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Full Length Research Paper

Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecal microflora and serum antioxidant activity of broiler chickens

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A feeding trial was conducted to investigate the effects of dietary oregano (*Origanum vulgare* L.) essential oil (OEO) on broiler performance, cecal microflora and serum antioxidant activity. One hundred and eighty (180) 1-day old broiler chicks were randomly divided into four groups. Group I was kept as normal control and received basal diet. Birds of groups II, III and IV were treated with basal diet supplemented with 300, 600 and 1200 mg/kg of OEO. Inclusion of 600 mg/kg of OEO in grower diet significantly increased body weight gain when compared with the control group ($P < 0.05$). Supplementation of 600 and 1200 mg/kg of OEO significantly improved feed conversion ratio compared with the control group in grower and overall experimental periods ($P < 0.05$). Although, populations of lactic acid bacteria remained unaffected ($P > 0.05$), populations of cecal *Escherichia coli* were significantly lower in 300 and 600 mg/kg OEO supplemented groups in comparison with the control and 1200 mg/kg OEO supplemented groups ($P < 0.05$). Although, serum antioxidant activity was not significantly affected by the treatments ($P > 0.05$), antioxidant activity of serum was higher in OEO supplemented groups. In conclusion, OEO exerted growth promoting effects and also displayed potent antibacterial effects against cecal *E. coli*.

Key words: Oregano essential oil, performance, cecal microflora, antioxidant activity.

INTRODUCTION

Supplementation of antibiotics in poultry diets at sub-therapeutic levels has long been shown to increase growth rate (Becker et al., 1955; Stahly et al., 1980; Cromwell, 2002). Furthermore, in the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease as well as to improve meat or egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), drug residues in the body of the birds (Burgat, 1999) and imbalance of normal microflora (Andremont, 2000). However, due to growing concerns over these mentioned problems, the use of antibiotics as growth promoters in animal diets became limited due to public or regulatory pressures. As a consequence, removal of antibiotic growth promoters from poultry diets has triggered researches for suitable natural alternatives to combat the increased potential for bacterial disease development in growing flocks. Actually, the utilization of antibiotics in poultry nutrition and its subsequent associated concerns has created efforts to use different plant compounds as possible natural alternatives (Cross et al., 2007).

Phytogenic feed additives or phytobiotics are plant-derived natural bioactive products used in animal feeding to improve the performance of animals and also affect their growth and health, positively. They are often applied to essential oils, botanicals and extracts derived from

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Abbreviations: DPPH, Diphenyl-2-picryl hydrazyl; **OEO,** oregano essential oil.

herbs. This class of feed additives has recently gained increasing interest, especially for use in poultry nutrition, as can be derived from a significant increase in the number of scientific publications since 2000. This appears to be strongly driven by the ban on most of the antibiotic feed additives within the European Union in 1999, a complete ban enforced in 2006 and ongoing discussions to restrict their use outside the European Union because of speculated risk for generating antibiotic resistance in pathogenic microbiota (Windisch et al., 2008). Selected herbs have long been used as complementary and alternative medicine to improve human health or to cure human disease. Recent advances in science have allowed for the identification of active components from selected phytobiotics and investigation into the underlying mechanisms of action relating to these components in the animal's body. Recently, animal nutritionists have attempted to use some phytobiotics as alternatives to in-feed antibiotics for young animals and birds (Mao et al., 2005; Kommera et al., 2006; Peeters et al., 2006; Yuan et al., 2006). Phytobiotics, however, are a relatively new class of feed additives and our knowledge is still rather limited regarding their modes of action and aspects of their application.

Oregano (*Origanum vulgare* L.) is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia (Vokou et al., 1993). The essential oil obtained from *O. vulgare* subsp. *hirtum* plant by a steam distillation process comprises more than 20 ingredients, most of which are phenolic antioxidants (Vekiari et al., 1993). Major components are carvacrol and thymol that constitute about 78 to 82% of the total oil (Adam et al., 1998). It has been suggested that the essential oil derived from oregano possess *in vitro* antimicrobial (Sivropoulou et al., 1996; Lambert et al., 2001), antifungal (Thompson, 1989), insecticidal (Karpouhtsis et al., 1998) and antioxidant (Botsoglou et al., 2002) properties. These properties are mainly attributed to carvacrol and thymol. The activity of other constituents such as the two monoterpene hydrocarbons, γ-terpinene and p-cymene, that often constitute about 5 and 7% of the total oil, respectively (Adam et al., 1998) is uncertain.

