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The Effect of Dietary Tarragon (*Artemisia dracunculus*) and Peppermint (*Mentha piperita*) Leaves on Growth Performance and Antibody Response of Broiler Chickens

Research Article

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ABSTRACT

A semi-field study was carried out to evaluate the effect of two medicinal herbs, individually and in combination, on growth performance, carcass traits, nutrient digestibility and immune response of broiler chickens. A total of 384 one-day-old straight-run Arbor Acres broiler chickens were allocated into 24 floor pens prepared in a commercial broiler house. Pen-groups were fed one of the following five diets for 42 days: a basal corn-soybean meal diet as control (5 pens), the same basal diet plus 200 ppm virginiamycin (V; 4 pens), the same basal diet supplemented with 0.4% peppermint (*Mentha piperita*) leaves (P; 5 pens), 0.4% tarragon (*Artemisia dracunculus*) leaves (T; 5 pens) or with 0.2% tarragon leaves + 0.2% peppermint leaves (P+T; 5 pens). The results showed that performance traits, including average body weight, body weight gain, feed intake and feed conversion ratio were not affected by dietary treatments (P>0.05). No significant differences were detected between the control and experimental groups in apparent digestibility of nutrients and antibody titer against newcastle disease virus (NDV). Slaughter traits of herb or antibiotic supplemented groups did not differ significantly from those of the non supplemented control group. In conclusion, the additives tested had no impact on broiler growth and health status.

KEY WORDS antibody titer, broiler chicken, carcass, peppermint, performance, tarragon.

INTRODUCTION

Public health issues regarding development of antibiotic-resistant bacteria led to ban the use of antibiotic growth promoters (AGPs) in animal nutrition (Windisch *et al.* 2009) prompting researchers to find effective and safe alternatives to AGPs. For this reason, a large variety of products such as prebiotics, probiotics and symbiotics (Ayasan, 2013; Houshmand *et al.* 2012; Patterson and Burkholder, 2003), organic acids (Chowdhury *et al.* 2009), antimicrobial peptides (Bao *et al.* 2009) and phytobiotics (Cross *et al.* 2007) have been tested and proposed for use in AGP-free animal diets. Phytobiotics are defined as plant-derived feed additives including integral organs of the plant, various kinds of extracts (aqueous, alcoholic and other types of extracts), as well as essential oils. They have been reported to strengthen useful non-pathogenic gut bacteria against potentially pathogenic ones (Mitsch *et al.* 2002; Mitsch *et al.* 2004; Bölükbaşi and Erhan, 2007) and to support digestion process either by increasing the endogenous secretion of enzymes, bile acids and pancreatic juice (Platel and Srinivasan, 2000; Platel and Srinivasan, 2001; Platel and Srinivasan, 2003) and elevating digestive enzymes activities (Hashemipour *et al.* 2013) or by improving absorptive

characteristics of gastrointestinal tract (Khattak *et al*. 2014). In addition, there are several publications unveiling immune-enhancing properties of certain herbal preparations in poultry (Daneshmand *et al*. 2012; Hashemipour *et al*. 2013; Li *et al*. 2013).

Peppermint (*Menthe piperita*), an aromatic herb belonging to the family *Lamiaceae*, has numerous activities such as anti-bacterial, anti-viral, antioxidant, anti-inflammatory, and detoxicant activities, as well as bronchodilator and stomachic effects (Duke *et al*. 2002). Peppermint has been investigated by several researchers as poultry feed additive (Al-Kassie, 2010; Sharifi *et al*. 2013) and proposed as a potential alternative to AGPs. According to herbal medicine texts (Duke *et al*. 2002), tarragon (*Artemesia dracunulus*), from family of *Asteraceae*, has stomachic, digestive stimulating, anti-microbial and anti-inflammatory properties. There are only limited experimental data about the use of tarragon in poultry feeding (Hosseinzadeh and Farhoomand, 2014; Hosseinzadeh and Moghaddam, 2014; Hosseinzadeh *et al*. 2014). Recently, tarragon and peppermint leaves and their associated essential oils were tested in broiler diets and some beneficial effects were observed in growth performance and slaughter traits. Most studies testing phytogenics in poultry diets have been conducted under hygienic challenge-free experimental conditions and produced results that may not be generalizable to stressful commercial circumstances. Hence, this study aimed to evaluate the effect of tarragon and peppermint leaves, alone and in combination, as feed additives on performance and health status of broilers grown under commercial housing conditions.

