Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online)
http://psj.gau.ac.ir
GUASNR

Effects of Drinking Thyme Essence (*Thymus vulgaris* L.) on Growth Performance, Immune Response and Intestinal Selected Bacterial Population in Broiler Chickens

Saki AA, Kalantar M & Khoramabadi V

Department of Animal Science, Bu-Ali Sina University, Hamedan, Iran.

Poultry Science Journal 2014, 2 (2): 113-123

Article history:
Received: August 5, 2014
Revised: September 9, 2014
Accepted: September 14, 2014

Corresponding author:
Ali Asghar Saki, Ph.D
Dralisaki@yahoo.com

Keywords:
Broiler
Performance
Thyme essence
Immune response
Bacterial population

Abstract

This study evaluated the effects of Drinking Thyme Essence (DTE) (Zero, 0.10, 0.15 and 0.20 mL/L) on growth performance, immune response and changing of intestinal bacterial population in broiler chickens. A total number of 500 day old male broiler chicks (Ross 308), were randomly assigned to 4 treatments with 5 replicates and 25 chickens per each, based on a completely randomized design (CRD). Growth performances were assessed during the range of 8-21, 22-42 and 8-42 d. At 21 and 42 d blood serum titers including: Newcastle Disease (ND), Avian Influenza (AI), Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease (IBD) were sampled. Bacterial populations in intestinal digesta were determined at the age of 21 and 42 d. DTE levels significantly ($P<0.05$) improved total weight gain and total feed conversion ratio as compared with the control group during 1-42 d of age. The titer of serum antibodies did not show significant differences between different treatments at the 21 or 42 d. Total count, *E. coli*, and Gram negative bacteria (GNB) at the age of 21 and 42 days showed a significantly ($P<0.05$) lower number compared with the control group. There was a significantly ($P<0.05$) higher number of Lactic Acid Bacteria (LAB) in DTE groups compared with control group at both ages of 21 and 42 d. In conclusion, different levels of DTE (especially at level of 0.20 mL/L) could improve the growth performance, immune response and intestinal lactic acid bacteria as a health index during different growth periods.

Please cite this article as: Saki AA, Kalantar M & Khoramabadi V. 2014. Effects of drinking Thyme essence (*Thymus vulgaris* L.) on growth performance, immune response and intestinal selected bacterial population in broiler chickens. Poult. Sci. J. 2 (2): 113-123.

© 2014 PSJ. All Rights Reserved

Introduction

Due to the detrimental effects of antibiotics in animal feeds as a growth promoter, a wide range of additives had been used to improve the performance of birds. Specifically, development of antibiotic resistant bacteria and antibiotic residues in poultry products persuades researches to follow other alternatives and substitutes (Grashorn, 2010; Hashemi and Davoodi, 2010).

Organic matters such as phytobiotics can improve performance (Ghazalah and Ali, 2008 ; Herawati, 2010), and exhibit antibacterial and antiviral activity (Dorman and Deans, 2000). Herbal products can also improve the health, immunity (Gulfraz *et al*., 2008; Al-Jaff, 2011) and antioxidant effects (Hoffman and Wu, 2010). Some consideration like reducing the number of certain pathogenic bacteria and its challenges, improving gut health, as well as better pictures of blood metabolites, are common beneficial effects of using organic matters in poultry nutrition (Griggs and Jacob, 2005).

Thyme (*Thymus vulgaris L.*) is a popular medicinal plant belonged to *Lamiaceae sp*. and mostly grown in Mediterranean regions (Chevallier, 1996). This herb has been paid more attention due to its antioxidant (Dorman and Deans, 2000; Bolukbasi and Erhan, 2007), antibacterial (Dorman and Deans, 2000) anticoccidial (Jamroz *et al*., 2003), and antifungal properties (Hertrampf, 2001). Volatile oils from thyme were assessed as inhibitors of microbial growth (Toghyani *et al*., 2010). The major derived components of thyme plant are thymol and carvacrol, the phenolic compounds which have shown antioxidant and antibacterial activities (Demir *et al*., 2008). These compounds exhibit beneficial effects in poultry health and production (Mitsch, 2004).

