

RESEARCH ARTICLE

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Effects of antibiotic growth promoter, probiotic and basil essential oil supplementation on the intestinal microflora of broiler chickens

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ABSTRACT

Effects of the probiotic 'Protexin', basil essential oil and the antibiotic growth promoter 'Avilamycin' were studied on the ileum microbial flora of broilers when these substances are used as broiler feed additives. A total of six hundred Arian broilers were divided into 6 treatment groups, with 4 replicates of 25 birds. Treatments have been performed with a plant essential oil at 3 levels (200, 400 and 600 ppm), the probiotic 'Protexin' (150 ppm), the antibiotic 'Avilamycin' (150 ppm) and a control group with no additives. Birds in different treatments received the same diets during the experimental period. The results showed that the probiotic treatment significantly decreased the total bacteria counts ($P < 0.05$). Any of supplementation did not affect colony-forming units of lactobacilli ($P > 0.05$). The lowest and highest lactic acid bacteria in ileum were obtained in the control group and in birds receiving 400 ppm basil essential oil, respectively. Moreover, addition of 600 ppm of basil essential oil into diet decreased the number of *E. coli* colonies as compared to other treatments ($P < 0.05$). It could be speculated that the basil essential oil and 'Protexin' could replace the antibiotics, which have been banned to use as growth promoter in animal feeds.

Key words: broiler, intestine, basil essential oil, antibacterial, probiotic

Introduction

The use of antibiotics with prophylactic characters in aviculture, started to be seen as a risk factor to human health, since there is a chance that their residues may be found in the tissues, and also by the probable induction of cross-resistance for pathogenic bacteria in humans, which could generate problems related to public health (Petrolli et al., 2012). Today, in the European Union only three antibiotics are still permitted as growth promoters (Salinomycin-Na, Flavophospholipol and Avilamycin) and a general ban is foreseen in some years from now, because of the increased occurrence of pathogens resistant against therapeutical antibiotics used in animals and humans (Wenk, 2003). With the restricted use or ban of dietary antimicrobial agents we must explore new ways to improve and protect the health status of farm animals, to guarantee animal performance and to increase nutrient availability (Wenk, 2003). The essential

oils, organic acids, enzymes, probiotics (*Lactobacilli*), prebiotics (oligosaccharides) and the herbs have received increased attention as possible antibiotic growth promoters replacement (Cho et al., 2006). Probiotics are viable microbial additives that assist in the establishment of a beneficial intestinal population antagonistic to harmful microbes. The action of probiotics can be explained by their production of antimicrobial substances that protect villi and absorptive surfaces against toxins produced by the pathogens, improvement of immunity stimulation, and ability to increase volatile fatty acids (Shams Shargh et al., 2012). Essential oils (also called volatile or ethereal oils) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, woods, fruits and roots) (Cho et al., 2006). Beneficial effects of essential oils on farm animals may arise from activation of feed intake and secretion of digestive secretions, immune stimulation, anti-bacterial, coccidiostatic, antiviral and antioxidant properties (Wenk, 2003).

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Gut microflora has significant effects on host nutrition, health, and growth performance by interacting with nutrient utilization and the development of gut system of the host. This interaction is very complex and, depending on the composition and activity of the gut microflora, it can have either positive or negative effects on the health and growth of birds (Yang et al., 2009). Chicks grown in a pathogen-free environment grow 15% faster than those grown under conventional conditions, where they are exposed to bacteria and viruses. The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria, so that the health, immune status and performance are improved (Yang et al., 2009). The pharmacological action of active plant substances or herbal extracts in humans is well known, but in animal nutrition the number of precise experiments is relatively low (Mohamed et al., 2011). Basil known as sweet and garden basil, a member of the *Lamiaceae* family, is commonly cultivated throughout Mediterranean region (Abbas, 2010). The leaves and flowering tops of sweet basil are used as carminative, galactogogue, stomachic and antispasmodic medicinal plant in the folk medicine (Sajjadi, 2006). However, recently the potential uses of *O. basilicum* essential oils, particularly as antimicrobial and antioxidant agents, have also been investigated. The chemical composition of basil oil has been the subject of considerable studies. There is extensive diversity in the constituents of the basil oils and several chemotypes have been established from various phytochemical investigations. However, methyl chavicol, linalool, methyl cinnamate, methyleugenol, eugenol and geraniol are reported as major components of the oils of different chemotypes of *O. basilicum*. (Sajjadi, 2006). We have no information about the relationship between *in vitro* antimicrobial potential and efficiency of essential oils in broiler chickens. Perhaps essential oils, which inhibit pathogenic and nonpathogenic bacteria, are more efficient in broiler chickens. The objective of the present study was to evaluate the antimicrobial activities of basil essential oil against pathogenic and non-pathogenic bacteria and their effects on broiler chickens.

