Organic acids for broilers: Effects on intestinal morphology and growth performance

Ácidos orgánicos para pollos de engorde: Efectos sobre la morfología intestinal y el rendimiento del crecimiento

Ácidos orgânicos para frangos de corte: Efeitos na morfologia intestinal e no desempenho de crescimento

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Abstract

Background: Organic acids and ammonium salts added to drinking water can optimize productivity of broiler chickens. **Objective:** To evaluate the effect of acidifying drinking water on productive performance, blood and intestinal pH, and intestinal morphology of broilers. **Methods:** 1,400 one-day-old broiler chicks were used to evaluate two pH levels (4 and 6) of drinking water during three periods (1-21, 1-28, and 1-42 days of age). The treatments consisted of water added with a blend of formic acid (31%), propionic acid (19%), and their salts ammonium formate (26%), and ammonium propionate (6%) compared to a control group (pH 8). **Results:** Compared to the control, the groups consuming water at pH 6 (0.038 moles) continuously for 42 days improved ($p \leq 0.01$) live weight (2.785 vs 2.691 kg), feed conversion ratio (1.430 vs 1.463 kg/kg), and increased the number of intestinal villi (59.0 vs 55.7). Additionally, blood and intestinal pH was reduced vs the control group (7.75 vs 7.89; 6.32 vs 6.41, respectively). **Conclusion:** The blend of formic and propionic acids and their ammonium salts in drinking water at pH 6 during the complete production cycle of broilers improves performance, increases the number of intestinal villi, and reduces the pH of blood, duodenum, and ileum.

Keywords: *ammonium propionate; ammonium formate; blood pH; broilers; drinking water; growth performance; intestinal morphometry; formic acid; propionic acid.*

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Resumen

Antecedentes: Agregar ácidos orgánicos y sus sales amoniacales en el agua de bebida de pollos de engorde puede optimizar su productividad. **Objetivo:** Evaluar el efecto de la acidificación del agua de bebida sobre el rendimiento productivo, pH sanguíneo e intestinal, y morfología intestinal del pollo de engorde. **Métodos:** Se utilizaron 1.400 pollos de engorde de un día de edad para evaluar dos niveles de pH (4 y 6) en agua de bebida durante tres períodos (1-21, 1-28 y 1-42 días de edad). Los tratamientos consistieron en agua con una mezcla de ácido fórmico (31%), ácido propiónico (19%) y sus sales formiato de amonio (26%) y propionato de amonio (6%) en comparación con un grupo control (pH 8). **Resultados:** En comparación con el control, los grupos que recibieron agua a pH 6 (0,038 moles) continuamente durante 42 días tuvieron mejor ($p \leq 0{,}01$) rendimiento en términos de peso vivo (2,785 vs 2,691 kg) y conversión alimenticia (1,430 vs 1,463 kg/kg) así como un mayor número de vellosidades intestinales (59,0 vs 55,7). Adicionalmente, el pH sanguíneo e intestinal disminuyó vs el grupo control (7,75 vs 7,89; 6,32 vs 6,41, respectivamente). **Conclusión:** La mezcla de ácidos fórmico y propiónico y sus sales de amonio en el agua de bebida a pH 6 durante todo el ciclo productivo mejora los parámetros productivos en pollos de engorde, aumenta el número de vellosidades intestinales y reduce el pH de la sangre, duodeno e íleon.

Palabras clave: *ácido fórmico; ácido propiónico; agua de bebida; crecimiento; formiato de amonio; morfometría intestinal; pH sanguíneo; pollos de engorde; propionato de amonio; rendimiento.*

