This unedited manuscript has been accepted for future publication. The manuscript will undergo copyediting, typesetting, and galley review before final publication. Please note that this advanced version may differ from the final version.

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

Magda Y Serrano-Gamboa1; José Arce-Menocal2; Ernesto Ávila-González3; Carlos López-Coello4; Luis Garibay-Torres2; José Herrera-Camacho1.

1Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, México.
2Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás Hidalgo, Morelia, México.
3Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México.


Received: August 11, 2021; accepted: May 20, 2022

*Corresponding author. Posta Zootecnia. Km 9.5 Carretera Morelia-Zinápécuaro. Colonia el Trébol, Tarímbaro, Michoacán CP 58880. Tel.: +52 4433223500, ext 5206. E-mail: jose.camacho@umich.mx

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

© 2022 Universidad de Antioquia. Published by Universidad de Antioquia, Colombia.
eISSN: 2256-2958

Rev Colomb Cienc Pecu
Abstract

Background: Recommendations for the application of organic acids blend and ammonium salts in drinking water are needed to optimize productivity in broiler chickens. Objective: To evaluate the effect of the acidification of drinking water on the productive performance, blood and intestinal pH, and intestinal morphology. Methods: 1,400 broiler chicks between the ages of 1- and 42-days-old were used to evaluate two pH levels (4 and 6) in drinking water between three periods (1-21, 1-28 and 1-42 days of age) of treatments in water with a blend of formic acid 31%, propionic acid 19%, and their salts ammonium formate 26%, and ammonium propionate 6% compared to control (pH 8). Results: Groups that consume water pH 6 (0.038 moles) continuously for 42 days improved vs the control (p≤0.01) live weight (2.785 vs 2.691 kg) and feed conversion ratio (1.430 vs 1.463 kg/kg), as well as increased numbers of intestinal villi (59.0 vs 55.7), blood and intestinal pH reduced vs control (7.75 vs 7.89; 6.32 vs 6.41). Conclusion: The blend of formic and propionic acids and their ammonium salts in drinking water at pH 6 during the complete production cycle improved production parameters in 42-day-old broilers and it increased the number of intestinal villi and a reduction in blood, duodenum and ileum pH.

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

Resumen

Antecedentes: Las recomendaciones para la aplicación de una mezcla ácidos orgánicos y sus sales amoniaca en el agua de bebida son necesarias para optimizar los resultados de producción en pollos de engorde. Objetivo: Evaluar el efecto de la acidificación del agua de bebida sobre el rendimiento productivo, pH sanguíneo e intestinal, y morfología intestinal. Métodos: Se utilizaron 1,400 pollos de engorde entre 1 y 42 días de edad para evaluar dos niveles de pH (4 y 6) en agua de bebida en tres períodos (1-21, 1-28 y 1-42 días de edad) de tratamientos en agua con una mezcla de ácido fórmico 31%, ácido propiónico 19% y sus sales formiato de amonio 26% y propionato de amonio 6% respecto al control (pH 8). Resultados: Los grupos que recibieron agua continuamente durante 42 días con un pH 6 (0,038 moles) tuvieron un mejor rendimiento en comparación con el control (p≤0.01) 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), el pH sanguíneo e intestinal disminuyó vs control (7,75 vs 7,89; 6,32 vs 6,41). Conclusión: La mezcla
de ácido fórmico y propiónico y sus sales de amonio en agua de bebida a pH 6 durante todo el ciclo productivo mejoró los parámetros productivos en pollos de engorde de 42 días, aumentó el número de velosidades intestinales y una reducción del pH de la sangre, duodeno e íleon.

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

**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 eintestinal 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 propiónico 19%, e seus sais formiato 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≤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 propiónico 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 de crescimento; formiato de amônio; frangos de corte; morfometria intestinal; pH sanguíneo; propionato de amônio.

**Introduction**

There is growing interest in the use of organic acids and their salts (OA+AS) as an alternative to antibiotic growth promoters in broiler production. However, studies to examine the potential effects of organic acids have produced contradictory results, 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 cell membranes in microorganisms that in turn affects bioenergetic pathways (Ricke, 2003). These effects could be beneficial for controlling a broad range of pathogens, including those of importance to 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).

The 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). Organic acids blend formic and propionic 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 gastrointestinal tract pH to acidify the crop, proventriculus and gizzard (Broom, 2015), while also enhancing activity of digestive enzymes and reducing chelate formation of electrostatically charged molecules in the diet with citric acid (Vieira et al., 2018). Together, these benefits improve digestive processes and nutrient absorption that translate to increased poultry performance.

