (NEU, 1992). Nowadays, antibiotic resistant strains of bacteria have increased the concern about the potential public health problems and food safety is more seriously considered than before. On the other hand, the economy of the poultry industry and food production are also factors which may not be overlooked. Therefore, poultry nutritionists are being challenged to develop an alternative for AGP and the search for alternative feed supplements has been stepped up. Considerable attention has been paid to medicinal herbs as replacements for AGP (IBRAHIM et al., 2005). Herbs or products including plant extracts, essential oils or the main components of the essential oil are among the alternative growth promoters that are already being used in practice (OCAK et al., 2008).

There is evidence suggesting that herbs, spices, and various plant extracts have appetizing, digestion-stimulating and antimicrobial properties. But there is only limited evidence about whether their inclusion as a solid herb material would have growth promoting effects in live birds. The aim of this study was to describe the effects of dietary inclusion of dry Peppermint (*Mentha piperita*), Cumin (*Cuminum cyminum*), Yarrow (*Achillea millefolium*) and Poley (*Teucrium polium*) as growth promoter supplementation on growth performance, small intestinal microflora, the immune system and some biochemical blood parameters in broiler chicks. Chemical investigations have shown that *Teucrium Polium* contains various active compounds, such as flavonoids, tannins, saponin, glycosides, terpenes and steroids (ABDOLAHI et al., 2003). The pinene, alpha-terpineol, apigenin, cuminaldehyde, P-cymene and flavonoids are the main active components in *Cuminum cyminum* (GULER, et al., 2006). Peppermint (*Mentha piperita*) contains menthol, menthone, and their derivatives: isomenthone, neomenthol, acetylmenthol, pulegone as active components (PATTNAIK et al., 1997). Several constituents including mainly chlorogenic acid and flavonoids, luteolin, and apigenin, have been identified in *Achillea millefolium* (RAIMONDAS et al., 2008).

Materials and methods

A total of 336 day-old Arbor Acres broiler chicks, obtained from a local hatchery, were randomly allocated to 24 wire floor battery type experimental cages (100 × 85 cm). The chickens were assigned to 1 of 6 dietary treatments (4 pen replicates; 14 chicks per cage) and raised under environmentally controlled conditions, following a standard temperature regimen that gradually decreased from 32 to 24 °C by 0.5 °C daily, and a 23L: 1D lighting program. Each cage contained an equal number of male and female birds. Feed intake and live BW were recorded weekly on a per pen basis, and feed conversion ratio (FCR) was then calculated. Dead chicks were collected daily and weighed at the time of carcass

removal; carcass weights were included in FCR calculations. The experimental diets were iso-caloric and iso-nitrogenous and formulated to meet or exceed the nutritional recommendations (Table 1; NRC, 1994).

Table 1. Composition (g/kg) and calculated analysis of experimental diets

Feed ingredient (g/kg)	Starter (0-14 d)	Grower (15-28 d)	Finisher (29-42 d)
Corn	577.5	662	702
Soy oil	15	23	25
Soybean meal	365	280	240
DCP	19.7	12	12.4
Limestone	15.8	13.8	11.6
Salt	3	3	3
Vitamin premix[a]	2.5	2.5	2.5
Mineral premix[b]	2.5	2.5	2.5
DL-methionin	1.5	1.2	1
L-Lysine	1	-	-
Calculated Analysis			
AMEn (Kcal/kg)	3005	3100	3150
Crude protein (%)	21.92	19.39	17.72
Ca (%)	0.95	0.87	0.79
Available P (%)	0.43	0.35	0.37

MEn: Nitrogen-corrected apparent metabolisable energy; [a] The mineral mixture supplied (mg per kg diet): Mn, 64.5; Zn, 33.8; Fe, 100; Cu, 8; I, 640; Co, 190; Se, 8, as support. [b] The vitamin mixture supplied the following vitamins (per kg diet): vitamin A 4400000IU; Vitamin D 72000 IU; vitamin E 14400 IU; Vitamin K 2000 IU; Cobalamin 640 mg; thiamine, 612 mg; riboflavin, 3000 IU; calcium pantothenate, 4896 mg; niacin, 12160; pyridoxine, 612 mg; folic acid, 1.5 mg; choline chloride, 260 g.

