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Effect of some medicinal plants as feed additive on total coliform count of ileum in Japanese quails (*Coturnix coturnix japonica*)

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Abstract

The study was performed to evaluate effects of turmeric powder, black pepper, cumin and coriander seeds on total coliform counts of ileum in quails. A total of 1820 one-day old unsexed quails were randomly allocated in 13 treatments with four replicates. The quails were fed a corn – soybean meal based diet containing different concentration of the medicinal plants (0, 0.05, 1 and 1.5%) supplemented to ration. At 42 days of age, eight birds from each treatments were killed and ileum part of small intestine sent to laboratory. Results showed that supplementation of basal diet with turmeric powder (1.5%) cumin (1.5%) and coriander seeds (1 and 1.5%) reduced bacteria counts, but black pepper in 0.5% level increased coliform counts in ileum of Japanese quails, significantly ($P<0.01$).

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Introduction

Poultry industry can produce very high quality proteins for human nutrition as well as a source of income for the community in many countries, therefore poultry production has very important role in economic development of any country (Tarhyel *et al.,* 2012).

So, in developing countries intensive brooding system is incresing. Unfortunately this system constraints very stress to poultry (Adams, 2004) and reduces livability and genetic performance of poultry. Thus, it is thought that some feed additives can moderate the stress, improve feed efficiency and economic indicators in intensive systems (Windisch *et al.*, 2008). These feed additives suche as antibiotics act through several mechanisms, moderation of gut microflora is one of them. But use of antibiotics as feed additive have some negative consequences. For example Sengül *et al.* (2008) reported that flavomycin consumption in quails nutrition resulted in DNA damage and increased oxidative stress. Also antibiotics as feed additive can increase abdominal fat in poultry and increase risk of heart disaese in consumers (Murwani and Bayuardhi, 2007). In other hand use of antibiotics in poultry nutrition can increase risk of antibiotic resistance in human society, therefore in some countries, such as the Europe Union use of antibiotic as feed additive has been banned (Schwarz *et al.*, 2001; Lee *et al.,* 2004). So, we need to quickly replace of antibiotics as feed additive with other options. These new options should be inexpensive and available in everywhere as well as healthy for human socitey. There are some alternatives to in-feed antibiotics such as fibre-degrading enzymes, prebiotics, probiotics, mannan oligosaccharides, symbiotics and phytobiotics or medicinal plants (Yang *et al.*, 2009; Ahmadi *et al.*, 2013).

It is thought that medicinal plants are a good alternative and candidate for this porpose, because since ancient times medicinal plants play important role in health management of traditional poultry production (Eevuri and Putturu, 2013). Also, in contrast to antibiotics, most active components of medicinal plants are readily absorbed in intestine and have short half life. Therefore risk of tissue accumulation of these components is probably minimal (kohlert *et al.*, 2000). But there are some inconsistent results about effects of medicinal plants on poultry health and performance (Windisch *et al.*, 2008). For example vogot (1990) used several medicinal plant extracts in broiler nutrition and found they had no effect on daily gain and feed conversion ratio (FCR). While Denli and *et al.* (2004) reported that using herb essential oils resulted in significantly higher body weight gains and better feed efficiency. So it was not surprising to find conflicting results between scientific articles that used medicinal plants as feed additives or microflora modulators. For example Dalkiliç and Güler (2009) showed that clove (*Syzygium aromaticum*) extract reduced coliform counts in small intestine of broiler, significantly. But Erener *et al.* (2010) found that black cumin (*Nigella Sativa* L.) seed or black cumin extract had no effect on coliform counts in caeca.

Turmeric (*Curcuma longa*) belongs *Zingiberaeceae* family and extensively used as spice, food preservative, coloring and medicinal applications. Curcumin is one of active components of this plant and has anti-inflammatory, antifungal, antiprotozoal and antibacterial activities. (Basavaraj *et al.,* 2010).Negi *et al* (1999) reported that curcumin have antibacterial activity. Also, Namagirilakshmi *et al* (2012) showed that use of turmeric as feed additive in broiler chickens reduced small intestinal total microbial count, significantly.

