# The influence of physical form of organic acid and antibiotics on the performance of broiler chickens under bacterial induced challenge

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## Abstract

The present study was conducted to evaluate the influence of physical form of organic acid and antibiotics on the performance of broiler chickens challenged with *Salmonella typhimurium* (*S. typhimurium*). Two hundred forty 1-d-old male broilers (Cobb 500) were equally distributed into 6 treatment groups with 10 blocks and 24 chicks each. The treatments were: group 1 (positive control, basal diet without any addition, non-medicated and unchallenged), group 2 (negative control, basal diet, non-medicated and challenged), group 3 (basal diet with 0.05 g antibiotic flavomycin /kg diet) and groups 4 and 5 (basal diet with 1 g of commercial mixture powder and coated of organic acids fumaric) and citric acids/kg diet), respectively. Group 6 is a blend coated of organic acids (fumaric and citric) and their ammonium salts 2.5 g/kg diet. At 16 days of age, using oral dose, birds were challenged with *S. typhimurium* ($2\times10^8$ CFU/ml). Average body weight of group 3 and 5 was significantly increased ($P < 0.05$) at 21, 28 and 35 days of age and daily weight gain in the overall period was compared to other treatment groups. The highest value of daily feed intake was observed in dietary supplementation compared to the negative control (group 2), but group 2 had lower significant effect ($P < 0.05$) compared to the positive control (group 1) during the 15-21 days of age. However, supplementing diets with experimental groups had no significant effect ($P < 0.05$) on daily feed conversion ratio, during all periods. The results revealed that the supplementation of antibiotics and organic acids as coated capsules into the broiler diets might improve the performance in challenged broiler chicks with *S. typhimurium.*

**Key words*:*** Broiler, *Salmonella typhimurium,* Organic Acids, Antibiotics, Performance.

## Introduction

Poultry is vulnerable to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella* ssp, and *Clostridium* ssp. This depresses growth performance and increases incidence of disease. Antibiotics have been given at sub therapeutic dosage (as feed additive) to stabilize the intestinal microflora, to improve the general performances and to prevent some specific intestinal pathology promote growth. The use of growth promoter antibiotics has been banned by the EU since 2006 because of increasing evidence of microorganisms becoming resistant to antibiotics in both animals and humans (8, 22). Thus, the development of alternatives to antibiotic growth promoters is needed in commercial poultry production. Poultry industry has advanced to the use of alternatives to antibiotics (14), have been proposed such as for example organic acids such as citric acid, acetic acid, propionic acid, formic acid (19), prebiotics, probiotics (7 , 9), and enzymes (6).

The use of organic acids create an acidic environment (pH 3.5 to 4.0) in the gut that favors the development of lactobacilli and inhibit the replication of *Salmonella*, and other gram-negative bacteria. Also, works by activation and functions of proteolytic enzymes stimulates feed consumption, reduces the production of ammonia and other growth-

depressing microbial metabolites, favors mineral absorption, and lowers the incidence of subclinical infections. Several studies support the statement that the addition of organic acids to broiler rations improves weight gain (2, 23), increases feed consumption (20), and improves feed efficiency (1) in broiler chicks. It also decreases pH of cecal digesta, crop and gizzard (3), and intestine (11) in broiler chicks. In addition, there observed improved immune responses by broilers (24, 1). Most previous studies have used a single organic acid as a dietary supplement. Few studies conducted, concerning the effect of mixtures of different organic acids and their capability of such mixtures to substitute antibiotic as growth promoters in broiler diets. This study was conducted to evaluation the effects of physical forms of three commercial sources of organic acids and one antibiotic on growth performance of broilers, under exposed to *Salmonella typhimurium* challenge.

## Materials and Methods

### *Experimental Birds, Diets, and Housing:*

A total of 240, one day-old (Cobb 500) broiler chicks, obtained from commercial hatchery, grown over 35-day period. Birds were individually weighed with almost the same body weight per treatment and randomly assigned to six treatment groups following completely randomized block design. Treatments were randomly allocated in cages within each block of four-tier system; there were 24 birds per blocks (10 blocks × 6 dietary treatments). The experience was in an environmentally controlled battery room. The temperature of environment in the first week of life was 35 $^{o}$C and decreased to 22 $^{o}$C until the end of the experiment. Chicks developed in cage pens were similar managerial and hygienic conditions, fed on a starter and finisher diets from 1- 14 and 15-35 days of age; respectively. Diets were formulated to meet or exceed the requirements suggested by (4) (Table 1)

