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Blood parameters and hepatic histopathology of broilers fed rations supplemented with essential oils

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ABSTRACT - This study aimed to evaluate the hematological profile, hepatic function, and histopathology of mixed-sex broilers fed rations supplemented with microencapsulated essential oils from *Cymbopogon flexuosus* (lemon grass) and *Lippia rotundifolia* (chá-de-pedestre). One hundred and fifty Cobb chicks were housed in cages from 1 to 42 days of age in a completely randomized design, with six replicates with five chickens in each of five treatments (150 total chicks): basal diet without antibiotic growth promoter (negative control), diet with enramicina and salinomycin (positive control), diet with lemon grass essential oil, diet with *L. rotundifolia* essential oil, and diet with a mixture of lemon grass and *L. rotundifolia* essential oils. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly lower in untreated broilers (negative control group). Gender was not associated with erythrogram values, but aspartate aminotransferase activity (AST) was higher in females. Gamma-glutamyl transferase (GGT) was higher in chicks in the group that received rations with lemon grass oil. Broilers that received *L. rotundifolia* oil developed more hepatic lesions, although no effect of sex was observed related to the lesion score. Biliary hyperplasia and fibroplasias were observed in all groups, with higher histopathology scores in broilers that received diets containing *L. rotundifolia* oil. Mixed-sex broilers fed rations supplemented with lemon grass and *L. rotundifolia* essential oils have normal complete blood counts and unspecific hepatic lesions and are characterized by lipidosis, hyperplasia of the bile ducts, and fibroplasia.

Keywords: animal production, bird, bird nutrition, essential oils, hematology, liver

Introduction

Antibiotics in small dosages added to the avian diet as growth promoters result in better utilization of nutrients by the birds and higher growth rates and feed conversion efficiency. In addition, antibiotics act on the intestinal health of chickens. However, this practice has contributed to the emergence of resistant strains of microorganisms (Attia et al., 2017a). Thus, some countries have banned the use of antibiotics as additives in animal feed to minimize the transmission and spread of resistant bacteria through the food chain (Attia et al., 2018).

In contrast, the use of organic acids, enzymes, pre- and probiotics, and essential oils may result in higher performance and better intestinal health without the inconvenience of antibiotic resistance (Dhama et al., 2015; Zeng et al., 2015; Cho et al., 2014; Krishan and Narang, 2014). Variable results have been obtained using oils for antimicrobial purposes, such as rosemary (Traesel et al., 2011), thyme (Saleh et al., 2014; Toghyani et al., 2011; Toghyani et al., 2010; Tollba et al., 2010; Attia et al., 2017b), and ginger (Mehr et al., 2014) oils. However, the possible toxic effects and/or changes in metabolism caused by essential oils still generate doubts regarding their safety.

Cymbopogon flexuosus (lemon grass) and *Lippia rotundifolia* (chá-de-pedestre) essential oils can be used as alternative performance enhancers due to their *in vitro* antimicrobial action, and they can promote diet stability (Assis et al., 2017; Azevedo et al., 2016; Souza et al., 2015). *Cymbopogon flexuosus*, also known as grass balm, is native to Asia (May et al., 2008). The major components present in lemon grass oil are myrcene, geraniol, and citral (Simões et al., 2010), with the latter being the most important. *Lippia rotundifolia* is popularly known in Brazil as chá-de-pedestre. Its main components are β-myrcene, farnesol, limonene, and myrcenal (Leitão et al., 2008). Although it is promising as a phytotherapeutic plant, its pharmacological activity is still uncharacterized (Resende et al., 2015). Souza et al. (2015) demonstrated its antimicrobial activity against *S. aureus* and *E. coli* isolated from avian intestines.

Even though previous studies have used medicinal plants as performance enhancers for poultry, the subsequent changes affecting the metabolism of birds have not been fully characterized. Current research aims at evaluating the effect of phylogenics and essential oils on biochemistry and blood components. Available results are promising, indicating no changes in physiological parameters and improvement of the health status of treated birds (Attia et al., 2017b; 2018; Bortoluzzi et al., 2018; Chowdhury et al., 2018; Mokhtari et al., 2018).

Thus, this study aimed to evaluate blood parameters and hepatic histopathology of mixed broilers fed rations supplemented with microencapsulated essential oils.

Material and Methods

The present study was conducted in Montes Claros, MG, Brazil (16°41'00" S, 43°50'00" W). All procedures were in accordance with ethical standards and were approved by the Ethics Committee on Animal Use under case number 102/2013. The adopted procedures followed Azevedo et al. (2017).

One hundred and fifty one-day-old mixed sex Cobb 500® chicks were housed in 30 cages (60×35×100 cm) containing drinkers and feeders. The experiment was conducted in a completely randomized 2×5 (sexes × treatments) factorial design with three replicates of 10 animals per treatment (five males and five females). The treatments included the following: negative control, rations without antimicrobials or anticoccidials; positive control, rations supplemented with 10 ppm of enramycinand 42 ppm of salinomycin; *C. flexuosus* essential oil, rations with 120 mg of essential oil per kg of live weight; *L. rotundifolia* essential oil, rations with 120 mg of essential oil per kg of live weight; and association, rations with a mixture of two essential oils (60 mg of each essential oil). Dosage was based on previously demonstrated *in vitro* antimicrobial activity by the oils using the disc-diffusion method, minimum inhibitory concentration, and minimum bactericidal concentration as described by Azevedo et al. (2016) and Souza et al. (2015) for *C. flexuosus* essential oil and *L. rotundifolia* essential oil, respectively.

