Use of an organic acid blend to control the spread of *Salmonella* Heidelberg and improve broiler performance

[*Utilização de mistura de ácidos orgânicos para controlar a disseminação de Salmonella Heidelberg e melhorar o desempenho em frangos de corte*]

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Abstract

The objective was to evaluate an organic acid blend supplied via water and feed to control the spread of *Salmonella* Heidelberg in broilers from 1–31 days of age, and improve growth performance in broilers. The design was a complete randomized block with five treatments and five replicates, 25 experimental units (13 birds per unit, density of 13 birds/m^2). A total of 325 one day old female chicks were randomly arranged in five treatments: T1 – Negative control (no acid blend via water and feed + 0.5 mL of physiological solution in water); T2 – Positive control (no acid blend via water and feed + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU); T3 – Acid blend via water + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU); T4 – Acid blend via feed + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU); T5 – Acid blend both via water and feed + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU) in drinking water and feed. The results were analyzed using Bayesian comparisons with 5% significance and a priori beta distribution. Significant effects of treatments were found on feed conversion at 21, 28, and 31 days, where treatments T3 and T5 had the best results. Regarding live weight, no significant effects were observed between treatments. There was no significant effect of using a mixture of organic acids in reducing the incidence of *Salmonella* Heidelberg among the treated groups, in intestines at nine days, using drag swab at 16 days, and crop collection at 31 days. There were no significant differences between the test groups and control groups.

Keywords: animal health; broiler performance; food safety.

Resumo

O objetivo foi avaliar uma mistura de ácido orgânico fornecida via água e ração para controlar a propagação de *Salmonella* Heidelberg em frangos de 1 a 31 dias de idade e melhorar o desempenho dos frangos de corte. O delineamento foi em blocos ao acaso com cinco tratamentos e cinco repetições, 25 unidades experimentais com 13 aves/unidade (densidade de 13 aves/m^2). Um total de 325 pintinhas de corte foi distribuído aleatoriamente em cinco tratamentos: T1 - Controle Negativo (sem ácidos via água de bebida ou ração + 0,5 mL de solução fisiológica em água de bebida); T2 - Controle positivo (sem ácidos via água de bebida ou ração + 0,5 mL de *Salmonella* Heidelberg (1.2 x 10^6 CFU); T3 - Ácido na água de bebida + inoculação de 0,5 mL de *Salmonella* Heidelberg (1.2 x 10^6 CFU); T4 - Ácido na ração + inoculação de 0,5 mL de *Salmonella* Heidelberg (1.2 x 10^6 CFU); T5-Ácido via água de bebida e ração + inoculação de 0,5 mL de *Salmonella* Heidelberg (1.2 x 10^6 CFU). Os resultados foram analisados

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usando comparações Bayesianas (5% de significância) e distribuição com priori beta. Efeitos significativos foram encontrados na conversão alimentar aos 21, 28 e 31 dias, onde os tratamentos T3 e T5 apresentaram os melhores resultados. Em relação ao peso vivo, não foram observados efeitos significativos entre os tratamentos avaliados. Não foi observado efeito significativo para o uso de mistura de ácidos orgânicos na redução da incidência de *Salmonella* Heidelberg entre os grupos tratados, em intestinos em nove dias, swab de arrasto aos 16 dias e a coleta de papo aos 31 dias. Não houve diferenças significativas entre os grupos testes e grupos controle.

Palavras-chave: sanidade animal; desempenho; saúde alimentar.

Introduction

Considering the problem of foodborne diseases, *Salmonella* appears as one of the greatest agents in outbreaks of food poisoning (Lake et al., 2002). This bacterium also stands out due to its wide occurrence in humans and animals (mammals, reptiles, and birds), with birds responsible for a significant role in the epidemiology of *Salmonella* as they act as large reservoirs (Borsoi et al., 2010).

The genus *Salmonella* belongs to the Enterobacteriaceae family and consists of two species: *Salmonella enterica* and *Salmonella bongori*. The species *Salmonella* enterica is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, and *indic houtenae* (Grimont and Weill, 2007), where the *enterica* subspecies includes the serovar Heidelberg (Davis et al., 2008).

According to the classic Kauffman-White scheme, based on the characterization of their H (flagellar), O (somatic), and occasionally Vi (capsular) antigens, each subspecies has different serovars, which currently total 2,610 (Guibourdenche et al., 2010).

Salmonellosis in humans is caused by various serotypes of *Salmonella*. *Salmonella* Heidelberg is one of the most prevalent serovars correlated with salmonellosis in humans, as well as infections caused by *Salmonella* in broilers (Gast et al., 2007).

