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Effect of thyme extract on *Escherichia coli* intestinal microbial load of broiler chickens

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ABSTRACT

Recently, uses of alternative strategies to prevent an imbalance in the intestinal microbial population (IMP) and the potential development of intestinal disorders in livestock, developed. Thyme extract have been known to exhibit antimicrobial activity against specific microbial species and could therefore be considered an alternative in controlling the IMP. This study was conducted to determine the effect of the addition of different levels of thyme, added to drinking water, on the gastrointestinal tract. Total bacterial count, of E.coli bacteria was determined in different region of intestine. Two hundred and forty, day-old broiler chicks (Ross-308) were divided into four equal groups (each group include 3 repetition). Experiment was as follow; a control group with no thyme and in other two groups, thyme extract was used 500 ppm and 1000 ppm and in last group feed was pellet and thyme was not used. Experiments were carried out for 42 days and thyme extract was used from day 7 to 42. Results showed that chicks fed with 1000 ppm thyme extract had significantly lower ($p<0.05$) mortality rate followed by chicks fed with 500 ppm thyme extract and pellet group compared with control group, which showed the lowest performance. The chicks fed with 500 and 1000 ppm thyme extract had reduced ($p<0.05$) total bacteria count (TBC) and in pellet group TBC was highest. In conclusion, thyme extract could be considered as a potential natural growth promoter and have the advantage of inhibiting the growth of potential pathogens for poultry at the level of 1000 ppm.

Keywords: Thyme extract, *E.coli*, Broiler, Total Bacterial load

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INTRODUCTION

The prophylactic use of antibiotics (as growth promoters) in broiler feeds lead to enhance growth rate and improved feed conversion ratio in poultry industry. In the European Union, the use of antibiotics for broilers has been limited to only antibiotics that are not used in human medicine. Therefore, in the last few years, uses of medicinal plants (essential oils) have been increased that because of their antimicrobial activity(9). Thyme (*Thymus vulgaris* L.) is a popular medicinal plant mostly grown in Mediterranean regions and possesses antioxidants (19) and antibacterial properties(27). The major components of thyme essential oil are phenoylic compounds such as thymol (44.4-58.1%), carvacrol (2.4-4.2%) and γ-terpipene (6.9-18.9%)(1, 4, 13, 16, 21). The antimicrobial activity of EOs has been recognized and they have been extensively tested *in vitro* and *in vitro* against a wide range of pathogenic bacteria and fungi(11, 15, 17, 24, 25). Animal trials have also demonstrated the promising effects of EOs against the colonization and proliferation of *Escherichia coli* (2, 5). Recently researchers showed that, thyme oil only at high concentrations (500 mg/l) effective against *Clostridium perfringens, Streptococcus epidermis, Salmonella* serovars and at low concentrations (50 mg/l) effective against *Escherichia coli* (23). It seems that main target of essential oils is cell membrane of bacterial cells (6) and because of that, gram-positive bacteria's are generally more sensitive to antibacterial activity of thyme oil than gram-negative bacteria's(17). It has been suggested that lipophilic properties and chemical structures of essential oils can play a role in antimicrobial mechanism of them(8, 12).

The purpose of this study was to investigate the effect of thyme extract on the load of *Escherichia coli* in intestinal contents of broiler chickens.

MATERIALS AND METHODS

Animals and dietary treatments

The experiment used 240 day-old broiler chicks (Ross-308) that were randomly divided into four equal treatment groups with 3 replicates of 20 birds based on a completely randomized design. The dietary treatments consisted of the basal diet as control group that does not receive thyme (group 1) and in two other treatment groups, thyme extract was used 500 ppm (group 2) and 1000 ppm (group 3) and in last group feed was pellet and thyme was not used (group 4). Experiments were carried out for 42 days and thyme extract was used from day 7 to 42.

Chicks were raised on floor pens (100 × 200 × 80 cm) for 42 days 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.

E. coli Population in the Intestinal Contents

For a determination of *Escherichia coli* in intestinal digesta, 3 birds per each replicate randomly selected and the contents of the ileum and caeca were separately collected, cooled and used for microbial assays. The populations of *Escherichia coli* were then estimated as CFU g-1. One gram of fresh faeces was added to 9 ml Sterilized PBS, and then subsequent dilutions prepared. *E. coli* was cultured on MacConkey agar (Merck, Germany) at 37°C for 24 hours, and the presence of *E. coli* then determined.

Statistical analysis

Data were analyzed by one-way ANOVA ($p<0.05$) with a completely randomized design. Parameters mean was compared with the Duncan's multiple range tests. PASW SPSS (Version 18.0) statistical package was used for analyzing data.

RESULTS

Mortality rate (MR) presented in Table 1. Results showed that there were significant difference between groups and chicks fed with 1000 ppm thyme extract had significantly lower ($p<0.05$) mortality rate followed by chicks fed with 500 ppm thyme extract and pellet group compared with control group, which showed the lowest performance. The chicks fed with 500 and 1000 ppm thyme extract had reduced ($p<0.05$) total bacteria count (TBC) and in pellet group TBC was highest. The highest BW was in pellet and 1000 ppm thyme group that was significantly different ($p<0.05$) from two other groups. The highest mortality rate was observed in control group (group 1), followed by the pellet group (group 4), and in group 3 (1000 ppm thyme) the mortality rate was lowest.

Table 1. Effects of thyme extracts on mortality rate. (Mean ± Standard error)

Group	MR
(control) 1	8.2 ± 0.11^{d}
(500 ppm)2	6.53 ± 0.28^{b}
(1000ppm)3	5.5 ± 0.08^{a}
(pellet)4	7.2 ± 0.05^{c}

**: Means within same column having different letters are significantly different (P<0.05).*

DISCUSSION

Recently essential oils are used as feed supplements to improve growth performance of broilers. It was expected that supplementing extracted thyme oil would stimulate growth performance in the broilers, but research on essential oil yielded contradicting results (3, 10, 22).

The data showed that in both groups that fed the thyme diet there was a significant decrease ($p<0.05$) of total bacteria count (CFU/gm) in intestine, and in group that fed pellet total bacterial count was highest ($p<0.05$). This may be considered as an indication to the fact that the addition of the 500, and 1000 ppm thyme led to a significant decrease in the total bacteria count. Our results are in agreement with that of Al-Kassie, (2010) and Burt, S. and Reinders, R. (2003) (2, 7). Al-Kassie (2010), noted that the addition of thyme and cinnamon has decrease the total bacteria count statistically ($p<0.05$), in jejunum and large intestine compared into the control. Thymol 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 (18), the antimicrobial effect of thymol on these bacteria played on vital membrane ions of potassium and hydrogen equilibrium pumps (5). Also

Friedman, M., et. al., (2002), confirmed thymol and carvacrol antibacterial activities against *E.coli, S.enterica, C.jejuni, and L.monocytogenes* in vitro, and they noted that carvacrol, cinnamaldehyde, and thymol were most active against *E.coli*(14).

The chicks that get 500 and 1000 ppm thyme in drinking water had decreased ($p<0.05$) heterophil-to-lymphocyte ratio, this results showed that, increase of TBC in other groups cause increase in heterophil numbers and due to that the heterophil-to-lymphocyte ratio increased in other groups and the highest increase was in pellet group which because of bacterial growth stress in that group. This results in agreement with Najafi, P. and Totki, M. (2010) that they reported chicks fed with thyme diet had significantly lower number of heterophils comparing to control group(20), and Toghyani, M. et. al., (2010), reported that the chicks fed with 5g/Kg thyme diet had lower heterophil to lymphocyte ratio comparing to control and antibiotic groups(26).

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