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Microencapsulated Eucalyptol and Eugenol as Growth Promoters in Broilers

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Abstract. Antibiotic growth promoter has been effectively used in poultry production for stimulating growth and preventing diseases. However, the use of antibiotics has been questioned due to the possible appearance of resistant bacteria. Special attention has been paid to the use of essential oils as natural alternatives for antibiotics. We investigated the use of different concentrations of spray dryer microencapsulated eucalyptol and eugenol in broiler chickens diet as alternative growth promoters for avilamycin. One-day-old male broiler chickens were submitted to 7 different dietary treatments. The eugenol demonstrated strong antioxidant activity and both eugenol and eucalyptol showed antimicrobial activity. The broiler performance showed no significant difference to the group with administration of 10 mg/kg of avilamycin when eucalyptol (500 mg/kg) or eugenol (500 mg/kg) were used separately in diet. Our findings suggest that the microencapsulated phytotherapic agents are a potential alternative to the use of avilamycin as growth promoter in broilers during the broilers growing phase.

Keywords: *antibiotic alternative, antimicrobial activity, antioxidant activity, phytotherapic agents.*

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INTRODUCTION

Antibiotic growth promoter has been effectively used in poultry feed for stimulating growth, treating sickness and preventing disease among the animals (KHAKSAR *et al*., 2012). However, the increasing concern over food safety leads to an apprehension over the use of antibiotics (ROSEN, 1996; CASEWELL *et al*., 2003; PHILLIPS *et al*., 2004). The major risk consists in the indiscriminate use of antibiotics and the consequent spread of resistant bacteria which can be harmful to human health due to the difficulty of clinical treatment (PHILLIPS *et al*., 2004; CASTANON, 2007).

In 2006, the European Union (EU) approved a resolution that removal the antibiotic growth promoter for animal feed (CROSS *et al*., 2007). Ever since, many countries around the world made efforts to finding substitutes for antibiotic growth promoters in poultry feed (CASEWELL *et al*., 2003; PHILLIPS *et al*., 2004; CASTANON, 2007). Alternatives are being introduced and special attention has been paid to the use of natural compounds, especially essential oils, as natural alternatives for antibiotics (CROSS *et al*., 2007; KHODAMBAS, *et al*., 2012). Many natural compounds used as substitutes for antibiotics in animal feed demonstrated positive effects on growth performance and in different health parameters (AGUILAR *et al*., 2013, JAMROZ *et al*., 2006, WINDISCH *et al*., 2008).

Essential oil from plant extracts has distinct biological functions, such as antimicrobial, antifungal or antioxidant activities (LEE and AHN, 1998). The supplementation of poultry diets with essential oil has favorable effects in digestive enzymes (LEE *et al*., 2003; JANG *et al*., 2004) and in the intestinal microflora since they inhibit harmful microbial growth in the gut and increase the digestibility (HELANDER *et al*., 1998). The essential oils have numerous

advantages over commercial antibiotics since they are residue free and they are also usually used in the food industry (VAREL, 2002; BRENES and ROURA, 2010). Eugenol and eucalyptol, the major chemical component of clove and eucalyptus oil, respectively, act as antioxidants on oleaginous foods, antiseptics in pharmacology, anti-inflammatories and as antimicrobial agents (FARAG *et al*., 1989; SAN MYINFF *et al*., 1996). Therefore, they are potential substitutes for antibiotic growth promoters.

The microencapsulation provides an important tool for the industry, enabling the protection and controlled release of various active agents. Microcapsules are small particles with a size between 1 and 1000 um comprising an active agent surrounded by a natural or synthetic polymeric membrane. The encapsulation of essential oils in core-shell has been used for several reasons, for example, protection against oxidative decomposition and evaporation, odor masking or merely to act as a support for ensuring control of the dose to be administered (MARTINS, *et al*., 2014).

In this article, we investigated the use of different levels of microencapsulated eucalyptol and eugenol in broiler chickens diet as alternative growth promoters for commercial antibiotic avilamycin.