Nutritional planning was divided into three phases: initial, from 1 to 21 days; growth, from 22 to 33 days; and finishing, from 34 to 42 days. Rations were provided *ad libitum* during all experimental periods in ground form following the nutritional levels recommended by Rostagno et al. (2011) and presented by (Azevedo et al., 2017). The major compound of *C. flexuosus* essential oils was citral (77.42%), while that of *L. rotundifolia* essential oils was β-myrcene (15.52%) (Azevedo et al., 2017). Essential oils were converted into microcapsules by the coacervation method with edible polymers.

Compounds were identified by gas chromatography-mass spectrometry analysis, which ensured the stability of the rations after microencapsulation as described by Azevedo et al. (2017).

At 43 days of age, two birds of each experimental treatment were selected (one male and one female); selection included birds weighing up to 10% above or below the average weight. After fasting for 8 h, blood samples were collected for complete blood count analysis and serum biochemistry. Blood samples (4 mL) from the cutaneous ulnar vein were obtained according to recommendations of the Pan American Health Organization/World Health Organization (PAHO/WHO) (Manual..., 2010) and transferred to tubes to perform complete blood count and serum biochemistry. Hematological profiles were assessed by flow cytometry. Triglycerides were measured by end point colorimetry, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by the ultraviolet kinetic technique, gamma-glutamyl transferase (GGT) by the kinetic colorimetric method, and glucose by kinetic methodology.

For histopathological analysis, sections of the liver were collected and fixed by immersion in 10% buffered formalin for 24h, followed by dehydration in increasing concentrations of ethanol, diaphonization in xylol, and embedding in paraffin. Sections of 5 μm were stained with hematoxylin and eosin (H&E), for histopathological analysis under optical microscope coupled to a camera, under magnification of 10X and 40X. Lesions were assessed according to intensity and given a score: 0 = no damage, 1 = mild damage, 2 = moderate damage, and 3 = severe damage (Andrade et al., 2014). Histopathological scores are described in Table 1.

Table 1 - Criteria for histopathological scores considering lipidosis, fibroplasia and bile duct hyperplasia

Score	Lipidosis[1]	Fibroplasia[2]	Bile ducts hyperplasia[3]
0.5	HLMD	FPMM	PDMM
1.0	HLMM	FPMD	PDBD
2.0	HLMO	FPMO	PDBO
3.0	-	FPMI	-

[1] HLMD: hepatocytes with micro and macro vacuoles, well delimited, compatible with discreet multifocal lipidosis; HLMM: hepatocytes with micro and macro vacuoles, well delimited, compatible with diffuse minimal lipidosis; HLMO: hepatocytes with micro and macro vacuoles, well delimited, compatible with moderate multifocal lipidosis.
[2] FPMM: periportal minimum multifocal fibroplasia; FPMD: periportal mild multifocal fibroplasia; FPMO: periportal moderate multifocal fibroplasia; FPMI: predominantly multifocal periportal intense fibroplasia.
[3] PDMM: minimum multifocal bile ducts proliferation; PDBD: multifocal discreet bile ducts proliferation; PDBO: moderate multifocal bile ducts proliferation.

Data were analyzed by analysis of variance in R Statistical software (R Core Team, 2011) with sex and diet as the main effect and pen as an experimental unit. When there were significant interaction effects (P<0.05), averages were compared with Tukey's test. The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

in which Y_{ijk} = set of observations of the dependent variable corresponding to the sex i, adding essential oil j and repetition k; μ = set of observations; α_i = effect of the sex; β_j = effect of addition of essential oils in the diet; $(\alpha\beta)_{ij}$ = effect of interaction between sex i and addition of essential oils in the order j; and ε_{ijk} = experimental error concerning the observation of the effect of sex i, adding essential oils in the diet j and repetition k.

Histopathological score data were non-parametric; therefore, a Kruskal-Wallis test was used to compare the means of each group using the program GraphPad Prism version 5.0.

Results

During the period in which the broilers were housed, no clinical or behavioral changes were observed, indicating normal behavior. In this study, we defined as standard normality reference the results of the analyses of the positive control group and reference standards values for the species.

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were higher in the treatments than in the negative control (P<0.05; Table 2). The other erythrogram parameters were similar between treatments (P>0.05) and were not influenced by gender. Mean heterophil (Table 3) values were lower in the treatments compared with the negative control.

Gender influenced the enzyme activity of AST (P<0.05) with increased activity in females (Table 4). Gamma-glutamyl transferase was higher in the group that received rations with lemon grass oil (P<0.05). Triglyceride levels were not influenced by the treatments (P>0.05). There was no difference in the mean total protein, albumin, globulin, serum albumin:globulin ratio, total cholesterol, or glucose levels between treatments and the positive control (P>0.05) (Table 4). However, a significant difference was observed between the sexes regarding albumin (P<0.05), with males exhibiting a greater concentration than females.

In relation to the histopathological analysis (Figure 1), lesions were observed in chickens of all treatments, although with different scores. Discreet lipidosis was observed in animals in the negative control. The birds in the other treatments received scores that ranged from 2-3 for

Table 2 - Erythrogram of mixed-sex broilers fed rations supplemented with essential oils