Resumo

Antecedentes: Recomendações para a aplicação de mistura de ácidos orgânicos e sais de amônio na água potável são necessárias para otimizar a produtividade em frangos de corte. **Objetivo:** Avaliar o efeito da acidificação da água de bebida no desempenho produtivo, pH sanguíneo e intestinal e morfologia intestinal. **Métodos:** 1.400 pintos de corte entre 1 e 42 dias de idade foram utilizados para avaliar dois níveis de pH (4 e 6) na água de beber entre três períodos (1-21, 1-28 e 1-42 dias de idade) de tratamentos em água com uma mistura de ácido fórmico 31%, ácido propiônico 19%, e seus sais formato de amônio 26% e propionato de amônio 6% em relação ao controle (pH 8). **Resultados:** Os grupos que receberam água continuamente pH 6 (0,038 mols), por 42 dias tiveram melhor desempenho em relação ao controle ($p \leq 0{,}01$) em termos de peso vivo (2,785 vs 2,691 kg) e conversão alimentar (1,430 vs 1,463 kg/kg), bem como aumento do número de vilosidades intestinais (59,0 vs 55,7), sangue e pH intestinal reduzidos vs controle (7,75 vs 7,89; 6,32 vs 6,41). **Conclusão:** A mistura dos ácidos fórmico e propiônico e seus sais de amônio na água de bebida em pH 6 durante o ciclo completo de produção melhorou os parâmetros de produção em frangos de 42 dias de idade e aumentou o número de vilosidades intestinais e uma redução no sangue, duodeno, e pH do íleo.

Palavras-chave: *ácido fórmico; ácido propiónico; água potável; desempenho; formato de amônio; frangos de corte; morfometria intestinal; pH sanguíneo; propionato de amônio.*

Introduction

There is growing interest on organic acids and their salts (OA+AS) as an alternative to antibiotic growth promoters in broiler production. However, research examining the potential effects of organic acids has been contradictory; some studies reporting positive effects (Paul *et al.*, 2007; Menconi *et al.*, 2014; Adhikari *et al.*, 2020), and others no significant improvement (Açıkgöz *et al.*, 2011; Araujo *et al.*, 2019). Different combinations of organic acids and their efficacy compared to other additives, such as probiotics and essential oils, have been tested. Organic acids have demonstrated to alter the bacterial cell membrane that, in turn, affects energy pathways (Ricke, 2003). These effects could be beneficial for controlling a broad range of pathogens, including those of importance for the poultry industry, such as *Campylobacter, Clostridium, E. coli* and *Salmonella* (Polycarpo *et al.*, 2017). The addition of organic acids to drinking water provided to broilers can be used to improve water quality, as well as control the microbiome population in the upper parts of the intestinal tract and reduce bacterial overload in the intestines. However, the amounts and conditions needed to maximize the beneficial effects of organic acids have not been established (Oviedo, 2006).

Ammonium salts used with the mixture of organic acids function as their vehicles, increasing the antimicrobial effect in the intestine, including the development of intestinal villi (Paul *et al.*, 2007). A blend of formic and propionic acids increased the population of beneficial bacteria, such as *Lactobacillus spp*. (Nava *et al.*, 2009), which produce lactic acid that provides nutrients for enterocytes and promotes trophic effects on the intestine. Furthermore, organic acids alter the gastrointestinal pH, acidifying the crop, proventriculus and gizzard (Broom, 2015) while also enhance digestive enzymes activity and reduce chelate formation of electrostatically charged molecules in the diet with citric acid (Vieira *et al.*, 2018). Together, these benefits improve nutrient digestion and absorption that translate to increased poultry performance.

The effects of titrating drinking water with organic acids to obtain different pH values and different time periods of inclusion during broiler production have not been fully studied. Therefore, the objective of this study was to evaluate production parameters of broilers offered organic acids and their salts in the drinking water to determine the pH value and the time for supplementation. Intestinal morphology and pH values in the intestinal tract and blood were evaluated to optimize a blend of organic acids (formic acid 31%, propionic acid 19%), and their ammonium salts (ammonium formate 26%, and ammonium propionate 6%) (OA+AS) (Novus International, Mexico) in water for improved broiler performance.

Materials and Methods

Ethical considerations

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Experimental Trial

Treatments evaluated the interaction between two drinking water pH values: 4 and 6; and three application periods of OA+AS, as follows: from day 1 through day 21, from day 1 up to day 28, and from day 1 up to day 42. A control diet with no organic acids supplementation in water was used; the control water was pH 8. A total of 1,400 one-day-old Cobb 500 broiler chicks were randomly distributed across seven treatments, with four replicates of 50 chicks per treatment. The study was conducted in a research house for broilers (11 x 20 m) on an experimental farm located in Michoacán state, Mexico. The house had a capacity of 28 (2.5 x 1.8 m) pens, for a density of 11 broilers/m^2. Each pen had two hanging feeders with a base of 45-cm diameter; the capacity of each feeder was 10 kg. Each pen also had one Plasson cup drinker (Plasson SA, Queretaro, Qro, Mexico) connected to a 20-L water reservoir that allowed measurement of water consumption. Wood shavings were used as new litter, and the recommended lighting program and temperature control for the genetic line were used (Vantress, 2012).

