The effects of the avilamycin, Protexin® and basil essential oil supplements on ileal bacteria of broiler chickens

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Abstract

The effects of probiotic (Protexin®, Protexin Veterinary, Somerset, UK), medicinal plant (basil essential oil) and an antibiotic growth promoter (avilamycin) as broiler feed additives on ileal bacteria of broilers were studied. A total of 600 Arian broilers were divided into 6 treatments, with 4 replicates of 25 birds. Treatments were a plant essential oil in 3 level (200, 400 and 600 ppm), the probiotic (150 ppm), an antibiotic (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 probiotic treatment significantly decreased total bacteria counts ($P<0.05$). Any of the supplements did not affect colony-forming units of lactobacilli ($P>0.05$). The lowest and highest lactic acid bacteria in ileum were obtained by 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 ($P<0.05$). Our results suggest that basil essential oil and Protexin could be of value to replace the antibiotics that have been banned as growth promoter in animal feeds.

Introduction

The use of antibiotics, with prophylactic characters in aviculture, started to be seen as a risk factor to human health. There is the chance that their residues are 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.[1] 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 therapeutic antibiotics used in animals and humans.[2] 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. This will guarantee animal performance and to increase nutrient availability.[2] The essential oils, organic acids, enzymes, probiotics (*lactobacilli*), prebiotics (oligosaccharides) and the herbs have received increased attention as possible antibiotic growth promoters replacement.[3] 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. They also improve immunity stimulation and ability to increase volatile fatty acids.[4] 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).[3] Beneficial effects of essential oils on farm animals may arise from activation of feed intake, secretion of digestive enzymes, immune stimulation, antibacterial, coccidiostatic, antiviral and antioxidant properties.[2]

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.[5] Chickens 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.[5] 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.[6] Basil known as sweet and garden basil, a member of the *Lamiaceae* family, is commonly cultivated throughout Mediterranean region.[7] The leaves and flowering tops of sweet basil are used as carminative, galactogogue, stomachic and antispasmodic medicinal plant in folk medicine.[8] 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*.[8] 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 nonpathogenic bacteria and their effects on broiler chickens.

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Materials and Methods

Six-hundred one-day-old broiler chickens (Arian strain) were purchased from a local hatchery. On arrival, birds were weighed and randomly assigned to one of 6 treatments with 4 replicates of 25 birds based on a completely randomized design. The dietary treatments consisted of the basal diet as control and 5 test diets containing: 150 ppm avilamycin as a antibiotic, 150 ppm Protexin as a probiotic (Protexin®, Protexin Veterinary, Somerset, UK), and 200, 400 and 600 ppm of basil essential oil. The experimental diets were offered from day 1 to the end of the experiment at 42 days of age.

Diets were fed in mash form. As basil essential oil (like other essential oils) is a lipophilic substance, firstly it was added to 200 mL of soybean oil, then to the remaining oil content of the diet and after that the oil was mixed with other diet ingredients.

Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers provided by Arian broiler Manual. Protexin is beneficial probiotics containing *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Aspergillus oryzae*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Candida pintolepesii*, with a minimum of 6×10^7 cfu/g of the product.

The basil essential oil was obtained from Ayat Esans Company. The oil obtained were immediately analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the chemical constituents present in the essential oil (Table 2). Chickens were raised on floor pens (120×120×80 cm) for 6 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.

Bacterial count of ileal digesta

Enumeration of selected group of in the ileal digesta was performed at 42 d 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 digesta 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, Darmstadt, 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.[9] Significant difference among treatment means were separated using Tukey's test at 5% probability.[10]

Results

The main chemical components of the basil essential oil evaluated by GC/MS are presented in Table 2. The main compounds of oil were Alpha-Pinene, Methyl Chavicol and Limonene. The effects of treatments on ileal bacteria are shown at Table 3. The additives used in the current study failed to induce any significant impact on colony-forming units of lactobacilli in ileum at 42 day of age (P>0.05). The lowest

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[a]	0.25	0.25	0.25
Mineral permix[b]	0.25	0.25	0.25
Sodium bicarbonate	0.20	0.15	0.15
DL-Methionine	0.27	0.17	0.18
fiL-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
Available 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

[a]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; vitaminB6, 20 mg; vitamin B12, 12 mg; niacin, 70 mg; pantothenic acid, 25 mg; folic acid, 10 mg. [b]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.

Table 2. Composition of basil essential oil.

Peak	Constituents	Area, %	Peak	Constituents	Area, %
1	Cycloheptasiloxane	0.01	13	Alpha-Terpinolene	0.42
2	Tricyclo heptane	0.10	14	Bicyclo hexane	0.04
3	Alpha-Pinene	39.47	15	Limonene oxide	0.03
4	Camphene	1.31	16	Isolimonene	0.06
5	Sabinene	0.27	17	4,8-epoxy-p-menth-1-ene	0.09
6	Beta-Pinene	2.05	18	Methyl Chavicol	27.05
7	Beta-Myrcene	0.78	19	Linalool	0.26
8	Gamma-Terpinene	0.65	20	2-Cyclohexen	0.61
9	Delta 3 Carene	0.35	21	Alpha-Fenchyl Acetate	0.06
10	Limonene	18.87	22	Naphthalene	0.06
11	Cis-Ocimene	3.22	23	Methyl eugenol	0.15
12	Beta-Ocimene	4.05	24	Spathulenol	0.04

and highest lactic acid bacteria in ileum were achieved in control and in birds receiving 400 ppm basil essential oil, respectively. 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 supplements decreased total bacteria counts of ileum of broilers at 42 day of the experiment. *E. coli*. bacteria counts were significantly lower (P<0.05) in birds receiving diets supplemented with 600 ppm basil essential oil. But there were no treatment effect observed between other levels of basil essential oil and control group.

Table 3. The effect of treatments on microbial population (log10 cfu/g) of the ileum of broilers at 42 d of age.

Treatment	*Lactobacilli*	*E.coli*	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). BEO, Basil essential oil

Discussion and Conclusions

Jamroz *et al.*[11] reported that the number of *Lactobacillus* colony-forming units was significantly increased after using a blend of plant extracts. In contrast, Jang *et al.*[12] 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 a 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.

Cross *et al.*[13] 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 that received 600 ppm basil essential oil compared with birds receiving other treatments. In the study of Jamroz *et al.,*[14] significant reductions of *E. coli* and *Clostridium* have been obtained after using natural plant extracts. Gunal *et al.*[15] observed that the probiotic decreased ileal and cecal negative bacterial counts at 21 or 42 d.

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.[16] Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of *E. coli* and *Clostridium perfringens* in ceca.[17] These results were in accordance with our results for antimicrobial activity of herbal essential oils in broilers.

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*.[18-21] The reduction of *E. coli* may be explained by the ability of essential oils to disrupt the bacterial cell membrane. Antimicrobial action of essential oils are mediated by lipophilic properties. It was suggested that terpenoids and phenylpropanoids can penetrate the bacterial membrane and reach the inner part of the cell because of their lipophilicity, which releases membrane components from the cells to the external environment.[22] 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.[14] In other experiment, results 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*.[23]

This study proved in vivo antimicrobial effect of basil essential oil with 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.*[24] who reported that a probiotic based *Enterococcus*, *Cylactin*, treatments to diets reduced aerobic and coliform bacteria counts. Similar observation 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.[15]

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 ileal bacteria population 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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