There is very little information relevant to the effect of dietary or drinking inclusion of thyme essence on performance and immunity of growing broiler chicks. Therefore, this experiment was performed to investigate the potential of supplemented thyme in drinking water on growth performance, immune response and intestinal selected bacterial population in broiler chickens.

Materials and Methods

Animals and treatments

This study was conducted at the research poultry farm of Qom's agricultural research center in tropical climate in Iran. A total of 500 day old male broiler chicks (Ross 308), were randomly assigned to 4 treatments with 5 replicates and 25 chickens per each, based on a completely randomized design (CRD). Treatments were: 1) Control group (plain water), 2) 0.10 mL/L (0.10%), 3) 0.15 mL/L (0.15%), and 4) 0.20 mL/L (0.2%) of thyme essence added to drinking water from 8 to 42 d. A corn-soy basal diet was used among treatments during different periods according to breeding manual recommendation which is reflected in (Table 1).

Table 1. Ingredients and composition of the basal diet

Ingredients (g/Kg)	Starter (0-7 d)	Grower (8-21 d)	Finisher (22-42 d)
Corn (7.88% CP)	525.0	533.5	602.5
Soy bean meal (44% CP)	409.4	381.5	316.0
Soy bean oil	10.0	30.0	30.0
Calcium carbonate	16.0	16.0	20.0
Dicalcium phosphate[1]	17.2	17.0	11.0
DL-Methionine	4.4	4.0	3.5
L-Lysine	10.0	10.0	9.0
Vitamin premix[2]	2.5	2.5	2.5
Mineral premix[3]	2.5	2.5	2.5
Salt	3.0	3.0	3.0
Calculated values			
ME (Kcal/Kg)	2867	3003	3064
Crude protein (g/Kg)	22.45	21.32	19.03
Calcium (g/Kg)	0.99	0.92	0.84
Available phosphorus (g/Kg)	0.45	0.43	0.33
Methionine + Cystine (g/Kg)	13.51	15.46	17.94
Lysine (g/Kg)	14.30	14.50	11.70

[1]Dicalcium phosphate contained: 16.5% phosphorous and 23% calcium.

[2]Vitamin premix supplied the following per Kg of diet: vitamin A (retinol), 8400 IU; vitamin D3 (cholecalciferol), 1800 IU; vitamin E (tocopheryl acetate), 150 mg; vitamin K, 24 mg; B1, 8 mg; B2, 16.6 mg; B6, 13 mg; B12, 5 mg; panthothenic acid, 12 mg; niacin, 36 mg; biotin, 10 mg; folic acid, 2.2 mg; choline chloride, 128.8 mg; antioxidant, 100 mg.

[3]Mineral premix supplied the following per Kg of diet: Fe (FeSO4, 20.1% Fe), 95 mg; Mn (MnSO4, 32.5% Mn), 120 mg; Zn (ZnO, 80.5% Zn), 120 mg; Cu (CuSO4, 30.3% Cu), 35 mg; I (KI, 58% I), 5 mg; and Se (NaSeO3, 45.5% Se), 2.2 mg.

Performance traits

Body weight gain, feed intake and feed conversion ratio were assessed during the range of 8-21, 22-42 and 8-42 days of age. Body weight and feed intake were measured every week. Body weight gain was calculated on a weekly basis throughout the mentioned trial period. The consumed amounts of feed were recorded every week and cumulative feed intake was calculated at the end of the trial. Feed conversion ratio was calculated as feed intake divided by body weight gain. Mortality was recorded as it occurred and data were adjusted base on it.

Immune response

In order to measure the antibody titer against common important diseases as immune responses, at 21 and 42 days of age blood samples were collected from the brachial vein of the wing in both heparinized and nonheparinized microcapillary tubes, which were kept at room temperature. Heparinized tubes were spun for 10 min in a microcapillary centrifuge within 1 h of collection. Nonheparinized tubes were centrifuged 12–15 h after collection. Plasma and serum were maintained at 4°C until analysis. Antibody responses to Newcastle disease

(ND), avian influenza (AI), infectious bronchitis virus (IBV) and infectious bursal disease (IBD) were measured (Czifra *et al.*, 1996).

