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RESEARCH ARTICLE

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Inhibitory action of *Lippia gracilis* Schauer essential oil on pathogenic bacteria and its effects as a growth promoter on quail

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Abstract

Aim of study: To examine the *in vitro* sensitivity of *Salmonella* sp. and *Escherichia coli* strains to the microbial activity of *Lippia gracilis Schauer* essential oil (LGSEO) and to determine the optimal level of LGSEO as a growth promoter in diets for Japanese quail up to 35 days of age.

Area of study: São Cristovão, Sergipe, Brazil.

Material and methods: A total of 504 female Japanese quails (*Coturnix coturnix japonica*) at an initial average body weights of 6.80□0.10 g was allotted to one of six treatments (0, 100, 200, 300, 400 mg/kg of LGSEO and a diet containing 500 mg/kg of bacitracin methylene disalicylate) in 7 replicates, using 12 birds per experimental unit.

Main results: In the age period of 21 to 35 days, feed intake declined linearly (p=0.04) and feed efficiency improved (p<0.01), whereas no changes were observed in production performance (p>0.05). The estimated (p=0.01) maximum relative weights of proventriculus and pancreas were obtained at the LGSEO inclusion levels of 196.5 and 251 mg/kg, respectively. Inclusion of 100 to 300 mg/kg of LGSEO in the diet reduced the total *Salmonella* sp. bacterial count.

Research highlights: The use of 196.5 mg/kg of LGSEO in the diet of Japanese quail improved production performance and organ development and demonstrated potential antimicrobial capacity against *Salmonella* sp. bacteria. Due its pharmacological composition, LGSEO can potentially substitute to antimicrobials, because contains thymol and carvacrol as main active constituents.

Additional key words: alternative antimicrobial, birds, carvacrol, nutrition, performance, thymol.

Abbreviations used: BMD (Bacitracin methylene disalicylate); FE (feed efficiency); FI (feed intake); LGSEO (*Lippia gracilis* Schauer essential oil); UFS (Federal University of Sergipe); WG (weight gain).

Authors' contributions: Conceived, designed and performed the experiments: GMOJ, GSCJ, HBJP, DMS. Contributed with reagents/materials/analysis tools: AFB, FHFB. Wrote the paper: GSCJ, CMS, CCL. Critical revision of the manuscript: GMOJ, CMS, PGR. Critical revision of language: GMOJ, CMS, PGR.

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Introduction

The ban on the use of antibiotics as performance-enhancing additives in livestock farming has forced producers to adapt to the new world demands concerning the production of animal protein. As a result, the poultry industry has sought alternative additives, especially natural products which work analogously to chemical growth promoters (Koiyama *et al.*, 2014). Among these alternative additives are essential oils, secondary plant metabolites that act against external agents such as insects, fungi and pathogenic bacteria (Cho *et al.*, 2014; Giannenas *et al.*, 2014; Dantas *et al.*, 2015).

In this scenario, the antimicrobial properties of *Lippia gracilis* Schauer essential oil (LGSEO) have been investigated due to their inhibitory effect on pathogenic microorganisms such as *Escherichia coli, Salmonella* sp. and *Staphylococcus aureus* (Horošová *et al.*, 2006; Oliveira *et al.*, 2007; Wang *et al.*, 2016), as the essential oil contains high concentrations of terpenoids such as thymol and carvacrol. These compounds have pharmacological potential against gram-positive and gram-negative microorganisms by acting on the cell membrane, dispersing polypeptide chains that will be part of the cell membrane constitution (Lobo *et al.*, 2011; Costa Júnior *et al.*,2016) and thereby reducing microbial growth (Bona *et al.*, 2012).

Essential oils act on the intestinal microbiota, where they reduce pathogenic bacteria and consequently alter the intestinal morphology and influence nutrient absorption (Brenes & Roura, 2010; Behnamifar *et al.*, 2015). When used as additives in quail diets, essential oils may affect feed intake, since they increase digestive enzymes secretions and intestinal motility (El-Hack *et al.*, 2015), these associated factors may lead to change the intestinal epithelium paremnquima, improving bird's immunity (Rocha *et al.*, 2020). In addition, their antioxidant activity allows to mitigate thermal stress effects, due to the reduction in body and rectal temperatures of treated animals (Daader *et al.*, 2018).