Black pepper (*Piper nigrum*) is a member of *Piperaceae* family and cultivated for its fruit, which is usually dried and used as a spice and seasoning (Moorthy *et al.*, 2009; Al-Kassie *et al.*, 2011). Piperine as active component of this plant stimulates the digestive enzymes and may be shows antimicrobial activity in poultry intestine (Ahmad *et al.*, 2012). For example Kumar Pundir and Jain (2010) reported that black pepper extracts had antibacterial activity against *Staphylococcus aureus*.

Coriander (*Coriandrum sativum* L.) is an aromatic plant and belongs to the *Parsley* family and native of Eastern Mediterranean region and Southern Europe, but it is found in many other parts of world. (Güler *et al.*, 2005). There are evidences that coriander seed has anti-fungal (Basilico and Basilico, 1999), antimicrobial (Elgayyar *et al.*, 2001; Delaquis *et al.*, 2002; Singh *et al.*, 2002) antidiabetic (Gray and Faltt, 1999) and hypolipidemic (Chithra and Leelamma, 1997) effects.

Also Cumin (*Cuminum cyminum* L.) as annual plant has antioxidant and antimicrobial effects (Aami-Azghadi *et al.*, 2010). This medical plant increased secretion of some digestive tract enzymes (Ramakrishna *et al.*, 2003) and effect on gastrointestinal transit time in rat (Platel and Srinivasan, 2001). DE *et al.* (2003) reported that antimicrobial activity of cumin may be in relation of cuminaldehyde and other organic compounds**.**

Unfortunately, there are few articles about effects of medicinal plants on bacteria count of intestinal in Japanese quails. There for the aim of this study was to evaluate the effects of different levels (0.5, 1 and 1.5%) of turmeric powder, black pepper, cumin and coriander seeds as feed additive on total coliform count of ileum in Japanese quails.

Materials and methods

Experimental birds and management

A total of 1820 one-day old Japanese quail chicks were purchased from commercial farm in East Azerbaijan, Iran. This study was conducted in completely randomized design (CRD) with 13 treatments and four replications (35 chicks per cage). The birds were kept in same terms and fed standard diets according to NRC (1994). The quails were kept in cubic plastic fruit baskets during 42 days as experiment period.

Temperature was maintained at 35°C for the first 5 days and then gradually reduced according to normal management practices until a temperature of 22°C was achieved. After 21 days the quails were sexed based on their breast feathers and the experiment was continued with male chicks (14 male chicks per cage).

Treatment and preparation of feed

The medicinal plants were bought from local market and chemical composition of them were analyzed by using AOAC (1990) procedure and presented in Table 1.

Basal diet supplemented with certain levels of the medicinal plants as 0.5,1 and 1.5% to get the isocaloric and isonitrogenous test treatments. The ingredients and chemical composition of the diets are presented in Table 2.

Experimental procedure

At the end of the experiment birds were fasted for eight hours and eight birds from each treatment (two birds per replicate; weights near the mean of each replicate) were slaughtered. Small intestine was immediately evicted and its ileum part sent to laboratory immediately to colony counts by Violet Red Bile agar. (Dalkiliç and Güler, 2009).

Statistical analysis

All the data obtained from the experiment were analyzed by version 16 of SPSS software (SPSS Inc., Chicago, IL, USA) and Duncan's multiple ranges test was used to mean comparison in the 5 and 1% probability levels (Steel and Torrie, 1980).

Results and discussion

Chemical composition of the medicinal plants

The proximate chemical composition of the medicinal plants in this study and some other reports are presented in Table 1. Harvesting season, geographical origin, stage of maturity, crop management and soil type can be considered as the main reasons of differences in chemical composition of medicinal plants in different studies (Hossain and Ishimine, 2005; Windisch *et al.;* 2008; El-Mekawey *et al.*, 2010).

In this study, crude protein of cumin and ME content of turmeric powder were better than other plants.

Also in this research we measured total tannins and total phenol compounds of the medicinal plants, because there is report that showed tannins act as antibacterial agent (Cowan, 1999). Levels of total tannins and total phenol compounds of the plants have been presented in Table 1. Also effects of these plants on coliform population are shown in Table 3.

Table 1. Chemical composition of the medicinal plants in the experiment and other reports.