Due especially to the *in vitro* antimicrobial property, the application of oregano essential oil (OEO) in poultry production would be expected to have both prophylactic and therapeutic results. This study aimed to assess the potential of oregano essential oil as a dietary supplement for broiler chickens. The effects of this essential oil on the growth, cecal microflora and serum antioxidant activity in broiler chickens were investigated.

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Chemicals Co. (USA). Selective agar media were purchased from Merck (Germany). Essential oil of aerial parts of oregano (*O. vulgare* subsp. *hirtum*) was purchased from Pars Imen Daru Herbal Medicine Co. (Tehran, Iran). All other chemicals were of analytical grade or purer.

Animals, diets and treatments

One hundred and eighty (180) 1-day old male broiler chicks (Ross 308) were obtained from a local commercial hatchery. The birds had an initial average weight of 40.45 g at day old and there were no differences on the initial body weight among the treatments. The birds were randomly divided into 4 groups with three replicates of 15 birds each. Birds were housed in floor pens of identical size (1 × 2 m) using wood shaving as litter. The birds were provided with one hour of darkness following a period of 23 h light, for the entire period of the experiment. The temperature was set at 34°C during the first day, 32°C during the first week and was gradually reduced by 3°C per week to reach a minimum of 23°C at 28 days of age for the rest of the experiment. Relative humidity was between 65 and 75%. Feed and fresh water were provided *ad libitum*.

The experiment lasted for 42 days. To meet the nutrient requirements of the broiler chickens, two basal corn-soybean meal diets for starter (day 1 to 21) and grower (day 22 to 42) period were formulated on the NRC recommendations (NRC, 1994) and contained no antibacterial or anticoccidial additives (Table 1). The dietary treatments were (1) basal diet (control); (2) basal diet supplemented with 300 mg/kg; (3) basal diet supplemented with 600 mg/kg; (4) basal diet supplemented with 1200 mg/kg of OEO. The essential oil was first mixed into the vegetable oil component of the basal diet and then the oil mixture was added to the experimental diets.

The essential oil and its composition

The essential oil was stored in a sealed container at 4°C before administration to experimental diets. Identification of essential oil components was carried out by gas chromatography/mass spectrometry. Analysis by GC/MS was performed using a chromatograph interfaced to a mass spectrometer (HP 5971, USA). Table 2 presents compositions of the essential oil and their associated yields.

Growth performance parameters

All birds were weighed after their arrival from the hatchery to the experimental farm (initial weight) and also at weekly intervals. Weight gain and feed intake of each treatment group were recorded at weekly intervals during the experiment and the feed conversion ratios were calculated subsequently. Mortality was recorded daily.

Bacterial enumeration

At the end of the experiment (day 42), 6 birds from each group (2 birds per replicate) were randomly selected and euthanized by cervical dislocation. The birds were immediately eviscerated for collection of cecal contents. The contents were collected into sterile containers, kept on ice and used for bacteriological measurements within an hour from collection. The samples were serially diluted in 0.85% sterile saline solution for enumeration of lactic acid bacteria and *Escherichia coli* by conventional microbiological techniques using selective agar media. Briefly, enumeration of lactic acid bacteria was determined using MRS agar after incubation in an anaerobic chamber at 37°C for 48 h. The *E. coli* were enumerated on MacConkey agar, after aerobic incubation at 37°C for 24 h.

Table 1. Ingredient and nutrient composition of the basal diets.

Item	Starter (0 to 21 day)	Grower (22 to 42 day)
Ingredient (%)		
Corn	55.59	61.07
Soybean meal	37.32	31.83
Soy oil	2.98	3.41
Limestone	1.21	1.42
Dicalcium phosphate	1.60	1.16
NaCl	0.23	0.18
$NaHCO_3$	0.27	0.23
Vitamin-mineral premix[1]	0.60	0.60
DL-Methionine	0.20	0.10
Calculated nutrient content[2]		
ME (kcal/kg)	2950	3050
CP (%)	21.20	19.16
Lysine (%)	1.14	1.01
Methionine (%)	0.50	0.39
Methionine + cysteine (%)	0.85	0.71
Calcium (%)	0.93	0.90
Available phosphorus (%)	0.44	0.35

[1]Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 µg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.18 mg; copper, 8 mg; selenium, 0.15 mg.
[2]Based on NRC (1994) feed composition tables.

