Journal of Animal and Veterinary Advances 9 (7): 1164-1168, 2010
ISSN: 1680-5593

Performance, Blood Metabolites and Immunocompetaence of Broiler Chicks Fed Diets Included Essentioal Oils of Medicinal Herbs

P. Najafi and M. Torki
Department of Animal Science, Faculty of Agricultural, Razi University,
Imam Avenue, Kermanshah, Postal Code 67155-1158, Iran

Abstract: This study was undertaken to investigate growth performance, blood metabolites and humoral immune response of broiler chicks fed diets supplemented with essential oils of thyme, cinnamon and clove. Two hundred and sixty four days old Ross-308 chicks were weighed and randomly allocated between 24 battery pens. Four iso-nitrogenous and iso-caloric diets including: basal diet (control) and basal diet included 200 mg kg^{-1} essential oils of Thyme (THY), Cinnamon (CIN) and Clove (CLO) were formulated to meet bird requirements and fed chicks in starting (1-21 days), growing (22-42 days) and finishing (43-49 days) periods. Each diet was fed to birds in six pens (replicate) with 11 chicks per pen. At 17 and 26 days of age, all 360 chicks were vaccinated against NDV. Blood samples were withdrawn from the wing vein at days 17, 26, 34, 41 and 47 of age. The non-heparinized blood samples (1.5 mL/chicken-one bird per pen) were placed at 37°C for 2 h, centrifuged (3000×g for 15 min.) to separate sera and stored at 20°C until analysis. The sera were applied to HI test to determine Antibody (Ab) to NDV expressed as reciprocal log 2 values for the highest dilution that displayed HI. The data was analyzed based on completely randomized design by GLM procedure of SAS. Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) were not affected by dietary treatment, except for BWG and FCR during the 22-42 days of age. Chicks fed on THY included diets showed improved FCR and BWG comparing to chicks fed other experimental dietary groups during the growing period. Plasma total cholesterol, triglyceride and HDL were not affected by dietary essential oil inclusion ($p>0.05$). The highest and lowest levels of RBC and HCT were observed in chicks fed on CLO and CIN-included diets, respectively ($p<0.05$). Primary antibody response of CON chicks was higher than THY and CIN chicks ($p<0.05$). Secondary antibody response were not affected by dietary inclusion of essential oil ($p>0.05$).

Key words: Essential oils, performance, immunity, broilers, Iran, blood metabolism

INTRODUCTION

A number of feed additives such as antibiotics have been widely used in the poultry industry for several decades. After the use of most antibiotic growth promoters as feed additives has been banned by the European Union due to cross-resistance against pathogens and residues in tissues, scientists have searched for alternatives to antibiotics. In this view, aromatic plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects and the stimulating effect on animal digestive systems. Aromatic plants have been used traditionally in the therapy of some diseases for a long time in the world. Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable. There are many records in literature working on including herbal plant powders or essential oils extracted from medicinal plants in animal diets (Lopez-Bote *et al*., 1998; Zheng and Wang, 2001; Botsoglou *et al*., 2002; Miura *et al*., 2002; Burt and Reinders, 2003; Jamroz *et al*., 2005).

Thymol, a major component of thyme-essential oils has been widely studied for its antimicrobial properties (Dorman and Deans, 2000). Carvacrol, an isomer of thymol is found in essential oils isolated from oregano and thyme. Like thymol, carvacrol also displays antimicrobial activity (Helander *et al*., 1998). Given their antimicrobial activity, it would be expected that thymol and carvacrol could have positive effects on growth performance in broilers.

Cinnamaldehyde, a main principle of cinnamon essential oil, amounts to approximately 60-75% of the total oil. Lee and Ahn (1998) found that cinnamaldehyde, derived from the cinnamon essential oil, strongly inhibits *Clostridium perfringens* and *Bacteroides fragilis* and

Corresponding Author: M. Torki, Department of Animal Science, Faculty of Agricultural, Razi University, Imam Avenue, Kermanshah, Postal Code 67155-1158, Iran

moderately inhibits *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from human feces. The selective inhibition by cinnamaldehyde of pathogenic, intestinal bacteria may have a pharmacological role in balancing the intestinal microbiota. The wide range of *in vitro* anti-microbial activities of essential oils derived from cinnamon and thyme have been published (Paster *et al*., 1990; Cosentino *et al*., 1999; Dorman and Deans, 2000), supporting their possible use as anti-microbial agents.