Materials and Methods

Six hundred one day-old broiler chicks (Arian strain) were purchased from a local hatchery. On arrival birds were weighed and randomly assigned to one of six treatments with four replicates of 25 birds based on a completely randomized

design. The dietary treatments consisted of the basal diet as control and five test diets containing: 150 ppm 'Avilamycin' as an antibiotic, 150 ppm 'Protexin' as a probiotic, and 200, 400 and 600 ppm of basil essential oil. Protexin is a mixture of advantageous bacteria containing *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Aspergillus oryzae*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Candida pintolopesii*, with a minimum of 6×10^7 cfu/g of the product.

The basil essential oil was obtained from Ayat Esans Company. Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers provided by Arian broiler Manual. Chicks were raised on floor pens (120×120×80 cm) for six weeks and had free access to food and water through the entire experimental period (1–42 days). The uniform light was provided 24 hours per day. The ambient temperature was gradually decreased from 33°C to 25°C on day 21 and was then kept constant.

Ileum microbiota count

Enumeration of the microbial population in the ileal contents was performed at 42 day in 8 birds per treatment. The small intestine was immediately exposed, and the contents of the lower half of the ileum were collected into sterile stomacher bag to microbial enumeration. The ileum was defined as that portion of small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction.

Ileum digested samples (1 g) were diluted with sterile 0.9% NaCl to 5–10 folds, homogenized, and then, a specific agar was used to culture bacteria as follows: Total count agar (Merck, Germany) medium was used to count total bacteria (incubation for 48 h at 37°C); MRS-agar (Merck, Germany) medium for *Lactobacillus* (48 h incubation at 37°C) and MacConkey-agar (Merck, Germany) medium for *E. coli* (48 h incubation at 37°C). Finally, the number of bacterial colonies was calculated. The numbers of colony forming unit (CFU) were expressed as log₁₀ CFU per gram.

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 (2001). Significant difference among treatment means were separated using Tukey's test (Tukey, 1949) at 5% probability.

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Table 1. *The nutritional composition of dietary treatments.*

Ingredients (%)	Starter (1-14 days)	Grower (14-28 days)	Finisher (28-42 days)
Corn	48.60	45.70	45.55
Wheat	6.78	15.00	20.00
Soybean meal (44% CP)	36.50	32.00	27.90
Fish meal	2.10	1.40	0.50
Soybean oil	1.60	2.10	2.00
Dicalcium phosphate	1.90	1.68	1.80
Oyster shell	1.25	1.05	1.10
Salt	0.25	0.25	0.25
Vitamin permix ¹	0.25	0.25	0.25
Mineral permix ²	0.25	0.25	0.25
Sodium Bicarbonate	0.20	0.15	0.15
DL-Methionine	0.27	0.17	0.18
L-lysine	0.05	-	0.07
Calculated Composition			
ME (kcalkg ⁻¹)	2851	2937	2965
Crude protein (%)	22.23	20.39	18.5
Calcium (%)	1.06	0.90	0.90
Avilable Phosphorus (%)	0.50	0.45	0.45
Methionine	0.63	0.49	0.47
Met+cys (%)	0.99	0.83	0.78
Lysine (%)	1.28	1.10	1.00
Threonine (%)	0.85	0.77	0.69
Tryptophan (%)	0.32	0.29	0.26

¹ Vitamin premix (content per kg): vitamin A, 12,500 IU; cholecalciferol, 62.5 µg; vitamin E, 20 IU; vitamin K3, 2.5 mg; vitamin B1, 20 mg; vitamin B2, 10 mg; vitamin B6, 20 mg; vitamin B12, 12 mg; niacin, 70 mg; pantothenic acid, 25 mg; folic acid, 10 mg.

² Mineral premix (content per kg): Fe, 160 mg; Zn, 90 mg; Cu, 16 mg; Mn, 150 mg; Se, 0.1 mg; I, 0.8 mg.

Results and Discussion

The effects of treatments on microflora population of ileum are shown in Table 2. No significant differences were observed in colony-forming units of lactobacilli in ileum at 42 day of age ($P > 0.05$). The lowest and highest lactic acid bacteria in ileum were achieved in control and in birds receiving 400 ppm basil essential oil, respectively. Jamroz *et al.* (2005) reported that the number of *Lactobacillus* colony-forming units was significantly increased after using a blend of plant extracts.

In contrast, Jang *et al.* (2007) observed no change in the number of colony-forming units of lactobacilli in the ileocecal digesta when using a commercial blend of essential oils in broiler diets. Since short-chain fatty acids, as the final product of fermentation, are generated by *Lactobacillus*, they

can lower the intestinal pH and make the environment unfavorable for gram-negative bacteria.