Results were expressed as base 10 logarithm colony-forming units per gram (cfu/g) of cecal contents.

Determination of serum antioxidant activity

In order to assess the effect of dietary OEO on antioxidant activity of broilers' serum, at the end of the experiment, 6 broilers were randomly selected from each treatment (2 birds per replicate) and 5 ml blood samples were taken from the brachial vein into heparinized sterile syringes. Blood samples were then centrifuged at 3,000 × *g* for 15 min. In order to precipitate the proteins of plasma, 1 ml of ethanol was added to the separated plasma and the blend was centrifuged again. The stable DPPH was used for the determination of free radical-scavenging activity of the serum samples. For this, 2 ml of the supernatant obtained after precipitation of plasma proteins, were added to 1 ml of 0.3 mmol/l DPPH methanolic solution. After 10 min incubation at room temperature, the absorbance was recorded at 517 nm using a double beam UV/Visible spectrophotometer (Perkin Elmer, USA). 2 ml of normal saline and 1 ml of DPPH solution were used as the control (Muller et al., 2010).

Statistical analysis

All data were subjected to GLM procedure of the SAS (SAS Institute, 2001) according to a completely randomized design. Differences among treatment group means were determined using Duncan's multiple range test (Duncan, 1955). A significance level of $P < 0.05$ was used.

RESULTS

Growth performance

Performance responses of the broiler chickens are summarized in Table 3. Although, body weight gain in the starter and overall experimental periods were not affected by the dietary treatments, supplementation of 600 mg/kg of OEO in the grower period significantly increased body weight gain compared with the control group ($P < 0.05$). Feed intake was not significantly influenced by dietary inclusion of OEO in any of the growth periods ($P > 0.05$) but as it can be seen in Table 3, broilers which received 300 and 600 mg/kg of OEO in their basal diet, had slightly greater feed intake in grower and overall experimental periods compared with the control group.

Feed conversion ratio was not affected by dietary supplementation of OEO in starter period but, inclusion of 600 and 1200 mg/kg of OEO in grower period significantly improved feed conversion ratio compared with control group ($P < 0.05$). Supplementation of broilers with 600 and 1200 mg/kg of OEO significantly improved feed conversion ratio compared with either the control or 300 mg/kg of OEO supplemented birds during the overall

Table 2. Composition of essential oils of oregano obtained by GC/MS.

Constituent	Yield (%)
α-Thujene	0.29
α-Pinene	0.18
Camphene	0.02
Sabinene	0.18
β-Pinene	0.45
Myrcene	0.27
α -Terpinene	0.82
p-Cymene	1.28
1,8-Cineole	0.96
Limonene	0.55
γ-Terpinene	1.27
Trans-sabinen-hydrate	0.19
Borneol	0.17
Terpinen-4-ol	0.76
α-Terpineol	0.09
Methyl thymyl ether	0.22
Thymol	3.29
Carvacrol	86.06
β-Caryophyllene	0.96
α-Humulene	0.04
Germacrene D	0.06
γ-Cadinene	0.05
β-Bisabolene	0.38
δ-Cadinene	0.10
α-Cadinol	0.03

period ($P < 0.05$).

Cecal microflora

Results of cecal bacterial analysis are shown in Table 4. There were no statistically significant differences among treatments regarding populations of cecal lactic acid bacteria ($P > 0.05$). On the other hand, supplementation of 300 and 600 mg/kg of OEO significantly lowered cecal *E. coli* populations compared with both the control and 1200 mg/kg of OEO supplemented groups ($P < 0.05$).

Serum antioxidant activity

The effect of dietary treatments on the antioxidant activity of serum is presented in Table 5. Before interpreting the results of this table, some aspects of used method should be clarified, firstly.

