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# Performance, carcass characteristics, and health parameters of growing and finishing pigs fed with diets supplemented with isoquinoline alkaloids and essential oils

# Desempenho zootécnico, características de carcaça e parâmetros de saúde de suínos em crescimento e terminação submetidos a dietas suplementadas com alcaloides isoquinolínicos e óleos essenciais

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## Highlights

Phytogenic additives meet today's demands for reduced antibiotic use.
Phytogenic additives can replace preventive programs with antibiotics.
Phytogenic additives reduce convictions for respiratory problems.

## Abstract

The goal of this study was to evaluate, in a commercial herd, the use of isoquinoline alkaloids and carvacrol versus a preventive antibiotic program, such as feed additives, on the performance, carcass traits, and health status of pigs in the growing and finishing phase. There were 576 PIC immunocastrated males and females, at 70 days of age and 28.429 ± 2.302 kg of initial weight used. The experimental design was a 4 × 2 factorial randomized block, with four preventive programs, two sexes, and six repetitions per treatment (the pen with 12 animals of the same sex was the replicate). The treatments were T1 (positive control program with antibiotic shocks at preventive level), T2 (negative control with the absence of antibiotics as a growth promoter or as preventive), T3 (isoquinoline alkaloids at 100 to 150 g $ton^{-1}$), T4 (isoquinoline alkaloids at 90 g $ton^{-1}$ + carvacrol essential oil at 1 kg $ton^{-1}$). T1 and T3 presented higher daily feed intakes, followed by T2, and T4 showed the worst feed consumption ($P < 0.05$). T1 showed higher daily weight gain compared to T4 ($P < 0.05$), without differences between T2 and T3. T2 showed better feed conversion than T1 and T3, but it was similar to T4. There were no effects of the treatments on the carcass traits. Intestinal crypt hyperplasia

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and crypt abscesses (lesions caused by *Lawsonia intracellularis*) were significantly higher for T2 and T3 compared to T1, which was similar to T4. T2 presented the highest carcass condemnation at slaughter (7%), differing (P < 0.05) from T1, T3, and T4 (1, 2, and 3%, respectively). Isoquinoline alkaloids are an alternative for antibiotic-free diets for pigs in the growing and finishing phase, preserving the performance and carcass indices and minimizing sanitary carcass condemnations at the slaughterhouse.
**Key words:** Antibiotics. *Lawsonia intracellularis.* Phytogenics. Plant extracts. Sanguinarine.

## Resumo

O objetivo deste trabalho foi avaliar, em um rebanho comercial, o uso de alcalóides isoquinolínicos e do carvacrol frente a um programa preventivo com antibióticos, como aditivos alimentares, sobre o desempenho zootécnico, características de carcaça e o status de saúde de suínos em fase de recria e terminação. Foram utilizados 576 suínos PIC, machos imunocastrados e fêmeas, com 70 dias de idade e 28.429 ± 2.302 kg de peso médio inicial. O delineamento experimental foi em blocos casualizados, modelo fatorial 4 x 2, com quatro programas preventivos e dois sexos e 6 repetições por tratamento (baia com 12 animais do mesmo sexo representou a unidade experimental). Os tratamentos foram: T1 - Controle Positivo (programa com choque antibiótico em nível preventivo), T2 - Controle Negativo (ausência de aditivos promotores de crescimento ou preventivos), T3 - Alcalóides isoquinolínicos (100 a 150 g $ton^{-1}$), T4 - Alcalóides isoquinolínicos (90 g $ton^{-1}$) + Óleo essencial - carvacrol (1 kg $ton^{-1}$). T1 e T3 apresentaram maior consumo diário de ração, seguidos de T2, sendo que T4 apresentou o pior consumo (P < 0,05). O ganho de peso diário foi maior para T1 em relação a T4 (P < 0,05), não sendo verificada diferença entre T1 versus T2 e T3. T2 apresentou melhor conversão alimentar que T1 e T3, não diferindo de T4. Não houve efeito dos tratamentos para as características da carcaça. A hiperplasia e o abscesso das criptas intestinais (lesões causadas pela *Lawsonia Intracellularis*) foram significativamente superiores para T2 e T3 comparado com T1, que foi semelhante a T4. T2 apresentou maiores condenações de carcaça ao abate (7%), diferindo (P < 0,05) de T1, T3 e T4, respectivamente, 1, 2 e 3%. Os alcaloides isoquinolínicos são uma alternativa para dietas isentas de antibióticos para suínos em fase de crescimento e terminação, preservando o desempenho e os índices de carcaça e minimizando as condenações sanitárias de carcaças no frigorífico.
**Palavras-chave:** Antibióticos. Extratos vegetais. Fitogênicos. *Lawsonia intracellularis*. Sanguinarina.