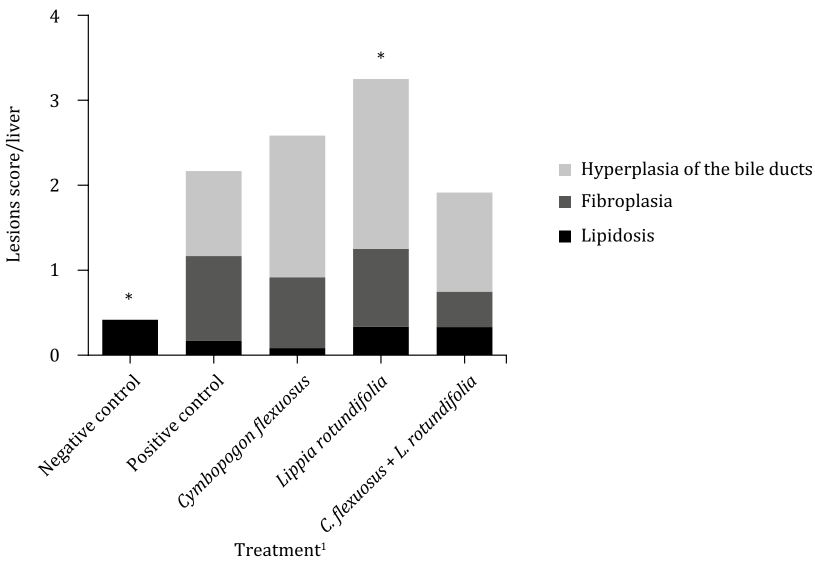
Treatment[1]	Sex	Erythrocytes (millions/mm^3)	Hemoglobin (g%)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (%)
	M	3.11±0.32	10.72±1.13	28.59±3.22	91.66±3.28	34.66±2.27	37.80±1.95
	F	3.15±0.57	10.03±1.26	28.15±4.95	90.00±4.05	32.98±3.60	36.80±3.70
Negative control		3.52±0.82	10.38±0.96	30.80±7.31	87.40b±3.21	30.32b±4.72	34.71±5.30
Positive control		3.05±0.36	11.00±1.54	28.28±4.45	92.32a±5.37	36.03a±2.82	38.85±2.36
C. flexuosus		3.08±0.23	9.90±1.75	28.03±2.58	90.99a±2.34	33.73a±0.93	37.07±0.86
L. rotundifolia		2.95±0.27	10.18±1.07	26.68±2.14	90.50a±1.76	35.02a±0.67	38.71±1.21
Association[2]		3.06±0.20	10.41±0.66	28.01±1.66	92.95a±3.17	34.00a±1.12	37.18±1.12
Negative control	M	3.26±0.60	10.40±0.81	29.13±4.92	89.53±2.36	32.34±3.58	36.09±3.53
	F	3.78±1.05	10.36±1.28	32.46±10.06	85.26±2.57	28.31±5.54	33.33±7.22
Positive control	M	3.28±0.24	12.23±1.15	31.56±3.68	95.93±4.31	37.22±1.02	38.83±1.70
	F	2.82±0.33	9.76±0.15	25.00±1.92	88.71±3.82	34.84±3.82	38.86±3.33
C. flexuosus	M	3.04±0.28	10.30±0.86	27.43±2.77	90.11±2.37	33.87±1.35	37.58±0.87
	F	3.12±0.22	9.50±2.55	28.73±2.76	91.88±2.39	33.59±0.55	36.56±0.57
L. rotundifolia	M	2.92±0.10	9.96±0.92	26.43±1.01	90.51±1.36	35.15±0.90	38.85±1.59
	F	2.98±0.42	10.40±1.37	26.93±3.20	90.48±2.43	34.88±0.51	38.56±1.03
Association	M	3.08±0.26	10.70±0.81	28.40±1.85	92.21±1.96	34.72±0.69	37.65±0.59
	F	3.04±0.19	10.13±0.45	27.63±1.73	93.70±4.43	33.29±1.06	36.71±1.46
Source of variation				P-value			
Treatment (T)		0.26	0.61	0.56	0.03	0.01	0.14
Sex (S)		0.83	0.13	0.77	0.14	0.08	0.36
T×S		0.49	0.32	0.34	0.06	0.67	0.93

MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.
[1] Treatment = ration negative control: without additives; positive control ration: with antimicrobial and anticoccidial; control diet essential oil of *C. flexuosus*; control diet essential oil of *L. rotundifolia*.
[2] Association: control diet essential oil of *C. flexuosus* and *L. rotundifolia*.
a,b - average followed by the same letter in the column does not differ among themselves by Tukey test (P<0.05).

Table 3 - Leukogram of mixed-sex broilers fed rations supplemented with essential oils

Treatment[1]	Sex	Leukocytes	Heterophil (Hf)	Hf:Lf	Eosinophil	Lymphocyte (Lf)	Monocyte
		mm^3					
	M	28.89±3.31	7.46±2.74	0.10±0.03	11.26±6.35	72.40±7.07	8.86±4.20
	F	27.58±4.23	8.00±5.43	0.12±0.12	12.73±6.39	69.46±10.80	9.80±4.66
Negative control		30.39±4.94	12.16b±7.52	0.20±0.17	11.83±7.33	65.66±12.51	10.33±6.68
Positive control		26.74±4.41	7.50a±2.88	0.10±0.04	12.50±7.44	74.33±10.09	5.66±2.58
C. flexuosus		28.30±3.80	7.16a±1.16	0.09±0.01	10.83±5.03	73.50±3.14	8.50±2.42
L. rotundifolia		28.56±3.31	6.16a±0.98	0.08±0.00	12.33±8.91	69.50±10.67	12.00±4.47
Association[2]		27.19±2.09	5.66a±2.25	0.08±0.03	12.50±3.78	71.66±6.31	10.16±2.63
Negative control	M	27.69±3.51	9.66±2.88	0.13±0.03	9.33±9.23	74.00±4.58	7.00±3.60
	F	33.09±5.18	14.66±10.69	0.28±0.23	14.33±5.50	57.33±12.74	13.66±8.08
Positive control	M	29.86±3.82	10.00±1.00	0.14±0.02	14.33±10.96	70.00±13.89	5.66±4.04
	F	23.62±2.18	5.00±1.00	0.06±0.01	10.66±2.88	78.66±2.30	5.66±0.57
C. flexuosus	M	28.59±5.87	6.66±1.15	0.09±0.01	11.66±5.03	72.33±2.88	9.33±3.21
	F	28.01±1.17	7.66±1.15	0.10±0.01	10.00±6.00	74.66±3.51	7.66±1.52
L. rotundifolia	M	29.75±2.55	6.33±1.15	0.08±0.00	9.00±4.00	73.00±8.88	11.66±6.35
	F	27.37±4.08	6.00±1.00	0.09±0.00	15.66±12.22	66.00±13.00	12.33±3.05
Association[2]	M	28.55±1.98	4.66±3.05	0.06±0.04	12.00±2.64	72.66±6.02	10.66±2.51
	F	25.83±1.23	6.66±0.57	0.09±0.01	13.00±5.29	70.66±7.76	9.66±3.21
Source of variation				P-value			
Treatment (T)		0.43	0.04	0.06	0.99	0.44	0.13
Sex (S)		0.32	0.69	0.44	0.57	0.36	0.54
T×S		0.10	0.25	0.17	0.69	0.15	0.45

