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EFFECTS OF INCLUSION OF AERIEL DRIED PARTS OF SOME HERBS IN BROILER DIETS

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ABSTRACT

This study determines possible antibacterial, antioxidant and growth-promoting effects of some selected herbs in broiler chickens. Ground aerial parts of *Thymbra spicata*, *Origanum minutiflorum*, *Rosmarinus officinalis*, *Mrytus communis*, *Salvia tomentosa* and ground seed of *Cumin* were added to broiler diets at 0.0, 0.25, 0.50, 1.00 and 1.50% (w/w). Volatile essential oil contents were analysed. Samples of blood, digesta and intestinal tissues were analysed to determine antioxidant activity, bacteria growth and intestinal histomorphology, respectively. The results showed that at 0-21 days of age the birds performed better on the diets up to 0.25% of herbal inclusion level. Increasing the inclusion level from 0.5 to 1.5% for *Origanum minutiflorum*, *Thymbra spicata* and partially for *Rosmarinus officinalis* increased feed consumption with lower weight gain and depressed efficiency of feed conversion, but not for *Salvia tomentosa, Mrytus communis* and *Cumin*. The results further showed that broilers could better perform on the diets up to 0.5% of inclusion level at 42 days. All levels (except 1.5%) for *Cumin* and *Salvia tomentosa* did not cause any impairment in performance of broilers. High inclusion levels (0.5 to 1.5%) reduced *E. coli* count. The selected herbs did not cause an oxidative stress at a given inclusion level. Of the plants *Rosmarinus officinalis* clearly demonstrated strong antioxidant effect. The high doses of *Origanum minutiflorum* caused depression in growth rate associated with shortened villus development of intestinal epithelium in broilers. However, increased growth rate with *Cumin* associated with longer villus and increased dry matter retention in broilers at 21 days were recorded. It was suggested that some of selected herbs can be incorporated up to 0.5% into broiler diets on account of their antibacterial and antioxidant properties.

Keywords: Herbs, broiler chicken, growth rate, antibacterial effect, antioxidant capacity, intestinal histomorphology.

INTRODUCTION

The European Union ban on the use of certain antibiotics as growth promoters in broiler production has lead to the search for some alternate feed supplements. The main objective of this study was to test possible antibacterial, antioxidant and growth stimulating effects of some herbs incorporated into broiler diets. The targeted herbs were those naturally grown or cultivated in Isparta, Turkey, and possesing an important place in the production and trade of medicinals herbs in the world. The flora of Isparta province was examined to have 190 plants with commercial medicinal and parfumery value and 160 plants with a commercial herbal value (Ozcelik and Serdaroglu, 2000).

The attention has mostly been focussed on the effects of herbs on their food improving properties as preservant, conservant and alternative health susbtances. Most of the experimental works have been conducted *in vitro* or in a rat model: The carvacrol, an extracted compound from *Thymus revolutus c.* which is endemic in Turkey were *in vitro* tested at various concentrations against 11 different bacteria species in food matrices and was found to have marked antibacterial and antifungal effects (Karaman *et al.,* 2001). Volatile essential oils extracted from *Cumin* were observed to stimulate *in vitro* acidity, and thereby increased development of *Lactobacillus pantarum* and *Leuconostoc mesenteroides,* while their high doses (300-600 ppm) had adverse effects. Similarly *Oregano* increased *in vitro* acidity, but inhibited bacteria developments (Kivanc *et al.,* 1991). *In vitro* growth of *Escherichia coli O157:H7* was examined under the influence of the extracts of *Cumin*, *Helichrysum compactum Boiss* (*HC*), *Laurel*, *Myrtle*, *Oregano*, *Sage* and *Thyme* (Sagdic *et al.,* 2002; Sagdic and Ozcan 2003). Important inhibitory effects were obtained from *Thyme* and *Oregano* while *laurel* and *HC* markedly stimulated *in vitro* growth of *E. coli O157:H7*. These effects mostly related to the concentration of extract used (Sagdic *et al.,* 2002). Some spice hydrosols were tested for their in vitro food preservative effects (Sagdic and Ozcan 2003). *Anise*, *Cumin*, *Oregano*, *Summer savory* and *Blackthyme* had antibacterial activity against *Bacillus amyloliquefaciens ATCC 23842*, *B. brevis FMC 3*, *B. cereus FMC 19*, *B. subtilis var. niger ATCC 10*, *Enterobacter aerogenes CCM 2531*, *Escherichia coli ATCC 25922, E. coliO157:H7 ATCC 33150, Klebsiella pneumoniae FMC 5, Proteus*

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vulgaris FMC 1, Salmonella enteritidis, S. gallinarum, S. typhimurium, Staphlococcus aureusATCC 2392, S. aureus ATCC 28213 and Yersinia enterocolitica ATCC 1501. *Oregano* and *Summer savory* were effective against all the bacteria species while *Anise*, *Cumin* and *Black thyme* hydrosols were only effective against some bacteria species. *Oregano* was found to be the most effective antifungusit (Akgul and Kivanc 1988; Per and Rodrigo 2000).

