Growth studies in commercial broiler birds offered citric acid in formulated feed with low mineral density

Shivani Katoch1 · Sumani Sharma1 · Varun Sankhyan1 · Daisy Wadhwa1 · Arun Sharma1 · Sanjiv Kumar1

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Abstract
Study of 35 days was conducted to evaluate citric acid (CA) as an additive in poultry broiler feed with lower mineral content of calcium (Ca) and total phosphorus (TP) in commercial broiler poultry birds for its effect on growth, nutrient utilization, carcass characteristics, and economics. Vancobb-400 strain day old broiler chicks were divided into four main treatment groups T0, T1, T2, and T3. Treatment groups were further divided into eight replicates with ten chicks in each. T0 served as control, given standard corn-soy flakes-based ration (Pre-starter %: Crude protein (CP)-23, Ca-1.00, TP-0.70; Starter %: CP-22, Ca-1.10, TP-0.72, and Finisher %: CP-20, Ca-0.99, TP-0.70). Treatment T1 served as positive control with added 0.5% CA (Pre-starter %: CP-23, Ca-1.00, TP-0.70; Starter %: CP-22, Ca-1.10, TP-0.72 and Finisher %: CP-20, Ca-0.99, TP-0.70). Treatment T2 was given feed containing 0.5% CA with low Ca and TP content (Pre-starter %: CP-23, Ca-0.90, TP-0.66; Starter %: CP-22, Ca-0.99, TP-0.71 and Finisher %: CP-20, Ca-0.90, TP-0.69), whereas treatment T3 was given feed containing 0.5% CA with moderately low Ca and TP content (Pre-starter %: CP-23, Ca-0.80, TP-0.65; Starter %: CP-22, Ca-0.88, TP-0.70 and Finisher %: CP-20, Ca-0.79, TP-0.68). Birds offered moderately low Ca and TP with 0.5% CA addition, exhibited higher growth rate ($P<0.05$), better nutrient utilization with positive influence on dressing percentage and forequarters weight. Economics of broiler feeding revealed that 0.5% CA supplementation fetched highest gross return above feed cost in broiler birds offered feed with moderately low Ca and TP whereas lowest profit was recorded in feed with low content of Ca and TP. In conclusion, supplementation of 0.5% CA in feed with low and moderately low Ca and TP content positively influenced overall growth, and carcass characteristics. Economics of broiler feeding with moderately low Ca and TP content revealed highest profit with CA (0.5%) supplementation.

Keywords Citric acid · Chicken · Growth · Nutrition · Economics · Calcium · Phosphorus

Introduction
Gut (or intestinal health) is a key factor that can affect the performance of poultry and thus play key role in profitable poultry production (Samik et al. 2007), and the composition of the intestinal micro flora has a major influence on intestinal health. Antimicrobial drug resistance has alarmed the scientists all over the world especially for the one used as livestock feed additives. Antibiotics added to/used in animal feeding such as tetracycline, bacitracin (Diarr and Malouin, 2014) which are given in preventive doses often lead to survival of pathogenic microbes which in turn develop drug resistance (Diarr et al. 2007; Furtula et al. 2010; Forgetta et al. 2012). Therefore, to avoid drug resistance, there is a search for alternatives to feed grade antibiotics. Moreover, the increasing broiler industry worldwide needs products or supplements which improve quality of produce and at the same time does not compromise yield; thus, there is continual search of such additives which can reduce dependence on antibiotics. Recent reports suggest that organic acids (OA) are being popularly used as non-antibiotic feed additives in poultry nutrition (Windisch et al. 2008). These acids potentially act and exhibit response by reducing pH of intestinal tract and thus favoring beneficial microbes which subsequently suppress the pathogenic microbes (Jin et al. 1997; Ghabdan, 2002), reducing the use of antibiotics. Breakdown of protein and fiber in the digestive system is enhanced with citric acid (CA) which is an organic acid
Mash into cubicles measuring 5 m × 3 m × 1.5 m each. The deep litter pen floor was divided using wire partitions. A schedule of 24 h per day was followed till the end of the experiment. The deep litter pen floor was divided using wire partitions. Each treatment had 80 birds. Birds were shifted from brooder house to deep litter system on the 7th day, and a lighting adaptation period of 3 days was provided to them adaptation time in the metabolic cages. After adaptation period of 3 days, the weighed quantity of feed for the same treatment feed for 3 days as in the growth study was given. 