The effects of using organic acids to titrate drinking water 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 given an organic acid 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
In this study all applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Experimental Trial

Treatments used in this study evaluated the interaction between two drinking water pH values: 4 and 6; and three application periods of OA+AS; 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 the 7 treatments, with 4 replicates of 50 chicks per treatment. The study was carried out in a broiler research house (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/m². Each pen had two hanging feeders with a base having a 45 cm diameter; each feeder had a 10 kg capacity. Each pen also had one cup Plasson (Plasson SA, Queretaro, Qro, Mexico), drinker 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 satisfy 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 for each organic acid was calculated in millimoles (mM) based on a published procedure (Brown, 2002). The required grams of solution were then divided by the molecular weight to obtain the moles per kg of solution. This result was divided by the specific gravity of the blended organic acids (1.1 kg/m³) to obtain the molar concentration in grams per L. To determine the quantity of moles used, the molar concentration in grams 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 per 1,000 L of product was prepared, resulting
in a concentration of 0.128 of moles; for pH 6 was 0.3 L per 1,000 L of product was prepared with a concentration of 0.038 moles.

Live Production Parameters

All treatments were evaluated for up to 42 days for their effect on live production parameters. Body weight (BW), feed consumption, water consumption, and mortality were recorded at 21, 28, 35 and 42 days of age. The 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

pH. 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).

Intestinal morphometry. 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 μ²) using the Motic Images Plus 2.0 program (Routine Software Series, Motic Asia, Hong Kong). The formula to determine the villi surface area was: (length villi x villi width x π) x number of villi / measurement area (1,000,000 μ²).

Statistical analysis

Live production 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 and those for which pH 4 water was discontinued at 21-days-old (p≤0.05).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Control pH 8</td>
<td>0.864 ± 0.012a</td>
</tr>
<tr>
<td>pH 4 -42*</td>
<td>0.819 ± 0.010a</td>
</tr>
<tr>
<td>pH 6 -42*</td>
<td>0.864 ± 0.008a</td>
</tr>
<tr>
<td>pH 4 -28*</td>
<td>0.818 ± 0.013a</td>
</tr>
<tr>
<td>pH 6 -28*</td>
<td>0.830 ± 0.005a</td>
</tr>
<tr>
<td>pH 4 -21*</td>
<td>0.833 ± 0.015a</td>
</tr>
<tr>
<td>pH 6 -21*</td>
<td>0.846 ± 0.015a</td>
</tr>
<tr>
<td>Probability</td>
<td>0.124</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Control pH 8</td>
<td>1.066 ± 0.022a</td>
</tr>
<tr>
<td>pH 4 -42*</td>
<td>1.041 ± 0.014a</td>
</tr>
<tr>
<td>pH 6 -42*</td>
<td>1.054 ± 0.005a</td>
</tr>
<tr>
<td>pH 4 -28*</td>
<td>1.037 ± 0.014a</td>
</tr>
</tbody>
</table>
pH 6 -28* 1.031 ± 0.006a 1.909 ± 0.014a 3.055 ± 0.027a 3.895 ± 0.024a
pH 4 -21* 1.031 ± 0.022a 1.877 ± 0.019a 3.043 ± 0.012a 3.923 ± 0.014a
pH 6 -21* 1.059 ± 0.012a 1.940 ± 0.014a 3.064 ± 0.017a 3.908 ± 0.013a
Probability 0.49 0.11 0.75 0.65

Similar letters within columns indicate no significant differences among treatment means (p≥0.05).

*42, 28, 21: days of consumption of water that included the OA+AS (organic acids and their salt). se= standard error.

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.

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Control pH 8</td>
<td>1.310 ± 0.019a</td>
</tr>
<tr>
<td>pH 4 -42*</td>
<td>1.331 ± 0.023a</td>
</tr>
<tr>
<td>pH 6 -42*</td>
<td>1.290 ± 0.013a</td>
</tr>
<tr>
<td>pH 4 -28*</td>
<td>1.329 ± 0.018a</td>
</tr>
<tr>
<td>pH 6 -28*</td>
<td>1.300 ± 0.006a</td>
</tr>
<tr>
<td>pH 4 -21*</td>
<td>1.295 ± 0.005a</td>
</tr>
<tr>
<td>pH 6 -21*</td>
<td>1.309 ± 0.018a</td>
</tr>
<tr>
<td>Probability</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Different superscript letters a, b within columns indicate significant differences among treatment means (p≤0.01).