Intestinal bacterial population

The intestinal digesta from 32 birds (8 birds per treatment) were used for determination of bacterial population. Total count of bacteria (TCB) was measured as a row index and some selected micro-organisms including:, *Escherchia coli* (*E.coli*), Lactic acid bacteria (LAB), Gram negative bacteria (GNB) and *Salmonella* were measured as specified indices. The content of the certain part of the small intestine (from the Meckel's apophysis until 10 cm to the junction with caecum) were separately collected, cooled and used for microbial analysis. The TCB was measured based on direct counting of dead cells (Black, 1996). Population of *E.coli* and LAB were estimated as CFU g^{-1} using subsequent dilution method (Black, 1996; Tortora and Funke, 1995). *E.coli* was cultured on MacConkey agar (Merck, Germany) at 37°C for 24 hrs. LAB was cultured on MRS (DeMan-Rogosa-Sharpe media) agar (Merck, Germany) after incubation under anaerobic condition for 72 hrs at 37°C. GNB was cultured on EMB (Eosin Methylene Blu) agar (Merck, Germany) at 37°C for 24 hrs. *Salmonella* was cultured on BGA (Brilliant Green Agar) specified media (Merck, Germany) at 37°C for 24 hrs.

Statistical analysis

Analysis of data was accomplished using the ANOVA model procedures of SAS institute (SAS, 2004). Comparisons of means were done using Duncan's multiple range tests, assuming error level of 0.05. Linear and quadratic relations of the studied traits with the experimental level of DTE were also analyzed.

Results and Discussion

Performance traits

The effect of different levels of Drinking Thyme Essence (DTE) on growth traits of broiler chickens at different growth period (8 to 21 and 22 to 42 days), and at the entire experimental period (8 to 42 days) are presented in Table 2.

Feed intake and weight gain during 8 to 21 days of age were significantly ($P<0.05$) affected by the inclusion of DTE at the level of 0.2 mL/L. The other levels of DTE had no significant differences compared to the control group. The gain obtained by the levels of 0.10 and 0.15 mL/L of DTE had no significant differences either with the level of 0.20 DTE or with the control group. Also during 8 to 21 days of age the feed conversion ratio was not affected by different levels of DTE. From 22 to 42 days of age similar findings in feed intake and weight gain were acquired, in contrast higher feed intake and lower gain were found by 0.15 mL/L of DTE than 0.20 mL/L. Feed conversion ratio in this period was significantly affected by the level of 0.2 mL/L of DTE, and the levels of 0.10 and 0.15 mL/L of DTE had no significant differences either at the level of 0.20 DTE or with the control group. Totally, during the entire experimental period (8 to 42 days), feed intake was significantly ($P<0.05$) higher than control but did not differ with the

other levels of DTE. Body weight gain was significantly higher by 0.15 mL/L DTE than 0.10 mL/L and control treatments, While no significant differences were found by 0.20 mL/L of DTE. A similar trend was observed in feed conversion ratio at the level of 0.15 mL/L of DTE. Therefore, different levels of DTE presented higher effects on feed intake and body weight gain, and in contrast had lower effects on feed conversion ratio when compared with the control group.

Table 2. Effect of different levels of DTE[1] on growth traits of broiler chickens

Items	Control	0.10 mL/L	0.15 mL/L	0.20 mL/L	*SEM*[2]	*P-value*
	8 to 21day					
Feed intake (g/d)	68.72^{b}	70.61^{b}	70.56^{b}	73.45^{a}	0.65	0.001
Weight gain (g/d)	51.76^{b}	52.29^{ab}	52.33^{ab}	54.46^{a}	0.72	0.001
Feed conversion ratio	1.33	1.35	1.35	1.35	0.02	0.001
	22 to 42 day					
Feed intake (g/d)	101.27^{b}	102.34 ^{b}	103.92^{a}	101.09^{b}	0.81	0.001
Weight gain (g/d)	54.44^{b}	57.04^{a}	57.66^{a}	58.13^{a}	0.42	0.001
Feed conversion ratio	1.86^{a}	1.79^{ab}	1.80^{ab}	1.74^{b}	0.02	0.001
	8 to 42 day					
Feed intake (g/d)	84.99 ^{b}	86.47^{ab}	86.76^{ab}	87.29^{a}	0.54	0.001
Weight gain (g/d)	48.11^{c}	50.67^{b}	52^{a}	51.30^{ab}	0.32	0.001
Feed conversion ratio	1.77 ^{a}	1.71^{ab}	1.67^{b}	1.70^{ab}	0.02	0.001

[1]Drinking Thyme essense, [2]Standard error of means.
Values in the same row not sharing a common superscript are significantly different ($P<0.05$).

