Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2021 / v.23 / n.2 / 001-008

http://dx.doi.org/10.1590/1806-9061-2020-1252

Original Article

Effect of a Combination of Propionic-Acetic Acid on Body Weight, Relative Weight of Some Organs, Lactic Acid Bacteria and Intestinal pH of Neonatal Broilers

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■Keywords

Broiler, organic acid, cecal beneficial bacteria, digestive and immune organ, intestinal pH.

Submitted: 27/August/2020
Approved: 04/January/2021

ABSTRACT

This study was designed to determine the effect of a combination of propionic-acetic acid on body weight, the relative weight of some organs, lactic acid bacteria, and intestinal pH of neonatal broilers. A total of 60 1-day-old Ross 308® broiler chickens were randomly placed in metabolic cages to two treatments, three replicates, and ten birds per replicates. The treatments consisted of a control diet (CD) and CD + 0.03% of propionic acid and acetic acid in the drinking water at a rate of 4 ml/L of water. The combination of organic acids depressed the body weight in neonatal broilers ($p<0.05$) and increased the relative weight ($p<0.05$) of gizzard, proventriculus, small intestine, and liver; also acidified the cecum with a significant decrease ($p<0.05$) of the pH. Also, these organic acids increased ($p<0.05$) the count of green bacilli with a white halo in the small intestine and decreased ($p<0.05$) the proliferation of irregular flat green bacilli in the cecum, although for both intestinal portions, the total lactic acid bacteria count was not different ($p>0.05$) between treatments. The combined use in the diet and drinking water of the propionic and acetic organic acids, respectively, reduced the bodyweight of neonatal broilers (10 days) and the cecal pH, as well as modified the relative weights of some digestive organs and the growth of some morphological groups of lactic acid bacteria.

INTRODUCTION

In recent years, global chicken production has been influenced by a growing demand due to the increase in the world population and the greater need for this animal protein, which is cheaper than red meat (FAO, 2017). In 2017, chicken meat accounted for 36.55% of world meat production. For 2020, the production was projected to reach 100.5 million tons of chicken meat, despite the global trade in meat being trimmed due to emerging threats from the spread of the COVID-19 virus, which makes this item an important part of world food production (USDA, 2020). In order to cover the demand, the poultry industry has technified and intensified its processes; however, this has caused an increase in the susceptibility, incidence and severity of bacterial diseases, attributed to the lack of biosecurity measures and the easy propagation of them under intensive conditions (Saleem *et al.* 2015).

For the prevention of diseases, from the 1950s to date, antibiotics have been used in sub-therapeutic doses as growth promoters to improve the daily weight gain of birds (Gadde *et al.*, 2017). Many studies have shown that the use of antibiotics causes bacterial resistance in broilers, and there is a potential risk that this resistance will be transmitted to humans (Fascina *et al.*, 2017). The poultry industry is increasingly facing legislative pressures to eliminate the use of antibiotics, since January 2006, the European Union banned the use of antibiotics in animal feed (Adil *et al*., 2010). The elimination of preventive antibiotics in Europe has led to problems in performance, in the feed conversion rate, and in the incidence of certain animal diseases. This topic has been a subject of discussion throughout the world; researchers and nutritionists have increased their interest in finding other alternatives that eliminate or minimize the use of antibiotics without depressing the growth performance of broiler (Martínez *et al.*, 2013; Gadde *et al*., 2017).

There are different growth promoters of natural origin that do not have residual effects on the final product, such as prebiotics, probiotics, plant extracts and organic acids that have been investigated in poultry, these reduce pathogens and improve the immune response (Vuong *et al*., 2016; Valenzuela-Grijalva *et al*., 2017; Yang *et al*., 2019). Organic acids have been used for more than 30 years, mainly because they have disinfecting effects on the digestive tract and are compounds that occur naturally in cellular metabolism, which have been used as growth promoters (Saleem *et al.*, 2015).

