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# Rosewood (*Aniba rosaeodora Ducke*) oil in broiler chickens diet

*Óleo essencial de pau rosa ("Aniba rosaeodora Ducke") em rações de frangos de corte*

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## SUMMARY

A study was conducted to evaluate the effects of Rosewood oil (RO) on performance, carcass and commercial cut yields and microbiology of the gastrointestinal tract of broiler chickens. Five hundred and forty one-day-old male broiler chicks were arranged in a completely randomized design with six treatments and six repetitions consisting of 15 broiler chicks each. The treatments were: inclusion levels of 0.00mL (0.00EO); 0.15mL (0.15 EO); 0.30mL (0.30 EO); 0.45mL (0.45 EO) and 0.60mL (0.60 EO) of RO/kg diet and control (commercial promoter virginiamycin). At 21 and 40 days old, no significant differences in body weight, feed intake, feed conversion and viability of birds were observed when comparing the controls with the different levels of inclusion of RO. The carcass yields of commercial cuts were not affected by treatments. The increased level of RO reduced the relative weight of the intestines. The broilers consuming growth promoter had the highest concentration of *Escherichia coli* in the intestinal contents, compared to 0.00 EO and 0.30 EO. It was concluded that, Rosewood oil does affect the performance and yield slaughter, but it does reduce the relative weight of the intestines. RO does not show a consistent antimicrobial activity *in vivo* against *Escherichia coli.*

**Keywords**: essential oil, growth promoter, performance

## RESUMO

O estudo foi conduzido para investigar os efeitos do óleo essencial de pau rosa sobre o desempenho, rendimento de carcaça e cortes comerciais e microbiologia do trato gastrintestinal de frangos de corte. Foram utilizados 540 pintos machos de um dia de idade, distribuídos num delineamento inteiramente casualizado com seis tratamentos e seis repetições de 15 aves cada. Os tratamentos consistiram em: níveis de inclusão de 0,00mL (0,00 EO); 0,15mL (0,15 EO); 0,30mL (0,30 EO); 0,45mL (0,45 EO) e 0,60mL (0,60 EO) de óleo essencial de pau rosa/kg de ração e controle (promotor comercial virginiamicina). Aos 21 e 40 dias de idade, não foram observadas diferenças significativas para peso corporal, consumo de ração, conversão alimentar e viabilidade das aves quando comparado o tratamento controle e os diferentes níveis de inclusão de RO. Os rendimentos de carcaça e de cortes comerciais não foram influenciados pelos tratamentos. O aumento do nível de inclusão de RO diminuiu o peso relativo dos intestinos. O promotor de crescimento teve maior concentração de *Escherichia coli* na digesta intestinal quando comparado com 0,00 EO e 0,30 EO. Conclui-se que o óleo essencial de pau rosa não afeta o desempenho e rendimento de abate, reduz o peso relativo dos intestinos. O RO mostra atividade antimicrobiana consistente contra *Escherichia coli*.

**Palavras-chave**: desempenho, óleo essencial, promotor de crescimento

## INTRODUCTION

Some herbs and spices are well known to exert antimicrobial actions *in vitro* against important pathogens, including fungi. In recent years, phytogenic feed additives have attracted increasing interest as an alternative feeding strategy to replace antibiotic growth promoters. Phytogenic feed additives usually have considerable variation in their chemical composition, depending on their ingredients and the influence of climatic conditions, location, harvest stage, or storage conditions. Hence, differences in efficacy between phytogenic products that are currently available on the market may be attributed mainly to differences in their chemical composition (WINDISCH et al., 2008).

Essential oils represent a particular subcategory of phytogenics material. They are odoriferous, secondary plant metabolites that contain most of the active substances of the plant, which include hydrocarbons (terpenes, and sesquiterpenes), oxygenated compounds (alcohols, aldehydes, and ketones), and a small percentage of nonvolatile residues (paraffin, and wax). They are usually obtained from the raw materials through steam distillation (APPLEGATE et al., 2010).

