Effect of Supplementation of Salts of Organic Acids on Serum and Haematological Parameters of Broilers

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A B S T R A C T

A trial was conducted to investigate the effects of dietary salts of organic acids for a period of 42 days on the serum parameters of broiler chicks. 300 commercial broiler chicks were randomly distributed into five treatments having six replicates consisting of ten birds each. A maize-soybean based diet was used as the basal diet (Control group). The basal diet was supplemented with salts of organic acids (sodium butyrate, calcium propionate @ 0.5% and 1%). Highest body weight gain (2338.2g.) was observed in the 1% sodium butyrate supplemented group and difference was significant (P<0.05) as compared to control group (1967.2g.). FCR was significantly improved in the supplemented groups as compared to control group. Inclusion of sodium butyrate and calcium propionate in the basal diet significantly increased serum phosphorus level while supplementation of organic acids showed no significant (P>0.05) difference in the concentration of serum cholesterol, triglycerides, HDL, LDL and calcium in all the treated groups. Dietary acidification significantly improved the haemoglobin value and RBC count and lowered the heterophils number.

Introduction

The growing demand for poultry products worldwide requires novel strategies that increase the efficiency of poultry production. Optimization of nutrient utilization is an essential element in such strategies. The growth is 10-12% in broilers per year and the per capita chicken consumption has gone up from 400 grams to 2.5 kg (APEDA, 2016). In previous years, the extensive uses of antibiotics in animal farms to promote growth rate, increasing feed efficiency and prevention of intestinal infections have led to the development of antibiotic-resistant bacteria in the gastrointestinal tract and drug residuals in meat. But, now a days, various researchers have developed a number of non-therapeutic alternatives of antibiotics to improve the production performance of the animals. One of such alternatives is use of organic acids as feed additives in the animal production. Organic acids are organic carboxylic acid including fatty acids and amino acids, of the general structure R-COOH such as formic, acetic, propionic and butyric acids or carboxylic acids with the hydroxyl group such as lactic, malic, tartaric and citric acids or short-chain carboxylic acids containing double bonds like fumaric and sorbic acids (Shahidi et al., 2014). Health of the gut is one of the major factors governing the
performance of birds and thus, the profile of intestinal microflora plays an important role in gut health (Soltan, 2008). Organic acids can improve bird’s performance due to their antimicrobial activity, which improve protein and energy digestibility thereby reducing microbial competition with the host for nutrients and endogenous nitrogen losses, as well as by lowering the incidence of subclinical infections and the secretion of immune mediators (Wang, 2010). Acidified diets had significant reduction in serum level of cholesterol while, serum calcium, phosphorus and magnesium concentrations were significantly increased (Kamal et al., 2014 and Taherpour et al., 2009). Therefore, the present work was conducted to investigate the effects of dietary supplementation of salts of organic acids on blood and serum analysis for cholesterol, lipoptorteins and minerals in broiler chickens.

Materials and Methods

Five diets were composed for each growth period. As per BIS (2007) maize based diet was figured to fulfill the metabolizable energy (ME), crude protein and limiting amino acids (methionine and lysine) requirements of birds, to serve as control (T1). Level of crude protein and ME content in starter (0-4 weeks) ration were 22 percent and 3100 kcal/kg and the corresponding values in finisher (4-6 weeks) ration were 20 percent and 3200 kcal/kg. Three hundred commercial day old broiler chicks were purchased from a local commercial hatchery. The chicks were individually weighed, wing banded and randomly distributed into 30 subgroups having five dietary treatments with six replicates per treatment having 10 birds in each replication. The first group was kept as a control (T1) and given the basal diet while, second (T2), third (T3), fourth (T4) and fifth (T5) groups given a 0.5% sodium butyrate, 1% sodium butyrate, 0.5% calcium propionate and 1% calcium propionate respectively in the diet. Birds were vaccinated against F1 strain of Ranikhet disease on 0, 7th day and Gumboro disease on 14th day.

The experimental chicks were reared under deep litter system. Proper ventilation of the house is important for the flow of fresh air into the house and for removal of other harmful gases from the house. The feeding programme consisted of a starter diet from 0 to 28 days and a finisher diet from 29 to 42 days. Weighed amount of feed was offered on paper sheets for first 3 days and thereafter, in the automatic feeders up to 28 days of age. Afterwards, the feeds were offered through hanging feeders maintained at appropriate heights. The chicks were provided ad libitum clean drinking water throughout the experiment. Ingredient and chemical composition of experimental diets are given in Table 1 and chemical composition of the feed ingredients has been given in Table 2.

