Effect of Organic Acid Blends in Drinking Water on Growth Performance, Blood Constituents and Immune Response of Broiler Chickens

Ernesto Marín-Flamand¹, Alma Vázquez-Durán² and Abraham Méndez-Albores³

¹National Autonomous University of Mexico-Superior Studies Faculty at Cuautitlan (UNAM-FESC), Master in Production Sciences and Animal Health. Km 2.5 Carretera Cuautitlan-Teoloyucan, Cuautitlan Izcalli, CP. 54714, Mexico
²Autonomous University of Nuevo Leon-Agronomy Faculty (UANL-FA), Agropecuary Sciences Campus. Av. Francisco Villa s/n, Colonia Ex Hacienda el Canada, General Escobedo, Nuevo Leon, CP. 66050, Mexico
³National Autonomous University of Mexico-Superior Studies Faculty at Cuautitlan (UNAM-FESC), Multidisciplinary Research Unit. Km 2.5 Carretera Cuautitlan-Teoloyucan, Cuautitlan Izcalli, CP. 54714, Mexico

This research was conducted to evaluate the effect of organic acid blends (OAB) of ascorbic (A), citric (C), malic (M), sorbic (S), and tartaric (T) acids provided through the drinking water on growth performance, blood constituents, and immune response of broiler chickens from hatch to 42 d of growth. Three-hundred 1-d-old Ross broiler chickens were randomly divided into 4 treatment groups (1 control and 3 experimental) of 5 replicates (15 birds per replicate). Control group was given ordinary water and treatments were given continuously the acidified water using three different blends consisting of: OAB1 = A:C:M, OAB2 = A:S:M, and OAB3 = A:T:M. Blends were prepared with ratio 35:60:5 and were used at a concentration of 0.5% (wt/vol). The results showed that all OAB had no significant effect on live body weight (LBW); however, an improvement on feed consumption (FC), feed conversion ratio (FCR), and survival rate (SR) were observed as compared to the control. Birds supplied with OAB1 presented the lowest FCR (1.803), FC (96.19 g/bird/day), and the highest SR (95.63%). Blood constituents (hematocrite, total protein, and albumin), enzymatic activity (gamma glutamyl transpeptidase and aspartate aminotransferase), immune response, organs weight, and pH values of different gastrointestinal tract segments were not affected by administering the OAB. However, reductions in the alanine aminotrasferase activity and an increment in the aspartate aminotransferase:alanine aminotrasferase ratio were observed in groups provided with the OAB1 and OAB3, respectively. From these results, it is concluded that OAB1 could be used as an alternative for improving FC, FCR, and SR in broiler chickens.

Key words: blood parameters, broiler, growth performance, immune response, organic acid blends


Introduction

With the removal of antibiotic growth promoters from the poultry industry in different areas of the globe, it is of interest to investigate potential alternatives to improve feed efficiency and animal health. Several organic acids (OA) protect young chickens by competitive exclusion (La Ragione and Woodward, 2003), improve growth performance, feed efficiency, mineral adsorption and nutrient utilization (Denli et al., 2003; Ao et al., 2009). In addition, OA reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall (Langhout, 2000; Ricke, 2003), enhance phytate-P utilization (Boling et al., 2000; Liem et al., 2008), and reduce the aflatoxin content in diet (Méndez-Albores et al., 2007). Their mode of action is believed to rely on the compound’s ability to acidify the diet and ultimately the content of the digestive tract, which is primarily important in young animals, in which endogenous acid production is limited (Cranwell and Titchen, 1974).

OA have a long history of being utilized as food/feed additives and preservatives. As a group, these compounds primarily include the saturated straight-chain monocarboxylic acids and their respective derivatives: unsaturated, hydroxyl, phenolic, and multicarboxylic (Cherrington et al., 1991). OA were originally added to feeds to serve as fungistats (Dixon and Hamilton, 1981), but in the past 30 years, various combinations have also been examined for their potential bacteriostatic or bactericidal activity.

Our recent studies indicated that dietary OA supplementation had also a detoxification effect when used to treat
Animal Ethics

This study was conducted according to the Internal Committee for Care and Use of Experimental Animals (ICCUEA), approved by the National Autonomous University of Mexico.