Tucker (2002a) reported that growth of *E. coli* and *C. perfringens* reduced in broilers, when blends of essential oils were fed in industry trials, while numbers of *Lactobacillus* spp. increased. Thus, essential oils may act differently compared with synthetic antimicrobials, which tend to depress bacterial numbers across species. A significant reduction ($P < 0.05$) in the number of *E. coli* colonies were found in digesta harvested from the ileum of birds receiving 600 ppm basil essential oil compared with birds receiving other treatments. In the study of Jamroz *et al.* (2006), significant reductions of *E. coli* and *Clostridium* have been obtained after using natural plant extracts. Gunal *et al.* (2006) observed that the probiotic decreased ileal and cecal negative bacterial counts at 21 or 42 day. However, Ozturk & Yildirim

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(2004) found that a probiotic containing *Lactobacilli* had no effect on ileal and cecal gram-negative bacteria counts.

A field study with a commercial preparation of essential oils showed a reduction of colony forming units of *Clostridium perfringens* as compared to the positive control diet containing zinc bacitracin at the level of 20 ppm (Dalkilic & Guler, 2009). Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of *E. coli* and *Clostridium perfringens* in ceca (Jamroz & Kamel, 2002). These results were in accordance with our results for antimicrobial activity of herbal essential oils in broilers.

Table 2. The effect of treatments on microbial population (log₁₀ cfu/g) of the ileum of broilers at 42 day of age.

Treatment	<i>Lactobacilli</i>	<i>E.coli</i>	Total bacteria
Control	6.49	5.54 ^a	7.56 ^a
Avilamycin	6.66	5.69 ^a	7.32 ^a
Protexin	6.50	4.49 ^b	6.41 ^c
BEO (200 mg/kg)	6.66	5.78 ^a	6.55 ^{bc}
BEO (400 mg/kg)	6.75	5.65 ^a	6.89 ^b
BEO (600 mg/kg)	6.56	3.87 ^c	6.63 ^{bc}
SEM	0.07	0.06	0.08
P value	0.0956	0.0001	0.0001

Means within a column with no common letters are significantly different ($P < 0.05$). ^aBEO, Basil essential oil

In our studies supplementing diets with 600 ppm basil essential oil were found to be effective against *E. coli*. Numerous *in vitro* studies demonstrated that major components of basil essential oils including methyl chavicol, linalool, alpha-pinene, methyleugenol, eugenol, displayed antimicrobial activity against intestinal microbes such as *C. perfringens*, *S. typhimurium* and *E. coli* (Prabuseenivasan et al., 2006; Sienkiewicz et al., 2013; Hanif et al., 2011; Suppakul et al., 2003). The reduction of *E. coli* may be explained by the ability of essential oils to disrupt the bacterial cell membrane. The antimicrobial action of essential oils is mediated by lipophilic property to perforate the bacterial membrane, which releases membrane components from the cells to the external environment (Helander et al., 1998). Moreover, it has been shown that essential oils stimulate the release of mucus into the small intestine which reduces the adhesion of pathogens to the epithelium (Jamroz et al., 2006). Tucker (2002b) indicated that the supplementation of a mixed botanical product containing

garlic, anise, cinnamon, rosemary and thyme to commercial pig diets significantly inhibited the number of *E. coli*.

The counts of total bacteria were affected ($P < 0.05$) by dietary treatments. Probiotic treatment decreased ($P < 0.05$) ileal bacteria counts at 42 day of age compared to the control and antibiotic diets. In general, basil essential oil and probiotic supplementation decreased total bacteria counts of ileum of broilers at 42 day of the experiment (Table 2). This study proved *in vivo* the antimicrobial effect of the basil essential oil, which was dose-dependent. Studies carried out with broilers seem to confirm the *in vitro* findings. It is important to remember that the *in vivo* antimicrobial property of essential oils in birds can be influenced by basal diet and environment conditions. In the present study, probiotic decreased on ileal total bacteria counts at 42 day of age ($P < 0.05$).

These results concur with the results of Ceylan et al. (2003) who reported that a probiotic based *Enterococcus*, *Cylactin*, treatments to diets reduced aerobic and coliform bacteria counts. A similar observation was reported by Ghadban et al. (1998). They reported that spray application of probiotic by water reduced *Salmonella* and *E. coli* colonizations in caecum from 38.8% to 9.72%, from 51.4% to 22.2% respectively.

Conclusion

In conclusion, the findings of this study suggest that increased level of basil essential oil lowers *E. coli* colonies and increases the number of *Lactobacillus* colonies; a process that may improve intestinal microflora and indirectly enhance the performance immune system through elimination of pathogens. Further research in this area is required to fully understand the associations between gut microbiota and broiler performance.

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