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Full Length Research Paper

Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter

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A research study was conducted to investigate the effect of dietary inclusion of thyme powder as an antibiotic growth promoter substitution on performance, immune responses, hematological and biochemical parameters in broiler chicks. In this study, 192 day old chicks (Ross 308) were allocated to four treatments with four replicates based on a completely randomized design. Dietary treatments included control, antibiotic (flavophospholipol), and 5 and 10 g/kg thyme powder. Supplementing the diet with antibiotic and 5 g/kg thyme resulted in a significant increase in body weight (P<0.05). Feed intake of broilers was not markedly influenced by treatments but birds fed diets containing antibiotic had the lowest FCR (P<0.05). None of the immune related parameters tested differed significantly among experimental treatments (P>0.05). Thyme powder at 10 g/kg level significantly (P<0.05) increased HDL-cholesterol concentration but protein, albumin, triglyceride, total and LDL cholesterol concentrations were not influenced. Treatments also failed to induce any statistical impacts on hematological parameters of broilers including red and white blood cell count, hemoglobin and hematocrit values. The obtained results suggest that supplementing broilers' diet with 5 g/kg thyme can indicate favorable influences of antibiotic growth promoter on performance without any detrimental impacts on immune responses and blood parameters.

Key words: Broiler, thyme, growth performance, immunity, serum biochemistry, hematology.

INTRODUCTION

Sub-therapeutic dosages of antibiotics have been used extensively as growth promoters in livestock feeds for many years. They have been reported to increase growth rate and feed efficiency of poultry and other livestock as a result of improved gut health, nutrient utilization and improved feed conversion efficiency (Visek, 1978). However, there is the fear that the continuous and sub therapeutic use of in-feed antibiotics could lead to the development of antibiotic resistant bacteria which are harmful to humans.

Thus efforts have been made in different parts of the world to ban the inclusion of all types of antibiotic growth promoters in animal feeds. As a result of this inhibition and growing pressure on livestock producers, alternative substances and strategies for animal growth promotion and disease prevention are being investigated, among which phytogenic and herbal products have received increased attention since they have acquired more acceptability among consumers as natural additives.

Thyme (*Thymus vulgaris* L.) is a popular medicinal plant mostly grown in Mediterranean regions and is among the herbal plants which have received increased attention due to its antioxidant and antibacterial properties. The herb has also been reported to have anti-bacterial activities against a wide range of pathogenic microbial organisms (Vincent, 2002). The major components of thyme essential oil are thymol and carvacrol, which have been both shown to possess potent antioxidant properties

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Abbreviations: HDL, High-density lipoprotein; **LDL,** low-density lipoprotein; **RBC,** red blood cell; **WBC,** white blood cell; **Hb,** hemoglobin; **FCR,** feed conversion rate; **HMG-CoA,** 3-hydroxy-3-methylglutaryl-coenzyme A; **SRBC,** Sheep red blood cell.

Table 1. Ingredients and composition of the basal diet.

Diet ingredients (g/kg)	Starter	Grower	Finisher
Corn (8% CP)	545.5	540	567
Soybean meal (43 % CP)	401	390	360
Soybean oil	11	33.2	39
Calcium carbonate	10.6	8.9	8.7
Dicalcium phosphate	19.1	17.3	15.7
DL-Methionine	3	2.1	1.6
L-lysine	1.3	-	-
Vitamin Premix[1]	2.5	2.5	2.5
Mineral Premix[2]	2.5	2.5	2.5
Salt	3.5	3.5	3
Calculated composition			
Metabolizable energy (MJ/kg)	11.76	12.47	12.76
Crude protein (g/kg)	215	210	200
Ca (g/kg)	9.7	8.6	8.1
Available phosphorous (g/kg)	4.6	4.3	4
Methionine + Cystine (g/kg)	10	9	8.2
Lysine (g/kg)	13.2	11.9	11.1

Dicalcium phosphate contained: 16% phosphorous and 23% calcium.
[1]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; and Antioxidant 100 mg.
[2]Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe), 50 mg; Mn (MnSO4.H2O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO4.5H2O), 10 mg; I (KI, 58% I), 1mg; and Se (NaSeO3, 45.56% Se), 0.2 mg.