All diets were formulated to meet the requirements for the Cobb genetic line and involved three feeding phases: 0-21 days for Starter, 22-35 days for Grower 1, and 36-42 days for Grower 2 (Avila, 2018). Diets were corn-SBM based with no added growth-promoting antibiotics. Health programs for all treatments were identical, including a Marek vaccine that was applied at the hatchery and Newcastle Disease vaccination at the farm when the chicks were 8- and 25- days-old. The OA+AS were included in the water reservoir for each pen.

To determine the molar concentration of the OA+AS, the molecular weight of each organic acid was calculated in millimoles (mM) based on a published procedure (Brown, 2002). The required amount of solution was then divided by the molecular weight to obtain moles per kg of solution. This result was divided by the specific gravity of the blended organic acids (1.1 kg/m^3) to obtain molar concentration (g/L). To determine the quantity of moles used, the molar concentration (g) was multiplied by the dose for each pH, divided by 1,000. The dose for pH value of 4 and 6 was determined in the reservoirs by titration using a potentiometer (HI-98127, Hanna Instruments SA de CV, Ciudad de México, México). For pH 4, 1.0 L/1,000 L of product was prepared, resulting in a concentration of 0.128 of moles; for pH 6, 0.3 L/1,000 L of product was prepared with a concentration of 0.038 moles.

Growth performance

All treatments were evaluated for up to 42 days for their effect on growth performance. Body weight (BW), feed consumption, water consumption, and mortality were recorded at 21, 28, 35 and 42 days of age. Feed conversion ratio (FCR) was calculated taking into consideration BW of mortality as the ratio between feed consumption and BW for the same age periods.

pH and intestinal morphometry

When the chicks were 40 days-old, the pH in blood (2 ml/sample) from the aortic artery was measured on one broiler per replicate for all treatments. At day 27 of age, in one broiler per pen the pH of the duodenum and ileum was evaluated. pH values were determined using a Fisher Scientific AB15/15+ potentiometer (Fisher Scientific, Waltham, MA, USA).

At 21 days of age, four broilers per pen were randomly selected from all treatment groups and slaughtered according to the Official Mexican Standard (NOM-033-ZOO-1995, NOM, 1995). Tissue samples (2 cm) were collected in the descending loop of the duodenum and for the ileum 1 cm prior to Mekel's diverticulum. The samples were fixed in standard 10% formalin and then stained with hematoxylin-eosin. For each sample, three intestinal villi and adjacent Lieberkühn crypts were evaluated in terms of length, width, and number (in 1,000,000 μ^2) using the Motic Images Plus 2.0 program (Routine Software Series, Motic Asia, Hong Kong). The following formula was used to determine villi surface area: (length villi x villi width x π) x number of villi / measurement area (1,000,000 μ^2).

Statistical analysis

Performance data were analyzed as a one-way random analysis of variance using the GLM procedure in StatSoft, Inc (2011) software, version 10.0 (St. Tulsa, OK, USA), and statistical significance among treatments was evaluated with a Tukey test using a level of 0.05. Variables measured as percentages were transformed to arccosine for analyses.

Results

At 28 days of age, broilers in the different treatment groups showed statistically significant differences in body weight gain (Table 1). Higher BW were observed for broilers given pH 6 water through 28 and 42 days compared to those receiving pH 4 water through 21 and 28 days of age. On day 35, the group that continuously received pH 6 water had increased BW compared to treatments receiving pH 4 water or the control pH 8 water lacking organic acid. Broilers receiving pH 6 drinking water had increased BW up to day 42 compared to those given control pH 8 water

Table 1. Body weight (kg; mean±SE) of broilers given acidified water at pH 4 or 6 for three time periods.

