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Immune response of broiler chickens supplemented with Thyme exteract (*thymus vulgaris*) in drinking water

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ABSTRACT

The recent study was conducted to investigate the effects of 0 (control), 0.2, 0.4 and 0.6% alcoholic extract of thymuse vulgarise in drinking water on immune response of broiler chickens. One hundred and sixty day old chicks (Ross 308) in four treatment and 4 replicate each based on a completely randomized design were used. Immune organs relative weight such as spleen and bursa fabriciuse not affected by using of thyme extract at 21 and 42 days of experimental period but higher bronchitis antibody titer for the birds consumed 0.2 and 0.6% of thymuse vulgarise showed (P<0.05) as compared with those consumed the 0.4% thymuse vulgarise extract and control birds at 21 days of age. Moreover, consumption of thymuse vulgarise extract in water increased the bronchitis antibody titer as compared to the control birds in orthogonal comparsions. But there were no significant differences between the treatments for bronchitis antibody titer at 42 days of age and Newcastle antibody titer at 21 and 42 days of age.

Key words; Thymuse vulgarise, immune system, broiler chickens.

INTRODUCTION

Antibiotic growth promoters (AGP) that had used for long terms in animal industry because of the microbial resistance in animal and probably transfer of this resistance via animal products to human [1] restricted or baned. The phasing out of antibiotic growth promoters (AGP) will affect the poultry and animal industry at large. To minimize the loss in growth, there is a need to find alternatives to AGP[2]. There are a number of non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immunostimulant and other management practices [3]. Medical plants and their principal secondary metabolits used extensively in food products, perfumery, and dental and oral products due to their different medicinal properties [4], are the most common materials that are applied instead of antibiotic growth promoters in poultry production[5]. As regards in current years, epidemic infectious diseases are important problam in throughout world and the cuase of the financial failure for the poultry producers. In addition, other factors such as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immunostimulants is one solution to

improve the immunity of animals and to decrease their susceptibility to infectious disease [6]. In some research, medical plants efficiency on broiler immunes system has been reported. Dietary birds with polysavone (alfalfa extract) improved the relative thymus, bursa and spleen weights and led to increase in proliferation of T and B lymphocytes compared with the control group (P <0.05). Moreover polysavone consumption resulted in a significant increase (P <0.05) in serum antibody titer of Newcastle disease virus [7]. Khaligh et al [8] indicated that addition of a 10 g/kg blend of alfalfa, liquorice root, great burdock, cinnamon to the broiler diet resulted in the most consistent improvement in antibody titer against Newcastle disease virus (p < 0.05) compared with the control group.

Thymus vulgaris is a medicinal herb in the *Lamiaceae* family, cultivated worldwide for culinary, cosmetic perennial and medical purposes. This species has special functions such as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant [9,10]. Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methyl phenol) are the main phenolic components in *Thymus vulgaris* [11] and antibacterial activity of thyme or main poly phenolic components against of *Clostridium botulinum*, *Clostridium perfringens, Bacillus subtilis, S. sonnei, E. coli, H. pylori, S. typhimurium*, *S. sonnei*, *Bacillus cereus, L monocytogenes, C. jejuni* and *S. enteric* reported in previous literatures [12, 13,14,15,16,17,18,19]. Performance promoting effects of essential oil, extract, powder or principal components of thyme have been demonstrated in poultry [20,21,22,]. But evidences about the effect of thyme extract on immunes responses in broiler chickes are rare and therefore the aim of this study was evaluate the effect of thymus vulgaris extract in drinking water on immunes responses

MATERIALS AND METHODS

A total of 160 day-old mixed sex broiler chicks (Ross 308) were weighed and based on completely randomized design assigned to 4 treatment groups with 4 replicate and 10 bird (5 male and 5 female) per each. Water and feed were provided *ad libitum* for consumption. All the chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form (Table 1), but the drinking water of the birds supplemented with 0.0 (ZT), 0.2 (LT), 0.4 (MT) and 0.6% (HT) alcoholic extract of thyme vulgaris during the whole of experimental period. Thymus vulgaris alcoholic extract was prepared using a standard maceration method [23]. For this purpose, vegetative parts of the shade dried thymus vulgaris full bloom stage were crushed and soaked in ethanol 80% in 1:5 ratios (w/v) for 72 h on a shaker then the extract strained and its thymol content was determined by TLC (thin layer chromatography) method.

All treatments (drinking water) were prepared daily. Bronchitis vaccination against Bronchitis virus was done on the 1th and 14 th days (as eye drop), and vaccination against Newcastle virus happened by injection in breast muscle at 8 th day of the experimental period. At day 21 and 42 of age, two birds per pen (a male and a female) were selected, weighed and killed by decapitation to obtain the immune organs relative weights such as spleen and bursa fabricius (percentage of live body weight). Blood samples were collected in anticoagulant tubes (citrate sodium 3.6% solution) during a forty minute period. After centrifugation (5000 rpm) for 7 min, blood serum was separated and at the consequent Newcastle and Bronchitis disease virus antibody titers were measured by using the elaisa reader (Ornest American staff, fax 3200) The data were subjected to SAS [24] statistical software (version 9.1) and analyzed based on a completely randomized design using the general linear model (GLM) procedure. When the overall model was statistically different (P<0.05), the Tukey-Kramer multiple comparison test was used to compare the mean values (P<0.05). Moreover, orthogonal contrasts were constructed

in order to compare the mean response variables for thyme extract received birds vs control birds.

