**Effect of dietary acidification in broiler chickens: 1. Growth performance and nutrients ileal digestibility**

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**Abstract**

An experiment was conducted to evaluate the effect of dietary Orgacids® (organic acid; OA) supplementation on the productive performance, nutrients ileal digestibility, relative weight of organs and serum enzyme activities in broiler chickens. One hundred-sixty Ross 308 male chicks were randomly allotted to 4 dietary treatments: a nutritionally balanced basal diet supplemented with 0, 1, 2 and 3 OA g kg⁻¹ of feed from 7 to 42 d of age. Each treatment had 4 replications with 10 broilers/replicate pen. As a result of this study, body weight, average daily gain and average daily feed intake increased (linear effect, P<0.05) at 3 g kg⁻¹ of OA inclusion, whereas feed conversion ratio was negatively affected by dietary treatments (quadratic, P<0.05) as inclusion of OA increased to 2 g kg⁻¹ and then decreased with further inclusion. Ileal digestibility of total phosphorus and relative weight of pancreas, heart and spleen increased (linear effect, P<0.05) with increasing inclusion of OA. Metabolizable energy corrected to zero nitrogen retention increased linearly and quadratically on increasing OA addition reaching a maximum at 2 g kg⁻¹ diet. The results indicated that serum enzyme activity of alkaline phosphatase and alanine aminotransferase increased (linear effect, P<0.05) with increasing inclusion levels of OA, but lactate dehydrogenase decreased. In conclusion, these findings demonstrate that the OA supplementation at 3 g kg⁻¹ of the diet resulted in optimal growth performance and nutrients digestibility.

**Introduction**

The poultry industry is continuously search-
from d 7 of age (BW=121 g ± 1; P<0.05). Birds had free access to feed and water.

Experimental procedure

The BW and feed intake for each cage were determined after withdrawing feed for 3 h before weighing on d 7, 21, and 42. ADG, ADFI and FCR were calculated for each phase. Dead birds were weighed and recorded daily. When calculating FCR, the body weight of the dead birds was taken into consideration.

At day 30 of age, titanium oxide (1 g/kg of diet) was added to all the diets for five days and was used as an analytical marker to determine the effect of treatments on digestibility of crude protein (CP), apparent metabolizable energy corrected to zero nitrogen retention (AMEn), calcium (Ca) and total phosphorous (tP). At day 35 of age, three birds per replicate (12 chicks per treatment) were randomly selected, weighed, killed and manually processed to collect the ileal contents and immediately frozen and stored at ~20°C until needed for determination of apparent nutrient retention. The ileum was defined as the segment of small intestine, which extended from the duodenum to 40 mm proximal to the ileo-caecal junction. At 42 d of age, two randomly chosen birds per replicate (8 birds per treatment) were randomly selected and electrically stunned, then slaughtered through cutting of jugular veins and carotid arteries, and processed manually and collections were made following a 4-h fast. The carcass, pancreas, liver, heart, bursa, and spleen were harvested, weighed, and expressed relative to the total body weight (g/100 g BW).

At day 42 of age, 5 ml blood were collected from two killed birds in non-heparinised tubes and kept on slush-ice until they were subjected to serum collection by centrifuging the whole blood sample at 2,500 g for 10 min. The serum samples were analyzed for the activity of alkaline phosphatase (ALP, EC 3.1.3.1) alanine aminotransferase (ALT, EC 2.6.1.2) aspartate aminotransferase (AST, EC 2.6.1.1) and lactate dehydrogenase (LDH, EC 1.1.1.27) using the Express Plus (Ciba-Corning Diagnostics Corp., Medfield, MA) automated clinical chemistry analyzer according to the manufacturer’s directions (Nagel et al., 1964).