Salmonella Heidelberg is the enteric pathogen originated from food most often related to the occurrence of human intoxication in the literature. The importance of this microorganism stems from its significant prevalence with worldwide distribution in poultry production and its implications for public health (Gast, 2003).

With the emergence of some types of bacterial resistance, the use of antibiotics and adjuvant vaccines restrictions has increased. Thus, other tools to control the incidence of *Salmonella* in chickens are being explored (Rahimi et al., 2007), as organic acids, that have been evaluated for this purpose with success (Stratford et al., 2009).

In this context, the objective of this work was to evaluate the effectiveness of a blend of organic acids, provided via drinking water and feed, to control the spread of *Salmonella enterica* subsp. *enterica* serovar Heidelberg and to improve growth performance in broilers.

Material and Methods

This experiment was conducted at the Laboratory of Experimental Aviary Poultry Innovations (LINAV) of Universidade Tecnológica Federal do Paraná, Campus Dois Vizinhos, Paraná State, Brazil. The study was conducted over a period of 31 days from August to September of 2014. A total of 325 Cobb 500 female broilers, vaccinated in the hatchery against Marek disease, avian infectious bronchitis, and yaws were included. The average live weight was 42.80 ± 0.30 g; at day one, the density was 13 birds/m². All boxes were equipped with a tubular feeder and four beaks of nipple type water fountains. The floor was made of concrete and the bed composed of pine wood shavings with a thickness of 8 cm.

The facility is equipped with automatic temperature control, which controlled heating and cooling systems. When the ambient temperature was below a set point, the heater was triggered. Similarly, the cooling was triggered when the ambient temperature was higher than desired, according to the age of the birds.

The feeding program was divided into two stages, starter feed (1–14 days) and growth phase feeding (15–31 days). The diet was composed of corn, soybean meal, bone meal, and meat. The rations did not contain any anticoccidial agent nor growth promoter. Diets were formulated according to the nutritional requirements indicated by Rostagno et al. (2017).

The birds were kept from 1 until –31 days of age under ideal temperatures according to their age, supplied with non-chlorinated water, and abundant feeding. The lighting program was that recommended for the lineage: first day 24L:0D (L: light and D: dark), from the second to seventh day 23L:1D, from the eighth to twenty-eighth day 18L:6D, and from the twenty-ninth to thirty-first day (slaughter) 23L:1D.

The experimental design used involved randomization in blocks with five treatments and five replicates each, with a total of 25 boxes as experimental units, and with 13 birds per box (density of 13 birds/m^2). The 325 birds were randomly distributed into the experimental units and the treatments were classified as: T1 – Negative control (no acid blend via water and feed + 0.5 mL of physiological solution in water); T2 – Positive control (no acid blend via water and feed + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU); T3 – Acid blend via water + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU); T4 – Acid blend via feed + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU); T5 – Acid blend both via water and feed + inoculation of 0.5 mL of *Salmonella* Heidelberg (1.2 x 10^6 CFU) in drinking water and feed.

The blend administered via feeding (trademark TART-450®) had as a basic composition: calcium formate; fumaric acid; silicon dioxide; bentonite; citric acid; calcium propionate; kaolin; oregano; citric acid. Fumaric acid and citric acid were the main components with 19 and 95 g/kg, respectively. The blend administered via drinking water had the trade name TART AQUAVITTA® and its elemental composition was as follows: fumaric acid; sodium bisulfate; citric acid; and silicon dioxide, with fumaric acid and citric acid being its main components at 243.8 and 248.7 g/kg, respectively. These products were used in treatments T3, T4, and T5 until the seventh day, 24 hours per day.

After this period, the birds were given feed and water *ad libitum*, without any treatment. This procedure was repeated until the last four days of age when again the birds received the blend of organic acids via drinking water and feeding, following the treatments for 24 hours, until the preslaughter fasting period, which was five hours.

The strain of *Salmonella* Heidelberg used in the experiment was previously isolated from a field sample and was resistant to nalidixic acid (100 μg/mL). It was recovered from lyophilized cultures. Five minutes before being placed in the bird pits, all birds from treatments T2, T3, T4, and T5 were inoculated with 0.5 mL of a solution containing 1.2 x 10^6 CFU *Salmonella* Heidelberg/mL. In T1 (negative control), the birds were inoculated with 0.5 mL of physiological solution through a 3 mL sterile pipette via the esophageal route. At the end of each experimental (7, 14, 21, 28 days) and slaughter (31 days) week, all birds and feed leftovers were weighed for performance evaluation: average food intake, middleweight, daily weight gain, and feed conversion. From the first day of life, the birds that died were weighed and discounted from the total number to obtain estimates of food conversion, as per the methodology described by Rostagno et al. (2017).

