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Different periods of inclusion of acetic acid in broiler drinking water

Diferentes períodos de inclusão de ácido acético na água de bebida de frangos de corte

Diferentes períodos de inclusión de ácido acético en el agua de bebida de pollos de engorde

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Abstract
The objective of this experiment was to evaluate the benefits of acetic acid in different periods of inclusion in broiler drinking water on broiler performance, pH, and microbiota, especially the morphometry of the jejunum of broiler chickens. For this, 240 chickens of the *Cobb*500® lineage of 21 days of age were used and distributed in boxes in an experimental broiler house, considering a completely randomized design, with four treatments and four replications of 15 birds each (experimental unit). The treatments were: T_1 - Control (chlorinated water at 3 ppm); T_2 - chlorinated and acidified water with acetic acid in the period from 21 to 42 days of age; T_3- chlorinated and acidified water with acetic acid in the period from 28 to 42 days of age; T_4 - chlorinated and acidified water with acetic acid in the period from 35 to 42 days of age. The results were analyzed using Analysis of Variance, followed by *Tukey*'s post-test, considering a significance level of 5%. No differences in performance were observed in the period from 21 to 42 days of age, pH, and *Lactobacillus* spp. counts of the crop at 42 days of age and height of villi and jejunum crypt depth in the same age. However, there was a reduction ($P<0.05$) in the enterobacteria count in the crop at 42 days of age, which could contribute to the reduction of intestinal enterobacteria counts and, in turn, reduce the contamination of the carcass of broiler chickens.
Keywords: Acidifiers; Performance; Enterobacteria; *Lactobacillus* spp.; Intestinal morphometry.

Resumo

O experimento objetivou avaliar os benefícios do ácido acético (total) em diferentes períodos de inclusão na água de bebida de frangos de corte sobre o desempenho, pH e microbiota do papo e a morfometria do jejuno de frangos de corte. Para isso, foram utilizados 240 pintos da linhagem *Cobb*500® com 21 dias de idade distribuídos em boxes em um aviário experimental, considerando um delineamento inteiramente ao acaso, com quatro tratamentos e quatro repetições de 15 aves cada (unidade experimental). Os tratamentos foram: T_1 - Controle (água clorada a 3ppm); T_2 - água clorada e acidificada com ácido acético no período de 21 a 42 dias de idade; T_3 - água clorada e acidificada com ácido acético no período de 28 a 42 dias de idade; e T_4 - água clorada e acidificada com ácido acético no período de 35 a 42 dias de idade. Os resultados foram analisados por meio de Análise de Variância, seguido de pós-teste de *Tukey*, considerando nível de significância de 5%. Não foram verificadas diferenças no desempenho no período de 21 a 42 dias de idade, pH e contagem de *Lactobacillus* spp. do papo aos 42 dias de idade e altura de vilo e profundidade de cripta do jejuno na mesma idade. No entanto, verificou-se redução ($P<0,05$) na contagem de enterobactérias no papo aos 42 dias de idade, o que poderia contribuir para a redução da contagem de enterobactérias do intestino e por sua vez reduzir a contaminação da carcaça de frangos de corte.

Palavras-chave: Acidificantes; Desempenho; Enterobactérias; *Lactobacillus* spp.; Morfometria intestinal.

Resumen

El experimento tuvo como objetivo evaluar los beneficios del ácido acético (total) en diferentes períodos de inclusión en el agua de bebida de pollos de engorde sobre el rendimiento, pH y microbiota del buche y la morfometría del yeyuno de pollos de engorde. Para ello, se distribuyeron 240 pollitos Cobb500® de 21 días de edad en corrales en piso en un aviario experimental, considerando un diseño completamente al azar, con cuatro tratamientos y cuatro repeticiones de 15 aves cada uno (unidad experimental). Los tratamientos fueron: T_1 - Control (agua clorada a 3ppm); T_2 - agua clorada acidificada con ácido acético en el período de 21 a 42 días de edad; T_3 - agua clorada acidificada con ácido acético en el período de 28 a 42 días de edad; y T_4 - agua clorada acidificada con ácido acético en el período de 35 a 42 días de edad. Los resultados se analizaron mediante Análisis de Varianza, y la prueba de rango múltiple de *Tukey*, considerando un nivel de significación del 5%. No se observaron diferencias estadísticas en los parámetros productivos, pH y recuento de *Lactobacillus*, altura de vellosidad y profundidad de cripta de yeyuno en el período de 21 a 43 días. Sin embargo, hubo una reducción ($P<0,05$) en el recuento de enterobacterias a los 42 días de edad, lo que podría contribuir a la reducción del recuento de enterobacterias en el intestino y, a su vez, reducir la contaminación de la canal de pollos de engorde.