Sample preparation and chemical analyses

The ileal samples were freeze-dried and finely ground in a grinder (CBGS Smart Grind, Applca Consumer Products Inc., Shelton, CT) to pass through a 0.5 mm screen and were thoroughly mixed before analysis. The samples were analyzed for gross energy, crude protein (N × 6.25), Ca, tP and Ti contents. Gross energy was determined by using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Loughborough, UK) and was standardized with benzoic acid. The CP (N×6.25) was determined by Kjeldahl method (Kjeltec 2000 Autoanalyzer, Foss Tecator AB) (AOAC, 1995; method no. 981.10). Samples for Ca analysis were ashed for 12 h and digested according to AOAC (1990) procedures (method 990.08) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc.). Total P was determined using the AOAC (1990) method 965.17. Titanium was determined according to the procedure described by Lomer et al. (2000) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA).

Calculations and statistical analysis

Nutrient retention was calculated using the following equation (Scott et al., 1982):

\[\text{Apparent nutrient retention} = 1 - \left(\frac{[\text{TiO}_2 \text{ diet}] - [\text{TiO}_2 \text{ excreta}]}{[\text{nutrient} \text{ excreta}] - [\text{nutrient} \text{ diet}]}\right)\]

The apparent metabolizable energy corrected to zero nitrogen retention (AMEn) values were calculated by subtracting GE excreted (adjusted to zero N balance) from GE intake and dividing this value by DM feed intake. For correction to zero N retention, a value of 34.39 kJ g⁻¹ of N retained was used (Hill and Anderson, 1958).

Data were analyzed using GLM procedure (SAS, 2006) in a completely randomized design. All of the treatment means were compared using Tukey-Kramer’s test. For the different statistical tests, significance was declared at P<0.05. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of OA addition on performance, relative weights of organs, serum enzyme activities and ileal digestion parameters.

Results and discussion

The results indicated that dietary supplementation with OA did not affect growth performance during the growth period of 7 to 21, but was significant on ADG, ADFI and FCR during 22 to 42 and 7 to 42 d periods (Table 2). The inclusion of OA at 3 g kg⁻¹ significantly increased (P<0.05) ADG and ADFI, whereas FCR increased both linearly and quadratically

Table 1. Feed ingredients and nutrient composition of basal diets for experimental broiler chicks. Based on Aviagen (2011).

| Ingredient, g/kg | Grower, d 7 to 21 | Finisher, d 22 to 42 |
|-----------------|------------------|---------------------|
| Corn            | 527              | 502                 |
| Soybean meal (43% CP) | 289           | 346                 |
| Wheat           | 80               | 50                  |
| Fish meal       | 30               | 30                  |
| Soybean oil     | 40               | 35                  |
| Dicalcium phosphate | 14             | 16                  |
| Oyster shell    | 10               | 11                  |
| Salt            | 2                | 2                   |
| Methionine      | 2                | 2                   |
| Lys             | 1                | 1                   |
| Premix°         | 5                | 5                   |

Calculated nutrient composition

| Metabolizable energy, MJ/kg | 12.98 | 12.56 |
| Crude protein, %            | 20.0  | 22.0  |
| Calcium, %                  | 0.9   | 1.0   |
| Available phosphorus, %     | 0.45  | 0.50  |
| Methionine, %               | 0.35  | 0.37  |
| Lysine, %                   | 1.1   | 1.25  |
| Methionine + Cystine, %     | 0.85  | 0.95  |

