Original Article

The effect of Antibiofin® on intestinal bacterial population in broiler chickens

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Summary

The recent study was performed to investigate the effects of Antibiofin® (including mostly *Thymus vulgaris*) in drinking water on intestinal bacterial population in broiler chickens. A total of 120 one day-old broiler chickens were purchased and divided into three equal groups. Each group divided into four subgroup of 10 chicks. Chickens of groups A and B received 0.1% and 0.2% of Antibiofin®, respectively in drinking water from one week before vaccination till two weeks after vaccination. Chickens of group C did not receive Antibiofin®. All groups were subcutaneously vaccinated with AI-ND killed vaccine (subtype H9N2) at neck back site at nine days old. The results of this study showed that the consumption of antibiofin® at 0.1% and 0.2% concentrations reduced colony forming units of *Escherichia coli* in group A and B compared to control group, though it was not statistically significant. The colony forming units of *Escherichia coli* in digesta of ileo-cecum in group A and B on Mac Conkey agar, nutrient agar and Eosin methylene blue agar showed a lower number compared to control group. However, there was no significant difference between all groups in *E. coli* counts.

Keywords: Antibiofin®, thyme extract, *E. coli*, intestine, broiler chickens.

Introduction

Nowadays, using antibiotics at sub-therapeutic levels has caused concerns about antibiotic residues in the animal production which consequently leads to the development of drug-resistant bacteria in animals and human. Thus, at the beginning of 2006, in the European Union, medical and public concerns focused on the complete omission of the antibiotics from animal food (Cervantes, 2006; Nollet, 2005; Wakeman, 2005). Accordingly, in poultry industry, it is important to replace antibiotic growth promoters in the food by other substances (Bach Knudsen, 2001). Application of feed additives has two objectives: controlling pathogenic microorganisms and enhancing beneficial microorganisms in the digestive content of the gut (Vahdatpour et al., 2011). Recently some substances such as phytogenic feed additives, prebiotics, and probiotics have been used instead of antibiotics (Patterson and Burkholder, 2003; Ricke, 2003). Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions. Isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract. The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism (Horton et al., 1991; Baratta et al., 1998; Jamroz et al., 2003). Volatile oil from thyme (*Thymus vulgaris*) was assessed for antibacterial and antiviral activity as inhibitors of microbial growth (Dorman and Deans, 2000). In older animals the effectiveness of plant extract supplementation was relatively low, but higher digestibility of nutrients and reduction of *Escherichia coli* (*E. coli)* and *Clostridium* sp.in intestinal content were stated (Jamroz et al., 2003). Some herbs that are full of flavonoids such as thyme (*Thymus vulgaris*) increase the activity of vitamin C and act as antioxidants and seem to improve the immune function (Manach et al., 1996; Cook and Samman, 1996). Carvacrol and thymol are the main phenolic components in *Thymus vulgaris* (Masada, 1976). This study was conducted to study the effect of different levels of Antibiofin® (including mostly *thymus vulgaris* and other extract such as *Salvia officinalis*, *Satureja hotensis*, and *Agastache foeniculum*) on intestinal bacterial population in broiler chickens.

Materials and Methods

Thyme extract:

Antibiofin® (including mostly *Thymus vulgaris* and other extract such as *Salvia officinalis*, *Satureja hotensis*, and *Agastache foeniculum*) was purchased commercially as solution from Pars Imen Daru Co., Iran.

Experimental design:

A total of 120 one day-old broiler chickens were purchased and divided into 3 equal groups. Each group divided into 4 subgroup of 10 chicks. Chickens of groups A and B received 0.1% and 0.2% of Antibiofin®, respectively in drinking water from one week before vaccination till two weeks after vaccination. Chickens of group C did not receive Antibiofin®. All groups were subcutaneously vaccinated with AI-ND killed vaccine (subtype H9N2) on neck back site at 9 days old.