	Turmeric powder					Black pepper		Cumin seed		Coriander seed			
Chemical analysis (%)	Hossain & Ishimine (2005) a	Hossain & Ishimine (2005) b	Hossain & Ishimine (2005) c	Lokhande et al. (2013)	The experiment	Al-Jassas and Al-Jasser (2012)	The experiment	Golian et al. (2010)	The experiment	Shahwar et al. (2012)	Platt (1962)	Wahed abaza (2001)	The experiment
DM				90.92	90.81	95.32	92.00		93.17	93.8			93.72
CP	3.36	3.40	5.20	8.66	8	6.9	11.3	21	19.80	12.58	14	14.83	13.58
EE	2.14	3.64	3.62	7.86	3.29	16.63	6.99	19.5	8.07	9.12	16	7.10	13
CF	3.50	3.51	4.19	7.83	8.40	11.47	20.50	9.6	33.07	37.14	32	23.63	46.43
Ash	4.02	5.13	5.30	6.5	9.08	5.97	6.99	7.6	9.32	8.59		8.91	9.01
NFE					62.02		46.21		22.9	26.37			11.69
Total phenol					1.759		1.223		1.263				0.847
Total Tannins					0.116		0.001		0.336				0.279
ME* (kcal /Kg)					3261.88		3029.99		2469.62				2186.87

DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, ME: metabolizable energy.
a: cultivated on red soil., b: cultivated on gray soil., c: cultivated on dark red soil.
*ME (Kcal/kg) = (35.3 X CP%) + (79.5 X EE%) + (40.6 X NFE%) + 199. (Al-Harthi and El-Deek, 2010).

Total coliform count of ileum

It is considered that four bacteria genus of *Enterobacteriaceae family as name as Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* are called total coliform (Brenner *et al.,* 1982).

In the present study coliform population of ileum were significantly (P<0.01) influenced by the addition of medicinal plants in diet. The colony forming units (CFU) of coliform in digesta of ileum in the turmeric powder (1.5%), cumin (1.5%) and coriander seeds (1 and 1.5%) treatments were significantly lower than control group.

These results are in agreement with Samarasinghe *et al.* (2003). They reported that turmeric powder reduced coliform bacteria in duodenum broiler chicks. Also Lawhavinit *et al.* (2010) showed that curcuminoids from turmeric inhibited some pathogenic bacteria. As well as Lacobellis *et al.* (2005) reported that cumin and carwey (*Carum carvi* L.) essential oils showed antibacterial activity against the genera *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia* and *Agrobacterium*. But Pokhrel *et al.* (2012) reported that ethanolic extract of coriander and turmeric was inactive against strains of *E.Coli.*

Also Al-Kassie and Mohammed (2008) reported that use of anise *(Pimpinella anisum* L.*)* and rosemary (*Rosemarinus official*) in broilers diet decreased total bacteria and coliform counts in crop, jejunum and large intestine. As well as Mohamed *et al.* (2011) showed that suplemetation of diet with cinnamon (*Cinnamomum zylenicum*) decreased total bacterial and *E. coli* counts in ileum of Japanese quails. In other report a mixture of essential oil from thyme (*Thymus vulgaris*) and anise reduced cecal coliform population in broilers (Dalkiliça and Güler, 2009). Mitsch *et al.* (2004) reported that essential oils of thyme can reduce concentration of *Clostridium perfringens* in feces of broiler chickens.

Table 2. Feed ingredients and nutrient contents of experimental diets of Japanese quails.

	Control	Turmeric powder			Black pepper			Cumin seed			Coriander seed		
Ingredients		0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5

Corn grain	52.89	52.45	51.95	51.46	52.51	52.08	51.64	52.66	52.38	52.11	52.64	52.32	52.01
Soybean meal	38.80	38.65	38.61	38.57	38.55	38.41	38.27	38.31	37.92	37.52	38.21	37.74	37.26
Sunflower oil	1.32	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Corn gluten meal	3.93	4.03	4.06	4.09	4.06	4.13	4.20	4.15	4.30	4.46	4.27	4.54	4.81
Medicinal plant	0	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Oyster shell	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.35	1.35	1.34
DCP	0.77	0.78	0.78	0.78	0.78	0.78	0.79	0.78	0.79	0.79	0.78	0.79	0.8
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit. Premixes*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Min. Premixes*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine mono hydrochloride	0.08	0.08	0.09	0.09	0.09	0.09	0.09	0.09	0.10	0.11	0.09	0.1	0.12
DL-methionine	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11
Nutrients (calculated)													
ME (Kcal/Kg)	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900
CP % (N × 6.25)	24	24	24	24	24	24	24	24	24	24	24	24	24
CF %	3.93	3.95	3.98	4.01	4.03	4.14	4.25	4.05	4.19	4.32	4.12	4.31	4.51
Ca %	0.80	0.8	0.80	0.80	0.80	0.80	0.8	0.8	0.8	0.80	0.80	0.80	0.80
Av. P %	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Met. %	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lys. %	1.3	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Cys. %	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Met+ Cys	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Na %	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
K %	0.95	0.94	0.94	0.94	0.94	0.94	0.93	0.94	0.93	0.92	0.93	0.92	0.92
Cl %	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
ME/ CP	120.83	120.83	120.83	120.83	120.83	120.83	120.83	120.83	120.83	120.83	120.83	120.83	120.83