Similarly, Clove (*Syzygium arimaticum L.*) has been used as an antiseptic (Robenorst, 1996) and antimicrobial (Dorman and Deans, 2000; Ouattara *et al*., 1997; Teissedre and Waterhouse, 2000; Valero and Salmeroj, 2003). In addition, it has appetizing and stimulating effect of digestion, antifungal (Velluti *et al*., 2003), antiparasitic (Kim *et al*., 2004) and antioxidant (Gülçin *et al*., 2004; Lee and Shibamoto, 2002).

There are very little information in literature evaluating effects of dietary inclusion of essential oils of thyme, cinnamon and clove on performance and immunity of growing broiler chicks at the single experiment, so the objective of the present study was to examine the effect of adding essentiaol oils of these herbal plants on growth performance, blood metabolites and humoral immune response of broilers.

MATERIALS AND METHODS

A total number of 264 days old unsexed Ross 308 broiler chicks were distributed in a completely randomized experimental design in cage batteries with four treatments and six replications of eleven chicks each. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4 the lighting schedule was 24 h light. At days 5-49 the dark time was increased to 1 h. Birds were fed diets (starter: 1-21 days, grower: 22-42 days and finisher 43-49 days). The composition of experimental diets is shown in Table 1. Diets prepared without additive as Control (CON) or with 200 mg kg^{-1} essential oils of thyme, cinnamon and clove. Feed and water were available *ad libitum*. Data were collected for Body Weight (BW) at 21, 42 and 49 days of age and for Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) during periods of 0-21, 22-42 and 43-49.

One bird of each replicate was randomly selected, weighed after feed deprivation for 12 h and killed by cervical dislocation at 47 days of age. The relative weights of breast, drumstick and thigh were calculated as organ weight (g/100 g BW). The visceral were then opened and the thymus, spleen, bursa of Fabreciuos, liver, pancreas gizzard, fat pad, heart and intestinal segments removed and weighed. Relative organ weights were calculated as organ weight (g/100 g BW).

Blood taking happened on the 34th day of age from on birds per each cage to determine HDL, TG and total cholesterol. Blood samples were centrifuged (15 min, 3000 rpm) and the obtained serum was preserved in -70°C to be analyzed latter. Serum cholesterol concentration, TG, and HDL were determined. To count WBC, a diluted sample of 1/100 was a sed on a counting champer, thin blood slides were made and after staining with Geimsa, differential count glucosides was performed.

Broiler vaccination against Newcastle virus happened in two turns as the following; the first on the 17th day of the experiment as an eye drop and the second or the booster on the 26th day as a drink. Blood samples were withdrawn from the wing vein on 17, 26, 34, 41 and 47th days of age. The non-heparinized blood samples (1.5 mL/

Table 1: Ingredients of experimental diets (g/100 g diet)

	Starting diets		Growing diets		Finishing diets	
Ingredients (%)	Control	EO[1]	Control	EO	Control	EO
Corn	59.00	58.95	67.36	67.32	72.01	71.99
Soybean meal	35.54	35.55	28.43	28.44	24.26	24.26
Soybean oil	1.56	1.58	0.65	0.67	0.56	0.56
Calcium carbonate	0.60	0.60	0.67	0.67	0.63	0.56
Dicalcium phosphate	1.41	1.41	1.02	1.02	0.84	0.84
Oyster shell	0.66	0.66	0.66	0.66	0.63	0.63
Common salt	0.50	0.50	0.50	0.50	0.50	0.50
Vit. and min. premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.13	0.13	0.06	0.06	0.02	0.02
Lysine-HCL	0.09	0.09	0.14	0.14	0.05	0.05
Essential oil	-	0.02	-	0.02	-	0.02
Calculated analyses						
ME (Kcal kg^{-1})	2900.00	2900.00	2950.00	2950.00	3000.00	3000.00
Crude protein (%)	20.84	20.84	18.43	18.43	16.87	16.87

[1]EO: Essential Oils, [2]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (*all-trans*-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (α-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg and antioxidant, 250 mg

chicken-one bird per pen) were placed at 37°C for 2 h, centrifuged (3000×g for 15 min) to separate sera and stored at 20°C until analysis. The sera were applied to HI test to determine Ab to NDV, expressed as reciprocal log 2 values for the highest dilution that displayed HI.

Data on diet-associated difference were analyzed by PROC-GLM of SAS procedures appropriate for completely randomized designs. Mean differences among dietary treatments were evaluated by the Duncan multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

According to the Table 2, BWG, FI and FCR were not affected by dietary treatment except for BWG and FCR during the 22-42 days of age. Chicks fed on THY-included diets showed improved FCR and BWG comparing to chicks fed other experimental dietary groups during the growing period.