°Provided (per kilogram of diet): vitamin A, 11,000 U; cholecalciferol, 1800 U; vitamin E, 11 mg; vitamin K, 5.7 mg; vitamin B₆, 2 mg; vitamin B₂, 3 mg; vitamin B₃, 9.24 mg; folic acid, 0.5 mg; niacin, 28 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; manganese, 100 mg; selenium, 0.02 mg; copper, 5 mg; iodine, 0.5 mg; cobalt, 0.5 mg; zinc, 62 mg.
(P<0.05) in days 22 to 42 of age. From days 7 to 42, BW, ADG and ADFI increased linearly, whereas FCR increased quadratically (P<0.05) with the increasing dietary levels of OA, and it was maximized at 2 g kg⁻¹ supplemental OA. Compared to the control group, the lowest BW and ADG being obtained with 2 g kg⁻¹ of OA. Throughout the experiment, there was only one case of mortality and it was in the control treatment. Because of this limited number of death cases no statistical analysis was performed. There are conflicting reports on the effect of OA on the performance of broilers. Some research has reported promising effects (Boling et al., 2000; Boling-Frankenbach et al., 2001), whereas other research has shown no significant effect (Biggs and Parsons, 2008; Woyengo et al., 2010), and still other research has explained negative effects of OA on the performance of broilers (Brenes et al., 2003). Recently, has been suggested that OA improves growth rate by regulating the intestinal microbial flora in the digestive organs, allowing the development of commensal bacteria and reducing pathogenic bacteria that can produce toxins (Woo et al., 2006; Mohammadiour et al., 2014). The beneficial effect of acidifiers, such as OA, on performance is related to a more efficient use of nutrients and digestibility improvement (Nourmohammadi et al., 2012). In the present study, all dietary treatments were isocaloric; therefore, the increased performance with 3 g kg⁻¹ of OA may be attributed partly to the effect of OA on increasing phosphorus availability. Boling-Frankenbach et al. (2001) investigated the effect of OA addition according to the available phosphorus (aP) level and found that lowering the aP level (0.2% or less) and feeding OA decreased productivity and the FCR. But supplementation of OA when the aP level was at 0.2% or higher improved productivity, similar to the results of the present experiment. Therefore, the results of the current study suggest that 3 g kg⁻¹ OA might be an appropriate dosage for diets containing adequate aP in broilers. The increased growth response observed in this study is in agreement with the results reported by Nourmohammadi et al. (2012), who found that adding OA to the diet improved broiler performance as compared with those fed un-supplemented diets. However, this observation was not found by the findings of Woo et al. (2006), who reported no significant effect on ADFI and FCR. In addition to the above results, Nourmohammadi and Afzali (2013) revealed that inclusion of dietary OA promoted cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus and consequently increased the digestive absorption and use of nutrients in the feed (Nourmohammadi et al., 2012).

The effects of OA on relative weights (g/100 g BW) of the pancreas, liver, heart, bursa and spleen are presented in Table 3. No significant differences were observed in carcass yields across treatment groups. Inclusion of 3 g kg⁻¹ of OA increased the relative weights of heart (linear), pancreas and spleen (linear and quadratic) compared to values for the control group (P<0.05). The OA may promote effective contact between the digestive enzymes and their corresponding substrates, leading to significant modifications of the structure and function of digestive organs (Cheng, 2009). To adapt to those changes, the activities of the intestinal secretory mechanisms may be enhanced. Thus, this may lead to increases in the size of the gastrointestinal tract (GIT) and pancreas. Lymphoid tissues play an important role in the body.

Table 2. Effect of organic acid supplementation on broiler performance from 7 to 42 days of age.

|                | Body weight, g | ADG, g/b/d | ADFI, g/b/d | FCR, g/g |
|----------------|---------------|------------|-------------|---------|
|                | 42 d          | 7 to 21 d  | 22 to 42 d  | 7 to 21 d | 22 to 42 d | 7 to 21 d | 22 to 42 d | 7 to 21 d | 22 to 42 d | 7 to 21 d | 22 to 42 d |
| BD             | 1931b         | 33.8       | 64.9b       | 35.5     | 113.4b    | 90.1b     | 1.63      | 1.75b     | 1.72b     |
| BD + 1 OA g kg⁻¹ | 1885b         | 29.7       | 62.4c       | 49.6b    | 50.3      | 111.9b    | 87.2b     | 1.69      | 1.79b     | 1.76b     |
| BD + 2 OA g kg⁻¹ | 1905b         | 32.5       | 63.3c       | 51.1ab   | 54.6      | 119.8b    | 92.9ab    | 1.68      | 1.89b     | 1.82ab    |
| BD + 3 OA g kg⁻¹ | 2012b         | 33.1       | 67.6c       | 53.8b    | 56.4      | 120.4b    | 94.8b     | 1.70      | 1.78b     | 1.76b     |
| Pooled SEM     | 42.1          | 0.62       | 0.76        | 0.41     | 0.38      | 1.17      | 0.79      | 0.02      | 0.01      | 0.06      |
| Orthogonal polynomials | Linear | 0.05 | ns | ns | 0.05 | 0.05 | ns | 0.05 | 0.05 | ns | 0.05 | 0.05 |
|                | Quadratic     | ns | ns | 0.05 | ns | ns | ns | ns | 0.05 | 0.05 |