In order to assess the presence of *Salmonella* in organs of the digestive tract, samples of the ileum pool, cecum, and cecal tonsils were obtained at nine days of age, 15 birds of each treatment were necropsied at 7:00 am. The organs were collected sterilely. The same procedure was used to obtain the craw samples at the time of slaughter at 31 days of life. These samples were stored in a Styrofoam box, packed with recyclable ice and forwarded on the same day to the laboratory responsible for the analyses.

For the isolation of *Salmonella* Heidelberg in the feces of the birds, drag swabs were collected at 16 days of age, in 25 boxes. The swabs were collected following act IN 126 from the Brazilian Agriculture Ministry, Livestock and Supply (MAPA) regulations (BRASIL, 1995). To avoid cross-contamination between the blocks, disposable plastic boots and a pair of gloves for each swab sample were used. These samples were stored in a Styrofoam box, packed with recyclable ice and forwarded the same day to the laboratory responsible for the analyses.

To assess the effectiveness of the treatments on colonization of *Salmonella* Heidelberg in the gastrointestinal tract, samples of ileum, cecal tonsils, and cecum were collected, and wattle samples were collected based on the legal act IN 126 from the MAPA regulations. The percentage of positivity was classified using the swab tests, using the same standard used by MAPA. These samples were processed in a private Animal

Health Laboratory, recognized by MAPA (BRASIL, 1995).

For the statistical analysis, the objective was to test the null hypothesis of equality between treatments with a 5% level of significance using Bayesian comparisons. For this, we used non-informative a priori distributions in the procedure.

It was considered that feed conversion and slaughter live weight followed normal distributions:

$$Y_i \sim Normal\ (\mu_i, \sigma_i^2) com\ i = 1, 2, 3, 4, and\ 5 \text{ (corresponding to treatments)}$$

Each μ_i and σ_i^2 were considered a priori non-informative distributions:

$$Y_i \sim Normal(0, 16^6)\ and\ \sigma_i^2 \sim Gama\ (10^3, 10^3)\ (conjugate\ family)$$

It was considered that isolation of *Salmonella* Heidelberg in swabs and necropsies followed a priori beta distribution:

$$Y_i \sim Beta(\mu_i, \sigma_i^2)\ where\ i = 1,2,3,4, and\ 5\ (corresponding\ to\ the\ treatments)$$

Each μ_i and σ_i^2 were considered a priori:

$$Y_i \sim Normal(0, 16^6)\ and\ \sigma_i^2 \sim Binomial\ (10^3, 10^3)\ (conjugate\ family)$$

Multiple comparisons were made among distributions a posteriori of the averages. With a 5% level of significance, the treatments were considered different when credibility intervals for the differences were not zero (Rossi, 2011). The results were obtained using the R2OpenBUGS packages, MASS, BRugs, and CODA from R® software (R Core Team, 2014).

Results and Discussion

Performance

According to Table 1, the addition of organic acids in treated groups did not significantly influence the average live weight and feed conversion at seven days of age.

It was observed that the inoculation of *Salmonella* Heidelberg, administered orally on the first day, adversely influenced ($p \leq 0.05$) feed conversion of the birds challenged within one to seven days. This was related to the interaction between the agent and the intestinal microbiota of inoculated birds. Chaves (2007) found similar results for broilers treated for *Salmonella* enteritidis.

According to Rocha (2008), the invasion of the intestinal microflora by *Salmonella* spp., which are considered pathogenic to birds and humans, probably caused the imbalance in natural colonization of the gut during the first week of life, reflecting negatively on the use of diet and nutrients and causing a reduction in bird performance.

Salazar et al. (2008) and Zanelato et al. (2008) evaluated the interaction of organic acids on performance variables and found significant differences for weight gain, feed conversion, and feed intake. Campo et al. (2004) stated that the first few weeks of life are crucial to the development of the digestive tract of birds. Similarly, Viola and Vieira (2008) showed benefits in weight gain and average weight of broilers in the first weeks of rearing using organic acids in feed.

Table 1. Bayesian estimates for feed conversion ratio (FCR) and middleweight (MW), a posteriori, at seven days of treatment.

Treatments	FCR posteriori	CV (%)	MW a posteriori (g)	CV (%)
T1	1.294 [a]	5.65	0.146[b]	9.46
T2	1.331[b]	5.40	0.148[ab]	10.06
T3	1.311[b]	5.90	0.147[b]	9.72
T4	1.403[bc]	3.60	0.134[bc]	10.67
T5	1.379[bc]	4.14	0.143[b]	10.34

[a, b] Means followed by different letters in the column are statistically different through Bayesian comparisons ($p \leq 0.05$)

*: Treatments: T1: Negative control; T2: Positive Control; T3: Acid blend via water; T4: Acid blend via feed; T5: Acid blend via water and feed.

