



## AGRARIAN SCIENCES

# Microencapsulated carvacrol and cinnamaldehyde replace growth-promoting antibiotics: Effect on performance and meat quality in broiler chickens

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**Abstract:** The aim was to evaluate the use of mixture of microencapsulated carvacrol and cinnamaldehyde as a replacement for growth-promoting antibiotics in broiler diets on performance, intestinal quality, organ development, carcass yields and cuts, and meat quality. In the trial were used 600 male chicks, allocated in a completely randomized design, with five treatments and eight replicates of 15 birds, reared up to 41 days of age. The treatments were: Negative Control (NC), Positive Control (PC) 30 mg/kg of virginiamycin, NC+100 mg/kg of essential oils, NC+200 mg/kg of essential oils and NC+400 mg/kg of essential oils. Essential oils were composed by a micro-encapsulated blend, with of 60% cinnamaldehyde, 30% carvacrol and 10% carrier. Birds received essential oils achieved performance equivalent to those birds received PC diets, having better development than NC broilers. No differences were found on relative organ weight, intestinal mucosa and meat quality parameters, however, higher villus:cript ratio was found in PC, NC+200 and NC+400 groups. Meat crude protein and yellowness were influenced by inclusion of carvacrol and cinnamaldehyde. It was concluded microencapsulated carvacrol and cinnamaldehyde can replace growth-promoting antibiotic in broiler diets, ensuring performance, intestinal integrity and broiler meat quality.

**Key words:** cinnamon, essential oils, oregano, bacterial resistance, villus.

## INTRODUCTION

The broiler meat production has grown substantially in recent decades, especially due to the intensive production systems, improving its importance for the global animal protein production and economy. The Food and Agriculture Organization of the United Nations (FAO 2017) estimates that, by 2022, chicken meat will be the most consumed animal protein worldwide. The increase in the consumption of chicken meat is directly associated with

consumer preference for healthier eating habits and with increased health and life expectancy (FAO 2017). Petracci et al. (2015) stated that the increase in the consumption of chicken meat is associated with its low cost as well as its nutritional profile.

To find the demand for chicken meat production, improvements in nutrition and sanitation are necessary, and the continuous use of antibiotics as growth promoters has been a tool of constant use, to maintain expected zootechnical indexes. Nevertheless, antibiotic

growth-promoters generate antibiotic resistant organisms (Muro et al. 2015), a substantial problem for the human population. Therefore, these growth promoters have been prohibited in countries such as Sweden, Denmark, Norway and the European Union (Diarra & Malouin 2014). In response to changes in the market for animal products, dietary strategies for broiler diets include replacement of traditional antibiotic-based growth promoters (Reis et al. 2018, Galli et al. 2020). Among these strategies, various essential oils, including carvacrol and cinnamaldehyde extracted from oregano and cinnamon, respectively, have become prominent due to these substances possess antimicrobial activity and may, therefore, substitute antibiotic-based growth promoters (Petrolli et al. 2012, Bastos-Leite et al. 2016, Reis et al. 2018, Zhai et al. 2018, Galli et al. 2020), in addition to their antioxidant actions, anti-inflammatory and analgesic properties (Suntres et al. 2015). Zeng et al. (2015) reported carvacrol acts on intestinal flora balance and stimulates intestinal mucosa to increase mitosis in villus crypts, increasing the number of cells and consequently increasing villus size, resulting in improved nutrient absorption.

On account of these anti-inflammatory and antioxidant properties, essential oils have been studied with the aim of improving chicken meat quality. Kuttappan et al. (2012) described consumers rejected meat with poor and visual-oxidated aspect, demonstrating that people prefer meat of particular quality and appearance at the time of purchase. Due to this behavior, alternatives to access better broiler meat quality are little studied and needs to be tested. Oregano essential oil in the diets of broiler chickens reduced lipid peroxidation in broiler chicks (Arieza Nieto et al. 2018). Luna et al. (2010) compared adequate BHT substitution by phytochemicals compounds (from oregano), on

thiobarbituric acid reactions (TBARS) evaluation in breasts of fresh-cut broilers. Thus, carvacrol and cinnamaldehyde may have beneficial effects in improving the quality of meat, preventing or delaying its oxidation. However, studies testing this potential effect are scarce, and its evaluation is strongly necessary.