(Aeschbach et al., 1994). In addition, these phenolic compounds exhibit considerable antimicrobial and antifungicidal activities (Basilico and Basilico, 1999). Thymol is currently used to inhibit oral bacteria (Twetman and Peterson, 1997). Furthermore, Allen et al. (1998), Denil et al. (2004) and Cross et al. (2007) reported the beneficial effects of thyme in poultry production. The current study was conducted to evaluate the potential of applying different levels of thyme in comparison with an antibiotic growth promoter on performance, some imm-une responses, biochemical and hematological para-meters in broiler chicks when used as supplements in the diet.

MATERIALS AND METHODS

Animals and dietary treatments

One hundred and ninety two (192) day-old broiler chicks (Ross 308) were purchased from a local hatchery. On arrival, birds were randomly assigned to one of four treatments with four replicates of 12 birds based on a completely randomized design. The dietary treatments consisted of the basal diet as control, the antibiotic group received 30 mg/kg Flavophospholipol, thyme powder at levels of 5 and 10 g/kg as natural growth promoter. Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers provided by Ross Broiler Manual (2002). Chicks were raised on floor pens (120 × 120 × 80 cm) for 6 weeks and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness. The ambient temperature was gradually decreased from 33 to 25°C on day 21 and was then kept constant.

Performance parameters

Body weight was determined at 14, 28 and 42 days of age. Feed consumption and weight gain were recorded at different periods and feed conversion ratio (feed intake: weight gain) was calculated. Mortality was recorded as it occurred.

Carcass components

At 42 days of age, two birds per replicate were randomly chosen, slaughtered and their abdominal fat, liver, pancreas, gizzard, heart, proventriculus small intestine, caecum and lymphoid organs (bursa of Fabricius and spleen) were collected, weighed and calculated as a percentage of live body weight. The length of small intestine and caecum were also measured and recorded.

Immunity parameters

In this experiment, antibody titer against Newcastle, Influenza viruses and sheep red blood cell, heterophil to lymphocyte ratio and albumin to globulin ratio were measured as immune responses. At day 24 of age, two birds per replicate were randomly chosen and their blood samples were collected from brachial vein and centrifuged to obtain serum. Antibody titers against Newcastle and Influenza (H2N9) viruses were measured using Hemagglutination

Table 2. Effect of experimental diets on performance of broilers at different ages

Performance Parameters	Dietary treatments				
	Control	Antibiotic	5g/kg Thyme	10 g/kg Thyme	SEM
Body weight (g)					
14 d	275.8	306.7	279.8	281.8	10.25
28 d	946.8	1043.7	966.8	971	29.09
42 d	1956[b]	2091[a]	2079[a]	1949[b]	36.09
Daily feed intake (g/d)					
0-14 d	28.9	30.5	29.8	30.8	0.99
14-28 d	73.9	74.9	75.3	74.5	1.76
28-42 d	171	173.9	177	177	5.05
0-42 d	90.8	93.8	94	94.8	1.21
Feed : gain (g:g)					
0-14 d	1.78	1.66	1.8	1.85	0.0111
14-28 d	1.43	1.32	1.43	1.41	0.012
28-42 d	2.37	2.32	2.28	2.54	0.0309
0-42 d	1.95[bc]	1.86[c]	1.9[bc]	2.03[a]	0.006

Values in the same row not sharing a common superscript differ significantly (P <0.05).

Inhibition Test.

At day 22 of age, two birds were randomly selected from each replicate, and were inoculated via the brachial vein with 1 mL of 1% SRBC suspension. At day 6 after inoculation, blood samples were obtained from the brachial vein and SRBC antibody titers were measured by the microtiter procedure of Wegmann and Smithies (1966). Titers were expressed as the log2 of the reciprocal of the highest dilution giving visible hemagglutination. At 42 days of age, 2 birds per replicate were selected and their blood samples were collected using syringes containing heparin to avoid blood clot formation. Blood samples were prepared on slides and painted by Gimsa methods. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope then heterophil to lymphocyte ratio was calculated and recorded (Gross and Siegel, 1983).