The organic acids have impacts on growth, nutrient utilization, mineral availability, gut microbiota, and disease resistance (Mohammed *et al*., 2018). Dehghani-Tafti & JaHanian (2016) found that the use of organic acids causes non-pathological intestinal acidification that reduces the population of Enterobacteriaceae and increases the secretion of gastric enzymes, the functionality of the intestine and the absorption of nutrients. Therefore, the development and health of the gastrointestinal tract is an essential part of good performance in broilers (Fascina *et al*., 2017). Organic acids, such as propionic and acetic, have been the most used individually in broiler production (Adil *et al*., 2010).

The individual use of propionic and acetic acids has been reported to improve the performance and the health status of birds, as an effective alternative to the use of sub-therapeutic antibiotics (Alshawabkeh & Tabbaa 2001; Haque *et al*., 2009; Attia *et al*., 2013). However, recent researches have focused on combining various organic acids to enhance the functional activity of these compounds. In this sense, studies of Gunal *et al.* (2006) reported a decrease in cecal gram-negative bacteria without positive effects on body weight when they used an organic acid mixture (propionic and formic acids) in broiler diets. Similarly, Dehghani-Tafti & JaHanian (2016) have shown an increase in performance using dietary supplementation with an organic acid mixture (citric + butyric). Meanwhile, Mohammed *et al*. (2018) and Beier *et al*. (2019) have shown that the use of acetic acid in drinking water and propionic acid in the diet improved the growth performance and the health condition of neonatal birds. Despite the beneficial effects of organic acids, to our knowledge, few studies have evaluated the combined use of these organic acids to improve the productivity and physiological activity of broilers. The objective of the study was to evaluate the effect of a combination of propionic-acetic acid on body weight, the relative weight of some organs, lactic acid bacteria, and intestinal pH of neonatal broilers.

MATERIALS AND METHODS

The study was carried out in July 2019, at the Poultry Research Center of the Zamorano Pan-American Agricultural School (Zamorano University), located 30 km southeast of Tegucigalpa, in the municipality of San Antonio de Oriente, department of Francisco Morazán, Honduras. The average annual temperature is 26 °C, and the average rainfall is 1100 mm per year.

A total of 60 1-day-old Ross 308® broiler chickens were randomly placed in metabolic cages to two treatments, three replicates, and ten birds per replicates. The treatments consisted of a control diet (CD) and CD + 0.30% of propionic acid and the addition of acetic acid in the drinking water at a proportion of 4 mL in a liter of water according to commercial house recommendations. We used propionic acid in the feed and acetic in drinking water of broiler according to the findings of Dittoe *et al*. (2008) and Mohammed (2018), respectively. The diets were formulated according to the requirements of the genetic line (Table 1).

The broilers in metabolic cages with dimensions of 0.70 m wide x 1 m long were housed, at the rate of 10 chickens per cage, with a density of 10 chickens/0.7 m^2. Feed and water were offered *ad libitum* in linear feeder and nipple drinkers, respectively. In the drinking water of the control group and propionic+acetic group, the temperature (26.33 ± 0.057 and 26.37 ± 0.057, respectively) and pH (7.42 ± 0.25 and 4.01 ± 0.06, respectively) was determined using portable Multiparameter Meter, Orion Star A3290 (Thermo Scientific) according to APHA methods (1995). Also, bacteriological analysis (negative results) was performed by the Petrifilms method (*E. coli*/Coliform Count Plates-3M™ Petrifilm™ Plates, Minneapolis, USA). During the 10 experimental days, the broilers received 23 hours of light with an intensity of 30-40 lux.

Table 1 – Ingredients and contributions (0-10 days; as fed).

