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Effect of Dietary Thyme (*Thymus vulgaris*) on Laying Hens Performance and *Escherichia coli* (E. coli) Concentration in Feces

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Abstract

The aim of the present study was to investigate the effect of dietary supplementation with Thyme (Thymus vulgaris) on performance of laying hens and E. coli concentrations in feces. Sixty four of 24 weeks old white Lohman LSL laying hens were randomly assigned to four groups equally (n = 16). Each treatment was replicated four times. Experimental diets were prepared by adding thyme at the levels of 0, 0.1, 0.5 and 1% to basal diet. Feed conversion and egg productions of laying hen were improved by thyme supplementation at level 0.1 and 0.5 %. Also, the usage of 0.1 and 0.5 % thyme in laying hens diets significantly ($P<0.05$) reduced E. coli concentration in feces.

Key words: laying hen, thyme, E. coli, egg production

INTRODUCTION

Recently, it has been reported that the usage of antibiotics as a growth promoter in chicken diets has caused some unwanted factors [1, 2-3]. Therefore, the searches for alternative feed supplements have been increased extensively and considerable attention has been given to the essential herbs as replacements for antibiotics growth promotants [4]. Langhout, [5] and Williams and Losa, [6] discovered that essential oils have a stimulating effect on animal digestive systems. They postulated that these effects could be due to the increased production of digestive enzymes and the improved utilization of digestive products through enhanced liver functions.

Hertrampf, [7] and Alçiçek et al. [8] demonstrated that essential oils improved animal performance, however, other researchers [9, 10-11] reported that these additives were not effective in this regard. Deschepper et al. [4] determined that essential oil from herbs have received considerable attention as replacements for antibiotic growth promotants. The antibacterial and anticoccidial effects of essential oils, or components from plant extracts, have received widespread attention and numerous reports exist in the literature. For example, Jamroz et al. [12] determined that plant extract (carvacrol, cinnamaldehyde and capsaicin) reduced the total *E. Coli* and *Clostridium perfringes* numbers in the intestines of broiler chickens; it has been reported that blends of essential oil components can control *Clostridium perfringens* colonization in the intestine and feces of broiler chickens [13].

As an essential oil, antibacterial, anticocidial, antifungal and antioxidant effects of thyme oil derived from thyme were reported by Hertrampf, [7]. Also, in vitro studies have shown that essential oils to have antibacterial properties against *Listeria monocytogenes*, *Salmonellatyphimurium*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* [14]. Smith-Palmer et al. [15] and Hammer et al. [16] 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*. Dorman and Deans, [19] reported that thymol (5-methyl-2-(1-methylethyl) phenol), a main component of the essential oil from thyme, has antimicrobial properties. Essential oils' antimicrobial mode of action consists of interactions with the cell membranes of microorganisms by changing permeability for cautions such as H^+ and K^+ [18]. Since, there has been yet any report dealing with the promoter and antimicrobial effect of dietary supplemented thyme on performance, the objective of this study was to evaluate the use of the thyme in hen feeding to promote performance and reduced *E. coli* concentration in feces.

MATERIALS AND METHODS

Sixty four, 24- wk-old lohman-LSL hybrid laying hens were used in this experiment. Birds were randomly assigned to 4 groups at equally (n=16), each of which included 4 cages (50 x 46 x 46 cm) with four animals. The treatments consisted of diets containing 0, 0.1, 0.5 or 1 % thyme powder. Composition of the experimental diets is presented in Tables 1. The diets were isoenergetic and isonitrogenous. Experiment lasted in 12 weeks at the beginning of laying period. During the experiment hens were fed and water *ad libitum*. Egg production, feed conversion rate and feed consumption amounts were recorded daily from each cage. Percentage of yolk, albumen and shell, egg weight and Hough unit values were measured biweekly using 8 eggs from each dietary treatment. At the end of experiment, feces samples were taken from each replicate cage in order to determine total *Coliform* and *E. coli*.

Bacteriology

Fecal samples were blended in a stomacher (Stomacher 400; AJ Seward, London, England) for 2 min in 50 mL of 0.85 % (w/v) salt water. A series of fermentation tubes that contain Fuluorocult lauryl sulfat broth were inoculated with the water sample and incubated for 48 hours at 35 °C. The fermentation tube contains an inverted tube to trap gases that were produced by the *Coliform* bacteria. After 48 hours, the fermentation tube was examined for gas production. After, the tubes were examined under a 366-nm Lampe UV for E. coli. Based on which dilutions showed positive for C*oliform* and *E. coli*, a table of most probable numbers was used to estimate the C*oliform* content of the sample. The results were reported as most probable number (MPN) of C*oliform* and *E. coli* per g [19].

Differences between groups were analyzed with analysis of variance (ANOVA) by using the statistical package SPSS for Windows (20), version 10.0. Significant means were subjected to a multiple comparison test (Duncan) at α = 0.01 and 0.05 level.