The effects of different levels of DTE on performance traits of broilers chickens during 1 to 42 days of age are presented in Table 3. Performance traits including total live weight, total weight gain, cumulative feed intake and total feed conversion ratio during 1 to 42 days of age were significantly ($P<0.05$) improved by different levels of DTE compared with the control group. All the DTE levels had a higher total live weight and total weight gain compared with the control group. Cumulative feed intake was significantly ($P<0.05$) lower for the levels of 0.15 and 0.20 but not for 0.10 mL/L of DTE compared with the control group. Total feed conversion ratioes for all of the DTE levels were significantly lower ($P<0.05$).

Immune response

The effects of the experimental treatments on titer of serum antibodies in broiler chickens at the age of 21 and 42 are presented in Table 4. The mean titer of serum antibodies of four disease including ND, AI, IBV and IBD did not show significant differences between different treatments at 21 or 42 days of age. In the other hand, antibody response to ND, AI, IBV and IBD at age 21 and 42, was unaffected by different levels of DTE. Although some numerical differences among the treatments were observed and sometimes the serum mean titers of antibodies for mentioned diseases were higher than control group, but these differences were not

significant. The levels of serum antibody titer for diseases were measured as the initial criterion at the age of 7 days to compare the differences between control and different levels of DTE.

Table 3. Effect of different levels of DTE[1] on Performance traits of broiler chickens during 1 to 42 days of age

Items	Control	0.10 mL/lit	0.15 mL/lit	0.20 mL/lit	*SEM*[2]	*P-value*
Total live weight (Kg)	2.07[b]	2.17[a]	2.22[a]	2.20[a]	0.02	0.001
Total weight gain (Kg)	2.02[b]	2.13[a]	2.18[a]	2.16[a]	0.02	0.001
Cumulative Feed Intake(Kg)	4.32[a]	4.31[a]	4.26[b]	4.24[b]	0.02	0.001
Total feed conversion ratio	2.09[a]	1.98[b]	1.95[b]	1.93[b]	0.02	0.001

[1]Drinking Thyme essense, [2]Standard error of means.
Values in the same row not sharing a common superscript are significantly different (*P<0.05*).

Intestinal bacterial population

The effects of inclusion of different levels of DTE on population of intestinal selected bacteria of broiler chicken at 21 and 42 days of age are presented in Table 5. The colony forming units from digesta of ileum with a view to count different items including total count, *E. coli*, and gram negative bacteria at the age of 21 and 42 showed a significantly (*P<0.05*) lower number compared with the control group. There was a significantly (*P<0.05*) higher number of LAB in DTE groups compared with the control group at both ages of 21 and 42 days. Salmonella bacteria were not detected through different treatments at both 21 and 42 days of age.

Table 4. Effect of different levels of DTE[1] on titer of serum antibodies of broiler chickens

Items	Control	0.10 mL/lit	0.15 mL/lit	0.20 mL/lit	*SEM*[2]	*P-value*
	At the age of 7					
ND[3]	2713.23	2644.41	2640.38	2795.44	59.65	0.651
AI[4]	334.60	331.87	365.80	345.53	14.71	0.768
IBV[5]	654	628.41	630.35	677.07	16.11	0.615
IBD[6]	620.40	633.05	637.59	669	16.40	0.678
	At the age of 21					
ND[3]	522.50	574.30	587.00	575.50	14.70	0.851
AI[4]	344.63	339.63	360.55	360.59	17.18	0.901
IBV[5]	197.20	197.40	215.80	177.80	13.60	0.748
IBD[6]	236	239.60	280.20	234	14.68	0.543
	At the age of 42					
ND[3]	1720.20	1719.40	1768.40	1697.40	25.85	0.515
AI[4]	1123.13	1112.61	1149.46	1083.81	25.98	0.615
IBV[5]	653.20	641.80	640.20	625.80	14.56	0.132
IBD[6]	7551.40	7588.40	7607	7541.60	18.49	0.584

[1]Drinking Thyme essense, [2]Standard error of means, [3]Newcastle disease, [4]Avian influenza, [5]Infectious bronchitis virus, [6]Infectious bursal disease.
Values in the same row not sharing a common superscript are significantly different (*P<0.05*).