[1] Treatments = ration negative control: without additives; positive control ration: with antimicrobial and anticoccidial; control diet essential oil of *C. flexuosus*; control diet essential oil of *L. rotundifolia*.
[2] Association: control diet essential oil of *C. flexuosus* and *L. rotundifolia*.
a,b - average followed by the same letter in the column does not differ among themselves by Tukey test (P<0.05)



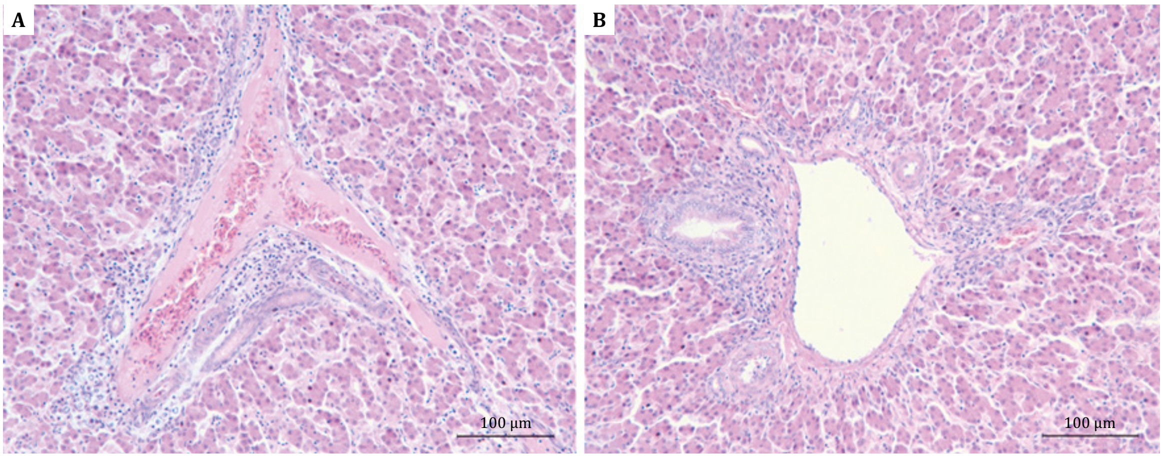
[1] Treatments: negative control: negative control diet without additives; positive control: positive control diet with antimicrobial and anticoccidial; *C. flexuosus*: control diet with essential oil of *C. flexuosus*; *L. rotundifolia*: control diet with essential oil of *L. rotundifolia*; association: control diet association between the essential oils of *C. flexuosus* and *L. rotundifolia*.
Kruskal-Wallis Test; * indicates (P<0.0162).

Figure 1 - Score of liver lesions of mixed-sex broilers fed rations supplemented with essential oils.

Table 4 - Liver enzymes activity and serum biochemistry of mixed-sex broilers fed rations supplemented with essential oils