Recent studies have shown that dietary inclusion of *Lippia javanica* leaf meal improves performance, carcass characteristics, intestinal health and immune development in quail and chickens reared in free-range systems (Mpofu *et al.*, 2016; Mnisi *et al.*, 2017; Matshogo *et al.*, 2018). However, there are few reports on the use of *Lippia gracilis* essential oil as a growth-promoting additive in quail diets and its potential as an inhibitor of pathogenic microorganism growth. It is important to emphasize that quails are more susceptible to enteropathogens than chickens, caused mainly by *Salmonella* sp. and *E. coli* bacteria (Dipineto *et al.*, 2014), showing the need for studies to assess the prevalence of these bacterial strains in these production systems.

In view of the above considerations, the present study proposes to examine the effect of LGSEO as a growth promoter on the performance and organ development of Japanese quail up to 35 days of age as well as its potential as an antimicrobial agent against *Salmonella* sp. and *E. coli* bacteria.

Material and methods

The experimental project was approved by the Ethics Committee on Animal Research of the Federal University of Sergipe (UFS) under the protocol no 09/2015.

Essential oil extraction

The plants used for the extraction of the LGSEO were cultivated at the Rural Campus of the UFS. Leaves of *Lippia gracillis* Schauer varieties LGRA 107 and 108 were used to obtain the oil. The oil was extracted by the hydrodistillation process using a Clevenger apparatus, following the methodology described by Silva *et al.* (2019). The oil yield was 3% and contains myrcene (2.49%), p-cymene (11.61%), 1.8-cineole (1.36%), ϒ-terpinene (11.17%), thymol methyl ether (5.07%), thymol (4.08%), carvacrol (45.17%) (Santos *et al.*, 2016).

Antibiogram test

The antibiogram test was performed in accordance with Pereira *et al.* (2017). *Salmonella* sp. and *E. coli* strains belonging to the collection at the Laboratory of Bacteriology at the Department of Microbiology, Federal University of Sergipe, Brazil, were used.

The inocula were seeded on the surface of Müller-Hinton agar; 2.0 mL of saline solution with the inocula (3×10^8 CFU inoculum per plate) were added and smears were performed using sterile swabs, covering the plate's surface with inoculum. Ten cultured petri dishes were used, five for each inoculum (*Salmonella* sp. or *E. coli*). Six wells were drilled in each plate (6 mm) using the "hole plate" technique; the control solution, based on sodium hypochlorite at 0.9% to confirm bacterial growth, was deposited in only one of the wells; and at the other five wells 20 µL of diluted LGSEO for 2.5% of dimethyl sulfoxide (Rocha *et al.*, 2020) were deposited. Finally, 25 repetitions were evaluated for each bacterial strain.

Lastly, the plates were incubated in a forced-air oven at a temperature of 37 ºC for 24 h for the first reading, which was repeated 48 h later to check for alterations in the inhibition halos. Readings were taken with a 150-mm digital caliper (Lee Tools).

Performance trial

A total of 504 female Japanese quail (*Coturnix coturnix japonica*) at an average initial weight of 6.80 ± 0.10 g was used in the study. The birds were evaluated in a completely randomized design with six treatments, namely, a diet with 500 mg/kg bacitracin methylene disalicylate (BMD) and diets with 0, 100, 200, 300 and 400 mg/kg of LGSEO. Seven replicates were performed, using 12 birds per experimental unit.

The animals were housed in suspended cages (50 cm wide × 50 cm long × 40 cm high), equipped with feeders and pressure-type drinkers until the 14th day of age. After this period, the pressure-type drinkers were replaced with nipple drinkers. The floor under the cages was adapted and covered with reused wood shavings bedding to increase the health challenge and simulate a commercial farming environment.

In the rearing period of 2 to 14 days, the recorded maximum and minimum temperatures were 36 and 28ºC, respectively, and air relative humidity was 37%. From 14 to 35 days, the maximum and minimum temperatures were 34 and 24ºC, respectively, and air relative humidity was 46%.

The diets were formulated to meet the nutritional requirements of Japanese quail as recommended by the Brazilian Tables for Poultry and Swine (2011) in the growth phase (Table 1). LGSEO and the growth promoter (BMD) were added to the diets replacing the inert material kaolin. The LGSEO was diluted in soybean oil prior to being mixed with the diets. Diets were prepared and stored in white buckets with lids for protection against sunlight. Water and feed were available *ad libitum*.