The digestibility of the feed was evaluated using the fecal collection method on the 24th day onwards. Two male birds selected randomly from each replicate having similar weight were shifted to electric battery brooders. Birds were offered the same treatment feed for 3 days as in the growth study to provide them adaptation time in the metabolic cages. After adaptation period of 3 days, the weighed quantity of feed for next five consecutive days was offered to each replicate, both during morning at 9:00 a.m. and in evening at 6:00 p.m. Actual consumption of feed was estimated by weighing the

Materials and methods

Experimental design and management

The experimental plan was approved by the Institutional Animal Ethics Committee of College of Veterinary and Animal Sciences (COVAS) Palampur. The experiment was carried out in an experimental chicken coop on a deep bed in 320–day-old-meat-type chicken (Vencobb-400). The poultry house was kept at 30 ± 1°C during the first 2 weeks and gradually reduced to 21 ± 1°C by the end of the experiment. The relative humidity ranged between 40 and 60% throughout the experimental period. Feed was offered ad libitum and water was always accessible for drinking.

Experimental plan

Chicks were reared in electric battery brooders during first week post hatch. Single battery brooder consists of ten compartments placed vertically measuring 36 × 72 in. per compartment. Each compartment was allocated to a specific replicate with ten chicks in complete randomized design during which, chicks were wing tagged, weighed, and randomly distributed according to the experimental plan into four groups T₀, T₁, T₂, and T₃. Each group was divided into eight replicates with ten chicks in each replicate; thus, each treatment had 80 birds. Birds were shifted from brooder house to deep litter system on the 7th day, and a lighting schedule of 24 h per day was followed till the end of the experiment. The deep litter pen floor was divided using wire mesh into cubicles measuring 5 × 4 × 6 ft each, covered with wood shaving up to 6-cm height. T₀ served as control diet and was given standard corn-soy flake-based ration (Pre-starter %; CP; Ca; TP-23, 0.70, 0.50, 0.50). Treatment T₁ served as positive control with added 0.5% CA (Pre-starter %; CP; Ca; TP-23, 0.80, 0.50, 0.50). Treatment T₂ served as positive control with added 0.5% CA but with low Ca and TP content (Pre-starter %; CP; Ca; TP-23, 0.80, 0.60; Starter 22, 0.99, 0.50; Finisher 20, 0.70, 0.60). Further treatment T₃ served as positive control with added 0.5% CA but with low Ca and TP content (Pre-starter %; CP; Ca; TP-23, 0.90, 0.60; Starter 22, 0.90, 0.60; Finisher 20, 0.70, 0.60). Further treatment T₄ served as positive control with added 0.5% CA but with low Ca and TP content (Pre-starter %; CP; Ca; TP-23, 0.90, 0.60; Starter 22, 0.90, 0.60; Finisher 20, 0.70, 0.60).

The physical and chemical composition of diet for different phases is presented in Table 1.