Organic acids and antibiotic were used as feed additives in this study. Chicken was injected *S. typhimurium* $2\times10^{8}$ CFU/ml (challenged) oral at 16 days of age, which was obtained commercially (MicroBiologics, Cloud); all groups given bacteria challenge except the group 1 (positive control). Chicks of group 1 (positive control) were fed the starter and finisher diets without any addition (non-medicated and unchallenged); group 2 (negative control) non-medicated and challenged; group 3 was dietary supplemented with antibiotic flavomycin 0.05 g/kg diet under bacterial challenge; group 4 was supplemented with commercial mixture powder of organic acids 1 g/kg diet (Fumaric and Citric acids), was purchased from a company of Intermedicavet under bacterial challenge; group 5 was supplemented with commercial mixture coated of organic acids (Formic, Citric acids) and essential oils 1 g/kg diet (Kemin), under bacterial challenge; group 6 is a blend coated of organic acids (Formic acid, Citric acid) and their ammonium salts 2.5 g/kg diet (Selko), under bacterial challenge. Light was provided 24 hs daily, during the experiment.

### *Performance Measurements:*

Measurements of broiler performance included body weight (g), weekly weight gain (g) and weekly feed intake (g). All birds in each group were weighed individually at hatch 1, 2, 3, 4 and 5 weeks of age. Daily weight gains were calculated for the periods: hatch-1, 2, 3, 4 and 5 weeks. The feed offered to each room was recorded daily with an automatic weighing machine. At the end of each week, feed residues were weighed, feed consumption was therefore recorded on a weekly basis, and then calculated as feed consumed per week over the periods: hatch-1 week, 2, 3, 4 and 5 week. The feed

conversion ratios could then be calculated for the periods: hatch-1 week, 2, 3, 4 and 5 week expressed as feed conversion ratio: feed consumed/weight gain.

***Statistical Analysis:***

Data were analyzed based on a completely randomized block design, using the General Liner Model of SAS (25). Significant differences among treatment means were separated by Duncan's new multiple rang test (13) with a 5% level of probability.

## Results

The effect of experimental treatments on the average body weight of broiler chickens, during the period between 1 to 35 days of age, is presented in Table 2. Dietary supplementation with organic acid and antibiotics had no significant effect ($P < 0.05$) in the average body weight, compared with that of control groups at 1, 7 and 14 days of age. However, dietary supplementation with antibiotic (group 3) was the highest significance ($P < 0.05$) in average body weight followed group 5 at 21, 28 and 35 days of age compared to the Group 4 and 2, whereas group 2 (negative control) was of lower significance ($P < 0.05$), compared with group 1 (positive control). Also no significant difference ($P < 0.05$) was found between the group 2 (negative control) and group 4 at 21, 28 and 35 days of age.

Data of daily weight gain at (1-7), (8-14), (15-21), (22-28), (29-35) and (1-35) days of age were listed in Table 3. The obtained data explained that supplementing broiler diet with organic acid and antibiotic had no significant effect ($P > 0.05$) on daily weight gain at (1-7), (8-14), (22-28) and (29-35) days of age. The daily weight gain was significantly ($P<0.05$) lower in birds that fed dietary organic acid and antibiotics at (15-21) days of age, particularly in group 4, as compared with the group 1 and 2 (positive control and negative control). Dietary groups 3 and 5 were significantly higher ($P < 0.05$) in daily weight gain in the overall periods, compared with groups 4 and 2, also no significant ($P > 0.05$) compared with the group 1.

The effect of experimental treatments on the daily feed intake of broiler chickens, during the period between 1 to 35 days of age, is given in Table 4. Dietary organic acid and antibiotics (group 3, 4, 5 and 6) did not affect daily feed intake at (1-7), (8-14), (22-28), (29-35) and (1-35) days of age of the broiler chickens, compared with control group2 and 1, whereas, during the 15-21 days of age, daily feed intake had the highest values for the positive control (group 1) compared to the negative control (group 2). Also, dietary supplementation was the significantly higher compared with negative control (group 2) except group 6 that had no significantly difference with group 2.

There were no significant differences for feed conversion ratio (FCR) among treatments groups during experimental periods (Table 5).