Ingredients	Percentage (%)
Cornmeal (CP, 7.79%)	49.57
Soymeal (CP, 48.0%)	39.54
Mineral and vitamin premix	0.50
Sodium chloride	0.50
Crude palm oil	6.15
Colin	0.08
DL-Methionine	0.38
L-Threonine	0.10
L-Lysine	0.25
Calcium carbonate	1.13
Biophos	1.58
Mycofix plus 5.0	0.12
Enzymes Lumis Lbzyme X50	0.05
Coccidiostato	0.05
Contributions (%)	
Metabolizable energy (kcal/MS)	3000
Crude protein	23.43
Crude Fiber	2.39
Ashes	6.36
Ca	0.96
P available	0.48
Methionine+Cystine	0.95
Threonine	0.86
Valine	0.91
Isoleucine	0.80
Leucine	1.60
Lysine	1.28
Histidine	0.51
Arginine	1.30
Tryptophan	0.24
Phenylalanine	0.80

[1] Each kg contains: vitamin A, 13,500 UI; vitamin D3, 3,375 UI; vitamin E, 34 mg; B2, 6 mg; pantothenic acid, 16 mg; nicotinic acid, 56 mg; Cu, 2,000 mg; folic acid, 1.13 mg; vitamin B12, 34 mg; Mn, 72 mg; Zn, 48 mg.

At 10 days- of age, 10 broilers per treatment were sacrificed by the bleeding method of the jugular vein after six hours of feed fasting (water was offered ad libitum) to collect samples. The viscera (liver and heart), immune organs (thymus, spleen, and bursa of Fabricius), and the intestines (small and large) were removed and a digital scale Truweigh Blaze digital scale BL-100-01-BK with accuracy ± 0.1 g were weighed (Martinez *et al.*, 2013). In the slaughter (10 days- of age), the small intestine and left cecum of 10 broilers per treatment were taken, and the pH was determined using an Oakton® digital pH potentiometer model 700, calibrated with pH buffer solutions at 1.68, 4.01, 7.00, 10.01, and 12.45 (Molina *et al.*, 2019).

Also, the small intestines and right cecums of 10 broilers per treatment were taken, and the mucosa with a scalpel was scraped for microbiological culture. Each sample's cecal content was placed in a sterile tube; weight was recorded and diluted with Butterfield's phosphate-buffered dilution water to a 1:9 ratio (w:v). Diluted cecal contents were homogenized, and serial dilutions (1/10) were made from it until dilution 10^5. Aliquots of 0.1 ml of each dilution were spread plated on the surface of MRS agar (Neogen Acumedia, Mich.) supplemented with methylene blue (0.016 g/1000 ml) at 37 °C with a pH of 5.6 for 48 hours in anaerobiosis (Gas Pak system, BBL, Cockeysville, USA). Counts of lactic acid bacteria were reported as Log CFU/g by colonies' morphology on MRS + MB agar. Gram stain and catalase activity was tested on each type of colonies reported (Molina et al., 2019). The microbiological tests were performed in the Food Microbiology Laboratory of the Zamorano University.

The results are expressed as mean and ± SEM. An unpaired T-student test was performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Table 2 shows the body weight and relative weight of the digestive organs, viscera, and lymphoids in broilers when acetic acid was used in the drinking water and propionic acid in broiler diet (10 days). The use of these combined organic acids significantly decreased ($p<0.05$) the body weight (10 days). However, this combination of organic acids increased ($p<0.05$) the relative weight of the proventriculus, gizzard, small intestine, and liver, although without significant changes ($p>0.05$) among treatments for the relative weight of the cecum, pancreas, bursa of Fabricius, heart, spleen, and thymus.

Table 2 – Effect of a mixture of propionic-acetic acid on relative weight of digestive organs, viscera and lymphoid organs in neonatal broilers (10 days).