This method actually is based on absorption spectrophotometry. In this method, the reaction of DPPH is monitored by the decrease in the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears (Brand-Williams et al., 1995). DPPH is a stable nitrogen-centered free radical color which changes from purple to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore, radical scavengers (Ebrahimzadeh et al., 2008; Nabavi et al., 2009). The more final solution become transparent, the absorption at 517 nm will be lower and this means that more amounts of DPPH were reduced by antioxidant compounds. Therefore, higher antioxidant contents of serum can be concluded.

As shown in Table 5, descending trend of the absorbance can be seen as levels of the supplementation increased. As it was mentioned earlier, decrease in the absorbance along with increase in the supplementation indicated that broilers which received higher amounts of OEO in their basal diet, had possessed higher antioxidant content in their serum. Although, there were no statistically significant differences for serum antioxidant activity among treatment groups ($P > 0.05$), chickens with OEO in their diets tended to show a slightly greater serum antioxidant activity than the control group in a dose dependent manner.

DISCUSSION

Results obtained for feed intake in this study are in agreement with those of Cross et al. (2007) who reported that feed intake of broilers fed 1 g/kg of OEO from 7 to 28 days of age remained unaffected. In this study, supplementation of OEO improved both feed conversion ratio and body weight gain. On a contrary, Cross et al. (2007) reported that dietary inclusion of 1 g/kg OEO in broilers' diet could not affect these performance parameters. They suggested that the lack of growth promoting action of OEO was related to the absence of thymol and carvacrol in the essential oil used in their study. Inconsistent with this study, Lewis et al. (2003) reported that performance of growing broilers was not affected by using an oregano-based supplement. Similarly, Botsoglou et al. (2002) reported that inclusion of 50 and 100 mg/kg OEO in the form of Orego-Stim (a commercial supplement based on oregano) could not exert any growth promoting action in experimental broilers. In agreement with this study, Lee et al. (2003) found that some bioactive components of essential oils especially carvacrol, improved feed conversion ratio in broiler chickens. They proposed that the effect of carvacrol on feed conversion ratio could be related to increased efficiency of feed utilization. Similarly, Marcincak et al. (2008) suggested that the effects of herbal essential oils on growth performance may be due to the greater efficiency in the utilization of feed, resulting in enhanced growth. There is evidence which suggests that herbs, spices, various plant extracts and especially

Table 3. Effects of dietary oregano essential oil on growth performance of chickens in different growth periods.

Item	Level of oregano essential oil (mg/kg)				SEM
	0	300	600	1200	
Weight gain (g)					
Starter (0 to 21 days)	631	611	603	613	0.01
Grower (22 to 42 days)	1131^{b}	1202^{ab}	1252^{a}	1200^{ab}	0.01
Overall (0 to 42 days)	1762	1813	1854	1813	0.02
Feed intake (g)					
Starter (0 to 21 days)	960	932	915	931	0.01
Grower (22 to 42 days)	2532	2645	2595	2503	0.03
Overall (0 to 42 days)	3493	3577	3510	3435	0.04
Feed conversion ratio (g/g)					
Starter (0 to 21 days)	1.52	1.53	1.52	1.52	0.001
Grower (22 to 42 days)	2.24^{a}	2.20^{ab}	2.07^{b}	2.09^{b}	0.005
Overall (0 to 42 days)	1.98^{a}	1.97^{a}	1.89^{b}	1.90^{b}	0.003

In each row, means with no common superscript are significantly different ($P < 0.05$).

Table 4. Effect of dietary oregano essential oil on cecal bacterial populations of broiler chickens at 42 days of age (log cfu/g).

Bacteria	Level of oregano essential oil (mg/kg)				SEM
	0	300	600	1200	
Lactic acid bacteria	6.25	6.10	6.24	6.29	0.04
Escherichia coli	5.73^{a}	5.10^{b}	5.11^{b}	5.60^{a}	0.02

In each row, means with no common superscript are significantly different ($P < 0.05$).

Table 5. Effect of dietary oregano essential oil on antioxidant activity of chickens.

Parameter	Level of oregano essential oil (mg/kg)				SEM
	0	300	600	1200	
Absorbance at 517 nm	1.197	1.192	1.139	1.025	0.03

herbal essential oils have appetite and digestion-stimulating properties (Hernandez et al., 2004). Windisch et al. (2008) indicated that phytogenic compounds may specifically enhance activities of digestive enzymes and nutrient absorption.