Observations and sampling

Weekly feed intake and residual amount per replicate was measured throughout the experiment to calculate the feed consumption per bird. The birds were weighed individually at weekly interval and the body weights were recorded to calculate body weight gain.

Blood samples were collected from the slaughtered birds in non-heparinised tubes and in EDTA tubes for haematology using auto analyser. The samples were centrifuged at 3000 rpm for 15 minutes and serum obtained was stored at -20°C until analysis. Serum parameters were determined by auto analyzer using commercially available kits purchased from Transasia Biomedical Ltd. Serum samples were analysed for different serum variables like – Total cholesterol, Triglyceride, HDL, LDL, Calcium and Phosphorus.
Data was analyzed statistically as described by Snedecor and Cochran, 1994. Analysis of variance was used to study the differences among treatment means and they were compared by using Duncan’s (1995) Multiple Range Test as further modified by Kramer (1956).

**Results and Discussion**

There was significant improvement in body weight gain on supplementation of salts of organic acids. Highest body weight gain was recorded in the T₃ group (2338.2g.) and difference was significant (P<0.05) as compared to control group (1967.2g) (Table 3).

Also 1% calcium propionate supplemented group showed significantly higher body weight gain in comparison to the control group. Improvement in the body weight gain recorded in the present study could be attributed to the improved digestibility of proteins, amino acids and the absorption of minerals on supplementation of organic acids (Mroz et al., 2000).

Similar results were also observed by Ragga et al., (2016) who reported that thyme or formic acid or formic acid plus thyme had significant effects on growth performance and carcass traits of broilers (P<0.05).

In case of feed intake sodium butyrate at its both levels showed higher feed intake (3759.84, 4161.50) than the control group (3612.92) followed by T₅ and these differed significantly with that in the control group. Mohammadagheri et al., (2016) also observed increased (P <0.05) feed consumption and body weight gain after vinegar supplementation. Similar findings were observed in other studies with supplementation of butyrate (Panda et al., 2009) and partially protected sodium butyrate (Mallo et al., 2010).

Besides these, highest improvement in the FCR was in T₃ group followed by T₅ group and both the groups were significantly differ from the control group. The results of FCR obtained in this experiment confirm those reported by Khosravi et al., (2008) in broilers fed diet supplemented with 2 g/kg propionic acid during 21 d period.

Better feed conversion ratio was found on 0.5% supplementation of organic acid during 0-5 weeks of age. The improvement in FCR in organic acid supplemented group could be possibly due to better utilization of nutrients resulting in increased body weight gain (Adil et al., 2008).

Data presented in Table 4 showed that supplementation of organic acids showed no significant (P>0.05) difference in the concentration of serum cholesterol, triglycerides, HDL, LDL and Calcium among all the groups while serum phosphorus level was significantly improved in all the treated groups. These results are in line with previous studies (Seifi et al., 2015; Hernandez et al., 2006).

Furthermore, formic acid addition to broilers diet did not alter the biochemical profile of blood under conditions of good hygiene (Hernandez et al., 2006). The increase of Ca and P levels in blood serum produced by addition of organic acids may be attributed by the lowered GI-tact pH which increases the absorption of such minerals from the gut into the blood stream.

The WBC count of birds receiving diet containing salts of organic acids were significantly (P<0.05) increased in T₃ and T₅ than the control group. Haemoglobin content and RBC count were recorded highest in the T₃ group, other treatments didn’t show any significant difference. Highest haematocrit value was observed in T₃ group (Table 5).
Table 1 Ingredient and chemical composition of experimental diets

| Ingredient (kg/100 kg feed) | 0-4 weeks | 4-6 weeks |
|----------------------------|-----------|-----------|
| Maize                      | 55        | 60        |
| Soybean meal               | 24        | 20        |
| Groundnut cake             | 10        | 10        |
| Fish meal                  | 5         | 3         |
| Vegetable oil              | 4         | 5         |
| Mineral mixture            | 2         | 2         |

**Feed additives (g/100kg feed)**

| Spectromix                  | 10        | 10        |
| Spectromix BE*              | 20        | 20        |
| Chlortetracycline           | 33.5      | 33.5      |
| Veldot                      | 50        | 50        |
| Choline chloride            | 50        | 50        |
| Lysine                      | 50        | 50        |
| DL-methionine               | 150       | 150       |
| Carbon tetrachloride        | 150       | 150       |
| Sodium butyrate             | 0.5,1%    | 0.5,1%    |
| Calcium propionate          | 0.5,1%    | 0.5,1%    |