The use of essential oils in diets for broilers is a new concept and there is a huge variety of products e the Rosewood oil (RO) represents an alternative to antibiotic growth promoters because of its antimicrobial properties (HAMMER et al., 1999). The RO is obtained commercially from the Lauraceous species *Aniba rosaeodora Ducke* (pau rosa), a large tree reaching up to 30 m in height, that occurs in the Amazon region. All parts of the tree are fragrant, although only the wood of the trunk is harvested and hydrodistilled. The oil is a colorless to pale yellow liquid with a woody floral fragrance. The main constituent of rosewood oil is linalool, a monoterpene alcohol commonly used for a number of derivatives to the flavor and fragrance industries (MAIA & ANDRADE, 2009).

Rosewood oil has antimicrobial activity, as evidenced in study in vitro against bacteria and fungi (HAMMER et al., 1999). However, in an *in vivo* study, the efficacy of essential oils in diets has been shown to be affected by intrinsic and extrinsic factors such as the nutritional status of animals, diet composition and environment (HIPPENSTIEL et al., 2011). Thus, the present study was conducted to evaluate the potential of Rosewood essential oil as a possible alternative to improve the performance of broilers.

## MATERIAL AND MÉTHODS

The experiment was performed using 540 male, one-day-old, Ross 308 chicks, distributed in a randomized design with six treatments and six replications; each replication consisted of fifteen birds. The treatments were: inclusion levels of 0.00mL (0.00 EO); 0.15mL (0.15 EO); 0.30mL (0.30 EO); 0.45mL (0.45 EO) and 0.60mL (0.60 EO) of Rosewood essential oil/kg of feed. The control was the commercial promoter virginiamycin. These additives were added to basal diets.

The birds were reared in 30 floor pens, 1.5m$^2$ each, with water troughs, hanging feeders and 250W infrared heating lamps. They received water and feed ad libitum. Bed of shavings were of 10cm deep. The birds were vaccinated against Newcastle and Gumboro at fourteen days old of age. A continuous lighting

program was used during the experiment.
The feeding program consisted of a starter diet (until 21 days old), a growing diet (from 22 to 35 days old) and a finisher diet (from 36 to 40 days old). The composition of the experimental basal diets is shown in Table 1. All diets for each period were prepared with the same batch of ingredients, and all diets within a period had the same composition.

Table 1. Composition of experimental diets

| Ingredients | Basal diet (%) | | |
|---|---|---|---|
| | Starter | Growing | Finisher |
| Corn (8% CP) | 57.281 | 65.208 | 66.518 |
| Soybean meal (45% CP) | 33.80 | 26.00 | 23.90 |
| Meat and bone meal (45% CP) | 5.50 | 5.20 | 4.80 |
| Limestone (37 % Ca) | 0.44 | 0.43 | 0.45 |
| Soy-oil | 2.00 | 2.20 | 3.50 |
| Sodium chloride | 0.36 | 0.28 | 0.31 |
| Sodium bicarbonate | 0.09 | 0.150 | 0.09 |
| DL-Methionine | 0.208 | 0.170 | 0.159 |
| L-Lysine | 0.071 | 0.107 | 0.123 |
| Vitamin-mineral premix[1] | 0.20 | 0.20 | 0.15 |
| Senduramicin sodium | 0.05 | - | - |
| Salinomicin | - | 0.055 | - |
| Calculated composition | | | |
| Metabolizable Energy (kcal/kg) | 3000 | 3100 | 3200 |
| Protein (%) | 22.45 | 19.45 | 18.42 |
| Total lysine (%) | 1.30 | 1.12 | 1.06 |
| Total methionine + Cystine (%) | 0.92 | 0.80 | 0.76 |
| Total threonine (%) | 0.88 | 0.76 | 0.72 |
| Total tryptophan (%) | 0.27 | 0.23 | 0.21 |
| Total arginine (%) | 1.50 | 1.27 | 1.19 |
| Calcium (%) | 1.00 | 0.94 | 0.88 |
| Available phosphorus (%) | 0.48 | 0.45 | 0.42 |
| Sodium (%) | 0.22 | 0.20 | 0.19 |
| Potassium (%) | 0.91 | 0.78 | 0.73 |
| Chloride (%) | 0.30 | 0.35 | 0.27 |
| Ether Extract (%) | 5.03 | 5.46 | 6.66 |
| Electrolytic Balance (mEq/kg) | 243 | 215 | 195 |

[1]The vitamin and trace mineral mix provided the following per kilogram of diet: retinyl palmitate, 6.000 IU; cholecalciferol, 1.000 ICU; DL-$\alpha$-tocopherol acetate, 10 IU; menadione sodium bisulfite, 1mg; thiamin, 1.8mg; riboflavin, 3.6mg; niacin, 25.0mg; pantothenic acid, 10.0mg; pyridoxine, 3.5mg; folacin, 0.5mg; biotin, 0.15mg; choline, 500mg; copper, 8mg; iron, 80mg; manganese, 60mg; selenium, 0.1mg; and zinc, 40mg.