Currently, molecules microencapsulation process has been subject of studies in poultry nutrition, since it allows the molecules to reach with better proportionality all areas of the intestine, mainly jejunum and ileum, which are sites of impacting microbial proliferation, and the microencapsulation allows phytochemical molecules to reach these regions more efficiently, generating a better intestinal health-enhancing effect, with better efficiency when compared to phytochemical molecules in free form.

Despite the fact that there have been studies on the replacement of growth promoters by essential oils, it remains unknown which essential oils and what levels of inclusion give the best results in terms of broiler meat quality. Therefore, in the present study, we aimed to evaluate the inclusion of carvacrol and cinnamaldehyde on zootechnical performance, gut histology and broiler meat quality.

## MATERIALS AND METHODS

### Animals and experimental design

This study was conducted in the Núcleo de Ciência e Pesquisa Aplicada à Monogástricos, from Universidade do Oeste de Santa Catarina - UNOESC Xanxerê, with a protocol submitted and approved by the animal ethics committee Comissão de Ética do Uso de Animais (CEUA/UNOESC) under protocol number 50/2017.

A total of 600 ROSS 308 male chicks were used, being vaccinated *in ovo* in hatchery, against Marek and Gumboro disease, and via spray on the first day of life, against infectious

bronchitis. Birds were weighed and distributed on the first day of life, in a completely randomized experimental design, consisting of five treatments and eight replicates, with 15 animals in each replicate: Negative Control (NC), Positive Control (PC) (virginiamycin 15 mg/kg), NC+100 mg/kg of essential oils, NC+200 mg/kg of essential oils and NC+400 mg/kg of essential oils. The additive evaluated was a commercial formulated product (Enterosan, Konkreta Nutrition Technology, Navegantes, Brazil), being a blend of micro-encapsulated essential oils consisted of 60% cinnamaldehyde from cinnamon, 30% carvacrol from oregano and 10% of limestone as vehicle. The microencapsulation process was performed by atomization process, as described by Pereira et al. (2018).

The animals were housed in boxes of 2-m<sup>2</sup> in a bed of wood and fed ad libitum according to Table I (Rostagno et al. 2017) in tubular feeders and water supply via nipple, pens were equipped with infrared heaters to maintain thermal comfort, connected to a thermostat, with all management recommendations being according to the norms and indications of commercial farms and of the strain manuals. To simulate a sanitary challenge, 25 g/liter of aviary reused bed broilers was supplied via drinking water on the third and fourth day of age, being the only source of drinking water provided in the period.

### **Performance, carcass and organ evaluation**

Evaluation of live weight, weight gain, feed intake and feed conversion were obtained by weighing all birds and residual feed at 7, 28 and 41 days of the experiment. The productive efficiency index (PEI) was calculated according to the following formula: ((live weight x viability) / (feed conversion x bird age)) \* 10. At 41 days of age, after 12 hours of fasting, two birds per

experimental unit were sacrificed by cervical dislocation and were manually bled, according to animal welfare norms and euthanasia norms according to the Conselho Nacional de Experimentação Animal (CONCEA) euthanasia practice guidelines (BRASIL/MCTI 2013). After slaughter, the birds were scalded, plucked, eviscerated, washed and then the carcasses were weighed to obtain the weight of the hot carcass without having undergone the cooling process in a refrigerator to avoid changes in meat quality. Carcass weighing, organs (liver, heart, ventricle, proventriculus and small intestine), and commercial cuts (breast with bone, leg, thigh, back and wings) were performed, to determine carcass yield and cuts, being calculated as the ratio of their respective weights and body weight, expressed as percentages. For the evaluation, two birds per replication, with average replicate weigh, being weighed individually 12 hours before slaughter and were weighed again after slaughter to determine carcass, cuts and organs yield.

### **Gut morphometry evaluation**

For intestinal villi and crypts evaluation, small intestine fragments were collected for intestinal histological analysis (Labiocel 2002). The collected intestinal samples were embedded in paraffin and sectioned between 4 and 6 µm using a microtome and fixed on histological slides. The slides were stained using a hematoxylin technique for further analysis using Imagepro Plus 1.3.2 (1994) (40x magnification) under an optical microscope. For each slide, 30 villi and 30 crypts were selected and measured to obtain the mean value of each segment. For the evaluation of the villus/crypt ratio, the value of the height of intestinal villi was divided by the depth value of the adjacent crypt.