Palabras clave: Acidificantes; Rendimiento; Enterobacteria; *Lactobacillus* spp.; Morfometría intestinal.

1. Introduction

Brazil invests in technology for the production of chicken meat, and this has been reflected in the increase in exports, which in 2020 reached 4,231 thousand tons (ABPA, 2021). However, the current production systems of broilers, in high density, favor the appearance of infections, demonstrating the need to search for alternatives, such as the supplementation of organic acids.

Organic acids have been used in feed or drinking water not only for improving immunity and digestibility of nutrients but also for contributing to the prevention of diseases of the gastrointestinal tract and, consequently, improve the performance of broilers (Yadav e Jha, 2019).

According to Maiorka et al. (2004), organic acids increase the performance of chickens by improving feed conversion and weight gain, mainly associated with growth promoters. As antimicrobial action, it may be controversial, as they do not present superior efficiency in relation to antibiotics, however, it can be enhanced with associations of other organic acids or with other additives such as oils, enzymes, etc.

The use of organic acid blends (formic acid, acetic acid, and ammonium formate) in drinking water 24 hours before the slaughter of broiler chickens decreased the percentage of broilers positive for *Escherichia coli* and *Hafnia alvei* in the crop and maintained the number of broilers positive for *Lactobacillus* spp when compared to the control group (Fernandes et al., 2014a), demonstrating benefits of its use.

Vinegar (acetic acid) has been used empirically in broilers, because of its low cost and its ability to reduce the pH of the gastrointestinal tract. However, it is necessary to verify if it has benefits in the periods before the slaughter of the animals, not only related to the reduction of pH, but also the improvement of the performance.

Thus, the objective of this study was to evaluate the benefits of acetic acid (vinegar) supplementation in broiler drinking water for the periods from 21 to 42, 28 to 42, and 35 to 42 days of age on the performance, pH, and microbiota of the crop and jejunum morphometry of broilers.

2. Methodology

The experiment was carried out at the Experimental Aviary of the Veterinary Hospital of the Paranaense University - Unipar, Umuarama, Paraná, located at latitude 23º 46'00,19 "and longitude 53º 16 '26,39", after approval in the Ethics and Research Committee Involving Experimentation Animal (CEPEEA) of Unipar under protocol number 29558/2016, for the period of October to November of 2016.

Two hundred forty 21-day-old *Cobb* 500 ® line chicks (mean weight 568g) were used for a 21-day experimental period. The chickens had been previously vaccinated against Marek's disease, Infectious bursal disease, *New Castle* disease and Infectious bronchitis. The experimental design was completely randomized with four treatments and four replicates of 15 birds each (experimental unit).

The treatments were: T_1 - Control (chlorinated water at 3ppm); T_2 - chlorinated and acidified water with acetic acid in the period from 21 to 42 days of age; T_3 - chlorinated and acidified water with acetic acid in the period from 28 to 42 days of age; and T_4 - chlorinated and acidified water with acetic acid in the period from 35 to 42 days of age.

The drinking water was acidified directly into the water box, using one for each treatment, weekly in the dilution of 0.1% vinegar (4% acetic acid). Once a day, the pH of the water was measured and it remained on average at 4.2 in treatments T_2, T_3, and T_4, and the control group – T_1 remained on average at 6.47.

The broiler chickens were housed in the experimental aviary with a positive ventilation system (six fans), composed of 16 boxes of approximately 2.8 m^2 (1.85 x 1.5 m) in wood shaving litter, tubular feeders, and nipple drinkers. The ration and water supply were *ad libitum*. Daily temperatures and humidity were recorded using a thermohygrometer placed inside the shed. Data were measured daily (8:00 am, 10:00 am, 11:00 am, 2:00 pm, 4:00 pm, 6:00 pm, and 8:00 pm). During the experimental period, the temperature and humidity registered were respectively 24.1ºC and 55.1%, with a maximum and minimum temperature of 29.6 and 22.5ºC, and maximum and minimum humidity of 69.2 and 40.6%, respectively.

The rations were formulated according to the composition of the feed proposed by Rostagno et al. (2011) to meet the nutritional requirements of broilers for the growth stages (21 to 35 days) and final (35 to 42 days) (Table 1).