Treatment	**Age (days)**			
	21	**28**	**35**	**42**
Control pH 8	0.864 ± 0.012[a]	1.477 ± 0.007[ab]	2.239 ± 0.006[b]	2.691 ± 0.015[b]
pH 4 -42*	0.819 ± 0.010[a]	1.439 ± 0.016[abc]	2.218 ± 0.012[b]	2.715 ± 0.017[ab]
pH 6 -42*	0.864 ± 0.008[a]	1.496 ± 0.009[a]	2.305 ± 0.013[a]	2.785 ± 0.018[a]
pH 4 -28*	0.818 ± 0.013[a]	1.415 ± 0.007[c]	2.232 ± 0.013[b]	2.701 ± 0.019[ab]
pH 6 -28*	0.830 ± 0.005[a]	1.457 ± 0.013[abc]	2.243 ± 0.009[ab]	2.714 ± 0.013[ab]
pH 4 -21*	0.833 ± 0.015[a]	1.431 ± 0.008[bc]	2.243 ± 0.011[ab]	2.683 ± 0.020[b]
pH 6 -21*	0.846 ± 0.015[a]	1.475 ± 0.006[ab]	2.266 ± 0.011[ab]	2.748 ± 0.011[ab]
Probability	0.124	0.001	0.001	0.004

Different superscript letters [(a, b, c)] within columns indicate significant differences among treatments ($p \leq 0.01$).
*42, 28, 21: days of consumption of water that included the OA+AS (organic acids and their salt). SE= standard error.

and those for which pH 4 water was discontinued at 21-days-old ($p \leq 0.05$).

No significant differences were found in feed consumption among treatments through all phases of evaluation (Table 2).

FCR were not different among treatments through 35-days-old (Table 3). At 42-days-old, the group that continuously received pH 6 water had a decreased FCR relative to the group that received pH 4 water through 21-days-old. However, the similar feed consumption in the groups translated to statistically similar FCRs among the group regardless of the increase in BW observed for some treatments.

Average mortality (3.29%) was not affected by treatments, suggesting the absence of specific challenges during the trial, which was expected based on the experimental conditions.

Water consumption was higher on day 21 for the group that received OA+AS with pH 6, however, at 42 days consumption of drinking water was similar ($p \geq 0.05$) between treatments, averaging 9.705 L per chicken. At 28 and 35 days of age, only broilers that continuously received water pH 6 - 0.038 mol had higher water consumption than those receiving the same pH with organic acids mixture for 21 days and those that received drinking water with pH 4 - 0.128 mol, until day 28.

The inclusion of the OA+AS in both pH 4 and 6 on water significantly reduced ($p \leq 0.05$) blood pH at 40 days of age compared to the control group with pH 8 (7.89 vs 7.75 on pH 4 and 6). Additionally, broilers receiving water at pH 4 or 6 containing the OA+AS during the first 28 days had higher blood pH at 40 days of age. At 27 days of age, the groups in which OA+AS was added to the water (pH 4 and 6, respectively), had a lower pH in duodenum (6.41, 6.30 and 6.32) and ileum (7.11, 6.94 and 6.95) compared to the control group with pH 8.

Intestinal villi (Table 4) were shorter in broilers in the control group that were given water lacking OA+AS (pH 8) compared to those receiving pH 6 water ($p \leq 0.05$). Meanwhile, villi were wider in the control group and in the group receiving pH 4 water than broilers receiving pH 6 water ($p \leq 0.01$). OA+AS supplementation increased crypt depth of pH 4 and 6 groups compared to the control group ($p \leq 0.01$). The number of villi in 1,000,000 μ^2 was increased in the pH 6 group compared to those receiving pH 4 or the control ($p \leq 0.01$). Meanwhile, there were no significant differences ($p \geq 0.05$) in surface area among treatment groups. In a comparison of intestinal segments, higher values were observed in duodenum samples for villi length and width as well as crypt depth compared to samples from the ileum, although the number of villi was higher in ileum compared to duodenum ($p \leq 0.01$).

Table 2. Feed consumption (kg; mean±SE) of broilers supplied with acidified water at pH 4 or pH 6 for three time periods.