**Table I. Ingredients and nutritional composition of the experimental diets.**

<b>Ingredient (kg/ton)</b>	<b>Initial (1-21 days)</b>	<b>Final (22-41 days)</b>
Corn	544	578.66
Soybean meal (46%)	361.65	309
Soybean oil	27.79	44.89
Dicalcium phosphate	18.3	18.64
Limestone	8.25	8.41
Salt	3.25	3.32
DL-Methionine (99%)	2.6	3.11
L-Lysine HCl	2.25	1.94
Choline chloride (60%)	1.0	1.0
Supplemental vitamins <sup>1</sup>	15	15.0
Supplemental minerals <sup>2</sup>	15	15.0
Calculated values		
Metabolic energy (kcal/kg)	2950	3100
Crude protein (g/kg)	215.00	194.00
Lysine dig. (g/kg)	12.00	10.50
Methionine dig. (g/kg)	5.44	5.05
Met. + Cys. dig. (g/kg)	8.39	7.75
Threonine dig. (g/kg)	7.55	6.84
Tryptophan dig. (g/kg)	2.46	2.13
Arginine dig. (g/kg)	14.14	12.27
Valine dig. (g/kg)	9.25	8.20
Calcium (g/kg)	9.02	8.24
Available phosphate (g/kg)	4.51	4.10
Sodium, g/kg	1.70	2.05
Potassium, g/kg	8.49	7.46
Chloride, g/kg	3.77	3.56

<sup>1</sup>Supplemental vitamins containing, per kg of product: Vit. A - 10.000.000 IU; Vit. D3 - 2.000.000 IU; Vit. E - 30.000 IU; Vit. B1 - 2.0 g; Vit. B2 - 6.0 g; Vit. B6 - 4.0 g; Vit. B12 - 0.015 g; Pantothenic acid - 12.0 g; Biotin - 0.1 g; Vit. K3 - 3.0 g; Folic acid - 1.0 g; Nicotinamide acid - 50.0 g; Selenium - 250.0 mg; and Excipient q.s.p - 1000 g;

<sup>2</sup>Supplemental mineral content per kg of product: Iron - 100.0 g; Cobalt - 2.0 g; Copper - 20.0 g; Manganese - 160.0 g; Zinc - 100.0 g; Iodine - 2.0 g; and Excipient q.s.p - 1000 g;

### Meat quality

After slaughter (approximately 30 minutes), pH evaluations were performed to obtain the initial pH and after rigor mortis to obtain the final pH, in the breast muscle at three points through direct insertion of an electrode (Hanna model HI99163). Meat quality parameters were also determined, including color (lightness  $L^*$ , intensity of red ( $a^*$ ) and intensity of yellow ( $b^*$ )), water retention capacity (WRC), cooking losses (CL), shear force (SF), lipid oxidation, moisture content, mineral matter (MM), crude protein (CP) and total lipids. After determination of pH, the breasts were dissected (removed from the bone) to determine the coloration of the breast muscle using a Minolta Chroma Meter CR-300 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan), previously calibrated, with readings in triplicate. The results were expressed in three dimensions,  $L^*$  (lightness),  $a^*$  (intensity of red), and  $b^*$  (intensity of yellow).

Water retention capacity (WRC) was determined as described by Hamm (1960), using approximately 2.0 grams of boned breast. Cooking losses (CL) were determined as recommended by Honikel (1998). The evaluation of the shear force (SF) of the meat was carried out using a texturometer (TA-Xt.plus), which samples of five replicates being obtained from each treatment and cut into cubes of 1.5 x 1.5 cm each and placed with the fibers perpendicular to a Warner-Bratzler Shear Force blade, to evaluate the shear force of the sample, operating at 30 cm/min and measuring the maximum shear force, expressed as kgf.