The performance of the groups in the different treatments was evaluated in the period from 21 to 42 days by determination of the means of weight gain (kg), feed intake (kg), and feed conversion (kg/kg). To evaluate the weight gain, the broilers were weighed using 10 grams precision scale in plastic boxes at the beginning and weekly to obtain the weight gain from the periods of 21 to 42 days of age. The feed conversion was calculated using the relation between the feed intake and the weight gain of the period.

Table 1. Centesimal and nutritional composition of experimental rations for broiler chickens for growth phases (21 to 35 days) and final (35 to 42 days).

Ingredient	Centesimal composition (%)	
	Growth**	Final***
Corn	65	69
Soybean meal	30	26
Nucleus*	5	5
TOTAL	100	100
	Nutritional composition	
Metabolizable Energy (Kcal/Kg)	2.921	2.954
Crude protein (%)	18,74	17,22
Total Calcium (%)	0,77	0,74
Available Phosphorus (%)	0,40	0,38
Total Lysine (%)	1,03	0,92
Methionine + Total Cystine (%)	0,73	0,60
Total tryptophan (%)	0,25	0,22

* Nucleus (Tectron, Toledo, PR, Brazil) containing salt, limestone, dicalcium phosphate, DL-methionine, vitamin-mineral premix, enzymes (xylanase, cellulase, protease, β-glucanase, amylase, and phytase), halquinol (antibiotic) and salinomycin (anticoccidial). The vitamin-mineral premix contained the following ingredients per kg of diet: growth**: vit. A, 5,500 UI; vit. D_3, 1,200 UI; vit. E, 10 UI; vit. K_3 1 mg; vit. B_1, 1 mg; vit B_2, 4 mg; vit. B_6, 2 mg; vit. B_{12}, 8 µg; niacin, 28 mg; pantothenic acid 9 mg; folic acid 1 mg; biotin, 40 mg; manganese, 60 ppm; zinc 50 ppm; iron, 40 ppm; copper, 8 ppm; iodine, 1 ppm; selenium, 0,3 ppm; final***: vit. A, 2,000 UI; vit. D_3, 500 UI; vit. E, 6 UI; vit. K_3 1 mg; B_2, 2 mg; vit. B_{12}, 5 µg; niacin, 20 mg; pantothenic acid 7 mg; manganese, 60 ppm; zinc 50 ppm; iron, 40 ppm; copper, 8 ppm; iodine, 1 ppm; selenium, 0.3 ppm. Source: Authors.

At the end of the experimental period (42 days of age), one broiler from each experimental unit (four broilers per treatment) was euthanized using an anesthetic overdose: intramuscular xylazine (IM) - 4 mg/kg and intravenous thiopental (IV) - 25 mg/kg, for collection of material from the crop and subsequent microbiological analysis (counting of enterobacteria and *Lactobacillus* spp.), pH determination and morphometric analysis of the jejunum (villus height and crypt depth).

The pH was measured using the Tecnal® pH-meter Tec-2 equipment. For this purpose, an incision was made in the crop and the electrode of the pH-meter was introduced inside it. After each calibration, the electrode was washed with distilled water and dried with a paper towel, and then it was recalibrated.

A gram of contents was collected for microbiological analysis of the crop and after that nine mL of sterile buffered peptone water (BPW) (dilution 10^{-1}) were added. From the 10^{-1} dilution, serial decimal dilutions were performed, always with the aseptic transfer of 1 mL of the sample to nine mL of BPW (10^{-2} to 10^{-3}) (Pickler et al., 2012 adapted).

The enterobacteria count was performed by inoculating 100 µL of the sample (dilution 10^{-1}) onto *MacConkey* agar, followed by incubation at 36°C for 24h (Brazil, 2003). Three to five typical colonies were selected for confirmation by the oxidase-negative and Gram-negative bacteria characteristics. This result was multiplied by the reciprocal of the dilution and expressed as the number of $CFU.g^{-1}$ (Colony Forming Units).

For counting of lactic bacteria in the crop, the De Man, Rogosa, and Sharpe medium (MRS) were used. Successive serial dilutions (10^{-2} to 10^{-4}) were performed and 1mL aliquots of each dilution were transferred to sterile Petri dishes containing the MRS medium. Then about 15-20 mL of the MRS medium, previously fused and cooled at a temperature of 40 to 45°C, was added. The plates were incubated at 37°C for three days. After incubation, plates were counted with between 30 and 300 colonies. This result was multiplied by the reciprocal of the dilution used and expressed as the number $CFU.g^{-1}$.