## Introduction

Restrictions on the use of antibiotics are ongoing around the world (Cardinal et al., 2020; Maron et al., 2013) and at the same intensity, a series of alternative additives, such as acidifiers (organic acids) (Denck et al., 2017), nucleotides (Jiao et al., 2019), and phytogenics (Li et al., 2020) have been filling this gap.

The class of phytogenics (represented by essential oils and plant extracts) hold a variety of active substances with numerous pharmacological activities, highlighting antibacterial and antioxidant actions, in addition to promoting the palatability of the diet and modulation of the microbiota and intestinal functions, resulting in the improvement in zootechnical performance (Fernandes et al., 2015).

*Macleaya cordata* plant extract (Li et al., 2020), a plant belonging to the *Papaveraceae* family, is a representative of this class of additives (Chen et al., 2018, 2019; Kosina et al., 2010). This compound has active substances called isoquinoline alkaloids (Croaker et al., 2016), the most prevalent principal components being sanguinarine (SG), chelerythrine (CHE), dihydrosanguinarine (DHSG), dihydrochelerythrine (DHCHE), protopine (PR), and allocryptopine (AL) (Kosina et al., 2010). Additionally, other active ingredients such as cryptopine and berberine are present in smaller amounts.

The use of *Macleaya cordata* extract in the diet of production animals has shown positive results in several species, such as birds (Karimi et al., 2014), fish (Imanpoor & Roohi, 2016), ruminants (Aguilar-Hernández et al., 2016), and pigs (Li et al., 2020). Studies with pigs have shown the safety of the active principles of this extract, providing results of improvement in systemic health and performance (Li et al., 2020). As a feed additive for weaned piglets, *Macleaya cordata* improved performance, nutrient digestibility, and intestinal morphology (Boroojeni et al., 2018; Chen et al., 2018, 2019).

Another virtue of *M. cordata* extract involves promoting feed consumption (Chen et al., 2018; Kantas et al., 2015). This benefit was verified in nursery piglets, being attributed to the modulation of the tryptophan-serotonin pathway, determined by sanguinarine, which binds to some neuroreceptors, including serotonin (5-HT2), the basis of this result (Schmeller et al., 1997). In this process, sanguinarine also inhibits the action of the enzyme aromatic amino acid decarboxylase (Držata et al., 1996), increasing the availability of aromatic amino acids, such as tryptophan. This inhibition promotes a better protein balance and, consequently, a higher percentage of lean meat in the carcass (Chen et al., 2018), which is related to the improvement of feed efficiency, two aspects of interest, especially in pigs in the growing-finishing phases.

Essential oils, another class of additives used in animal production, are present in different parts or structures of some aromatic plants, such as in orange and clove flowers, in eucalyptus and mint leaves, in the rhizome of ginger, and in the seeds of coriander as well as anise and fennel fruits (Dhifi et al., 2016). The main active compounds of essential oils are classified into two chemical groups. These are terpenoids (limonene, thymol, carvacrol, linalool), which are derived from an unsaturated hydrocarbon (isoprene), and phenylpropanoids (cinnamaldehyde, eugenol, anethole), derived mainly from phenylalanine, corresponding to compounds with a three-carbon chain linked to a benzene ring (Simitzis, 2017).

Essential oils additionally demonstrate antibacterial activity against the main respiratory bacterial pathogens of swine, such as *Streptococcus suis* and *Actinobacillus pleuropneumoniae* (Lebel et al., 2019). In addition, they have high antioxidant capacity, mainly attributed to phenolic compounds, which help to prevent diseases caused by cell damage caused by free radicals (Dhifi et al., 2016), and anti-inflammatory activity (Caldefie-Chézet et al., 2006), inhibiting histamine release or reducing the production of inflammation mediators. Particularly, carvacrol is a phenolic monoterpene with high antibacterial and antioxidant activity (Dhifi et al., 2016).

When provided in the diet of weaned piglets, essential oils improved weight gain, nutrient digestibility, and diarrhea index (Li et al., 2012). These results are associated with the effects of active ingredients on the promotion of morphology and modulation of the intestinal microbiota, with positive repercussions on the immune status, measured through fecal scores, immunological indices, and antioxidant activities (Zeng et al., 2015).

Supported by the trends to replace antibiotics as growth-promoting and preventive additives in animal production, and in compliance with legal and consumer demands, this work was carried out to evaluate, in commercial conditions, the effects of the use of *Macleaya cordata* extract (isoquinoline alkaloids) and carvacrol on the health, zootechnical performance, and carcass characteristics of swine in the growing and finishing phases.