For the evaluation of lipid oxidation, 5 grams of chicken breast meat samples were used; 25 ml of 7.5% trichloroacetic acid were added, homogenizations were carried out for 2 minutes, 5 ml of the filtrate were pipetted and transferred to test tubes with 5 ml of 0.02 M thiobarbituric acid solution. The tubes were capped and

brought to boiling temperature in a water bath for 40 minutes. After this period, they were cooled in running water and read at absorbance 538 nm. To determine the centesimal composition of the meat, after obtaining the weight of the wet and frozen samples, they were lyophilized (LS 3000, Terroni) for 72 hours at  $-40^{\circ}\text{C}$  under vacuum. Subsequently, the samples were again weighed to obtain dry weight and then samples were ground. The residual moisture content was removed by oven drying at  $105^{\circ}\text{C}$  for at least 8 hours and ash was obtained by incineration in a muffle at  $600^{\circ}\text{C}$  for 4 hours (Silva & Queiroz 2009). To determine quantities of meat protein, we used the methodology of the Association of Official Analysis Chemists (AOAC 2016) and values were expressed as natural matter. To quantify total lipid content of the meat, we used the extraction method of Bligh-Dyer (1959).

### Statistical analysis

All variables were subjected to the normality test (Shapiro-Wilk), the variables that did not have a normal distribution were transformed into logarithms. All data were subjected to analysis of variance (ANOVA) and in cases of significant difference, means were subjected to the Tukey test at  $p < 0.05$  of significance, using the statistical platform R.

## RESULTS

### Zootechnical performance

Birds fed PC diet presented higher LW and WG ( $p < 0.05$ ) when compared to birds received 100, 200 and 400 mg/kg of essential oils and birds fed NC diet in 1 - 7-day old phase (Table II). Chickens fed diet containing essential oils, in all doses evaluated, showed same performance when compared to birds consuming the NC diet. No differences were observed ( $p > 0.05$ ) on FI and FC in 1-7 day-period. At 1 to 28 and 1 to 41 days of

age, birds showed different performance ( $p < 0.05$ ) for LW, WG, FI, FC and PEI parameters, which birds fed NC diet showed lower performance when compared to birds fed PC diet and birds consuming diets containing essential oils. Were also observed birds fed essential oils obtained same performance as PC birds.

### Organ, carcass and commercial cuts yield

Data analyzed for small intestine (SI), ventricle, proventriculus, liver and heart relative weight (Table III) and for carcass yield (CR) and commercial cuts of breast, thigh, overcoat, wing and back (Table IV), showed no alterations

( $p > 0.05$ ) on these parameters. No alterations were found ( $p > 0.05$ ) between broilers fed NC, PC or NC supplemented essential oils diets.

### Gut morphometry

Results showed in Table V demonstrated no difference ( $p > 0.05$ ) of villus height or crypt depth among broilers fed all different diets. The villus/crypt ratio differed ( $p < 0.05$ ), which birds received NC diet presented smaller villus/crypt ratios than those who received PC diets. The villus/crypt ratio of the birds receiving essential oils did not differ from PC and NC birds.

**Table II. Effects of inclusion of carvacrol and cinnamaldehyde in diets of broiler chickens on live weight (LW), weight gain (WG), feed intake (FI), feed gain ratio (FGR) and productive efficiency index (IPE).**

		Treatments					P-value	CV (%)
		NC	PC	NC+100	NC+200	NC+400		
1 to 7 days	LW (g)	136 b	147 a	140 ab	137 b	138 b	0.004	3.88
	WG (g)	89 b	100 a	93 ab	90 b	91 b	0.008	6.62
	FI (g)	135	139	134	133	135	0.669	7.26
	FGR (g)	1.506	1.391	1.440	1.475	1.489	0.535	9.61
1 to 28 days	LW (g)	1110 b	1229 a	1196 a	1176 a	1201 a	0.000	3.13
	WG (g)	1062 b	1182 a	1148 a	1129 a	1153 a	0.000	3.62
	FI (g)	1595 b	1658 a	1645 ab	1632 ab	1675 a	0.000	2.13
	FGR (g)	1504 a	1402 b	1432 b	1446 ab	1453 ab	0.003	3.35
1 to 41 days	LW (g)	2089 b	2309 a	2265 a	2245 a	2279 a	0.000	3.59
	WG (g)	2040 b	2262 a	2217 a	2198 a	2231 a	0.000	3.60
	FI (g)	3340 b	3513 a	3513 a	3502 a	3513 a	0.001	2.61
	FGR (g)	1.636 a	1.553 b	1.584 b	1.593 b	1.575 b	0.000	1.57
	IPE	308 b	362 a	343 a	341 a	350 a	0.000	6.25

\* Different letters on the same line differ according to the Tukey test at 0.05 significance.