Table 5. Effect of different levels of DTE[1] on population of intestinal selected bacteria

Items (CFU/g)	Control	0.10 mL/lit	0.15 mL/lit	0.20 mL/lit	*SEM*[2]	*P-value*
	At the age of 21					
Total count	9.41[a]	8.95[b]	8.96[b]	8.65[b]	0.09	0.001
E. coli	5.16[a]	5.00[b]	5.10[a]	4.71[c]	0.04	0.001
LAB[3]	1.2[b]	1.25[a]	1.24[a]	1.25[a]	0.01	0.001
GNB[4]	9.00[a]	8.66[b]	7.83[c]	6.5[d]	0.05	0.001
Salmonella detection	negative	negative	negative	negative	-	-
	At the age of 42					
Total count	9.21[a]	8.60[b]	8.50[b]	8.47[b]	0.06	0.001
E. coli	5.79[a]	5.32[b]	5.31[ab]	5.26[b]	0.03	0.004
LAB[3]	1.34[b]	1.34[b]	1.46[ab]	1.54[a]	0.04	0.014
GNB[4]	8.33[a]	7.13[b]	6.46[c]	6.33[c]	0.05	0.001
Salmonella detection	negative	negative	negative	negative	-	-

[1]Drinking Thyme essense, [2]Standard error of means, [3]Lactic acid bacteria, [4]Gram negative bacteria. Values in the same row not sharing a common superscript are significantly different ($P<0.05$).

Discussion

Results presented in Table 2 have shown that chickens receiving different levels of DTE had a significant ($P<0.05$) higher feed intake and weight gain during the first growth period (8 to 21days) compared with the control group. There were no significant differences in feed conversion ratio between control and different levels of DTE at the first growth period (8 to 21 days). These results have shown that the effects of DTE on appetite and feed conversion ratio in this period could not be appeared yet, and needs more experiences on these items. During the second growth period (22 to 42 days) or the entire experimental period (8 to 42 days), feed intake, weight gain and feed conversion ratio were significantly affected by the inclusion of different levels of DTE compared with control. Stimulatory effect of herbal products on growth and digestion is the main reason for these results, so essential oils and related components derived from thyme have been widely used for the appetizing and stimulating effect on digestion. Such materials have traditionally been used to stimulate the production of endogenous secretions in the small intestine mucosa, pancreas and liver, and thus aid digestion (cross *et al*, 2007). The major components of the essential oil of thyme are thymol (5-methyl-2-isopropylphenol) and carvacrol (5-isopropyl-2-methyl-phenol), which improve animal performance (Hertrampf, 2001; Alcicek *et al*., 2003). Langhout (2000), as well as Williams and Losa (2001) discovered that essential oils of thyme have a stimulating effect on the animal digestive system, due to the increase of digestive enzymes and improve nutrients utilization through the enhanced liver function (Safa and Al-Beitawi, 2009).

As it was shown in Table 4, antibody responses to ND, AI, IBV and IBD at the ages of 21 and 42day were unaffected by inclusion of different levels of DTE in drinking water. Immune stimulant effects of other factors were more evident than DTE levels. Some factors such as environmental, nutritional and physiological situations, as well as the variation of active compounds in the experimental treatments could directly influence the results. Improving immunity in poultry production is very important to prevent common important diseases. A variety of different factors such as vaccination quality or vaccination failure, effect of some immune suppressive diseases and the content of experimental diets and materials can induce immunodeficiency. The studies of the immune system have shown that some herbs such as coneflower (*Echinacea purpurea*) were most effective in immune system improvement, because this herb increased stimulation of non-specific immune system. It is though that immune enhancement of *Echinacea* is provided by certain polysaccharides, flavonoids, and isobutylamides (Rehman *et al.*, 1999). Herbs like thyme (*Thymus vulgaris*) that are rich in active compounds such as flavonoids extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Cook and Samman, 1996 ; Manach *et al*., 1996).