## Material and Methods

The work was carried out in a commercial Finishing Unit (UT) with a clinical history of porcine proliferative enteropathy (PPE), located in the municipality of Veranópolis, state of Rio Grande do Sul, Brazil. This study was approved by the Ethics Committee on the Use of Research Animals of Akei Animal Research (protocol number 022/20) and international animal welfare guidelines.

A total of 576 PIC genetic pigs, immunocastrated males and females, aged approximately 70 days and with a mean initial weight of 28.429 ± 2.302 kg were used. The animals were housed in masonry pens with an area of 10 $m^2$, equipped with a partially slatted floor, front tilting feeder, pendular nipple drinker, partitions (sidewalls of the pens) in cast iron and thermal control carried out through the management of side curtains.

The experimental design was in randomized blocks in a 4 × 2 factorial scheme, with four preventive programs, two sexes (immunocastrated males and females), and six replications per treatment, with a pen with 12 animals of the same sex being the experimental unit. The animals were blocked according to their initial weight (light, medium, and heavy) and randomly distributed in the pens. Preventive programs are shown in Table 1.

The active principles of treatments 3 and 4 corresponded, respectively, to isoquinoline alkaloids (10 mg $kg^{-1}$ of Macleaya cordata extract) with at least 1 to 1.5% sanguinarine (Sangrovit®; PhytobioticsGmbH, Eltville, Germany); and essential oils (carvacrol 3000 mg $kg^{-1}$, Vigoramax®).

**Table 1**
**Experimental preventive programs according to production phases**

| Phases | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|---|---|---|---|---|
| Housing (70-79 d) | Florfenicol 100 ppm (200 g) | - | Sangrovit 100 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Growth I (80-101 d) | - | - | Sangrovit 100 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Growth II (102-111 d) | Tiamulin 220 ppm (275 g) | - | Sangrovit 100 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Growth III (112-127 d) | - | - | Sangrovit 150 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Growth IV (128-136 d) | Florfenicol 100 ppm (200 g) | - | Sangrovit 150 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Finishing I (137-152 d) | - | - | Sangrovit 150 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Finishing II (153-158 d) | Tilmicosin 400 ppm (800g)<br>Tiamulin 120 ppm (150 g) | - | Sangrovit 150 g | Sangrovit 90 g<br>Vigoramax 1.000 g |
| Finishing III (159-176d) | - | - | Sangrovit 100 g | Sangrovit 90 g<br>Vigoramax 1.000 g |

The animals were submitted to a commercial feeding program consisting of eight diets, represented by phases (Table 1). For all treatments, the rations were isonutritious and isoenergetic, being supplied in a restricted way during the entire experimental period (70 days to 176 days of age), and with weekly adjustments based on the genetic consumption curve and the gender used. Water supply was ad libitum throughout the experimental period.

The animals were weighed individually at the beginning of the evaluation (D0, corresponding to 70 days of age), when the feed was changed from Growth IV to Finishing I (D67, corresponding to 137 days of age), and at the end of the experimental period. (D106, corresponding to 176 days of age). At the same intervals, the total feed consumption per pen was calculated. With these data, the daily feed intake, daily weight gain, and feed conversion ratio were calculated in the periods and over the entire experiment.

The probable clinical causes of death and the mortality rate were also determined, as well as eventual withdrawals of animals from the evaluation and their respective causes.

In the development of the evaluation, to minimize the clinical signs of respiratory problems, verified by the increase in coughing and sneezing episodes, a medication via water with the antibiotics spectinomycin and lincomycin (Spectomix®) was administered for all treatments between days D78 and D84 (61.6 g for 1000 L of water) and between D93 and D97 (66 g for 1000 L of water) of the experiment.

For the evaluation of immune responses to treatments, 96 piglets were randomly selected at the beginning of the experiment, 24 animals from each experimental group. On experimental days D3, D48, and D93, they were submitted to blood collection by puncture of the vessels in the neck region (5 mL in Vacutainer® tubes without anticoagulant). After deseruming the samples, the sera were stored in Eppendorf tubes and preserved under freezing (-20°C) until serology was performed to detect IgG anti-*L. intracellularis,* using the cell monolayer immunoperoxidase (IPMA) technique (Guedes et al., 2002).