Diets were formulated to meet requirements by the "Brazilian tables for poultry and swine – Feed composition and nutritional requirements" (ROSTAGNO et al., 2011) for broilers of those ages. The antibiotic growth promoter virginiamycin was added at 10ppm. Rosewood essential oil, at different concentrations, was included in the basal diet, replacing the growth promoter virginiamycin. The anticoccidial ionophores, salinomycin and senduramycin were included in the starter and growing rations, respectively. A trunk wood sample was collected from an exemplar of *Aniba*

*rosaeodora Ducke* growing in the Zoobotanical Park of Emilio Goeldi Museum, located in the city of Belém, state of Pará, Brazil. A voucher (17.710) of the plant was deposited in the herbarium of the same institution.

The trunk wood sample (500g) was dried at room temperature (5 days), grinded and hydrodistilled using a Clevenger-type apparatus (3h). The oil was dried over anhydrous sodium sulfate, stored in dark flasks and kept refrigerated. The qualitative analysis of the oil was performed on a Thermo Focus DSQ GC-MS instrument, with the following conditions: a WCOT DB-5ms (30 m x 0.25mm; 0.25 μm film thickness) fused silica capillary column; temperature programmed: 60 - 240$^{o}$C (3$^{o}$C/min); injector temperature: 220ºC; carrier gas: helium, adjusted to a linear velocity of 32cm/sec (measured at 100$^{o}$C); injection type: splitless (2 L, of a 1:1000 hexane sol.); split flow was adjusted to give a 20:1 ratio; septum sweep was a constant 10ml/min; EIMS: electron energy, 70 eV; ion source temperature and connection parts: 180$^{o}$C. The rosewood (*Aniba rosaeodora Ducke*) provided an oil yield of 1.5%. The quantitative data on the oil were obtained by peak area normalization using a Thermo Focus GC/FID operating under the same GCEMS conditions, except for the use of nitrogen as a carrier gas. Individual components were identified by comparison of both mass spectrum and GC retention data, with authentic compounds previously analyzed and stored in the data system. Other identifications were made by comparing of mass spectra with those existing in the data system libraries and cited in the literature (ADAMS 2007). The retention indices were calculated for all volatiles constituents using an n-alkanes homologous series.

In each experimental unit, body weight, feed intake, feed conversion and viability (live birds/total birds x 100) were evaluated at 21 and 40 days old. At 40 days old, two birds per replicate were selected (a media of the two birds was the value of the replicate) and, after a fast of six, they were killed to determine yields of carcass and commercial cuts (breast, thigh and wings), which were expressed as percentage of body weight before slaughter. In addition, the proventriculus, gizzard, intestines (small and large), pancreas, spleen and liver were removed to determine the relative weight of the internal organs (% of body weight). Broiler chickens were killed at similar times to avoid the influence of the period of feed withdrawal on the weight of birds.

For microbiological evaluation, the gastrointestinal tract (small and large intestines) was collected from one bird per replicate. The broilers were subjected to a two hour ration fast . For this purpose, the birds were euthanized through cervical dislocation and a cut in the abdominal cavity was made immediately, to expose the intestines. Three ligatures were made with suture material, one at the beginning of the small intestine, one at the rectum and one at the ileocecal junction. Cuts were made on both extremities and at the ileocecal junction, and the intestinal sections were immediately placed into zip lock bags (10cm x 14cm x 0.1cm). Possible residues of cuts on the extremities were removed with distilled water.

