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Use of *Lippia rotundifolia* and *Cymbopogon flexuosus* essential oils, individually or in combination, in broiler diets

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ABSTRACT - This study investigated the effects of *Cymbopogon flexuosus* and *Lippia rotundifolia* microencapsulated essential oils on broiler performance and carcass yield. One hundred and fifty mixed-sex Cobb broiler chicks were used, from one day up to 42 days of age, in a completely randomized design, with five treatments and three replicates of ten birds each. The treatments were: negative control (basal diet), positive control (diet with enramycin and salinomycin), and three diets with microencapsulated essential oils from lemongrass, *L. rotundifolia*, and combination with 50% of both. The performance and carcass yield were not affected by the treatments. The intestine absolute weight was lower in the combination treatment compared with the negative control treatment and the lemongrass essential oil. The intestine relative weight was higher in the treatments with lemongrass and *L. rotundifolia* essential oils in relation to the combination. The liver relative weight was lower with the lemongrass essential oil and the combination compared with the treatment with the *L. rotundifolia* essential oil. The trial could not find results enough to recommend the use of the lemongrass and *L. rotundifolia* essential oils as an additive in broiler diets.

Key Words: antimicrobials, essential oils, lemongrass, performance

Introduction

Antimicrobials have been used as additives in poultry for many years, contributing to improved growth performance and feeding efficiency of birds (Agostini et al., 2012). However, concern about the increase in cases of bacterial resistance and residues in products have resulted in the prohibition of performance enhancers by the European Union (Santos et al., 2005). Thus, Brazilian exporters must confirm to the standards, stimulating studies on substitutes for antibiotics, such as plant essential oils and other additives such as organic acids, probiotics, and prebiotics.

Essential oils and their main compounds can be used as additives and improve broiler performance and intestinal health (Cho et al., 2014). In general, they act by promoting selection for the beneficial microorganisms in the intestine (Traesel et al., 2011).

Cymbopogon flexuosus essential oil is extracted from the leaves of the plant also known as lemongrass. The mixture between neral isomers (citral b or cis-citral) and geranial (citral a or trans-citral) form citral, which is the major component of lemongrass and gives the essential oil its characteristic aroma, besides various long-known medicinal properties utilized in popular culture, including antimicrobial activity (Adukwu et al., 2012; Desai and Parikh, 2012). Lemongrass essential oil, due to the effect of the citral, can be cytotoxic and genotoxic to human lymphocytes, but is safe if used at low concentrations, under 400 µg/mL (Sinha et al., 2014).

Lippia rotundifolia is an endemic plant to the Brazilian Cerrado, from the area known as the "Serra do Espinhaço". Although little studied, it is a promising species that belongs to the family Verbenaceae. According to Gomide et al. (2013), the major component of its essential oil is β-myrcene. It also has proven antimicrobial activity (Souza et al., 2015). There are no reports in the literature of the use of these two species as additives in animal feed. Also, when used

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together, the plants can show synergistic effects on the performance of broilers.

Microencapsulation can be made to increase the stability of the essential oils (to circumvent volatility problems) and protect them during processing and feed passage through the stomach (Barreto et al., 2008; Traesel et al., 2011). The objective of this study was to evaluate the effect of the microencapsulated essential oils of lemongrass and *L. rotundifolia* as additives in the diet on performance, carcass yield, and organ weights of broilers.

Material and Methods

The research was conducted in Montes Claros, Minas Gerais, Brazil, between January and February, 2015. The procedures were performed in accordance with ethical standards and approved by the Ethics Committee on Animal Usage of the Universidade Federal de Minas Gerais.

The 150 one-day-old Cobb 500® chicks were used, mixed, housed in 30 cages (60 × 35 × 100 cm) with feeders and waterers. The experiment was conducted in a completely randomized design, with five treatments and three replicates of 10 animals. The treatments were: negative control, without antimicrobials and anticoccidials; positive control - feed supplemented with 10 ppm enramycin and 42 ppm salinomycin; lemongrass - feed containing 120 mg of lemongrass essential oil for each kg^{-1} of animal body weight; *L. rotundifolia* - feed containing 120 mg of *L. rotundifolia* essential oil for each kg^{-1} of animal body weight; combination - feed with the mixture of the two essential oils. The dose used was defined from the antimicrobial activity presented by the essential oils of *L. rotundifolia* (Souza et al., 2015) and lemongrass (Azevedo et al., 2016) in preliminary *in vitro* tests.