MATERIALS AND METHODS

Birds and Diet

A total of 392 male, one-day-old male broiler chicks (Ross) were housed in metal cages with 100 W lamps for heating, trough feeders and nipple-type water supply. There were seven dietary treatments; each treatment consisted of 8 repetitions and 7 chicks per experimental unit. The birds were randomly assigned to the corresponding experimental diet treatment supplemented with none (A: control/basal feed); 10 mg avilamycin/kg (B); 500 mg eucalyptol/kg (C); 1000 mg eucalyptol/kg (D); 500 mg eugenol/kg (E); 1000 mg eugenol/kg (F); 500 mg eucalyptol /kg plus 500 mg eugenol/kg (G). The essential oils in these diets were in microencapsulated form. The crumbled basal diets (Table 1) and water were provided ad lib from 1 to 21 days. We evaluated the performance of the chicks using the variables: initial weight, feed consumption (FC), weight gain (WG), food conversion (FC), mortality rate and final broiler weight. All the chicks were weighed at 1, 10 and 21 days of age.

The compounds avilamycin, halquinol, lincomycin, tiamulin, tylosin, ferulic acid, rutin, caffeic acid, chlorogenic acid, quercetin, and galic acid, BHA (butylated hydroxyanisole) and

Table 1 –Composition of the dietary feeds used in the experiments.

Item (g/100g)	Treatments[1]						
	A	B	C	D	E	F	G
Corn	52	52	52	52	52	52	52
Soy bran	40.1	40.1	40.1	40.1	40.1	40.1	40.1
Soy oil	3.52	3.52	3.52	3.52	3.52	3.52	3.52
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Limestone	1.24	1.24	1.24	1.24	1.24	1.24	1.24
Dicalcium phosphate	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Methionine	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Premix [2]	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Eucalyptol [3]	-	-	0.245	0.49	-	-	0.245
Eugenol [3]	-	-	-	-	0.245	0.49	0.245
Avilaycin	-	0.001	-	-	-	-	-
Kaolin	0.65	0.649	0.405	0.17	0.41	0.17	0.165

[1] Treatments: A = control; B = Avilamycin (10 mg/kg); C = eucalyptol (500 mg/kg); D = eucalyptol (1000 mg/kg); E = eugenol (500 mg/kg); F = eugenol (1000 mg/kg); G = mixture of eugenol (500 mg/kg) and eucalyptol (500 mg/kg).
[2] Composition of premix (100 Kg): Vit. A: 1057500 IU, vit. D3: 255375 IU, vit. K: 180 mg, vit. B2: 450 mg, vit. B12: 1200 µg, vit. E: 787 mg, niacin: 3000 mg, pantothenic acid: 1173 mg, Vit B1: 201 mg, Vit B6: 249 mg, iron: 5589 mg, copper: 1101 mg, zinc: 5578 mg, manganese: 7203.6 mg, iodine: 84 mg, selenium: 43.5 mg, antioxidant (BHT): 378 mg. Calculated composition: Metabolizable energy (kcal/kg): 2950, Protein (%): 22.5, Methionine (%): 0.35, Methionine + cystine (%): 0.71, Calcium (%): 0.95, Available phosphorus (%): 0.45.
[3] Microcapsules.

DPPH (2,2-diphenyl-1-picryl-hydrazyl) were acquired from Sigma-Aldrich (St. Louis, MO, USA) or Absolute Standards, Inc (Hamden, CT, USA).

The purity of the fractions of eucalyptol and eugenol were determined as described by Scherer *et al*. (2010) on a gas chromatography coupled to flame ionization and mass spectrometry detectors (GC-17A-QP5000, Shimadzu, Japan). The antioxidant activity of the fractions was determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl) assay as described by Scherer and Godoy (2009). To determine the antimicrobial activity, we use the following microorganisms: *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (isolated from swine), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 13388) and *Clostridium perfringens* (ATCC 1324). The minimal inhibitory concentration (MIC) was determined in culture plates with 96 wells, according to the NCCLS (National Committee of Laboratory standards, 2003) norms. All the analyses were carried out in triplicate.

