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Use of organic acids in water and its effect on productive performance in broiler chicks

Empleo de ácidos orgánicos en el agua de bebida y su efecto en el desempeño productivo en pollos de engorda

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ABSTRACT

In order to evaluate the productive performance, gut health, pH, digestive morphology, blood pH, jejunum resistance (JR) and skin pigmentation (SP) on the employment of an additional mixture of organic acids (OA) into the water, in broilers from 1 to 42 days of age, 1080 chickens were used, distributed in three treatments with nine repetitions of 40 birds. The treatments used: non-acidified drinking water (pH 8) control group (T1), acidification with an OA mixture (formic acid 31%, propionic acid 19 %, ammonium format 26% and ammonium propionate 6%) in a dose of 1.0 L/1000 L of water (T2) to obtain a pH of 4, and 0.3 L / 1000 L of water (T3) for a pH of 6. The results showed ($p \leq 0.01$) higher body weight, lower feed intake, and better feed efficiency for the birds from T3. There were no differences ($p \geq 0.05$) on the values of digestive pH, SP, and intestinal morphology; only showed effects ($p \leq 0.01$) in the width of villi and in the digestive area. Blood pH and JR showed effects ($p \leq 0.01$) among treatments. It is concluded that the acidification of the drinking water in broiler chickens, with the OA mixture at 0.3 L/1000 L is sufficient to obtain better performance.
Keywords: Broilers, organic acids, performance.

RESUMEN

Se evaluó el empleo de ácidos orgánicos (AO) en el agua de bebida en pollo de 1 a 42 días de edad en el desempeño productivo, salud intestinal, pH, morfología digestiva, pH sanguíneo, resistencia de yeyuno (RY) y pigmentación de piel (PP). Se utilizaron 1080 pollos distribuidos en tres tratamientos con nueve repeticiones de 40 aves: Los tratamientos fueron: Uso de agua de bebida sin acidificar (pH 8) grupo testigo (T1), acidificación con una mezcla AO (ácido fórmico 31%, ácido propiónico 19%, formato de amonio 26% y propionato de amonio 6%) en dosis de 1.0 L/1000 L de agua (T2) para obtener un pH de 4 y 0.3 L/1000 L de agua (T3) para un pH de 6. Los resultados mostraron ($p \leq 0.01$) mayor peso corporal, menor consumo de alimento y mejor eficiencia alimenticia, para las aves del T3. No existieron diferencias ($p \geq 0.05$) en los valores del pH digestivo, PP y morfología intestinal; únicamente mostraron efectos ($p \leq 0.01$) en el ancho de vellosidades y en el área digestiva. El pH sanguíneo y la RY, mostraron efectos ($p \leq 0.01$) entre los tratamientos. Se concluye que la acidificación en el agua de bebida en pollos de engorda, con la mezcla AO en 0.3 L/1000 L es suficiente para lograr un mejor desempeño productivo.
Palabras clave: Pollo de engorda, ácidos orgánicos, rendimiento productivo.

INTRODUCTION

The compounds known as organic acids (OA), used in the livestock industry, are aliphatic carboxylic acids formed by carbon, oxygen and hydrogen, they are also called short chain fatty acids (SCFA) or volatile fatty acids; whose structural chain is composed of less than 7 carbon molecules. These compounds are used as one of the alternatives to the use of growth-promoting antibiotics in the poultry industry (Ricke, 2003; Khan and Iqbal, 2016). Commercially, OAs such as propionic acid have been used for more than 30 years to reduce bacterial and fungal growth in food, in order to hygienically preserve its quality; as well as preventing and controlling infections by *Salmonella* spp and *E. coli K88* in birds and their derivatives (Freitag, 2007; Emami *et al.,* 2017). On the other hand, due to the prohibition on antibiotic use in animal feed by regulations in the European community and the United States (Smith, 2011), OAs have proven to be an alternative in f the enteric microbiota modulation and inhibition from intestinal pathogenic bacteria, such as *E coli*, *Salmonella Typhimurium*, and *Campylobacter coli*; in addition to promoting the colonization of beneficial flora such as lactobacilli (Roth *et al.,* 2017; Roth *et al.,* 2019; Bourassa *et al.,* 2018; Mortada *et al.,* 2020).

