Iranian Journal of Applied Animal Science

Intestinal Morphology and Microbiology of Broiler Chicken Fed Diets Containing Myrtle (*Myrtus communis*) Essential Oil Supplementation

Research Article

S. Ghazanfari[1*], M. Adib Moradi[2] and M. Mahmoodi Bardzardi[1]

[1] Department of Animal Science, College of Abouraihan, University of Tehran, Tehran, Iran
[2] Department of Basic Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Received on: 8 Oct 2013
Revised on: 16 Dec 2013
Accepted on: 30 Dec 2013
Online Published on: Sep 2014

*Correspondence E-mail: shghazanfari@ut.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

This experiment was conducted to determine the effects of dietary myrtle essential oil on small intestinal morphology and microbiology of broiler chickens. Two hundred day-old broiler chickens (Ross 308) were allocated to 5 treatments, 4 replications with a completely randomized design. The diets were supplemented with: no myrtle essential oil (control), each of myrtle essential oil at levels of 100, 200 and 300 mg/kg and 600 mg/kg flavophospholipol antibiotic of diet. On day 42 days of age, one bird from each replicate was selected and then slaughtered and the intestinal microflora contents as well as morphology were analyzed. Dietary myrtle essential oil and antibiotic supplementations consistently resulted in significantly longer villus height, lower epithelial thickness and lower goblet cell number of the small intestinal at 42 day of age compared with the control treatment (P<0.05). Also, lower *Escherichia coli* count and higher *Lactobacillus* count of the caecum was found by inclusion of myrtle essential oil in the diet (P<0.001). The results of this study suggested that myrtle essential oil may be considered as an alternative to antibiotic growth promoter may improve poultry performance.

KEY WORDS broiler, gut morphology, microbiology, myrtle essential oil.

INTRODUCTION

Use of antibiotic growth promoters to improve animal performance and health has been practiced during the last 50 years. Antibiotics have generated resistant strains by transferring resistance to other species, especially in shared strains between humans and animals, and made serious problems in public health and livestock (Jang *et al.* 2007). Recently, the use of essential oil has become popular due to antimicrobial properties. Essential oils may specifically enhance the activities of digestive enzymes and nutrient absorption, which may improve the values of feed (DI Pasqua *et al.* 2007). Also, the efficient feed utilization of broiler chickens such as essential oil could be associated with changes in the intestinal functions, for example villi structure. It is well documented that the structure and morphology of villi play a substantial role in the digestion and absorption of nutrients in the gastrointestinal tract. Nutrient absorption in the gastrointestinal tract is more effective to increase in the size and height of intestinal villi. Factors, such as diet were found to influence the morphology of the intestinal villi. In various domestic birds, there was a correlation between the morphology of the intestinal villi and food habits (Zulkifli *et al.* 2009). Digestive microflora populations affect broiler performance and health. These effects may be due to the complex interactions that influence the intestinal environment and the development and responses of the host immune system against pathogenic and nonpathogenic antigens (Jang *et al*. 2007). The understanding and monitoring of the dynamics of gut microbial ecology are important to the development of alternative methods to modulate the microbial communities under situations of debilitating stress and disease, such as during coccidial infection in poultry (Oviedo-Rondo´n *et al*. 2006). *Myrtus communis* (Myrtaceae family and subfamily Myrtoideae) is a plant that has been used since ancient times for medicinal, food and spice purposes (Romani *et al*. 1999). This aromatic plant grows wild in the coastal areas of Tunisia, Morocco, Greece, Turkey and France and it is cultivated in these countries as well as in Iran (Weyerstahi *et al*. 1994). The fruits mostly contain volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids. Also the leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (Romani *et al*. 1999). Ozek *et al*. (2000) reported the most important constituents of myrtle essential oil are myrtenol, myrtenol acetate, limonene, linalool, α-pinene, 1, 8-cineole, β-caryophyllenein, p-cymene, geraniol, nerol, phenylpropanoid and methyleugenol.

The purpose of this study was to determine the effects of increasing dietary levels of myrtle essential oil supplementation in comparison with an antibiotic growth promoter on microbial populations of *Lactobacilli* and *Escherichia coli* and small intestinal histological characteristics in broiler chickens.