## Discussion

The harmful affected to the integrity of the intestinal epithelium a result to strong inflammatory that induced by *Salmonella* strains (18). These negative effects of *Salmonella* colonization lead to poor digestion and absorption thus bad performance of chickens (12, 21, 15) and this was confirmed in-group 2 in this study. Antibiotics has strong activity against *S. typhimurium,* especially Neomycin, and this was observed in antibiotics (group3) that had the highest improvement for bird's body weight (BW) in our study under *S. typhimurium*. In addition, the results of our study were similar with (1), who found that the addition of dietary citric acid in the form of coated increased live BW of

broiler chicks, as compare with those chickens fed unsupplemented diets. However, a decreased body weight in treatment group 4 may be an unaccepted feed taste because of increasing the dose according to the company 0.4/1kg, where feed intake was tended to decrease and as a result influenced by digestive secretions in GI tract of chickens. Positive effects of organic acids might increase gastric tract microflora activity of broiler chickens. The results of the present study showed a positive improvement of BWG by antibiotics (group 3) and organic acids (group 5) added, when compared with negative control (group 2). Also, our results in this study are compatible with (3, 17) who stated that dietary supplements with antibiotics and organic acids were growth performance improved compared with control under challenge, probably due to the beneficial effect of antibiotics and organic acids on *S. typhimurium*. When compared with the positive control (group 1) not has a positive effect with feed additives (group 3, 5 and 6), where results of this study with respect to effect of organic acids on BWG differed with results of (16). The results of the studies showed that feed additives in order to improve poultry diets BWG, compared with the unsupplemented diets, and this maybe because of the effect of bacteria that irritate the gut mucosa, thereby inhibiting nutrient absorption, so feed additives are working on improving the nutrient absorption.

Regarding feed intake values in this study, they were found similar with (23), who reported no significant effect on feed intake in broiler chicks fed a diet supplemented with citric acid and disagreed with (20, 5). Feed intake did not differ significantly among treatments during the entire period. However, this is in agreement with other researchers (16, 10) who showed no significance for feed intake when adding organic acids to feed additives of broilers; while group 2 was significantly lower in feed intake, compared to all the dietary groups, probably due to the impact of *salmonella* on the bird at (15-21) days of age of the broiler chickens.

Also, our results for FCR was similar with (3, 2), where group 5 had the best FCR but there were no significant differences, compared with other groups during the entire period. On the contrary, they reported that organic acids improved FCR (1).

## Conclusions

A combination of antibiotic (flavomycin) and organic acids (coated capsules form) supplemented to the broiler chickens diet has beneficial effects on live BW and BWG, but without beneficial effects on FI and FCR under induced challenge condition with *S. typhimurium*. In addition, the activity of coated capsules form of organic acids is better than powder form against *S. typhimurium*. Further researches should be carried out to fully explore the positive effects on gut microflora, blood biochemistry and intestinal morphology of broiler chickens.

**Table1.** Diet composition and calculated nutrient content

| ----------Treatment Period (1 -35) days ---------- | | |
|---|---|---|
| **Finisher diet (15- 35d)** | **Starter diet (1-14d)** | **Ingredients** |
| 70.09 | 63.01 | Yellow corn |
| 23.08 | 31.15 | Soybean meal (48% CP) |
| 2.98 | 1.72 | Palm oil |
| 1.87 | 1.96 | Di-calcium phosphate |
| 0.59 | 0.73 | Ground limestone |
| 0.04 | 0.05 | Choline chloride |
| 0.25 | 0.25 | DL-methionine |
| 0.17 | 0.18 | L- lysine |
| 0.25 | 0.25 | Salt |
| 0.11 | 0.12 | Sodium bicarbonate |
| 0.07 | 0.07 | Threonine |
| 0.50 | 0.50 | Vitamin & Mineral premix [a] |
| 100 | 100 | Total |
| | | Calculated analysis |
| 3150 | 3000 | ME (kcal/kg) |
| 17.28 | 20.5 | Crude protein (%) |
| 0.38 | 0.41 | Non phytate P (%) |
| 0.85 | 0.95 | Calcium (%) |
| 0.98 | 1.20 | Lysine (%) |
| 0.51 | 0.55 | Methionine (%) |
| 0.80 | 0.89 | SAA (%) |
| 0.73 | 0.85 | Threonine (%) |

[a] Containing by kg of diets: manganese –10,000 mg; zinc–7500 mg; copper–800 mg; iron–10,000 mg; iodine–45 mg; selenium –44.7 mg; vitamin A –1,100,000 IU; vitamin D –3,360,000 IU; vitamin E–3600 IU; vitamin K–3260 mg; thiamine hydrochloride –260 mg; riboflavin –960 mg; DL–calcium pantothenate–1300 mg; nicotinic acid –8000 mg; sinkaline – 66,000 mg; vitamin B1– 22.7 mg; folic acid –120 mg; pyridoxine hydrochloride –530 mg; biotin–24 mg.