On the other hand, some studies show the positive impact on the productive parameters, intestinal integrity, immune response and intestinal microbiota (Emami *et al.,* 2017; Polycarpo *et al.,* 2017; Araujo *et al.,* 2018; Nguyen *et al.,* 2018; Adhikari *et al.,* 2020; Al-Mutairi *et al.,* 2020), benefits that have initially been attributed to the effect they have on the environment of the gastrointestinal tract (GIT), in the digestion process and as a source of energy; but mainly as protection of the same. OAs are found naturally in GIT of birds; which include lactic acid in a higher proportion in the small intestine; while propionic, acetic and butyric acids are found mainly in the blind bags; this is due to the fermentation process (Meimandiopur *et al.,* 2011; Khan and Iqbal, 2016; Rhot *et al.,* 2017). The chemical properties, the pH-lowering effects, and the microbial inhibition effectiveness of an acid depend on its pKa value, which is the pH at which OAs are 50% dissociated. The pKa value defines the power of action that OAs can have; the lower its value, it is considered to have a greater capacity to acidify (Freitag, 2007; Khan and Iqbal, 2016).

The most accepted theory on the action mechanism in the bacterial growth inhibition by OAs considers their liposolubility in media with acidic pH. A pH close to 4.5 maintains the compound liposolubility, which allows them to penetrate the bacterial cell and dissociate in its cytoplasm, generating a metabolic imbalance that ends with the microorganism death (Nakal and Siebert, 2003; Immerseel *et al.,* 2006). Propionic acid combined with formic acid when used in food, have shown synergy in the control of *Salmonella spp*, *Campylobacter spp* and *E. coli* (Roth *et al.,* 2017; Emami *et al.,* 2017; Adhikari *et al.,* 2020); as well as zootechnical benefits. The inclusion of formic acid in combination with propionic acid via drinking water (Nhuyen *et al.,* 2018; Adhikari *et al.,* 2020), showed favorable results in the parameters in broilers from 1 to 35 days of age, where included 0.02, 0.03, 0.04, 0.05, and 0.06% organic acids.

However, the information does not mention pH values. Other investigations in chickens used a mixture of organic acids (propionic acid, formic acid and butyric acid), to acidify the water to a pH of 3 to 4.5; The results showed benefits in the productive parameters, intestinal microbiota, immune response, nutrient digestibility and carcass quality (Ghulam *et al.,* 2013; Emami *et al.,* 2017; Polycarpo *et al.,* 2017; Araujo *et al.,* 2018; Nguyen *et al.,* 2018; Adhikari *et al.,* 2020; Al-Mutairi *et al.,* 2020); however, the literature available on the use of these OAs used in drinking water and aimed at demonstrating their benefits on zootechnical performance and intestinal pH is scarce.

The objective of the present work was to evaluate the zootechnical performance of the broiler chicken, to the addition of an OA mixture in the drinking water during its productive life; as well as the digestive pH and morphology of the duodenum, blood pH, resistance to jejunum traction and yellowing of the skin.

MATERIAL AND METHODS

Location

The work was carried out in an experimental poultry farm located in Charo municipality, Michoacán state, at an altitude of 1,940 meters above sea level, with a minimum annual temperature of 16 ºC and a maximum of 18 °C; the maximum rainfall is 800 mm and the minimum is 600 mm (Municipio de Charo, 2020).

Animals

All the procedures used in the handling of animals were approved by the animal care and welfare committee of Veterinary Medicine and Zootechnics Faculty from UNAM.

Experimental design

1080 chicks of both sexes were used (50% male and 50% female), 1 day old of the Ross 308 lineage from a commercial incubator (El Avión), located in Tepic, Nayarit, which were kept until 42 days of age in the months of April and May. They were randomly distributed in three treatments, with nine replications of 40 birds. The treatments consisted in the administration of a mixture of OA, to the drinking water to reach different pH, from the arrival of the chick to the end of the test in a continuous way. The OAs administered were a commercial mixture manufactured by Novus International (Acidomix® AFL, Querétaro, Mexico), composed of a combination of 31% formic acid, 19% propionic acid, 26% ammonium format and 6% ammonium propionate. Prior to the experiment, the water quality of the farm was analyzed by the "National Water Commission" (located in Morelia, Michoacán, Mexico), to know source physicochemical conditions and to corroborate its viability for its use in domestic animals (Table 1). The pH of the water was measured with a Hanna HI-98127 portable potentiometer.