ME: metabolizable energy, CP: crude protein, CF: crude fiber, Ca: calcium, Av. P: available phosphorus, Met: methionine, Cys: cysteine.
*Vitamin and mineral mix supplied/ kg diet: vitamin A, 11000 IU; vitamin D3, 1800 IU; vitamin E, 11 mg; vitamin K3, 2 mg;
Vitamin B2, 5.7 mg; Vitamin B6, 2mg; vitamin B12, 0.024 mg; Nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg;
choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; Co, 0.5 mg.

The interesting point in our study is that supplementation of ration with black pepper increased coliform population in ileum, so that black pepper in 0.5% level increased coliform counts, significantly ($P<0.01$). We could not find a reason for this result. But Tekeli *et al.* (2006) reported that ration supplementation with essential oil of *Zingiber officinale* increased coliform counts in jejunum digesta of broiler chicks significantly.

Also in some reports medicinal plants had no effect on microbial population (Erener *et al.,* 2010). Therefore, there are some inconsistencies about medicinal plants effects on modulation of gut bacteria population. This discrepancy can be related to several factors. For example Brenes and Roura (2010) noted that antibacterial properties of essential oils were affected by ration and environment. Also it is noticeable that, gut microflora population is effected by flock density, age, nutrition management, pathogens and individual differences (Bjerrum *et al.,*

2006). On the other hand, amount of active substances of medicinal plants depended on plant part used in ration (e.g., seeds, leaf, root or bark), crop management, harvesting season, and geographical origin (Windisch *et al.,* 2008).

It should be noted that, the beneficial effects of medicinal plants are not just related to manipulation of gut microflora. Erener *et al.* (2010) reported that use of black cumin seed (*Nigella sativa* L.) as feed additive did not effect on coliform bacteria population, but improved feed conversion ratio (FCR) in broiler chicks.

Table 3. Effect of medicinal plants on ileum coliform counts (Log cfu/g).

Control	Turmeric powder			Black pepper			Cumin seed			Coriander seed			SEM	P
	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5		
6.04cde	5.64cde	5.88cde	3.47a	6.85f	6.07cde	6.24ed	5.38cde	4.63abc	3.97ab	5.17bcd	3.89ab	3.36a	0.18	**

Means with different superscripts in the row indicate statistically different (P < 0.01).

The mode of action of medicinal plants as feed additive is not completely clear, but there are evidences to suggest that midicinal plants or their extracts stimulate the secretion of endogenous digestive enzymes, (Williams and Losa, 2001; Lee *et al.,* 2004), modulate micro flora population, antiviral, antibacterial (Elgayyar *et al.,* 2001) and antioxidant activity (Ertas *et al.,* 2005; Cross *et al.,* 2007). As well as aromatic plants with changing ions diffusion by distribance in bacteria cell walls and decreased harmfull bacteria population in digestive tract (Al-Kassie and mohammad, 2008). Also it is possible that increase of digestive enzymes secretion by medicinal plants limits bacteria growth or their toxins (Baba *et al.*, 1992). As well as secondary compounds of plants may have a role in this field as Twetman and Peterson (1997) reported that phenolic compounds of medicinal plants probably exhibit antimicrobial and antifungical activities.

Conclusion

Supplementation of quails ration with turmeric powder (1.5%), cumin (1.5%) and coriander seeds (1 and 1.5%) reduced coliform counts, significantly. While black pepper in 0.5% level increased bacteria counts in ileum of Japanese quails.

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