The nutrition plan was divided into three phases: starter (1-21 days), grower (22-33 days), and finisher (34-42 days). The diets were formulated to meet nutritional levels recommended by Rostagno et al. (2011) and offered *ad libitum* throughout the experimental period in a mashed form (Table 1).

The lemongrass essential oil was acquired from the Ferquima Industry and Trade LTD company (Vargem Grande Paulista, SP, Brazil) and the *L. rotundifolia* oil was purchased from producers of the Fundação Universidade do Vale do Jequitinhonha Cooperativa (Serro, district of São Gonçalo do Rio das Pedras, Minas Gerais, Brazil), both extracted by the steam distillation method and packaged in 500-mL bottles.

Analysis of the chemical composition of the oils was conducted by gas chromatography mass spectrometry (GC-MS) using an Agilent Technologies system (7890A) interfaced with a mass spectrometer (MS 5975C) equipped with DB-5ms capillary column (30 m × 0.25 mm × 0.25 μm) with helium (1 $mL \cdot min^{-1}$) as carrier gas. The injector was maintained at 220 °C, following the temperature program of 60 °C-240 °C (3 °C min^{-1}), operating at an electron impact of 70 eV in a range of 45 to 550 m/z. The identification of essential oil constituents was made using the MSD Chemstation software with the library (National Institute of Standards and Technology, NIST 2002). The relative abundance (%) of constituents was calculated from the peak area of the chromatogram (GC) and organized according to the elution order.

Table 1 - Composition of experimental diets

	Starter	Grower	Finisher
Ingredient (g kg^{-1})			
Corn	541.00	571.89	602.64
Soybean meal 46%	374.90	337.50	302.00
Soy oil	44.90	51.53	56.52
Dicalcium phosphate	15.71	13.38	11.18
Calcitic limestone	9.10	8.65	7.78
Common salt	4.83	4.58	4.46
DL-methionine	1.47	1.42	1.32
L-lysine	0.64	0.75	1.02
Choline chloride	0.55	0.50	0.37
Mineral Premix[1]	0.50	0.50	0.50
Vitamin Premix[2]	0.40	0.30	0.20
Inert	6.00	9.00	12.00
Total	1000.00	1000.00	1000.00
Additives (mg kg^{-1})			
Coccidiostat[3]	500	500	500
Antimicrobial[4]	125	125	125
Capim-limão essential oil[5]	2100	5000	6330
Lippia rotundifolia essential oil[5]	2100	5000	6330
Capim-limão/*Lippia rotundifolia* essential oil[6]	1050	2510	3170
Calculated content			
Crude protein (%)	21.25	19.84	18.43
Metabolizable energy (kcal kg^{-1})	3069.92	3165.93	3215.51
Available phosphorus (%)	0.40	0.35	0.30
Calcium (%)	0.84	0.75	0.66
Sodium (%)	0.21	0.20	0.20
Lysine (%)	1.22	1.13	1.06
Methionine (%)	0.48	0.45	0.43

[1] Mineral Premix containing, per 1 kg of product: Cu (min), 15 $g.kg^{-1}$; Fe (min), 100 $g.kg^{-1}$; Mn (min), 140 $g.kg^{-1}$; Zn (min), 100 $g.kg^{-1}$; I (min), 2,400 $mg.kg^{-1}$; Se (min), 400 $mg.kg^{-1}$; inclusion of 500 g/t of feed.

