

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

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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: OAB<sub>1</sub>=A:C:M, OAB<sub>2</sub>=A:S:M, and OAB<sub>3</sub>=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 OAB<sub>1</sub> 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 aminotransferase activity and an increment in the aspartate aminotransferase:alanine aminotransferase ratio were observed in groups provided with the OAB<sub>1</sub> and OAB<sub>3</sub>, respectively. From these results, it is concluded that OAB<sub>1</sub> 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

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### 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, hydroxylic, 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

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aflatoxin-contaminated poultry feed (Salgado-Tr nsito *et al.*, 2011). At present, effects of the addition of OAB to drinking water on growth performance, blood constituents, and immune response are still meager, since research has been focused principally on the prevention of *Salmonella* or *Campylobacter* colonization in broilers (Byrd *et al.*, 2001; Chaveerach *et al.*, 2001, 2004). Consequently, the present research was conducted with the objective to evaluate the effect of three different OAB administered through the drinking water on the performance, blood constituents, and immune response of broiler chickens in an attempt to find out new growth promoting substances to reduce antibiotic supplementation in farm animals.

## Materials and Methods

### 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: OAB<sub>1</sub>=A:C:M, OAB<sub>2</sub>=A:S:M, and OAB<sub>3</sub>=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, Conductronic 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 (1–21 d) and grower (21–42 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-

Table 1. **Ingredients and nutrient composition of the experimental diets**

Ingredient (%)	Starter (1 to 21 d)	Grower (21 to 42 d)
Sorghum	50.33	56.28
Soybean meal (48)	41.52	35.59
Sunflower oil	3.46	3.11
Orthofosfate	1.86	1.90
Calcium carbonate	1.56	1.92
Salt (NaCl)	0.41	0.41
Alimet 88 <sup>1</sup>	0.35	0.35
L-Lisine HCl	0.19	0.11
Choline chloride	0.10	0.10
Vitamine premix <sup>2</sup>	0.10	0.10
Mineral premix <sup>3</sup>	0.05	0.05
Sugar + zinc	0.05	0.05
L-Threonine	0.03	0.03
<i>Calculated</i> <sup>4</sup>		
ME (kcal/kg) <sup>5</sup>	3010	3111
Crude protein	24.00	22.50
Calcium	1.00	1.00
Phosphorus (total)	0.50	0.50
Methionine + Cystine	1.60	1.40
Lysine	1.50	1.30
Threonine	0.97	0.85
Zinc (mg/kg)	45	45
<i>Analyzed</i>		
Crude protein	23.75	22.39
Calcium	1.10	1.10
Phosphorus (total)	0.57	0.53

<sup>1</sup> Aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid (HMTBA).

<sup>2</sup> Vitamine premix supplied/kg diet: Vitamin A, 12,000 IU; Vitamin D<sub>3</sub>, 2500 IU; Vitamin E, 15 IU; Vitamin K<sub>3</sub>, 2 mg; Vitamin B<sub>1</sub>, 2.25 mg; Vitamin B<sub>2</sub>, 7.5 mg; Vitamin B<sub>6</sub>, 3.5 mg; Vitamin B<sub>12</sub>, 0.020 mg; folic acid, 1.5 mg; pantothenic acid, 12.5 mg.

<sup>3</sup> Mineral premix supplied/kg diet: Cu, 8 mg as CuSO<sub>4</sub> 5H<sub>2</sub>O; Mn, 100 mg as MnO; Fe, 80 mg as FeSO<sub>4</sub> H<sub>2</sub>O; I, 1 mg as ethylenediamine dihydroiodide (EDDI); Se, 0.15 mg as Na<sub>2</sub>SeO<sub>3</sub>.

<sup>4</sup> Data on dry matter.

<sup>5</sup> ME: Starter, 12.58 MJ, Grower 13.00 MJ.

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 treatments were given the acidified water using the 3 different 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).

### 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, DJB Labcare, UK) for 7 min. The serum gamma glutamyltranspeptidase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were determined according to Reitman and Frankel (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 intra-ocular 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 × 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 Dunnett procedure using the Statistical Analysis System (SAS Institute, 1998). A significance value of  $\alpha=0.05$  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. OAB<sub>1</sub> was the group that presented the lowest FCR (1.803), followed by groups supplied with OAB<sub>3</sub> and OAB<sub>2</sub>, which presented FCR values of 1.831 and 1.860, respectively (Table 2). In addition, OAB<sub>1</sub> 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 OAB<sub>1</sub> and OAB<sub>3</sub> 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 OAB<sub>1</sub>, followed by the group treated with the OAB<sub>3</sub> (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

Parameter	Control	Treatment		
		OAB <sub>1</sub>	OAB <sub>2</sub>	OAB <sub>3</sub>
LBW (g)	2259.62±29.15	2240.71±37.24	2219.04±37.00	2236.43±35.01
FC (g/bird per day)	107.12±3.1 <sup>a</sup>	96.19±2.3 <sup>b</sup>	98.27±2.2 <sup>b</sup>	97.49±2.5 <sup>b</sup>
FCR (g feed/g gain)	1.991±0.063 <sup>a</sup>	1.803±0.037 <sup>b</sup>	1.860±0.028 <sup>c</sup>	1.831±0.083 <sup>c</sup>
SR (%)	90.66 <sup>a</sup>	95.63 <sup>b</sup>	94.66 <sup>c</sup>	93.33 <sup>c</sup>

Mean±standard error.