*42, 28, 21: days of consumption of water that included the OA+AS (organic acids and their salt). se= standard error.

The 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 the OA+AS with pH 6, however, at 42 days the consumption of drinking water was similar (p≥0.05) between the treatments with an average of 9.705 L per chicken. At 28 and 35 days of age, only broilers that
continuously received water pH 6 - 0.038 mol had a higher water consumption than those that received 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≤0.05) the 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 a 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 the 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 that lacked OA+AS (pH 8) compared to those receiving pH 6 water (p≤0.05). Meanwhile, villi were wider in the control group and in the group receiving pH 4 water than broilers that received pH 6 water (p≤0.01). OA+AS supplementation increased the crypt depth of the pH 4 and 6 groups compared to the control group (p≤0.01). The number of villi in 1,000,000 µ2 was increased in the pH 6 group compared to those that received pH 4 or the control (p≤0.01). Meanwhile, there were no significant differences (p≥0.05) in surface area among the 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 the ileum than in the duodenum (p≤0.01).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Crypt Depth (µm)</th>
<th>No. of Villi in 1,000,000 µ²</th>
<th>Area (µm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control pH 8</td>
<td>702.0 ± 48.8b</td>
<td>73.5 ± 0.7a</td>
<td>104.5 ± 4.4b</td>
<td>55.7 ± 2.0b</td>
<td>8.13 ± 0.4a</td>
</tr>
<tr>
<td>pH 4</td>
<td>719.2 ± 49.2ab</td>
<td>73.7 ± 1.5a</td>
<td>114.1 ± 5.6a</td>
<td>54.8 ± 2.0b</td>
<td>8.41 ± 0.5a</td>
</tr>
<tr>
<td>pH 6</td>
<td>735.8 ± 46.2a</td>
<td>68.8 ± 0.9b</td>
<td>116.9 ± 5.4a</td>
<td>59.0 ± 2.1a</td>
<td>8.61 ± 0.4a</td>
</tr>
<tr>
<td>Probability</td>
<td>0.044</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.170</td>
</tr>
</tbody>
</table>

Intestinal segment

<table>
<thead>
<tr>
<th>Segment</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
<th>Crypt Depth (µm)</th>
<th>No. of Villi in 1,000,000 µ²</th>
<th>Area (µm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>1,042.2 ± 9.9a</td>
<td>77.2 ± 0.6a</td>
<td>146.0 ± 1.6a</td>
<td>43.8 ± 0.5b</td>
<td>11.03 ± 0.2a</td>
</tr>
<tr>
<td>Ileum</td>
<td>395.7 ± 5.0b</td>
<td>66.8 ± 0.6b</td>
<td>77.6 ± 0.7b</td>
<td>69.3 ± 0.7a</td>
<td>5.73 ± 0.1b</td>
</tr>
<tr>
<td>Probability</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Different superscript letters (a, b) within columns indicate significant differences among treatment means ($p \leq 0.001$).

*Measurement area ($1,000,000 \text{ } \mu\text{m}^2$). 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 indicated 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. Açıkgoz 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 and found that at 2.0% organic acid the 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), which involved supplementation with formic acid and a reduction in 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 acids, affected feed consumption and concluded that only propionic and acetic acids tend to depress appetite. Together, these results highlight the importance of adhering to recommendations for each product and of not exceeding the recommended dose in the water or feed.

In terms of gastrointestinal tract 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, 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 the 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 zootechnical results (pH 6), despite presenting a lower value in the width of the villi, which could be related to the degree of cellularity, with a lower infiltration of cells of the inflammatory type located mainly in the lamina propia (Maisonnniers-Gomez et al., 2003), which suggests little cellularity, characteristics 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 this work, several structures of the villus were considered to obtain the absorption area or capacity; that would be the important value of the intestinal morphology as a whole.

The results obtained are consistent with previous studies that reported 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, it was not part of the objective of this work. These microorganisms produce short-chain fatty acids that promote the 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 effect of the growth promoter 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 applied 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, and reduces 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. These authors 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 the FCR.
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 production 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.

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

Acknowledgments
The authors are grateful for the collaboration of Dr. Ingrid Y Martinez Rojas in the analysis of the final manuscript and data.

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

Funding
Integración y Desarrollo Agropecuario S.A. de C.V. provided economic support to conduct the study.
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.

References