[2] Initial vitamin Premix containing per kg^{-1} of product: vitamin A (min), 14,000,000.00 $IU.kg^{-1}$; vitamin D3 (min), 4,400,000.00 $IU.kg^{-1}$; vitamin E (min), 22,000.00 $IU.kg^{-1}$; vitamin. K3 (min), 3,200.00 $mg.kg^{-1}$; vitamin B1 (min), 4,000.00 $mg.kg^{-1}$; vitamin B2 (min), 10,000.00 $mg.kg^{-1}$; vitamin B6 (min), 6,000.00 $mg.kg^{-1}$; vitamin B12 (min), 24,000.00 $mcg.kg^{-1}$; niacin (min), 70 $g.kg^{-1}$; pantothenic acid (min), 26 $g.kg^{-1}$; folic acid (min), 1,600.00 $g.kg^{-1}$; inclusion of 500 g/t of feed. Growth Vitamin Premix containing per kg^{-1} of product: vitamin A (min), 12,000,000.00 $IU.kg^{-1}$; vitamin D3 (min), 4,000,000.00 $IU.kg^{-1}$; vitamin E (min), 20,000.00 $IU.kg^{-1}$; vitamin K3 (min), 3,200.00 $mg.kg^{-1}$; vitamin B1 (min), 2,800.00 $mg.kg^{-1}$; vitamin B2 (min), 8.000.00 $mg.kg^{-1}$; vitamin B6 (min), 4,000.00 $mg.kg^{-1}$; vitamin B12 (min), 20,000.00 $mcg.kg^{-1}$; niacin (min), 60 $g.kg^{-1}$; pantothenic acid (min), 22 $g.kg^{-1}$; folic acid (min), 1,200.00 $g.kg^{-1}$.

[3] Salinomycin coccidiostat for all phases, at 500 g/t of feed.

[4] Antimicrobial enramycin for all phases, at 125 g/t of feed.

[5] Essential oil of *Cymbopogon flexuosus*/*Lippia rotundifolia* in the corresponding treatments.

[6] Number of each of the essential oils in the association treatment.

Subsequently, the oils were converted into microcapsules by the Croma Microencapsulados company (São Paulo, SP, Brazil) via the coacervation method with edible polymers.

After microencapsulation and inclusion to diet, new analysis was done to verify the permanence of the essential oil compounds. For the analysis of the volatiles, the feed was transferred to headspace-type glass vials (20 mL) and placed in the autosampler system (HS combi-PAL). The vial contents were homogenized (500 rpm), incubated (75 °C for 5 min) and subsequently injected (1000 µL) with a pre-heated syringe (75 °C). For identification of volatiles, analysis was performed using Agilent Technologies system (7890A), coupled with mass spectrometry (MS 5975C), fitted with a DB-5ms fused silica capillary column (30 m × 0.25 mm × 0.25 µm) and helium (flow of 1 mL·min^{-1}) as carrier gas. The programming was 35 °C for 2 min, at 2 °C/min up to 80 ºC, then 4 °C min^{-1} up to 150 °C, for a total chromatographic run of 42 min. The column was heated at 300 °C during 1 min for cleaning. The system operated in scan mode (monitoring) with electron impact at 70 eV, in a range of 45 to 550 m/z. The identification of volatiles was performed by MSD Chemstation software for comparison of the mass spectrum with the library (National Institute of Standards and Technology, NIST 2002).

The birds and feed leftovers were weighed on the first day of the experiment and at 7, 21, 33, and 42 days of age. The variables analyzed were average body weight (BW), weight gain, feed intake, and feed conversion ratio (the relationship between intake and gain, calculated by BW gain/feed intake) of each phase. In the total period of the experiment, we calculated the production viability (VC), by the difference between the total number of housed birds and the number of dead birds, divided by the total birds housed and multiplied by 100, and productive efficiency index (PEI), through the daily average weight gain multiplied by the production viability and divided by the feed conversion multiplied by 10.

After 43 days, two birds of each experimental treatment were selected (one male and one female, weighing up to 10% above or below the average weight), after fasting for 8 h, then slaughtered by bleeding from the jugular vein, scalded, plucked, eviscerated, and then the commercial cuts were separated. Yields assessed were: carcass; breast; thighs + drumsticks; wings; back; feet; relative and absolute weight of the organs (intestine, caecum, liver, pancreas, gizzard, bursa, spleen, heart, and abdominal fat), plus the length of the intestines. Carcass yield was obtained by the ratio of hot carcass weight (gutted) and the fasting weight of the bird. The remaining yields were obtained by the ratio of the weight of the parts and the hot carcass weight.