Table 1. Water physicochemical analysis*

Parameters	Units	Admissible levels in birds	Municipal intake	Compliance degree
Temperature	°C		25	
Hydrogen potential	pH	6.5-8.5	8.0	Complies
Electrical conductivity	µohms/cm		526	
Turbidity	UTN		1.4	
Color	Pt-Co		5	
Dissolved oxygen	mg/L		6.4	
Biochemical oxygen demand	mg/L		2.4	
Chemical oxygen demand	mg/L		5	
Settleable solids	mg/L		0	
Total solids	mg/L		415	
Total suspended solids	mg/L		20	
Total dissolved solids	mg/L	1000	395	Complies
Nitrates	mg/L	10	0.2	Complies
Ammoniacal nitrogen	mg/L	60-80	0.5	
Total hardness	mg/L		118.9	Complies
Calcium hardness	mg/L		82	
Magnesium Hardness	mg/L		36.9	
Total alkalinity	mg/L		200	
Alkalinity to phenolphthalein	mg/L		0	
Chlorides	mg/L	200	46.7	Complies
Sulfates	mg/L	125	24.2	Complies
Carbonates	mg/L		0	
Bicarbonates	mg/L	60	200	
Calcium	mg/L		32.8	Complies
Magnesium	mg/L	32	8.9	
Sodium	mg/L	0	66.3	Complies
Fecal coliforms	UFC/100ml		0	Complies

*National Water Comission

Treatments are described, such as:
1) A control without OA or growth promoter; the drinking water network from Charo municipality, Michoacán was used for its consumption, which had a pH of 8.0 without the addition of the OA mixture.
2) Drinking water from the control treatment plus the addition of the OA mixture at a rate of 1.0 liters per 1000 liters of water, to establish 0.128 moles and obtain a pH of 4.0
3) Drinking water from the control treatment plus the addition of the OA mixture at a rate of 0.3 liters per 1000 liters of water, to establish 0.0038 Moles and obtain a pH of 6.0

To calculate the molar concentration of organic acid combination used (Table 2), the molecular weight of each of them was identified, and they were expressed in millimoles (mM) described by Brown, 2002. The grams per kilo of the solution used and divided by the molecular weight to obtain the moles per kilo of the solution, which was divided by 1.1, which is the specific density of acid mixture, (consider that a liter weighs more than a kilo). The molarity in grams per liter of the acid mixture was obtained, which helped to calculate the dose used to lower the pH to 4.0 and 6.0 of the water with a pH of 8.0. The calculation was established by adding a thousand liters of water from the control treatment (pH of 8.0); increasing amounts until pH 4.0 and 6.0 are achieved. The dose of 1.0 liters of the mixture of organic acids used for pH 4.0 and 0.3 liter for pH 6.0 was found. To know the amount of moles used, it was obtained from the total sum of molarity acid mixture multiplied by the dose used divided by 1000. Thus, it was obtained that for pH 4.0 the value was 0.128 and 0.0038 mol for pH of 6.0.

A house 11 m wide x 40 m long was used, with a galvanized sheet roof; with a capacity of 27 floors each, fitted with a bed of wood shavings; two hopper feeders with a diameter of 45 cm at the base with a capacity of 10 kilos each; as well as a round automatic Plasson type drinker, which was connected to a 20-liter graduated jug with a capacity; in which the described amount of OA was added to maintain the pH required daily and which also served to evaluate water consumption per bird. The diets were formulated in a similar way for each of the treatments with a flour presentation; Corn-soybean was used as a base, in three stages (0-21; 22-35 and 36-42 days of age), where the established needs for the broiler lineage were covered (Ávila, 2018), provided to free access (Table 3), without the addition of antibiotics as growth promoters (AGP).
Los tratamientos se describen, como:

Table 2. Calculation of the molar concentration of organic acid combination used to obtain the desired pH in water

Compound	Molecular weight in Millimoles *	g per Kg of solution	Moles x Kg of solution	Molarity g x lt of solution	pH 6 (0.3 lt AFL* / 1000 lt of water)	pH 4 (1.0 lt AFL* / 1000 lt of water)
Propionic Acid	74	190	2.567	2.336		
Formic Acid	46	310	6.739	6.127		
Ammonium Format	63	260	4.126	3.754		
Ammonium Propionate	91	60	0.659	0.600		
total OA		820	14.091	12.817	**0.0038**	**0.0128**

Described by Brown (1) AFL. Product used

The sanitary program was similar for all treatments. On the first day of age in the hatchery the vaccine against Marek was applied, and in the experimental farm two vaccines were applied against Newcastle disease, using La Sota strain administered ocularly (at day 8 and 25 days of age). During the first four weeks the birds were raised in a house equipped with automatic infrared gas brooders, and received a program of natural light. Food and drinking water were freely available.

At 42 days of age, the data from the records of the mixed birds (males and females) of body weight (g), accumulated feed consumption (g), feed efficiency (g/g), and water consumption (liters) were summarized. and mortality (%). 18 males were randomly selected per treatment (2 per replicate), to evaluate the yellow skin pigmentation (in the fat vein) in live birds, with the CR-400 reflactance photocolorimeter (Kónica Minolta Sensing, NJ, USES); under the CIELab scale of the International Committee for Colorimetry.

A blood sample was obtained from each bird from the jugular vein, to evaluate the blood pH. Subsequently, birds´ slaughter was carried out in accordance with the provisions of the Official Mexican Standard, by separating the head from the body, with a sharp object; through a single firm and accurate movement NOM-033-ZOO-1995, Official Mexican NORMA Humanitarian Sacrifice of Domestic and Wild Animals (NOM, 1995). To evaluate the pH *in situ* of the gastrointestinal tract, which was performed immediately after sacrifice, with a Fisher Scientific brand potentiometer model AB15/15 +.

Table 3. Calculated analysis of the diets

Nutrients	1-21 days	22-35 days	36-42 days
Crude Protein (%)	22.00	20.1	18.5
EM. Kcal./Kg.	3025	3185	3210
Lysine (%)	1.38	1.17	1.05
Methionine (%)	0.64	0.59	0.52
Methionine + Cystine (%)	1.00	0.94	0.83
Threonine (%)	0.84	0.78	0.68
Tryptophan (%)	0.27	0.25	0.23
Calcium (%)	1.0	0.94	0.85
Available Phosphorus (%)	0.46	0.40	0.38
Sodium (%)	0.20	0.18	0.17

The evaluation of the histological morphology of the duodenum was performed with 5 cm cross sections of the duodenal loop, which were fixed in 10% formalin for histological processing and stained by the hematoxylin and eosin technique. Once the slide was prepared, the length and width of 5 villi of each sample were measured in microns (μ); in addition to the adjacent Lieberkuhn crypt supported by the Motic Images Plus 2.0 program (Routine Software Series, Motic Asia, Hong Kong). The estimated formula of the area in the duodenum was, length x width of the villi at the middle level of the same (μ^2), divided by 1000. Likewise, the resistance to rupture by traction of the jejunum was evaluated, with the help of a digital dynamometer (IMADA MV 110), in 10 cm sections prior to Meckel's diverticulum; Values expressed in kilograms-force and transformed to the international unit Pascal per square meter.

The means resulting from the productive parameters and mortality, as well as the other variables, were analyzed under an analysis of variance design. The general linear model was used and when there were significant differences ($p \leq 0.05$), between the treatments, the comparison of means was performed by the Tukey test (SAS Institute Inc, 2012). The results expressed in percentages were transformed to the arc-sine proportion, for their analysis.