At 180 days of age (D110), four days after the final weighing, 400 animals, 100 from each experimental group (half females and half immunocastrated males), were sent for slaughter. The animals had previously fasted for 8 hours before loading and 4 hours in the resting pens before slaughter. After this period, they were subjected to 3-point electronarcosis stunning, followed by bloodletting of the vessels in the neck region. Carcasses and viscera were evaluated following the rules of the regulation of industrial and sanitary inspection of products of animal origin (Decreto nº 10.468, 2020) and records of findings and deviations/ condemnations were recorded. In parallel, samples of ileum, cecum, and colon were collected from 18 animals from treatments T2 and T4, 17 animals from T1, and 15 animals from treatment T3. The samples were placed in flasks with 10% formalin and submitted to histological evaluation to identify lesions caused by *Lawsonia intracelluaris* (hyperplasia and crypt abscess).

For the typification of the carcasses, 96 pigs were used, 24 from each treatment, 12 males and 12 females, represented by animals from the same experimental blocks. The evaluation was carried out on the left half carcasses, which were first subjected to a cross-section at the height of point P2, followed by manual measurement, with a pachymeter, of the backfat thickness (BT) and the depth of the longissimus thoracis et lumborum muscle (LD). (Bridi & Silva, 2009). With these data, the equation was applied to predict the lean meat percentage in the carcass (LMP): = 54.449 - (0.5623 × BT) + (0.198 × LD).

Parametric data were submitted to analysis of variance and means to Tukey's test. Nonparametric data were evaluated using the chi-square test. For the analyses, the statistical program R (version 3.5.0) was used. The data referring to the samples that presented lesions (crypt hyperplasia and crypt abscess) were submitted to nonparametric statistical analysis using the Kruskal Wallis test and correction with Dunn's test. Non-normal quantitative data related to carcass condemnations were compared with the Kruskal-Wallis test.

## Results and Discussion

The animals of the positive control group (T1) showed better results of zootechnical performance (Table 2) in the first phase (growth phase, between 70 and 137 days of age), demonstrating superior daily weight gain (DWG) and better final weight in compared to the other treatments ($P < 0.05$). Daily feed intake (DFI) in this period was also higher ($P < 0.01$) for T1 compared to the negative control (T2) and the group treated with isoquinoline alkaloids plus essential oils (T4), but did not differ from T3 (isoquinoline

alkaloids). Feed conversion ratios (FCRs) did not differ between treatments. Regarding the sex factor, males showed lower DFI, higher DWG, and better FCRs compared to females (P < 0.01). There was no interaction effect between the factors.

In the second experimental phase (finishing, between 137 and 176 days of age) there was no interaction effect between programs and sex (Table 2), with a higher DFI (P < 0.01) being observed for treatments T1 and T3 relative to T4, while treatment T2 was similar to all treatments. For the FCRs, treatments T2 and T4 were better relative to T1, while T3 was similar to all treatments. As for the final weights, they were higher for T1 than for T4, not differing from the others. For DWG, there was no difference between treatments in this phase. Regarding the sex factor, the immunocastrated males showed higher values of DFI, DWG, and final weight as well as better FCRs than females (P < 0.01).

**Table 2**
**Means of the zootechnical performance of swine in the different experimental phases and in the total period according to treatments and sex (values expressed in kg)**