Table 2 - Relative abundance (%) of the compounds detected in the essential oils of *Cymbopogon flexuosus* and *Lippia rotundifolia* by gas chromatography coupled to mass spectrometry

Number of peak	Retention time (minutes)	Compound	% Total ions chromatogram[1]	
			Cymbopogon flexuosus	*Lippia rotundifolia*
1	6.3	Camphene	0.03	ND
2	6.9	Myrcene	ND	15.52
3	7.2	6-methyl-5-hepten-2-one	0.07	ND
4	7.4	α-phellandrene	ND	1.65
5	10.1	Unknown	ND	5.57
6	10.7	β-linalool	1.17	1.68
7	12.6	2,4 dimethyl 2,6-octadiene	ND	13.69
8	12.8	Tagetone	ND	11.86
9	14.6	Unknown	0.68	ND
10	16.1	Verbenone	ND	3.50
11	16.5	Berbenone	ND	3.82
12	17.2	β-citral	33.80	ND
13	17.6	Trans-geraniol	6.06	ND
14	18.5	α-citral	43.62	ND
15	23.1	Geraniol acetate	4.20	ND
16	24.7	Caryophyllene	2.15	6.54
17	27.1	γ-elemene	ND	5.60
18	27.7	α-cedrene	ND	4.20
19	28.5	γ-cadinene	0.33	ND
20	29.2	Elemol	ND	2.81
Total			92.10	76.50
Trace[2]			7.80	23.49

[1] Relative area obtained of the total ions chromatogram.
[2] Trace compounds correspond to those under 1% of the peak area of the total ion chromatogram.
ND - compound not detected in the analysis.

Data of performance, carcass yield, and organ weights were subjected to analysis of variance and means were compared by Tukey test at 0.05 probability.

Results and Discussion

The major component of lemongrass was citral (77.42%), a mixture of the neral (β-citral) and geranial (α-citral) isomers detected in less abundance in the works of Adukwu et al. (2012) and Agnolin et al. (2014), who observed 80% and 90.6% citral, respectively.

The compound found in highest abundance in *L. rotundifolia* oil was β-myrcene (15.52%) (Table 2). The results agree with those obtained by Gomide et al. (2013), who detected β-myrcene (18.48%) as the major component of *L. rotundifolia* originating from Juiz de Fora, MG.

Analysis by gas chromatography using the headspace system was carried out to evaluate the persistence of the components in the feed (Table 3). The compounds detected in the essential oils prior to microencapsulation remained after the process, as well as after incorporation in the feed. Even storage for ten days did not impair the composition of the oils. In the feed with combination of the two essential oils, component characteristic of both species used were detected.

Animal health is directly linked to the balance of intestinal microflora. Naturally, beneficial and pathogenic microorganisms colonize the gastrointestinal tract of

Table 3 - Main compounds detected in the essential oils of *Cymbopogon flexuosus* and *Lippia rotundifolia* after microencapsulation and incorporation in feed