On the contrary, some studies reported beneficial effects of some herbs in animal *in vivo* models: *Rosemarry* is shown to have very strong antioxidant effect in farm animals (Scheeder et al., 2000). The rats received leaves or extract of *Rosemary* (*Rosmarinus officinalis*) at rate of 0.5% of the diet (w/w), especially the characterised hydrosol extract had an enhanced both cytochrome P450 and detoxication enzymes (Debersac et al. 2001). Therefore, *Rosemary* keeps a potentiality as antioxidant in animals. A mixture of herbs added to the broilers diet at a rate 0.5, 1.0 and 1.5 kg/tonne was tested against flavomycin or a probiotic additive of Paciflor, and better carcass quality parameters in broiler chicken were obtained (Ristic et al., 2001). The levels of 0.5 and 0.75 g/kg of the ground *anise* seed produced only better body weight gain in broiler chicken compared to a control diet, whereas a level of 1.5 g/kg *anise* seed depressed growth performance (Soltan et al., 2008). The doses of 100 and 200 ppm essential oils extracted from *thyme* and *cinnamon* produced high feed intake, weight gain and feed efficiency ratio, compared to a control broiler diet (Al-Kassie 2009). A dose of 200 ppm essential oils of *thyme*, *cinnamon* and *clove* did not affect the performance of broiler chickens, expect that the diets of *thyme* essential oil caused significant improvement in weight gain and feed efficiency ratio during the growing period from 22 to 42 days of age (Najafi and Torki 2010). Essential oils of *cinnamon* and *cloves* with or without organic acid salts did not affect the growth rate and feed efficiency ratio in broiler chicken, except that the relative breast weight was high with the diets containing essential oils (Isabel and Santos 2009). A dose of 48 mg/kg of an essential oil mixture was found to improve the birds' performance when compared to an antibiotic (Alcicek et al., 2003). In contrary, no improvement in the birds' performance was reported by the doses of 25 or 50 mg/kg of an essential oil extract in comparision to the diet containing an antibiotic (Jang et al., 2007). The doses of 0.2, 0.4 and 0.8 g/kg of *cumin* essential oil in broiler diets did not affect the performance characteristics of broilers, except that the lowest dose of *cumin* essential oil caused higher body weight gains in growing period from 15 to 35 days of age (Aami-Azghadi and Golian 2010). However, the dietary inclusion levels of an essential oil extract showed a decreased *E. coli* population in ileo-cecal digesta, and its 50 mg/kg dose resulted in a significant increase in certain digestive enzyme activities in growing broiler chickens (Jang et al., 2007). Herbs and their essential oils were seen to differently affect both the birds' performance and the gut health and secretions. The best performance was obtained by the diets of *thyme* oil and *yarrow* herb whereas lowest performance was with the diets of *oregano* herbs and *yarrow* oil (Cross et al., 2007). On the other hand, the improvement in bird' performance was greater with the diets with *oregano* herb than the control diet (Florou-Paneri et al., 2006). Supplementation of broiler diets with 0.5 and 1.0% *oregano* or *clove* herbs significantly increased feed intake and improved feed conversion ratio (Borazjanizadeh et al., 2011). Recently *oregano* essensial oil and a plant extract *hop* were reported to improve broiler live performance, whereas a mixture of these plant extracts did not have an additive effect on the performance (Bozkurt et al., 2009). *Rosemary* leaves was shown to produce better weight gain and efficiency of feed utilization when added to broiler diets at 0.5%, and its high levels, more than 0.5% lowered growth, efficiency and nutrient utilization (Ghazalah and Ali 2008). This indicated that the medicinal and herbal plants and/or their essential oil extract may have potential growth-promoting and health effects in animals.

Keeping above in view, six herbs (*Thymbra spicata*, *Rosmarinus officinalis*, *Origanum minutiflorum*, *Mrytus communis*, *Salvia tomentosa* and *Cumin*) were identified through literature review and their antibacterial, antioxidant and growth promoting effects were investigated in broiler chicken fed on a dried and ground aerial parts of these plants (*cumin* as ground whole seed) at an inclusion rate varying from 0.25 to 1.5% (w/w).

MATERIALS AND METHODS

Collection of Herbs and Diet Formulation: Six different herbs were collected from the nature in Isparta-Sutculer-Antalya region, however, *cumin* was procured from a local market in the form of ground seed. The aerial parts of these herbs were separated by hand and left for drying under the shadow. A small homogenous sample was analysed for dry matter and ash content.

The total volatile essential oil content (v/w) was determined by extracting homogenous mixture of the collected dried herbs in a Clevenger distillation apparatus for 3 h (Baydar et al., 2004, Gulluce et al., 2003). The collected extracts were analysed for approximate active components at the central laboratory of Suleyman Demirel University using Gas Chromotograpgy (Perkin Elmer Autosystem XL GC) with FID Detector - flame ionization detector (Baydar et al, 2004, Gulluce et al., 2003). The employed chromatographic conditions were set up as follows: colon- CP-Wax 52 CB (50 m x 0.32 mm), detector temperature of 240°C, enjector temperature of 240°C, carrying He, gas burning H_2 gas,

velocity of 400 ml/min for air, 40 ml/min for H_2, Split ratio of 1/20 ml/min., enjector capacity of 5 microlitter.

Broiler diets were formulated containing herbs with inclusion levels of 0.0, 0.25, 0.5, 1.0 or 1.5% of each herbs. Isocaloric and isonitrogenous diets were prepared for the starter (0-21 d-old) and the finisher (21-42 d-old) periods (Table 1).

Experimental trial: A total of 900 of one day-old commercial Ross PM3 broiler chicks were randomly assigned to 150 cages, 6 chicks each, in an environmentally controlled poultry house. Each herb (in total 6) was incooporated into broiler diets with 5 inclusion levels, providing a total of 30 treatments. Each treatment was allocated to 5 replicate cages. The chicks were fed *ad libitum* broiler starter diets from 0-21 days and the finisher diets from 21 to 42 days of age. The experimental birds were maintained under recommended optimal management conditions. Daily feed intake (per cage) and weekly body weights (per bird) were recorded throughout the experimental periods. For each inclusion level of one herb the feacal outputs were collected from 3 cages for a period of three days per week for determining the retained amount of feed dry matter in the digestive tract.

Table 1: Composition of experimental diets containing herbs.