Table 1. Calculated nutrient contents of the diet and additionally

Ingredients and analyses	Composition (%)
Corn	48
Soybean meal	19.5
Wheat	12
Meat and bone meal	3
Sunflower meal	5
Limestone	8.5
Soybean oil	3
Dicalcium phosphate	0.40
Vitamin premix[1]	0.25
Mineral premix[2]	0.1
Salt	0.05
DL-methionine	0.14
Calculated analysis	
CP	16
ME, kcal/kg	2710

[1] Per kilogram of vitamin premix: 4800000 IU vitamin A; 960000 IU vitamin D;1200 IU vitamin E; 1 g vitamin K_3; 1.2 g vitamin B_1; 2.8 g vitamin B_2; 8 g niacin; 3.2 g calcium D-pantothenate; 1.6 g vitamin B_6; 6 mg vitamin B_{12}; 400 mg folic acid; 18 mg D-biotin; 20 g vitamin C; 50 g choline chloride

[2]per kilogram of diet**:** 80 g manganese; 80 g iron; 60 g zinc; 5 g copper; 200 mg cobalt; 500 mg iodine; 150 mg selenium

RESULTS

In the present study, the effects of dietary treatment on feed intake and egg production were significant (P< 0.05). Supplementation of thyme at levels of 1% diet significantly decreased feed intakes. Addition of 0.1% and 0.5% of thyme to the diets significantly improved feed conversion ratios. But, supplementation of 1% thyme decreased feed conversion compared to control. Egg production in laying hens was increased significantly (P<0.05) with the supplementation of dietary 0.1 and 0.5% thyme. However, egg weight did not change during the experimental period from the birds receiving the control diet and the others with thyme (Table 2).

There were significant effects of dietary treatments on yolk rate of egg (Table 3). Hens receiving the diets containing 1 % thyme had significantly lower yolk rate compared to those fed the control and the diet containing 0.1 and 0.5% thyme. However, there were no differences (P > 0.05) in albumen, shell and Hough unit between the treatments in this study.

The *coliform* count in the feces did not differ (P > 0.05) by any of the supplemental treatments (Table 4). The control group and 1% thyme group showed the highest average concentration of *E. coli* in the feces. The group fed with the 0.1% and 0.5% thyme had significantly lower *E. coli* count than the control group and 1% thyme group. Average *E. coli* concentrations significantly differed (P < 0.05) from each other 0.1% thyme group had the lowest concentration. The inhibitory effect o on the proliferation on *E. coli* seemed to be stronger for 0.1% thyme.

Table 4. Influence of dietary thyme herb on total *Coliform* bacteria and *E. coli* in fecal samples of laying hens (MPN/g)

Groups	*coliform*	*E.coli*
Control	110	110[a]
0.1% Thyme	73	46[c]
0.5% Thyme	110	73[b]
1% Thyme	110	110[a]
P	NS	*

NS: not significant *: P<0.05

Table 2. Influence of dietary thyme *(Thymus vulgaris)* on performance of laying hens

Groups	Feed intake (g)	Feed conversion (g/g)	Egg production %	Egg Weight (g)
Control	150.66[a]	2.95[b]	89.81[b]	50.90
0.1% Thyme	150.55[a]	2.88[c]	95.37[a]	51.62
0.5% Thyme	147.57[ab]	2.79[d]	94.44[a]	49.07
1% Thyme	145.59[c]	3.04[a]	87.96[b]	52.69
SEM	0.46	0.04	1.15	0.94
P	*	*	*	NS

NS: not significant *: P< 0.05 [a,b,c] :Column means with no common superscript differ significantly

Table 3. Influence of dietary thyme on egg quality of laying hen.

Groups	Yolk %	Albumen %	Shell %	Hough Unit
Control	26.25[a]	62.65	11.08	81.25
0.1% Thyme	26.67[a]	61.94	11.38	83.03
0.5% Thyme	26.35[a]	62.25	11.34	83.76
1% Thyme	23.44[b]	63.46	12.19	81.57
SEM	0.30	0.38	0.36	1.32
P	*	NS	NS	NS

NS: not significant *: P< 0.05 [a,b,c] :Column means with no common superscript differ significantly

DISCUSSION

Recently, scientists discovered that essential oils have a stimulating effect on animal digestive systems. They postulated that these effects could be due to the increased production of digestive enzymes and the improved utilization of digestive products through enhanced liver functions [5- 6]. Hertrampf, [7] reported that essential oils derived from spices and herbs could be successfully used as growth promoters, since they increased the feed intake due to their aromatic characteristics in chickens.

In this study, the *coliform* counts in the feces of the laying hens were not affected by any of the supplemental treatments. But, Cross et al. [21] demonstrated that thyme reduced the numbers of *coliforms*. The 0.1% thyme group showed the significantly lowest average *E. coli* concentration. Some studies reported that thyme (*Thymus vulgaris*) was among the most active from this respect against to strains of *E. coli* [14, 16, 17-22]. Jamroz et al. [12] reported that plant extract (carvacrol, cinnamaldhyde and capsaicin) reduced the total *E. coli* numbers in intestine of broilers chickens. Sarıca et al. [22] found that the broilers fed with thyme (0.1%) had significantly lower *E. coli* count than the control diet in the small intestine. Tucker [23] reported that the supplementation of a mixed herbal product containing garlic, anise, cinnamon, rosemary and thyme to commercial pig diets significantly inhibited the number of *E. coli* in the digestive tract.

In conclusion, the data of the present study showed that feeding laying hens with diet containing 0.1 and 0.5 % thyme improved egg production and feed conversion and significantly reduced *E. coli* concentrations in the feces compared to the basal diet.

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