| Phases | Treatment | | | | Sex | | P-value | | | CV,% |
|---|---|---|---|---|---|---|---|---|---|---|
| 70-137 d | T1 | T2 | T3 | T4 | Males | Females | Treat | Sex | Inter | |
| Initial weight | 28.391 | 28.458 | 28.450 | 28.416 | 28.662 | 28.195 | 0.999 | 0.217 | 0.980 | 4.53 |
| DFI | 2.103$^{a}$ | 1.976$^{b}$ | 2.032$^{ab}$ | 1.966$^{b}$ | 1.982$^{b}$ | 2.052$^{a}$ | <0.001 | 0.007 | 0.435 | 4.30 |
| DWG | 1.032$^{a}$ | 0.980$^{b}$ | 0.978$^{b}$ | 0.971$^{b}$ | 1.011$^{a}$ | 0.970$^{b}$ | <0.001 | <0.001 | 0.221 | 3.69 |
| FCR | 2.040 | 2.016 | 2.067 | 2.026 | 1.959$^{a}$ | 2.115$^{b}$ | 2.239 | <0.001 | 0.397 | 3.11 |
| Final weight | 97.715$^{a}$ | 94.195$^{b}$ | 94.018$^{b}$ | 93.665$^{b}$ | 96.544$^{a}$ | 93.252$^{b}$ | 0.014 | 0.001 | 0.525 | 3.44 |
| 137-176 d | | | | | | | | | | |
| DFI | 2.866$^{a}$ | 2.776$^{ab}$ | 2.868$^{a}$ | 2.686$^{b}$ | 2.912$^{a}$ | 2.686$^{b}$ | <0.001 | <0.001 | 0.742 | 4.04 |
| DWG | 0.967 | 0.996 | 1.002 | 0.959 | 1.050$^{a}$ | 0.913$^{b}$ | 0.150 | <0.001 | 0.897 | 5.50 |
| FCR | 2.969$^{b}$ | 2.789$^{a}$ | 2.873$^{ab}$ | 2.814$^{a}$ | 2.780$^{a}$ | 2.942$^{b}$ | 0.013 | <0.001 | 0.901 | 4.81 |
| Final weight | 135.686$^{a}$ | 133.230$^{ab}$ | 133.484$^{ab}$ | 131.330$^{b}$ | 137.927$^{a}$ | 128.937$^{b}$ | 0.055 | <0.001 | 0.610 | 2.79 |
| Total 70-176d | | | | | | | | | | |
| DFI | 2.384$^{a}$ | 2.270$^{bc}$ | 2.334$^{ab}$ | 2.231$^{c}$ | 2.324 | 2.285 | <0.001 | 0.129 | 0.721 | 3.77 |
| DWG | 1.008$^{a}$ | 0.986$^{ab}$ | 0.987$^{ab}$ | 0.967$^{b}$ | 1.025$^{a}$ | 0.949$^{b}$ | 0.027 | <0.001 | 0.518 | 3.22 |
| FCR | 2.367$^{b}$ | 2.303$^{a}$ | 2.365$^{b}$ | 2.311$^{ab}$ | 2.266$^{a}$ | 2.407$^{b}$ | 0.041 | <0.001 | 0.461 | 2.93 |

$^{a,b,c}$ Means followed by distinct letters in the line indicate difference by Tukey's test (<0.05).
T1 - positive control (commercial program with antibiotic shocks at preventive level); T2 - negative control (absence of growth promoters); T3 - isoquinoline alkaloids; T4 - isoquinoline alkaloids + essential oil.
DFI - daily feed consumption; DWG - daily weight gain; FCR - feed conversion ratio.

Considering the entire experimental period, there was no interaction between the factors (Table 2). The DFI was higher (P < 0.01) for T1 and T3 compared to T4, which had the lowest consumption, a negative aspect that limits weight gain. Animals in the T2 group showed intermediate DFI results, which differed only from T1. The DWG of the animals in the T1 group was 4% better (P < 0.05) than T4, and the DWG of the T2 and T3 animals did not differ from the others. Animals in the T2 group presented FCRs 2% better than T1 and T3 (P<0.05), while T4 did not differ for all treatments. For the sex factor, males showed better DWG and FCRs (P<0.05).

Mortality and animal removal for health reasons were not different (P > 0.05) between treatments. The numbers of dead animals verified for T1, T2, T3, and T4 were 4, 2, 1 and 3, respectively. The causes attributed to the deaths mainly comprised sudden death and respiratory problems. Only one animal (T2) was excluded from the evaluation due to problems in the locomotor system.

The percentage of positive and negative animals in serology for anti-*Lawsonia* IgG (Table 3) indicated that the prevalence of positive animals remained high throughout the experimental period for all treatments, but without significant differences between treatments at any time of the evaluation.

In terms of intestinal lesions, of the 68 samples evaluated, 12 (17.65%) had crypt hyperplasia and 16 (23.53%) had crypt abscesses. Additionally, 10 samples (14.70%) had both lesions. Of the 12 samples with crypt hyperplasia, classified as grade 1 (with the lowest number of hyperplastic crypts), 4 belonged to the T2 group, 5 to the T3 group, and 3 samples to the T4 group. No grade 2, 3, or 4 lesions (related to damage with a greater number of hyperplastic crypts) were observed in the samples evaluated. Of the 16 samples with crypt abscess, 8 belonged to the T2 group, 6 to the T3 group and 2 samples belonged to the T4 group (Table 4). There was no difference between treatments for the variable crypt hyperplasia. However, for the variable crypt abscess, statistical differences of 44.44% and 40% were observed between groups T1 and T2 (P = 0.0126) and T1 and T3 (P = 0.0494), respectively, while T4 did not differ between treatments.