Treatment[1]	Sex	AST	ALT	GGT	TP	Albumin	Globulin	Triglycerides	TC
		---------------- IU/L ----------------			--------------- g/L ---------------			--------- mg/dL ---------	
	M	297.40b±96.48	9.40±0.82	22.66±6.22	3.20±0.62	1.68a±0.15	1.51±0.70	19.06±9.76	115.40±18.82
	F	400.86a±178.23	10.20±1.47	20.46±4.27	2.82±0.39	1.53b±0.25	1.28±0.37	17.00±12.07	105.26±18.42
Negative control		257.66±9.22	9.00±1.09	21.33b±3.66	2.97±0.31	1.44±0.24	1.53±0.26	14.33±8.52	96.33±12.17
Positive control		335.83±200.98	10.00±1.54	17.50b±2.81	2.79±0.62	1.62±0.15	1.17±0.54	25.00±16.58	127.66±14.12
C. flexuosus		301.00±95.34	9.50±0.83	27.16a±5.56	2.83±0.50	1.64±0.19	1.19±0.43	25.33±5.78	105.33±19.28
L. rotundifolia		431.00±174.94	10.00±0.89	20.66b±5.46	3.26±0.51	1.58±0.23	1.67±0.54	15.83±6.24	110.00±14.99
Association[2]		420.16±154.18	10.50±1.51	21.16b±5.11	3.18±0.72	1.74±0.21	1.43±0.88	9.66±5.75	112.33±22.83
Negative control	M	256.66±12.66	8.66±1.15	22.33±4.50	3.08±0.32	1.60±0.06	1.48±0.26	14.33±12.50	106.33±7.63
	F	258.66±7.02	9.33±1.15	20.33±3.21	2.87±0.34	1.29±0.27	1.58±0.30	14.33±5.03	86.33±3.51
Positive control	M	263.33±32.33	9.33±0.57	19.00±3.46	2.93±0.87	1.74±0.07	1.19±0.80	27.00±9.16	129.00±21.70
	F	408.33±290.12	10.66±2.08	16.00±1.00	2.65±0.37	1.50±0.10	1.14±0.27	23.00±24.33	126.33±4.72
C. flexuosus	M	266.00±9.64	9.33±0.57	29.66±7.50	3.22±0.36	1.68±0.10	1.54±0.27	25.33±8.62	114.33±20.55
	F	336.00±137.69	9.66±1.15	24.66±1.52	2.44±0.21	1.59±0.28	0.84±0.19	25.33±3.05	96.33±16.25
L. rotundifolia	M	284.66±28.53	9.66±0.57	21.33±7.37	3.50±0.57	1.69±0.20	1.81±0.75	15.66±7.37	114.00±10.53
	F	577.33±107.05	10.33±1.15	20.00±4.35	3.02±0.40	1.48±0.25	1.53±0.34	16.00±6.55	106.00±20.07
Association[2]	M	416.33±189.43	10.00±1.00	21.00±5.29	3.26±1.05	1.71±0.30	1.54±1.33	13.00±6.24	113.33±31.72
	F	424.00±153.29	11.00±2.00	21.33±6.11	3.10±0.44	1.77±0.14	1.33±0.36	6.33±3.21	111.33±17.15
Source of variation					P-value				
Treatment (T)		0.14	0.32	0.04	0.51	0.19	0.55	0.06	0.06
Sex (S)		0.04	0.09	0.23	0.07	0.04	0.30	0.59	0.12
T×S		0.32	0.96	0.90	0.86	0.55	0.82	0.96	0.84

AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - gamma-glutamyl transferase; TP - total protein; TC - total cholesterol.
[1] Treatment = negative control ration: without additives; positive control ration: with antimicrobial and anticoccidial; control diet essential oil of *C. flexuosus*; control diet essential oil of *L. rotundifolia*.
[2] Association: control diet essential oil of *C. flexuosus* and *L. rotundifolia*.
a,b - average followed by the same letter in column does not differ among themselves by Tukey test (P<0.05).



A - Bile ducts hyperplasia and inflammatory infiltrate; B - Fibroplasia, hyperplasia of the bile ducts and inflammatory infiltrate.
Hematoxylin-eosin staining (H&E); Bar = 100 µm.

Figure 2 - Lesions in the liver of mixed-sex broilers fed rations supplemented with essential oil of *L. roduntifolia*).

lipidosis, hyperplasia of the bile ducts, and fibroplasia. Broilers that received chá-de-pedestre oil in the diet exhibited higher damage scores in comparison with those in other groups (P<0.05). No effect of gender was observed on lesion scores (P>0.05). The occurrence of lesions characterized by hyperplasia of the bile ducts (Figure 2A) and fibroplasia (Figure 2B) was found in birds of all treatments, except the negative control, and broilers that received rations containing chá-de-pedestre oil had the highest scores.

Discussion

Productivity parameters of experimental batches in this study were within the range considered normal for this commercial lineage as previously described by Azevedo et al. (2017), because the survival was 100% throughout the experimental period and experimental animals remained healthy. Studies with other phytogenic and essential oils added to the diet are in good agreement with our results. The beneficial effects have been associated with the modulating activity of the metabolic pathways of the cecal microbiota, by stimulating the immune system, and improving intestinal digestibility (Attia et al., 2017b; 2018; Bortoluzzi et al., 2018; Chowdhury et al., 2018; Mokhtari et al., 2018).

The hematological parameters results obtained in this research may also be associated with the health of birds and corroborate those of studies conducted with *Thymus vulgaris* (Toghyani et al., 2010; Ali, 2014; Saleh et al., 2014) and physiological criteria (Attia et al., 2017b; 2018).

The mean heterophil values (Table 3) and mean total protein, albumin, globulin, serum albumin:globulin ratio values (Table 4) indicated that the birds remained within normal ranges during the experiment. Attia et al. (2017b), Attia et al. (2018), Mokhtari et al. (2018), and Traesel et al. (2011) observed similar results with other phytogenics and essential oils, suggesting an improvement in general health. The possible action of essential oils on the avian immune system is still unknown. However, immunomodulating activity has been described for other essential oils in different experimental models (Brenes and Roura, 2010; Dhama et al., 2015). Essential oils acting as immune stimulants have been demonstrated by the use of thyme oil and ginseng in broiler diets (Toghyani et al., 2010; Toghyani et al., 2011; Saleh et al., 2014; Attia et al., 2017b; 2018). According to these authors, thyme oil and ginseng may stimulate innate immunity, increasing the phagocytic activity of heterophils.

Gender influencing AST levels has not been described in studies using phytogenic or essential oils in birds. High AST levels may be related to hepatocyte membrane damage and lesions in bile ducts. However, generation of AST from other tissues, such as muscles, must be considered (Tennant and Center, 2008; Grunkemeyer, 2010; Attia et al., 2017a). High environment temperatures may have affected these results. Notably, broilers and laying hens can suffer liver damage from unfavorable environmental conditions (Attia et al., 2017a).