For the morphometric analysis of the jejunum of the broiler at 42 days of age, a two-centimeter sample of the jejunum, cut transversally, was collected and washed with saline solution (0.9% sodium chloride) to remove intestinal contents. The samples were fixed in buffered formalin solution (10%), pH 7.2, for processing by the usual histopathology techniques, andstained by hematoxylin and eosin method (HE) (Beçak and Paulette, 1976).

For the morphometric analysis of the jejunum, the images were captured by a digital camera coupled to a trinocular light microscope[1] with 4 x objective, connected to an image analysis system[2] in the Laboratory of Microbiology and Morphology of the Post-graduate Program in Animal Science. Were measured 30 to 60 villi and 30 depth crypts for each repetition and the average of these values was obtained. The villi height was determined having as a parameter the basal region of the villi, coincident with the upper portion of the crypts to their apex. The crypt depth was measured from the base to the crypt transition area: villi. The resulting values were expressed in μm.

The results were tabulated in the Excel 2007 program. The results of the zootechnical performance (weight gain, feed intake, and feed conversion) in the period from 21 to 42 days of age, the pH of the crop at 42 days, the villi height and crypt depth of the jejunum at 42 days of age were first analyzed for their normality and homogeneity of variance. After confirming the normality of the data and homogeneity of variance, the studied variables were submitted to analysis of variance (ANOVA). The counts of enterobacteria and lactic bacteria of the crop at 42 days of age were transformed into Log_{10} and later compared using ANOVA. When pertinent, the *Tukey* test was used to compare averages, after identifying significant differences between treatments in ANOVA. The statistical program used was BioEstat 5.3 (Ayres et al., 2007). For all tests, a significance level of 5% was considered.

3. Results and Discussion

There were no differences in weight gain, feed intake, and feed conversion of broiler chickens from 21 to 42 days of age receiving drink water supplemented with acetic acid in different periods (21 to 42, 28 to 42, and 35 to 42 days of age) (Table 2).

Table 2. Average weight gain (WG) (kg), feed intake (FI) (kg) and feed conversion rate (FC) (kg/kg) of broiler chickens receiving drinking water supplemented with acetic acid at different periods (21 to 42, 28 to 42 or 35 to 42 days of age).

Treatments	WG (kg)	FI (kg)	FC (kg/kg)
T_1 – 3 ppm chlorine control	1.28	3.17	2.38
T_2 – acetic acid in 21 to 42 days	1.39	3.06	2.23
T_3 – acetic acid in 28 to 42 days	1.36	3.08	2.25
T_4 – acetic acid in 35 to 42 days	1.40	2.97	2.12
Mean Standard Error	0.025	0.292	0.0625
P value*	0.1433	0.6269	0.2383

* = Not significant by ANOVA. Source: Authors.

Organic acids have been used in feed or drinking water not only for improving immunity and digestibility of nutrients but also for contributing to the prevention of diseases of the gastrointestinal tract and, consequently, improve the performance of broilers (Yadav e Jha, 2019).

Currently, there are different formulations of organic acids available on the market, which could justify differences in performance results found by different authors.

Studies using blends of different organic acids, with improvements in the performance of broilers were obtained by Fernandes et al. (2014b), Marín-flamand et al. (2014), and Srinivas et al. (2018).

[1] Nikon Eclipse E200. New York, USA.
[2] Motic Image Plus 3.0.B British Columbia, Canada.

Cruz-Polycarpo et al (2020) in their research using citric acids, noticed an increase in the number of gram-positive bacteria in jejunum, concluding that citric and benzoic acids, isolated or associated, do not benefit the nutrition of broilers challenged with E. acervulin.

Concerning acetic acid, Jha and Berrocoso (2015) reported that the acid can be carried to the liver as an energetic substrate for muscle tissue, which would contribute to the improvement of performance. However, in the present work, no differences were observed in the performance of broiler chickens, when acetic acid was supplemented in broiler drinking water in different periods.

Similarly, Seifi et al. (2015) did not observe improvement in the performance of broiler chickens from one to 42 days of age, with the supplementation of different levels of acetic acid (1, 2, 4, and 8%).

Under challenge conditions with *Salmonella Enteritidis*, Pickler et al. (2012) also found no difference in broiler performance (FI, WG, and FC) of broilers from one to 21 days of age that received a commercial organic acid mixture (14% lactic acid, 4.5% fumaric acid, 5.0% citric acid and 1% formic acid) in the feed and a mixture of commercial organic acid in the feed and drinking water (27% fumaric acid, 28% citric acid) concerning negative control group.