**Table 3**
**Percentage of pigs positive for IgG anti-Lawsonia, according to different periods (ages) of evaluation and treatments**

| Treatments | Evaluation periods (age, days) | | | Mean |
|---|---|---|---|---|
| | 73 | 118 | 163 | |
| T1 | 96% | 88% | 96% | 93% |
| T2 | 96% | 100% | 100% | 99% |
| T3 | 92% | 100% | 100% | 97% |
| T4 | 90% | 92% | 92% | 91% |
| Mean | 93% | 95% | 97% | 95% |

T1 - positive control (commercial program with antibiotic shocks at preventive level); T2 - negative control (absence of growth promoters); T3 - isoquinoline alkaloids; T4 - isoquinoline alkaloids + essential oil.

**Table 4**
**Number (n) and percentage (%) of pigs positive for crypt hyperplasia and crypt abscesses according to the experimental treatments**

| Treatments | Crypt hyperplasia | Crypt abscess |
|---|---|---|
| T1 (n = 17) | 0 (0%) | 0 (0%)[a] |
| T2 (n = 18) | 4 (22.23%) | 8 (44.44%)[b] |
| T3 (n = 15) | 5 (33.34%) | 6 (40%)[b] |
| T4 (n = 18) | 3 (16.67%) | 2 (11.11%)[ab] |

[a,b,] distinct letters indicate a significant difference by nonparametric statistical analysis using the Kruskal Wallis test and correction with Dunn's test.
T1 - positive control (commercial program with antibiotic shocks at preventive level); T2 - negative control (absence of growth promoters); T3 - isoquinoline alkaloids; T4 - isoquinoline alkaloids + essential oil.

There were no interaction effects for carcass characteristics (Table 5) and no differences (P > 0.05) between treatments. The only difference between the sexes was for the measurement of backfat thickness, in which females presented higher averages than immunocastrated males.

**Table 5**
**Means of backfat thickness (BT), loin depth (LD) and lean meat percentage (LMP) in the carcass of swine according to experimental treatments and sex**

| Parameters | Treatment | | | | Sex | | P-value | | | CV,% |
|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | Males | Females | Treat | Sex | Inter | |
| BT, mm | 13.38 | 13.55 | 14.40 | 13.60 | 13.97 | 13.48 | 0.755 | 0.517 | 0.117 | 23.14 |
| LD, mm | 76.88 | 81.45 | 77.40 | 77.30 | 77.78 | 78.76 | 0.245 | 0.608 | 0.681 | 10.26 |
| LMP, % | 62.14 | 62.95 | 61.67 | 62.10 | 61.99 | 62.46 | 0.333 | 0.376 | 0.320 | 3.58 |

No statistical difference was observed by the Tukey test.
T1 - positive control (commercial program with antibiotic shocks at preventive level); T2 - negative control (absence of growth promoters); T3 - isoquinoline alkaloids; T4 - isoquinoline alkaloids + essential oil.

The total carcass condemnations, determined by the evaluation of the technicians of the Federal Inspection Service, were caused by respiratory problems and lung injuries, being observed 7% for T2 and 1, 2, and 3%, respectively, for treatments T1, T3, and T4, with T2 being different (P < 0.05) from the other treatments (Table 6).

**Table 6**
**Number and percentage of carcasses condemned to slaughter and respective classes of condemnation according to treatments**

| | T1 | T2 | T3 | T4 | P-value |
|---|---|---|---|---|---|
| Number of animals evaluated (n) | 100 | 100 | 100 | 100 | NS |
| Condemnations directed at sausage (n) | 1[b] | 6[a] | 1[b] | 2[b] | 0.0727 |
| Condemnations directed to grease (n) | 0 | 1 | 1 | 1 | NS |
| Total condemnations (n) | 1[b] | 7[a] | 2[b] | 3[b] | 0.0560 |
| Total condemnations (%) | 1% | 7% | 2% | 3% | - |

[a,b,] distinct letters indicate tendency by Kruskal Wallis (P<0.10).
T1 - positive control (commercial program with antibiotic shocks at preventive level); T2 - negative control (absence of growth promoters); T3 - isoquinoline alkaloids; T4 - isoquinoline alkaloids + essential oil.

In the first phase of the experimental period (70-137 d) the best DWG and final live weight verified for the animals of the group submitted to antibiotic shocks (T1) endorse the virtues of this class of additives, whose effects are commonly more direct, differing in part of some additives called alternative, in this case, represented by isoquinoline alkaloids, and by carvacrol, whose actions are also indirect, such as antioxidant, anti-inflammatory or having microbial and immune modulating character (Boroojeni et al., 2018; Caldefie-Chézet et al., 2006; Chen et al., 2018, 2019; Dhifi et al., 2016) and, therefore, with effects that are often noticeable within a longer term of use. This condition is noticed in the second experimental phase (137-176 d), in which the DWG was similar between the treatments, as well as the final weight in the phase, which was only worse for the T4 group compared to T1, which nevertheless presented better FCRs relative to T1.

