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The Effect of Essential Oil of Thyme (*Thimus vulgaris*) on to Performance and Humoral Immune Response Broilers Chicken

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Abstract: A trial was conducted to investigate the effects of adding different levels essential oils of thyme on growth performance and humoral immune response of chicken broilers. A 350 days old broiler chicks from a commercial hybrid (ROSS 308) were divided into 35 groups of 10 birds each. Seven diets were provided with T1 (without supplemented), T2 (45 mg), T3 (90 mg), T4 (135 mg), T5 (180 mg), T6 (225 mg) and T7 (270 mg) essential oils of thyme per kilogram. Each diet was randomly assigned to 5 replicates of birds for 48 days. Birds were fed the experimental diet as *ad libitum* throughout the experiment. The experiment lasted 48 days. At 8 and 18 days of age, all 350 chicks were vaccinated against NDV and at 10th day of age for vaccinated against Avian Influenza (AI). The sera were applied to Haemagglutination Inhibition (HI) test to determine Antibody (Ab) to NDV and AI expressed as reciprocal log 2 values for the highest dilution that displayed HI. Feed Intake (FI) and Feed Conversion Ratio (FCR) were calculated in each pen on weekly bases. Body weight, FCR and feed intake was recorded for the corresponding periods. Results showed Body Weight Gain (BWG) was not affected by dietary treatments. The differences among the groups were statistically significant for FI, FCR and final weight ($p<0.05$). Evaluation Antibody (Ab) for NDV showed among experimental diets was different significantly ($p<0.05$) but for AI not affected by dietary treatments. The best level of essential oil of thyme was 180 mg kg^{-1} and addition of >180 mg kg^{-1} to diet decreased performance.

Key words: Thyme, essential oil, broiler, performance, humoral immune, Iran

INTRODUCTION

During the last decade, phytogenic compounds have attracted a lot of attention for their potential role as alternatives to antibiotic growth promoters in animal nutrition. The efficacy of phytogenic applications in broiler nutrition depends on many factors such as composition and feed inclusion level of phytogenic preparations, bird genetic, diet composition and farm management. 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 (Steiner, 2009).

Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable (Lee *et al*., 2004). Thymol and Carvacrol, a major component isolated from essential oil of thyme that has been widely studied for its antimicrobial properties (Najafi and Torki, 2010). Thymol and carvacrol could have positive effects on growth performance in broilers and antibacterial, anticocidial, antifungal and antioxidant effects were reported by Hagmuller *et al*. (2006). Growth promoting feed additives relieve the host animal from immune defense stress during critical situations, raise the intestinal availability of essential nutrients for absorption and thus assist the animal to grow better within the framework of its genetic potential (Windisch *et al*., 2008). There is a clear lack of studies regarding the immune modulatory potential of phytogenic components. A wide range of phytogenic feed inclusion levels have been reported. In particular when aromatic plant parts were used, feed inclusion levels ranged from (0.01-30 g kg^{-1} diet). Examples include oregano addition at 30 g kg^{-1} feed (Young *et al*., 2003) and rosemary at 0.5 g kg^{-1}

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feed. Lower feed inclusion levels have been reported for EO. Examples include; rosemary and sage extracts at 500 mg kg^{-1} of feed oregano EO at 50-100 mg kg^{-1} of feed (Botsoglou *et al*., 2002) and thymol and cinnamaldehyde at 100 mg kg^{-1} feed (Lee *et al*., 2003; Al-Kassie and Jameel, 2009). The objective of the present research was to examine the effect of adding different levels essential oils of thyme on growth performance and humoral immune response of broilers.