MATERIALS AND METHODS

Myrtle essential oil was obtained from Zardband Pharmaceutical Company, (Tehran, Iran) that myrtle essential oil obtained from the leaves *Myrtus communis* by steam distillation. The myrtle essential oil was analyzed by a gas chromatograph (9-A-Shimadzu) and GC/MS (Varian-3400) column (DB-1, 60 mm×0.25 mm fused silica capillary column, film thickness 0.25 µm) using a temperature program of 40 ˚C-220 ˚C at a rate of 4 ˚C/min, an injector temperature of 260 ˚C and using the carrier gas helium. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data. The study took place in the poultry research station at University of Tehran, Iran, in 2012. Two hundred day-old broiler chicks (Ross 308) were weighed and randomly assigned into five treatments, each treatment was further sub-divided into four replicates of 10 birds per cage. The experimental diets were based on corn-soybean meal containing vegetable oil. Feed and water were consumed *ad libitum*. Temperature and relative humidity were maintained within the optimum range. The lighting program consisted of a period of 23 h light and 1 h of darkness during the trial. The ambient temperature was gradually decreased from 33 to 20 ˚C on day 42. The birds were kept in 20 pens (1×1.1 m) at total of period. The dietary treatments consisted of a basal diet (Ross 308 recommendation) (Aviagen. 2002), considered as control, an antibiotic treatment received 600 mg/kg (Recommended level for growth promotion by animal drug company) of flavophospholipol (antimicrobial growth promoter), myrtle essential oil at levels of 100, 200 and 300 mg/kg feed. The ingredients and the composition of control diet are presented in Table 2. The myrtle essential oil was mixed in a carrier (soybean oil), which was then added to the basal diet. All diets were prepared freshly every week and diets were in mash form. Gas chromatograph-MS analysis of the myrtle essential oil is presented in Tables 1.

Table 1 Chemical composition of the essential oil of *Myrtus communis*

No	Compound	Retention index	%
1	Isobutyl isobutyrate	890	0.8
2	α-thujene	920	0.24
3	α-pinene	932	30.1
4	Myrcene	981	0.2
5	p-cymene	1019	0.5
6	Limonene	1023	20.4
7	1, 8-cineole	1027	18.1
8	Y-terpinene	1057	0.5
9	Terpinolene	1084	0.4
10	Linalool	1087	9.8
11	α-campholenal	1120	0.3
12	Terpinene-4-ol	1168	0.4
13	α-terpineole	1179	3.3
14	Trans-carveole	1213	0.5
15	Cis-carveole	1218	0.1
16	Geraniol	1244	1.0
17	Linalyl acetate	1249	4.2
18	Methyl geranate	1312	0.2
19	α-terpinyl acetate	1342	1.2
20	Methyl eugenol	1370	1.7
21	ß-caryophyllene	1432	0.3
22	α-humulene	1464	0.1
23	Spathulenol	1564	0.1
24	Caryophylleneb epoxide	1588	0.3

All chicks were fed starter diets from 1 to 10 days of age, grower diets from 11 to 24 days of age and finisher diets from 25 to 42 days of age. At 42 days of age, one bird from each pen (closest to the mean pen body weight) was selected. The birds were sacrificed by cervical dislocation and the digestive tract was carefully excised. After removing the intestinal contents, approximately 3 cm lengths of duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after Meckel's diverticulum) were removed for gut morphological measurements. Intestinal samples from each section were immersed in formaldehyde, before fixation in Bouin's solution and paraffin embedding. Histological examinations were carried out according to the method of Iji *et al.* (2001).

Table 2 Composition of basal diet as fresh matter basis and analyzed contents of main nutrients (%)

Feed ingredients (g/kg)	Starter (1-10 d)	Grower (11-24 d)	Finisher (24-42 d)
Maize, yellow	55.9	57.7	63.0
Soybean meal 44%	36.7	34.3	29.2
Soybean oil	2.79	4.17	4.00
Dicalcium phosphate	1.79	1.45	1.35
Calcium carbonate	1.33	1.16	1.14
Common salt	0.20	0.20	0.29
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-methionine	0.39	0.32	0.27
L-lysine HCl	0.40	0.22	0.20
Total	100	100	100
Metabolizable energy (kcal/kg)	2938	3055	3100
Crude protein	21.48	20.37	18.57
Calcium	1.01	0.91	0.82
Available phosphorus	0.51	0.44	0.41
Lysine	1.39	1.19	1.06
Methionine	0.72	0.65	0.57
Sodium	0.11	0.11	0.14
chlorine	0.24	0.20	0.25