Conversely, the highest levels of GGT observed in birds of the diet group with lemon grass essential oil (Table 4) may be associated with liver lesions. Gamma-glutamyl transferase is a primary marker for hepatobiliary diseases and can be indicative of intra-hepatic and extra-hepatic cholelithiasis (Tennant and Center, 2008; Grunkemeyer, 2010). The effect of essential oils on the hepatic function of broilers is variable. Cinnamon, oregano, pepper, thyme (Toghyani et al., 2011; Attia et al., 2017b; 2018), and ginseng (Catalan et al., 2013) oils do not influence hepatic biochemical components, such as serum protein, albumin, triglyceride, and AST. However, considering the conditions of this study, GGT levels (Table 4) may indicate hepatic damage in broilers, which may be associated with hepatic fibroplasia and hyperplasia of the bile ducts observed in groups that received rations with essential oils (Figure 1).

Biochemical profile results from a study on the association between hepatic lesions and alterations of hepatic enzymes such as GGT may indicate a loss in the synthesis capacity of the liver or hepatocellular damage, which reflects the ability of the liver to employ proper metabolism of lipids, proteins, and carbohydrates (Grunkemeyer, 2010). The author also notes that GGT changes are nonspecific and may be observed in association with various liver changes.

The results obtained in the present study are similar to those of studies with clove essential oils (Mehr et al., 2014) and thyme (Toghyani et al., 2010). This pattern may be indicative of a balance between intestinal absorption, synthesis, and secretion in hepatocytes and absorption in adipose tissue (Lumeij, 2008).

Lipidosis, as observed in this study, should not be interpreted as a possible effect of essential oils, since it was also observed in broilers that received diets without these oils. However, in other studies, a difference in the degree of lipidosis has been observed, depending on the origin of oils used in the diets (Tufarelli et al., 2015), even when this difference has been reversible, depending on the extent of lipidosis (Blevins et al., 2010). According to Schmidt et al. (2003), lipidosis is frequent in broilers and may be associated with diet, mycotoxins, or the metabolism of hepatic enzymes; however, in this study, only the broilers in the treatment containing lemon grass oil exhibited higher levels of GGT.

In broilers, hyperplasia and fibroplasia of the bile ducts are classified as nonspecific lesions and are associated with changes in hepatic metabolism that occur systematically after lesion of the liver parenchyma. These lesions are of various origins, and most of the time, they are not observed as clinical manifestations prior to death, making it difficult to determine the exact causes (Hochleithner et al., 2005).

Previous studies on the toxicity of *C. flexuosus* and *L. rotundifolia* specifically were not found in the literature. However, plants of the same genus have been shown not to be toxic. Costa et al. (2011) analyzed oral toxicity and genotoxicity in *Cymbopogon citratus* at a dose of 3,500 mg/kg in mice and found it not to be toxic, and Andrade et al. (2014) tested the effects of *Lippia origanoides* at a dosage of 120 μL/mL, and, likewise, it did not induce acute oral toxicity or subchronic signs in rats.

Despite being used in veterinary and human medicine, there is little information about the absorption of essential oil compounds after oral administration (Kohlert et al., 2000). Few studies have focused on the toxicity of essential oils; however, it has been confirmed that they have toxic effects on eukaryotic cells with variable effects. Raut and Karuppayil (2014) showed the safety of essential oils at low concentrations. However, studies that assess the toxic concentrations for each oil are still needed.

Conclusions

Mixed-sex broilers fed rations supplemented with *C. flexuosus* and *L. rotundifolia* essential oils had normal complete blood counts and unspecific hepatic lesions characterized by lipidosis, hyperplasia of the bile ducts, and fibroplasia.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, E.R. Martins, I.L. Azevedo and A.C. Almeida. Data curation: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, E.R. Martins and A.C. Almeida. Formal analysis: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, N.J.F. Oliveira, E.R. Martins, T.F. Carvalho and A.C. Almeida. Funding acquisition: E.R. Martins and A.C. Almeida. Investigation: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, N.J.F. Oliveira, E.R. Martins, I.L. Azevedo, T.F. Carvalho and A.C. Almeida. Methodology: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, N.J.F. Oliveira, E.R. Martins, I.L. Azevedo, T.F. Carvalho and A.C. Almeida. Project administration: E.R. Martins and A.C. Almeida. Resources: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, N.J.F. Oliveira, E.R. Martins, I.L. Azevedo, T.F. Carvalho and A.C. Almeida. Writing-original draft: V.K.F.R. Santos, W.C.L. Nogueira, R.L. Santos, N.J.F. Oliveira, E.R.

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References

Ali, A. H. H. 2014. Productive performance and immune response of broiler chicks as affected by dietary thyme leaves powder. Egyptian Poultry Science Journal 34:71-84. https://doi.org/10.21608/epsj.2014.5307

Andrade, V. A.; Almeida, A. C.; Souza, D. S.; Colen, K. G. F.; Macêdo, A. A.; Martins, E. R.; Fonseca, F. S. A. and Santos, R. L. 2014. Antimicrobial activity and acute and chronic toxicity of the essential oil of *Lippia origanoides*. Pesquisa Veterinária Brasileira 34:1153-1161. https://doi.org/10.1590/S0100-736X2014001200002

Attia, Y. A.; Bakhashwain, A. A. and Bertu, N. K. 2017a. Thyme oil (*Thyme vulgaris* L.) as a natural growth promoter for broiler chickens reared under hot climate. Italian Journal of Animal Science 16:275-282. https://doi.org/10.1080/1828051X.2016.1245594

Attia, Y. A.; Al-Harthi, M. A. and Hassan, S. S. 2017b. Turmeric (*Curcuma longa* Linn.) as a phytogenic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks. Revista Mexicana de Ciencias Pecuarias 8:11-21. https://doi.org/10.22319/rmcp.v8i1.4309

Attia, Y. A.; Bakhashwain, A. A. and Bertu, N. K. 2018. Utilisation of thyme powder (*Thyme vulgaris* L.) as a growth promoter alternative to antibiotics for broiler chickens raised in a hot climate. European Poultry Science 82. https://doi.org/10.1399/eps.2018.238

Azevedo, I. L.; Almeida, A. C.; Martins, E. R.; Nogueira, W. C. L.; Faria Filho, D. E.; Oliveira, S. P.; Prates, J. P. B. and Souza, C. N. 2016. Eficácia *in vitro* do óleo essencial de capim-limão (*Cymbopogon flexuosus* steud. wats.) frente a bactérias entéricas de origem avícola. Acta Veterinaria Brasilica 10:25-31.