Microencapsulation of Eucalyptol and Eugenol

The microencapsulation of eucalyptol and eugenol was made using malt dextrin (20 DE), modified starch, the essential oils and water in the proportion of 1.5:1.0:1.5:3.5, respectively. The mixture was homogenized in a turrax and dried in a Lab Plant SD-05 double fluid (1.5 mm diameter) spray dryer (L.P. Technology, UK) at 180 ºC; air flow of 73 $m^3.h^{-1}$, air inlet temperature: 180 ± 2 °C, air outlet temperature: 115 ± 3 °C and flow rate: 0.7 $L.h^{-1}$.In order to assure the quality of the microcapsules, the amount of core material was determined by hydro-distillation in triplicate 5 g with 200 mL distilled water in a Clevenger system, being then analyzed by gas chromatography as described above.

Statistical Analysis

The statistical analyses were carried out using the software Statistic 6.0. The differences were considered significant with $P<0.05$ (ANOVA/ Tukey's test).

RESULTS AND DISCUSSION

The fraction eugenol which was 99.90% pure according to the chromatographic analyses showed elevated in vitro antioxidant activity with an Antioxidant Activity Index (AAI) significantly ($P<0.05$) higher than those of rutin and ferulic acid, but lower ($P<0.05$) than those of gallic acid and quercetin (Table 2). The results found for eugenol fraction suggest that its use as substitute for antibiotics can promote additional benefits to the broilers. The AAI of eugenol revealed no significant difference to chlorogenic acid, caffeic acid and BHA. In contrast, eucalyptol which was 99.95% pure did not show DPPH-reducing capacity even at the highest concentration assessed (0.8 mg/mL).

In order to define the concentrations of eugenol and eucalyptol for the in vivo experiment, we investigated the antimicrobial activity of both fractions, comparing these results with those of the main antibiotics used as growth promoters. There was no difference in the antimicrobial activity between eucalyptol and eugenol for the microorganisms *E. coli*, *P. aeruginosa* and *S. typhimurium*. The antimicrobial action of essential oils on the intestinal flora can inhibit the growth of pathogens and consequently promote the development of benefic microorganisms which increase animal performance (BRENES and ROURA, 2010). *S. aureus* and *C. perfringens* were more sensitive to eugenol than to eucalyptol. Considering the classification of antimicrobial activity established by Duarte *et al.*, (2005) both eucalyptol and eugenol showed from moderate to strong antimicrobial action. This result indicated a great potential for the eucalyptol and eugenol as substitutes for antibiotic growth promoters.

Considering the antimicrobial activity results (Table 3), for *E. coli*, the mean values for avilamycin, tilosin and halquinol were about 7, 27 and 100 times lower, respectively, than the mean values of eucalyptol and eugenol. We compare the antimicrobial activity for

Table 2- Results of antioxidant activity of the eucalyptol and eugenol compared to other chemicals antioxidantes.

Compound	IC_{50}[1]	AAI[2]
Gallic acid	2.83 ± 0.07	27.15 ± 0.68[a]
Quercetin	4.88 ± 0.56	15.92 ± 1.76[c]
Eugenol	6.99 ± 0.32	11.02 ± 0.49[d]
Chlorogenic acid	7.44 ± 0.12	10.34 ± 0.17[d]
Caffeic acid	8.21 ± 0.31	9.37 ± 0.35[d]
BHA	8.23 ± 0.41	9.36 ± 0.46[d]
Rutin	12.09 ± 0.70	6.38 ± 0.37[e]
Ferulic acid	14.45 ± 0.66	5.33 ± 0.25[e]
Eucalyptol	NA	NA

[1] inhibitory concentration of 50% of the DPPH radicals (µg/mL)
[2] AAI = antioxidant activity index
[a−e] Means within a column with different superscripts differ (P<0.05). NA: no activity

Table 3- Minimal inhibitory concentration of eucalyptol and eugenol compared to avilaycin, halquinol, lincomycin, thiamulin and tylosin.