However, Calaça et al. (2019) in their studies, evaluated the action of a compound of organic acids (acetic, formic, and propionic) in the proportion of 4 kg per ton of feed of birds challenged with *Salmonella* Enteritidis and *Eimeria tenella*, found little bacterial action in their isolation of SE in the spleen and cecal tonsil compared to groups that did not receive acids in the feed, promoting improvements to intestinal health with positive actions in the control of *Salmonella* Enteritidis together with *Eimeria tenella*.

In broilers of the *Label Rouge* lineage, Frank et al. (2016), evaluated the supplementation of 0.6% of a commercial acidifier (lactic acid 30%, benzoic acid 25.5%, formic acid 7%, citric acid 8%, and acetic acid 7%) in the ration in the period from one to 63 days and found no effect of the acidifier supplementation on FI, WG and FC in diets containing salinomycin as anticoccidial and virginiamycin as a performance enhancer.

The absence of a significant effect when using acetic acid in the present work can be due to the use of the growth promoter during the growth phase (21 to 35 days of age) in all treatments, in the low inclusion (0.1%) of acetic acid in drinking water and in the absence of sanitary challenge during the experimental period. However, it should be noted that in the study (Seifi et al., 2015) of the evaluation of higher levels of inclusion of organic acids (1 to 8%), the authors did not observe improvement in the performance in free rations of growth promoters and anticoccidials.

Concerning the pH of the crop at 42 days of age, no differences were observed about acetic acid supplementation in broiler drinking water in different periods (Table 3).

Table 3. Mean pH of the crop of broiler chickens at 42 days of age receiving drinking water supplemented with acetic acid at different periods (21 to 42, 28 to 42, or 35 to 42 days of age).

Treatments	Crop pH
T_1 - Control 3ppm chlorine	4.74
T_2 – acetic acid 21 to 42 days	4.71
T_3 – acetic acid 28to 42 days	4.70
T_4 – acetic acid 35 to 42 days	4.82
Mean Standard Error	0.207
P value*	0.9892

Source: Authors.

In broilers, the pH of the crop is of great importance in the formation of the bacterial microbiota, since it acts as a barrier for the colonization of the digestive tract (Hinton Júnior et al., 2000).

The reduction in crop pH by organic acids is related to the pH of the acid and the pH of the medium Kim et al., (2005). This low pH in the crop allows the maintenance of lactic bacteria and inhibit the growth of bacteria such as *E. coli*, *Proteus* spp., *Salmonella* spp., *Campylobacter* spp., this being more efficient in the crop than in the proventriculus and gizzard (Hinton Júnior et al., (2000); Chaveerach et al. (2004); Fernandes et al., (2014). This demonstrates the importance of its evaluation as a way to verify possible modifications in the microbiota of the crop. However, in the present study, no differences were observed in the pH of the crop of the broiler chickens that received acetic acid in drinking water.

On the other hand, there was a reduction in the enterobacteria count of the crop (Table 4) for the groups that received acetic acid supplementation in drinking water, but no differences were observed between treatments in relation to the *Lactobacillus* spp count.

Table 4. Mean (Log_{10} mL^{-1}) of the enterobacteria and *Lactobacillus* spp. of broiler chickens' crop at 42 days of age receiving drinking water supplemented with acetic acid in different periods (21 to 42, 28 to 42 or 35 to 42 days of age).

Treatments	Enterobacteria	*Lactobacillus* spp.
T1 - Control 3ppm chlorine	5.69[a]	8.30
T2 – acetic acid 21 to 42 days	1.78b	8.57
T3 – acetic acid 28 to 42 days	3.05b	8.35
T4 – acetic acid 35 to 42 days	3.11b	8.43
P value	0.014	0.6612*

Means followed by different letters differ significantly (P <.05) by the *Tukey* test. * not significant by ANOVA. Source: Authors.

Dibner and Buttin (2002) reported little antimicrobial activity of organic acids at pH 7.3; however, at pH 4.0, all acids (lactic, formic, 2-hydroxy-4- (methylthio) butanoic acid (HMB) had some activity against inoculated *Escherichia coli*.

It should be noted that the intestinal microbiota may vary depending on the number and species of bacteria present, thus a balanced microbiota facilitates the development of beneficial bacteria such as lactic bacteria and inhibits the growth of pathogenic bacteria (Saavedra e Tschermia, 2002).