The performance results considering the entire experimental period indicate that isoquinoline alkaloids, when administered alone in the diet, exerted effects similar to the antibiotic program (T1), which may be associated with antibacterial activities (Kosina et al., 2010) and anti-inflammatory properties (Chaturvedi et al., 1997) that they hold. For this reason, there are reports that piglets in the nursery phase that received isoquinoline alkaloids showed better digestibility, performance, and intestinal morphology (Boroojeni et al., 2018; Chen et al., 2018, 2019).

Considering the entire study period, the absence of difference between T1 and T3 groups is identified with the results obtained by Chen et al. (2019), who found that animals supplemented with isoquinoline alkaloids (50 mg $kg^{-1}$), compared with animals supplemented with APC (flavomycin and aeromycin) did not show differences in zootechnical performance.

In the literature, there are several effects reported on the action of these alkaloids for swine, such as improved digestibility (Boroojeni et al., 2018) and the stimulation of feed consumption through the tryptophan-serotonin pathway, since sanguinarine binds to neuroreceptors, including serotonin (5-HT2) (Schmeller et

al., 1997), and also inhibits the action of the aromatic amino acid decarboxylase enzyme (Dršata et al., 1996), increasing the availability of aromatic amino acids, such as tryptophan.

This additive is also able to beneficially modulate the intestinal microbiota, increasing the amount of *Lactobacillus* spp. and decreasing the amounts of *Escherichia coli* in the ileum, in addition to reducing the presence of *Salmonella* spp. in the ileum and cecum (Chen et al., 2018). This beneficial modulation of the microbiota, in addition to reducing the competition for nutrients between pathogenic and non-pathogenic microorganisms, has effects on the intestinal barrier function, on the immune system, as well as on the absorption and metabolism of nutrients, affecting the performance of the animals (Nowland et al., 2019).

These effects, however, are not clearly explained in the results of the zootechnical indices of this work. Perhaps these have been masked by the therapeutic approach/program with antibiotics that was used via water on two occasions in this work, to reduce the number of animals with clinical respiratory problems. However, this procedure may have improved the health status of the animals, lessening the inherent endemic challenge (Zotti et al., 2009), which is common in commercial fattening systems, thus masking the effects of isoquinoline alkaloids.

The results of DWG and FCRs observed in the negative control group (T2), compared with the group submitted to drug pulses with antibiotics (T1), indicate that these animals, unlike the T1 animals, may not have gone through a process of intestinal dysbiosis (Nowland et al., 2019). Dysbiosis is a condition that is sometimes present in animals that receive high doses of antibiotics, leading to a reduction in the diversity of the intestinal microbiota, with negative local and systemic consequences. Looft et al. (2012) and Kim et al. (2012) demonstrated that antibiotics in feed to piglets compromised the establishment of the microbiome and reduced the amount and diversity of the intestinal microbial population. This dysbiosis, reported by the cited authors, causes a decrease in IgA-producing cells and hypoplasia of Peyer's patches, impairing the animals' immune system, in addition to negatively influencing the absorption of nutrients (Nowland et al., 2019).

Adversely, the association of isoquinoline alkaloids with essential oils (T4) did not potentiate the results of zootechnical performance, characterizing the group with the worst results. It is observed that in all phases, with repercussions in the total period (Table 2), this group always presented worse feed intake. Although some essential oils are associated with increased feed consumption, promoting improved palatability and, consequently, increased feed consumption, especially when the target species is swine (Zeng et al., 2015). In addition to acting in minimizing oxidative processes in the diet, which would result in the presence of unpleasant odors and loss of palatability (Sola-Oriol et al., 2011), there are some secondary metabolites of phytogenic additives that can affect the sensory characteristics of the feed (Alves et al., 2017), in which, depending on the level of inclusion or the mixtures between different oils, they can generate strong odors and unpleasant flavors, resulting in a decrease in palatability and lower feed intake (Yan et al., 2012). Although the presence of compounds that can antagonize an effective feed intake has not been evaluated sensorially

or chemically, it is possible to predict that this condition may justify the worse results for this group, because when ingesting a smaller amount of feed, the weight gain was consequently compromised.

Considering the sex factor, there was coherence in the evaluated indices, with advantages that adequately characterize the superior performance of immunocastrated males over females. Immunocastrated males have greater benefits imprinted by endogenous steroid hormones, being typically leaner and more efficient in feeding compared to females and castrated males (Martins et al., 2013).