Number of peak	Compound	Retention time	Retention index	Sample				
				LrEO	CfEO	Control feed + CfEO	Control feed + LrEO	Control feed + LrEO + CfEO
1	3-methyl cyclohexane	5.6		ND	D	ND	ND	ND
2	4-heptanone	10.2	872	ND	D	ND	ND	ND
3	Tricyclene	12.9		ND	D	ND	ND	ND
4	α-pinene	13.7	917	D	D	ND	ND	ND
5	Canfene	14.8	954	ND	D	D	ND	D
6	6-methyl-5-hepten-2-one	17.3	988	ND	ND	D	ND	D
7	β-myrcene	19.3	988	D	D	D	D	D
8	4-carene	19.3	1002	ND	D	ND	ND	ND
9	α-phellandrene	19.7	1007	D	ND	ND	D	ND
10	ρ-cymene	19.9	1021	ND	D	ND	ND	ND
11	2-carene	20.0	1021	D	ND	ND	ND	ND
12	D-limonene	20.3	1028	ND	ND	ND	ND	ND
13	o-cymene	20.7	1028	D	ND	ND	D	ND
14	β-phellandrene	21.0	1030	D	ND	ND	ND	ND
15	(E)-β-ocimene	21.2	1037	D	D	ND	ND	ND
16	(Z)-β-ocimene	22.0	1044	D	D	ND	ND	ND
17	γ-terpinene	22.6	1060	D	ND	ND	ND	ND
18	Perylene	25.2	1101	ND	D	ND	ND	ND
19	β-linalool	25.9	1104	D	D	D	ND	D
20	Trans-p-menta-2,8-dienol	26.7	1118	ND	D	ND	ND	ND
21	(4E,6Z)-2,6-dimethyl-2,4,6-octatriene	27.2	1131	ND	D	ND	ND	ND
22	Trans-tagetone	29.1	1144	D	ND	ND	ND	D
23	p-menth-1-en-4-ol	30.1	1177	D	ND	ND	ND	ND
24	p-menth-1-en-8-ol	30.8	1190	D	ND	ND	ND	ND
25	D-verbenone	32.5	1205	D	ND	ND	ND	ND
26	Hxenyl valerate	33.0	1244	D	ND	ND	ND	ND
27	Neral	33.1	1249	ND	D	D	ND	D
28	Geraniol	33.2	1267	D	D	ND	ND	ND
29	Geranial	33.9	1269	D	D	D	ND	ND
30	δ-elemene	36.4	1324	D	ND	ND	ND	ND
31	β-elemene	36.5	1387	D	ND	ND	ND	ND
32	α-cubebene	37.0	1364	D	ND	ND	ND	ND
33	Copaene	38.0	1377	D	ND	ND	ND	ND
34	β-cubebene	38.4	1388	D	ND	ND	ND	ND
35	α-bergamotene	39.0	1438	D	ND	ND	ND	ND
36	Cryophyllene	39.6	1444	D	D	ND	D	D
37	α-humulene	40.7	1452	D	ND	ND	ND	ND
38	α-curcumene	41.6	1483	D	ND	ND	ND	ND

LrEO - microencapsulated essential oil of *Lippia rotundifolia*; CfEO - microencapsulated essential oil of *Cymbopogon flexuosus*; D - compound detected in the analysis; ND - compound not detected in the analysis.

poultry. The beneficial microorganisms can collaborate in the synthesis of vitamins, reduce gas production, improve digestion and nutrient absorption, and inhibit the growth of pathogenic bacteria. However, the imbalance of microflora leads to an increase of pathogenic microorganisms that can cause much harm to the animal (diseases, mucosal lesions, digestion process deterioration) (Furlan et al., 2004). Essential oils can ensure the balance of the microflora and, consequently, the animal health (Cho et al., 2014).

Body weight, weight gain, feed intake, feed conversion, and productive efficiency indices (Table 4) were not statistically different among treatments (P>0.05). Good hygiene and management practiced during animal rearing may have contributed to their low quantity of pathogenic microorganisms in the intestine, not worsening even in the negative control. Mortality (0.0%) and production viability (100%), which were similar among treatments, confirm the good experimental conditions. According to Freitas et al. (2001), the significant effects of the use of performance enhancers are best perceived when animals are subjected to hygiene-challenge conditions, with high population density, high contamination risk, poor hygiene, and exposure to diseases. The same applies to the use of essential oils. As a result, the lack of effect on performance

Table 4 - Performance of broilers fed diets containing essential oils of *Cymbopogon flexuosus* and *Lippia rotundifolia*