**Calculated Chemical composition (% DM basis)**

| Moisture   | 11.84 | 11.58 |
| DM         | 88.16 | 88.42 |
| CP         | 22.01 | 20.10 |
| EE         | 4.81  | 5.02  |
| CF         | 4.47  | 5.05  |
| Ash        | 9.10  | 9.20  |
| NFE        | 47.77 | 49.05 |

*Spectromix BE: Each gm. Contained Vit.B1- 8mg, Vit.B6- 16mg, Vit.B12- 80mg, niacin-120mg, calcium pentothenate-80mg, Vit. E-160 mg, Lysine hydrochloride-10 mg, DL-methionine-10 mg and calcium – 260 mg. Mixing rate: 20g/100kg of feed

Table 2 Chemical composition of feed ingredients (% DM basis)

| Ingredient       | CP (%) | EE (%) | CF (%) | TA (%) | Lysine* (%) | Methionine* (%) | ME* (kcal/kg) | Calcium (%) | Phosphorus (%) |
|------------------|--------|--------|--------|--------|-------------|----------------|--------------|-------------|----------------|
| Maize            | 9.1    | 3.44   | 2.44   | 2.25   | 0.18        | 0.15           | 3300         | 0.90        | 0.45           |
| Soyabean meal    | 45.2   | 3.16   | 3.93   | 8.47   | 2.57        | 0.76           | 2230         | 0.20        | 0.65           |
| Groundnut cake   | 40     | 1.52   | 7.23   | 6.62   | 1.82        | 0.49           | 1180         | 0.21        | 0.54           |
| Fish meal        | 50     | 13.5   | 1.79   | 39.62  | 1.42        | 1.42           | 2600         | 3.40        | 2.20           |

*Calculated values (Singh and Panda., 1998)
### Table 3: Effects of different levels of salts of organic acids on growth performance of broiler chickens at 42 d of age

| Treatment | Total body weight gain (g.) | Total feed intake (g.) | FCR |
|-----------|----------------------------|------------------------|-----|
| **T₁**    | 1967.2 ± 23.2              | 3612.92 ± 31.7         | 1.84 ± 0.01 |
| **T₂**    | 2026.2 ± 17.1              | 3759.84 ± 17.0         | 1.86 ± 0.01 |
| **T₃**    | 2338.2 ± 16.4              | 4161.50 ± 28.9         | 1.78 ± 0.01 |
| **T₄**    | 2011.2 ± 11.2              | 3751.50 ± 12.9         | 1.87 ± 0.01 |
| **T₅**    | 2084.6 ± 19.6              | 3808.67 ± 35.5         | 1.83 ± 0.02 |

Means bearing different superscripts in a column differ significantly (P<0.05)

### Table 4: Serum cholesterol, triglycerides, HDL, LDL Calcium and Phosphorus under different dietary treatments

| Treatment | Cholesterol (mg/dl) | Triglycerides (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | Calcium (mg/dl) | Phosphorus (mg/dl) |
|-----------|---------------------|-----------------------|-------------|-------------|-----------------|-------------------|
| **T₁**    | 159.67 ± 2.53       | 86.48 ± 0.68          | 120.95 ± 0.47 | 34.57 ± 0.64 | 11.38 ± 0.25    | 5.40 ± 0.28       |
| **T₂**    | 158.33 ± 2.69       | 86.00 ± 1.03          | 120.82 ± 0.79 | 34.44 ± 0.28 | 11.87 ± 0.20    | 6.06 ± 0.23       |
| **T₃**    | 157.00 ± 1.84       | 85.92 ± 0.82          | 121.33 ± 0.60 | 34.39 ± 0.63 | 12.43 ± 0.19    | 7.11 ± 0.23       |
| **T₄**    | 159.50 ± 2.64       | 86.28 ± 0.99          | 120.35 ± 0.61 | 35.52 ± 0.51 | 11.42 ± 0.23    | 6.11 ± 0.08       |
| **T₅**    | 158.50 ± 3.04       | 86.27 ± 0.44          | 120.55 ± 1.24 | 34.43 ± 0.59 | 11.85 ± 0.10    | 6.16 ± 0.13       |

Means bearing different superscripts in a column differ significantly (P<0.05)