Ingrediends, %	Starter diets	Finisher diets
Herbal plants	0 to 1.5	0 to 1.5
Ground maize	54	58
Soybean meal	29.75	25
Meat-bone meal	8.20	8.0
Vegetable oil	6.25	7.50
Limestone	0.25	0.05
Salt	0.25	0.25
Vitamin-mineral premix	0.35	0.35
Methionine	0.20	0.10
Total	100	
Nutrient composition		
Dry matter, %	89	89
Crude protein, %	22	20
Metabolisable energy, kcal/kg	3100	3200
Crude fat, %	9.3	10.5
Crude fibre, %	3.4	3.1
Calcium, %	1.2	1.1
Phosphour, %	0.9	0.8
Available P, %	0.6	0.6
Methionine+cystine, %	0.9	0.7
Lysine, %	1.2	1.1
Linoleic acid, %	4.9	5.6

Biochemical, microbiological and histological determinations: At the end of 21 and 42 days of the study, one bird from each cage was slaughtered to collect blood, digesta and intestinal samples, providing 5 replicates per each inclusion rate within each plant. Homogenats followed by centrifugation of the samples were collected for the determination of antioxidant capacity based on the analyses of Thiobarbituric acid reactive substances (TBARS) levels, antioxidant potential (AOP) and activities of superoxide dismutase (SOD), gluatiton peroxidase (GSH-Px) and catalase (CAT).

TBARS was determined by the method of Hoyland and Taylor (1991) as follows: 0.5 ml tissue homegenates mixed with 2.5 ml of 10% TCA (trichloroacetic acid) were left in boiled water for 15 min and cooled by centrifugation under tap water. 1 ml of 0.67% TCA was added to the supernatants and left in boiled water again. After that clear supernatants were measured for absorbance readings at 532 nm using a spectrophotometer. The measured levels of TBARS in tissues are an indirect indicator of oxidative stress (Draper and Hadley, 1990). Protein contents were analysed according to methods of Lowry *et al.* (1951). AOP was determined according to method developed by (Durak *et al.,* 1998). The reaction media were enriched by fish oil. Unsaturated fatty acids are more susceptible to free radical attacks. The difference is inversely proportionate to AOP. SOD was determined using Ransod kit and GSH-Px by the method of Paglia and Valentine, (1967) and CAT by the method of Aebi (1984).

The intestinal tissues were processed to obtain histological slides to examine the epithelium development by measuring the heights of villus, depths of crypt layer and tunica muscularis under a light microscope (Yasar, 1998). Ileal contents of the experimental birds were collected in sterilized tubes placed in ice. Pre-prepared nutrient agars were inoculated with the digesta and bacterial counts were made thereafter. 10 mg digesta were meshed in a 90 ml physiological saline solution containing 0.85% NaCl. Dilutions were made until 10^8 (Gurgun and Halkman, 1998). Total *Mesophilic aerobic bacteria* were counted on plate count PCA agar (Merck) at 30 °C for 48 h. Total *Psychrophilic aerobic bacteria* count on PCA (Merck) at 10 °C for 7 days. *Coliform bacteria* count on violet red bile agar VRBS (Merck) at 37°C for 24 h, *Enterobactericiae* group counts on violet red bile dextrose agar VRBDA (Merck) at 37°C for 24 h, *E. coli* count on EMB agar (Merck) at 37°C for 24 h, *Lactic acid bacteria* count on MRS agar (Merck) at 30°C for 48. All the counts were expressed as colony forming unit per g of diegsta. The studied antibacterial paramaters were the colony forming unit (c.f.u per gram digesta sample) of the studied bacteria species: total *Mesophilic aerobic bacteria* counts (PCA30), total *Psychrophilic aerobic*

bacterial (PCA10), total *Coliform bacteria* counts (VRBA), *Enterobacteriae* counts (VRBDA), *E. coli* count (EMB) and *Lactic acid bacteri* counts (MRSA).

The effects of each diet on the studied parameters were statistically analysed by an analysis of variance using the GLM (general linear model) procedure of Statistical Package of Social Sciences (SPSS, 2006), where the effects of 6 plants and 5 inclusion rates were fixed. Differences between experimental groups were compared by Duncan's multiple range test of the same package (SPSS, 2006). The data obtained from microbiological measurements were log transformed before statistical analysis.

RESULTS AND DISCUSSION

Chemical Analysis: Herbs were analysed for the content of dry matter and crude ash, and the results are presented in table 2.

Active components of essential oils obtained from the tested herbs are shown in table 3. The main constitutes substantially varried amongst herbs studied: *Origanum minutiflorum* and *Thymbra spicata* show somehome similar pattern of constitutes in terms of carvacrol component. Carvacrol appears to be main constitute in all herbs essential oil content except in the *cumin* seed.

Table 2: The percentage of dry matter and ash content of tested plants.

Plants	Dry matter content, % (average ± stdev)	Ash content, % (average ± stdev)
Thymbra spicata	91±0.7	8.2±1.5
Origanum minutiflorum	91±0.2	8.0±2.2
Rosmarinus officinalis	94±0.8	9.9±1.0
Mrytus communis	94±0.2	7.7±0.9
Salvia tomentosa	95±0.3	8.7±1.7
Cumin (Cuminum cyminum L. – Apiaceae)	90±0.8	13.2±1.1

Table 3: Chemical composition of the essential oils (% total peak area).