Variable	Negative control[1]	Positive control[2]	Feed + CfEO[3]	Feed + LrEO[4]	Feed + CfEO + LrEO[5]	P-value	CV (%)
			Initial (1-21 days)				
Body weight (g)	707.33NS	708.90NS	712.27NS	727.40NS	689.73NS	0.09	6.51
Feed intake (g)	883.33NS	961.73NS	839.20NS	908.93NS	912.00NS	0.28	7.14
Weight gain (g)	671.12NS	672.44NS	676.22NS	692.19NS	654.33NS	0.89	6.83
Feed conversion ratio (g:g)	1.31NS	1.42NS	1.25NS	1.31NS	1.39NS	0.38	8.17
			Growth (1-33 days)				
Body weight (g)	1664.6NS	1736.20NS	1771.00NS	1796.07NS	1753.17NS	0.10	3.08
Feed intake (g)	2514.2NS	2566.20NS	2394.90NS	2603.70NS	2468.80NS	0.49	5.94
Weight gain (g)	1628.45NS	1699.74NS	1734.95NS	1760.86NS	1717.76NS	0.10	3.15
Feed conversion ratio (g:g)	1.54NS	1.51NS	1.38NS	1.47NS	1.43NS	0.26	6.04
			Final (1-42 days)				
Body weight (g)	2502.67NS	2510.67NS	2407.07NS	2475.83NS	2332.67NS	0.13	3.51
Feed intake (g)	4135.40NS	4130.20NS	3933.20NS	4250.60NS	3979.10NS	0.42	5.30
Weight gain (g)	2466.45NS	2474.20NS	2371.28NS	2440.62NS	2297.25NS	0.12	3.54
Feed conversion ratio (g:g)	1.67NS	1.66NS	1.66NS	1.74NS	1.73NS	0.24	3.04
Productive efficiency index (%)	350.40NS	353.28NS	340.67NS	333.81NS	315.90NS	0.05	4.15

[1] Feed negative control without additives.
[2] Feed positive control with antimicrobials and anticoccidials.
[3] Control feed + essential oil of *Cymbopogon flexuosus*.
[4] Control feed + essential oil of *Lippia rotundifolia*.
[5] Control feed + association of *Cymbopogon flexuosus* and *Lippia rotundifolia* essential oils.
CV - coefficient of variation; NS - not significant by the Tukey test at 5% probability.

Table 5 - Carcass and cut yields of broilers fed diets containing essential oils of *Cymbopogon flexuosus* and *Lippia rotundifolia* at 43 days of age

Variable	Negative control[1]	Positive control[2]	Feed + CfEO[3]	Feed + LrEO[4]	Feed + CfEO + LrEO[5]	P-value	CV (%)
Weight after fasting (g)	2321.67NS	2406.67NS	2338.67NS	2392.67NS	2302.00NS	0.77	7.13
Hot carcass weight (g)	1809.75NS	1933.40NS	1800.57NS	1853.51NS	1787.06NS	0.37	7.53
Carcass yield (%)	78.35NS	80.35NS	77.08NS	77.52NS	77.55NS	0.77	6.13
			Weight of the cuts in relation to the carcass (%)				
Breast	31.85NS	31.89NS	32.90NS	33.35NS	33.92NS	0.08	4.42
Thigh + drumstick	26.79NS	26.64NS	25.45NS	27.88NS	27.26NS	0.38	7.91
Wings	8.95NS	9.23NS	9.73NS	9.42NS	9.27NS	0.26	6.29
Back	19.05NS	18.67NS	17.55NS	16.66NS	18.43NS	0.17	9.95
Feet	4.86NS	4.68NS	4.87NS	5.00NS	4.70NS	0.90	12.87

[1] Feed negative control without additives.
[2] Feed positive control with antimicrobials and anticoccidials.
[3] Control feed + essential oil of *Cymbopogon flexuosus*.
[4] Control feed + essential oil of *Lippia rotundifolia*.
[5] Control feed + association of *Cymbopogon flexuosus* and *Lippia rotundifolia* essential oils.
CV - coefficient of variation; NS - not significant by the Tukey test at 5% probability.

is common among works. Silva et al. (2011) also obtained similar results using essential oil of mastic-red, compared with the negative control (without feed additives) and the positive control (ration with added zinc bacitracin and salinomycin). Shiva et al., 2012 did not detect differences between the performance of animals receiving essential oil of oregano and those receiving antibiotics (bacitracin methylene disalicylate and colistin sulfate) or no additive. Both authors highlight the lack of hygiene challenge as a cause of the resemblance. However, Cho et al. (2014) improved feed efficiency in broilers challenged with *Clostridium perfringens* fed diets with thyme and star anise, compared with positive (avilamycin) and negative controls.

Treatments with essential oils did not differ statistically from the positive (with antimicrobials and anticoccidials) and negative (without additives) controls (P>0.05) for carcass and cut yields (Table 5). Rizzo et al. (2010) analyzed broiler chickens fed diets plus commercial products containing various essential oils and also found no differences in carcass and cut yields at 44 days in animals receiving essential oils and fed diets containing avilamycin or no additive. On the other hand, Khattak et al. (2014) observed higher carcass and breast yields in animals treated with a commercial combination of essential oils of basil, cumin, bay leaf, lemon, oregano, sage, thyme, and tea, compared with the diet without additives.