The chemical composition of the diets was determined at the Animal Nutrition Laboratory of the Animal Science Department (DZO/UFS) Sergipe, Brazil. Crude protein and minerals were analyzed following AOAC (2012) recommendations.

Performance and organ development

Birds and experimental diets were weighed weekly to determine final body weight (g), weight gain (WG, g/bird), feed intake (FI, g/bird) and feed efficiency (FE, g/g).

Table 1. Composition of the experimental diets.

Ingredients (kg)[1]	**LGSEO**[3] **(mg/kg)**					**BMD**[4]
	0	**100**	**200**	**300**	**400**	**500**
Corn	57.12	57.12	57.12	57.12	57.12	57.12
Soybean meal[a]	38.35	38.35	38.35	38.35	38.35	38.35
Soybean oil	1.07	1.07	1.07	1.07	1.07	1.07
Phosphate[b]	1.38	1.38	1.38	1.38	1.38	1.38
Limestone	1.19	1.19	1.19	1.19	1.19	1.19
Common salt	0.39	0.39	0.39	0.39	0.39	0.39
L-lysine HCl	0.03	0.03	0.03	0.03	0.03	0.03
DL-methionine	0.16	0.16	0.16	0.16	0.16	0.16
Premix[c]	0.20	0.20	0.20	0.20	0.20	0.20
L-threonine	0.03	0.03	0.03	0.03	0.03	0.03
Inertd	0.05	0.04	0.03	0.02	0.01	-
LGSEO	-	0.01	0.02	0.03	0.04	-
BMD	-	-	-	-	-	0.05
Total	100	100	100	100	100	100
Analyzed composition (%)						
Crude protein	21.75	21.75	21.75	21.75	21.75	21.75
Calcium	0.756	0.756	0.756	0.756	0.756	0.756
Available P	0.365	0.365	0.365	0.365	0.365	0.365
Sodium	0.176	0.176	0.176	0.176	0.176	0.176
Potassium	0.754	0.754	0.754	0.754	0.754	0.754
Calculated composition (%)[2]						
ME, kcal/kg	2900	2900	2900	2900	2900	2900
SID Met	0.457	0.457	0.457	0.457	0.457	0.457
SID Met+Cys	0.760	0.760	0.760	0.760	0.760	0.760
SID Lys	1.120	1.120	1.120	1.120	1.120	1.120
SID Thr	0.790	0.790	0.790	0.790	0.790	0.790
SID Trypt	0.251	0.251	0.251	0.251	0.251	0.251

[1] [a]Soybean meal with 45% of crude protein. [b]Dicalcium phosphate. [c] Minimum composition per kilogram: folic acid 150 mg, pantothenic acid 6000 mg, biotin 40 mg, copper 1400 mg, iron 6000 mg, iodine 915 mg. manganese 17 g, niacin 13 g, selenium 300 mg, vitamin A 5000000 IU, vitamin B12 6500 mg, vitamin B2 2000 mg, vitamin B6 250 mg, vitamin D3 1600000 IU, vitamin E 4000 IU, vitamin K3 1000 mg, zinc 38 g. dKaolin. [2] ME: metabolizable energy. SID: standardized ileal digestibility (Brazilian Tables for Poultry and Swine, 2011). [3] LGSEO: *Lippia gracilis* Schauer essential oil. [4] BMD: Bacitracin methylene disalicylate.

At 35 days of age, two birds whose weight was closest to the average of the experimental unit were slaughtered by cervical displacement, to evaluate organ development. The proventriculus, gizzard, heart, liver, pancreas, intestines and abdominal fat were weighed on a digital scale (0.01-g precision). The length of the small intestine and ceca were measured using a tape measure. The relative weight of the organs was calculated in relation to the weight of the bird (Gul *et al.*, 2018).

$$Relative\ organ\ weight = \frac{Organ\ weight}{Bird\ weight} * 100$$

Quantitative microbiology

For the microbiological analysis of *Salmonella* sp. and *E. coli* counts, another two birds per experimental unit were slaughtered by cervical displacement. The small intestine's content was harvested, and one pool was formed per experimental unit. The collected material was weighed and packed in Falcon tubes containing buffered saline solution (5.61 g NaCl, 1 g KH_2PO_4, 2 g Na_2PO_4 and 0.11 g KCl in 1000 mL distilled water) and 10% glycerol, which was used as a cryoprotectant agent, and frozen at –4 ºC. Subsequently, the samples were sent to the Laboratory of Bacteriobiology at UFS for analyses (Rocha *et al.*, 2020).