Items	Experimental groups		SEM±	*p* value
	Control	Propionic+Acetic		
Body weight (g)	187.97	153.13	7.810	0.005
Proventriculus (g/kg)	0.94	1.30	0.056	<0.001
Gizzard (g/kg)	5.54	6.72	0.261	0.005
Small intestine (g/kg)	8.50	10.22	0.459	0.016
Cecum (g/kg)	1.26	1.51	0.118	0.143
Pancreas (g/kg)	0.66	0.60	0.040	0.336
Heart (g/kg)	0.70	0.73	0.030	0.518
Liver (g/kg)	2.84	3.43	0.12	0.003
Spleen (g/kg)	0.07	0.10	0.009	0.059
Thymus (g/kg)	0.13	0.15	0.017	0.457
Bursa of Fabricius (g/kg)	0.17	0.13	0.013	0.058

The goal of this study was to determine whether the combination of propionic and acetic as one of the most frequent organic acids in the poultry industry could influence any biological indicators in broilers.

The results showed that the group with organic acids depressed the body weight (Table 2), perhaps due to the excessive acidification of the drinking water (4.01) and the cecum of the broilers (Table 3). Authors as Gunal *et al*. (2006), Houshmand *et al*. (2012) and Fascina *et al.* (2017) found no benefits on body weight and intestinal histomorphometry of broilers supplemented with mixtures of organic acids such as fumaric+propionic, formic+lactic+citric, and lactic+benzoic+formic+citric+acetic, respectively. According to Jiang *et al*. (2012) the intestinal villus and crypt are correlated with gut health and growth in birds and are affected by diet and intestinal health. In this sense, Sayrafi *et al.* (2011) when using butyric acid in the diets of chickens found no changes in the gain and villi height in the duodenum and jejunum. However, Adil *et al.* (2010) indicated that the use of fumaric acid and lactic acid improved the performance and increased villus height in the small intestines of broiler chickens. Likewise, Panda *et al.* (2009) reported that the dietary use with 0.4% butyric acid increased the body weight and villus development in broilers; however, a higher supplementation of this organic acid reduced the productive response due to an excessive reduction in intestinal pH and a lower activity of digestive enzymes. Authors such as Kum *et al*. (2010) demonstrated that the use of organic acids increased the villi width, villus height, villus area, and goblet cells in the small intestine. Apparently, a positive response to the use of organic acids in chickens is mediated by intestinal health, especially by changes in the structures of the villi and crypts.

On the other hand, Pinchasov *et al*. (1994) conducted a study to evaluate the anorexic effect of propionic and acetic acids in chicks from 7 to 21 days. The authors found that these organic acids significantly decreased the voluntary intake; the higher the dose, the more the intakes were depressed. It is observed that the combination of the two organic acids caused some digestive disorders, which reduced the use of nutrients in the intestinal lumen, despite the fact that these organic acids acidified the cecum and modified the relative weight of the small intestine, liver, cecum, and gizzard and proventriculus (Tables 2 and 3).

Generally, the positive effects of organic acids reported in the literature are inconsistent. Factors such as the environment, feed palatability, buffer capacity of the diet, concentration of organic acid used, management, gut health, presence of other antimicrobial compounds, water pH, and genetic expression of poultry are factors responsible for the variability of the results (Houshmand *et al*., 2012; Fascina *et al*., 2017). Moreover, Angel (2005) claims that, in a favorable environment, and in totally healthy animals, organic acids have no effect. It is known that the proventriculus has a glandular function and the gizzard a muscle function; both sections are directly related, since, together, they are integral parts of the gastrointestinal tract (Martínez et al., 2013). In this sense, Svihus (2011) indicated that the use of feed additives improves the disposition of nutrients, which promotes the development of the proventriculus and gizzard in broilers. According to Van Immerseel *et al*. (2006), the supplemental acids are most likely to affect in the proventriculus and gizzard rather than the intestines; these authors showed that formic acid and propionic acid increased the activity of these organs, being similar to the results shown in Table 2. However, Dehghani-Tafti & JaHanian (2016) have reported a decrease in the relative weight gizzard in broilers due to the dietary supplementation with a mixture of organic acids (citric+butyric acids). Likewise, Abdel-Fattah *et al*. (2008) found no notable differences in the relative weight of the gizzard when they used lactic, acetic, and citric acid on broiler diets. It is important to note that, although the combined use of organic acids (propionic acid + acetic acid) increased the relative weight of some digestive organs (proventriculus, gizzard, small intestine and liver), this did not promote the growth of broilers. Similar results were found by Martínez *et al*. (2013) and Savón *et al*. (2015), who indicated that in apparently healthy birds, a higher relative weight of some digestive organs does not always translate into a greater productive response, especially since these organs increase their activity to maintain homeostasis due to extrinsic factors such as high fiber content, antinutritional factors, feed granulometry and intrinsic factors such as enzymatic activity, gut dysbiosis, pH and inflammation in the gastrointestinal tract, apparently these organic acids caused an intestinal disturbance (with lower pH and lower cecal lactic acid bacteria count) which directly affected this productive indicator (body weight), however more research is needed to justify this hypothesis.