MATERIALS AND METHODS

Preparing herbal treatments

Shadow-dried peppermint and tarragon leaves were purchased from medicinal herb suppliers in Mashhad (northeast Iran) and Qom (north-central Iran), respectively. The herbs were then ground to pass through a No. 18 (1 mm) sieve.

Birds, housing condition, dietary treatments

Three hundred eighty four day-old Arbor Acres Plus sexed broiler chickens (192 females and 192 males) with an initial body weight of 46.18 ± 0.17 grams ($\overline{X}\pm S_{\overline{X}}$) were distributed into 24 wood shavings-bedded pens. A commercial Arbor Acres flock from the same source and age was also reared in the remaining area of the house in which the floor pens were assembled. This was to expose the tested birds to challenges commonly occurring in commercial poultry houses. Each pen-confined group was fed one of the following five diets for 42 days; a basal corn-soybean meal diet (Table 1) (control group, 5 pens), the basal diet supplemented with 200 ppm antibiotic virginiamycin (positive control group, 4 pens), the basal diet supplemented with 0.4 % peppermint leaves (P group, 5 pens), the basal diet supplemented with 0.4% tarragon leaves (T group, 5 pens) and the basal diet supplemented with 0.2% peppermint leaves + 0.2% tarragon leaves (P+T group, 5 pens). The basal diets were formulated to meet or exceed Abrbor Acres Plus ashatch broilers nutrition requirements (Aviagen 2009a). The formulations were made using feedstuff information of NRC (1994), however, the crude protein contents of diets presented in Table 1 are based on chemical analysis of diet samples. All birds had *ad libitum* access to feed and water and were exposed to a 23 L:1D lighting program after a 48-hour continuous initial lighting period. Other management practices were based on a strain-specific management guide with minor modifications (Aviagen, 2009b).

Data collection

Total live body weight and total feed intake of the experimental groups were recorded at 14, 28 and 42 days of age and data of death body weight and death time of dead birds were noted and used to estimate adjusted performance statistics including daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR). All birds were vaccinated against newcastle disease virus (NDV; Lasota strain) via drinking water at 16 days of age. On the 23rd day of the experiment two birds per pen (one female+one male) were selected and dye-marked. Blood samples were collected from the brachial vein of the dye-marked birds. The same birds were used for blood collection on the 30th day of the study. Blood samples were allowed to clot at room temperature then test tubes containing clotted bloods were centrifuged at 5000 rpm for 15 minutes. After centrifugation the upper clear sera were removed and transferred to 1.5 mL micro-tubes and stored at -20 ˚C until evaluation of antibody titers against NDV by hemagglutination inhibition (HI) assay (Allan and Gough, 1974). The geometric mean titer was reported as reciprocal log2 of the highest serum dilution showing complete hemagglutination inhibition.

At 18 days of age, all groups were exposed to feed deprivation for 2 hours. Then each group received its associated diet containing 0.3% chromic oxide (98.5% purity) (CAS No: 1308-38-9. SAMCHUN PURE CHEMICAL CO., LTD. Mogok-dong, Pyeongtaek City, Gyeonggi-do-Korea.T. (031)668-0700/3, F. (031) 665-7482) for 72 hours. Excreta samples were collected in well-sealed plastic containers 48 hours after the start of the re-feeding period until achieving an adequate sample size. Furthermore, representative feed samples were also taken before and after the addition of chromic oxide.

Table 1 Feed ingredients and nutrient composition of diets used in this experiment

Ingredients (%)	Starter (0 to14 days of age)	Grower (15 to28 days of age)	Finisher (29 to 42 days of age)
Corn	58.17	63.48	67.87
Soybean meal (44% crude protein)	36.62	31.20	26.34
Vegetable oil	1.00	1.50	2.00
Common salt	0.38	0.38	038
Sodium bicarbonate	0.10	0.10	0.10
Limestone	1.16	1.06	1.04
Di calcium phosphate	1.74	1.54	1.46
Vitamin premix*	0.25	0.25	0.25
Mineral premix**	0.25	0.25	0.25
DL-methionine	0.24	0.18	0.19
L-lysine (HCl)	0.19	0.16	0.22
Nutrient composition* **			
ME (kcal/kg)	2861	2954	3035
Crude protein (%)	20.69	18.81	17.10
Calcium (%)	0.94	0.85	0.80
Available phosphorus (%)	0.47	0.42	0.40
Sodium (%)	0.20	0.20	0.20
Met (%)	0.57	.49	0.47
Met + Cys (%)	0.91	0.80	0.76
L-lysine (%)	1.27	1.11	1.04
Dietary cation-anion difference (mEq/kg)	233	211	186