MATERIALS AND METHODS

Birds and diets: Total 350 days old broiler chicks from a commercial hybrid (Rass 308) were divided into 35 groups of 10 birds each. Seven diets were provided with of T1 (without supplemented), T2 (45 mg), T3 (90 mg), T4 (135 mg), T5 (180 mg), T6 (225 mg), T7 and (270 mg) essential oils of thyme per kg. Each diet was randomly assigned to 5 groups of birds for 48 days. Birds were housed in deep litter pens (1×2 m^2). The birds were housed in environmental controlled room and they had allowed giving access to feed (mash) and water *ad libitum*. Birds were given starter diet from 0-21 days and from 22-48 finisher diet. The diets formulated according to the National Research Council recommendations (NRC, 1994) (Table 1).

Performance parameters and humoral immune response: Feed Intake (FI) and Feed Conversion Ratio (FCR) were calculated in each pen on weekly bases. Body weight, FCR and feed intake was recorded for the corresponding periods. Mortality was recorded a daily basis, FI data were corrected for body weight of dead birds. At 8 and 18th day of age, all 360 chicks were vaccinated against NDV and at 10th day of age for vaccinated. The sera were applied to Haemagglutination Inhibition (HI) test to determine Antibody (Ab) to NDV and AI expressed as reciprocal log 2 values for the highest dilution that displayed HI. Blood samples were withdrawn from the wing vein at days 48 of age. The non-heparinized blood samples (1.5 mL/chicken, 1 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.

Statistical analysis: The experiment was a completely randomized design with 5 replications. Data were analyzed by variance analysis using the procedures described by the SAS (2002). Significant differences among treatments were determined according to GLM procedure. For significant differences ($p<0.05$) means were compared by using the Duncan's method of the same statistically package.

Table 1: Composition of the basal experimental diets

	Days	
Ingredients (g kg^{-1})	0-21	22-49
Yellow maize	583.0	300.0
Soybean meal	331.0	200.0
Canola meal	-	70.0
Animal oil	-	25.0
Fish meal	50.0	-
wheat	-	292.0
Triticale	-	70.0
Malase	5.0	10.0
Limeston	12.0	10.5
Dicalcium phosphate	11.0	12.0
Vitamin premix1	2.5	2.5
Mineral premix[2]	2.5	2.5
Salt	1.5	1.0
DL-methionine	1.5	1.0
Lysine	-	0.5

[1]Supplied the following per kg of vitamin premix: Vitamin A 2700 IU; cholecalciferol 94000 IU; vitamin E 40 mg; menadione 681 mg; thiamine 454 mg; riboflavin 1.5 mg; niacin 13 mg; pantothenic acid 3.17 mg; pyridoxine 908 mg; folic acid 363 mg; biotin 30 mg. [2]Supplied the following per kg of mineral premix: Mn: 150 mg; Zn: 120 mg; Fe: 40 mg; Cu: 13 mg; I: 1 mg

RESULTS AND DISCUSSION

The effects of different levels thyme essential oil on broiler performance are shown in Table 2. Results showed Body Weight Gain (BWG) from 0-21, 21-48 and 0-28 days were not affected by dietary treatments. The differences among the groups were significant for Feed Intake (FI) from 22-48 and 0-48 days but was not significant from 0-21 days. Results showed that chicks fed with higher level essential oils had significantly decrease FI ($p<0.05$) for treatments T6 and T7 compared with control group. The birds were fed diet contain 180 mg kg^{-1} essential oils had the highest values FI and lowest FCR. With increased level to 180 mg kg^{-1} supplementation increased the FI and therefore improved the FCR. Final weight was not affected by dietary treatments from 0-21 days. The results showed that with increase level of essential oil to 180 mg, the highest final weight increase ($p<0.05$).

Improved body weight, feed intake and feed conversion ratio which may be due to active materials (thymol and carracerol) in these plants which are considered as digestion stimulating factors, in addition to their antimicrobial activity against bacteria found in the intestine resulting in enhanced growth. There is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects. These results agree with the research of Lee *et al*. (2004) who found that adding the cinnamon to the diet of broilers improved their growth performance. Some other researchers as Botsoglou *et al*. (2002) reported that essential oils were not effective in improving animal performance.