NC – Negative Control; PC – Positive Control; NC+100 -- Negative Control with inclusion of 100 mg/kg of essential oils; NC+200 - Negative Control with inclusion of 200 mg/kg of essential oils; NC+400 - Negative Control with inclusion of 400 mg/kg of essential oils.

**Table III.** Effect of inclusion of carvacrol and cinnamaldehyde in broiler diets on the relative weight (%) of the small intestine (SI), ventricle, proventriculus, liver and heart compared to live weight expressed as a percentage.

	Treatments					P-value	CV (%)
	NC	PC	NC+100	NC+200	NC+400		
SI	2.62	2.41	2.63	2.55	2.50	0.113	10.27
Ventricle	1.80	1.78	1.83	1.59	1.91	0.064	13.28
Proventriculus	0.48	0.43	0.44	0.43	0.43	0.084	14.49
Liver	2.06	2.05	2.11	2.15	2.05	0.534	9.78
Heart	0.50	0.46	0.48	0.49	0.47	0.337	14.20

\*Different letters on the same line differ according to the Tukey test at 0.05 significance. NC – Negative Control; PC – Positive Control; NC+100 -- Negative Control with inclusion of 100 mg/kg of essential oils; NC+200 - Negative Control with inclusion of 200 mg/kg of essential oils; NC+400 - Negative Control with inclusion of 400 mg/kg of essential oils; CV - Coefficient of variation.

**Table IV.** Effects of inclusion of carvacrol and cinnamaldehyde on carcass yield (CY) and commercial cuts (breast, leg, thigh, wing and back) of broiler chickens expressed as percentages.

	Treatments					P-value	CV (%)
	NC	PC	NC+100	NC+200	NC+400		
CY	73.86	74.74	73.87	74.48	73.98	0.424	1.88
Breast	33.91	34.24	33.48	34.61	34.07	0.764	7.03
Leg	12.84	12.60	12.50	12.47	12.66	0.811	7.33
Thigh	14.60	14.58	14.74	14.57	14.75	0.989	9.00
Wing	11.08	10.95	11.42	10.68	11.09	0.300	8.65
Back	26.94	25.49	26.32	26.11	26.14	0.445	8.20

\*Different letters on the same line differ according to the Tukey test at 0.05 significance. NC – Negative Control; PC – Positive Control; NC+100 -- Negative Control with inclusion of 100 mg/kg of essential oils; NC+200 - Negative Control with inclusion of 200 mg/kg of essential oils; NC+400 - Negative Control with inclusion of 400 mg/kg of essential oils.

**Table V. Effect of the inclusion of carvacrol and cinnamaldehyde in broilers diets on villus height, crypt depth and villus/crypt ratios.**

	Treatments					P-value	CV (%)
	NC	PC	NC+100	NC+200	NC+400		
Villus height ( $\mu\text{m}$ )	389	442	372	424	433	0.2271	15.36
Crypt depth ( $\mu\text{m}$ )	73.23	61.76	60.80	62.20	65.07	0.0615	12.95
Villus/crypt ratio	5.35c	7.15a	6.06bc	6.84ab	6.70ab	0.0001	10.00

\*Different letters on the same line differ according to the Tukey test at 0.05 significance. NC – Negative Control; PC – Positive Control; NC+100 -- Negative Control with inclusion of 100 mg/kg of essential oils; NC+200 - Negative Control with inclusion of 200 mg/kg of essential oils; NC+400 - Negative Control with inclusion of 400 mg/kg of essential oils.