Serology confirmed the presence of the bacterium *L. intracellularis* throughout the research period (Table 3) and the histopathological analysis showed that this bacterium caused intestinal lesions, such as crypt hyperplasia (Table 4), a characteristic damage of this proliferative enteropathy (McOrist et al., 1996). These results confirm that the experimental unit (farm), which historically had clinical signs of ileitis, now has confirmation of the presence of the agent. However, no clinical symptoms characteristic of this enteric disease, such as anorexia, diarrhea, bloody diarrhea, and poor performance, were observed, which might suggest that the animals presented a subclinical picture of the disease (Kroll et al., 2005). This scenario, associated with the observed mortality rate, 1.73% for the entire experimental period, considering all treatments together, may indicate that the health challenges were not high. This condition may have minimized the effects of the additives on the parameters of zootechnical performance and carcass characteristics evaluated, which are known to have better action in situations of greater challenge (C. A. Silva et al., 2003). According to Holman and Chénier (2013), when pigs are in good health, some antimicrobial agents do not result in consistent improvements in performance.

It is important to emphasize that the anti-inflammatory activity of sanguinarine is due to the inhibition of the activation pathway of the nuclear factor kappa B (NF-κB), which is a transcription factor involved in the control of the expression of several genes linked to the inflammatory response (Chaturvedi et al., 1997). Thus, the anti-inflammatory action of this additive may not have been effective against *L. intracellularis* in this subclinical situation. We hypothesized that in a more challenging condition, where the agent was expressing clinical symptoms, such as bloody diarrhea (Guedes et al., 2002), the effects of isoquinoline alkaloids could be more evident, minimizing the inflammatory processes that the agent determines, possibly with more positive consequences on performance.

Regarding the best carcass condemnation results (Table 6), which were obtained for treatments with antibiotics in the form of pulses (T1) and for groups treated with isolated isoquinoline alkaloids (T3) or associated with essential oils (T4), relative to the negative control group (T2), it is possible to associate them with the effects of the additives on the health of the animals, considering that all of them have antibacterial and antioxidant activities.

The prevalent causes of condemnation were determined by respiratory problems and lung injuries, which is in line with data recorded in Brazil and in international histories, where lung injuries are the main cause of carcass condemnation (Alberton &

Mores, 2008). According to Ê. C. Silva et al. (2020), pleural adhesion was the main cause of condemnation of swine carcasses in Brazilian slaughterhouses registered with the Brazilian federal inspection service between 2012 and 2017, corresponding to 37.3% of all convictions.

Comparing the rate of carcass condemnations in Brazilian slaughterhouses, registered by the Federal Inspection Service (SIF) between the years 2012 and 2017, which was 2.16% (Ê. C. Silva et al., 2020), with the condemnation rate observed in this study, we found that the groups that used antibiotics in the form of pulses (T1) or isoquinoline alkaloids associated or not with carvacrol (T3 and T4, respectively) had a similar rate of condemnations (between 1% and 2%) to those recorded in the year references mentioned above. On the other hand, the negative control group (T2) presented the worst ($P < 0.05$) result of carcass condemnations (7%).

These results indicate that the use of isoquinoline alkaloids, associated or not with carvacrol, and of antibiotics used in the pulse form, minimized the conditions that determined the recorded condemnations. All these additives have actions that justify these benefits, highlighting the systemic immune modulatory action determined by isoquinoline alkaloids (Li et al., 2020), and the antibacterial effect of essential oils against the main respiratory pathogens of swine: *Streptococcus suis* and *Actinobacillus pleuropneumoniae* (Lebel et al., 2019).

In this matter, the economic damage, estimated in Brazil, for the industry and the producer, resulting from carcass condemnations, can exceed US$ 27 million per year (Ê. C. Silva et al., 2020). At the industrial level, in Brazil, the partially or totally condemned carcasses are destined, respectively, for the production of cooked sausage products and rendering. In this sequence, these have a devaluation of 12% and 92% (Alberton & Mores, 2008), very significant values that reflect the relevance of the economic damage recorded by the authors above, thus valuing the participation of additives evaluated as improvers of these lesions.

## Conclusion

Isoquinoline alkaloids can replace preventive programs with antibiotics for growing and finishing pigs, with positive repercussions on performance and carcass characteristics.

Diets supplemented with isoquinoline alkaloids associated or not with carvacrol reduce intestinal lesions caused by *Lawsonia intracellularis* and decrease the frequency of carcass condemnation at slaughter, signaling an improvement in the animal's systemic health, with the potential to replace programs with antibiotics in the condition of preventive shocks.

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