* Supplied per kilogram of diet: vitamin A: 22500 IU; vitamin D_3: 5000 IU; vitamin E: 45 IU; vitamin K_3: 5 mg; B_1: 4.375 mg; B_2: 16.5 mg; B_3: 24.5 mg; B_5: 74.25 mg; B_6: 7.35 mg; B_9: 2.5 mg; B_{12}: 0.0375 mg; H_2: 0.25 mg; Choline chloride: 625 mg and Antioxidant: 2.5 mg.
** Supplied per kilogram of diet: Mn: 248 mg; Fe: 125 mg; Zn: 211.75 mg; Cu: 25 mg; I: 2.475 mg and Se: 0.5 mg.
*** Proportions of all nurtients were calculated on the basis of feedstuff information of NRC (1994), except for crude protein (CP) which was measured by Kjeldahl method.

All samples were stored at -20 ˚C until analysis. The frozen samples were dried (60 ˚C for 48 hours), ground (1 mm) and analyzed for crude protein by the Kjeldahl method. A 1 g portion of each ground sample was ashed in a muffle furnace to measure ash and organic matter contents as well as to determine chromic oxide concentration (Fenton and Fenton, 1979). Then, nutrient digestibility was calculated by the following formula (Scott *et al.* 1976):

Nutrient digestibility (%)= 100 – (% chromic oxide in feed/% chromic oxide in feces) × (% nutrient in feces/% nutrient in feed)

At 42 days of age two birds from each replicate (1 female+1 male) with body weight close to the replicate average body weight were slaughtered for carcass analysis.

Statistical analysis

Data were analyzed using GLM procedure of SAS 9.1 software (SAS, 2002). Duncan's multiple range test was used to detect the differences between treatments. Differences were considered significant when the probability value (P) was less than or equal to 0.05. Before analysis, all data were tested for normality (UNIVARIATE procedure of SAS). For digestibility data, some outliers were identified and removed from the dataset according to Tukey (1977).

RESULTS AND DISCUSSION

Performance

The effects of the dietary treatments on performance traits have been summarized in Table 2. Generally, dietary treatments had no significant effect on average body weight (ABW) and average daily gain (ADG) at the end of the experiment (P>0.05). Birds fed with diets containing herbal and antibiotic supplements had significantly (P<0.05) higher daily feed intake (DFI) compared to the birds fed with the control diet during the starter period (from 0 to 14 days of age). The same groups also tended to consume larger quantities of feed compared to their control counterparts from 14 to 28, 0 to 28, 28 to 42 and 14 to 42 and 0 to 42 days of age, however, the differences were not statistically significant. The highest (99.49 g) and lowest (95.87 g) 42-day DFI values were recorded in T and control groups, respectively. No significant difference was found in feed conversion ratio (FCR; P>0.05); however, the best FCR estimation was obtained in P group (1.87 *vs.* 1.90 in the control group) at the whole study period.

Slaughter traits

Statistical analysis showed no considerable differences between dietary treatments regarding carcass yield and relative weights of liver, heart, spleen, pancreas, gizzard, small intestine and abdominal fat (Table 3).

Table 2 The effect of dietary treatments on growth performance[a]