The samples were identified within and on the outside of the zip lock bags and placed into polystyrene boxes, on ice. Sample collection lasted approximately one hour. After the collections were completed, a 50 liter thermal polystyrene box containing the samples was filled completely with ice and

enveloped to transport via airway to the JFLAB SP laboratory. The samples were collected sterilely, using and with sterilized material. The broilers were subjected to a two hour ration fast. The period between the collection and the processing of the samples in the laboratory was approximately 20 hours.
Samples of 25 g of intestinal contents were placed in vials containing 225mL of saline solution at 0.85%, resulting in a $10^{-1}$ dilution. From this dilution, consecutive decimal dilutions were performed, using the same proportion. The presumptive test was made from $10^{-1}$ to $10^{-7}$ dilutions of samples of intestinal contents. For each dilution inoculums, 1.0mL was used in the petri dish to evaluate each microorganism. Escherichia coli were enumerated on MacConkey Agar (Difco) after aerobic incubation at 37°C for 24h. For the growth of Clostridium perfringens, each dilution was inoculated into fluid Thioglycolate media. For assessment of Salmonella spp, intestinal contents of birds were inoculated in test tubes containing enrichment media Rappaport Vassiliadis, Selenite Novobiocin and Tetrationate and incubated for 24h. The colony count data were expressed as the number of colony forming units (CFU) per gram of intestinal contents. These values were transformed into log10 for analysis and interpretation of results (APHA, 2001).
The data were analyzed through the GLM SAS procedure (STATISTICAL ANALYSIS SYSTEM, 2000). Comparisons between the control and each of the treatments were performed using a Dunnett test at 5%. Regression analysis was used to determine linear and quadratic responses to 5%. In this analysis, the control group was excluded. The microbiological count data (CFU of Escherichia coli) were transformed into the logarithm (X), to achieve data with normal distribution.

## RESULTS AND DISCUSSION

In recent years, aromatic plants and their extracts have received attention as growth and health promoters. Most of these properties are due to essential oils and other secondary plant metabolites. Essential oils enhance production of digestive secretions, stimulate blood circulation, exert antioxidant properties, reduce levels of pathogenic bacteria and may enhance immune status (BRENES & MOURA, 2010).
The constituents of Rosewood oil are shown in Table 2. The main component was linalool (84.8%), followed by minor oxygenated sesquiterpenes (3.4%), α-terpineol (2.9%) and geraniol (1.0%). Oil composition obtained from the trunk a Rosewood tree confirms the claim by Maia & Andrade (2009) that linalool is the most abundant component of RO.
The performance parameters of broilers at 40-days-old are shown in Table 3. The results show that body weight, feed intake, feed conversion and viability of the birds at this age were similar between the control group and the different levels of inclusion of RO ($P>0.05$).
This response is consistent with studies that claim that healthy and well-nourished birds are less responsive to antibiotics when they are housed in environments that have been carefully cleaned and disinfected (HERNANDEZ et al., 2004). This is the first study to evaluate the inclusion of RO in the diet of birds, and have been no parameters to compare the results with the current literature. Furthermore, the inclusion levels of phytogenic material included

do not appear to be toxic to birds. Other experiments with broiler chickens have also resulted in no significant difference in performance variables of animals supplemented with different species, concentrations or combinations of plant extracts in the diet. Windisch et al. (2008), reported that the absence of effect on broiler performance may be related to the composition of basal diet provided and/or environmental conditions in which the experiment was conducted.

Table 2. Constituents of Rosewood oil

| Constituents | Retention index | Rosewood oil (%) |
|---|---|---|
| α-pinene | 939 | 0.1 |
| Benzaldehyde | 959 | 0.2 |
| Limonene | 1031 | 0.7 |
| 1,8-cineol | 1033 | 0.3 |
| (Z)-β-ocimene | 1039 | 0.2 |
| Trans-linalool oxide (furanoid) | 1074 | 0.6 |
| Cis-linalool oxide (furanoid) | 1088 | 0.7 |
| Linalool | 1098 | 84.8 |
| α-terpineol | 1188 | 2.9 |
| Nerol | 1228 | 0.3 |
| Geraniol | 1255 | 1.0 |
| α-copaene | 1376 | 0.4 |
| β-elemene | 1391 | 0.3 |
| alloaromadendrene | 1462 | 0.1 |
| β-selinene | 1489 | 0.7 |
| α-selinene | 1496 | 0.4 |
| (E)-nerolidol | 1564 | 0.2 |
| caryophyllene oxide | 1581 | 0.1 |
| Minor oxigenated sesquiterpenes | 1655-1726 | 3.4 |
| Benzyl benzoate | 1762 | 0.6 |
| Total | | 98.5 |