[1] Contained per kilogram: vitamin A: 5500000 IU; vitamin D_3: 1500000 IU; vitamin E: 15000 mg; vitamin K: 800 mg; vitamin B_{12}: 15 mg; Thiamine: 1000 mg; Riboflavin: 4000 mg; Niacin: 25000 mg; Biotin: 30 mg; Folic acid: 500 mg; Pantothenic acid: 5000 mg and Pyridoxine: 1500 mg.
[2] Contained per kilogram: Cu: 12000 mg; Fe: 35000 mg; Zn: 25000 mg; Co: 150 mg; I: 500 mg; Co: 150 mg; Se: 120 mg and Mn: 38000 mg.

Paraffin sections at 6 µm thickness were made from each sample, stained with hematoxylin and eosin, and examined by light microscopy. Villus height, crypt depth, goblet cell number and epithelial thickness were analyzed from each preparation. The length of the intestinal villi and the depth of the intestinal crypt were measured with linear scaled graticule. The number of goblet cells/μm^2 area of the villus and crypts was measured by 25 squared graticule. Also, digest content samples from the cecum of 2 birds from each replicate were collected into glass containers at slaughter. Samples put on ice till they were transported to the laboratory for enumeration of microbial populations. The populations of *Escherichia coli* and *Lactobacilli* bacteria were then estimated as the log 10 of colony forming units (cfu) per gram of caecum content. Sterilized PBS (99 mL) was added (1:100) to 1 g of fresh material, and then subsequent dilutions prepared. *Escherichia coli* was cultured on MacConkey agar (Merck, Germany) at 37 ˚C for 24 hours, and the presence of *Escherichia coli* then determined. *Lactobacilli* was enumerated on MRS (Merck, Germany) agar after incubation for 48-72 h at 37˚.

A completely randomized design (CRD) was employed. Data were analyzed by General Linear Models procedure of Statistical Analysis System (SAS, 2004).

Logarithmic ($\log_{10}$) transformation was applied for microbial colony forming unit (cfu). Duncan's multiple range test were used for comparison of means ($P<0.05$).

RESULTS AND DISCUSSION

The major components were α-Pinene (30.1%), Limonene (20.4%), 1, 8-cineole (18.1%), Linalool (9.8%), Linalyl acetate (4.2%) and α-terpineole (3.3%).

Morphometric measurements made in the small intestinal (duodenum, jejunum and ileum) of broiler chicken are presented in Table 3. It was found that dietary myrtle essential oil and antibiotic supplementations consistently resulted in significantly longer villus height of the jejunum ($P<0.05$) and ileum ($P=0.06$) at 42 day of age compared with the control treatment.

No differences were found in crypt depth and villous height to crypt depth ratio of small intestinal at 42 days of age by the inclusion of myrtle essential oil and antibiotic supplementations in the diet. Myrtle essential oil and antibiotic supplementations significantly influenced epithelial thickness in this study may suggest an enhanced rate of nutrient absorption. Myrtle essential oil and antibiotic supplementations significantly decreased epithelial thickness of small intestinal ($P<0.05$) at 42 days of age compared with the control treatment. At 42 days of age, the goblet cell number of small intestinal tended to be decreased ($P<0.05$) for broiler chickens fed the antibiotic supplemented diet than the myrtle essential oil supplement. In addition to, dietary myrtle essential oil supplement had a lower number of goblet cell ($P<0.05$) compared with the control treatment.

Animals of all treatments had a normal intestinal structure; the highest villi were in duodenum followed by lower villi in jejunum and the lowest being in ileum. The intestinal villi were slim and finger-shaped and the intestinal mucosa revealed no histopathological changes in animals from all treatments (Figure 1 and 2).