Traits[C]	Age (in day)	Treatments[b]					SEM	P-value
		C	V	P	T	P + T		
ABW (g)	14	300.50[ab]	327.79[a]	308.38[ab]	291.95[b]	308.17[ab]	4.2711	0.121
	28	1105.00	1121.41	1082.03	1110.47	1101.03	6.7327	0.502
	42	2170.75	2240.10	2250.45	2258.92	2200.45	14.5050	0.248
DWG (g/bird/d)	0 to14	18.18[ab]	20.08[a]	18.72[ab]	17.45[b]	18.55[ab]	0.3076	0.116
	14 to 28	57.46[ab]	56.69[ab]	54.96[b]	58.43[a]	56.67[ab]	0.4883	0.565
	0 to 28	37.82	38.24	36.79	37.74	37.47	0.2647	0.565
	28 to 42	76.13	79.91	83.46	81.17	78.50	1.1470	0.319
	14 to 42	66.79	68.30	69.15	69.71	67.58	0.5072	0.369
	0 to 42	50.59	52.05	52.22	51.95	51.09	0.2668	0.224
DFI (g/bird/d)	0 to14	26.05[b]	27.80[a]	27.80[a]	27.21[a]	27.52[a]	0.1953	0.009
	14 to 28	94.99	97.31	96.37	99.19	96.30	0.6334	0.299
	0 to 28	60.51	62.28	61.97	62.84	61.67	0.3414	0.264
	28 to 42	166.60	170.71	169.85	174.60	167.79	1.5370	0.535
	14 to 42	130.79	134.01	132.99	136.63	132.05	1.0023	0.428
	0 to 42	95.87	98.22	97.69	99.49	96.87	0.6225	0.433
FCR (g/g)	0 to14	1.44[ab]	1.39[b]	1.49[ab]	1.57[a]	1.49[ab]	0.0214	0.097
	14 to 28	1.65[b]	1.72[ab]	1.75[a]	1.70[ab]	1.70[ab]	0.0121	0.103
	0 to 28	1.60[a]	1.63[ab]	1.69[c]	1.67[bc]	1.65[abc]	0.0094	0.012
	28 to 42	2.19	2.14	2.04	2.17	2.14	0.0298	0.530
	14 to 42	1.96	1.97	1.92	1.96	1.95	0.0151	0.9178
	0 to 42	1.90	1.89	1.87	1.92	1.90	0.0117	0.8254

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
[a] Data are means of 5 replicate pens of 16 birds, except for the antibiotic group which had 4 replicate pens.
[b] C: control; V: virginiamycin (200 ppm); P: peppermint (0.4%); T: tarragon (0.4%) and P + T: peppermint (0.2%) + tarragon (0.2%).
[c] ABW: average body weight; DG: daily weight gain; DFI: daily feed intake and FCR: feed conversion ratio.
SEM: standard error of the means.

Table 3 The effect of dietary treatments on carcass yield and relative weights of some visceral organs in 42 days old broiler chickens[a]

Traits (% of live body weight)	Treatments[b]					SEM	P-value
	C	V	P	T	P + T		
Carcass yield	71.91	70.48	70.28	72.68	69.86	0.5439	0.4347
Liver	2.61	3.08	2.63	2.76	2.80	0.0740	0.2750
Spleen	0.12	0.11	0.10	0.11	0.10	0.0040	0.5623
Pancreas	0.27	0.28	0.28	0.24	0.25	0.0056	0.1716
Abdominal fat	1.71	1.69	1.84	1.68	1.81	0.0552	0.8584
Gizzard	1.68	1.62	1.64	1.58	1.61	0.0227	0.6679
Heart	0.48	0.44	0.44	0.49	0.49	0.0104	0.2577
Bursa of fabricius	0.079[ab]	0.067[b]	0.108[a]	0.063[b]	0.069[b]	0.0058	0.0799
Small intestine	2.91	3.33	2.91	3.31	3.15	0.0659	0.0841

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
[a] Data are means of 10 birds (5 replicate pens per treatment×2 birds per pen), except for the antibiotic group which had 4 replicate pens with 8 slaughtered birds.
[b] C: control; V: virginiamycin (200 ppm); P: peppermint (0.4%); T: tarragon (0.4%); P + T: peppermint (0.2%) + tarragon (0.2%).
SEM: standard error of the means.

Birds receiving P had higher relative weight of bursa of Fabricius than those receiving T, T + P and V in their feed (P<0.05).

Nutrient digestibility and humoral immune response against newcastle disease virus

Digestibility estimates of selected nutrients are presented in Table 4. Dry matter, protein, ash and organic matter digestibilities were not affected by dietary treatments under current experimental conditions. Likewise, according to HI assay outputs (Table 5), additives used in this study had not any effect on humoral immune response against NDV (P>0.05). Positive effects of growth enhancer feed additives (*i.e.* AGPs or their natural non-antibiotic alternatives), which have been established under experimental conditions, are expected to be potentiated in commercially grown poultry owing to their exposure to the stressors and challenges rarely occurring in experimental environments. In an early unpublished study, powdered tarragon leaves, peppermint leaves and caraway seeds and their associated essential oils were tested in broiler feeding and relatively better growth performance was observed in tarragon- and peppermint-treated birds. These findings increased our enthusiasm to evaluate the effect of both herbs as broiler feed additives under a semi-commercial environment in the direct vicinity of commercially grown broilers. In the present study, all birds, were provided from the same breeder flock and fed with the same basal diets.