Table 3. Effects of Rosewood oil on performance of broilers at 40 days of age

| Treatments | Variables | | | |
|---|---|---|---|---|
| | Body weight (g) | Feed Intake (g) | Feed: gain (g:g)[1] | Viability (%) |
| 0.00EO | 2604 | 4393 | 1.715 | 90.0 |
| 0.15EO | 2658 | 4453 | 1.702 | 82.2 |
| 0.30EO | 2659 | 4481 | 1.712 | 90.0 |
| 0.45EO | 2639 | 4347 | 1.674 | 87.8 |
| 0.60EO | 2635 | 4459 | 1.720 | 85.6 |
| Control | 2737 | 4532 | 1.688 | 88.9 |
| CV (%) | 3.69 | 2.62 | 2.93 | 14.01 |

Means within a column did not differ (P>0.05) by Dunnett test.

Rations with highly digestible ingredients limit the proliferation of bacteria in the intestinal tract because there is no substrate available for bacterial growth, thereby reducing the antimicrobial potential of plant extracts. The same can be observed if the birds have been raised in places with low immune challenges and strict sanitary control (HIPPENSTIEL et al., 2011).

The carcass and commercial cuts yields of broilers fed different inclusion levels of Rosewood oil in the ration are shown in Table 4. The results show no influence of treatments on carcass, breast, thigh and wing yields (P>0.05). The carcass and commercial cuts yields were not affected by treatments. The literature shows divergent results in regards to carcass and commercial cuts yields. While some studies show positive results in carcass yield with the use of virginiamycin (SINGH et al., 2008) and a mixture of essential oils in the diet (ALÇIÇEK et al., 2004), other studies show that these variables were not affected by dietary inclusion of avilamycyn and phytogenic material (GARCIA et al., 2007). Therefore, there is not enough evidence to support that the use of dietary phytogenic material improves the carcass and commercial cuts yields. The absence of effect may be due to high performance, which does not allow space for additives to increase yields.

Table 4. Yield of carcass and commercial cuts of broilers (% of body weight) fed with Rosewood essential oil in the diet

| Treatments | Variables | | | |
|---|---|---|---|---|
| | Carcass | Breast | Thighs | Wings |
| 0.00EO | 82.9 | 24.2 | 21.8 | 7.4 |
| 0.15EO | 82.1 | 23.5 | 21.4 | 8.2 |
| 0.30EO | 82.6 | 24.7 | 21.8 | 7.9 |
| 0.45EO | 82.7 | 24.4 | 21.7 | 8.1 |
| 0.60EO | 82.5 | 24.5 | 21.6 | 7.9 |
| Control | 82.8 | 24.4 | 21.9 | 8.0 |
| CV (%) | 1.75 | 7.25 | 5.97 | 12.7 |

Means within a column did not differ (P>0.05) by Dunnett test.

The relative weight of internal organs of broilers (% body weight) fed with the Rosewood oil is shown in Table 5. The results show that the relative weight of internal organs was not affected significantly (P>0.05) by the inclusion of different levels of essential oil in the diet. These observations are similar to those encountered by Hernandez et al. (2004), who found no differences in gizzard or liver weights of broiler chickens fed a diet containing essential oil extracts from oregano, cinnamon and pepper and labiatae extracts from sage, thyme and rosemary.

The relative weight of internal organs data were subjected to regression analysis and were observed for linear (Figure 1) and quadratic (Figure 2) effects for the gizzard and intestines weights, respectively. The weight of the gizzard decreased with increasing level of inclusion of phytogenic material. The relative weight of the intestines was higher in birds fed the control diet, as well as with 0.60mL/kg of RO.

Table 5. Relative weight of internal organs (% of body weight) of broilers fed with Rosewood oil in the diet

| Treatment | Variable (% of body weight) | | | | | |
|---|---|---|---|---|---|---|
| | Proventriculus | Gizzard | Intestines | Pancreas | Spleen | Liver |
| 0.00EO | 0.36 | 1.30 | 3.57 | 0.17 | 0.07 | 1.76 |
| 0.15EO | 0.38 | 1.27 | 3.39 | 0.15 | 0.07 | 1.74 |
| 0.30EO | 0.34 | 1.28 | 3.38 | 0.17 | 0.08 | 1.61 |
| 0.45EO | 0.33 | 1.24 | 3.35 | 0.20 | 0.07 | 1.58 |
| 0.60EO | 0.34 | 1.23 | 3.54 | 0.15 | 0.07 | 1.62 |
| Control | 0.34 | 1.21 | 3.14 | 0.18 | 0.08 | 1.70 |
| CV (%) | 24.2 | 12.3 | 12.3 | 31.2 | 25.2 | 14.3 |

Means followed by distinct letters in the same column are different (P<0.05) by Dunnett test.