Birds were fed a starter diet from day 0 to 14 followed by grower and finisher diets from 15 to 28 days and 29-42 days of age, respectively. The cumin (*Cuminum cyminum),* peppermint (*Mentha piperita),* Yarrow (*Achillea millefolium)* and poley (*Teucrium polium)* were purchased commercially as dried herbs and were added at 15, 3, 2 and 2 g/kg diet, respectively. Two dietary treatments were considered as negative (containing no medicinal plant or antibiotic) and positive (containing Flapol® [Flavophospholipol (flavomycin); Damyaran Arak Co. Iran], at 0.5g/kg) control groups. Feed and water were provided ad-libitum. At day 35, two birds (including one of each sex) per treatment cage (n = 8 birds/treatment) were randomly selected, and blood samples were taken from their jugular vein. The concentration of serum lipids (HDL, LDL, total cholesterol and triglyceride) were determined calorimetrically, using commercial kits as previously described (QURESHI et al., 1983). Then the birds were killed by cervical dislocation. The abdominal cavity was opened and the total gastrointestinal tract immediately exposed.

From each euthanized bird, the ileum, starting from the Meckel's diverticulum to 4 cm above the ileo-caecal junction, was quickly dissected and the digesta contents of this intestinal segment (1 g) were collected and homogenized with 10 mL phosphate buffer solution (PBS, pH 7). The ileal digesta specimens were sent packed on ice to the laboratory (Microbiological laboratory, Faculty of Veterinary Medicine, University of Tehran) for enumeration of total bacteria, *E. coli*, *Clostridia* spp. and *Lactobacilli* spp.

At the end of the experimental period (day 42) 2 birds, including one of each sex, per treatment cage (n = 8 birds/treatment) were randomly selected, individually weighed, and slaughtered. The abdominal cavity was opened and the total gastrointestinal tract exposed. Total gut tract, liver, ceca and abdominal fat were removed and weighed, and the carcass yield was concluded.

All data were subjected to ANOVA using the General Linear Models Procedure of SAS software (SAS, 2002). Treatment means were tested using the Duncan's multiple-range test, and statistical differences declared at $P<0.05$.

Results

The effects of the dietary treatments on growth performance are presented in Table2. During the starter period, only birds fed Poley supplemented diet had significantly, lower body weight gain(BWG) as compared to the control treatment ($P<0.05$). Birds fed diets containing Flavomycin had the highest weight gains during the starter diet ($P<0.05$). No significant differences were observed between the BWG of birds receiving Flavomycin, cumin, peppermint in their diets and the control, during the starter period. Feed intake (FI) and FCR did not differ between dietary treatments.

A significant growth promoting effect was observed from flavomycin and peppermint feed additives during the grower and finisher periods ($P<0.05$). No significant differences were observed between the FI and BWG of birds fed on the flavomycin and peppermint diets during the grower periods. However, during the finisher period the FI of birds on the peppermint diet and the BWG of birds on thein Flavomycin diet were higher than those of the birds in the other treatment groups ($P<0.05$). However, broilers fed on the Flavomycin and peppermint diets had higher FI and BWG than birds in the control group ($P<0.01$). Birds receiving the diet containing poley and yarrow exhibited the lowest BWG and highest FCR during both grower and finisher phases. Body weight gain, FI and FCR did not differ among birds fed the cumin supplemented and control diets during the grower phase. Although birds consumed more of the cumin diet and exhibited higher FCR during the finisher period, their BWG did not differ from the control.

No significant differences were observed between the FI of birds under different dietary treatments at the end of the rearing period. Dietary Flavomycin significantly increased BWG in contrast to the other dietary treatments during the overall rearing

period (P<0.05). Also, peppermint and cumin supplementation of the diet increased the BWG of broiler chickens, whereas dietary poly and yarrow significantly reduced BWG and increased FCR compared with broilers fed the control diet (P<0.05).

Table 2. Effects of dietary supplementation of medicinal plants on WG (g), FI (g) and FCR of broiler chicks