Table 4 The effect of dietary treatments on apparent nutrient digestibility (%) in 21 days old broiler chickens[a]

Nutrient	Treatments[b]					SEM	P-value
	C	V	P	T	P + T		
Crude protein	62.55	64.93	59.00	57.35	61.10	2.163	0.9269
Organic Dry matter	69.38	65.38	66.76	67.62	64.58	2.109	0.9648
Ash	38.80	36.88	34.69	35.48	39.08	2.837	0.9941
Dry matter	68.07	66.68	67.13	62.90	65.77	1.446	0.8921

[a] Data are means of 3 or 4 replicate pens of 16 birds.
[b] C: control; V: virginiamycin (200 ppm); P: peppermint (0.4%); T: tarragon (0.4%); P + T: peppermint (0.2%) + tarragon (0.2%).
SEM: standard error of the means.

Table 5 The effect of dietary treatments on newcastle disease virus (NDV) antibody titer (log_2) measured by hemagglutination inhibition (HI) assay[a]

Age (day)	Treatments[b]					SEM	P-value
	C	V	P	T	P + T		
23	3.25	2.89	2.89	2.63	2.86	0.1704	0.8674
30	3.00	2.22	2.67	2.10	2.50	0.1547	0.3653

[a] Data are mean values of 10 birds (5 replicate pens per treatment×2 birds per pen) except for antibiotic treatment which consisted of 8 birds (4 replicate pens×2 birds per pen).
[b] C: control; V: virginiamycin (200 ppm); P: peppermint (0.4%); T: tarragon (0.4%); P + T: peppermint (0.2%) + tarragon (0.2%).
SEM: standard error of the means.

In contrary to our hypotheses, direct exposure of experimental birds to commercial ones could not magnify growth enhancing properties of additives used in the current study. In the same manner, nutrient digestibility and antibody responses against NDV were not influenced by dietary treatments. These results are in agreement with Ocak *et al.* (2008) who studied supplemental dry peppermint and thyme (*Thymus vulgaris*) leaves in broiler diets (2 g dry herb per kg of diet) and observed no positive effect on growth performance and slaughter traits at the end of the experiment (42 days of age). They also reported that the herb-supplemented birds deposited significantly more fat in their abdominal cavity as compared to the control birds. In contrast, Khodambashi Emami *et al.* (2012) reported that dietary supplementation with 200 ppm peppermint oil resulted in the significantly enhanced FCR and CP digestibility, but at higher inclusion level (400 ppm) this oil was not effective. The same authors demonstrated that the addition of 400 ppm of peppermint oil to broiler diet had a negative impact on secondary antibody response again sheep red blood cell (SRBC). Antibiotic and non antibiotic growth enhancers exert their beneficial effects primarily by manipulating the gut microflora. Dosage of herbs and spices used as integral ingredients in foods (or feeds) may be insufficient for their antimicrobial properties to be significant (Dorman and Deans, 2000). Dorman and Deans (2000) suggested that active terpenes in plants may be trapped within secretory gland structures, making them unavailable and useless for the animal. The problem may be highlighted in chickens due to the relatively short transit time of digesta. The discrepancy between the results of numerous experiments which have used herbs as growth enhancers could be attributed to the dependence of investigated traits on a variety of factors such as gender and genetic source of birds tested, composition of experimental diets and source of herbal materials used (Mountzouris *et al.* 2009).

Our previous study was accomplished on Ross-308 male broiler chickens and herbs used were provided from a traditional medicinal herb supplier in east Azarbayjan, Iran, whereas, in the present study as hatch Arbor Acres broilers were used and the herbal materials were provided from two separate sources; peppermint from Mashhad in northeast Iran and tarragon from Qom in the center of Iran. There is evidence that the content of active substances in phytogenic products vary substantially, depending upon the plant part used (e.g., seeds, leaf, root and bark), geographical origin, harvesting season (Steiner, 2006) and on processing and storage conditions (Vienna *et al.* 2005; Arabhosseini *et al.* 2007).

CONCLUSION

Since even the virginiamycin treated-birds (positive control) showed no improvement in growth rate or nutrient digestibility, it could be concluded that challenges coming from the direct vicinity of a healthy commercial flock were probably not sufficient to make the experimental birds show clear responses to the additives tested. Thus, further investigation needed with known preparations of tarragon and peppermint under a more challenging condition.

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