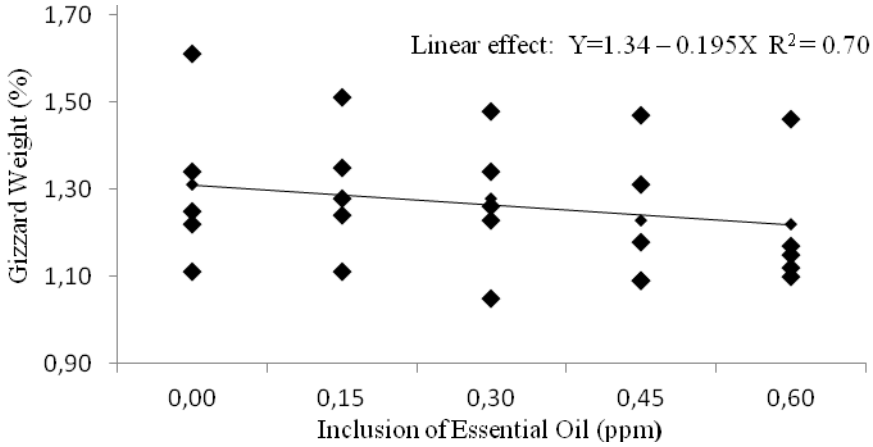


Figure 1. Relative weight of gizzard (% of body weight) of broilers fed with Rosewood oil in the diet

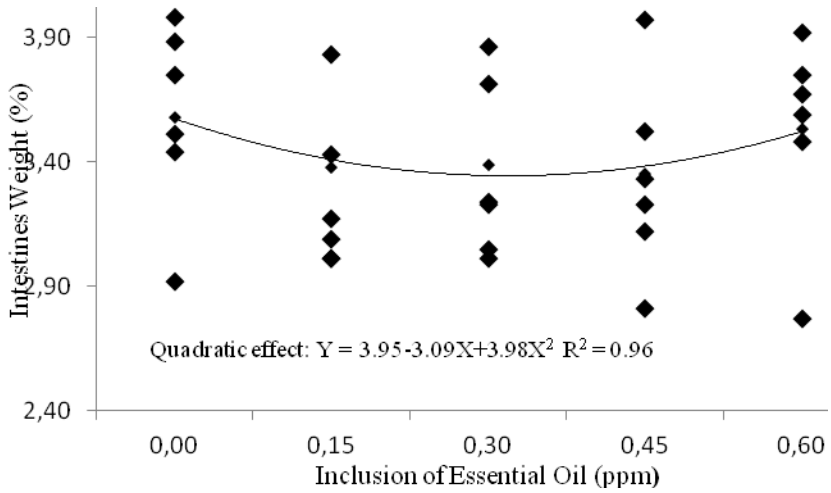


Figure 2. Relative weight of intestines (% of body weight) of broilers fed with Rosewood oil in the diet

Research shows firstly that there is no relationship between the weight of the gizzard and the inclusion of essential oils in the diet (ÇABUK et al., 2004) however; conversely, other studies show increase in gizzard weight with the addition of these phytogenic materials in the feed of broilers (AMERAH et al., 2011). The greater weight of the gizzard is the result of a higher accumulation of feed due to reduced transit time for ingesta passage (PLATEL & SRINIVASAN, 2004). This finding indicates a larger area of gastric secretion, and hence, a greater enzymatic attack would result in better utilization of nutrients in the ration (JANG et al., 2007).

The lower gizzard weight observed in this study may be associated with increased rates of intestinal transit in chickens, not reflected in improved performance. However, more studies are needed to precisely determine the mechanisms by which this occurs to increase or decrease the gizzard weight.

One of the modes of action of antimicrobial agents is related to reduction in the amount of toxin-producing microorganisms adhered to the intestinal epithelium and consequently, reduction in the thickness of the intestinal wall (ANDERSON et al., 1999) and changes in intestinal morphology, such as shorter villi and deeper crypts (GARCIA et al., 2007). Alternatively, additional effects of growth promoters on parameters other than performance in healthy animals include a reduction in gut size, including thinner intestinal and total gut wall, which result in a sparing use of nutrients and an improvement in performance (JAMROZ et al., 2006).