### Broiler meat quality

As demonstrated in Table VI, no alteration were found in broiler meat ( $p>0.05$ ) fed all diets on water cooking losses (WCL), Shear Force (SF) and lipid oxidation or reaction to thiobarbituric acid (TBARS). For water retention capacity (WRC) in chicken breasts, there was a reduction ( $p<0.05$ ) on meat of broilers fed CN+100 diet when compared to birds fed CN, CN+200 and CN+400 diets. The nutritional composition of broiler chicks (crude protein, lipids, moisture and mineral matter) under different treatments (Table VI) showed no alterations ( $p>0.05$ ) on fat, moisture and mineral matter among groups. However, crude protein meat levels showed differences ( $p<0.05$ ) which meat of broilers fed NC and NC+400 diets showed higher CP content than broilers fed PC diet. Data obtained for lightness ( $L^*$ ), intensity of red ( $a^*$ ) and intensity of yellow ( $b^*$ ) are presented in Table VI, having no difference ( $p>0.05$ ) for lightness and intensity of red parameters, however, the intensity of yellow was higher ( $p<0.05$ ) in PC and NC+100 groups than NC and NC+400 broiler groups. The initial and final pH evaluation were not affected ( $p>0.05$ ) by the treatments tested.

### DISCUSSION

Inclusion of essential oils improved performance of birds when we compared broilers consuming NC diet, and essential oils may substitute for growth-promoting antibiotics without loss of performance. As substitutes for antibiotic-based growth promoters, carvacrol and cinnamaldehyde have been analyzed in several studies (Petrolli et al. 2012, Alarcon 2017, Reis et al. 2018, Galli et al. 2020), yielding conflicting results. The live weight and weight gain in the first phase evaluated (1 to 7 days) presented in Table III, demonstrated 100 mg/kg essential oils inclusion improved the indices compared to the NC treatment and the levels of greater inclusion of essential oils. The performance of the birds that received 100 mg/kg of essential oils was equal to the performance of the birds that received the PC diet, an equivalence also found by Alarcon et al. (2017). The lower bird performance of that received greater inclusion of essential oils can be explained by the possible irritability of the intestinal mucosa of the birds, with reduction of the intestinal surface and consequent lower absorptive area, according to the hypothesis of Windisch et al. (2009).

In LW, WG, FI and FC evaluation, there was influence of the essential oil blend inclusion in the phases 1 to 35 and 1 to 41 days of age when compared to the birds that received the NC diet in the same periods. Birds supplemented with NC+100, NC+200 and NC+400 obtained an equivalent performance to the PC diet birds.

These results confirm the beneficial action of essential oils on improving performance, as substitutes for growth promoters, corroborating the results found of Petrolli et al. (2012) and Reis et al. (2018).

In the present study, essential oil blend presented satisfactory results in the substitution

**Table VI. Effect of inclusion of carvacrol and cinnamaldehyde in diets on broiler meat quality and nutritional content.**

	Treatments					P-value	CV (%)
	NC	PC	NC+100	NC+200	NC+400		
WCL (%)	13.54	12.10	13.00	12.66	11.31	0.651	19.99
WRC (%)	79.13 <sup>a</sup>	74.11 <sup>ab</sup>	71.39 <sup>b</sup>	78.09 <sup>a</sup>	78.78 <sup>a</sup>	0.000	4.12
SF kgf/cm <sup>2</sup>	3.535	3.088	2.434	3.158	3.382	0.128	21.70
TBARS TMP/kg	9.492	9.203	9.560	10.105	9.781	0.440	7.91
CP (%)	26.886 <sup>a</sup>	25.745 <sup>b</sup>	26.418 <sup>ab</sup>	25.918 <sup>ab</sup>	26.788 <sup>a</sup>	0.013	2.02
Fat (%)	2.464	1.657	2.210	1.670	2.678	0.136	32.97
Moisture (%)	63.338	63.422	61.206	60.844	60.336	0.173	3.93
MM (%)	4.618	4.142	4.902	4.408	4.670	0.716	19.33
L*	50.18	50.53	53.42	51.01	49.54	0.173	4.90
a*	2.320	2.897	2.632	2.857	2.824	0.453	18.17
b*	3.416 <sup>b</sup>	6.448 <sup>a</sup>	6.442 <sup>a</sup>	5.192 <sup>ab</sup>	3.700 <sup>b</sup>	0.004	27.91
Initial pH	6.14	6.12	6.12	6.18	6.07	0.893	3.11
Final pH	5.90	5.94	5.84	5.87	5.98	0.262	1.80