RESULTS

The results of the zootechnical parameters are shown in Table 4. An effect was found ($p \leq 0.05$) of greater body weight (2.7%), in T2 with respect to the control T1. On the other hand, food consumption decreased ($p \leq 0.01$) in treatment 3, by 3.1% compared to T1 Control and 2.7%, compared to T2; which improved significantly ($p \leq 0.01$), in the feeding efficiency values in relation to T1 in 5.9% and in 3.8% to T2 with pH of 4. Water consumption was lower ($p \leq 0.01$) in the control group (T1), where the OA were not added to the drinking water in relation to the birds that were consuming water with OA (T2 and T3); which represented an increase of 2.23 and 2.42% respectively. There were no effects ($p \geq 0.05$) in the percentage of mortality between the evaluated treatments.

The gastrointestinal pH values did not show significant effects ($p \geq 0.05$), among the treatments studied in the different evaluated segments. As shown in table 5, in general values are seen at the level of the average and/or below, those birds that consumed water with the addition of OA (T2 and T3).

Table 4. Means and standard error of zootechnical parameters and mortality in broilers with OA, in drinking water at 42 days of age

OA (L/1000L)	Body weight (kg)	Feed consumption (kg)	Feed efficiency	Water consumption (L)	Mortality (%)
T1.- 0	2.46 ± 0.011^{b}	4.76 ± 0.022^{a}	0.51 ± 0.01^{b}	7.40 ± 0.015^{a}	6.3 ± 0.8^{a}
T2.- 0.3	2.53 ± 0.007^{a}	4.62 ± 0.016^{b}	0.54 ± 0.0^{a}	7.57 ± 0.029^{b}	5.4 ± 1.5^{a}
T3.- 1.0	2.49 ± 0.016^{ab}	4.75 ± 0.019^{a}	0.52 ± 0.0^{b}	7.58 ± 0.028^{b}	5.8 ± 1.8^{a}
AVERAGE	**2.50 ± 0.009**	**4.71± 0.018**	**0.52 ± 0.01**	**7.51 ± 0.024**	**5.8± 0.8**
Probability	0.006	0.001	0.001	0.001	0.919

a, b. Different literals between the columns show significant differences ($p \leq 0.01$)

Histological measurements of the duodenum (Table 6) did not show differences ($p \geq 0.05$) in the length of villi and in the depth of Lieberkuhn crypts, between the evaluated treatments. In the width of the villi, differences were observed ($p \leq 0.01$), with the highest values in treatment 3, with differences of 23 and 42 microns, in relation to T1 and T2; This effect influenced the results of the calculated digestive area, with the highest values ($p \leq 0.01$), the morphology of the Control treatment with pH of 8 and 4.

Table 5. Gastrointestinal pH values in broilers with the use of OA, in drinking water at 42 days of age

OA (L/1000L)	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Íleon	Cecum
T1.- 0	5.3±0.1	3.1±0.2	3.2±0.2	5.8±0.0	6.0±0.1	7.0±0.1	6.3±0.2
T2.- 0.3	4.8±0.2	3.1±0.3	3.1±0.2	5.6±0.1	5.8±0.1	6.7±0.1	6.0±0.1
T3.- 1.0	5.0±0.3	2.9±0.3	2.6±0.3	5.7±0.0	5.7±0.2	6.8±0.2	6.0±0.1
AVERAGE	**5.0 ± 0.1**	**3.1± 0.2**	**3.0 ± 0.1**	**5.7 ± 0.0**	**5.8± 0.1**	**6.8± 0.1**	**6.1 ± 0.1**
Probability	0.189	0.857	0.219	0.099	0.347	0.325	0.331

PH average ± standard error

Table 6. Histological measurements of the duodenum in broilers with the use of OA, in drinking water at 42 days of age.

Treatments	**Length (μ)**	**Width (μ)**	**Crypts (μ)**	**Area (μ^2)/1000**
OA (L/1000L)	**Average ± standard error**			
	1500 ± 12	121 ± 5[b]	169 ± 8	182 ± 8.0[a]
T1.- 0	1493 ± 36	102 ± 5[c]	150 ± 4	153 ± 8.2[b]
T2.- 0.3	1422 ± 41	144 ± 7[a]	153 ± 9	203 ± 8.9[a]
T3.- 1.0	**1476 ± 18**	**121 ± 4**	**158 ± 4**	**177 ± 5.4**
Probability	0.182	0.001	0.134	0.001

[* * a, b, c.] Different literals mark significant differences ($p \leq 0.01$)

Blood pH was higher ($p \leq 0.02$) in T1, in relation to treatments 3 and 2 respectively, which showed lower blood pH; likewise, the jejunum tensile strength showed effects ($p \leq 0.03$), among the evaluated treatments; being treatment 1 the one that showed the lowest resistance than treatments 3 and 2 respectively, without showing effects ($P \leq 0.05$) among the treatments evaluated in the values of yellow pigmentation in skin, as observed in table 7.