In this study, the relative weights of the intestines were higher in the control and 0.60 EO diets. According to the results obtained in this study, RO at 0.45mL/kg reduces the weight of the intestines, and the highest level used does not have an effect on that variable. There is likely a limit on the concentration of phytogenic material in a diet that would still produce positive effects, and levels above that threshold would not show changes in the weight of the intestines.

The results of the microbiological analysis of the intestines are shown in Table 6. The results show that the inclusion of growth promoter in feed correlated with a higher concentration of *Escherichia coli* in the gastrointestinal tract of birds, when compared with RO at 0.00 EO and 0.30 EO. The microbiological analysis was negative for *Clostridium perfringens,* and *Salmonella* spp. strains were not detected in the material analyzed.

Table 6. Results of microbiological analyses of the intestinal content of broiler chickens fed Rosewood oil at 40 days of age

| Treatment | Variables | | |
|---|---|---|---|
| | *Escherichia coli* (UFC/g) | *Clostridium perfringens* | *Salmonella ssp* |
| 0.00EO | 2.7167[b] | Negative | Absent |
| 0.15EO | 4.1667[a] | Negative | Absent |
| 0.30EO | 3.6833[b] | Negative | Absent |
| 0.45EO | 4.4500[a] | Negative | Absent |
| 0.60EO | 4.5500[a] | Negative | Absent |
| Control | 5.0000[a] | Negative | Absent |
| CV (%) | 25.3 | - | - |

Means followed by distinct letters in the same column are different (P<0.05) by Dunnett test.

Antimicrobial activity has been recognized as the major beneficial effect of plant extracts on animal production, although the exact antimicrobial mechanism has not been discovered. *In vitro* studies have demonstrated that essential oils display antimicrobial activity against intestinal microbes such as *Clostridium perfringens*, *Salmonella typhimurium* and *Escherichia coli* (HAMMER et al., 1999). However, in an *in vivo* study, it seems that the effect of plant extracts on gastrointestinal microflora is not consistent, even though essential oils have been generally recognized as antimicrobial agents (HIPPENSTIEL et al., 2011). In the evaluation of a blend of essential oils in the diet of chickens, Tiihonen et al. (2010) observed higher body weight gain in birds fed with the phytogenic material and, moreover, observed an increase in the concentration of *Escherichia coli* in the cecum. However, Coduk et al. (2008) tested a blend of essential oils in chickens and observed a greater weight gain in birds fed the phytogenic material, but the concentration of *E. coli* in the intestine remained unchanged. Additionally, Jang et al. (2007) reported a decrease in the concentration of *E. coli* in the intestines of birds fed with diets containing essential oils when compared to birds fed without additives, but found no influence of phytogenic material on broiler performance. Finally, Kirkpinar et al. (2011) observed no significant effect on the performance of birds or on the concentration of *Escherichia coli* in the ileum when a blend of essential oils was included in the diet.

According to these observations, it seems that there is not a clear relationship between the concentration of *Escherichia coli* in the gut and broiler performance; therefore, different concentrations of that bacterium in the intestinal tract do not seem to impact the performance of birds under experimental conditions.

*In vivo* studies on antimicrobial action of essential oils are rare and results are difficult to compare due to different methods used. Antimicrobial activity can be tested in all parts of the intestinal tract and methods to determine activity vary. Although it has already been demonstrated that phytogenic materials act as antimicrobial agents *in vitro*, these results sometimes do not appear in *in vivo* studies. This depends on several other factors such as environment and basal diet. If the birds are housed in clean and healthy conditions and if the diets are highly digestible, it is possible that the antimicrobial effect of essential oils does not show. There are no improvements if the microflora is already in a state of equilibrium (HIPPENSTIEL et al., 2011).

There appears to be a range of concentration of Escherichia coli in intestinal content that is does not produce a negative effect on broiler performance. It is likely that in this age there is a balance in intestinal microbes that does not affect the health of birds.

Rosewood oil does not affect the performance or carcass and commercial cuts yields, but it does reduce the relative weight of the intestine. Finally, it does not show consistent antimicrobial activity *in vivo* against *Escherichia coli.*

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