Table 2: The influence of dietary difference levels of thyme essential oil on the performance of broilers

Variables	T1[1]	T2	T3	T4	T5	T6	T7	p-value	SEM
Weight gain (g/bird/day)									
0-20 (days)	33.64	33.33	33.37	33.15	35.01	33.32	32.65	NS	0.35
21-48 (days)	73.01	73.08	73.09	73.52	74.01	72.65	71.57	NS	0.69
0-48 (days)	55.79	55.69	55.71	55.86	56.95	55.44	54.55	NS	0.49
Feed intake (g/bird/day)									
0-20 (days)	58.2.0	57.33	57.07	56.36	58.46	57.25	56.12	NS	0.51
22-48 (days)	147.76^{a}	147.53^{a}	147.64^{a}	147.77^{a}	147.57^{a}	146.03^{ab}	145.1^{b}	*	0.91
0-48 (days)	103.84^{ab}	103.96^{ab}	103.90^{ab}	103.36^{ab}	104.42^{a}	102.36^{b}	101.3^{b}	**	0.75
Feed efficiency (g g^{-1})									
0-20 (days)	1.73^{a}	1.72^{a}	1.71^{ab}	1.70^{ab}	1.67^{b}	1.72^{ab}	1.72^{ab}	*	0.01
22-48 (days)	2.02^{ab}	2.02^{ab}	2.02^{ab}	2.01^{b}	1.99^{b}	2.01^{ab}	2.03^{a}	*	0.02
0-48 (days)	1.86^{a}	1.87^{a}	1.86^{a}	1.85^{ab}	1.83^{b}	1.85^{ab}	1.86^{a}	*	0.01
Final weight (g bird^{-1})									
0-20 (days)	706.42	699.98	700.85	696.23	735.17	699.73	685.71	NS	35.21
22-48 (days)	1971.26^{b}	1973.25^{ab}	1973.41^{ab}	1984.98^{a}	1998.26^{a}	1961.53^{b}	1932.52^{c}	**	101.20
0-48 (days)	2677.68^{b}	2673.23^{b}	2674.26^{b}	2681.21^{a}	2733.43^{b}	2661.26^{bc}	2618.23^{d}	*	88.25
Mortality (%)	4.40	4.30	4.50	4.80	5.00	5.80	6.30	NS	0.26

Table 3: Humoral immune response against NDV and AI (log 2) of broiler chickens fed control diet (T1) and the diets containing different level essential oil of Thyme

Titers	T1	T2	T3	T4	T5	T6	T7	SEM	p-value
NDV	3.4^{b}	5.2^{ab}	5.4^{ab}	6.8^{a}	5.0^{ab}	6.0^{ab}	5.0^{ab}	0.92	*
AI	9.0	10.0^{a}	10.0	8.0	10.0	9.0	8.4^{b}	1.20	NS

T1 (without supplemented); T2 (45 mg); T3 (90 mg); T4 (135 mg); T5 (180 mg); T6 (225 mg) and T7 (270 mg) of essential oils of thyme per kilogram. $^{a-c}$Means in the same row for every trait with no common superscript differ significantly NS>0.05, *p≤0.05

Lee *et al*. (2004) reported that thymol did not improve poultry performance. Hertrampf, however noted that thyme oil supplementation in the drinking water of chickens increased weight gain. Al-Kassie and Jameel (2009) reported supplementation diet with 100 and 200 mg kg^{-1} resulted improved final gain, FCR and feed intake. The resulted contradictory with find Najafi and Torki (2009) that showed that high level supplement essential oils thyme adverse effect on FI, FCR and final weight. The effects of different levels thyme essential oil on humoral immune showed in Table 3. Secondary antibody response anti-NDV was affected by dietary essential oil inclusion levels. The level of 135 mg kg^{-1} essential oil showed the highest titer response anti-AI was not affected. This resulted contrast with resulted by Najafi and Torki (2010).

CONCLUSION

In this study, addition of >180 mg kg^{-1} essential oil of thyme to diet decreased performance and suggested level to 180 mg kg^{-1} to diet.

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