<sup>a-c</sup> 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.

**Table 3. Effect of organic acid blends (OAB) in drinking water on some blood constituents and immune response in broiler chickens**

Parameter	Control	Treatment		
		OAB <sub>1</sub>	OAB <sub>2</sub>	OAB <sub>3</sub>
Hematocrite (%)	33.83±2.12	31.12±3.99	32.00±2.47	33.25±2.50
Total protein (g/L)	30.28±3.03	31.18±3.19	30.46±3.35	28.91±5.28
Albumin (g/L)	11.29±3.54	13.97±3.70	12.33±3.22	12.69±3.53
GGT (IU/L)	46.50±4.56	44.29±3.48	43.00±2.48	47.44±2.56
AST (IU/L)	77.24±4.87	79.37±7.23	74.04±9.73	78.67±9.83
ALT (IU/L)	53.25±8.10 <sup>a</sup>	16.93±4.58 <sup>b</sup>	48.52±7.11 <sup>a</sup>	30.09±5.62 <sup>c</sup>
AST: ALT ratio	1.5 <sup>a</sup>	4.7 <sup>b</sup>	1.5 <sup>a</sup>	2.6 <sup>c</sup>
Antibody titers <sup>1</sup> (14d)	4.50±1.82	4.67±1.75	4.50±1.22	4.73±1.63
Antibody titers (35d)	5.00±0.89	5.83±1.03	5.35±0.52	5.33±0.98

Mean of 15 replicates±standard error.

<sup>1</sup> Antibody titers expressed in log<sub>10</sub>

<sup>a-c</sup> Means, within the same row, not sharing a common superscript differ significantly ( $p<0.05$ ).

GGT=gamma glutamyltranspeptidase; AST=aspartate aminotransferase; ALT=alanine aminotransferase.

**Table 4. Effect of organic acid blends (OAB) in drinking water on some organs as a percentage of body weight in broiler chickens**

Parameter	Control	Treatment		
		OAB <sub>1</sub>	OAB <sub>2</sub>	OAB <sub>3</sub>
Proventriculus	0.513±0.109	0.486±0.086	0.513±0.087	0.491±0.105
Gizzard	2.34±0.332	2.24±0.226	2.45±0.375	2.10±0.242
Liver	2.90±0.561	2.78±0.217	3.01±0.323	2.82±0.192
Intestine	5.77±0.652	5.41±0.450	5.36±0.759	5.55±1.550
Spleen	0.118±0.037	0.122±0.040	0.151±0.060	0.114±0.038
Bursa	0.185±0.046	0.184±0.039	0.198±0.074	0.217±0.033

Mean of 15 replicates±standard error.

**Table 5. Effect of organic acid blends (OAB) in drinking water on pH values of some gastrointestinal tract segments in broiler chickens**

Parameter	Control	Treatment		
		OAB <sub>1</sub>	OAB <sub>2</sub>	OAB <sub>3</sub>
Proventriculus	3.44±0.06	3.35±0.16	3.40±0.13	3.39±0.17
Gizzard	2.61±0.24	2.46±0.12	2.46±0.08	2.50±0.13
Duodenum	6.33±0.11	6.29±0.07	6.19±0.14	6.11±0.16
Jejunum	6.42±0.08	6.37±0.09	6.37±0.10	6.32±0.07
Ileum	6.50±0.14	6.44±0.09	6.48±0.12	6.46±0.14

Mean of 15 replicates±standard error.

## 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-Tr  nsito *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 OAB<sub>1</sub>, OAB<sub>2</sub>, and OAB<sub>3</sub>, 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 cm<sup>3</sup>/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, hematocrite, 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 OAB<sub>1</sub> and OAB<sub>3</sub>, 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: Aktaş *et al.* (2003) reported that citric acid (LD<sub>25</sub>=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  $\geq 2$  is strongly suggestive of liver disease. In this case, the AST:ALT ratio was a useful indicator of the toxic effect of OAB<sub>1</sub> and OAB<sub>3</sub> 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 OAB<sub>1</sub> 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, OAB<sub>1</sub> and OAB<sub>3</sub> 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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