Treatment	Age (days)			
	21	28	35	42
Control pH 8	1.066 ± 0.022[a]	1.920 ± 0.020[a]	3.047 ± 0.020[a]	3.884 ± 0.023[a]
pH 4 -42*	1.041 ± 0.014[a]	1.879 ± 0.013[a]	3.043 ± 0.012[a]	3.926 ± 0.023[a]
pH 6 -42*	1.054 ± 0.005[a]	1.919 ± 0.014[a]	3.081 ± 0.015[a]	3.928 ± 0.013[a]
pH 4 -28*	1.037 ± 0.014[a]	1.906 ± 0.017[a]	3.057 ± 0.019[a]	3.914 ± 0.013[a]
pH 6 -28*	1.031 ± 0.006[a]	1.909 ± 0.014[a]	3.055 ± 0.027[a]	3.895 ± 0.024[a]
pH 4 -21*	1.031 ± 0.022[a]	1.877 ± 0.019[a]	3.043 ± 0.012[a]	3.923 ± 0.014[a]
pH 6 -21*	1.059 ± 0.012[a]	1.940 ± 0.014[a]	3.064 ± 0.017[a]	3.908 ± 0.013[a]
Probability	0.49	0.11	0.75	0.65

Similar letters within columns indicate no significant differences among treatment means ($p \geq 0.05$).
*42, 28, 21: days of consumption of water that included the OA+AS (organic acids and their salt). SE = standard error.

Table 3. Feed conversion ratio (kg/kg; mean±SE) of broilers supplied with acidified water at pH of 4 and 6 for three time periods.

Treatment	Age (days)			
	21	28	35	42
Control pH 8	1.310 ± 0.019a	1.334 ± 0.018[a]	1.384 ± 0.009[a]	1.463 ± 0.009[ab]
pH 4 -42*	1.331 ± 0.023[a]	1.340 ± 0.017[a]	1.396 ± 0.009[a]	1.466 ± 0.006[ab]
pH 6 -42*	1.290 ± 0.013[a]	1.316 ± 0.012[a]	1.359 ± 0.005[a]	1.430 ± 0.006[a]
pH 4 -28*	1.329 ± 0.018[a]	1.384 ± 0.007[a]	1.393 ± 0.008[a]	1.469 ± 0.007[ab]
pH 6 -28*	1.300 ± 0.006[a]	1.344 ± 0.003[a]	1.385 ± 0.012[a]	1.455 ± 0.010[ab]
pH 4 -21*	1.295 ± 0.005[a]	1.347 ± 0.016[a]	1.379 ± 0.007[a]	1.483 ± 0.012[b]
pH 6 -21*	1.309 ± 0.018[a]	1.349 ± 0.009[a]	1.375 ± 0.009[a]	1.442 ± 0.007[ab]
Probability	0.44	0.059	0.10	0.004

Different superscript letters [a, b] within columns indicate significant differences among treatment means ($p \leq 0.01$). *42, 28, 21: days of consumption of water that included the OA+AS (organic acids and their salt). SE = standard error.

Table 4. Intestinal morphometric parameters (mean±SE) in 20 days-old broilers supplied with acidified water.

Treatment	Length (µm)	Width (µm)	Crypt Depth (µm)	No. of Villi/ 1,000,000 μ^2	Area (μm^2)*
Control pH 8	702.0 ± 48.8[b]	73.5 ± 0.7[a]	104.5 ± 4.4[b]	55.7 ± 2.0[b]	8.13 ± 0.4[a]
pH 4	719.2 ± 49.2a[b]	73.7 ± 1.5[a]	114.1 ± 5.6[a]	54.8 ± 2.0[b]	8.41 ± 0.5[a]
pH 6	735.8 ± 46.2[a]	68.8 ± 0.9[b]	116.9 ± 5.4[a]	59.0 ± 2.1[a]	8.61 ± 0.4[a]
Probability	0.044	0.001	0.001	0.001	0.170
Intestinal segment					
Duodenum	1,042.2 ± 9.9[a]	77.2 ± 0.6[a]	146.0 ± 1.6[a]	43.8 ± 0.5[b]	11.03 ± 0.2[a]
Ileum	395.7 ± 5.0[b]	66.8 ± 0.6[b]	77.6 ± 0.7[b]	69.3 ± 0.7[a]	5.73 ± 0.1[b]
Probability	0.001	0.001	0.001	0.001	0.001

Different superscript letters (a, b) within columns indicate significant differences among treatment means ($p \leq 0.001$).
*Measurement area (1,000,000 μm^2). SE= standard error.