Over the period from one to 31 days, no differences in growth performance among the treatments evaluated ($p \geq 0.05$) were observed (Tables 2–5). The use of a blend of organic acids positively influenced the feed conversion ratio in the groups treated with organic acids at 21 days old, as shown in Table 3.

Feed conversion in the groups treated with organic acids at the age of 14 days was better only when compared to the negative control group, not demonstrating an effectiveness in improving this indicator for this age group. Similar results were observed by Maiorka et al. (2004), who used a mixture of organic acids based on fumaric acid and citric acid, and Garcia et al. (2000), who used isolated butyric acid and an association of butyric acid and formic acid in broiler diets and did not observe differences in weight gain, feed conversion, or feed intake between the treated groups when evaluating zootechnical indices at 14 days.

Regarding average weight, the group treated with a blend of organic acids via drinking water had a better numerical result when compared with other groups. This result agrees with those obtained by Viola and Vieira (2008), who observed better results for feed conversion and average weight when using organic acids via drinking water.

Table 2. Bayesian estimates for the feed conversion ratio (FCR) and middleweight (MW) posteriori at 14 days of age of treatments.

Treatments	FCR a posteriori	CV (%)	MW a posteriori (g)	CV (%)
T1	1.569[b]	2.14	0.343[b]	6.06
T2	1.449[ab]	6.46	0.325[bc]	6.27
T3	1.433[ab]	6.90	0.351[ab]	4.47
T4	1.449[ab]	5.92	0.319[bc]	5.32
T5	1.464[b]	4.67	0.337[b]	4.62

[a, b] Means followed by different letters in the column are statistically different through Bayesian comparisons ($p \leq 0.05$)
*: Treatments: T1: Negative control; T2: Positive Control; T3: Acid blend via water; T4: Acid blend via feed; T5: Acid blend via water and feed.

Table 3. Bayesian estimates for the feed conversion (FCR) and middleweight (MW) posteriori at 21 days of age of treatment.

Treatments	FCR a posteriori	CV (%)	MW a posteriori (g)	CV (%)
T1	1.666 [a]	1.51	0.699[b]	3.63
T2	1.996[b]	6.54	0.659[bc]	4.77
T3	1.582[a]	5.49	0.699[b]	2.67
T4	1.734[b]	8.92	0.667[bc]	4.48
T5	1.604[ab]	4.65	0.689[b]	2.71

[a, b] Means followed by different letters in the column are statistically different through Bayesian comparisons ($p \leq 0.05$)
*: Treatments: T1: Negative control; T2: Positive Control; T3: Acid blend via water; T4: Acid blend via feed; T5: Acid blend via water and feed.

Other authors also report improvements in the performance of poultry over the growth phase, due to the increase of organic acids in the diet, such as Daskiram et al. (2004), who used citric acid as an additive in the diet during the first week of life of birds and had significantly better zootechnical results in the treated group. Zanelato et al. (2008) used a mixture of formic acid and butyric acid in the feed as an additive in the first week of life and obtained significantly better average live weight and feed conversion at 14 days.

Evaluating the period from 1 to 21 days of age, differences were observed ($p \leq 0.05$) for feed conversion ratio when comparing the groups treated with the blend of organic acids with the control groups (Table 3).

Concerning feed conversion, the group treated with the blend of organic acids via drinking water was significantly better when compared to the other evaluated groups. In the evaluation performed at 21 days of rearing, the group treated with acids via water and feed also had better results than the positive control group.

Related to the average live weight of the birds, evaluated under one to 21 days, the groups treated with the blend of organic acids via drinking water and via drinking water and feed had a better numerical response when compared to other groups. Salazar et al. (2008) also showed an improvement in the growth performance of birds by acidifying the diet, when assessing the period from one to 21 days. On the other hand, Vale et al. (2004) used 0.5% of a mixture of organic acids based on formic acid and propionic acid in feed and did not observe a significant difference in average live weight or feed conversion during the same time interval.

Evaluating the period from one to 28 days of age, differences were observed for feed conversion ratio, when comparing the groups treated with the blend of organic acids used in treatments with the control (Table 4).

The feed conversion of all groups treated with the blend of organic acids was better ($p \leq 0.05$) when compared with control groups, which proves the effectiveness of the product in improving performance, over the period from 1 to 28 days. On the other hand, Maiorka et al. (2004) observed no difference in feed conversion between treatments when using a mixture of organic acids in the diet of broilers.

The average live weight value evaluated in the period from one to 28 days, showed better numerical results in the group treated with the blend of organic acids via drinking water when compared to control groups.

Calaça et al. (2019) observed better performance in birds of the groups that received organic acids via drinking water, which agrees with the results obtained in the present study.