The effect of dietary myrtle essential oil supplementation on *Escherichia coli* and *Lactobacilli* numbers in cecum contents of broilers are shown in Table 4. The counts of *Escherichia coli* in the cecum of broilers fed on diets with myrtle essential oils at levels of 200 and 300 mg/kg feed and antibiotic were decreased significantly ($P<0.0001$) as compared with the control treatment. On the other hand, the counts of *Lactobacilli* were significantly higher in birds fed the diet containing myrtle essential oil and antibiotic than those given the control diet ($P<0.0001$).

In the present study, The major components myrtle essential oil were α-pinene (30.1%), Limonene (20.4%), 1,8-Cineole (18.1%), Linalool (9.8%), Linalyl acetate (4.2%) and α-terpineole (3.3%).

Table 3 Effect of dietary myrtle essential oil supplementation on small intestine morphology of broiler chickens at 42 days of age

Item	Treatments					±SEM	P-value
	100 mg/kg	200 mg/kg	300 mg/kg	Control	Antibiotic		
Duodenum							
Villous height (µm)	1754	1765	1757	1737	1760	23.7	0.55
Crypt depth (µm)	150^{a}	147^{ab}	150^{a}	142^{b}	149^{a}	2.13	0.1
Villous height to crypt depth ratio	11.7	12	11.7	12.2	11.8	0.18	0.23
Epithelial thickness (µm)	43^{b}	45^{ab}	46.5^{ab}	49.2^{a}	43^{b}	1.37	0.02
Goblet cell number	9^{ab}	9^{ab}	8.2^{ab}	10.2^{a}	7.2^{b}	0.66	0.05
Jejunum							
Villous height (µm)	859^{a}	864^{a}	860^{a}	844^{b}	860^{a}	3.95	0.02
Crypt depth (µm)	153	153	155	145	148	3.37	0.23
Villous height to crypt depth ratio	5.62	5.66	5.55	5.83	5.8	0.12	0.44
Epithelial thickness (µm)	38.7^{ab}	37^{abc}	35.2^{bc}	41^{a}	34^{c}	1.31	0.01
Goblet cell number	8.7^{a}	8^{ab}	8.2^{ab}	9.7^{a}	6.5^{b}	0.67	0.04
Ileum							
Villous height (µm)	804^{a}	800^{a}	791^{ab}	775^{b}	789^{ab}	6.6	0.06
Crypt depth (µm)	104	108	108	99.5	109	4.12	0.43
Villous height to crypt depth ratio	7.77	7.43	7.36	7.84	7.25	0.34	0.68
Epithelial thickness (µm)	32.5^{ab}	27.7^{bc}	26.2^{bc}	37.7^{a}	25.7^{c}	2.05	0.004
Goblet cell number	8.5^{ab}	7.5^{b}	7^{bc}	9.5^{a}	5.75^{c}	0.54	0.002

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Rasooli *et al.* (2002) reported that the major components of *Myryus communis*. Essential oil are α-pinene (29.4%), Limonene (21.2%), 1, 8-cineole (18%), Linalool (10.6%), Linalyl acetate (4.6%) and α-terpineole (3.1%). Chryssavgi *et al.* (2008) reported that the major components of *Myrtus communis*. Essential oil are myrtenyl acetate (39%), 1, 8-cineole (13.5%), α-pinene (10.9%) and linalyl acetate (3.6%).

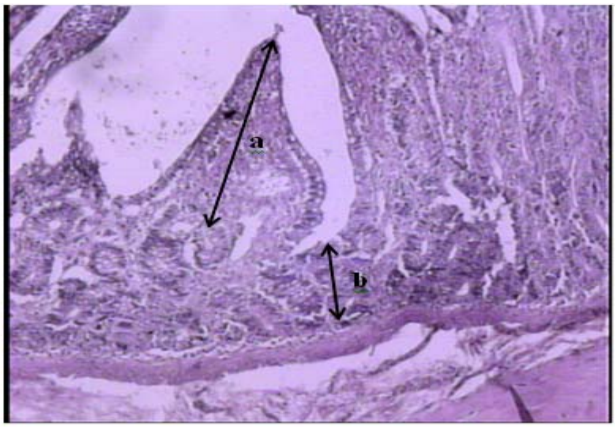

Figure 1 Photomicrograph of cross section of jejunum full thickness from control animal
(a: indicating villous height and b: indicating crypt depth as measured) H and E, X 100

However, Akin *et al.* (2010) did not find α-pinene and 1, 8-cineole in myrtle oil. Romani *et al.* (1999) has previously reported this composition, but they have also suggested that environmental factors such as geography, temperature, day length and nutrients, may modify the chemical composition of the myrtle oil. These factors influence plant biosynthesis pathways and consequently, the relative proportions of the main characteristic compounds (Biricik *et al.* 2012).