Discussion

The addition of OA+AS to drinking water given to broiler chickens may be beneficial for enhancing growth and could be a viable alternative to the use of other growth promoters like antibiotics. The ammonium salts used with the mixture of organic acids are a complement that facilitate their inclusion in water or feed by not reacting with metals; in this way, when the free organic acids and salts reach the proventriculus, the acidic pH of the gastric juice triggers a reaction that transforms them into free acids and, from this point on, follow the same metabolic route as free organic acids, maintaining a synergistic effect on intestinal health (Paul *et al.*, 2007).

However, the optimal OA+AS needed to maximize growth has not been fully determined. Al-Mutairi *et al.* (2020) reported that broilers given water containing either 2% formic acid or 2% butyric acid exhibited reduced BW, but the combination of these two organic acids did not negatively impact growth performance. Results of another study indicate that supplying formic and propionic acids in their ammonium salts (formate and propionate), at up to 3% in feed, did not impact broiler performance by 42 days of age (Paul *et al.*, 2007). In the present study, the percentage of OA+AS given in the water was 0.03%, which is substantially lower than that used in previous studies.

Other studies showed that changes in BW are related to the type of organic acid used and the pH of the water obtained by titration. Açıkgöz *et al.* (2011) and Ao (2005) reported negative responses in BW in broilers given water containing formic and citric acids to yield a pH of 4.5 and 3.26, respectively. In the current study, we used a blend of formic and propionic acids at a molecular concentration of 0.128 and 0.038 moles for pH 4 and 6, respectively, and saw similar positive results to those reported in a study by Eftekhari *et al.* (2015), which evaluated the effects of the same blend, but did not report the molecular concentration of organic acids.

Vale *et al.* (2004) examined the effects of including 0.5, 1.0, 1.5 and 2.0% of a mixture of propionic and formic acid in feed, reporting that at 2.0% organic acid growth was negatively impacted, but 0.5, 1.0 and 1.5% had no substantial effect on broiler performance up to 42 days.

In terms of mortality, a meta-analysis of 121 studies by Polycarpo *et al.* (2017) showed that antibiotics, but not organic acids, demonstrated significantly reduced mortality rates compared to treatments that lacked antibiotic growth promoters. They further found that inclusion of organic acids did not produce substantial differences in mortality relative to control group and diets that included antibiotics, despite the use of organic acids in the field to support flocks under challenging conditions. It is possible that organic acids incorporated in water rather than feed could have different effects on mortality rates in the field, and the type of microorganism under challenge. The water management program could also impact mortality. Highly acidic water can be unpalatable, and thus the pH can affect water consumption (Tabler *et al.*, 2013). However, Ao (2005) reported no effect on consumption of pH 3.26 water containing citric acid. Furthermore, in the present study we observed that OA+AS supplementation had no effect on feed consumption, similar to that reported by Açıkgöz *et al.* (2010), who supplemented with formic acid and reduced pH from 7.4 to 4.5. Similar results were also reported by Bourassa *et al.* (2018) with the same organic acid. Pinchasov and Jensen (1989) studied how several molecules such as organic acids - including formic and propionic acid- affected feed consumption, concluding that only propionic and acetic acids tend to depress appetite. Together, these results highlight the importance of adhering to recommendations for each product, not exceeding the recommended dose in water or feed.

In terms of gastrointestinal pH, Al-Tarazi and Alshawabkeh (2003) found a reduction in pH in the crop and cecum of broilers given water supplemented with formic and propionic acid,

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whereas Açıkgöz *et al.* (2011) saw no reduction in crop pH following supplementation of formic acid in the drinking water.

The results of the present study demonstrate that including OA+AS in water could reduce pH in blood, which contributes to the physiological normal status of the bird and could be considered as a tool to mitigate the effects of heat stress (Sandercock *et al.*, 2001).