Evaluating the period from one to 31 days old (slaughter), differences were observed ($p \leq 0.05$) for feed conversion ratio when comparing those treated with the blend of organic acids and the control groups (Table 5).

Table 4. Bayesian estimates for the feed conversion ratio (FCR) and middleweight (MW) posteriori at 28 days of age of treatment.

Treatments	FCR a posteriori	CV (%)	MW a posteriori (g)	CV (%)
T1	1.752^{b}	2.50	1.093^{b}	2.54
T2	1.872^{bc}	7.56	1.061^{bc}	3.52
T3	1.674^{a}	3.48	1.108^{ab}	1.53
T4	1.743^{ab}	5.67	1.078^{bc}	3.37
T5	1.660^{a}	3.91	1.097^{b}	2.30

$^{a,\,b}$ Means followed by different letters in the column are statistically different through Bayesian comparisons ($p \leq 0.05$)
*: Treatments: T1: Negative control; T2: Positive Control; T3: Acid blend via water; T4: Acid blend via feed; T5: Acid blend via water and feed.

Table 5. Bayesian estimates for the feed conversion ratio (FCR) a posteriori and middleweight (MW) posteriori at 31 days old of treatments.

Treatments	FRA a posteriori	CV (%)	MW a posteriori (g)	CV (%)
T1	1.882^{b}	1.72	1.278^{b}	3.23
T2	1.942^{b}	6.49	1.204^{bc}	3.30
T3	1.784^{ab}	2.80	1.280^{ab}	2.21
T4	1.843^{ab}	3.92	1.208^{bc}	3.28
T5	1.762^{a}	3.44	1.275^{b}	2.11

$^{a,\,b}$ Means followed by different letters in the column, are statistically different through Bayesian comparisons ($p \leq 0.05$)
*: Treatments: T1: Negative control; T2: Positive Control; T3: Acid blend via water; T4: Acid blend via feed; T5: Acid blend via water and feed.

Performing the comparison for feed conversion in the period from one to 31 days, all groups treated with the blend of organic acids were significantly better when compared to control groups. Compared to the average live weight, in the same period, the group treated with the blend of organic acids via drinking water obtained better results among the treatments.

Rahmani and Speer (2005) investigated the effects of an organic acid as a growth promoter in a broiler diet and noted an improvement in performance when compared to the group free from additives in all phases.

Microbiological analysis

Concerning the isolation of *Salmonella* Heidelberg in broilers, all challenged groups showed positivity in pool collection of intestines to nine days. In drag swab collection at 16 days, there was isolation of *Salmonella* Heidelberg in challenged groups, except for the T5 group, which did not show any positives. In the wattle collection at 31 days (slaughter), isolation of *Salmonella* Heidelberg in all challenged groups was observed.

Positivity for *Salmonella* Heidelberg among challenged groups proves the effectiveness of the inoculation, since the negative control group had obtained only one positive, representing 6.66% of the tests. The positive control group had 86% of positivity, proving the effectiveness of inoculating with *Salmonella* Heidelberg on the first day of life.

In Table 6, results of evaluations made among treatments are shown. A significant difference was observed only when comparing the groups challenged with *Salmonella* Heidelberg and treated with organic acids with the negative control group, in the evaluation of pools of intestines collected at nine days of age.

In the evaluation of positivity for *Salmonella* Heidelberg assessed in the craw during necropsy at 31 days of age, a significant difference was observed only when comparing the negative control with the other treatments (Table 6).

Table 7 shows the results of isolation of *Salmonella* Heidelberg in drag swabs in all treatments and replications as well as comparisons between treatments. Swab samples were collected at 16 days of age, and there was no significant difference among treatments. The analysis of the characteristics of *Salmonella* Heidelberg, especially the formation of biofilm and low excretion of bacteria in the stool, suggests that the low number of positive samples in the swab noticed in this study may also be related to these two factors.

Table 6. Bayesian a posteriori estimates for *Salmonella* Heidelberg research in ileum pool samples, cecal tonsils and ceca in necropsies performed at nine days and craw samples in necropsies performed at 31 days of age.

Days	Contrast *	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
9	Delta	-0.7051	-0.5305	-0.6472	-0.4711	0.1747	0.058	0.2341	-0.1167	0.0593	0.1760
	P-value *	0.1181	0.1348	0.1251	0.1389	0.1428	0.1336	0.1460	0.1501	0.1599	0.1522
	Significance *	s	S	s	s	NS	NS	NS	NS	NS	NS
31	Delta	-0.2935	-0.0592	-0.0585	-0.1185	0.2342	0.234	0.1749	0.0006	-0.0593	-0.0600
	P-value	0.1254	0.0937	0.0935	0.1063	0.1354	0.135	0.1443	0.1065	0.1187	0.1178

*: Contrast corresponds to the comparisons carried out on statistical analysis. Being C1: (T1 xT2); C2: (T1 x T3); C3: (T1 x T4); C4: (T1 x T5); C5: (T2 X T3); C6: (T2 x T4); C7 (T2 x T5); C8: (T3 x T4); C9 (T3 x T5); C10 (T4 x T5).