Active components (%)	*Origanum minutiflorum*	*Thymbra spicata*	*Rosmarinus officinalis*	*Mrytus communis*	*Salvia tomentosa*	*Cumin*
Alfa pinen	0.52	4.10	1.66	10.84	22.98	0.2
Sabinene	1.71					
Alpha-terpinene		3.26				
Beta-pinene		2.68		0.34	2.68	4.0
Beta-terpinene	0.77	18.74		0.47		
Borneol	7.34	9.05			10.40	
Borneol acetate		1.94				
Carvacrol	**78.73**	**55.03**	**24.45**	**16.72**	**32.24**	
Carvyl-Acetate				0.33		
Caryophyllene	2.05	1.81				
Cineole				14.53	25.25	
Cumin-Alcohol						0.3
Cuminaldehyde						31.06
Fenilalcohol				9.87		
Gamma-Terpinene						9.0
Isocaryophyllene						0.2
Limonene			0.93	8.32		0.2
Linalool	1.18		8.54	12.57		
Linalyl acetate				4.52	1.05	
Nerol/geraniol				3.05		
P-cymene		16.18		0.49		3.8
Terpinolene	1.24		4.43			
Thymol	0.6	0.3				
Essential oil contents ppm	**1950**	**1400**	**800**	**300**	**300**	**1200**

Performance of broiler chicken: The experimental birds fed on different experimental diets gained similar body weights at 21 days of age, showing non- significant effect ($p>0.05$) of the diets containing no herb or low (0.25%) inclusion level. However the control (0.0%) and the low inclusion groups (0.25-0.50%) gained higher ($P<0.05$) body weight than experimental groups containing 0.5-1.5% herbs. This was apperant for the diet containing *Origanum minutiflorum* and *Thymbra spicata*, but not for the remaining diets (Table 4). The difference in feed intake between the diets was significant ($p<0.05$) during the starter period (21 days), whereas, the effects of various inclusion rates and their interaction effect were also significant ($p<0.05$). The control (0.0%) and the low

inclusion groups (0.25%) consumed significantly ($p<0.05$) less amount of feed as compared to those with higher inclusion levels (0.5-1.5%) except for those supplemented with *Salvia tomentosa*.

The feed conversion ratio (FCR) was significantly ($p<0.05$) affected by the experimental diets and not by the inclusion rates during the starter period (0-21days). The interaction between the diets and their inclusion levels were also observed to be significant ($p<0.05$). The control (0.0%) and the low inclusion group (0.25%) showed significantly ($p<0.05$) better FCR for *Origanum minutiflorum*, *Thymbra spicata* and *Rosmarinus officinalis*, but not for *Mrytus communis*, *Salvia tomentosa* and *Cumin*. The poorest FCR with high inclusion levels (0.5 to 1.5%) could be attributed to decreased weight gain associated with increased feed consumption.

Table 4: Body weights (g/bird), feed intakes (g/bird) and feed efficiency rates of broiler chickens of 21 d-old age fed on diets containing various inclusion levels of herbs.

Inclusion rate, %	*Origanum minutiflorum*	*Thymbra spicata*	*Rosmarinus officinalis*	*Salvia tomentosa*	*Mrytus communis*	*Cumin*	SEM
Body weights (g/bird) of broiler chickens at 21 d-old age							
0.00	1048a	1070a	1059a	1055a	1050a	985a	27
0.25	1042a	1065a	1045a	1050a	1041a	1000a	20
0.50	995ab	1050a	1030a	1040a	1038a	975ab	27
1.00	970b	1010a	1018a	1020a	1010a	1020ab	17
1.50	980ab	980a	1025a	1045a	1045a	1050a	30
*SEM	32	35	15	12	14	27	
Significance	Plants p >0.05		Doses p >0.05		P x D p >0.05		
Feed intakes (g/bird) of broiler chickens at 21 d-old age							
0.00	1520a	1490a	1589a	1688a	1628a	1480a	76
0.25	1646ab	1500ab	1672ab	1670a	1603a	1500ab	73
0.50	1701b	1550ab	1803ab	1685a	1661ab	1550ab	88
1.00	1678ab	1575ab	1802ab	1703a	1636ab	1575b	79
1.50	1701b	1590b	1900b	1710a	1714b	1580b	105
SEM	68	40	109	14	38	40	
Significance	Plants p >0.05		Doses p <0.05		P x D p <0.05		
Feed efficiency rates of of broiler chickens at 21 d-old							
0.00	1.40a	1.39a	1.50a	1.60ab	1.55a	1.50a	0.08
0.25	1.58ab	1.40ab	1.60ab	1.59a	1.54a	1.50a	0.07
0.50	1.71b	1.46ab	1.75ab	1.62ab	1.60a	1.59a	0.09
1.00	1.73b	1.56b	1.77ab	1.67b	1.62a	1.51a	0.09
1.50	1.74b	1.62b	1.85b	1.64ab	1.64a	1.50a	0.11
SEM	0.13	0.09	0.13	0.03	0.04	0.04	
Significance	Plants p <0.05		Doses p >0.05		P x D p <0.05		

* Standard error of means. Similar letters indicate no significant differences between the means across the inclusion levels within each plant.

During the finisher period (42 days), body weights of broilers differed non-significantly between the diets and their inclusion levels (Table 5). The differences in feed intakes of broilers fed on various diets were observed to be significant ($p<0.05$), however, the effect of the different inclusion rates on feed intake was found to be non- significant ($p>0.05$). The effect of diet x inclusion rate interaction was significant ($p<0.05$). The birds significantly ($p<0.05$) consumed less feed with low inclusion rates (0 to 0.25%) in comparison to those fed on high inclusion rates (0.5 to 1.5%) for *Origanum minutiflorum*, *Thymbra spicata*, *Rosmarinus officinalis* and *Mrytus communis*, but not for the remaining herbs. The results in respect of FCR followed the similar pattern of feed intakes of birds at 42 d-old age. The birds fed on low inclusion rates of 0 and 0.25% or 0.5% showed better FCR for *Origanum minutiflorum*, *Thymbra spicata*, *Rosmarinus officinalis* and *Mrytus communis*, but not for the remaining herbs. The improvement in FCR of the broilers could be attributed to the lesser amount of feed consumed associated with increased weight gain. The differences in survial rates and feacel blood output were found to be non- significant.