The absolute and relative weights of the intestines and liver were affected by the treatments (Table 6). The values for the absolute intestine weight were lower in the treatment with the combination of essential oils, compared with treatment without additives and treatment with the lemongrass essential oil (P<0.05). The relative weight of intestine was higher in treatments with lemongrass essential oil and *L. rotundifolia* essential oil in relation to their combination (P<0.05). The reduction in intestinal weight is expected, since the use of such additives reduces the thickness of the intestinal wall. Silva et al. (2011) also observed a reduction in weight of the intestines of birds supplemented with antimicrobials and essential oil of mastic-red. However, animals that received the control diet and the diet with lemongrass essential oil presented the highest absolute and relative intestinal weights.

The relative liver weight was lower with the essential oil of lemongrass and the mix of essential oils compared with treatment with essential oil of *L. rotundifolia* (P<0.05). This decrease may be due to some kind of toxicity, although the animals had normal livers without macroscopic lesions.

Table 6 - Absolute and relative weight of organs of broiler chickens fed diets containing essential oils of *Cymbopogon flexuosus* and *Lippia rotundifolia* at 43 days of age

Variable	Negative control[1]	Positive control[2]	Feed + CfEO[3]	Feed + LrEO[4]	Feed + CfEO + LrEO[5]	P-value	CV (%)
			Absolute weight (g)				
Intestine	89.167a	71.15ab	93.74a	73.09ab	62.26b	0.00	19.26
Caecum	11.877	13.50	13.93	13.73	11.95	0.81	30.33
Liver	46.180	41.39	42.50	43.91	38.76	0.21	12.72
Pancreas	5.537	3.21	4.24	5.78	5.58	0.08	36.13
Gizzard	32.548	29.52	32.66	35.05	33.71	0.40	14.94
Bursa	3.677	3.18	4.57	5.24	5.34	0.37	47.79
Spleen	2.378	1.27	3.15	2.96	3.01	0.06	46.51
Heart	10.797	11.21	11.80	13.10	11.55	0.67	23.89
Abdominal fat	38.197	40.06	44.61	44.91	37.33	0.88	37.69
Intestine (cm)	168.420	175.13	178.92	178.72	176.67	0.86	10.71
			Relative weight (%)				
Intestine	4.955ab	3.66bc	5.16a	3.95abc	3.52c	0.00	18.29
Caecum	0.652	0.69	0.77	0.74	0.67	0.83	29.86
Liver	2.543a	2.13b	2.35ab	2.37ab	2.17b	0.01	8.53
Pancreas	0.307	0.16	0.23	0.32	0.31	0.05	38.29
Gizzard	1.804	1.52	1.81	1.89	1.89	0.15	15.75
Bursa	0.206	0.16	0.25	0.29	0.29	0.35	50.63
Spleen	0.1298	0.06	0.17	0.16	0.17	0.04	47.44
Heart	0.596	0.57	0.65	0.71	0.64	0.49	22.31
Abdominal fat	2.109	2.07	2.49	2.43	2.07	0.85	37.52

Means followed by different letters in the same row differ by the Tukey test (P<0.05).
[1] Feed negative control without additives.
[2] Feed positive control with antimicrobials and anticoccidials.
[3] Control feed + essential oil of *Cymbopogon flexuosus*.
[4] Control feed + essential oil of *Lippia rotundifolia*.
[5] Control feed + association of *Cymbopogon flexuosus* and *Lippia rotundifolia* essential oils.
CV - coefficient of variation.

Data are consistent with those found by Barreto et al. (2008), who also observed lower liver weight relative to the control in chickens receiving red pepper essential oil.

The relative spleen weight was highest (P = 0.044) in the animals fed the essential oils (lemongrass, *L. rotundifolia*, and combination of the two oils), showing that there was a likely effect on the immune system.

Conclusions

The trial could not find results enough to recommend the use of the lemongrass and *Lippia rotundifolia* essential oils as additives in broiler diets.

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