Table 7. Bayesian estimates retrospectively for *Salmonella* Heidelberg, made by drag swab, performed at 16 days old.

	Contrast *	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Swab 16 days	Delta	-0.4293	-0.2846	-0.1442	0.1363	0.1448	0.2851	0.4293	0.1404	0.2846	0.1442
	P-value	0.2135	0.2146	0.2009	0.1714	0.2489	0.2383	0.2136	0.2355	0.2131	0.2009

*: Contrast corresponds to the comparisons carried out on statistical analysis. Being C1: (T1 xT2); C2: (T1 x T3); C3: (T1 x T4); C4: (T1 x T5); C5: (T2 X T3); C6: (T2 x T4); C7 (T2 x T5); C8: (T3 x T4); C9 (T3 x T5); C10 (T4 x T5).

Alali et al. (2013) tested the effectiveness of organic acids through drinking water against birds with *Salmonella* Heidelberg. To verify the effectiveness of these organic acids in the control of *Salmonella*, trawl swabs were collected at 14 days of age. As a result of these analyses, they observed that organic acids were not efficient in controlling *Salmonella* Heidelberg infection in broilers, results similar to this work.

Bassan et al. (2008), while adding organic acids and Mananoligossacharides (MOS) in feed and treated chickens challenged with *Salmonella* enteritidis, observed similar results to those found in this study. Their research found a 40% reduction in positivity in the group treated with organic acids and a 20% reduction in the group treated with MOS, in the evaluation of feces and cecal tonsils, at the age of 25 days old.

Pickler et al. (2012), when assessing the presence/absence of *Salmonella* enteritidis in swabs after 48 h of inoculation in groups treated with organic acids, found that there was no significant difference, but after seven days, the mixture of organic acids proved effective in controlling Salmonella enteritidis colonization in the cecum of birds.

The result of the analysis of biological material from the necropsy performed at 31 days of age was positive for *Salmonella* Heidelberg in all challenged groups. No statistical difference was observed among them. Byrd et al. (2001), evaluating the effectiveness of a blend of organic acids administered during the preslaughter fasting on birds previously challenged with Salmonella Typhimurium, found a significant reduction in positive samples, as well as in bacterial count of *Salmonella* Typhimurium in the groups treated with a blend of organic acids compared to the control group. This highlights the importance of using organic acids in the last days of the lot, and also during the preslaughter fasting on these lots when there is positive confirmation for any serotype of *Salmonella*.

Similar results were found by Piva et al. (2007), who observed that using a mixture of organic acids (malic acid, citric acid, and sorbic acid) protected via the microencapsulation process of fat, showed a slow release of the active ingredients in the animals' intestines, preventing rapid dissociation of organic acids.

Most of the literature regarding control of other *Salmonella* serotypes have shown that organic acids are effective in control, especially for *Salmonella* enteritidis (Sterzo et al., 2007).

According to Gauthier (2005), essential oils can be used in association with organic acids to increase the action of organic acids. Essential oils act by damaging the bacterial cell membrane, facilitating the penetration of organic acids.

In the same way, the results of swab tests obtained in this work were not significant to control *Salmonella* Heidelberg compared to the positive control group. Oliveira et al. (2000) reported that the organic acids added to the rations were unable to prevent infection of birds with the serovars of *Salmonella* enteritidis, *Salmonella* Agona, *Salmonella* Infantis, and *Salmonella* Typhimurium when collecting swabs at 15 days of age.

Rúbio et al. (2009) reported that the use of butyric acid protected with vegetable fat was efficient to control fecal excretion of *Salmonella* enteritidis in broilers, compared to the use of organic acid only.

Conclusion

There was a positive effect when using the blend of organic acids added to drinking water and feed, with an improvement in feed conversion of broilers challenged with *Salmonella* Heidelberg after 21 days of age. Regarding live weight, there was no significant effect observed among the treatments.

Based on the results observed in the pool of organs, in wattle samples, and in swab samples, there was no significant effect observed among the test and control groups.

Conflict of Interest

The authors report no conflicts of interest.

Ethics Committee

For the handling of the animals, the norms of the Ethics and Animal Research at the university in question followed the determinations of the Brazilian College of Animal Experimentation (COBEA).

References

Alali, W.Q.; Hofacre, C.L.; Mathis, G.F; Faltys, G. Effect of essential oil compound on shedding and *Salmonella* enteric serovar Heidelberg of colonization in boilers. **Poultry Science**, 92: 836-841, 2013.