Good intestinal health will lead to a better growth rate and feed efficiency in poultry. Intestinal morphology characteristics are affected by dietary treatments. In this experiment, the results showed that the use of myrtle essential oil improved intestinal morphology characteristics such as longer villus height and lower epithelial thickness, which this reaction can lead to increase feed utilization and improve performance.

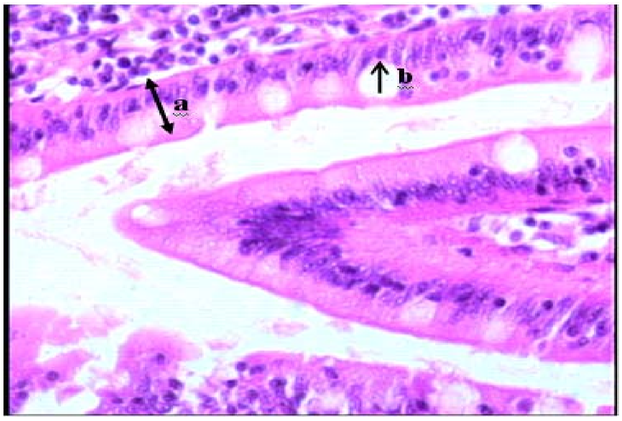

Figure 2 Photomicrograph of cross section of jejunum full thickness from control animal
(a: indicating epithelial thickness and b: indicating goblet cell as measured) H and E, X 400
(Bar=500 µm)

Similar observations were reported by Garcia *et al.* (2007), who showed that using medicinal plants in feed causes a higher villous in chickens. They suggest that medicinal plants decrease the total harmful bacteria in the intestinal wall and cause a reduction in production of toxic compounds and damage to intestinal epithelial cells such as shorter villi and deeper crypts.

Table 4 The effect of dietary myrtle essential oil supplementation on *Escherichia coli* and *Lactobacilli* numbers in cecum contents of broilers at 42 days of age

Item	Treatments						
	100 mg/kg	200 mg/kg	300 mg/kg	Control	Antibiotic	±SEM	P-value
Escherichia coli (log cfu g^{-1})	5.62[a]	4.54[b]	3.53[c]	5.74[a]	3.33[c]	0.09	< 0.0001
Lactobacilli (log cfu g^{-1})	5.68[b]	5.64[b]	5.78[b]	4.46[c]	6.25[a]	0.06	< 0.0001

SEM: standard error of the means.

This function could lead to a conversion in intestinal morphology. Giannenas *et al.* (2010) reported a shorter villus and a deeper crypt when the counts of pathogenic bacteria increase in the gastrointestinal tract, which result in fewer absorptive and more secretory cells. The size and height of the villi are important for intestinal function and diet is one of the important factors that could alter the morphology of intestinal villi.

In this experiment, shows that villi height increased significantly in birds given diet containing myrtle essential oil and this is an important factor in the efficiency of digestion and absorption of nutrients. The height of the villi may reflect the surface area. The increase in villi height suggests an increase in the absorptive surface area and greater absorption of available nutrients and thus, feed efficiency will be improved. Also, it was reported that the active principles of herbs, essential oils or plant extracts may act as digestibility enhancers, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (Cross *et al.* 2007).

In this study, higher villus height could be related to antibacterial property of antibiotic and myrtle oil. The mucus layer in the small intestine plays an important role in protection of the small intestinal epithelial cells and in transport between the lumen and the brush border membrane and thus, the ontogeny of its development has extensive implications for intestinal function. Mucin is secreted by goblet cells along the villi within the epithelium. The present study has indicated that number of goblet cells was decreased in the small intestine at 42 days of age for birds fed the myrtle essential oil and antibiotic supplementations. The study has shown that evidence of enhanced mucin secretion in response to differing intestinal microbial populations has been presented. As antibiotics and myrtle essential oil supplementations could control colonization of numerous pathogenic and nonpathogenic species of bacteria in chicks' gut (Bedford, 2000). We suggested that may lead to a lower mucus secretion, resulting in a decreased number of goblet cell. Uni *et al.* (2003) showed that delayed access to feed for 48 h post hatch resulted in an increase in goblet cell density in broiler. In contrary, Jamroz *et al.* (2006), who observed qualitative increases in the number of goblet cells and in mucin secretion at the surface of the villi of the jejunum when feeding broilers a mixture of 5, 3 and 2 mg/kg of carvacrol, cinnamaldehyde, and capsicum oleoresin.