Chemicals

Organic acids: ascorbic (A) [2-(1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3-one], citric (C) [2-hydroxypropane-1,2,3-tricarboxylic acid], DL-malic (M) (hydroxybutanedioic acid), sorbic (S) (2,4-hexa-2,4-dienoic acid), and tartaric (T) (2,3-dihydroxybutanedioic acid), were obtained from Mallinckrodt Baker (JT Baker, Xalostoc, Mexico). All other chemicals used were analytical reagent grade.

Organic Acid Blends (OAB)

Based on OA properties such as solubility, three different blends were prepared: OAB1 = A:C:M, OAB2 = A:S:M, and OAB3 = A:T:M. Blends were prepared with ratio 35:60:5 and were administered continuously through the drinking water at a concentration of 0.5% (wt/vol). Because ascorbic acid is a potent antioxidant compound, and malic acid is an important intermediate (malate anion) in the citric acid cycle, both OA were maintained constant in the blends. Ordinary drinking water presented a pH value of 7.79, and the resulting acidified water using the three OAB presented average pH values of 2.68, 2.58, and 2.73, respectively. The pH was determined using a pH meter (Model PC45, Condustronic S.A., Puebla, Mexico).

Experimental Diets

Typical sorghum-soybean meal diets were prepared based on National Research Council recommendations (NRC, 1994). The compositional and chemical analysis of the starter (1 to 21 d) and grower (21 to 42 d) diets are presented in Table 1. No antibiotic growth promoters nor anticoccidial drugs were used in the diets. The final average moisture content of the diet was approximately 12%, determined by drying replicate portions of 5–10 g each of feed at 103°C for 72 h, with percentages calculated on a wet-weight basis.

Birds and Housing

For the experiment, 300 1-d-old Ross 308 broiler chicks were divided into 3 experimental and 1 reference group (such that the average weight of the birds differed by less than 1 g). Fifteen birds of mixed sex (5 replicates) were housed in plastic cages, 113 cm (l) × 90 cm (w) × 60 cm (h), in a light-cycled room (12 h cycle), maintained within the temperature range of 30 to 32°C for the first week and then lowered to 27°C for the remainder of the study. The feeding program consisted of a starter diet until 21 d and a grower diet until 42 d of age. Control group was given ordinary water and treated with OAB at a concentration of 0.5% (wt/vol). The floor was covered with 5 cm deep wood shavings and two 2 L capacity chick opaque cup drinkers were placed per cage. When birds were 14-d-old, the cup drinkers were replaced by trough drinkers. During the first 7 days of age, chicks were fed in a tray feeder, over which a 1 cm mesh plastic screen was placed to prevent feed wastage. After 7 days of age, feed was offered in trough feeders 91.5 cm (l) × 11.5 cm (w) × 5.4 cm (h).

Table 1. Ingredients and nutrient composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Starter (1 to 21 d)</th>
<th>Grower (21 to 42 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>50.33</td>
<td>56.28</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>41.52</td>
<td>35.59</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>3.46</td>
<td>3.11</td>
</tr>
<tr>
<td>Orthofosfate</td>
<td>1.86</td>
<td>1.90</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.56</td>
<td>1.92</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>Alimet 88</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>L-Lisine HCl</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Vitamine premix</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Sugar + zinc</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td></td>
<td>3111</td>
</tr>
<tr>
<td>Crude protein</td>
<td></td>
<td>22.50</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Phosphorus (total)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Methionine + Cystine</td>
<td>1.60</td>
<td>1.40</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.50</td>
<td>1.30</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.97</td>
<td>0.85</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>45.00</td>
<td>45.00</td>
</tr>
</tbody>
</table>

1 Aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid (HMTBA).
2 Vitamine premix supplied/kg diet: Vitamin A, 12,000 IU; Vitamin D3, 2500 IU; Vitamin E, 15 IU; Vitamin K3, 2 mg; Vitamin B1, 2.25 mg; Vitamin B2, 7.5 mg; Vitamin B6, 3.5 mg; Vitamin B12, 0.02 mg; folic acid, 1.5 mg; pantothenic acid, 12.5 mg.
3 Mineral premix supplied/kg diet: Cu, 8 mg as CuSO4·5H2O; Mn, 100 mg as MnO; Fe, 80 mg as FeSO4·H2O; I, 1 mg as ethylenedi-amine dihydroiodide (EDDI); Se, 0.15 mg as Na2SeO3.
4 Data on dry matter.
5 ME: Starter, 12.58 MJ, Grower 13.00 MJ.
Collection of Samples and Measurements