Proximate analysis

The chemical composition of feed and feed ingredients were analyzed in agreement with AOAC (2000, 2005). The dry matter was determined by oven drying for 16 h at 105 °C (AOAC International, 2000; method no: 930.15), total ash (AOAC International, 2000; method no: 942.05) by muffle burning, fat by Soxhlet extraction with petroleum ether (AOAC International, 2005; method no: 990.03) employing the equipments (Pelican Kel plus Ultimate duo Dist-TS E-Tamil Nadu, India distillation assembly; titration using Titro Line-7000 titrator, SI Analytics, Germany), crude fiber (AOAC International, 2000; method no: 2003.05) using (Socs Plus 08AS DLS, Pelican equipment’s, Tamil Nadu, India) nitrogen as per (AOAC International, 2000; method no:990.03) employing the equipments (Pelican Kel plus KES 12L R-Tamil Nadu, India; digestion assembly-Pelican p Kel plus Ultimate Duo Dist-TS E-Tamil Nadu, India distillation assembly; titration using Titro Line-7000 titrator, SI Analytics, Germany), crude fiber (AOAC International, 2000; method no: 978.10) by the use of (Pelican FIBRA Plus FES4-Tamil Nadu, India). Ca was estimated by atomic absorption spectrophotometry method (AA-8000 LABINDIA, Mumbai, India) whereas TP in ashed samples were estimated calorimetrically by (Spectronic 200, Thermo Fisher Scientific, US) as per the method proposed by Parks & Dunn (1963). Similarly, metabolizable energy (ME) was calculated using the equation as proposed by Lodhi et al. (1976).
residual feed on the 5th day in each replicate. The excreta voided by birds from each replicate were collected in the morning and weighed on daily basis, screened for the presence of feathers and feed particles which were removed manually. Around 10% of the total excreta was taken as final sample. The samples were mixed carefully in plastic tray and further divided into two similar portions for the subsequent determination of nitrogen and dry matter. Twenty mL of 5% sulfuric acid was mixed in the feces collected for nitrogen estimation to avoid nitrogen loss, and the feces collected for dry matter estimation was dried separately at 105 °C in hot air oven till the constant weight was achieved, and then pooled samples were ground and analyzed for various proximate parameters in the laboratory of the Department of Animal Nutrition. The digestibility of nutrients viz dry matter, ether extract, crude fiber, and retention of nitrogen, Ca, and P was calculated by total fecal collection method, which involves measurement of feed intake and excreta voided.

**Growth performance**

Pre-weighed formulated feed was offered twice daily as per the experimental plan to the broiler birds from 0 day till 35 days of age. Various parameters like gain in body weight (GIW), feed intake (FI), feed conversion ratio (FCR), GIW/day, FI/day, and mortality % were recorded. Body weight

### Table 1 Physical and chemical composition of broiler Pre-starter, Starter, and Finisher diet (per cent dry matter basis)

| Attribute                  | Pre-starter (0–14 days) | Starter (15–21 days) | Finisher (22–35 days) |
|---------------------------|-------------------------|----------------------|-----------------------|
|                           | T₀  | T₁  | T₂  | T₃  | T₀  | T₁  | T₂  | T₃  | T₀  | T₁  | T₂  | T₃  |
| Ingredient composition (%)|     |     |     |     |     |     |     |     |     |     |     |     |
| Yellow corn-9%             | 52  | 52  | 52  | 52  | 52  | 52  | 52  | 52  | 52  | 52  | 52  | 52  |
| Soy flakes-45%             | 41.5| 41.5| 41.5| 41.5| 41.5| 41.5| 41.5| 41.5| 41.5| 41.5| 41.5| 41.5|
| Palm oil                   | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   | 3   |
| Dicalcium phosphate        | 1.0 | 1.0 | 0.9 | 0.8 | 2.0 | 2.0 | 1.8 | 1.6 | 2.0 | 2.0 | 1.8 | 1.6 |
| DL-Methionine              | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Lysine                     | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Limestone powder           | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Salt                       | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Citric acid (CA)           | 0.0 | 0.5 | 0.5 | 0.5 | 0.0 | 0.5 | 0.5 | 0.5 | 0.0 | 0.5 | 0.5 | 0.5 |
| Sodium bentonite           | 0.0 | 0.0 | 0.1 | 0.2 | 0.0 | 0.0 | 0.2 | 0.4 | 0.0 | 0.0 | 0.2 | 0.4 |
| Premix*                    | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

Chemical composition (% DM basis-calculated)

| Attribute                  | Pre-starter (0–14 days) | Starter (15–21 days) | Finisher (22–35 days) |
|---------------------------|-------------------------|----------------------|-----------------------|
| DM (dry matter)           | 90                      | 90                   | 90                    |
| CP (crude protein)        | 23                      | 23                   | 23                    |
| EE (ether extract)        | 3.1                     | 3.1                  | 3.1                   |
| CF (crude fiber)          | 5.4                     | 5.4                  | 5.4                   |
| TA (total ash)            | 4.05                    | 4.05                 | 4.05                  |
| AIA (acid insoluble ash)  | 3                       | 3                    | 3                     |
| Ca (calcium)              | 1.00                    | 1.00                 | 1.00                  |
| TP (total phosphorus)     | 0.70                    | 0.70                 | 0.70                  |
| NFE (nitrogen free extract) | 64.45                  | 64.45               | 64.45                 |
| ME (metabolizable energy-Kcal/kg) | 3077               | 3077                | 3077                  |
| Methionine                | 0.55                    | 0.55                 | 0.55                  |
| Lysine                    | 1.39                    | 1.39                 | 1.39                  |
| Tryptophan                | 0.25                    | 0.25                 | 0.25                  |
| ME: CP                    | 134:1                   | 134:1                | 134:1                 |