Period	Traits	Treatments						
		Control	Flavomycin	Cumin	Peppermint	Yarrow	Poley	SEM
Starter								
	FI	397.6	404.6	396.1	399.6	387.7	385.5	11.51
	WG	309.1^{ab}	319.1^{a}	308^{ab}	313.6^{ab}	297.2^{bc}	290^{c}	11.53
	FCR	1.28	1.26	1.28	1.27	1.3	1.33	0.053
Grower								
	FI	1196^{c}	1242.3^{a}	1200.7b^{c}	1222^{ab}	1209.3^{bc}	1195.9^{c}	15.07
	WG	760.1b^{c}	776.4^{a}	753.5^{c}	767.4^{ab}	712.4^{d}	712.9^{d}	8.82
	FCR	1.57^{b}	1.60^{b}	1.59^{b}	1.59^{b}	1.69^{a}	1.67^{a}	0.031
Finisher								
	FI	2033.1^{d}	2163.5^{b}	2156.9^{b}	2196.4^{a}	2093^{c}	2157.5^{b}	21.88
	WG	980. 6^{c}	1030.1^{a}	982.2^{c}	1005.3^{b}	899 ^{d}	869. 2^{e}	12.82
	FCR	2.07^{d}	2.10^{d}	2.19^{c}	2.18^{c}	2.35^{b}	2.48^{a}	0.026
Total								
	FI	3570.1	3601.1	3548.2	3504.5	3598.8	3532.5	76.31
	WG	1936.7^{c}	2043.6^{a}	1982 ^{bc}	2008.5^{b}	1857. 5^{d}	1821.3^{e}	22.13
	FCR	1.79^{b}	1.75^{b}	1.80^{b}	1.79^{b}	1.91^{a}	1.92^{a}	0.04

$^{a-e}$Means in each row with different superscripts are significantly different (P<0.05). SEM: Standard error of mean.

Table 3. Effect of dietary supplementation of medicinal plants on eviscerated carcass yield and relative weight of gastro-intestinal organs (as a percentage of live body weight) at d 42

Treatments	Carcass yield (%)	Abdominal fat (%)	Liver (%)	Ceaca (%)	Gut (%)
Control	57	1.37	2.37	0.59	9.40
Flavomycin	60	1.38	2.22	0.49	9.1
Cumin	57	1.50	2.2	0.56	8.79
Peppermint	59	1.41	2.57	0.58	8.76
Yarrow	58	1.51	2.66	0.70	10.28
Poley	57	1.99	2.54	0.60	10.07
SEM	1.40	0.21	0.16	0.07	0.47

$^{a-e}$Means in each column with different superscripts are significantly different (P<0.05). SEM: Standard error of mean.

Table 4. Effect of dietary supplementation of medicinal plants onhe blood lipids of broiler chicks

Treatments	Triglyceride (mg/dL)	Cholesterol (mg/dL)		
		Total	HDL	LDL
Control	79.33[c]	152.66[b]	104.33[c]	33.23[c]
Flavomycin	98[a]	163.66[a]	102[c]	37.367[a]
Cumin	91.33[b]	160.33[a]	103[c]	35.7[ab]
Peppermint	93[b]	160[a]	103.66[c]	35.5[ab]
Yarrow	91.66[b]	151.66[b]	112.33[b]	30.33[cd]
Poley	91.33[b]	151.33[b]	120.16[a]	29.06[d]
SEM	9.83	5.181	1.59	1.39

[a-c]Means in each column with different superscripts are significantly different (P<0.05). SEM: Standard error of mean.

Table 5. Effect of dietary supplementation of medicinal plants on ileal microflora of broiler chicks (Log10/g)

Treatments	*Bifidobacterium*	*Lactobacillus*	*Clostridium*	Coliform
Control	7.6[a]	8.05	7.74[a]	5.81
Flavomycin	5.22[b]	8.10	5.01[c]	5.03
Cumin	7.29[a]	7.92	5.51[b]	5.96
Peppermint	5.38[b]	7.99	5.17[c]	5.21
Yarrow	5.88[b]	7.44	6.02[b]	5.58
Poley	5.91[b]	7.88	6.10b	5.33
SEM	0.56	0.38	0.15	0.23

[a, e] Means in each column with different superscripts are significantly different (P<0.05). SEM: Standard error of mean.

There were no effects of dietary treatments on eviscerated carcass yields and the relative weight of gastro intestinal organs at day 42 (Table 3). However, broilers had numerically enlarged gut, ceca and abdominal fat when fed diets containing poley and yarrow in comparison with the control or Flavomycin, peppermint and cumin diets.

The results for triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein HDL) and total cholesterol of the serum of broiler chicks are presented in Table 4. Dietary Flavomycin and peppermint tended to increase concentrations of TG, LDL and total cholesterol in serum compared to control (P<0.05). The serum TG and HDL levels were higher in chicks fed diets containing poley and yarrow compared with those given cumin and also the control group (P<0.05). The poly and yarrow had no significant effect on the total cholesterol, but decreased the serum LDL level (P<0.05).