Feed and water were provided ad libitum during the whole period of the experiment. Broilers were individually weighed at the beginning of the experiment, then at weekly intervals until the end of the experiment. Live body weight (LBW), feed consumption (FC), feed conversion ratio (FCR), and survival rate (SR) were recorded during these periods. After 42 days, blood was drawn by cardiac puncture under anesthesia (the bird was exposed for 1 minute to 40% carbon dioxide, 30% oxygen, and 30% nitrogen) from 15 randomly selected birds from each treatment (3 birds per replicate), and serum prepared. Total protein and albumin were determined using commercially available kits (Wiener Lab, Rosario, Argentina). For hematocrite measurements, blood was taken up in heparinized capillary tubes and centrifuged in a Hettich Microliter centrifuge (Mikro 220R, DBJ Labcare, UK) for 7 min. The serum gamma glutamiltranspeptidase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were determined according to Reitman and Franked (1970). The bled chickens were then exposed to 80% carbon dioxide, 5% oxygen, and 15% nitrogen for euthanasia (Coenen et al., 2000). Proventriculus, gizzard, liver plus gall bladder, spleen, and bursa were excised, washed in cold saline and their relative percentages estimated. The intestinal weight was also considered and pH values in different parts of the gastrointestinal tract (proventriculus, gizzard, duodenum, jejunum and ileum) were also registered immediately by using a digital pH meter (model HI 99163, HANNA, Romania).

Immune Response

Hitchner B1 Newcastle Disease Virus (NDV) live vaccine was obtained from MAVER Laboratories (Coyoacan, Distrito Federal, Mexico). The vaccine was administered via intraocular route in accordance with the recommendations for field applications. Birds were vaccinated against NDV on 7 and 21 d of experiment. On the mornings of days 14 and 35, a total of 20 birds (5 birds per treatment) were randomly selected for estimation of antibody titers against NDV. Blood samples were kept at room temperature for 2 h, then overnight at 4°C in refrigeration and centrifuged (1,500 x g, 15 min). Serum was inactivated at 56°C for 30 min and stored at −20°C until analysis. The titers of antibody against NDV were estimated by the haemagglutination inhibition test (HI) method described by Hu and Liu (1997).

Experimental Design and Statistical Analysis

The experiment was conducted as a completely randomized design with 5 replicates. Data were assessed by analysis of variance (ANOVA) and means were separated by the Dunnet procedure using the Statistical Analysis System (SAS Institute, 1998). A significance value of α = 0.05 was used to distinguish significant differences between treatments.

Results

The effects of administering OAB in drinking water on growth performance and survival rate of broiler chickens are summarized in Table 2. The results indicated that LBW was not significantly improved in birds supplied with the OAB. Moreover, FC significantly differ (P < 0.05) between control and OAB provided birds. Control birds presented a FC value of about 107.12 g, significantly different to the FC average value (97.32 g) of OAB treated birds. Regarding FCR, control birds was the group that presented the highest value (1.991); however, birds supplied with OAB had significantly (P < 0.05) better FCR values compared to the control. OAB1 was the group that presented the lowest FCR (1.803), followed by groups supplied with OAB2 and OAB3, which presented FCR values of 1.831 and 1.860, respectively (Table 2). In addition, OAB1 was the group that presented the better SR value (95.63%).

OAB did not significantly affect the values of hematocrite and the serum concentrations of total protein, albumin, GGT, AST, and antibody titers against NDV (Table 3). However, significant differences (P < 0.05) were found for the enzymatic activity of ALT as well as for the AST:ALT ratio. Control birds presented an average ALT value of 53.25 IU/L. Nevertheless, birds supplied with OAB1 and OAB3 decreased its serum concentration to 16.93 and 30.09 IU/L, respectively. The maximum increment in the AST:ALT ratio (4.7) was recorded in birds supplied with the OAB3, followed by the group treated with the OAB1 (2.6).