Dry Matter Retention: During the starter period (21 days), the retention of dry matter in the digestive tract of broilers was not significantly ($p>0.05$) affected by the experimental diets and their inclusion levels. The feeding of diets with *Thymbra spicata* resulted in lower retention of dry matter as compared to other experimental diets (Table 6).

Table 5: Body weights (g/bird), feed intake (g/bird) and feed efficiency of broiler chickens of 42 d-old age fed on diets containing various inclusion levels of herbs.

Inclusion rate, %	*Origanum minutiflorum*	*Thymbra spicata*	*Rosmarinus officinalis*	*Salvia tomentosa*	*Mrytus communis*	*Cumin*	SEM
Body weights (g/bird) of broiler chickens at 42 d-old age							
0.00	2201a	2190a	2224a	2216a	2205a	2110ab	38
0.25	2188ab	2175a	2200ab	2205a	2186a	2180a	10
0.50	2100b	2169a	2163ab	2184a	2180a	2100ab	36
1.00	2110b	2170a	2140b	2173a	2190a	2095ab	35
1.50	2120ab	2100a	2175ab	2190a	2200a	2050b	52
*SEM	42	31	29	15	9	42	
Significance	Plants p >0.05		Doses p >0.05		P x D p >0.05		
Feed intakes (g/bird) of broiler chickens at 42 d-old age							
0.00	4787a	4700a	5004a	5317ab	5127ab	5130a	211
0.25	5186a	4810ab	5280ab	5259a	5050a	5100a	158
0.50	5387b	4820ab	5678b	5307ab	5232bc	5145a	258
1.00	5475b	4950ab	5682b	5443b	5322bc	5180a	232
1.50	5480b	5100b	5570ab	5400ab	5500c	5120a	18
SEM	260	137	263	67	157	27	
Significance	Plants p <0.05		Doses p >0.05		P x D p <0.05		
Feed efficiency of of broiler chickens at 42 d-old							
0.00	2.17a	2.15a	2.25a	2.40a	2.33ab	2.43ab	0.11
0.25	2.37ab	2.21ab	2.40ab	2.39a	2.31a	2.34a	0.06
0.50	2.57b	2.22ab	2.63ab	2.43a	2.40ab	2.45ab	0.13
1.00	2.59b	2.28ab	2.66b	2.50a	2.43ab	2.47ab	0.12
1.50	2.58b	2.43b	2.56b	2.47a	2.50b	2.50b	0.05
SEM	0.16	0.10	0.15	0.04	0.07	0.05	
Significance	Plants p <0.05		Doses p >0.05		P x D p <0.05		

*Standard error of means.Similar letters indicate no significant differences between the means across the inclusion levels within each plant.

Table 6: Retention of dry matter in broiler chicken of 21 and 42 d-old age fed on diets based on herbs.

Retention at 21 d-old age	*Origanum minutiflorum*	*Thymbra spicata*	*Rosmarinus officinalis*	*Salvia tomentosa*	*Mrytus communis*	*Cumin*	Mean	SEM
0.00	65.0a	60.0ab	62.0a	66.0a	63.0a	69.0a	55.0	3.0
0.25	63.0a	49.0a	61.0a	67.0a	63.0a	66.0a	53.0	6.0
0.50	66.0a	60.0ab	60.0a	64.0a	65.0a	67.0a	55.0	3.0
1.00	63.0a	62.0b	60.0a	67.0a	60.0a	64.0a	54.0	3.0
1.50	52.0a	52.0ab	60.0a	61.0a	59.0a	68.0a	50.0	5.0
Mean	62.0ab	57.0b	61.0ab	65.0ab	62.0ab	67.0a		
*SEM	5.0	5.0	1.0	2.0	2.0	1.0		
Significance	Plants p <0.05		Doses p >0.05		P x D p >0.05			
Retention at 42 d-old age								
0.00	70.0a	66.0a	69.0ab	72.0a	66.0a	72.0a	69.0	2.0
0.25	71.0a	61.0ab	70.0ab	73.0a	69.0a	70.0ab	69.0	4.0
0.50	69.0a	59.0b	70.0ab	69.0ab	70.00a	69.0b	68.0	4.0
1.00	70.0a	57.0b	73.0a	68.0ab	65.0a	69.0b	67.0	5.0
1.50	68.0a	60.0ab	68.0b	67.0b	65.0a	71.0ab	66.0	3.0
Mean	70.0a	60.0b	70.0a	69.0a	67.0a	70.0a		
SEM	1.0	3.0	2.0	2.0	2.0	1.0		
Significance	Plants p <0.05		Doses p >0.05		P x D p <0.05			

*Standard error of the means. Similar letters indicate no significant differences between the means across the inclusion levels within each plant.

During the finisher period (42 days), the dry matter retention in the digestive tract of broilers was significantly ($p<0.05$) influenced by the experimetal diets, but not by their inclusion levels. The birds fed on *Thymbra spicata*, *Rosmarinus officinalis* and *Salvia tomentosa* at the level of 0 and 0.25% in the diets had significantly ($p<0.05$) higher retention values as compared to those fed on diets with levels of 0.5 to 1.5%. However, dry matter retention differed non- significantly between the dietary inclusion levels of the remaining diets. *Thymbra spicata* resulted in lower retention rate of dry matter. Dry matter retention improved in 42 days-old broilers as compared to that at 21 days of age.