**Table 2**: Effect of dietary treatments on average body weight (g/b/d) in broiler chickens under Induced challenge at 1, 7, 14, 21, 28 and 35 days of age

| ----------(Age (days))---------- | | | | | | |
|---|---|---|---|---|---|---|
| 35 | 28 | 21 | 14 | 7 | 1 | Treatments |
| 1827.28[a] | 1316.15[a] | 732.25[ab] | 337.05 | 124.36 | 43.60 | Group 1 |
| 1713.93[bc] | 1171.40[bc] | 650.13[c] | 325.80 | 111.46 | 43.65 | Group 2 |
| 1879.50[a] | 1325.70[a] | 750.28[a] | 323.33 | 137.60 | 43.67 | Group 3 |
| 1671.43[c] | 1141.23[c] | 641.65[c] | 347.13 | 126.03 | 43.75 | Group 4 |
| 1878.75[a] | 1314.48[a] | 714.30[ab] | 342.43 | 128.64 | 43.77 | Group 5 |
| 1799.98[ab] | 1260.03[ab] | 686.88[bc] | 322.33 | 128.08 | 43.75 | Group 6 |
| 33.806 | 31.709 | 14.552 | 13.754 | 5.474 | 0.1186 | SEM[1] |
| 0.0021 | 0.0022 | 0.0004 | 0.7034 | 0.0865 | 0.8824 | *P*-value |

[a- c] Means values within a columns with different superscripts are significantly different ($P < 0.05$)
[1]Standard error of mean.

**Table 3**: Effect of dietary treatments on daily weight gain (g/b/d) broiler chickens under Induced challenge during 1 to 35 days of age

| ----------(Periods (days))---------- | | | | | | |
|---|---|---|---|---|---|---|
| 1-35 | 29-35 | 22-28 | 15-21 | 8-14 | 1-7 | Treatments |
| 50.95[a] | 75.500 | 84.32 | 57.32[a] | 30.55 | 11.52 | Group 1 |
| 47.75[bc] | 73.425 | 76.57 | 46.25[a] | 29.83 | 9.75 | Group 2 |
| 52.42[a] | 79.125 | 82.32 | 59.62[bc] | 26.32 | 13.35 | Group 3 |
| 46.50[c] | 74.550 | 68.00 | 42.57[c] | 31.75 | 11.77 | Group 4 |
| 52.45[a] | 81.425 | 82.50 | 54.12[ab] | 31.10 | 12.15 | Group 5 |
| 50.15[ab] | 79.125 | 85.37 | 51.20[abc] | 27.82 | 12.05 | Group 6 |
| 0.971 | 5.024 | 4.331 | 3.336 | 2.413 | 0.796 | SEM[1] |
| 0.0023 | 0.8470 | 0.0981 | 0.0195 | 0.5995 | 0.1134 | *P*-value |

[a- c] Means values within a columns with different superscripts are significantly different ($P < 0.05$).
[1]Standard error of mean.

**Table 4**: Effect of dietary treatments on daily feed intake (g/b/d) in broiler chickens under Induced challenge during 1 to 35 days of age

| ----------(Periods (day))---------- | | | | | | |
|---|---|---|---|---|---|---|
| 1-35 | 29-35 | 22-28 | 15-21 | 8-14 | 1-7 | Treatments |
| 85.77 | 159.25 | 126.77 | 91.47[a] | 54.67 | 21.42 | Group 1 |
| 83.77 | 150.25 | 117.95 | 73.92[c] | 49.78 | 19.37 | Group 2 |
| 84.00 | 138.75 | 127.00 | 85.72[ab] | 47.78 | 22.85 | Group 3 |
| 78.65 | 134.98 | 118.25 | 84.02[ab] | 44.99 | 20.65 | Group 4 |
| 80.35 | 144.23 | 120.50 | 87.35[ab] | 41.09 | 21.12 | Group 5 |
| 83.85 | 149.53 | 120.20 | 80.15[bc] | 45.82 | 20.52 | Group 6 |
| 2.076 | 9.225 | 3.011 | 3.024 | 4.226 | 0.863 | SEM[1] |
| 0.2075 | 0.5060 | 0.1654 | 0.0156 | 0.3583 | 0.1788 | *P*-value |

[a- c] Means values within a columns with different superscripts are significantly different ($P < 0.05$).
[1]Standard error of mean.