	Minimal inhibitory concentration (mg/mL)				
Compound	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhimurium*	***C. perfringens***
Eucalyptol	0.600	0.40	0.40	0.600	0.800
Eugenol	0.400	0.40	0.40	0.600	0.600
Avilaycin	0.004	0.06	0.06	0.060	0.060
Halquinol	0.004	0.004	0.03	0.015	0.015
Lincomycin	*	0.03	0.03	0.030	0.030
Thiamulin	*	0.03	0.06	0.060	0.060
Tylosin	*	0.015	0.06	0.060	0.060

* < 0.002 mg/mL.

avilamycin with the eucalyptol and eugenol values. We find that 70 to 100 mg/kg of the compounds tested would be required for equivalence effect to the commercial antibiotic, considering that the frequently concentration of avilamycin used is 10 mg/kg. It is known that are differences between in vitro and in vivo trials; therefore, the values for these fractions were super-estimate. We used concentrations of 500 and 1000 mg/kg for the eugenol and eucalyptol in the experiments with the broilers.

The chromatography analyses of the eucalyptol and eugenol in the microcapsules exhibited values of 20.4 ± 0.2 g/100g and 20.8 ± 0.9 g/100g, respectively. Consequently, there were losses of both eucalyptol and eugenol during the spray-drying process, probably due to the volatility of the compounds. The hydrodistilled fraction extracted from the microcapsules was analyzed by gas chromatography and the results revealed no significant alteration, with values for purity of 99.5 and 99.1% for eucalyptol and eugenol, respectively.

Only three deaths occurred during the experiment, one with the treatment with 1000 mg/kg eugenol and one with 1000 mg/kg eucalyptol, both at 7 days of age, and one after 19 days of treatment with 500 mg/kg eugenol. There was no significant difference in the initial weights of the broilers between any of the treatments (Table 4). Treatment G (500 mg/kg eucalyptol and 500 mg/kg eugenol) showed the lowest feed consumption, smallest weight gain, lowest food conversion and lowest weight after 21 days (P<0.05). The mixture of the two compounds may have contributed to the formation of an unpleasant odor in the diet, since the birds in this group had significantly lower feed intake. In contrast, the treatments with 10 mg/kg of avilamycin, 500 mg/kg of eucalyptol, 500 mg/kg of eugenol and 1000 mg/kg of eucalyptol showed the best food conversion indexes (Table 4).

In the period from 1 to 21 days of growth, no significant differences were detected in the feed consumption, weight gain and final weight between the broilers in the control group, 10 mg/kg avilamycin, 500 mg/kg eucalyptol and

Table 4- Effects of different dietary treatments on broilers performance.