*Premix was prepared by mixing the following in 500-g maize flour

- TRAYMIX- 9 g (vitamin A-82,500 IU, B₂-50 mg, D₃-12,000 IU, K-10 mg/g), MERIPLEX-18 g (vitamin B₁-8 mg, B₆-16 mg, B₁₂ -80 mcg, E-80 mg, Niacin-120 mg, Calcium D: Pantothenate-80 mg, Ca-88 mg), E-care Se forte-10 g (vitamin E-0.5 g, Se-1 mg), QUINACOX-27 g (Diclazuril-1 mg)
- Trace minerals-60.4 g (Ferrous oxide-19.8 g, Copper sulfate-2.64 g, Manganese sulfate-19.8 g, Zinc sulfate-18 g, Ferrous sulfate-19.8 g, Potassium iodate-0.130 g, Magnesium sulfate-19.8 g, Choline chloride-135 g)
- Phytase (5 g/quintal: Activity 5000 FPU/g)
of broilers and feed refusals were recorded on days 1, 7, 14, 21, 28, and 35 of the experimentation periods. GIW was calculated by subtracting weight of the previous week from the preceding week. FI per bird of each group was computed by subtracting the feed refusal from the total feed supplied.

FCR was determined at weekly intervals by formula: FI (gm)/GIW (gm).

Mortality % was calculated by keeping the record of dead birds during the experiment.

**European efficiency factor**

Formulae (Bera et al., 2010; Lup et al., 2010) for calculation of economic factors viz. viability and European efficiency factor (EEF) along with cost of 1 kg live weight (cm) production is given below:

Viability = \{1 − (n of dead birds/total birds)\} × 100

EEF = \{BW × viability × 100/(FCR × age)\}

BW refers to (average body weight in kg), units of age were days, and the calculation of the price for 1 kg live weight production is therefore as follows:

\[ C_m = FCR \times Cf \]

where \( C_m \) is the cost of producing 1 kg of live weight, FCR is the feed conversion ratio, and \( Cf \) is the cost of feed per kg. (Mohammad et al. 2018).

**Carcass parameters**

At the end of feeding trial, two birds from each replicate of same weight were slaughtered to record carcass weight, eviscerated weight, dressing percentage, abdominal fat, and weight of the heart, liver, and gizzard. The birds were fasted over night to drain the intestinal content and sacrificed to assess the effect of dietary treatments on the carcass weight, dressing percentage, muscle yield, and weight of the heart, liver, and gizzard. At the time of slaughter, pH of the contents of duodenum, jejunum, ileum, caecum, and colon were recorded with the help of pH meter (perfit digital pH meter).

**Microbial count**

The caecum was removed from birds of respective experimental group at time of slaughter and was collected in glass jars and placed along with ice packs to arrest microbial growth. Caecal content was removed with the help of scalpel blade and forceps into sterilized petri plates. The samples of caecal content were processed by preparing 1:100 dilutions with double distilled water. The dilutions are prepared by thorough mixing of the sample and the diluents. One milliliter of dilution was transferred to the sterile petri dishes from the tube with the highest dilution 10⁻⁷. Then to each plate, 15–20 mL molten agar cooled to temperature 50–55 °C was transferred by plate inoculation method. Petri plates were kept inverted for incubation at 37 °C for 24–48 h. After incubation, the colonies were counted with the help of Quebec colony counter at the Department of Veterinary Public Health, COVAS (H.P). Twenty different colonies from each respective treatment group were taken from the petri plates with the help of inoculation loop on the glass slide and were stained with the help of gram staining. Then these slides were observed under the microscope, and the shape of microorganism along with gram stain whether positive or negative was recorded.