Organ weights were not affected due to the use of OAB (Table 4). Furthermore, the results indicated that OAB had no significant effect on pH values of different gastrointestinal tract (GIT) segments, such as: proventriculus, gizzard, duodenum, jejunum, and ileum (Table 5).

### Table 2. Effect of organic acid blends (OAB) in drinking water on growth performance and survival rate of broiler chickens

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>OAB1</th>
<th>OAB2</th>
<th>OAB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBW (g)</td>
<td>2259 ± 29.15</td>
<td>2240 ± 37.24</td>
<td>2219 ± 37.00</td>
<td>2236 ± 35.01</td>
</tr>
<tr>
<td>FC (g/bird per day)</td>
<td>107.12 ± 3.1a</td>
<td>96.19 ± 2.3b</td>
<td>98.27 ± 2.2b</td>
<td>97.49 ± 2.5b</td>
</tr>
<tr>
<td>FCR (g feed/g gain)</td>
<td>1.991 ± 0.063a</td>
<td>1.803 ± 0.033b</td>
<td>1.860 ± 0.028c</td>
<td>1.831 ± 0.083c</td>
</tr>
<tr>
<td>SR (%)</td>
<td>90.66a</td>
<td>95.63b</td>
<td>94.66c</td>
<td>93.33c</td>
</tr>
</tbody>
</table>

Mean ± standard error.

** and *** Means, within the same row, not sharing a common superscript differ significantly (P < 0.05).

LBW = live body weight; FC = feed consumption; FCR = feed conversion ratio; SR = survival rate.
**Discussion**

The OA have a positive effect on growth performance, since dietary acidification increases gastric proteolysis and protein-amino acid digestibility by enhancing digestive enzyme activities (Langhout, 2000; Salgado-Tránsito et al., 2011). The reason why protein is better used when OA is added to diet is due to the fact that pepsinogen is converted to pepsin, which increases pepsin activity and improves protein digestibility. Moreover, peptides arising from pepsin proteolysis trigger the release of hormones (including gastrin and cholecystokinin) which regulate the digestion and absorption of protein. The improvement of FCR and the increase of LBW have already been demonstrated in broilers fed a diet supplemented with acidifiers (Denli et al., 2003). Conversely, test results for the inclusion of certain OA or blends in
drinking water remain limited and controversial. Chaveerach et al. (2004) reported no differences in LBW for the control and treated chickens with a commercial OA product in drinking water. Parker et al. (2006) reported that water acidification (0.08% acid blend) led to a significant improvement in FCR, but had no effect on LBW or mortality. In this context, the results of this research are in close agreement with the above mentioned authors. In this research FC, FCR, and SR were significantly improved in the groups supplied with the OAB (Table 2). This phenomenon could be possible related to the benefits of OAB, since it is well known that acidification reduce the numbers of Enterobacteriaceae and Campylobacter in the water and increase the total aerobic bacteria from the cecae, being possible that OAB provided an extra-energy source for bacterial growth. OA have a low tendency to free their H⁺ ions and a strong taste is commonly associated with them. Consequently, high amounts of OA supplementation may reduce water consumption. In this work, the addition of the three different OAB was successfully tolerated by the chickens (even when pH was below 3). Consumption of water was recorded for the 4 treatments, and no significant differences were observed between control and treated groups (data not shown). SR also shows that there was a significant difference between groups (Table 2). The observed mortality during the 42 d period was: 7 chicks from the control group, and 3, 4, and 5 chicken from the acidified treatments provided with the OAB1, OAB2, and OAB3, respectively. It is important to note that mortality was only observed during the first 2 wk of the trial. Majewska et al. (2009) reported SR of 95% and 95.2% for unsexed Ross 308 broilers chicks provided twice a week with undiluted fresh acid whey and diluted 50% lactic acid (4 cm³/L water), respectively. These results are in close agreement with the values found in this research (Table 2). In summary, the better FC, FCR, and SR in some treatments may be due to the effect of OAB by controlling pathogenic bacteria, or maintaining the health of the GIT, considering that the effect of OAB might not be due to pH reduction only, since the beneficial effect of OAB on performance is related to a more efficient use of nutrients (proteins, calcium, phosphorus, magnesium, and zinc), which in turn results in an improved FCR. Consequently, the use of combinations of OA would be more effective in view of the synergistic activity between them.