Table 7. Averages of blood pH, jejunum tensile strength and yellow skin pigmentation in broilers with OA use, at 42 days of age

	Blood Values	Jejunum resistance	Yellowing
Treatments	**pH**	**Pascal/m²**	**Deltas**
OA (L/1000L)		**Average ± standard error**	
	7.29 ± 0.05[b]	2.60 ± 0.017[b]	19.17 ± 1.76
T1.- 0	7.06 ± 0.02[a]	3.37 ± 0.019[a]	18.04 ± 1.99
T2.- 0.3	7.16 ± 0.03[ab]	3.30 ± 0.023[ab]	19.45 ± 0.72
T3.- 1.0	**7.16 ± 0.03**	**3.11 ± 0.014**	**18.84 ± 0.909**
Probabilityd	0.002	0.033	0.812

[a, b] = Different literals between the columns show significant differences (p≤ 0.01)

DISCUSSION

The zootechnical benefits obtained with the addition of OA have been verified with different compounds, such as fumaric, formic, acetic and propionic acids, (Ghulam *et al.,* 2013; Broom, 2015; Emami *et al.,* 2017; Al-Mutairi *et al*., 2020); as well as in the mixture of these; where the formic acid combination with acetic acid and propionates has been shown to have a positive effect on productive performance, intestinal morphology, immune response and on the intestinal microbiota (Adil *et al.,* 2011; Polycarpo *et al.,* 2017; Araujo *et al.,* 2018; Nguyen *et al.,* 2018). Historically, these benefits have been initially associated with the native and pathogenic microflora modulation of the intestinal tract, demonstrated by the effect that OAs have in drinking water and food, to counteract the negative effect of pathogenic bacteria, such as *Salmonella spp*. , *E*. *coli*, *Campylobacter spp* and *Clostridium spp*., (Immersel *et al.,* 2006; Emami *et al.,* 2017; Adhikari *et al.,* 2020; Bourassa *et al.,* 2018; Mortada *et al.,* 2020), through colonization of the digestive tract by *Lactobacillus*. These are considered to be resistant to acidic pH, having a protective role in the gastro-intestinal tract (Jin *et al.,* 1998). Broom (2015) mentions that in an acid medium of pH 4.6 the effect of OAs as antibacterials is more effective, based on evaluations carried out *in vitro*, where they conclude that the minimum inhibitory concentration for OAs may vary with the type of bacteria and acid used. Other *in vitro* studies with *Escherichia coli* cultures, (Dibner and Buttin, 2002), add different acids, including hydrochloric, formic, lactic and hydroxy-methionine analog (HMTBa), to acidify the culture medium to pH 4 and pH 7, showed that the antibacterial effect is more efficient with pH 4; in addition to making evident the low microbicidal activity of hydrochloric acid. By using a commercial product that included formic and propionic acid in concentrations

of 50 mM formic acid, bacteria could be sensitized by an osmotic process (Sánchez *et al.,* 2009); however, it can be deduced that the pH level is more important as a criterion in the dosage of acids than their molar concentration.
This behavior in chickens of T3, contrasts with the primary role of the aforementioned microbial modulation arguments, which suggests as more important the effect that OAs have on the digestive process, since the birds were not challenged to any pathogens, nor did they present no pathological process during the test period. First, in the digestive process, the medium acidification allows a greater transformation of pepsinogen into pepsin at the proventriculus and gizzard level (Cuca et al., 2009) and protein digestion is more efficient; in the same way, the work at pancreas level is improved by increasing its secretions, and in its case, better activity of some exogenous enzymes, such as phytases and mananasses (Rafacz *et al.,* 2005).