In this study, cecal populations of lactic acid bacteria were not affected by dietary treatments. On the other hand, supplementation of experimental broilers with 300 and 600 mg/kg OEO significantly lowered viable counts of cecal *E. coli* compared with both the control and 1200 mg/kg OEO supplemented groups. In agreement with this study, Cross et al. (2007) showed that supplementation of 1 g/kg OEO in broilers' diet, could not affect cecal populations of lactic acid bacteria. Generally, there are limited numbers of *in vivo* studies about the effects of OEO on the intestinal microflora of broiler chickens. Nevertheless, Penalver et al. (2005) in their *in vitro* study showed that essential oil of oregano incredibly exerted antibacterial effect against poultry origin strains of *E. coli*. They also suggested that this potent antibacterial activity can widely be attributed to the presence of two major active components of OEO that is, thymol and carvacrol. Helander et al. (1998) investigated the antibacterial mechanism of two major components of OEO, carvacrol and thymol on *E. coli* and reported both carvacrol and thymol, in a similar mechanism; disintegrate the membrane of bacteria, leading to the release of membrane-associated materials to the external medium. They also suggested that thymol and carvacrol are able to penetrate the bacteria and may thus, be able to influence their proliferation.

Although, serum antioxidant activity was not

significantly affected by dietary treatments, a dose dependent increase in antioxidant activity can be observed in the serum of broilers which received OEO in their basal diet. High concentration of unsaturated fatty acids in poultry meat increases susceptibility of chicken meat lipids to oxidative deterioration during storage (Botsoglou et al., 2002). Results of this study show that essential oil of oregano potentially can exert antioxidant property and it may be necessary to incorporate higher levels of this essential oil into diet in order to reach significant antioxidant properties on the serum. Since antioxidant compounds present in OEO can enter the circulatory system, be distributed and finally, retained in muscle and other tissues (Botsoglou et al., 2002), more antioxidant activity of serum may result in higher antioxidant content of meat.

It was suggested that the high antioxidant activity of thymol (a bioactive component presents in essential oil of oregano), is due to the presence of phenolic OH groups which act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation, thus, retarding the hydroxy peroxide formation (Farag et al., 1989). Botsoglou et al. (2002) reported that OEO exerted antioxidant property in meat and abdominal fat of broiler chickens. They also indicated that the antioxidant effect was dose dependent.

Conclusions

In conclusion, the results of this study indicated that OEO might be able to exert growth promoting effects on broiler chickens. Supplementation of broiler diets with 600 and 1200 mg/kg OEO significantly improved feed conversion ratio compared with the control in either grower or overall experimental periods. These effects may be attributed to the better efficiency of feed utilization. Furthermore, this essential oil displayed a potent antibacterial effect and significantly lowered populations of cecal *E. coli*. Moreover, OEO tended to increase serum antioxidant activity in a dose dependent manner. However, higher levels of this essential oil in the diet might be needed in order to reach significant antioxidant properties in the serum. Overall, OEO might be a potential alternative for antibiotic growth promoters as pressure to eliminate these growth promoters in animal feed, increases.

REFERENCES

Adam K, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M (1998). Antifungal activities of *Origanum vulgare* ssp. *Hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human patho- genic fungi. J. Agric. Food. Chem., 46: 1739-1745.

Andremont A (2000). Consequences of antibiotic therapy to the intestinal ecosystem. Ann. Fr. Anesth. Reanim., 19: 395-402.

Becker DE, Terrill SW, Notzold RA (1955). Supplementary protein and the response of the pig to antibiotics. J. Anim. Sci., 14:492-498.

Botsoglou NA, Florou-Paneri P, Christaki E, Fletouris DJ, Spais AB (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br. Poult. Sci., 43: 223-230.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

Burgat V (1999). Residues of drugs of veterinary use in food. Rev. Prat. 41:985-990.

Cromwell GL (2002). Why and how antibiotics are used in swine production. Anim. Biotechnol., 13:7-27.

Cross DE, McDevitt RM, Hillman K, Acamovic T (2007). The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br. Poult. Sci., 48: 496-506.