Cross *et al*. (2007) who investigated the effect of the dietary inclusion of 5 culinary herbs (thyme, oregano, marjoram, rosemary or yarrow) or their essential oils on the growth, digestibility and intestinal microflora status in female broiler chicks reported that dietary thyme oil or yarrow herb inclusion had the positive effects on chick performance, while oregano herb and yarrow oil were the poorest supplements. Some other researchers (Botsoglou *et al*., 2003; Papageorgiou *et al*., 2003) reported that essential oils were not effective in improving animal performance. In addition, *in vitro* studies have shown that essential oils to have antibacterial properties against *Listeria monocytogenes*, *Salmonellatyphimurium*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* (Cosentino *et al*., 1999).

Some researchers have shown that essential oils of rosemary (*Rosmarinus officinalis*), sage (*Salvia sclarea*), thyme (*Thymus vulgaris*) were among the most active in this respect against strains of *E. coli* (Smith-Palmer *et al*., 1998; Hammer *et al*., 1999).

The results for visceral measurements are presented in Table 3. Among the visceral organs, only the relative weights of drumstick and pancreas were affected from dietary inclusion of essential oils. Chicks fed on CIN and THY included diets shown the highest and the lowest relative weight of drumstick, respectively. However, only the difference between these two groups was significant ($p<0.05$).

Chicks fed on THY and CIN included diets shown the highest and lowest relative weight of drumstick, respectively. However, only the difference between these two groups was statistically significant ($p<0.05$). The results for relative weight of intestinal segments and the viscosity of ileal digesta are presented in Table 4. Dietary addition of essential oils of medicinal herbs in this experiments did not affect the relative weight of intestinal segments and the viscosity of ileal digesta ($p>0.05$).

Plasma total cholesterol, triglyceride and HDL were not affected by dietary essential oil inclusion (Table 5). Thehighest and lowest levels of RBC and HCT were observed in chicks fed on CLO and CIN included diets,

Table 2: Feed intake, body weight, body weight gain and feed conversion ratio of chicks fed the control diet (CON) and the diets containing 200 mg kg^{-1} essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO)

	Treatments[1]					
Days of age	CON	THY	CIN	CLO	SEM	p-value
Feed intake (g/chick)						
1-21	1113.77	1120.26	1117.48	1106.26	39.501	0.93
22-42	3236.32	3233.13	3087.38	3106.73	167.104	0.30
43-49	1212.78	1230.63	1180.00	1160.00	80.463	0.44
1-49	5562.9	5570.0	5384.9	5373.0	207.143	0.20
Body weight (g/chick)						
21	471.90	486.29	496.81	467.73	44.642	0.66
42	1491.14^{ab}	1513.47^{a}	1482.90^{b}	1468.86^{b}	21.241	0.01
49	2440.42	2495.76	2445.92	2387.41	73.705	0.12
Body weight gain (g/chick/day)						
1-21	22.47	23.15	23.65	22.27	2.125	0.66
22-42	71.00^{ab}	72.07^{a}	70.61^{b}	69.94^{b}	1.011	0.01
43-49	68.19	70.85	66.60	64.40	7.270	0.48
1-49	49.80	50.93	46.91	48.72	1.502	0.12
Feed conversion ratio (g feed: g gain)						
1-21	2.36	2.32	2.27	2.39	0.231	0.82
22-42	2.17^{a}	1.99^{b}	2.08^{ab}	2.11^{ab}	0.101	0.05
43-49	2.57	2.51	2.55	2.58	0.344	0.98
1-49	2.28	2.23	2.20	2.25	0.115	0.72

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$). [1]CON (Control), THY (Thyme essential oil, 200 mg kg^{-1} diet), CIN (Cinnamon essential oil, 200 mg kg^{-1} diet) and CLO (Clove essential oil, 200 mg kg^{-1} diet)

Table 3: Relative weights (% BW) of visceral organs of broilers fed the control diet (CON) and the diets containing 200 mg kg^{-1} essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO) in 47 day of age

	Treatments[1]					
Items	CON	THY	CIN	CLO	SEM	p-value
Breast	19.11	20.93	20.34	19.05	0.023	0.43
Thigh	9.65	9.20	10.30	10.46	0.010	0.14
Drumstick	8.91^{ab}	8.03^{b}	9.19^{a}	8.80^{ab}	0.008	0.05
Liver	2.11	2.04	2.15	2.10	0.002	0.82
Pancreas	0.22^{ab}	0.23^{a}	0.19^{b}	0.22^{ab}	<0.001	0.04
Heart	0.53	0.51	0.52	0.52	<0.001	0.97
Gizzard	2.49	2.46	2.55	2.39	0.003	0.86
Fat pad	2.83	2.63	2.30	2.55	0.005	0.39
Bursa fabrecious	0.47	0.43	0.40	0.40	0.441	0.06
Thymus	0.19	0.18	0.18	0.19	<0.001	0.95
Spleen	0.10	0.11	0.12	0.12	<0.001	0.64