Among the different blood constituents measured, hematoctrite, serum total protein, and albumin concentrations were not significantly affected at the end of the experiment in broilers receiving the acidified water (Table 3). These results are consistent with those obtained in broiler chicks due to acetic acid inclusion (Abdo, 2004). Abdel-Fattah et al. (2008) also reported that total protein and albumin were not affected due to the inclusion of 1.5 or 3% citric acid in broiler chickens fed during a 42 d period. Whereas the increment of the enzymatic values in birds varies with the different species, the elevation of the enzymatic activity has been correlated with hepatocellular damage. The most frequent cause of the elevation of the AST activity in birds is hepatic disease; birds with AST values in the upper 230 IU/L range are considered abnormal (Campbell and Coles, 1986). A moderate increase (2 to 4 fold) in the AST enzyme is observed when there is a soft weave injury, whereas in the hepatic necrosis, a more remarkable elevation is caused. In the present study, the results demonstrated that the inclusion of different OAB in the drinking water has no effect on the GGT and AST enzyme activities. Adil et al. (2010) reported no significant differences in the serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGPT) in broilers due to the dietary supplementation of OA (butyric, fumaric, and lactic acid). Conversely, in the case of ALT activity, lower values were registered due to the inclusion of OAB1 and OAB3, respectively (Table 3). These results are in accordance with those of Brenes et al. (2003) who reported a decrease in ALT values in broiler chickens fed a diet containing 20 g citric acid/kg at different levels of available phosphorous. In this context, some toxic effects of OA have been reported: Aktaç et al. (2003) reported that citric acid (LD₅₀ = 480 mg/kg BW) applied intraperitoneally to mice increased serum AST level (from 177.8 to 307.2 IU/L), and decreased ALT activity (from 695 to 101 IU/L). Those findings are in agreement with the results found in the present investigation. The AST:ALT ratio has been used in some studies with human patients and poultry (Sorbi et al., 1999; Valdivia et al., 2000). The AST: ALT ratio appears to be a useful index for distinguishing liver disease and may be helpful diagnostically. In particular, a ratio of ≥ 2 is strongly suggestive of liver disease. In this case, the AST:ALT ratio was a useful indicator of the toxic effect of OAB1 and OAB3 in birds, confirming that citric and tartaric acids exert some toxic effects; however, broilers does not show any harmful effects of hepatic damage during histological examination. It seems that enzyme activity determination can be of some help in the diagnosis of hepatotoxicity cases before major clinical symptoms appear.

Regarding antibody titers against NDV, OAB slightly increased the titers (14 and 35 d); nevertheless, no significant differences were observed (Table 3). Sadeghi et al. (2012) reported values between 6.69 to 8.75 for antibody titers in male broilers (Ross 308) vaccinated against NDV. These results are consistent with the values found in this research. As shown in Table 4, no significant differences were also noted among all treatments in the relative proventriculus, gizzard, liver, intestine, spleen, and bursa percentages. These results confirmed those of Denli et al. (2003) who found that dietary OA had no effect on carcass yield and liver weight of broiler chickens at 42 d old.

The effect of drinking water acidification on pH values of different GIT segments is presented in Table 5. The results indicate that OAB slightly reduced proventriculus, gizzard, duodenum, jejunum, and ileum pH values, compared with the control group; however, the differences were not significant. Hernández et al. (2006) reported no effect on intestinal pH with the use of a product containing a combination of propionic-formic acids. These authors attributed this insignificant effect to the strong buffering action of the GIT in broiler
chickens.

In conclusion, our study showed that OAB1 provided in drinking water may be beneficial, since the blend significantly improved, in the absence of antibiotics growth promoters, FC, FCR, and SR in broiler chickens. Moreover, OAB1 and OAB3 decrease the serum ALT level and increase the AST:ALT ratio. The exact mechanism by which serum enzyme activity is apparently modified is unclear; therefore, further studies on the consequence of these organic acid blends on hepatocellular effects, needs to be conducted.

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