\*Different letters on the same line differ according to the Tukey test at 0.05 significance. NC – Negative Control; PC – Positive Control; NC+100 -- Negative Control with inclusion of 100 mg/kg of essential oils; NC+200 - Negative Control with inclusion of 200 mg/kg of essential oils; NC+400 - Negative Control with inclusion of 400 mg/kg of essential oils. CP – Crude protein. MM – Mineral matter.

of the growth promoters for the indices mentioned in Table III, without performance reduction compared to the birds receiving diets containing growth promoters during the 1- to 41-day phase. The explanation for this equivalence of zootechnical performance between the birds that received the essential oil blend and the birds that received growth promoter is explained by the equilibrium of the intestinal microbiota of the birds by essential oils, as found by Reis et al. (2018) and Galli et al. (2020), giving better intestinal mucosa development, stimulation of the enzymatic secretion and better nutrient digestion and absorption. Our results agree with those of Petrolli et al. (2012) who evaluated the same parameters at 1 to 41 days of age with the inclusion of essential oils and demonstrated the same efficiencies with the substitute growth promoters.

PEI is an index used by commercial meat producers to generate value for the integrated producers in agroindustry. This evaluation is a general summary of flock development, being an integration of body weight, viability, age of birds and feed conversion. The PEI is an important tool of analysis for the use of the essential oil blend as a substitute growth promoter. In the present study, the PEI demonstrated that essential oils could indeed serve as substitutes for antibiotic-based growth promoters; the results were similar to those obtained by the birds that received the PC diet, and in both diets the birds obtained better results than birds consuming NC diets. The results obtained in this study are in accordance with those found by Petrolli et al. (2012) for the evaluation of PEI, where the diets with essential oils showed similar indexes as those consuming positive control diets, both being superior to indexes generated by the negative control diet, reinforcing the hypothesis that essential oils may be used as substitute growth promoters.

Health challenges in non-ruminant production systems mainly involve the digestive tract, including villus height, crypt depth, and villus/crypt ratio. In the present study, villus height and crypt depth were not influenced by the various types of diets. Greater villus height correlates with greater absorptive area will in the intestinal mucosa, as described by Galli et al. (2020), with consequent better nutrient absorption and rates of zootechnical performance, including weight gain and feed conversion. We observed differences in villus/crypt ratios, where the birds that received the PC, NC+100, NC+200 and NC+400 diets had better LW and FC performance than birds consuming the NC diet. Infectious intestinal agents cause destruction at the level of the small intestine, decreasing the size of the villi. By contrast, in the crypts there is an increase in the production of enterocytes in order to increase the cells in the intestinal mucosa. In the present study, there were no enteric challenges of infectious agents, which explains our results.

Considering the influence of the blend of essential oils on the villi and crypt, it was expected that there would be some influence on the small intestine, ventricle, proventriculus, liver and heart organs. In the present study, we did not observe changes in organ and viscera weight of broilers supplemented with essential oils. This fact may be related to the inclusion levels of the essential oils and because the birds did not face major health challenges. Similar results to that found in this study were also found by Bastos-Leite et al. (2016).

Higher carcass yield and meat cuts are the objectives of genetic improvements and animal nutrition, both of which are important for the poultry sector in general. Differences in carcass yields and cuts may be influenced by temperature, nutrition, genetics and hygiene. In our trial, there was no difference for carcass,

breast, leg, thigh, wing and dorsum yields. The inclusion of essential oils in broiler diets may improve the deposition of muscle tissue by stimulating pancreatic enzymes, resulting in better digestion of amino acids; therefore, there were expected differences in carcass yields and cuts, which did not occur in the present study. The data found for carcass yields and commercial cuts are similar to those found by Rizzo et al. (2010) and Zamora et al. (2017).

Characteristics of meat composition, as well as visual quality, are important to obtain products that are more acceptable to the consumer market. Some quality analyses were carried out to identify the color of the meat, to help prevent inadequate management during transport and slaughter, as well as to identify myopathies that may cause consumers to reject the meat on the basis of appearance. The rates of water cooking losses (WCL), water retention capacity (WRC) and shear force (SF) are related to the meat content indexes. The values obtained from WCL and SF did not show any difference between the treatments, suggesting that the inclusion of carvacrol and cinnamaldehyde in the broiler diet does not affect meat tenderness. Variations in temperature, caloric stress and housing densities affect WCL and SF, on account of altered cellular integrity and increased membrane osmotic activity. In the present study, the birds did not show the variations mentioned above, possibly explaining the non-differentiation of the values obtained. Due to WRC evaluation, there was a difference between the diets, with the inclusion of 100 mg/kg of essential oils in the diet showed lower water retention capacity, suggesting lower yield and lower final quality of the product.