Serial dilutions (10^{-1}, 10^{-3}, 10^{-5} and 10^{-7}) were prepared with buffered saline solution. One 1-mL aliquot of each dilution was used for seeding in a Petri dish containing Rappaport Vassiliadis medium for *Salmonella* sp. growth and EC broth for *E. coli* growth. After the inoculum was spread with Drigalski's spatula, the dishes were incubated in an oven at 37 ºC for 24 h and then a bacterial count was performed (CFU/g of feces) (Pereira *et al.* 2017).

The count was determined by the following formula (Rocha *et al.*, 2020): *Number of colony forming units* × *Mean serial dilution value.*

Statistical analysis

The results of the antibiogram test and quantitative microbiology were analyzed descriptively. Performance (WG, FI and FE) and organ-development variables were subjected to analysis of variance by the polynomial regression model procedure of SAS statistical software version 9.0 (2002), at the 5% significance level. Dunnett's test was applied to compare the treatment containing BMD to the other treatments.

Results

Antibiogram test

After seeding, standard growth was observed for the gram-negative *E. coli* and *Salmonella* sp. bacteria in Müller-Hinton medium (Table 2). In the treatments with LGSEO addition, inhibition halos of 27.57 mm and 20.55 mm were observed for *E. coli* and *Salmonella* sp., respectively.

Performance and organ development

During the starter phase (2-21), increasing LGSEO levels resulted in a linear increase in FI (p<0.01), which can be represented by the following equation: FI = 168.204667 – 0.038151 LGSEO (R^2=71.28%). The WG, however, decreased, as shown by the equation: WG = 69.842462 – 0.014001 LGSEO (R^2=93.94%). FE was not influenced (p=0.87) by the LGSEO levels (Table 3).

Table 2. *In vitro* inhibitory potential of *Lippia gracilis* Schauer essential oil (LGSEO) against *Escherichia coli* and *Salmonella* sp. bacteria.

Treatment	*E. coli*	*Salmonella* sp.	Control
Control	30.00 mm	21.00 mm	6.00 mm
LGSEO 100 mg/kg	28.35 mm	18.10 mm	6.00 mm
LGSEO 200 mg/kg	25.10 mm	20.80 mm	6.00 mm
LGSEO 300 mg/kg	24.65 mm	21.10 mm	6.00mm
LGSEO 400 mg/kg	32.20 mm	22.20 mm	6.00 mm
BMD 500 mg/kg	24.40 mm	18.00 mm	6.00 mm
Mean	27.45 mm	20.20 mm	6.00 mm

Inhibition halo (in mm) against *Escherichia coli* and *Salmonella* sp. bacteria from a solution of DSMO + LGSEO by the "hole plate" technique after 24-48 h of exposure at a temperature of 37 ºC. LGSEO: solution with LGSEO + 2.5% dimethyl sulfoxide. Control: solution with 0.9% sodium hypochlorite.

Table 3. Performance of Japanese quail as a function of additive use from 2 to 21 and from 2 to 35 days of age.

Variable[1]	LGSEO[2] level					BMD[3]	CV%[4]	*p* value
	0	100	200	300	400	500		
				2-21 days				
IW (g)	6.66	6.95	6.71	6.86	6.77	6.82	6.50	0.81
FI (g/bird)	170.0^{a}	166.2^{a}	155.8^{b}	153.5^{b}	157.3^{b}	175.2^{a}	4.90	<0.01
WG (g/bird)	70.5^{a}	68.0^{b}	66.44^{b}	65.6^{b}	64.7^{b}	72.6^{a}	3.62	<0.01
FE (g/g)	0.412	0.425	0.424	0.422	0.414	0.409	6.58	0.87
				2-35 days				
IW (g)	6.66	6.95	6.71	6.86	6.77	6.82	6.50	0.82
FI (g/bird)	400.4^{b}	385.9^{b}	376.0^{b}	373.1^{b}	365.4^{b}	440.4^{a}	5.67	<0.01
WG (g/bird)	114.52	114.86	113.58	113.76	114.87	114.45	2.21	0.68
FE (g/g)	0.285^{a}	0.291^{a}	0.3^{a}	0.315^{a}	0.318^{a}	0.261^{b}	5.28	<0.01

[1] IW: initial weight; FI: feed intake; WG: weight gain; FE: feed efficiency. [2] LGSEO: *Lippia gracilis* Schauer essential oil. [3] BMD: bacitracin methylene disalicylate. [4] CV: coefficient of variation. Different letters in the same row represent differences between the other treatments and control according to Dunnett's test.