On the other hand, the fast-growing and small bowel development is one of the factors that define the genetic expression of broilers (Martínez *et al*., 2013). In their first days of age (until 10 days), the length of the intestine increases, however, few are efficient to digest nutrients due to the immaturity of the digestive tract, however a higher development of their villi improves the digestibility of nutrients (Abdel *et al.*, 2012). The results showed that the use of organic acids increases the relative weight of the small intestine (Table 2). In this regard, Paul *et al.* (2007) found similar results when they were using organic acid salt on broiler diets. According to Fascina *et al*. (2017), the functionality and absorption of nutrients in the intestine may be influenced by the slightly acidic conditions of this organ. Also, Peng *et al*. (2016) and Dittoe *et al*. (2018) reported that propionic and acetic acids decrease pH throughout the gastrointestinal tract, inhibiting pathogen growth and improving cellular production, which facilitates the absorption of nutrients transported to the bloodstream. According to Ruhnke *et al*. (2014) and Lv *et al*. (2015), an increase in the epithelial surface leads to a higher capacity when transporting nutrients, which could stimulate the development of the digestive organs. In this sense, Murry *et al*. (2004) determined that volatile fatty acids produced by *Lactobacillus salivarius* and *Lactobacillus plantarum* decrease the pH of the intestinal environment, which influences the relative weight of the small intestine. However, Tahmiz *et al*. (2015) found that excessive acidification in the gastrointestinal tract causes intestinal disturbances, with a decrease in the absorption of nutrients and enzymatic activity. The changes in the relative weight of the digestive organs due to the use of organic acids (acetic and propionic) seem to decrease the functionality of the digestive organs and the ability to absorb nutrients, which reduced the body weight in the neonatal broilers.

The function of the cecum in broilers is to ferment nutrients such as starch, protein, and fiber that was not digested in the small intestine and absorb some of the water contained in the digested feed (Martínez *et al*., 2013). Once these nutrients enter the cecum, fermentation begins to produce volatile fatty acids (AGV), transfer them to the bloodstream and be used as energy (Svihus *et al*., 2013). Studies by Gunal *et al*. (2006) have reported that mixture with organic acids, antibiotics, and probiotics modified the cecum relative weight in broilers. However, we did not find changes in the relative weight of this organ (Table 2); apparently, the combination with acetic acid in drinking water and propionic acid in the diets was not enough to modify the relative weight of this organ.

The liver is the largest gland in the endocrine system, its function in birds is to secrete bile fluid for the digestion of lipids and proteins; besides, it eliminates toxic agents and degrades residual and hormonal products. Liver growth may be associated with a higher metabolic rate also caused by the increase in the relative weight of the small intestine, which has a stimulating effect on the production of bile acid for the digestion of lipids (Adil *et al*., 2010). The results of this study are consistent with those obtained by Ullah *et al*. (2016), who obtained significant differences ($p<0.05$) in liver development when using acetic acid. They also agree with those obtained by Mohammadi *et al*. (2012), where they found significant differences ($p<0.05$) in favor of organic acids in the liver relative weight.