Duncan DB (1955). Multiple range and multiple F tests. Biometrics, 11:1–42.

Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR (2008). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol., 7(18): 3188-3192.

Farag RS, Badei AZMA, Hewedi FM, El- Baroty GSA (1989). Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. J. Am. Oil Chem. Soc., 66: 792-799.

Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, Von Wright A (1998). Characterization of the action of selected essential oil components on gram negative bacteria. J. Agric. Food Chem., 46: 3590- 3595.

Hernandez F, Madrid J, Garcia V, Orengo J, Magias MD (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci., 83:169-174.

Karpouhtsis I, Pardali E, Feggou E, Kokkini S, Scouras ZG, Mavragani-Tsipidou P (1998). Insecticidal and genotoxic activities of oregano essential oils. J. Agric. Food. Chem., 46: 1111-1115.

Kommera SK, Mateo RD, Neher FJ, Kim SW (2006). Phytobiotics and organic acids as potential alternatives to the use of antibiotics in nursery pig diets. Asian-Australian J. Anim. Sci., 19:1784-1789.

Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol., 91: 453-462.

Lee KW, Everts H, Kappert HJ, Yeom KH, Beynen AC (2003). Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. J. Appl. Poult. Res., 12: 394-399.

Lewis MR, Rose SP, Mackenzie AM, Tucker LA (2003). Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. Br. Poult. Sci., 44: S43-S44.

Mao XF, Piao XS, Lai CH, Li DF, Xing JJ, Shi BL (2005). Effects of α-glucan obtained from the Chinese herb *Astragalus membranaceus* and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic responses of weanling pigs. J. Anim. Sci., 83:2775-2782.

Marcincak S, Cabadaj R, Poplka P (2008). Antioxidative effect of oregano supplemented to broilers on oxidative stability of poultry meat. Slov. Vet. Res., 45: 61-66.

Muller L, Theile K, Bohm V (2010). *In vitro* antioxidant activity of tocopherols and tocotrienols and comparison of vitamin E concentration and lipophilic antioxidant capacity in human plasma. Mol. Nutr. Food Res., 54: 1-12.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B (2009). In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Pharmacog. Mag., 4: 122-126.

NRC (National Research Council) (1994). Nutrient requirements of poultry, 9th rev. edn (Washington, DC, National Academy Press).

Peeters E, Driessen B, Geers R (2006). Influence of supplemental magnesium, tryptophan, vitamin C, vitamin E, and herbs on stress responses and pork quality. J. Anim. Sci., 84:1827-1838.

Penalver P, Huerta B, Borge C, Astorga R, Romero R, Perea A (2005). Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS, 113: 1- 6.

SAS Institute (2001). User's manual, version 8.0. SAS Instituite Inc., Cary, NC.

Sivropoulou A, Paranikolaou E, Nikolaou C, Kokkini S, Lanaras T,

Arsenakis M (1996). Antimicrobial and cytotoxic activities of Origanum essential oils. J. Agric. Food. Chem., 44: 1202-1205.
Sorum H, Sunde M (2001). Resistance to antibiotics in the normal flora of animals. Vet. Res., 32:227-241.
Stahly TS, Cromwell GL, Monegue HJ (1980). Effects of the dietary inclusion of copper and (or) antibiotics on the performance of weanling pigs. J. Anim. Sci., 51:1347-1351.
Thompson DP (1989). Fungitoxic activity of essential oil components on food storage fungi. Mycologia, 81: 151-153.
Vekiari SA, Oreopoulou V, Tzia C, Thomopoulos CD (1993). Oregano flavonoids as lipid antioxidants. J. Am. Oil. Chem. Soc., 70: 483-487.
Vokou S, Kokkini S, Bessiere JM (1993). Geographic variation of Greek oregano (*Origanum vulgare* subsp. *hirtum*) essential oils. Biochem. Syst. Ecol., 21: 287-295.
Windisch W, Shedle K, Kroismayr A (2008). Use of phytogenic products as feed additives for swine and poultry. J. Anim Sci., 86: E140-E148.
Yuan SL, Piao XS, Li DF, Kim SW, Lee HS, Guo PF (2006). Effects of dietary Astragalus polysaccharide on growth performance and immune function in weaned pigs. Anim. Sci., 82:501-507