**Table 5**: Effect of dietary treatments on daily feed conversion ratio (g: g) in broiler chickens under Induced challenge during 1 to 35 days of age

| ----------(Periods (day))---------- | | | | | | |
|---|---|---|---|---|---|---|
| 1-35 | 29-35 | 22-28 | 15-21 | 8-14 | 1-7 | Treatments |
| 1.70 | 2.10 | 1.47 | 1.52 | 1.81 | 1.87 | Group 1 |
| 1.77 | 2.00 | 1.50 | 1.65 | 1.65 | 1.97 | Group 2 |
| 1.62 | 1.77 | 1.57 | 1.40 | 1.82 | 1.67 | Group 3 |
| 1.70 | 1.92 | 1.67 | 1.97 | 1.42 | 1.82 | Group 4 |
| 1.52 | 1.72 | 1.47 | 1.65 | 1.35 | 1.85 | Group 5 |
| 1.65 | 1.97 | 1.52 | 1.70 | 1.69 | 1.75 | Group 6 |
| 0.061 | 0.132 | 0.068 | 0.126 | 0.151 | 0.158 | SEM[1] |
| 0.1505 | 0.3815 | 0.3255 | 0.0935 | 0.1975 | 0.8204 | *P*-value |

[a- c] Means values within a columns with different superscripts are significantly different ($P < 0.05$).
[1]Standard error of mean.

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# تأثير الشكل الفيزيائي للأحماض العضوية والمضادات الحيوية على الأداء الإنتاجي لبداري التسمين المُعرضة للتحدي البكتيري

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## الملخص

أجريت هذه الدراسة لتقييم تأثير الشكل الفيزيائي للأحماض العضوية والمضادات الحيوية على الأداء الإنتاجي لبداري التسمين المعرضة للتحدي البكتيري بسالمونيلا التيفوئيد. تم توزيع 240 كتكوت بعمر يوم واحد من سلالة كوب (Cobb 500)، بالتساوي الى ستة مجموعات (معاملات) احتوت كل مجموعة على 10 قطاعات في كل قطاع 24 كتكوت. كانت معاملات الدراسة كالآتي المجموعة 1(السيطرة الموجبة، العليقة الأساسية من دون أي إضافة ،من دون معالجة ومن دون تحدي بكتيري)، المجموعة 2 (السيطرة السالبة، العليقة الأساسية، دون معالجة، تحت التحدي البكتيري)، المجموعة 3 (العليقة الأساسية، مع إضافة 0.05 جرام مضاد حيوي (فلافومايسين)/كجم عليقة)، المجموعة 4 و5 (العليقة الأساسية مع إضافة 1 جم/كجم عليقة خليط من مسحوق وحبيبات مغلفة لحمضي الفوماريك والستريك، على التوالي)، المجموعة 6 (العليقة الأساسية مع إضافة خليط من حبيبات مغلفة لحمضي الفورميك والستيريك بالإضافة 2.5 جم/كجم عليقة من أملاح الأمونيوم). تم تعريض الطيور للتحدي البكتيري بسالمونيلا التيفوئيد بالتجريع عن طريق الفم ($2\times10^8$ وحدة مستعمرة نامية/مل) عند يوم 16 من العمر. وجدت زيادة معنوية ($P<0.05$) في زيادة وزن الجسم في المجموعتين 3 و5 خلال 21، 28 و35 يوم من العمر، وفي الزيادة الوزنية اليومية طوال فترة التجربة بالمقارنة مع بقية المجموعات. و لوحظت أعلى القيم في مقدار الغذاء المأكول في كل مجموعات التجربة بالمقارنه بالمجموعة 2 (السيطرة السالبة)، في حين لوحظ انخفاض معنوي ($P<0.05$) في مقدار الغذاء المأكول في المجموعة 2 مقارنة بالمجموعة 1 خلال 15-21 يوم من العمر. في حين لم تلاحظ أية فروق معنوية ($P < 0.05$) في معامل التحويل الغذائي فيما بين المجموعات خلال كل مراحل التجربة. كشفت النتائج أنَ إضافة المضادات الحيوية والأحماض العضوية بشكل كبسولات مغلفة إلى علائق بدارى التسمين يمكنها تحسين الأداء الإنتاجي لبداري التسمين المعرضة للتحدي البكتيري بسالمونيلا التيفوئيد.

**الكلمات المفتاحية**: بداري التسمين، سالمونيلا التيفوئيد، الأحماض العضوية، المضادات الحيوية، الأداء الإنتاجي.