And as mentioned previously, the pH of the crop is of great importance for the formation of the bacterial microbiota, in which lactic bacteria, which are gram-positive facultative anaerobes, predominate (GUAN et al., 2003; Hinton Júnior et al., 2000).

According to Brazil (2011) and Gonsalvez et al. (2016) pathogenic enterobacteria such as *Salmonella* grow at an optimum pH of 7.0 to 7.5 and the minimum growth pH of enterotoxigenic and enteropathogenic *Escherichia coli* is 4.0, which could justify a reduction in enterobacteria count in the present work.

The result of the maintenance of lactic bacteria and the inhibition of enterobacteria can be explained by the principle of competitive exclusion or another mechanism not elucidated, such as the production of bacteriocins produced by *Lactobacillus* sp, which are peptides resistant to high temperatures that have antibacterial spectrum (OGAKI et al., 2015).

The count of enterobacteria and *Lactobacillus* spp. in the intestine was not evaluated, since they might have been modified with the use of acetic acid in the present study.

Regarding the results of the jejunum morphometry (Table 5), there were no significant differences ($p>0.05$) in the villi height and crypt depth of the animals receiving acetic acid in different periods.

Table 5. Mean ± standard error of villi height (µm) and crypt depth (µm) of the jejunum of broilers at 42 days of age receiving drinking water supplemented with acetic acid in different periods (21 to 42, 28 to 42, or 35 to 42 days of age).

Treatments	Villi height (µm)	Crypt depth (µm)
T_1 - Control 3ppm chlorine	1150.05 ±103.8	110.52 ± 10.5
T_2 - acetic acid 21 to 42 days	1221.46 ±74.3	109.86 ±10.4
T_3 - acetic acid 28 to 42 days	1136.10 ± 101.4	136.49 ±10.4
T_4 - acetic acid 35 to 42 days	1476.52 ± 343.1	112.5 ±21.1
P value*	0.781*	0.4128*

* not significant by ANOVA. Source: Authors.

The absence and inconsistency of response in the jejunum morphometry with acetic acid supplementation in the present study may be due to the absence of sanitary challenge since under these conditions there could be a stimulus for intestinal epithelial proliferation. The justifications for the low sanitary challenge are related to the high viability of the lot, as the lot obtained 100% viability.

Similar results were obtained by Frank et al. (2016), who did not find a significant difference for villus height and crypt depth in the duodenum in broilers of the *Label rouge* line at 63 days of age during the evaluation of the different levels of inclusion of acidifier in relation to the control group.

Similarly, Salazari et al. (2008) did not find a significant effect on jejunum height and crypt depth in broilers of the *Ross* lineage at 39 days of age who received acidifiers (lactic acid, butyric and butyric + lactic acid) when compared to the negative control group or the group that received performance improvement (avilamycin).

Maiorka et al. (2004) also found no significant difference in villi height and crypt depth when evaluating a mixture of organic acids (fumaric, lactic, citric, and ascorbic acid) of male broilers from the *Cobb*® lineage at seven days of age.

Barbieri (2015) did not observe a significant difference in villi height and crypt depth when evaluating the intestinal morphometry of the jejunum of broilers treated with a *blend* of lactic, acetic, and butyric acid and without organic acid at 14, 21, and 42 days of age.

On the other hand, Viola and Vieira (2007) obtained a positive answer regarding the use of two different *blends* of organic acids (1 = lactic acid 52%, formic 1%, acetic 2%; 2 = lactic acid 22%, formic 4%, acetic 2%, citric 4%, orthophosphoric 2.5%, and benzoic 29%) in different proportions in the diet for each stage of broiler chicken when they verified an increase ($p < 0.05$) in the villi height of the duodenum in relation to the negative control group without addition of additives and antibiotics at 14 days of age. According to the authors, the organic acids can control the pathogenic microbiota, and with that, reduce the inflammation and the cellular desquamation, contributing to the increase of the villus height.

4. Conclusion

Supplementation of 0.1% vinegar, containing 4% acetic acid in the periods from 21 to 42, 28 to 42, and 35 to 42 days of age, reduced the enterobacteria count and maintained the Lactobacillus spp. In addition, there was no improvement in poultry performance, neither any change in crop pH or in broiler chickens jejunum morphometry. However, the low cost of the product used justifies further studies. Further studies are needed with the use of different concentrations, treatment periods, and blends in drinking water or feed to evaluate the benefits of organic acids and alternative mechanisms of action, such as the production of bacteriocins in the reduction of enterobacteria, and as a consequence, the modulation of the intestinal microbiota of birds.

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