In this study, the supplementation of myrtle essential oil decreased the colony unit forming of *Escherichia coli* and increased the colony unit forming of *Lactobacilli* (P<0.05). This was in accordance with the finding of some researchers who mentioned that essential oil (Jang *et al.* 2007) restrains pathogen bacteria as *Escherichia coli* by increasing non-pathogens. Jang *et al.* (2007) found that, CFU of *Escherichia coli* decreased and CFU of *Lactobacilli* increased by addition of essential oils at the level of 50 ppm. Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of *Escherichia coli* and *Clostridium perfiringensin* ceca (Jamroz and Kamel, 2002). Essential oil basically consists of two classes of compound, the terpens and phenylpropenes. Terpens and phenylpropenes are synthesized by the mevalonic and shimic pathway, respectively. Generally, the essential oil possesses the strongest antibacterial properties against pathogens. Essential oil contains a high percentage of phenolic compounds such as carvacrol, eugenol and thymol (Brenes and Roura, 2010). Improvements in treatments containing myrtle essential oil, probably is due to the myrtle oil antibacterial properties. Rodriguez-Vaquero *et al.* (2007) demonstrated the antimicrobial properties of phenolic compounds against *Escherichia coli*.

It is clear that controlling the microflora could positively influence birds' performance and dietary feed additives such as essential oils may enrich the diversity of *Lactobacillus* (Mandel *et al.* 2000). *Lactobacillus* species may repress pathogenic microorganism as aerobic bacteria, *Coliform*, *Escherichia coli*, *Enterococcusin* chicken jejunum and caecum, by this the microbial balance may be restored and the natural stability of jejunal and caecal microbiota. *Lactobacilli* bacteria in the small intestine banded to receptors located on villus and show a very close relationship between intestinal bacteria and villus. So, it could be assumed that the bacteria can cause changes in the villi of the small intestine (Lan *et al.* 2004).

CONCLUSION

The morphology and functionality of the different regions of the intestinal tract seem to be a flexible system that is able to adapt to the needs of the organism. However, little information is available on how phytogenic compounds may affect gastrointestinal morphology and functionality. In the present experiment, supplementation with the myrtle essential oil had the most notable effects on small intestinal morphology, causing an increase in villus height as well as a decrease in epithelial thickness and total number of goblet cells. Adding myrtle essential oil to broiler diets improved gut microflora (as measured by changes in populations of *Escherichia coli* and *Lactobacillus*). The results of this study suggested that myrtle essential oil may be considered as an alternative to antibiotic growth promoter may improve poultry performance.

ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support of University of Tehran for this research under grant number 27341 / 07.

REFERENCES

Akin M., Aktumsek A. and Nostro A. (2010). Antibacterial activity and composition of the essential oils of eucalyptus camaldulensis dehn and *Myrtus communis* growing in nothern Cyprus. *African J. Biotechnol*. **9,** 531-535.

Aviagen. (2002). Ross Broiler Management Manual. Aviagen Ltd., Newbridge, Scotland.

Bedford M. (2000). Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimize subsequent problems. *World Poult. Sci. J.* **56,** 347-365.

Biricik H., Yesilbag D., Gezen S.S. and Bulbul T. (2012). Effects of dietary myrtle (*Myrtus communis*) supplementation on growth performance, meat oxidative stability, meat quality and erythrocyte parameters in quails. *Rev. Med. Vet.* **163,** 131-138.

Brenes A. and Roura E. (2010) Essential oils in poultry nutrition: main effects and modes of action. *Anim. Feed. Sci. Technol*. **158,** 1-14.