In order to determine albumin to globulin ratio, blood samples (2 birds per replicate) were collected at 42 days of age and after serum separation, albumin and protein concentrations were determined using spectrophotometer and the kit package (Pars Azmoon Company; Tehran, Iran). Globulin concentration in serum was computed by subtracting albumin concentration from proteins.

Serum biochemical parameters

After 12 h fasting, blood samples were collected in non-heparinised tube at day 42 of age from 8 birds in each treatment by puncturing the brachial vein and the blood was centrifuged to obtain serum. Individual serum samples were analyzed for total protein, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglyceride using the kit package (Pars Azmoon Company; Tehran, Iran).

Hematological parameters

At day 42 of age, blood samples were collected from 8 birds in each treatment into vials containing EDTA. The red blood cell (RBC) and white blood cell (WBC) counts were determined by a hemocytometer method using Natt-Herrick solution; hematocrit (HCT) values and hemoglobin (Hb) values were measured by microhematocrit and cyanmethemoglobin methods respectively (Kececi et al., 1998).

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (1997). Mean scores were compared using the Duncan multiple range test. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

Performance and carcass traits

Data on performance parameters are summarized in Table 2. Broilers receiving antibiotic and 5 g/kg thyme had significantly higher body weights compared to other treatments at day 42 of age (P<0.05). Treatments failed to induce any marked effect on daily feed intake though it tended to increase in birds fed on thyme diets (P>0.05). Supplementing the diet with antibiotic resulted in a significant (P<0.05) improvement of FCR considering the entire period (0 – 42 d) but FCR of broilers in other periods was not affected. Antibiotics may control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chicks gut (Ferket, 2004). A more balanced biota population in gut could lead to a greater efficiency in digestibility and utilization of food, resulting in an enhanced growth and improved FCR (Bedford, 2000). The improvement in body weight of broilers achieved with 5 g/kg thyme could be attributed to its positive effect on nutrient digestibility, as reported by Langhout (2000). Other factors which could have contributed to the beneficial effects of the herbal products on

Table 3. Effect of experimental diets on carcass characteristics and organ weight of broilers at day 42.

Carcass traits	Dietary treatments				
	Control	Antibiotic	5 g/kg Thyme	10 g/kg Thyme	SEM
Carcass yield*	73.8	73.5	75	73.6	0.69
Abdominal fat*	1.76	1.25	1.68	1.55	0.18
Liver*	2.17	2.33	2.24	2.17	0.14
Gizzard*	2.05	2.08	3.23	2.38	0.16
Heart*	0.591	0.588	0.601	0.544	0.048
Pancreas*	0.228	0.244	0.236	0.245	0.021
proventriculus*	0.411	0.409	0.395	0.441	0.033
Small intestine*	3.22	3.34	3.4	3.71	0.18
Cecum*	0.618	0.685	0.705	0.732	0.07
Small intestine**	173	177	179	182	7.14
Cecum**	38	38	40	38	1.85

*Percentage of live body weight; ** cm.

the growth performance of birds are their probable anti-oxidant and antibacterial effects in the intestine (Nascimento et al., 2000). Mechanism of action of herbal products is not very clearly defined yet, but there are suggestions that they alter the permeability of the cell membranes and cause a destruction of the pathogenic bacteria (Skandamis and Nychas, 2001). For example, Helander et al. (1998) investigated how two isomeric phenols, carvacrol and thymol exert their antibacterial effects on *E. coli* O157 and S. typhimurium. Both carvacrol and thymol disintegrated the membrane of bacteria, leading to the release of membrane-associated materials from the cells to the external medium.

A higher dosage of thyme in the diet may have had an adverse effect on some beneficial microbial populations such as lactobacillus, preventing the herb from exhibiting its positive influence on performance and resulting in a poorer FCR. A number of reports have also provided evidence of the enhancement of FCRs through the dietary addition of carvacrol (Lee et al., 2003a) and a mixture of essential oils including oregano, cinnamon, thyme and capsicum (Zhang et al., 2005). Similar to our results, Denli et al. (2004) reported a significant improvement in body weight gain of broilers offered diets containing flavomycin at 10 mg/kg feed or thyme essential oil at 60 mg/kg and no differences in feed intake between treatment groups were observed. On the contrary, these scientists observed a positive influence of thyme essential oil on feed efficiency, which was not evident in our trial. Ciftci et al. (2009) showed that an increasing level of thyme oil upto 400 mg/kg in the diet of broilers depressed feed intake and birds receiving avilamycin antibiotic and 200 mg/kg thyme oil had the highest BW and the lowest FCR. However, Demir et al. (2005) indicated no differences in body weight gain, feed intake and feed efficiency of broilers fed diets supplemented with antibiotic growth promoter and five herbal natural feed additives from day 0 to 42 of age. No significant differences in body weight gain of broilers were observed when thyme powder (Sarıca et al., 2005) and a blend of extracts of sage, thyme and rosemary (Hernandez et al., 2004) were added to diets.