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$). [1]CON (Control), THY (Thyme essential oil, 200 mg kg^{-1} diet), CIN (Cinnamon essential oil, 200 mg kg^{-1} diet) and CLO (clove essential oil, 200 mg kg^{-1} diet)

Table 4: Relative weight (% body weight) of intestinal segments and the viscosity of ileal digesta in broilers fed the control diet (CON) and the diets containing 200 mg kg^{-1} essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO) in 47 day of age

	Treatments[1]					
Items	CON	THY	CIN	CLO	SEM	p-value
Duodenum weight	0.48	0.50	0.46	0.50	0.001	0.91
Jejunum weight	1.24	1.17	1.01	1.16	0.010	0.06
Ileum weight	1.04	0.88	0.95	1.01	0.001	0.15
Viscosity (cPs)	2.21	2.21	2.67	1.82	0.582	0.12

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$). [1]CON (Control), THY (Thyme essential oil, 200 mg kg^{-1} diet), CIN (Cinnamon essential oil, 200 mg kg^{-1} diet) and CLO (Clove Essential Oil, 200 mg kg^{-1} diet)

Table 5: Total cholesterol, triglyceride and high density lipoprotein of serum, red blood cell, Hematocrit (HCT) and white blood cell counts on day 34 of age in broilers fed the control diet (CON) and the diets containing 200 mg kg^{-1} diet essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO)

	Treatments[1]					
Items	CON	THY	CIN	CLO	SEM	p-value
Total cholesterol (mgr dL^{-1})	130.17	128.67	134.83	133.83	13.943	0.84
Triglyceride (mgr dL^{-1})	115.50	120.83	113.00	113.50	27.585	0.95
HDL (mgr dL^{-1})	77.67	77.00	83.17	79.17	7.879	0.54
RBC	2.24^{ab}	2.35^{ab}	2.23^{b}	2.50^{a}	0.158	0.05
HCT (%)	32.78^{ab}	32.65^{ab}	31.01^{b}	34.85^{a}	2.168	0.04
Heterophile (%)	39.67^{a}	30.17^{bc}	23.50^{c}	31.67^{ab}	0.065	0.005
Lymphocyte (%)	59.33^{b}	68.33^{a}	73.00^{a}	66.00^{ab}	0.054	0.02
Monocyte (%)	0.83	1.50	1.33	1.83	0.088	0.87
Basophile (%)	0.33	0.00	0.00	0.50	0.040	0.21
Eosinophile (%)	0.17	0.00	0.00	0.00	0.020	0.41

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$). [1]CON (Control), THY (Thyme essential oil, 200 mg kg^{-1} diet), CIN (Cinnamon essential oil, 200 mg kg^{-1} diet) and CLO (Clove essential oil, 200 mg kg^{-1} diet)

respectively so that the differences between these two dietary groups were significant ($p<0.05$) but no statistically significant differences was found with CON and THY ($p>0.05$). Among the white blood cell counts, only heterophiles and lymphocytes were significantly affected from dietary treatments. Chicks fed on THY and CIN included diets had significantly lower number of heterophile comparing to control group. In the case of lymphocyte numbers, chicks fed on THY and CIN included diets had significantly higher number of heterophile comparing to control group.

As it was shown in Fig. 1, primary antibody response (Anti-NDV titers 9 days after first vaccination) of CON chicks was higher than THY and CIN chicks ($p<0.05$) but no statistically significant differences was found between CON and CLO chicks. Secondary antibody response (Anti-NDV titers 8, 15 and 21 days after second vaccination) were not affected by dietary essential oil inclusion ($p>0.05$). Diet inclusion of essential oils of THY caused an accelerating increase in secondary antibody response comparing to other dietary groups.

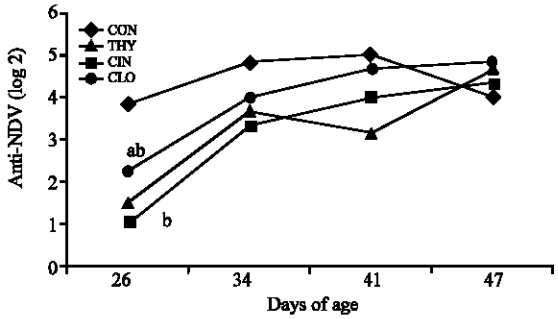

Fig 1: Humoral immune response against Newcastle disease virus (log 2) of broiler chickens fed the Control diet(CON) and the diets containing 200 mg kg^{-1} diet essential oil of Thyme (THY), Cinnamon (CIN) and Clove (CLO)

CONCLUSION

In this study, it can be concluded that adding essential oils of thyme to broiler diets may improve FCR with no adverse effect on other biochemical of immunological traits of birds.