Treatment[1]	Initial weight (g)	Feed consumption (g)	Weight gain (g)	Food conversion (g/g)	Final weight (g)
			1 to 10 days		
A	45 ± 1^{a}	228 ± 4^{a}	198 ± 4^{ab}	1.18 ± 0.05^{b}	243 ± 4^{a}
B	45 ± 1^{a}	233 ± 9^{a}	206 ± 8^{a}	1.10 ± 0.03^{bc}	252 ± 8^{a}
C	45 ± 1^{a}	224 ± 9^{a}	205 ± 7^{a}	1.08 ± 0.09^{bc}	251 ± 8^{a}
D	45 ± 1^{a}	222 ± 12^{a}	201 ± 9^{a}	1.12 ± 0.08^{bc}	246 ± 9^{a}
E	45 ± 2^{a}	229 ± 7^{a}	201 ± 8^{a}	0.99 ± 0.08^{c}	246 ± 8^{a}
F	45 ± 1^{a}	222 ± 9^{a}	186 ± 3^{ab}	1.19 ± 0.05^{b}	232 ± 5^{b}
G	44 ± 1^{a}	200 ± 13^{b}	142 ± 11^{c}	1.6 ± 0.14^{a}	187 ± 11^{c}
			1 to 21 days		
A	45 ± 1^{a}	1164 ± 38^{ab}	985 ± 30^{b}	1.18 ± 0.02^{c}	1031 ± 31b^{c}
B	45 ± 1^{a}	1161 ± 30^{ab}	1001 ± 18^{ab}	1.16 ± 0.01^{cd}	1046 ± 19^{ab}
C	45 ± 1^{a}	1146 ± 21^{b}	1014 ± 12^{ab}	1.13 ± 0.02^{d}	1060 ± 12^{ab}
D	45 ± 1^{a}	1170 ± 37^{ab}	1020 ± 25^{ab}	1.15 ± 0.02^{d}	1066 ± 25^{ab}
E	45 ± 2^{a}	1221 ± 55^{a}	1039 ± 40^{a}	1.17 ± 0.01^{c}	1085 ± 41^{a}
F	45 ± 1^{a}	1182 ± 25^{ab}	971 ± 22^{b}	1.22 ± 0.02^{b}	1016 ± 22^{c}
G	44 ± 1^{a}	977 ± 81^{c}	721 ± 64^{c}	1.36 ± 0.03^{a}	766 ± 65^{d}

Different letters in the same column correspond to significant differences (P < 0.05).
[1] Treatments: A = control; B = Avilamycin (10 mg/kg); C = eucalyptol (500 mg/kg); D = eucalyptol (1000 mg/kg); E = eugenol (500 mg/kg); F = eugenol (1000 mg/kg); G = mixture of eugenol (500 mg/kg) and eucalyptol (500 mg/kg).
$^{a-c}$ Means within a column with different superscripts differ (P<0.05).

1000 mg/kg eucalyptol groups (Table 4). Botsoglou *et al*., 2002 tested the addition of two levels of essential oil from oregano to the diet of broilers (50 and 100 mg/kg), however no performance improvement was observed when compared with the control group. On the other hand, Mathlouthi *et al*. (2011) assessed the effects of essential oils of rosemary, oregano and a commercial blend of essential oils in broiler chickens diet, and the results exhibited that the three essential oils tested can substitute growth promoter antibiotics.

Zakeri and Kashefi (2011) studied effects of avilamycin, protexin, nutracid focus, mannanoligosaccharides and vitacel in broiler chicken feed on humoral immunity, growth performance, mortality and feed intake. The birds fed with mannanoligosaccharides and protexin had superior (P<0.05) serum antibodies at the age of 25 days old. Falaki *et al*., (2011) assessed the effects of different levels of probiotic and prebiotic on growth performance and carcass characteristics of broiler chickens. The highest value of body weight gain, carcass and breast was verified for broilers fed with mixed of probiotic (900 mg/kg) and prebiotic (2000 mg/kg) supplementation. The authors related that supplemented diets with mixed of probiotic and prebiotic (symbiotic) as growth promoters improve broiler chickens growth indices.

The treatments C (500 mg/kg eucalyptol) and E (500 mg/kg eugenol) showed no difference (P<0.05) with the 10 mg/kg avilamycin treatment in the tested conditions. The treatments C and E presented better (P<0.05) food conversion and weight gain, respectively, as compared to the control (Table 4).

CONCLUSIONS

The eugenol demonstrated strong antioxidant activity and both eugenol and eucalyptol showed antimicrobial activity. The broiler performance showed no significant difference to the group with administration of 10 mg/kg of avilamycin when eucalyptol (500 mg/kg) or eugenol (500 mg/kg) were used separately in diet. Our findings strongly suggest that the use of microencapsulated eucalyptol and eugenol in the tested conditions could be considered as effective alternatives for the commercial antibiotics growth promoters in broilers during the growing phase.

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