Table 3 indicates that broilers fed diets supplemented with antibiotic significantly had a lower percentage of abdominal fat in comparison with control birds ($P<0.05$). Other carcass traits evaluated including liver, pancreas, gizzard, heart, proventriculus, small intestine and caecum weights, small intestine and caecum lengths were not markedly affected by dietary treatments. Although birds receiving 5g/kg thyme had the highest carcass yield percentage, the differences were not statistically significant. Small intestine length was measured to be longer in birds fed diets supplemented with antibiotic and thyme ($P >0.05$).

Similar to the results obtained in this study, Denli et al. (2004) also showed that carcass percentage was not influenced by the dietary treatments but the supplementation of the diet with thyme essential oil significantly decreased abdominal fat weight and abdominal fat percentage. Also the reports by Ocak et al. (2008) indicated no statistical variations in carcass weight, carcass yield, the relative weights of the edible inner organs and whole gut, and the relative length of the whole gut of broilers fed diets supplemented with peppermint and thyme.

Immune responses

The effect of experimental diets on humoral immune responses is listed in Table 4. Treatments failed to have any statistical effect on antibody titer against Newcastle and influenza viruses and also SRBC at 28 day of age ($P>0.05$). Heterophil to lymphocyte and albumin to globulin ratios did not differ significantly ($P>0.05$) among treatments. Table 5 indicates the effect of treatments on

Table 4. Effect of experimental diets on antibody titers against Newcastle and Influenza viruses at d 24 and SRBS at d 28, heterophil to lymphocyte ratio and albumin to globulin ratio at d 42.

Variables	Dietary treatments				
	Control	Antibiotic	5 g/kg Thyme	10 g/kg Thyme	SEM
New castle (log_2)	5.5	5	5.3	5.6	0.6
Influenza (log_2)	3.62	4.75	5	4.38	0.58
SRBC (log_2)	6	5.12	5.25	4.37	0.89
Heterophil to lymphocyte ratio	0.365	0.422	0.3	0.392	0.054
Albumin to globulin ratio	0.85	0.78	0.49	0.94	0.21

Table 5. Effect of experimental diets on lymphoid organs weight at d 42.

Lymphoid organs	Dietary treatments				
	Control	Antibiotic	5 g/kg Thyme	10 g/kg Thyme	SEM
Bursa*	0.0982	0.0905	0.0884	0.0909	0.011
Spleen*	0.187	0.139	0.162	0.134	0.026

*Percentage of live body weight; SEM, standard error of mean.

Table 6. Effect of experimental diets on serum biochemical parameters of broilers at d 42.

Biochemical parameters	Dietary treatments				
	Control	Antibiotic	5g/kg Thyme	10g/kg Thyme	SEM
Protein*	3.4	3.5	3.9	3.01	0.34
Albumin*	1.2	1.5	1.3	1.3	0.33
Triglyceride*	142	125	124	117	19.55
Total cholesterol*	112	116	117	132	12.61
LDL-cholesterol*	32	35	32	37	3.64
HDL-cholesterol*	71^b	74^b	69^b	85^a	7.23

Values in the same row not sharing a common superscript differ significantly (P <0.05). * (mg/100 mL). SEM = Standard error of mean.

lymphoid organs' weight at 42 day. As is shown, the differences in lymphoid organs' weight were not statistically significant.