### Table 5: Effects of different levels of salts of organic acids on haematological parameters

| Treatment | RBC (x10⁶/µL) | WBC (x10³/µL) | HB (mg/100ml) | Haematocrit (HCT%) | Heterophils (%) | Lymphocytes (%) | H:L Ratio |
|-----------|---------------|---------------|---------------|-------------------|-----------------|-----------------|-----------|
| **T₁**    | 2.90 ± 0.02   | 24.14 ± 0.36  | 11.38 ± 0.18  | 29.68 ± 0.23      | 30.12 ± 0.42    | 65.95 ± 0.44    | 0.46 ± 0.004 |
| **T₂**    | 2.86 ± 0.05   | 24.90 ± 0.30  | 11.29 ± 0.20  | 29.90 ± 0.44      | 28.64 ± 0.21    | 67.39 ± 0.38    | 0.42 ± 0.006 |
| **T₃**    | 2.95 ± 0.03   | 26.42 ± 0.25  | 11.55 ± 0.25  | 30.36 ± 0.36      | 27.44 ± 0.38    | 67.40 ± 0.27    | 0.41 ± 0.004 |
| **T₄**    | 2.93 ± 0.02   | 24.96 ± 0.19  | 11.29 ± 0.23  | 28.90 ± 0.33      | 28.50 ± 0.41    | 68.20 ± 0.27    | 0.42 ± 0.008 |
| **T₅**    | 3.06 ± 0.03   | 26.63 ± 0.33  | 12.18 ± 0.19  | 28.74 ± 0.44      | 25.65 ± 0.46    | 68.26 ± 0.35    | 0.38 ± 0.008 |

Means bearing different superscripts in a column differ significantly (P<0.05)

### Table 6: Economics of broiler production under different dietary treatments

| Treatment | Cost of chick (Rs.) | Feed intake (kg) | Cost of feed/kg (Rs.) | Cost of feed (Rs.) | Cost of additive (Rs.) | Total cost (Rs.) | Weight of bird (kg) | Sale price/bird (Rs.) | Profit (Rs.) |
|-----------|---------------------|------------------|-----------------------|-------------------|------------------------|-----------------|---------------------|----------------------|---------------|
| **T₁**    | 30                  | 3.612            | 29.59                 | 121.42            | -                      | 151.42          | 1.965               | 176.85               | 25.43         |
| **T₂**    | 30                  | 3.761            | 29.59                 | 126.12            | 1.62                   | 157.74          | 2.198               | 197.82               | 40.08         |
| **T₃**    | 30                  | 4.160            | 29.59                 | 141.27            | 3.54                   | 174.81          | 2.490               | 224.10               | 49.29         |
| **T₄**    | 30                  | 3.751            | 29.59                 | 125.88            | 1.69                   | 157.57          | 2.156               | 194.04               | 36.47         |
| **T₅**    | 30                  | 3.836            | 29.59                 | 130.45            | 3.45                   | 163.90          | 2.191               | 197.19               | 33.29         |
Supplementation of salts of organic acids significantly reduced the number of heterophils and lowest value was observed in T5 group followed by T3 group and significantly improved the lymphocytes count in different treatments as compared to control group. Similarly ratio of heterophils to the lymphocytes followed the trend as in heterophils count. However Hassan and Abdel Raheem (2016) revealed that there were no significant differences between different experimental groups in hemoglobin concentration, WBCs, RBCs count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration and lymphocyte percentage on feeding of benzoic acid to broiler Chickens. Moreover, dietary inclusion of benzoic acid didn't affect the total leucocytic count and differential leucocytic count at days 21 and 42 of broilers life (Abdalla et al., 2013).

The economics of broiler production was calculated by taking into consideration the cost of day old broiler chicks and feed consumed. The total feed cost/bird for treatment T1, T2, T3, T4 and T5 was Rs. 121.42, 126.45, 139.85, 126.11 and 128.96 respectively (Table 6).

Total cost of production together with cost of chicks, rearing of chicks, feed and growth promoter per bird that was Rs. 151.42, 158.07, 173.39, 157.80 and 162.41 for treatment T1, T2, T3, T4 and T5 respectively.

The profit was 25.43, 40.08, 49.29, 36.47 and 33.29 for treatment T1, T2, T3, T4 and T5 respectively. The relative profit of organic acid supplementation contrast to control was highest in treatment T3 which was Rs 49.29.

It is concluded that sodium butyrate and calcium propionate at 1% level had significantly improved the haematological and serum constituents. So these can be incorporated in the broiler’s diet without any harmful effect.

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