There were differences in the length, width, crypta and number of villi between treatments and anatomical regions evaluated; however, when analyzing the calculated area, a numerical difference, although no statistically, to a greater surface area (p=0.17) was observed in the group with better performance (pH 6) despite presenting a lower villi width, which could be related to the degree of cellularity, with lower infiltration of inflammatory cells in the lamina propia (Maisonniers-Gomez *et al.*, 2003). This suggests little cellularity, characteristic of a functional structure and no inflammatory processes, coinciding with the results of Arce *et al.* (2020). However, it is important to point out that a greater height of the intestinal villi is not synonymous with a greater absorption surface (Yamauchi, 2007). Therefore, in the present study, several traits were considered to obtain the absorption area or capacity of the intestinal morphology as a whole.

The results obtained are consistent with previous studies reporting that formic acid alone or in combination with propionic acid in feed was associated with positive effects on intestinal morphometry (García *et al.*, 2007; Senkoylu *et al.*, 2007). This effect has been related to the ability of organic acids to promote beneficial microorganisms in the gastrointestinal tract; however, this was not an objective of this work. These microorganisms produce short-chain fatty acids that promote proliferation and growth of enterocytes (Dibner and Buttin, 2002).

Organic acids and their salts promote intestinal health by acidifying the cytoplasm of pathogens (Ricke, 2003). Additionally, they may have positive effects on digestion by increasing enzyme secretion and improving absorption of dietary minerals (Dibner and Buttin, 2002), which together enhance weight gain.

These findings would explain the growth promoting effect found in the present study for groups that received water with pH 6 up to 42 days of age. Sanitization of water in a holistic approach can decrease microbial concentrations through use of correct cleaning procedures and sanitizers such as chlorine, which is commonly used in field conditions. However, chlorine must be present as hypochlorous ions to act as a strong sanitizer. In this form, chlorine affects membrane permeability of microorganisms, particularly Gram negative bacteria, reducing the pathogenic activity of endotoxins and exotoxins (Rosen and Klebanoff, 1982). Reductions in pH can increase the proportion of bound chlorine, and thus acidification is a useful strategy to reduce the amount of chlorine required for sanitization. Furthermore, a drop in pH with the use of organic acids can stabilize chelating molecules present in feed as phytic acid, which in turns improves the efficiency of phytase, trace minerals and vitamins (Vieira *et al.*, 2018).

Hamid *et al.* (2018) reported that a discontinuous supply of formic and propionic in water produced the same performance in broilers as a continuous supply for up to 42 days. They concluded that acidification of drinking water from pH 7.8 to 4.2 achieved comparable production results to antibiotic growth promoters, mainly by establishing gastric acidity and reducing total aerobic bacteria count. In the present study, pH 6 water (0.038 moles) given continuously for 42 days resulted in better weight gain with no change in feed consumption and improved FCR. Intestinal morphological results demonstrated a positive trophic effect on intestinal cells in groups given pH 6 water, even relative to pH 4 water, which could probably reflect modulation of the microbiome, since pH 6 would allow selection of beneficial populations that promote intestinal cell growth as demonstrated (Nava *et al.*, 2009), with a blend of formic and propionic acid.

This intestinal cell growth promotes maturation of intestinal cells that produce essential enzymes for digestive processes and nutrient absorption that also improves feed conversion efficiency. Also, a physiological benefit was obtained using pH 6 water through a decrease in blood pH without any effect on water and feed consumption. Water having pH 4-5 is commonly used in the field based on *in vitro* studies that demonstrated an inhibitory effect on bacterial populations like *Campylobacter* (Chaveerach *et al.*, 2002), and in an *in vivo* evaluation of *Salmonella* (Bourassa *et al.*, 2018). However, under experimental conditions that involve no microbial challenges and reduced stress factors than experienced in commercial conditions, our findings indicate that better results could be obtained with pH 6 water and demonstrate that different recommendations should be followed according to the OA+AS used.

In conclusion, inclusion of OA+AS in drinking water at pH 6 (concentration of 0.038 molar) for 42 days improves body weight gain and FCR in broilers not exposed to challenge conditions. The beneficial effects of the OA+AS mixture on performance are related to improvement in length and number of intestinal villi, as well as in absorption area, and reduction in blood, duodenum and ileum pH.

Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest with regard to the work presented in this report.

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Author contributions

MYSG, JAM, EAG and CLC participated in the experimental design and financial support of the experiment; MYSG, LGT and JHC contributed to sample collection and measurement of performance variables, MYSG, JAM, EAG, CLC and JHC contributed to the preparation and final revision of the manuscript.

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