Results presented in Table 5 showed that the lowest total count, *E.coli* and GNB counts were related to DTE especially at the level of 0.20 mL/L. Instead the highest LAB counts were related to the DTE groups than the control. There was no difference observed among different treatments with respect to salmonella detection. In other studies, the significant reduction of unfavorable bacteria has been obtained following an application of natural plant extract (Bolukbasi and Erhan, 2007). Thymol has been shown to reduce the number of coliforms within the digsta of chickens (Cross *et al.*, 2004). Herb derivatives may have an effect through an increase in production of lactic acid bacteria, thus increasing the population of beneficial bacteria and reducing the presence of gram negative bacteria (Savage *et al*., 1996). Carvacrol has a stimulating effect on *Lactobacillus* proliferation (Toghyani *et al*., 2010). Jamroz *et al.* (2005) reported that plant extract supplements also significantly increase the *Lactobacillus* numbers. Decreasing the number of viable gram positive bacteria such as *Lactobacilli* and *Bifidobacteria*, may increase the presence of gram negative species. Coli form bacteria is an indicator tool for intestinal performance, so that thyme and cinnamon with complex mechanisms affect pathogenic bacteria by changing cell wall bacterial permeability leading to pore formation and osmotic shock and leakage of cytoplasm and its active contents outside the cell leading to death of them (Dorman and Deans., 2000). The antimicrobial effect of thymol is played on vital membrane ions of potassium and hydrogen equilibrium pumps (Bolukbasi and Erhan, 2007).

Conclusion

In conclusion, the results of this study indicated that inclusion of thyme essence as drinking water at levels of 1, 1.5 and especially at 2 mL/L in both growth period

or at the entire experimental period could significantly improve growth indices and performance traits. But the DTE levels could not significantly improve the antibodies titer levels of diseases (ND, AI, IBV, IBD) compared with the control group. The colony forming units of ileum with regard to the parameters including total count, *E. coli*, and GNB at the age of 21 and 42 showed a significantly lower number compared with the control group. For LAB, there was a significantly higher number compared with control group both at ages of 21 and 42. Therefore, using drinking thyme essence improves the growth and performance indices as well as lowers the number of unfavorable bacteria, whilst increases the number of desirable bacteria. So it has positive a effect on the health status with no detrimental effect on the birds.

Acknowledgement
We wish to express our thanks to Poultry Association of Qom Province (Qoam, Iran) for supporting the study. Furthermore, we wish to thank Bu-Ali lab director for performing and conducting the trials.

References

Alcicek A, Bozkurt M & Cabuk M. 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. South African Journal Animal Science, 33: 89-94.

Al-Jaff FK. 2011. Effect of coriander seeds as diet ingredient on blood parameters of broiler chicks raised under high ambient temperature. International Journal of Poultry Science, 10: 82-86.

Black JG. 1996. Microbiology: Principles and Applications. 3rd Ed. Prentice Hall. New Jersey. 790 Pages.

Bolukbasi SC & Erhan MK. 2007. Effect of dietary thyme (*Thymus Vulgaris*) on laying hens performance and *Escherichia coli* (*E.coli*) concentration in feces. International Journal of Natural and Engineering Sciences, 1 : 55-58.

Chevallier A. 1996. The Encyclopedia of Medicinal Plants. DK Publishing. Michigan. 336 Pages.

Czifra G, Nilsson M, Alexander DJ, Manvell R, Kechskemeti S & Engstrom BE. 1996. Detection of PMV-1 specific antibodies with a monoclonal antibody blocking enzyme-linked immunosorbent assay. Avian Pathology, 25: 691–703.

Cook NC & Samman S. 1996. Flavonoids chemistry, metabolism, cardio protective effects and dietary sources. Journal of Nutrition and Biotecnology, 7: 66-76.

Cross DE, Hillman K, Fenlon D, Deans SG, McDevitt RM & Acamovic T. 2004. Antibacterial properties of phytochemicals in aromatic plants in poultry diets. In: Acamovic T, Stewart C & Pennycott TW. (Eds). Poisonous Plants and Related Toxins. CAB International. Wallingford. Oxon. Pages, 175-180.