The treatments containing 200, 300 and 400 mg/kg of LGSEO led to a lower FI ($p<0.01$) than the treatment containing BMD. The use of LGSEO also resulted in lower ($p<0.01$) WG when compared to the BMD treatment. No treatment effect was observed on FE ($p=0.87$).

In the entire experimental period (2-35 days), LGSEO inclusion was found to linearly reduce FI ($p=0.04$), which is represented by the following equation: FI = 396.698400 – 0.082770 LGSEO ($R^2=94.28\%$). The FE, in turn, improved linearly ($p<0.01$), as shown by the following equation: FE = 0.286087 + 0.000075 LGSEO ($R^2=94.72\%$) (Table 3). However, the increasing levels of essential oil did not affect WG ($p=0.68$).

On the other hand, the treatment containing BMD provided the highest FI ($p<0.01$), but lower FE ($p<0.01$) when compared to the treatments containing LGSEO. No differences were observed ($p=0.68$) for WG between the conventional growth promoter and the others.

The relative weight of the proventriculus responded quadratically ($p<0.01$) to the increasing LGSEO levels, as shown by the equation: Relative weight of proventriculus = 0.484629 + 0.001179LGSEO – 0.000003LGSEO^2 ($R^2=84.82\%$), with a maximum estimated weight obtained at the LGSEO level of 196.5 mg/kg (Table 4). The pancreas weight was also affected ($p<0.01$), as represented by the equation: Relative weight of pancreas = 0.199367 + 0.001004LGSEO – 0.000002LGSEO^2 ($R^2=97.35\%$). The maximum relative weight of this organ was estimated at the LGSEO level of 251 mg/kg. Intestine weight increased linearly ($p<0.01$) with the LGSEO inclusion levels, as shown by the equation: Relative weight of intestine = 4.031714 + 0.003156LGSEO ($R^2=82.27\%$). The same was found for the gizzard, as represented by the following equation: Relative weight of gizzard = 3.419714 + 0.001176LGSEO ($R^2=55.96\%$).

Nevertheless, the relative weights of abdominal fat ($p=0.41$), heart ($p=0.25$) and liver ($p=0.11$) as well as the lengths of the intestine ($p=0.11$) and ceca ($p=0.60$) were not affected by the LGSEO levels.

The BMD treatment provided a higher relative weight of the proventriculus ($p<0.01$) than the treatments including 200 and 300 mg/kg of LGSEO. The relative weight of the gizzard, in turn, was higher in the treatment with 300 mg/kg of the oil than with BMD. Compared to the BMD treatment, the LGSEO levels of 200, 300 and 400 mg/kg provided a higher relative weight of the intestine ($p<0.01$).

Quantitative microbiology

Birds fed the diets containing LGSEO at levels between 100 and 300 mg/kg presented a lower total count of *Salmonella* sp. bacteria (Table 5). The animals which received BMD showed a lower *Salmonell*a sp. count than those which were fed with control treatment. However, numerically, there was observed greater growth of *Salmonella* sp strains on control treatment and treatment with 400 mg/kg of LGSEO.

Only the LGSEO concentration of 400 mg/kg and the BMD treatment resulted in death of the *E. coli* strains.