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; BD, basal diet; OA, organic acid. Means represent 4 replicates of 10 birds per replicate for each of the 4 experiments (n=4). b Mean values within a column with no common superscript differ significantly from each other (P<0.05); ns, not significant.

Table 3. Effect of organic acid supplementation on carcass yield and relative weight of organs (g/100 g BW) at 42 days of age.

|                | Carcass yield | Pancreas | Liver | Heart | Bursa | Spleen |
|----------------|---------------|----------|-------|-------|-------|--------|
| BD             | 61.33         | 2.41b    | 19.74 | 5.67ab| 1.07  | 0.81ab |
| BD + 1 OA g kg⁻¹ | 60.64         | 2.10b    | 19.49 | 4.89b | 1.27  | 0.68b  |
| BD + 2 OA g kg⁻¹ | 60.32         | 1.73b    | 19.54 | 5.18ab| 1.04  | 1.04*  |
| BD + 3 OA g kg⁻¹ | 52.25         | 2.79b    | 18.82 | 6.51a | 1.54  | 1.07*  |
| Pooled SEM     | 1.05          | 0.28     | 1.99  | 0.39  | 0.56  | 0.15   |
| Orthogonal polynomials | Linear | ns | 0.05 | ns | 0.05 | ns | 0.05 |
|                | Quadratic     | ns | 0.05 | ns | ns | ns | 0.05 |

BD, basal diet; OA, organic acid. Each value represents the mean of 8 observations (four replicates × 2 birds/replicate). b Mean values within a column with no common superscript differ significantly from each other (P<0.05); ns, not significant.
defense against microorganisms. Broilers have central (thymus and bursa) and peripheral (spleen and all the lymphoid tissue associated to the intestinal mucosa) lymphoid tissues (Akter et al., 2006). A higher relative weight of pancreas and spleen indicates that the broilers were healthy and possessed higher immune status to fight against pathogens and infectious diseases (Abdel-Fattah et al., 2008). Thus, the addition of OA to the diets increased the immune status of the broilers.

OA supplementation significantly affected the retention of AMEn and tP (Table 4). The results indicated that ileal digestibility of tP increased linearly, whereas AMEn increased quadratically (P<0.05) with the increasing dietary levels of OA, and it was maximized at 2 g kg\(^{-1}\) supplemental OA. Inclusion of OA supplementation did not have a significant effect on the ileal digestibility of CP and Ca. Similarly to this study, Nourmohammadi et al. (2012) pointed out that the addition of OA to broiler chicken diets increased AME digestibility. Son et al. (2002) indicated that addition of OA to the broilers diet slowed the passage rate of feed through the gastro-intestinal tract and that the raised digestion time improved the usability of nutrients in broiler chickens. Inclusion of dietary OA may improve energy value of diets by various mechanisms. For instance, OA can acidify the gastro-intestinal tract contents, leading to increased digestive enzymes activity (Moghadam et al., 2006). Nourmohammadi et al. (2012) have reported positive effects of OA on tP digestibility in broilers fed corn- and soybean-based diets. Improved retention of tP might be due to the chelating effects of OA on Ca, resulting in increased phytate solubility and susceptibility to hydrolysis, subsequently increasing tP availability in the gut (Centeno et al., 2007).