Cross DE, McDevitt RM, Hilman K & Acamovic T. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut

microflora in young chicks from 7 to 28 days of age. British Poultry Science, 48: 496-504.

Dorman HJD & Deans SG. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of Application Microscopy, 88: 308-316.

Griggs JP & Jacob JP. 2005. Alternatives to antibiotics for organic poultry production. Journal of Applied Poultry Research , 14: 750-756.

Ghazalah AA & Ali AM. 2008. Rosemary leaves as a dietary supplement for growth in broiler chickens. International Journal of Poultry Science, 7: 234-239.

Gulfraz M, Mehmood S, Minhas N, Jabeen N, Kausar R, Jabeen K & Arshad G. 2008. Composition and antimicrobial properties of essential oil of *Foeniculum Vulgare*. African Journal of Biology, 7 : 4364-4368.

Grashorn MA. 2010. Use of phytobiotics in broiler nutrition – an alternative to infeed antibiotics. Jounal of Animal and Feed Science, 19: 338-347.

Hertrampf JW. 2001. Alternative antibacterial performance promoters. Poultry International, 40: 50-52.

Hoffman PD & Wu C. 2010. The effect of thymol and thyme oil feed supplementation on growth performance, serum antioxidant levels and cecal *Salmonella* population in broilers. Journal of Applied Poultry Research, 19: 432-443.

Hashemi SR & Davoodi H. 2010. Phytogenic as new class of feed additive in poultry industry. Journal of Animal and Veterinary Advances, 9: 2295-2304.

Herawati T. 2010. The effect feeding red ginger as phytogenic on body weight gain, feed conversion and internal organs condition of broiler. International Journal of Poultry Science, 9: 963-967.

Jamroz D, Orda J, Kamel C, Wiliczkiewicz A, Wertelecki T & Skorupinska J. 2003. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and maicrobial status in broiler chickens. Journal of Animal and Feed Sciences, 12: 583-596.

Jamroz D, Williczkiewicz A, Werteleck T, Orda J & Skorupinska J. 2005. Use of active substances of plant origin in chicken diet based on maiz and locally grown cereals. British Poultry Science, 46: 458-493.

Langhout P. 2000. New additives for broiler chickens. World Poultry, 16: 22-27.

Manach F. Regerat F & Texier O. 1996. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutrition Resserch, 16: 517-544.

Mitsch P, Kohler B, Gabler C, Losa R & Zitterl-Eglseer K. 2002. CRINA Poultry reduces the colonisation and proliferation of *Clostridium perfrigens* in the intestine and feces of broiler chickens. Proceedings of the 11th European Poultry Conference. Bremen. Page, 113

Najafi P & Torki M. 2010. Performance, blood metabolites and immunocompetaence of broiler chicks fed diets included essential oils of medicinal herbs. Journal of Animal and Veterinary Advances, 9: 1164-1168.

Rehman J, Dillow JM, Carter SM, Chou J, Le B & Maisel AS. 1999. Increased production of antigren-specific immunoglobulins G and M following *in vivo* treatment with medicinal plants *Echinacea angustifolia* and *Hydratis canadensis*. Immunity Letter, 68: 391-395.

Safa SEG & AL-Beitawi NA. 2009. The effect of feeding of crushed thyme (*Thymus valgaris*) on growth, blood constituents, gastrointestinal tract and carcass characteristics of broiler chickens. Journal of Poultry Science, 4: 100-104.

SAS (Statistical Analysis System). 2004. SAS/STAT® 9.2. User's Guide. SAS Institute Inc. Cary, North Carolina.

Savage TF, Cotter PF & Zakrzewska EI. 1996. The effect of feeding mannan oligosaccharide on immunoglobulins, plasma IgG and bile IgA of Wrolstad MW male turkeys. Poultry Science, 75: 143-149.

Toghyani M, Tohidi M, Gheisari AB & Tabeidian SA. 2010. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. African Journal of Biology, 9: 6819-6825.

Tortora GJ, Funke BR & Case CL. 1995. Microbiology. An Introduction. 5th Ed. Benjamin-Cummings Publishing Company. Redwood. 801 Pages.

Williams P & Losa R. 2001. The use of essential oils and their compounds in poultry nutrition. World Poultry, 17: 14-15.