Antioxidant effects: It is well known that when a cell is unable to eliminate free radicals unsaturated free fatty acids easily oxidized and subsequently the concentration of TBARS can increase. Using this reaction system it is possible to obtain precise information on the total enzymatic and non-enzymatic antioxidant potential of tissue and cells. For this purpose, TBARS level of reaction medium was measured before and after free radical attacks. TBARS values, expressed as nmol/ml of homogenate proteins, were not significantly ($p<0.05$) affected in broilers fed on diets supplemented with the herbal plants and their dietary inclusion levels, indicating that the experimental birds were not subjected to metabolic oxidative stress under the dietary manipulations (Table 7). SOD values were found to be significantly ($p<0.05$) influenced by the experimental diets and their inclusion levels. The levels of 0.5 to 1.5% increased SOD activity in the birds. The enzymatic activities of CAT and GSH-Px in broilers fed on various inclusion levels of herbal plants differed non-significantly ($p>0.05$). The mean values were 6.80 ± 0.03 k/mg Hb for CAT and 2.50 ± 0.01 U/mg Hb for GSH-Px, respectively. AOP activity was not significantly ($p>0.05$) affected by the diets and their inclusion rates (Table 7). In all diet groups the inclusion levels of 0.5% and 1.0% increased AOP level. The highest increase was obtained by the *Rosmarinus officinalis,* followed by *Origanum minutiflorum*, *Thymbra spicata* and *Salvia tomentosa*.

Table 7: Oxidative stress and antioxidant potential in broilers fed on diets based on herbs.

Inclusion levels	*Origanum minutiflorum*	*Thymbra spicata*	*Rosmarinus officinalis*	*Salvia tomentosa*	*Mrytus communis*	*Cumin*	Mean	SEM
TBARS at 42 days of age (nmol/g Hb)								
0.00	260	262	263	258	258	260	260	4
0.25	260	262	263	259	255	260	260	5
0.50	264	260	261	264	260	258	261	3
1.00	262	258	260	258	258	260	259	3
1.50	257	257	260	263	262	260	260	4
Mean	261	260	261	260	259	260		
SEM	2	2	1	3	2	1		
Significance	Plants p >0.05		Doses p >0.05		P x D p >0.05			
SOD at 42 days of age (U/mg Hb)								
0.00	4.5a	4.0a	6.0a	4.9a	5.4a	4.5a	4.2	0.5
0.25	4.7a	4.5ab	6.0a	5.5ab	5.5a	4.8a	4.4	0.8
0.50	5.0ab	4.8ab	6.5ab	5.8ab	5.8ab	5.2a	4.7	0.8
1.00	6.0ab	5.5ab	6.8b	6.0b	5.9ab	5.8a	5.1	0.7
1.50	6.5b	6.0ab	6.5ab	5.8ab	6.3b	5.6a	5.2	0.7
Mean	5.3	5.0	6.3	5.6	5.8	5.2		
*SEM	0.8	0.7	0.3	0.4	0.3	0.5		
Significance	Plants p < 0.05		Doses p < 0.05		P x D p < 0.05			
AOP at 42 days of age (1/nmol/g Hb.h)								
0.00	0.21	0.22	0.21	0.24	0.23	0.23	0.19	0.05
0.25	0.22	0.23	0.24	0.26	0.21	0.24	0.20	0.05
0.50	0.34	0.31	0.38	0.32	0.29	0.27	0.27	0.04
1.00	0.37	0.34	0.41	0.39	0.31	0.30	0.30	0.03
1.50	0.29	0.37	0.39	0.37	0.35	0.31	0.30	0.05
Mean	0.29	0.29	0.33	0.32	0.28	0.27		
SEM	0.1	0.1	0.1	0.1	0.1	0.1		
Significance	Plants p >0.05		Doses p >0.05		P x D p >0.05			

*Standard error of the means. Similar letters indicate no significant differences between the means across the inclusion levels within each plant.

Table 8: Intestinal bacteria status (log_{10} c.f.u/g digesta) in broiler chicken of 21 and 42 d-old age fed on diets based on herbs.