Azevedo, I. L.; Martins, E. R.; Almeida, A. C.; Nogueira, W. C. L; Faria Filho, D. E.; Santos, V. K. F. R. and Lara, L. J. C. 2017. Use of *Lippia rotundifolia* and *Cymbopogon flexuosus* essential oils, individually or in combination, in broiler diets. Revista Brasileira de Zootecnia 46:13-19. https://doi.org/10.1590/s1806-92902017000100003

Assis, Y. P. A. S.; Almeida, A. C.; Nogueira, W. C. L.; Souza, C. N.; Gonçalves, S. F.; Silva, F. E. G.; Santos, V. K. F. R. and Martins, E. R. 2017. Antibacterial activity and stability of microencapsulated lemon grass essential oil in feeds for broiler chickens. Revista Brasileira de Saúde e Produção Animal 18:587-593. https://doi.org/10.1590/s1519-99402017000400009

Blevins, S.; Siegel, P. B.; Blodgett, D. J.; Ehrich, M.; Saunders, G. K. and Lewis, R. M. 2010. Effects of silymarin on gossypol toxicosis in divergent lines of chickens. Poultry Science 89:1878-1886. https://doi.org/10.3382/ps.2010-00768

Bortoluzzi, C.; Rothrock, M. J.; Vieira, B. S.; Mallo, J. J.; Puyalto, M.; Hofacre, C. and Applegate, T. J. 2018. Supplementation of protected sodium butyrate alone or in combination with essential oils modulated the cecal microbiota of broiler chickens challenged with coccidia and *Clostridium perfringens*. Frontiers in Sustainable Food Systems 2:72. https://doi.org/10.3389/fsufs.2018.00072

Brenes, A. and Roura, E. 2010. Essential oils in poultry nutrition: Main effects and modes of action. Animal Feed Science and Technology 158:1-14. https://doi.org/10.1016/j.anifeedsci.2010.03.007

Catalan, A. A. S.; Avila, V. S.; Lopes, L. L.; Montagner, P.; Vargas, G. D.; Xavier, E. G. and Roll, V. F. B. 2013. Perfil metabólico, hematológico e comportamental de poedeiras suplementadas com *Panax ginseng*. Archivos de Zootecnia 62:89-100. https://doi.org/10.4321/S0004-05922013000100010

Cho, J. H.; Kim, H. J. and Kim, I. H. 2014. Effects of phytogenic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with *Clostridium perfringens* in broilers. Livestock Science 160:82-88. https://doi.org/10.1016/j.livsci.2013.11.006

Chowdhury, S.; Mandal, G. P.; Patra, A. K.; Kumar, P.; Samanta, I.; Pradhan, S. and Samanta, A. K. 2018. Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. Animal Feed Science and Technology 236:39-47. https://doi.org/10.1016/j.anifeedsci.2017.12.003

Costa, C. A. R. A.; Bidinotto, L. T.; Takahira, R. K.; Salvadori, D. M. F.; Barbisan, L. F. and Costa, M. 2011. Cholesterol reduction and lack of genotoxic or toxic effects in mice after repeated 21-day oral intake of lemongrass (*Cymbopogon citratus*) essential oil. Food and Chemical Toxicology 49:2268-2272. https://doi.org/10.1016/j.fct.2011.06.025

Dhama, K.; Latheef, S. K.; Mani, S.; Samad, H. A.; Karthik, K.; Tiwari, R.; Khan, R. U.; Alagawany, M.; Farag, M. R.; Alam, G. M.; Laudadio, V. and Tufarelli, V. 2015. Multiple beneficial applications and modes of action of herbs in poultry health and production - A review. International Journal of Pharmacology 11:152-176. https://doi.org/10.3923/ijp.2015.152.176

Grunkemeyer, V. L. 2010. Advanced diagnostic approaches and current management of avian hepatic disorders. Veterinary Clinics of North America: Exotic Animal Practice 13:413-427. https://doi.org/10.1016/j.cvex.2010.05.005

Hochleithner, M.; Hochleithner, C. and Harrison, L. D. 2005. Evaluating and treating the liver. p.441-450. In: Clinical Avian Medicine - Volume 1. Harrison, G.; Lightfoot, T., eds. Spix Publishing, Palm Beach.

Krishan, G. and Narang, A. 2014. Use of essential oils in poultry nutrition: A new approach. Journal of Advanced Veterinary and Animal Research 1:156-162. https://doi.org/10.5455/javar.2014.a36

Kohlert, C.; Van Rensen, I.; März, R.; Schindler, G.; Graefe, E. U. and Veit, M. 2000. Bioavailability and pharmacokinetics of natural volatile terpenes in animal and humans. Planta Medica 66:495-505. https://doi.org/10.1055/s-2000-8616

Leitão, S. G.; Oliveira, D. R.; Sülsen, V.; Martino, V.; Barbosa, Y. G.; Bizzo, H. R.; Lopes, D.; Viccini, L. F.; Salimena, F. R. G.; Peixoto, P. H. P. and Leitão, G. G. 2008. Analysis of the chemical composition of the essential oils extracted from *Lippia lacunosa* Mart. & Schauer and *Lippia rotundifolia* Cham. (Verbenaceae) by gas chromatography and gas chromatography-mass spectrometry. Journal of the Brazilian Chemical Society 19:1388-1393.