RESULTS AND DISCUSSION

The effect of dietary thymus vulgarism extract supplementation in drinking water on Immune system of broiler chickens is showed in table 2. Organs relative weight as like spleen and bursa fabriciuse not affected by using of thyme extract at 21 and 42 days of experimental period. but higher bronchitis antibody titer for the birds consumed 0.2 and 0.6% of thyme extract showed (P<0.05) as compared with those consumed the 0.4% thymuse vulgarise extract and control birds at 21 days of age. Moreover, consumption of thymuse vulgarise extract in water increased the Bronchitis antibody titer as compared to the control birds in orthogonal comparisions. But there were no significant differences between the treatments for Bronchitis antibody titer at 42 days of age.

Ingredients (%)	Starter (0-21 d)	Grower (21-42 d)		
Corn	54.87	61.78		
Soybean meal (44 % protein)	36.72	26.36		
Fish meal	1.31	4.50		
Vegtable oil	3.00	4.00		
Limestone	1.15	1.05		
Dicalcium phosphate	1.94	1.49		
Vit. and min. premix ¹	0.50	0.50		
Salt	0.30	0.30		
DL-methionine	0.21	0.02		
Total	100.00	100.00		
Calculated analysis				
ME (kcal/kg)	2937	3100		
CP (%)	21.44	19.37		
Calcium (%)	1.05	1.00		
A. Phosphorus (%)	0.51	0.50		
Sodium (%)	0.16	0.14		
Arginine (%)	1.41	1.23		
Methionine + Cystine (%)	0.91	0.69		
Lysine (%)	1.20	1.10		
Tryptophan (%)	0.31	0.26		

Table 1. Composition of experimental diets

¹ provide per kilogram of diet: vitamin A, 15000 IU; vitamin D₃,8000 IU; vitamin K3, 3 mg; B₁₂, 15 μg; niacin, 32 mg; choline, 840 mg; biotin, 40 μg; thiamine, 4 mg; B₂ (riboflavin), 6.6 mg; pyridoxine, 5 mg; folic Acid, 1 mg; Zn, 80 mg; Mn, 100 mg; Se, 200 mg; Fe, 80 mg; Mg (magnesium oxide), 12; Cu, 10 mg; Ca (calcium pontatenate), 15 mg; iodeine, 1 m

In recent experiment, thyme extract not stimulated the immune response significantly, although Bronchitis antibady titer affected by thyme consumption on 21 day of age (P<0.05). In agreement with our results, Teymouri Zadeh et al [25] reported that immune factors such as bursa and spleen relative weight, and also antibody responses to red blood cell and Newcastle disease viruse no significantly difference between 0.1% thymus vulgaris extract received birds and control group. None of the immune related parameters such as antibody titer against Newcastle, Influenza viruses and sheep red blood cell, heterophil to lymphocyte ratio and albumin to globulin ratio were differed significantly in broilers treated with 5 and 10 g/kg thyme powder while compared with control birds [26]. Furthermore, Rahimi et al [27] reported that dietary thyme extract (0.1%) soluble in water increased performance and lactic acid counts and reduced *E.coli* numbers but did not affect immune system compared with control group (P<0.05). In the same result, serum antibody titer level against NDV in broilers that supplemented with 0.2, 0.4, 0.6, 0.8 and 1% garlic powder did not differe with control birds at

14, 28 and 42 days of age [28]. The beneficial effects of thyme plant on bacterial and fungal activities and also potent antioxidant properties of major components of thyme essential oil as thymol and carvacrol has been reported [29, 30]. Considring the thyme characteristics, we anticipated that an increase in immune response of chicks would be observed. The lower results of thyme extract on immune system is probably related to the dose of additives, type of thyme, posses and preparation period and also vaccination program times and stimulator material that used in our study. Regarding this fact that a few reports are available on the impact of thyme or thyme component on bird immune response, more studies will be needed to investigate thyme extract immonomodulatory properties and principal components (Thymol and carvacole) on broiler health.

In conclusion, results of the present study showed that supplementation of 0.2, 0.4 and 0.6% thyme extract in drinking water did not improve the immune status in broiler chickens in the whole experimental period.

Table 2. Effects of different levels of thyme extract supplemented in drinking water on immune organs and serum antibady titer against Newcastl disease viruse and infectious Bronchites viruse of broiler chickens at 21 and 42 days of age $A = thyme \ extract \ received \ birds; B = control \ birds$

Parameter	Antibady titer against NDV		Antibady titer against IBV		Bursa fabricius (%)		Spleen (%)	
Treatment	21 d	42 d	21 d	42d	21d	42d	21d	42d
Control (0.0)	524	884	490 ^b	578	0.51	0.11	0.9	0.1
LT(0.2%)	445	1749	1036 ^a	506	0.53	0.1	0.1	0.13
MT(0.4%)	956	813	467 ^b	671	0.55	0.09	0.11	0.08
HT(0.6%)	654	1737	1072^{a}	506	0.54	0.09	0.11	0.12
P value	0.66	0.46	0.0003	0.71	0.99	0.44	0.46	0.11
Orthogonal comparisions								
A versus B	0.65	0.39	0.003	0.9	0.81	0.6	0.24	0.63

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