Selected bacterial population in the intestinal contents:

For determination of populations of *E. coli* in intestinal digesta of 12 birds (4 birds per treatment), the contents of the distal part of the small intestine (10 cm anterior to the junction with caecum and rectum) and whole caeca of one bird per replicate pen were separately collected, and used for microbial assays. The populations of *E. coli* were estimated as CFU g^{-1}. Sterilized phosphate buffered saline (PBS) (99 mL) was added (1:100) to 1 g of fresh material, and then subsequent dilutions prepared. Samples were cultured on Mac Conkey agar (Merck, Germany), nutrient agar (Merck, Germany), and eosin methylene blue agar *(*EMB*)* (Merck, Germany), at 37°C for 24 hours, and the presence of *E. coli* then determined.

Statistical analysis

The original data for microbial counts were transformed to log10 CFU g^{-1} of intestinal content for statistical analysis. The data were submitted to analysis of variance using the Statistical Package for Social Sciences (SPSS) version 18.0. Mean differences among treatments were evaluated through one way ANOVA and pair tests were performed using LSD Test at P< 0.05 level.

Results

According to Table 1, the results of this study showed that consumption of antibiofin® at 0.1% and 0.2% concentrations reduced colony forming units of *E. coli* in group A and B compared to control group, though it was not statistically significant.

Table 1. The effect of Antibiofin® on *E.coli* numbers in ileo-cecal contents of broilers**.**

Groups	**Medium**		
	NA	**Mac**	**EMB**
A (0.1%)	**8.42±0.88***	**7.73±0.74**	**8.12±0. 45**
B (0.2%)	**8.45±.0.92**	**8.13±0.9**	**8.23±.0.74**
C(control)	**8.88± 0.95**	**8.26± 0.98**	**8.28± 0.64**

* log CFU g^{-1} ± standard deviation of means

The colony forming units of *E. coli* in digesta of ileo-cecum in group A and B on Mac Conkey agar, nutrient agar and Eosin methylene blue agar showed a lower number compared to control group. However, there were no statistically significant differences between all groups.

Discussion

This study showed that lower *E. coli* counts were related to group A and B, but there was no significant difference between all groups. Significant reduction of *E. coli* number has been obtained following an application of natural plant extract in earlier studies (Jamroz et al., 2005). It has been documented that garlic extracts exert a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Rees et al., 1993). Inhibition observed in *E. coli* was more than 10 times greater than that seen in *Lactobacillus casei* for the same garlic dose (Skyrme, 1997). Exactly why this differential inhibition should occur is not clear, but it may be due to differing composition of bacterial membranes and their permeability to allicin (Miron et al., 2000). Garlic extract and allicin have been shown to exert bacteriostatic effects on some vancomycin-resistant enterococci. Decreasing number of such viable Gram-positive bacteria, as *Lactobacilli* and *Bifidobacteria* may increase the presence of Gram-negative species. Pollystilenes from coneflower showed bacteriostatics against *E. coli* (Schulte et al., 1967). Canan Bolukbasi and Kuddusi Erhan (2006) showed that control group and the 1% thyme group had the highest average concentration of *E. coli* in feces. The group fed 0.1% and 0.5% thyme had a significantly lower *E. coli* count than the control and the 1% thyme group. Average *E. coli* counts has significantly (P< 0.05) differed in two groups, with the 0.1% thyme group bearing the lowest concentration. In agreement with our results thymol (from thyme essential oil) has been shown to reduce the number of coliforms within the digesta of chickens (Cross et al., 2004). Furthermore, it has been suggested that supplementation with oligosaccharides may have a prebiotic effect through an increase in production of lactic acid, thus increasing the proliferation of beneficial bacteria and reducing the presence of Gram-negative bacteria (Savage et al., 1996).

In conclusion, the colony forming units of *E. coli* in digesta of ileo-cecum in group A and B on Mac Conkey agar, nutrient agar and Eosin methylene blue agar showed a lower number compared to control group, but there were no statistically significant differences between all groups. However, more trials are needed to clarify the effect of different medicinal plants on intestinal bacterial population in broiler chickens.

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