Chryssavgi G., Vassiliki P., Athanasios M., Kibouris T. and Komaitis M. (2008). Essential oil composition of *Pistacia lentiscus* and *Myrtus communis*: evaluation of antioxidant capacity of methanolic extracts. *Food Chem*. **107,** 1120-1130.

Cross D.E., Acamovic T., Deans S.G. and Cdevitt R.M. (2007). The effects of dietary inclusion of herbs and their volatile oils on the performance of growing chickens. *Br. Poult. Sci.* **43,** 33-35.

Di Pasqua R., Betts G., Hoskins N., Edwards M., Ercolini D. and Mauriello G. (2007). Membrane toxicity of antimicrobial compounds from essential oils. *J. Agric. Food Chem.* **55,** 4863-4870.

Garcia V., Catala-Gregori P., Hernandez F., Megias M.D. and Madrid J. (2007). Effect of formic acid and plant extracts on growth, nutrient, digestibility, intestine mucosa morphology and meat yield of broilers. *J. Appl. Poult. Res*. **16,** 555-562.

Giannenas I., Tontis D., Tsalie E., Chronis E.F., Doukas D. and Kyriazakis I. (2010). Influence of dietary mushroom agaricus bisporus on intestinal morphology and microflora composition in broiler chickens. *Res. Vet. Sci.* **89,** 78-84.

Iji P.A., Saki A.A. and Tivey D.R. (2001). Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. *Anim. Feed Sci. Technol.* **89,** 175-188.

Jamroz D. and Kamel C. (2002). Plant extracts enhance broiler performance*. J. Anim. Sci.* **80,** 41-46.

Jamroz D., Wertelecki T., Houszka M. and Kamel C. (2006). Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *J. Anim. Physiol.* **90,** 255-268.

Jang I.S., Ko Y.H., Kahn S.Y. and Lee C.Y. (2007). Effect of commerical essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler ckickens. *Anim. Feed Sci. Technol.* **134,** 304-315.

Lan P.T., Sakamoto M. and Benno Y. (2004). Effects of two probiotic *Lactobacillus* strains on jejunal and caecal microbiota of broiler chicken under acute heat stress condition as revealed by molecular analysis of 16S rRNA genes. *Microbiol. Immunol*. **8,** 917-929.

Mandel L., Biswas T. and Sarkar S.K. (2000). Broilers perform well on herbs or enzymes in maize diet. *World Poult.* **5,** 19-21.

Oviedo-Rondon E.O., Clemente-Hernandez S., Salvador F., Williams P., Losa R. and Stephen F. (2006). Essential oils on mixed coccidian vaccination and infection in broilers. *Int. J. Poult. Sci.* **5,** 723-730.

Ozek T., Demirci B. and Baser K.H.C. (2000). Chemical composition of Turkish myrtle oil. *J. Essent. Oil. Res.* **12,** 541-544.

Rasooli I., Moosavi M.L., Rezaee M.B. and Jaimand K. (2002). Susceptibility of microorganisms to *Myrtus communis* essential oil and its chemical composition. *J. Agric. Sci. Technol.* **4,** 127-133.

Rodriguez-Vaquero M.J., Alberto M.R. and Manca De Nadra M.C. (2007). Antibacterial effect of phenolic compounds from different wines. *Food Contr*. **18,** 93-101.

Romani A., Mulinacci N., Pinelli P., Vincieri F.F. and Tattini M. (1999). Identification and quantation of polyphenols in *Myrtus communis* leaves. *Chromatographia*. **49,** 17-20.

SAS Institute. (2004). SAS®/STAT Software, Release 8. SAS Institute, Inc., Cary, NC.

Uni Z., Smirnov A. and Sklan D. (2003). Pre and post hatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. *Poult. Sci.* **82,** 320-327.

Weyerstahi P., Marschall H. and Rustaiyan A. (1994). Constituents of the essential oil of *Myrtus communis* from Iran. *Flavour Frag. J*. **9,** 333-337.

Zulkifli I., Iman Rahayu H.S., Alimon A.R., Vidyadaran M.K. and Babjee S.A. (2009). Gut microflora and intestinal morphology of commercial broiler chickens and Red Jungle Fowl fed diets containing palm kernel meal. *Arch. Geflügelkd*. **73,** 49-55.