Lumeij, J. T. 2008. Avian clinical biochemistry. p.839-872. In: Clinical biochemistry of domestic animals. 6th ed. Kaneko, J.; Harvey, J. and Bruss, M., eds. Academic Press.

May, A.; Bovi, O. A.; Maia, N. B.; Moraes, A. R. A.; Pinheiro, M. Q. and Mario, M. 2008. Influência do intervalo entre cortes sobre a produção de biomassa de duas espécies de capim limão. Horticultura Brasileira 26:379-382. https://doi.org/10.1590/S0102-05362008000300017

Mehr, M. A.; Hassanabadi, A.; Nassiri Moghaddam, H. and Kermanshahi, H. 2014. Supplementation of clove essential oils and probiotic to the broiler's diet on performance, carcass traits and blood components. Iranian Journal of Applied Animal Science 4:117-122.

Manual veterinário de colheita e envio de amostras: Aves. 2010. OPAS/OMS, Rio de Janeiro.

Mokhtari, S.; Rahati, M.; Seidavi, A.; Haq, Q. M. I.; Kadim, I.; Laudadio, V. and Tufarelli, V. 2018. Effects of feed supplementation with lavender (*Lavandula angustifolia*) essence on growth performance, carcass traits, blood constituents and caecal microbiota of broiler chickens. European Poultry Science 82. https://doi.org/10.1399/eps.2018.249

R Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.r-project.org/>. Accessed: June 12, 2018.

Raut, J. S. and Karuppayil, S. M. 2014. A status review on the medicinal properties of essential oils. Industrial Crops and Products 1:250-264. https://doi.org/10.1016/j.indcrop.2014.05.055

Resende, C. F.; Bianchetti, R. E.; Oliveira, A. M. S.; Braga, V. F. and Peixoto, P. H. P. 2015. *In vitro* propagation and acclimatization of *Lippia rotundifolia*, an endemic species of Brazilian Campos Rupestres. Revista Ciência Agronômica 46:582-589. https://doi.org/10.5935/1806-6690.20150041

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos: Composição de alimentos e exigências nutricionais. 3.ed. Viçosa, MG.

Saleh, N.; Allam T.; El-latif, A. A. and Ghazy, E. 2014. The effects of dietary supplementation of different levels of Thyme (*Thymus vulgaris*) and Ginger (*Zingiber officinale*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Global Veterinária 12:736-744.

Schmidt, R. E.; Reavil, D. R. I. and Phalen, N. D. 2003. Liver. In: Pathology of pet and aviary birds. Schmidt, R. E.; Reavil, D. R. I. and Phalen, N. D., eds. Iowa State Press, Ames, Iowa.

Simões, C. M. O.; Schenkel, E. P.; Gosmann, G.; Mello, J. C. P.; Mentz, L. A. and Petrovick. P. R. (Orgs). 2010. Farmacognosia: da planta ao medicamento. 6th ed. Editora da UFRGS, Porto Alegre; Editora da UFSC, Florianópolis.

Souza, D. S.; Almeida, A. C.; Andrade, V. A.; Marcelo, N. A.; Azevedo, I. L.; Martins, E. R. and Figueiredo, L. S. 2015. Atividade antimicrobiana do óleo essencial de *Lippia origanoides* e *Lippia rotundifolia* frente à enterobactérias isoladas de aves. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 67:940-944. https://doi.org/10.1590/1678-4162-7580

Tennant, B. C. and Center, S. A. 2008. Hepatic function. p.379-412. In: Clinical biochemistry of domestic animals. 6th ed. Kaneko, J.; Harvey, J. and Bruss, M., eds. Academic Press.

Toghyani, M.; Toghyani, M.; Gheisari, A.; Ghalamkari, G. and Eghbalsaied, S. 2011. Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. Livestock Science 138:167-173. https://doi.org/10.1016/j.livsci.2010.12.018

Toghyani, M.; Toghyani, M.; Gheisari, A.; Ghalamkari, G. and Mohammadrezaei, M. 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint *(Mentha piperita*). Livestock Science 129:173-178. https://doi.org/10.1016/j.livsci.2010.01.021

Tollba, A. A. H.; Shabaan, S. A. M. and Abdel-Mageed, M. A. A. 2010. Effects of using aromatic herbal extract and blended with organic acids on productive and physiological performance of poultry. Egyptian Poultry Science 30:229-248.

Traesel, C. K.; Lopes, S. T. A.; Wolkmer, P.; Schmidt, C.; Santurio, J. M. and Alves, S. H. 2011. Óleos essenciais como substituintes de antibióticos promotores de crescimento em frangos de corte: perfil de soroproteínas e peroxidação lipídica. Ciência Rural 41:278-284. https://doi.org/10.1590/S0103-84782011000200016

Tufarelli, V.; Bozzo, G.; Perillo, A. and Laudadio, V. 2015. Effects of feeding different lipid sources on hepatic histopathology features and growth traits of broiler chickens. Acta Histochemica 117:780-783. https://doi.org/10.1016/j.acthis.2015.08.001

Zeng, Z.; Zhang, S.; Wang, H. and Piao, X. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of Animal Science and Biotechnology 6:7. https://doi.org/10.1186/s40104-015-0004-5