As thyme has been reported to have antibacterial and antifungicidal activities (Vincent, 2002; Basilico and Basilico, 1999) and the major components of thyme essential oil thymol and carvacrol have been indicated to possess potent antioxidant properties, an increase in immune responses of chicks was anticipated. Although the dietary treatments did not induce any significant effect on immune related parameters measured in this study, no deleterious impact was also detected as a result of thyme or antibiotic addition to the diet. It is probably due to the levels of additives applied in our study. To the best of our knowledge, unfortunately, no reports are available on the impact of thyme or thyme derivatives on bird immune responses. The results of the study of Nilforoushzadeh et al. (2008) showed that hydroalcoholic extracts of *Thymus vulgaris*, was significantly more effective than systemic glucantime or alcohol for the treatment of leishmaniais in Balb/c mice. Dietary supplmentation with 5000 mg/kg of thyme essential oil significantly decreased paw oedema and ear swelling and caused a significant inhibition of total mRNA IL-1B expression in the mouse colon; it markedly decreased the macroscopic and microscopic scores of colitis (Juhás et al., 2008).

Serum biochemical parameters

Table 6 summarizes data obtained on the effect of experimental treatments on serum biochemical parameters. No significant influence of experimental diets on protein, albumin, triglyceride, total and LDL- cholesterol was observed (P>0.05). The feeding of the broilers with 10 g/kg thyme resulted in a marked (P>0.05) increase in HDL- cholesterol concentration compared to other treatments. The differences in triglyceride concentration did not reach statistical significance but it tended to decrease

Table 7. Effect of experimental diets on blood parameters and indices of broilers at d 42.

Blood parameters	Dietary treatments				
	Control	Antibiotic	5 g/kg Thyme	10 g/kg Thyme	SEM
RBC (×10^6/µl)	2.58	2.58	2.51	2.52	0.16
WBC (×10^3/µl)	18.05	20.65	20.806	18.78	1.88
Hemoglobin (mg/100 mL)	10.6	11.01	10.2	10.8	0.49
Hematocrit (%)	30	32	31	31	1.3

SEM = Standard error of mean.

in serum of broilers fed thyme supplemented diets.

Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors. Triglyceride concentration tended to decrease in a dose dependent manner by addition of thyme, although the changes did not reach statistical significance; HDL-cholesterol level significantly increased on 10 g/kg thyme compared to other treatments. Similarly, Radwan et al. (2008) reported that the addition of 1% thyme to broiler diet resulted in a marked decrease in plasma total lipids. The results obtained do not agree with those reported by Ali et al. (2007) who found that adding thyme to hen's rations significantly decreased plasma HDL, total cholesterol, triglycerides and total lipids. The reduction of triglycerides and cholesterol noticed with thyme in animal studies was attributed to the lowering effect of thymol or carvacrol on HMG-Co A reductase the rate-limiting enzyme of cholesterol synthesis (Case et al., 1995; Lee et al., 2003b). But more compatible to our results Lee et al. (2003b) indicated that dietary carvacrol, and not thymol, reduces plasma triglycerides and phospholipids and suggested that carvacrol may have more impact on lipogenesis than on cholesterol biosynthesis. Bolukbası et al. (2006) reported that dietary thyme oil increases plasma level of triglycerides, LDL-cholesterol and HDL-cholesterol in broilers. Thus, further study is needed to clarify the mechanism of hypolipidemic actions of thyme.

Hematology parameters

Effects of experimental diets on broilers' blood parameters are presented in Table 7. The hematological parameters tested in current study include red and white blood cell counts, hemoglobin concentration and hematocrit percentage did not differ significantly among treatments. Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in hematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. For example, leucocytes are known to increase sharply when infection occurs, as they are one of the first lines of defense of the body (Ganong, 1999).

The hematological values obtained in this study indicated no detrimental impact of thyme on RBC and WBC counts, hemoglobin content and hematocrit percentage. Reports on the effect of thyme supplementation on blood hematological parameters are very scarce. Unlike our observation, Al-Kassie (2009) showed that feeding diets were supplemented with oil extract derived from thyme and cinnamon to broilers, which significantly increased RBC, HCT, Hb and WBC values compared with the control group. In conclusion, the results suggest that supplementing broilers diet with 5 g/kg thyme could indicate favorable influences of antibiotic growth promoter effect on performance of broilers without any detrimental impact on immune responses and blood parameters.

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