The results for microbiological counts of the ileum are presented in Table 5. Supplementing broiler diets with additives (Flavomycin or medicinal plants) did not alter the population of *Lactobacilli* and coliform bacteria in the ileum. However, a slight numerical reduction, but not significant (P>0.05), was observed in Coliform bacteria when the birds were fed with a Flavomycin or peppermint supplemented diet compared with the control and other treatments. However, birds fed Flavomycin or peppermint had the lowest number of *Clostridia* of all the treated birds; these bacteria were also significantly lower in cumin, yarrow or poley-fed birds than those fed the control diet. The ileal *Bifidobacteria* were not different in birds fed on cumin or control diets. However the addition of Flavomycin, peppermint, yarrow or poley to diets, significantly decreased the number of *Bifidobacteria* in the ileum compared to the control or cumin diets (P<0.05).

Discussion

Medicinal plant supplements are used commonly as dietary additives for humans. They are chosen for their non-toxic chemical composition, relatively low cost and easy availability. Also, over the past few years, medicinal plants and their extracts have been used in animal diets as feed additives in order to improve their performance, health and the quality of their products. This use of aromatic plants is based on their wide range of antimicrobial (SIVROPOULOU et al., 1996), antioxidant (BOTSOGLOU et al., 1997) or even appetite and digestion stimulative properties (KAMEL, 2001).

As a general finding of this study, the antibiotic (Flavomycin) caused significant growth-promoting effects, superior feed conversion, and reduced the amount of *Clostridia* in the ileum. In addition to superior FCR, the serum triglyceride and cholesterol were also increased when broilers were fed antibiotic diets. There is considerable evidence regarding the growth promoting effects of antibiotics in broilers (STUTZ et al., 1983; ESTEIVE et al., 1997; MILES et al., 2006) which have in part been attributed to AGP's effects in reducing the size of intestinal microflora (FULLER et al., 1984).

The results of this study indicate that feeding broilers peppermint led to significant improvements in growth, which was the result of increased digestion and utilization of nutrients, and a reduction in the intestinal populations of bacteria, such as *Clostridia* and *Bifidobacteria*. Also, there was a trend towards increased body weight and the amount of *Bifidobacteria*, and a decrease in the ileal *Clostridia* count in birds fed with the cumin diet compared to the control diet. There is evidence to suggest that herbs, spices, and various plant extracts have appetizing and digestion-stimulating properties and antimicrobial effects (HERNANDEZ et al., 2004). The results of the present study are in agreement with the observations made by OCAK et al. (2008) and HERNANDEZ et al. (2004) on peppermint and GULER et al. (2006) on cumin.

In this study, the ileal populations of *Bifidobacteria* and *Clostridia*, significantly, and *Lactobacilli*, numerically, were lower in the birds given any of the herbal treatments compared to those fed the control diet, which may suggest that these dietary herbs may possess antibacterial activity *in vivo*, and may preferentially inhibit gram positive bacteria such as *Bifidobacteria* and *Clostridia*. A similar pattern was reported for conventional antimicrobials used in broiler production, which have preferentially targeted Gram-positive bacteria, especially C. perfringens (ENGBERG and PETERSEN, 2001). Each medicinal plant contains an extensive variety of phytochemicals with variable bioactivity, but terpenes are the main constituents of the herbs and essential oils, and are responsible for the bulk of their antimicrobial activity (CHARAI et al., 1996). BISHT et al. (2009) reported that terpene composition in peppermint plants changes over time. Thus, this may be important when selecting the best plant supplements for use in poultry feeding trials. Therefore, the differences in antibacterial activity of medicinal plants used in this study may possibly be attributed to differences in the chemical composition of their terpenes.

Although yarrow and poley had antibacterial activity *in vivo*, when added to the broiler diet, body weight gain decreased significantly during the 42 day feeding trial. These results are contradictory since addition of antibacterial compounds to broiler diets generally increases body weight gain (SHARIFI et al., 2010). These results are inconsistent with the results of previous studies whereby yarrow administration caused significant growth improvement in broilers (FRITZ et al., 1993; LEWIS et al., 2003). LEWIS et al. (2003) reported that the benefits of yarrow inclusion may be dependent on dietary quality and yarrow inclusion hac no positive effect on undiluted diets.