Bassan, J.D; Flores, M.L; Antoniazzi, T.; Bianchi, E.; Kuttel, J; Trindade, M.M. Controle da infecção por *Salmonella* Enteritidis em frangos de corte com ácidos orgânicos e mananoligossacarídeo. **Ciência Rural**, 38(7): 1961-1965, 2008.

Byrd, J.A.; Hargis, B.M.; Caldwell, D.J.; Bailey, R.H.; Herron, K.L.; McReynolds, J.L.; Brewer, R.L.; Anderson, R.C.; Bischoff, K.M.; Callaway, T.R; Kubena, L.F. Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. **Poultry Science**, 80: 278-283, 2001.

Borsoi, A.; Moraes, L.L.S.; Salle, C.T.P; Nascimento, V.P Número mais provável de *Salmonella* isoladas de carcaças de frango resfriadas. **Ciência Rural**, 40(11): 2338-2342, 2010.

BRASIL. **Portaria nº 126 de 03 de novembro de 1995. Ministério da Agricultura, Pecuária, do abastecimento e Reforma Agrária. Normas de Credenciamento e Monitoramento de Laboratórios de Diagnóstico das Salmoneloses Aviárias (*S. Enteritidis*, *S. Gallinarum*, S. *Pullorum* e *S. Typhimurium*)**. Diário Oficial da União, 1995.

Calaça, G.M.; Café, M.B.; Andrade, M.A.; Stringhini, J.H.; Araújo, I.C.S.; Leandro, N.S.M. Frangos desafiados experimentalmente com *Salmonella enterica* sorovar Enteritidis e *Eimeria tenella* e tratados com ácidos orgânicos. **Ciência Animal Brasileira**, 20(1): 1-10, 2019.

Campo, M.P.A.; Rabello, C.B.V.; Sakomura, N.K.; Long, F.A.; Kuana, S; Gut, F. Use of fumarínico acid in chicken diets with low energy metabilizável. ACTA **Scientiarum Animal Sciences,** 26(1): 35-39, 2004.

Chaves, L.S. **Frangos de corte de crescimento lento e rápido, oriundos de ovos inoculados com probióticos, submetidos a desafio de *Salmonella* Enteritidis, e jejum após eclosão**. 52 f. Dissertação (Mestrado). Universidade Federal de Goiás, Goiânia-GO. 2007.

Daskiram, M.; Teeter, R.G.; Vanhooser, S.L.; Gibson, M.L.; Roura, E. Effect of dietary acidification on mortality rates, general performance, carcass characteristics, and serum chemistry of broilers exposed to high ambient temperature cycling stress. **Journal Applied Poultry Research**,13: 605-613, 2004.

Davis, A.L.; Curtis, P.A.; Conner, D.E.; **McKee**, S.R.; Kerth, L.K. Validation of cooking methods using shell eggs inoculated with *Salmonella* serotypes Enteritidis and Heidelberg. **Poultry Science**, 87: 1637-1642, 2008.

Garcia, R.G.; Ariki, J.; Mathew, V.M.B.; Kronka, S.N.; Borges, S.A.; Murata, L.S; Field, L.A. Isolated or combined action of organic acids and growth promoter in broiler rations. **Brazilian Journal of Poultry Science**, 2(2):149-154. 2000.

Gast, R.K. *Salmonella* infections. In: Saif, Y.M.; Barnes, H.J.; Fadly, A.M.; Glisson, J.R.; McDougald, L.R.; Swayne, D.E. (Eds.). **Diseases of poultry**. 11th. Ames: Iowa State University Press, 2003. p. 567-613.

Gast, R.K.; Guard-Bouldin, J.R.G.; Holt, P.S; Die, W.R. United States Department of Agriculture, Agricultural Research Service, Egg Safety and Quality Research Unit, Russell Research Center. **Summarized from Avian Diseases**, 51(1): 40-44, 2007.

Gauthier, R. Organic acids and essential oils, the realistic alternative to antibiotic growth promoters in poultry. Anais do I Forum Internacional de Avicultura. Foz do Iguaçu, Paraná: 2005.

Guibourdenche, M; Mikoleit Roggentin, P.M; Fields, P.I.; Grimont, J.; Bockemuhl, P.A.D; Weill, F.X. Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. **Research in Microbiology**, 161: 26-29, 2010.

Grimont, P.A.D; Weill, F.X. Antigenic formula and of the *Salmonella* serovars. **WHO Collaborating Center for Reference and Research on *Salmonella*. Paris: Pasteur Institute**, 9th ed., 2007. 166p.