Contrary to the results of this study (Table 2), Abdel-Fattah *et al*. (2008) reported an increase in the relative weight of the pancreas of broilers by adding acetic acid in the drinking water, in the same way, these authors affirm that the development of the small intestine is correlated with a higher enzymatic activity of the pancreas and liver. However, Fascina *et al*. (2017) reported that a mixture of organic acids decreased the relative weight of the pancreas in broilers. On the other hand, the relative weight of the heart did not show significant changes with the use of organic acids in drinking water (acetic acid) and feed (propionic acid) (Table 2). Other authors working with mixtures of organic acids on bird diets did not find significant variations in the relative weight of this organ (Abdel-Fattah *et al*., 2008; Maty & Hassan, 2020).

Birds during evolution have developed a unique immune system characterized by the activity of lymphoid organs, such as bursa of Fabricius, thymus, and spleen (Verduzco *et al*., 2010; Senthilkumar *et al*., 2018). As observed in Table 2, the addition of organic acids did not affect the development of lymphoid organs; these results are consistent with those obtained by Fascina *et al*. (2017), who found no effect with the use of organic acids in the development of immune organs. However, Abdel-Fattah *et al*. (2008) reported a mild hyperplasia in the lymphoid organs of broilers supplemented with citric acid with an increase in the immune response. According to Senthilkumar *et al*. (2018), the increase in the relative weight of lymphoid organs is an indicator of a better immune response and disease resistance. However, Martínez *et al*. (2013) did not find a relationship between a higher relative weight of the immune organs and weight gain, which could be related to an increase in energy expenditure for the production of immune cells, which reduces the body weight in pullets (Table 2).

Table 3 shows the effect of acetic (drinking water) and propionic (diets) acids on pH and lactic acid bacteria (BAL) in the small intestine and cecum in neonatal broilers (10 days). No significant differences

(p>0.05) were found between the treatments for the pH of the small intestine; however, a significant decrease in the cecal pH (p<0.05) was found with the use of the organic acids. Likewise, these natural products increased (p<0.05) the count of the green bacilli with the white halo in the small intestine. On the contrary, a significant decrease in irregular flat green bacilli (p<0.05) was observed in the cecum. The other morphological groups of lactic acid bacteria did not differ (p>0.05) between the experimental treatments.

Table 3 – Effect of a mixture of propionic-acetic acid on pH intestinal and lactic acid bacteria in neonatal broilers (10 days).

Items (log 10 CFU/g)	Experimental groups		SEM±	*p* value
	Control	Propionic+Acetic		
Small intestine				
pH	6.02	5.73	0.314	0.554
Bacilli[1]	7.91	8.16	0.224	0.485
Bacilli[2]	5.74	6.90	0.547	0.209
Bacilli[3]	6.80	6.49	0.884	0.816
Bacilli[4]	6.40	7.71	0.103	0.002
Total	8.02	8.39	0.126	0.102
Cecum				
pH	7.11	6.16	0.249	0.050
Bacilli[1]	8.16	8.21	0.189	0.851
Bacilli[2]	7.36	5.22	0.884	0.164
Bacilli[3]	6.86	4.77	0.565	0.050
Bacilli[4]	7.17	6.58	0.411	0.370
Total	8.32	8.25	0.196	0.822

[1] Greens; [2] white; [3] Greens with irregular planes; [4] Greens with white halo.

It is known that the material that reaches the cecum, undergoes anaerobic degradation that produces mainly volatile fatty acids (VFA) that influences the entire digestive tract, the majority of VFAs are found in the cecum. According to Jozefiak *et al*. (2011), the predominant acids in the cecum of the broilers are acetic acid (65%), butyric acid (16%) and propionic acid (12%). Thus, due to the large amount of volatile fatty acid (VFA), the pH is usually slightly acidic, below 6.5. The efficient production of AGV in the cecum occurs after seven days-old in broilers, and because of their immaturity, they do not reach the full fermentation capacity before 28 days of age when a neutral pH is observed in the cecum (Svihus, 2013).