Inclusion levels	Origanum minutiflorum	Thymbra spicata	Rosmarinus officinalis	Salvia tomentosa	Mrytus communis	Cumin	Mean	SEM
Total *Psychrophilic aerobic bacteria* (PCA10) counts (c.f.u per g digesta)								
0.00	6.5a	6.3a	6.4a	6.3a	6.2a	6.4a	5.4a	0.1
0.25	5.6ab	5.5ab	5.5ab	5.3ab	5.6ab	5.8ab	4.8b	0.1
0.50	5.4ab	5.2ab	5.4ab	5.7ab	5.4ab	5.5b	4.7b	0.1
1.00	4.7b	4.8b	4.5ab	4.7b	4.3b	5.7ab	4.2b	0.4
1.50	4.6b	4.9b	4.3b	4.3b	4.0b	5.5b	4.6b	0.5
Mean	5.4a	5.3a	5.2a	5.3a	5.1a	5.8a		
SEM	0.7	0.5	0.8	0.7	0.8	0.3		
Significance	Plants p >0.05		Doses p<0.05		P x D p<0.05			
Total *Coliform bacteria* (VRBA) counts (c.f.u per g digesta)								
0.00	5.1	5.0	4.5	5.3	4.5	5.4	4.3	0.4
0.25	4.5	4.7	3.5	4.9	3.7	5.0	3.8	0.6
0.50	4.3	4.5	4.0	4.2	4.6	4.5	3.8	0.2
1.00	3.9	3.6	4.5	4.7	4.4	4.8	3.8	0.4
1.50	4.0	3.9	4.1	4.0	4.6	4.7	4.2	0.3
Mean	4.4	4.3	4.1	4.6	4.4	4.9		
SEM	0.4	0.5	0.4	0.5	0.3	0.3		
Significance	Plants p >0.05		Doses p >0.05		P x D p >0.05			
***Enterobacteriae* counts (VRBDA)**								
0.00	5.2ab	5.3ab	5.1a	4.9	5.3a	5.4a	4.5a	0.2
0.25	5.4ab	5.6ab	5.0a	5.1	4.8ab	4.9ab	4.4a	0.3
0.50	5.6b	5.2b	4.5ab	5.2	4.4b	4.6ab	4.3a	0.4
1.00	5.2ab	5.0ab	3.1b	5.0	5.0ab	5.0ab	4.2a	0.7
1.50	5.1a	4.9ab	3.5ab	4.6	4.3b	4.7b	4.5a	0.5
Mean	5.3a	5.2a	4.2b	5.0	4.8ab	4.9ab		
SEM	0.2	0.2	0.8	0.2	0.4	0.3		
Significance	Plants p <0.05		Doses p >0.05		P x D p <0.05			
***E. coli* count (EMB)**								
0.00	1.6a	1.7a	1.5a	1.6ab	1.4a	1.8a	1.4a	0.1
0.25	1.1ab	1.3ab	1.2ab	1.1a	1.1ab	1.5ab	1.1ab	0.1
0.50	1.0ab	1.0ab	1.1ab	0.9b	1.0ab	1.0ab	0.9b	0.1
1.00	0.9b	0.7b	1.1ab	0.6ab	0.9b	0.9b	0.9b	0.2
1.50	0.6b	0.8b	1.0b	1.0ab	0.8b	0.8b	0.8b	0.1
Mean	1.0a	1.1a	1.2a	1.0ab	1.0a	1.2a		
SEM	0.3	0.4	0.2	0.3	0.2	0.4		
Significance	Plants p >0.05		Doses p <0.05		P x D p <0.05			
***Lactic acid bacteri* counts (MRSA)**								
0.00	5.2ab	5.5a	5.6ab	6.0a	6.1ab	5.5a	4.8a	0.3
0.25	5.1a	5.3ab	6.0a	5.7a	6.5a	4.9ab	4.8a	0.5
0.50	5.5b	5.3ab	6.0a	5.1ab	5.8ab	5.3a	4.8a	0.3
1.00	5.3ab	5.3ab	5.9a	5.9ab	5.4b	5.0ab	4.8a	0.3
1.50	5.4b	5.0b	4.9b	5.5ab	5.0b	4.2b	5.0a	0.4
Mean	5.3ab	5.3ab	5.7b	5.6ab	5.8ab	5.0a		
SEM	0.1	0.2	0.4	0.3	0.5	0.4		
Significance	Plants p <0.05		Doses p >0.05		P x D p >0.05			

*Standard error of the means. Similar letters indicate no significant differences between the means across the inclusion levels within each plant.

Antibacterial effects: The bacterial counts in 21 and 42 days-old broilers did not significantly differ ($p<0.05$), therefore these values were merged and presented together (Table 8). Non- significant ($p>0.05$) difference

was observed between the herbal plant based diets and also between the various inclusion levels for total *Mesophilic aerobic bacterial* counts, the mean value of 9,3 (log_{10} per g digesta) remained almost the same over the diets of different herbs and their inclusion levels. Non-significant difference was observed in *Psychrophilic aerobic bacteria, Coliform bacteria, Enterobacteriae and Lactic acid bacterial* counts between the tested herbal plants and their inclusion levels. However, significant reduction in E. *Coli* count was observed with increasing inclusion levels of these herbal plants from 0,5 to 1.5% (Table 8).

Intestinal Histomorhology: The broilers during starter period (21 days) fed on *Cumin*, *Salvia tomentosa*, *Thymbra spicata* and *Mrytus communis* had significantly ($p<0.05$) longer villus of ileum epithelial, compared to those fed on *Origanum minutiflorum* and *Rosmarinus officinalis*. The differences in villus heights between the diets was not significant ($p>0.05$) in 42 days-old broilers. Both at 21 and 42 days of age, the thickness of crypt layer and tunica muscularis did not significantly ($p>0.05$) differ between the tested diets, except that the thickness of crypt layer in broilers fed on *Origanum minutiflorum* and *Thymbra spicata* was significantly ($p<0.05$) different.

Table.9: intestinal histomorphologic status of broiler chicken of 21 and 42 d-old age fed on herbs based diets.

Parameters	Villus Height (micrometer)		Crypt layer thickness (micrometer)		Tunica Muscularis thickness (micrometer)	
Age	21 d	42 d	21 d	42 d	21 d	42 d
Control diet	93.3ab	121.0a	43.0a	62.0a	45.0a	76.0a
Origanum minutiflorum	91.0a	122.0a	51.0b	65.0a	45.0a	79.0a
Thymbra spicata	95.0ab	119.0a	49.0b	63.0a	43.0a	77.0a
Rosmarinus officinalis	92.0ab	129.0a	46.0a	59.0a	46.0a	79.0a
Salvia tomentosa	96.0 ab	121.0a	45.0a	60.0a	44.0a	76.0a
Mrytus communis	95.0ab	125.0a	45.0a	61.0a	45.0a	75.0a
Cumin	104.0 b	130.0a	46.0a	64.0a	41.0a	73.0a
*SEM (pooled)	5.0	6.0	2.0	4.0	3.5	4.0

*Standard error of the means. Similar letters indicate no significant differences between the means across the inclusion levels within each plant.

None of the tested herbal plants and the dietary inclusion levels caused a significant increase in oxidative stress in broiler chickens. Similarly no significant antioxidant effects were observed for any of the tested herbal plants, except that the inclusion levels of 0.5 and 1.0% of mostly *Rosmarinus officinalis* and partially of *Origanum minutiflorum*, *Thymbra spicata* and *Salvia tomentosa* increased AOP in broiler chickens with no reflection in the bird's performance of this improvement. These results were supported by previous in vitro studies on *Thyme* and *Rosemary* (Deans *et al.* 1993; Scheeder *et al.* 2000). In vivo results supporting our finding indicated that the addition of thyme extract (Florou-Paneri *et al.* 2005) and rosemary extract (Lopez-Bote *et al.* 1998) increased oxidative stability of poultry meat.