REFERENCES

Botsoglou, N.A., P. Florou-Paneri, E. Christaki, D.J. Fletouris and A.B. Spais, 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.

Botsoglou, N.A., A. Govaris, E.N. Botsoglou, S.H. Grigoropoulou and G. Papageorgiou, 2003. Antioxidant activity of dietary oregano essential oil and alpha-tocopheryl acetate supplementation in long-term frozen stored Turkey meat. J. Agric. Food Chem., 51: 2930-2936.

Burt, A.S. and R.D. Reinders, 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157: H7. Lett. Applied Microbiol., 36: 162-167.

Cosentino, S., C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia and F. Palmas, 1999. *In vitro* antimicrobial activity and chemical composition of sardinian thymus essential oils. Lett. Applied Microbiol., 29: 130-135.

Cross, D.E., R.M. Mc Devitt, K. Hillman and T. Acamovic, 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.

Dorman, H.J. and S.G. Deans, 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol., 88: 308-316.

Gülçin, I., I.G. Sat, S. Beydemir, M. Elmastas and O.I. Küfrevioglu, 2004. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem., 87: 393-400.

Hammer, K.A., C.F. Carson and T.V. Riley, 1999. Antimicrobial activity of essential oils and other plant extracts. J. Applied Microbiol., 86: 985-990.

Helander, I.M., H.L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm and I. Pol *et al*., 1998. Characterization of the action of selected essential oil components on gram-negative bacteria. J. Agric. Food Chem., 46: 3590-3595.

Jamroz, D., A. Wiliczkiewicz, T. Wertelecki, J. Orda and J. SkorupinSka, 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Br. Poult. Sci., 46: 485-493.

Kim, S.I., J.H. Yi, J.H. Tak and Y.J. Ahn, 2004. Acaricidal activity of plant essential oils against *Dermanyssus gallinae* (Acari: Dermanyssidae). Vet. Parasitol., 120: 297-304.

Lee, H.S. and Y.J. Ahn, 1998. Growth-inhibiting effects of *Cinnamomum cassia* bark-derived materials on human intestinal bacteria. J. Agric. Food Chem., 46: 8-12.

Lee, K.G. and T. Shibamoto, 2002. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. J. Agric. Food Chem., 50: 4947-4952.

Lopez-Bote, L.J., J.I. Gray, E.A. Gomaa and C.I. Flegal, 1998. Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. Br. Poult. Sci., 39: 235-240.

Miura, K., H. Kilkuzaki and N. Nakatani, 2002. Antioxidant activity of chemical components from sage (*Saliva officinalis* L.) and oregano (*Thymus vulgaris* L.) measured by the oil stability index methods. J. Agric. Food Chem., 50: 1845-1851.

Ouattara, B., R.E. Simard, R.A. Holley, G.J. Piette and A. Begin, 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol., 37: 155-162.

Papageorgiou, G., N. Botsoglou, A. Govaris, I. Giannenas, S. Iliadis and E. Botsoglou, 2003. Effect of dietary oregano oil and á-tocopheryl acetate supplementation on ironinduced lipid oxidation of turkey breast, thigh, liver and heart tissues. J. Anim. Physiol. Anim. Nutr., 87: 324-335.

Paster, N., B.J. Juven, E. Shaaya, M. Menasherov, R. Nitzan, H. Weisslowicz and U. Ravid, 1990. Inhibitory effect of oregano and thyme essential oils on moulds and foodborne bacteria. Lett. Applied Microbiol., 11: 33-37.

Robenorst, J., 1996. Production of methoxyphenol-type natural aroma chemicals by biotransformation of eugenol with a new *Psedomonas* sp. Applied Microbiol. Biotechnol., 46: 470-474.

Smith-Palmer, A., J. Stewart and L. Fyfe, 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett. Applied Microbiol., 26: 118-122.

Teissedre, P. and A. Waterhouse, 2000. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. J. Agric. Food Chem., 48: 3801-3805.

Valero, M. and M.C. Salmeroj, 2003. Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. Int. J. Food Microbiol., 85: 73-81.

Velluti, A., V. Sanchis, A.J. Ramos, J. Egido and S. Marin, 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and pahharose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. Int. J. Food Microbiol., 89: 145-154.

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.