The evaluation of TBARS is an index of lipid peroxidation evaluated through the formation of products from the oxidation reaction, used to determine the degree of tissue damage;

increasing in these levels suggest greater damage to cell membranes (Bozkurt et al. 2016). The inclusion of essential oils did not influence TBARS values. The inclusion of essential oils may have an antioxidant action (Galli et al. 2020), which stimulates the production of the enzymes superoxide dismutase and glutathione S-transferase and catalase (Hashemipour et al. 2013), possibly influencing the oxidation rates of meat. The values found in the evaluation of the TBARS may be explained by correct management during the rearing of the broilers. These values are different from those found by Masouri et al. (2015) and Kanani et al. (2017), who found different TBARS values in broilers fed with essential oils.

The world's population is increasingly demanding meat with lower percentages of fat, because of heart disease rate increased heart disease rate. The inclusion of the blend of essential oils in the diet of broilers did not interfere with the parameters of fat, moisture and mineral matter in broiler meat at 41 days of age, suggesting that the supply of a source of essential oils in the diet of birds did not affect the deposition of fat in breast meat. Furthermore, we observed that crude protein levels in the meat of the birds that received 400 mg/kg of essential oils and the NC diet were higher than those of the PC treatment, suggesting greater protein synthesis.

The coloration of fresh chicken meat is considered a significant factor in the moment of consumer acquisition of meat (Kuttappan et al. 2012). These authors found, in a study with chicken breasts with three degrees of white stripping, consumers rejection above 50%. The color of the meat is an indicative of meat quality. It may range from pale red to shades of gray. Meat color is related to the type of muscle fibers, myoglobin and hemoglobin in the blood. Both iron-associated substances and those

reacting with oxygen may alter the coloration of the meat (Petracci et al. 2015). The inclusion of carvacrol and cinnamaldehyde in broiler diets did not influence the lightness parameters and the redness ( $a^*$ ) of the chicken meat, however, birds received PC, NC+100 and NC+200 mg/kg essential oils differed from the others, presenting higher yellowness  $b^*$ . This higher value observed breast meat may be explained by the greater body development of the birds that received the PC diet, lower WRC and lower CP, however, this compromises the aesthetic quality of the marketed product (Kuttappan et al. 2013), and may also be related to a myopathy called white striping.

The pH evaluation is used as parameter of meat quality, with pH 5.8 to 5.9 considered normal at six hours after the slaughter. In chicken meat is used to indicate meat quality, and six-hour post-mortem chicken meat should have pH between 5.8 to 5.9 (Petracci et al. 2015). In the present study (Table VI) the values obtained for pH were between 5.84 and 5.98 at four hours after slaughter, explaining the values slightly above the desired parameters. pH values after 24 hours of slaughter above 6.2 can be an indicative of DFD (Dark, firm, dry) meat due to the retention of large amounts of water, affecting shelf life. However, values below 5.8 at less than 4 hours after slaughter may indicate poor water retention in addition to the soft and pale appearance, suggesting meat called PSE (pale, soft, exudative) (Guerrero et al. 2013).

The inclusion of microencapsulated carvacrol and cinnamaldehyde in broiler diets replaces adequately antibiotic growth promoters, maintaining performance, carcass and commercial cuts yield. Additionally, ensures broiler intestinal morphometry and chicken meat quality parameters up to 41 days of age.

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Gilnei Elmar Bosetti, Edegar Aniecevski, Caroline Schmidt Facchi, Gabriel Rossatto, Alicia Dal Santo, Felipe Leite and Fernanda Danieli Antoniazzi Valentini assisted in conducting the experiment, preparing experimental diets, weighing, data collection and tabulation. Cintiamara Baggio, Heloísa Pagnussatt, Letieri Griebler and Marcel Manente Boiago assisted in the analysis of meat quality, antioxidants, with data tabulation and statistical analysis. Tiago Goulart Petrolli was the advisor of the work, leading the project in all its stages.