In contrast to in vitro findings (Kivan *et al.* 1991; Karaman *et al.* 2001; Sagdic *et al.* 2002: Sagic and Ozcan 2003; Sagdic *et al.* 2005; Jamroz *et al.* 2005) no significant beneficial antibacterial effects (in terms of *Psychrophilic aerobic bacteria*, *Coliform bacteria*, *Enterobacteriae* and *Lactic acid bacteri*a counts) of the same tested herbs and spices were in vivo obtained from the present case despite of significant reductions in the counts of *E. Coli* by increased level of herb inclusion from 0.5 to 1.5% in broiler diet, similar to the previous results (Sivropoulou *et al*. 1996). It can be speculated that in vivo conditions are more complicated than and different from the conditions set up as in vitro. Therefore, in vitro beneficial antibacterial effects of an extract or herbal plant would not always be refelected same as in vivo. In addition, the tested experimental conditions were upmost environmentally controlled and hygienic conditions where herbal plants may not exert these beneficial effects, whereas there is a high possibility of these beneficial effects to be obtained in practically harsh conditions (commercial animal production).

As overall body weights of broiler chickens at 21 and 42 d-old age did not significantly differ between and within the herb based diets. Birds fed on the diets at an inclusion level from 0 to 0.5% had better weight gains compared to the remaining levels. This was so apperant at 21 d-old, but differences became insignificant ($p>0.05$) at 42 d-old. There was a tendency in reduced weight gain by increasing levels of herbs in the diets, particularly for *Origanum minutiflorum* and *Thymbra spicata*. In contrary increasing the dietary inclusion levels from 0.5 to 1.5% of herbs in the broiler diets significantly ($p<0.05$) increased amount of feed consumption, thereby a worsened efficiency of feed conversion ratio, particularly for *Origanum minutiflorum*, *Rosmarinus officinalis*, *Salvia tomentosa* and for *Thymbra spicata* at 21 d-old age and for *Origanum minutiflorum*, *Thymbra spicata* and *Rosmarinus officinalis* at 42 d-old age. Impairment in bird performance with the increased dosages of herbs of *Origanum minutiflorum*, *Thymbra spicata*, *Rosmarinus officinalis*, *Salvia tomentasa* and for *Mrytus cummunis* was highly correlated with their contents of carvacrol, active agent (Table 2). In addition increasing the amount of herbs in broiler diets does not only cause to increased

amount of ingested carvacrol, but also a probable increased amount of ingested antinutritional factors present in these plants.

In contrary, some studies (Florou-Paneri *et al.* 2005; Basmacioglu *et al.* 2004; Bampidis *et al.* 2005) did not report impairment of performance of turkeys fed on increasing level of carvacrol from *Thyme* and *Thyme* itself as herb. It can be speculated that the size of digestive tract in turkey which is greater than in broiler chicken can play important role to overcome any antinutritional effects of carvacrol and antinutritional factors present in these herbs. Similarly we observed that the broiler chickens at 42 d-old age with a greater size of digestive tract managed to overcome these negative effects of herbs, compared to the birds at 21 d-old age. Our histological findings have also confirmed that the epitheal surface area determined by longer and higher villus remarkably increased at 42 d-old age (Table 9). Moreover, dry matter retention at 21 d-old tended to decrease by increasing the dietary inclusion rate of herbs in broiler diets. This was more apperant for the diet based on *Thymbra spicata* and *Origanum minutiflorum*, which have high content of carvacrol.

The present results suggested the addition of tested herbs more than a level of 0.25% in all cases and 0.5% in some cases caused depression in bird's performance. This has been confirmed by previous results obtained with the high inclusion levels (>0.25%) of *Rheum rhabarbarum* (Wenk 2000). The same author did not report any changes in animal performance by the use of spicy herbs of *Clove*, *Pepper* and *Thyme*. This result was in parallel with a *Cumin* spice used in the present case. The spices such as *Cumin* may introduce to the broiler diets appreciable amount of nutrients for absorption and metabolism (protein and oil). This result was supported by the longer villus obtained with *Cumin* in 21 d-old age obtained from the present case. Improved epithelial surface area were obtained with the garlic powder with no significant improvement in bird' performance (Abdullah et al., 2010), whereas another study reported improved growth rate and FCR with 0.25% garlic powder based diet in broiler chicken (Onu, 2010). Excessive dosages of herbs and their essential oils can diminish the growth performance in broiler chickens. *Anise* seed can improve weight gain in the broiler diets up to 0.75 g/kg, but the depressed performance was induced by the inclusion rate of 1.5 g/kg (Soltan et al., 2008). Similarly an inclusion rate of more than 0.5% of *rosemary* leaves depressed growth and lowered nutrient utilization (Ghazalah and Ali, 2008). The essential oils or plants part of herbs can have growth-promoting effects in some studies (Alcicek et al., 2003, Florou-Paneri et al., 2006, Ghazalah and Ali, 2008, Al-Kassie 2009, Bozkurt et al., 2009, Borazjanizadeh et al., 2011) whereas no significant effects were observed in some other studies (Jang et al., 2007, Isabel and Santos 2009, Bozkurt et al., 2009, Najafi and Torki 2010, Aami-Azghadi and Golian 2010). Amongs the herbs the best performance was obtained by the diets of *thyme* oil and *yarrow* herb whereas lowest performance was with the diets of *oregano* herbs and *yarrow* oil (Cross et al., 2007), which implied that the variability in chemical composition of different herbs is the major reason for having sporadic effect on the growth performance as to we have observed the similar findings.

In conclusion the tested herbs can be incorporated into broiler diets up to a level of 0.25-0.5% without causing any changes in performance of animals due to their potential beneficial antibacterial (reduced c.f.u of *E.coli*) and antioxidant (improved antioxidant potential) effects.

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