Angel et al., (2013) refer to a work where they analyze the pH water effect on GIT pH, when comparing two levels (8.1 vs. 5.8), where an effect was had on the acidic environment of each one of the intestine segments, and as a consequence the group with acidified water at pH 5.8, positively affects the digestion of dry matter; as well as the apparent ileal phosphorus digestibility. The authors explain that part of the lower digestion at alkaline pH is due to the lower effectiveness of the phytases included in the diet. When the pH rises above 4, their efficiency tends to decrease due to their optimal operating pH and due to the precipitation of phytate-calcium chelates. On the other hand, calcium absorption is favored by acidification of the intestinal environment (by keeping calcium salts in solution), carboxylic acids such as propionic and formic, react with carbonates to form soluble salts in water and carbonic acid (Brown, 2002), which favors the growth and strength of the bone system (Nourmohammadi, 2013).

In the present work, a greater resistance in jejunal rupture was observed with acidification in T3 at a pH of 6. The benefits of better mineral absorption have been reported; firstly, calcium for the case of bone tissue and trace minerals such as zinc, copper and magnesium; which play an important role in the connective tissue formation. Without forgetting other functions that OAs play in a natural way in metabolism, as a source of energy to intestinal epithelium cells (Boroojeni *et al.,* 2014; Broom 2015; Yang *et al.,* 2019), and their beneficial effects on the immune system of the gastrointestinal tract, such as the activation of lymphocytes and macrophages; as well as a greater development of the thymus and Fabricius bursa (Ghulam *et al.,* 2013; Kim *et al.,* 2013; Emami *et al.,* 2017; Al-Mutairi *et al.,* 2020).

The results of the present study with respect to the intestine histo-morphology, do not show a clear relationship between the differences of each treatment with respect to the evaluated lengths (length, width and crypt), however when analyzing the calculated area of the duodenum, A smaller surface area was appreciated in the group with better zootechnical results (T3), in this regard it is noted that the effect that OAs have on the morphology of the small intestine has been demonstrated by several researchers, most of whom have found a greater length of the villi in different segments of the gastrointestinal tract, with an important correlation with body weight and feeding efficiency (Nourmohammadi, 2013; Emami *et al.,* 2017; Yang *et al.,* 2019), however, García *et al.,* 2007, reported greater height of the villi in the groups added with OA without changes in the calculated area of the villi, but with a significant effect (P <0.01) on efficiency to nutritional and digestibility. It is important to point out that a greater height of the intestinal villi is not synonymous with a greater absorption surface (Yamauchi, 2007); the variations in the width are usually related to cellularity degree, since the greater the width; greater infiltration of inflammatory cells located mainly in the lamina propria (Maisonnier *et al.,* 2003). So in the present study, the birds that were treated with acidified drinking water with a pH of 6, showed a lower calculated area of the villi, which coincides with the zootechnical results of the work of García *et al.*, 2007; which suggests little cellularity, characteristics of a functional structure and no inflammatory processes. Acidification in drinking water also affected blood pH levels among the evaluated treatments (p <0.02); since the values were lower in the birds that were with OA. The influence that food can have on blood pH has been shown, although there is a mechanism that promotes balance to neutrality. In the case of acids in solution, they allow the passage of hydrogen ions through biological membranes, this phenomenon is based on the mechanisms of digestive absorption and ionic exchanges between the digestive and blood compartments (Sturkie, 2000; Meschy, 2015). This possibility of the passage of hydrogen ions opens up an alternative in the control of metabolic alkalosis problems caused by birds' hyperventilation in heat stress states (Sturkie, 2000).

CONCLUSIONS AND IMPLICATIONS

The acidification of drinking water with 0.3L/1000 L of OA (formic acid 31%, propionic acid 19%, ammonium format 26% and ammonium propionate 6%), had a positive effect on the productive behavior in the chicken of fattening; Its beneficial effect lies in primarily facilitating the digestion process, where moderate acidification at a pH of 6 is sufficient to achieve better body weight and feed efficiency. The acidification in the drinking water with OA at 0.03 L/1000 L decreased the blood pH, thus opening an alternative in the control of metabolic alkalosis problems, which can be caused by birds' hyperventilation in heat stress states.

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