Discussion

The LGSEO demonstrated that the bacteria tested *in vitro* are highly sensitive to this additive. According to the Clinical and Laboratory Standard Institute (Patel *et al.*, 2015) greater halation than 20 mm of inhibition, indicate that the bacteria is highly sensitive to the product tested,

Table 4. Relative organ weight and intestinal length and weight of quail as a function of additive use

Variable	LGSEO [1] level					BMD [2]	CV% [3]	*p* value
	0	100	200	300	400	500		
Proventriculus (%)	0.37^{a}	0.45^{a}	0.49^{b}	0.49^{b}	045^{a}	0.37^{a}	25.9	<0.01
Gizzard (%)	3.53^{a}	3.30^{a}	3.69^{a}	3.94^{b}	3.79^{a}	3.34^{a}	15.2	<0.01
Abdominal fat (%)	0.37	0.48	0.44	0.57	0.35	0.34	68.9	0.37
Heart (%)	1.07	1.22	1.11	1.13	1.07	0.88	16.9	0.61
Liver (%)	3.05	3.18	3.09	3.33	3.16	2.36	16.4	0.71
Pancreas (%)	0.19^{a}	0.29^{a}	0.32^{a}	0.33^{a}	0.33^{a}	0.25^{a}	30.3	<0.01
Intestine (%)	4.12^{a}	4.01^{a}	4.95^{b}	5.00^{b}	5.21^{b}	4.08^{a}	17.7	<0.01
Intestine (cm)	48.21	51.2	51.34	52.47	50.61	51.87	8.2	0.11
Cecum (cm)	14.41	15.67	15.14	14.79	14.92	14.55	14.1	0.60

[1] LGSEO: *Lippia gracilis* Schauer essential oil. [2] BMD: bacitracin methylene disalicylate. [3] CV: coefficient of variation. ^{a,b}Different letters in the same row represent differences between the other treatments and control according to Dunnett's test.

which corroborates the current study results. These may be associated to the LGSEO chemical composition that, in this study, showed about 3.28% of thymol and 38.8% of carvacrol (Santos *et al.*, 2016). According to Santos *et al.* (2014), rosemary essential oil can exhibit up to 77% of carvacrol and up to 10.3% of thymol in its composition. Thus, the sum of these percentages gives the oil great potential for inhibition, as it acts on the structure of bacteria, increasing membranes permeability causing loss of ions, deregulating the proton pump action and, consequently, promoting cell denaturation and protein coagulation, resulting in death of pathogenic bacteria (Brenes & Roura, 2010; Ramos *et al.*, 2017). For these reasons, *Lippia gracillis* Schauer essential oil (LGSEO) shows itself as potential product capable of inhibiting bacteria growth evaluated in the present study.

Other researchers evaluated the *in vitro* antimicrobial activity of undiluted LGSEO and also observed inhibitory potential against gram-negative *Salmonella* sp. and *E. coli* bacteria. Dantas *et al.* (2015) evaluated the addition of 5 µL of undiluted LGSEO and observed inhibition for a *Salmonella* sp. strain in tilapia filets with a halo of 30 mm, while Sarrazin *et al.* (2012) used 10 µL of undiluted LGSEO and observed an inhibition halo of 29 mm for an *E. coli* strain. These results imply that, when evaluated *in vitro*, LGSEO has great inhibition potential against the tested *E. coli* and *Salmonella* sp. strains.

FI linear reduction was observed during the initial production phase, up to 21 days of age, when LGSEO inclusion levels increased. This can be associated with the volatile constituents of the oil that, as reported by Basmacioğlu Malayoğlu *et al.* (2010), affect negatively the FI and, consequently, the WG. However, the results here obtained did not affect FE during the whole production period (2 to 35 days of age). Therefore, it can be inferred that there was a better use of diet ingredients by birds, fact also observed by Windisch *et al.* (2008), since as reported in LGSEO *in vitro* test inhibiting growth of *Salmonella* sp. strains. It is also verified that the commercial growth promoter (BMD) was not able to improve quails performance, deteriorating the FE of birds that consumed diets with BMD during the 35 days, since FI of birds was increased, with no effect on WG.

Therefore, LGSEO can influence quails FI without losses in WG, acting positively on intestinal microbiology (Zhai *et al.*, 2018), as suggested by Rocha *et al.* (2020) that supplied LGSEO for quails and verified inhibition of many *E. coli* strains with significant increase in the intestinal population of *Lactobacillus* spp.