Poley herb may possess active compounds that produce antibacterial activity, but it may also possess an active compound such as tannin, which is responsible for reducing body weight. ODUGUWA et al. (2007) reported a reduction in AWG in birds fed diet supplemented with oregano, and suggested that there may have been bound tannin which may have had some influence, as well as other components of the herb. There is no report on the effect of poley or its extract in broiler diet on performance and ileal bacteria count under the same experimental conditions. But, in traditional medicine, it has been used as a palative, antispasmodic and anti-lipidemic spice (ABDOLLAHI et al., 2003; RASEKH et al., 2001).

There was a tendency for Flavomycin, peppermint and cumin to reduce the relative weight of the gastro-intestinal organs of broilers. Although not statistically different from the control diets, the effects were comparable to that of poley and yarrow. The low relative weight of the caecal and gut in broilers given dietary Flavomycin, peppermint and cumin may be explained by the fact that these additives had antibacterial activity against different bacteria populations of the intestinal tract. These data are consistent with the findings of MILES et al. (2006), who indicated that dietary inclusion of antibiotic growth promoters reduced intestinal weight by thinning the intestinal wall and shortening the gut.

The results revealed that the addition of Flavomycin, peppermint and cumin increased the concentration of TG (compared to the control), LDL and total cholesterol in serum when compared with all other dietary treatments. This may be due to a reduction in the growth and activity of the intestinal microflora responsible for bile salt catabolism. Therefore, by reducing the intestinal bacteria populations, Flavomycin, peppermint and cumin also lowered the deconjugation of bile salts that led to reduced impairment of fat emulsification and lipid absorption (GUBAN et al., 2006), hence resulting in TG, total cholesterol and LDL increments. Results showed a depression in LDL cholesterol and an increase in the level of HDL in birds fed on dietary poley and yarrow as compared with the other groups. This may attributed to the inhibition of the active enzyme hepatic 3-hydroxyl-3 methyglutaryl

Coenzyme A (HMG-CoA) which is responsible for cholesterol synthesis in the liver (QURESHI et al., 1983; CROWELL, 1999). These data are consistent with the findings of RASEKH et al. (2001).

In conclusion, adding peppermint to broiler diets improved gut microflora (as measured by changes in populations of *Clostridia*) and growth performance. Under the conditions of this study, peppermint failed to improve growth performance when compared with an antibiotic diet. However, feeding broilers a peppermint diet resulted in significant improvements in growth performance compared to the control. The addition of peppermint to the diet could be an alternative to the use of antibiotics as a growth promoter in poultry production.

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SAŽETAK

Istraživanje je poduzeto s ciljem da se procijene učinci dodatka u hranu četiriju biljaka od medicinskog značenja na proizvodnju, sadržaj lipida u krvi i mikrofloru u ileumu. U pokus je bilo uzeto 336 jednodnevnih tovnih pilića linije Ross, nasumce raspoređenih, od kojih je svaki prošao šest tretmana s četiri ponavljanja. Hrana je bila jednake kalorične vrijednosti i dušičnog sastava, a sadržavala je 15 g/kg suhog kumina, 3 g/kg peperminta, 2 g/kg stolisnika i 2 g/kg biljke dubačac. Dva pripravka hrane smatrana su negativnima (nisu

sadržavala ljekovito bilje ili antibiotike) i pozitivnima (sadržavali su flavomicin 0,4 g/kg). Dodatak flavomicina i peperminta hrani povećao je unos hrane i prirast tjelesne mase tovnih pilića u usporedbi s kontrolom ($P<0,01$). Dodatak flavomicina značajno je povećao prirast tjelesne mase u odnosu na dodatak drugih biljaka ($P<0,05$). Dodatkom peperminta i kumina u hranu također se povećala tjelesna masa tovnih pilića, dok su dubačac i stolisnik značajno smanjili tjelesnu masu i povećali omjer konverzije hrane u usporedbi s kontrolnim pilićima ($P<0,05$). Flavocin i pepermint u hrani povećali su koncentraciju triglicerida, lipoproteina niske gustoće i ukupnog kolesterola u serumu ($P<0,05$). Dodavanje flavocina ili peperminta u hranu, značajno je smanjilo količinu ilealnih *Bifidobacteria* i *Clostridia* ($P<0,05$). Može se zaključiti da je pepermint pojačao rast tovnih pilića te da njegovo dodavanje hrani može biti zamjena za antibiotike kao promotore rasta u proizvodnji peradi.

Ključne riječi: tovni pilići, ljekovito bilje, flavocin, promotori rasta