Also, authors such as García *et al*. (2008) indicate that a higher presence of cecal lactic acid bacteria decreases the pH due to the greater presence of VFAs. In this research, the addition of organic acids reduced the cecal pH to 6.16 (Table 3). The pH variations in the cecum are related to the volatile fatty acids produced and the different additives used in the diet (Paul *et al*., 2007). In this sense, Molina *et al*. (2019) reported a cecal pH of 6.10 at 10 days- of age with the use of *Ganoderma lucidum* mushroom in broiler diets.

The main effect of propionic and acetic acids is to reduce the pH of the gastrointestinal tract, although the pH of the small intestine decreased with organic acids from 6.02 to 5.73, this was not enough to find statistical differences (Table 3). A decrease in pH in this intestinal portion stimulates the pancreatic secretion of digestive enzymes and the segregation of bile acid for lipid emulsification, which optimizes the digestion and absorption of nutrients and minerals (Panda *et al*., 2009). Hamied *et al*. (2018) found that the discontinuous supply of acidified water improved the body weight of broilers; however, Ranmani *et al*. (2005) reported that a rapid, significant decrease in pH in the small intestine from 6.2 to 5.8 affected broiler performance, similar to our trial that the combined use of the most commonly used organic acids in the poultry industry significantly reduced body weight until 10 days (Table 2).

Gram-negative bacteria are more sensitive to short-chain acids (organic acids) that have less than eight carbon atoms, while Gram-positive bacteria are sensitive to longer chain acids. Lactic acid bacteria are Gram-positive bacteria that are mostly resistant to pH changes, they can tolerate a significant difference between internal and external pH and can even improve their growth (Wang *et al*., 2018). Studies have been conducted in which a decrease in the growth of E. coli, Salmonella spp. and Clostridium perfringens in broilers when the growth of lactic acid bacteria is added or promoted because these ferment carbohydrates and the products of this fermentation are lactic, acetic, and propionic acid (Samaniego *et al*., 2009).

The results showed that the use of organic acids in broilers increased the count of lactic acid bacteria in the small intestine of broiler (Table 3). However, the opposite happened in the cecum; in this intestinal portion, the lactic acid bacteria decreased their population (Table 3). In this sense, the cecum or ¨cecum pouch¨ is the first portion of the large intestine that is primarily responsible for intestinal health, nutrient fermentation, and modulation of the intestinal microbiota (Svihus *et al*., 2013). Yu *et al*. (2007) concluded that chickens inoculated with *Lactobacillus* spp. had a higher concentration of lactic acid and a decrease in the pH of the cecum pouch. Despite changes in cecum pH, the use of acetic acid in drinking water and propionic acid in the diet reduced cecal BALs (Table 3).

Apparently, the combined use of two sources of organic acids decreased the cecal BAL population (mainly irregular flat green bacilli), which shows that a decrease in cecal pH (Table 3) is not always related to the growth of beneficial bacteria in this intestinal portion. Studies by Martínez *et al*. (2013) found that the use of natural products lowered the intestinal pH without an improvement in the productive response of young birds. A decrease in cecal BALs (Table 3) considerably reduces the production of VFAs (Peng *et al*., 2016), which are energy sources for the cell; however, other studies are necessary to corroborate this hypothesis.

The combination of propionic (diets) and acetic (drinking water) acids reduced the body weight in neonatal broilers and modified the relative weight of the proventriculus, gizzard, small intestine, and liver and the growth of some morphological groups of lactic acid bacteria. In addition, organic acids used acidified the cecal pH, with no noticeable changes in the pH of the small intestine.

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