The effects of OA on serum enzyme activities are summarized in Figure 1. The results indicated that inclusion of 3 g kg\(^{-1}\) OA significantly increased enzyme activity of ALP and ALT, whereas significantly decreased LDH (linear effect, P<0.05). OA supplementation did not affect AST activity in serum. These results are in agreement with those of Nourmohammadi et al. (2011) who found that addition of OA caused a significant decrease in LDH and ALT enzymes, and also, significant increase in AST activities. Other authors, for example, Brenes et al. (2003), have reported significant effects of OA on the serum enzyme activities in broilers fed corn- and soybean-based diets. Increased activity of ALP might be reflected from the increase in the availability of phosphorus (Huff et al., 1998). Elevated activity is usually an indication of liver or muscle damage. Even though, ALT activity has been reported to be low in all tissues of chickens (Bogin and Israeli, 1976), ALT activities often are increased due to damage in many tissues (Zantop, 1997). Moreover, there are five LDH isoenzymes in birds; each occurring in several tissues, including skeletal muscle, cardiac muscle, liver, kidney, bone and red blood cells that is found to increase in LDH activity which could be related to liver diseases.

Figure 1. Effect of organic acid (OA) supplementation on serum enzyme activity of alkaline phosphatase (a), aspartate aminotransferase (b), alanine aminotransferase (c) and lactate dehydrogenase (d) in broiler chickens. Mean values represent the average of 4 replicates of 4 groups (n = 4). Values are means ± SEM. Within the graph, bars with different letters (a, b) are significantly different (P<0.05).

Table 4. Effect of organic acid supplementation on ileal digestibility of crude protein, apparent metabolizable energy corrected to zero nitrogen retention, calcium, and total phosphorus in broiler chickens.

|            | AMEn, MJ kg\(^{-1}\) | CP, % | Ca, % | tP, % |
|------------|----------------------|-------|-------|-------|
| BD         | 10.21\(^{a}\)        | 82.9  | 61.8  | 39.8\(^{a}\) |
| BD + 1 OA g kg\(^{-1}\) | 11.32\(^{b}\) | 82.4  | 62.7  | 42.9\(^{a}\) |
| BD + 2 OA g kg\(^{-1}\) | 12.08\(^{a}\) | 83.4  | 65.3  | 42.8\(^{a}\) |
| BD + 3 OA g kg\(^{-1}\) | 11.36\(^{b}\) | 83.5  | 62.3  | 44.4\(^{a}\) |
| Pooled SEM | 0.42                 | 1.34  | 2.05  | 1.49  |

| Orthogonal polynomials | AMEn, MJ kg\(^{-1}\) | CP, % | Ca, % | tP, % |
|------------------------|----------------------|-------|-------|-------|
| Linear                 | 0.05                 | ns    | ns    | 0.05  |
| Quadratic              | 0.05                 | ns    | ns    | ns    |

AMEn, apparent metabolizable energy corrected to zero nitrogen retention; CP, crude protein; Ca, calcium; tP, total phosphorus; BD, basal diet; OA, organic acid. Each value represents the mean of 12 observations (four replicates x 3 birds/replicate). \(^{a-b}\) Mean values within a column with no common superscript differ significantly from each other (P<0.05); ns, not significant.
because this enzyme increases quickly as the disease progresses (Zantop, 1997). This study indicates that the birds were apparently healthy throughout the experimental period that could also be correlated to livability.

Conclusions

In the present study, we found that dietary supplementation of OA can improve broiler performance. However, FCR was negatively affected by dietary treatments at higher levels. Dietary OA (Orgacids®) can be used at higher dosage than by manufacturer’s recommended usage rate (1.5 OA g kg⁻¹) to improve growth and ileal digestibility in broilers. To the best of our knowledge no similar work that could be reflected on broiler growth performance and ileal digestibility in broilers. A need to conduct more research in order to establish the suitability of such combinations to enhance satisfactory feed utilization results that could be reflected on broiler growth performance.

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