Lake, R.; Hudson, A.; Cressey, P. Risk profile: *Salmonella* (non typhoid) in poultry (whole and pieces). **Institute of Environmental Science and research Limited**, 212: 1-63, 2002.

Maiorka, A.; Santin, A.M.E.; Borges, S.A.; Opalinski, M.; Silva, A.V.F. Use of a mixture of fumaric acids, citric, ascorbic, latico in initial diets of broilers. **Archives of Veterinary Science**, 9(1): 31-37, 2004.

Oliveira, G.H.; Berchieri, J.R.A.; Barrow, P.A. Prevention of *Salmonella* infection contact intestinal flora using of adult birds and a mixture of organic acids. **Brazilian Journal of Microbiology**, 31(2): 116-120, 2000.

Pickler, L.; Hayashi, R.M.; Lourenço, M.C.; Miglino, L.B.; Caron, L.F.; Beirão, C.B.; Silva, A.V.F.; Santim, E. Evaluation, microbiological histological. And immunologic of broiler challenged with *Salmonella* Enteritidis and Minnesota and treated with organic acids. **Pesquisa Veterinária Brasileira**, 32: 27-36, 2012.

Piva, A.; Pizzmiglio, M.; Morlacchini, M.; Todeschi, M.; Piva, G. Lipid microencapsulation allows slow release of organic acids and natural identical flavors along the swine intestine. **Journal of Animal Science**, 85: 486-493, 2007.

Rahmani, H.R.; Speer, W. Natural additives influence the performance and humoral immunity of broilers. **International Journal of Science**, 4: 713-717, 2005.

Rahimi, S.Z.M.; Shiraz, T.Z.; Salehi, M.A.K.; Torshizi, E.J.; Grimes, L. Prevention of *Salmonella* infection in poultry by specific egg-derived antibody. **International Journal Poultry Science**, 6: 230-235, 2007.

R Core Team. A: the language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2014. Available at: <http://www.R-project.org/>.

Rocha, T.M. **Controle de *Salmonella* Typhimurium em frangos de corte, utilizando composto com ácido benzoico, fumárico, e 2-hidroximetiltilbutanóico**. Dissertação (Mestrado). 65 f. Universidade Federal de Goiás. Goiânia, GO, 2008

Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Abreu, M. L. T.; Rodrigues, P. B.; De Oliveira, R. F. M. Barreto, S. L. T.; Brito, C. O. **Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional**

Requirements. 4th ed. Viçosa: Editora UFV, 2017. 488p.

Rossi, R.M. **Introdução aos métodos Bayesianos na análise de dados zootécnicos com uso do Win BUGS e R.** Maringá: Eduem, 2011. 191p.

Rubio, C.F.; Orfonez, C.; Gonzalez, J.A.; Gallego, A.G.; Honrubia, M.P.; Mallo J.J.; Fouce, R. B. Butyric acid-based feed additives help protect broiler chickens from *Salmonella* Enteritidis infection. **Poultry Science**, 88: 943-948, 2009.

Salazar, P.C.R.; Albuquerque, R.; Takaeda, P.; Trinity Neto, M.A.; Araujo, L.F. Effect of lactic acid and butyric acid, isolated and associates, on performance and intestinal morphology in broilers. **Brazilian Journal Veterinary Research Animal Science**, 45(6): 463-471, 2008.

Sterzo, E.V.; Paiva, J.B.; Mesquita, A.L.; Freitas Neto, O.C.; Berchieri Jr, A. Organic acids and/or compound with defined to control *Salmonella enterica* serovar microorganisms Enteritidis experimental infection in chickens. **Brazilian Journal of Poultry Science**, 9(1): 69-73, 2007.

Stratford, M.; Plumridge, A.; Nebe-Von-Caron, G.; Archer, D.B. Inhibition of conidia by acetic acid mould spoilage and sorbic acid involves different modes of action, requiring modification of the classical weak-acid theory. **International Journal of Food Microbiology**, 136: 37-43, 2009.

Vale, M.M.; Menten, J.F.M.; Morais, S.C.O.; Brainer, M.M.A. Mistura dos ácidos orgânicos fórmico e propiônico em rações para frangos de corte. **Scientia Agricola**, 61(4): 371-375, 2004.

Viola, E.S.; Vieira, S.L. Acidifying organic and inorganic supplementation in diets of broilers under lactic acids supplementation, formic, acetic and phosphoric acid in food or water. **Brazilian Journal of Animal Science**, 37(2): 296-302, 2008.

Zanelato, E.A.; Favero, A.; Oliveira, R.; Huber, M; Borges, S. Ácidos orgânicos como substitutos a antibióticos promotores de crescimento para frangos de corte na fase de crescimento. **Revista Brasileira de Ciência Avícola**, 26(1): 35-39, 2008.