The literature also shows that essential oils act on the digestive physiology, intestinal microbiota, antimicrobial activity and as performance enhancers (Franz *et al.*, 2010). Thus, it is possible that LGSEO led to an increase in enzyme and biliary salt production, which allowed the

Table 5. Mean values of total intestinal microflora count (*Salmonella* sp. and *Escherichia coli*) in quail as a function of LGSEO levels and BMD

Variable	LGSEO [1] level					BMD [2]
	0	100	200	300	400	500
Salmonella sp.	2.9×10^{6}	3.6×10	9.0×10^{3}	6.0×10^{4}	6.7×10^{6}	5.8×10^{4}
Escherichia coli	7.19×108	5.2×108	7.1 ×108	3.0 ×108	ND	ND

[1] LGSEO: *Lippia gracilis* Schauer essential oil. [2] BMD: bacitracin methylene disalicylate value/g of feces. ND: no bacterial growth.

birds to better express their genetic potential and better utilize the nutrients and energy (El-Hack *et al.*, 2015).

The results obtained were different from those found by Koiyama *et al.* (2014), who evaluated the production performance of broilers fed diets containing 150 mg/kg of a blend of essential oils (blend A: rosemary, clove, ginger and oregano; blend B: cinnamon, sage, white thyme and copaiba oil-resin; blend C: 50% of A + 50% of B) from 1 to 42 days of age. Those authors observed no effects on FI, WG or feed conversion, in comparison to a control diet containing virginiamycin (antibiotic). Instead, Kheiri *et al.* (2018) observed that Japanese quails fed diets containing 2 mg/kg of the thyme showed minor feed conversion than the group submitted to the commercial antibiotic.

Based on the present results for organs weights, the birds which consumed the diets containing LGSEO showed greater development of the organs involved in nutrient digestion and absorption. This might have favored their FE; therefore, we can assume that their digestive system was better prepared for the laying phase. The same was not true for the commercial growth promoter (BMD), considering that the relative weight of those organs was similar to those found with control treatment and the treatment including 100 mg/kg of LGSEO. According to Andrade *et al.* (2012), when antibiotics are added to the animal diet, the toxin-producing intestinal microbiota is reduced. As a consequence, the density of the intestinal mucosa decreases, causing alterations in intestine weight.

Similar results were found by Cho *et al.* (2014), after supplementing diets for replacement broilers with 250 mg/kg of a blend phytogenic feed additive containing essential oils, those researchers observed no differences in organ weight. The essential oil possibly also stimulates enzyme and biliary salt production and pancreatic and gastric juice secretion (El-Hack *et al.*, 2015).

The lower number of *Salmonella* sp. strains observed in the material collected from the small intestine of the birds subjected to the treatments with 100 to 300 mg/kg of LGSEO reinforced the efficiency of the essential oil in controlling those bacteria in quail. However, the population increase of these strains on treatment containing 400 mg/kg of LGSEO has not been clarified.

Low levels supply of LGSEO has not been able to combat *E. coli* strains, and the lack of effect may indicate bacterial resistance to low doses of LGSEO. Despite this, there was no growth of these bacteria in the quail's intestines fed with 400 mg/kg of LGSEO and in birds submitted to BMD treatments, which allows to infer mortality of the existing strains due these treatments. El-Hack *et al.* (2015) found a reduction in the total count of *E. coli* of the cecum and ileum of quail fed with increasing levels of a blend of essential oils.

In general, little is known about the effect of LGSEO on the physiology of birds. This is especially true for quail, which are more resistant to pathogens than broiler chickens (Guo *et al.*, 2018), warranting further investigation on this additive. In the current study, we found that LGSEO has inhibitory activity on the main pathogenic microorganisms observed in the digestive tract of birds, in addition to presenting results that suggest improved digestion and absorption of the dietary nutrients, since it provided an increase in the weight of the organs responsible for those actions in the gastrointestinal tract, which might explain the improved FE.

Thus, LGSEO is a good alternative to be used in the diet of growing quail as a substitute for commercial antimicrobials. It is also emphasized that additional studies should be conducted also employing nutrigenomics, to evaluate the interaction between the essential oil and production and secretion of digestive enzymes, besides the gene expression of the membrane transporters related to nutrient absorption. Likewise, further research is warranted to determine whether the positive effects observed during this growth phase also affect egg production.

In conclusion, based on internal organ development, it is recommended to use 251 mg/kg of LGSEO in the diet of Japanese quails from 2 to 35 days old. LGSEO can be characterized with antimicrobial capacity against *Salmonella* sp. In situations of greater health challenge, up to 400 mg/kg of LGSEO can be included in the diet to combat *Escherichia coli.*

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