**Blood profile**

Approximately 3 to 5 mL of blood from randomly chosen birds of each replicate was collected from ulnar vein with the help of 24-gauge (24G) needle and collected in 10% heparin-coated centrifuge tubes. Whole blood after collection was centrifuged at 2000 x g for 15 min; thereafter, the separated plasma after collection was transferred into clean polypropylene tube using a Pasteur pipette. Samples were stored at −20 °C for biochemical analysis in the lab (CHEM 5 χ plasma analyzer, Erba Mannheim-India) with the help of the kits (Agape diagnostics, India). The analysis of whole blood for hematology was performed immediately after collection of the blood in heparin-coated tubes using fully automated hematology analyzer (BC 2800 vet-Agape diagnostics, India). The hematological analysis includes red blood cells (RBC), white blood cells (WBC), hematocrit (Hct), and platelet count.

**Tibiae bone analysis**

Tibiae bones of two birds from each replica were collected separately in labelled plastic bags at the time of slaughter. The plastic bags containing samples of tibiae bones were stored in freezer till analysis of mineral content. Processing of bone was done as per the method given by Hall et al. (2003) for the estimation of Ca and P by employing methods as discussed above.

**Statistical analysis**

All the recorded and calculated data were subjected to analysis of variance (ANOVA). Duncan multiple range test (Duncan, 1995) was used for determining the significant difference (at 5% level of significance) using statistical analysis software (version 9.3).
**Results**

GIW, FI, FCR, GIW/day, FI/day, and mortality % were studied (Table 2). Results of the experiment revealed no significant ($P > 0.05$) difference for GIW among control T0, positive control (PC) T1, and treatment groups T2 and T3 during 1–14 days and 14–21 days. Growth pattern during 21–35 days and 1–35 days revealed significantly ($P < 0.05$) higher GIW in treatment group T3 compared to control T0. Similarly, PC T1 also exhibited significantly ($P < 0.05$) higher GIW for the overall period 1–35 days compared to control T0. On an average, feed intake in treatment T1, T2, and T3 was recorded to be 11.8% higher than control T0. FCR value for the period 1–35 days, varied in between 1.65 and 1.81 with lowest in control T0 followed by treatment T3 exhibiting significant ($P < 0.05$) difference as compared to T2 which recorded highest FCR of 1.81. Highest mortality (Table 2) of 11.25% was exhibited in T0 which exceeded the normal management norms whereas treatment T1, T2, and T3 had mortality % varying between 3.75 and 7.50% acceptable as per management norms. 0.5% CA-supplemented treatments T3 exhibited higher EEF (Table 5) of 301 compared to 261 and 283 in control T0 and PC T1, whereas treatment T3 exhibited EEF of 260 similar to control T0. Overall, birds in treatment T3 exhibited lowest cost (Rs.51.90) of feed per kg live weight gain.

Digestibility studies (Table 3) revealed non-significant ($P > 0.05$) difference for calcium and phosphorous retention. Positive control T1 and treatment groups T2 and T3 exhibited higher calcium retention, whereas T2 and T3 exhibited higher P retention compared to control T0. Microbial count (Table 3) in caeca of broiler chicks exhibited a significant ($P < 0.05$) reduction in PC T1 and treatment groups T2 and T3 compared to control T0. Plasma calcium (mg/dL) (Table 4) level varied between 9.94 and 11.31 mg/dL and was significantly ($P < 0.05$) higher in control T0 compared to other groups.

### Table 2: Overall growth performance of broiler birds (0 to 35th day)

| Parameter | Days | Treatments | Control T0 | Control T1 | 0.5%CA + Low Ca and TP T2 | 0.5%CA + Mod. Low Ca and TP T3 | SEM | $P$ value |
|-----------|------|------------|------------|------------|---------------------------|----------------------------|-----|-----------|
| GIW (g)   | 1–14 days | 354.48 | 355.81 | 369.17 | 366.45 | 7.16 | 0.36 |
| FI (g)    | 1–14 days | 377.14 | 420.95 | 391.83 | 381.58 | 14.38 | 0.14 |
| FCR       | 1–14 days | 1.33 | 1.39 | 1.30 | 1.31 | .03 | 0.14 |
| ADWG (g)  | 1–14 days | 25.32 | 25.42 | 26.37 | 26.18 | .38 | 0.23 |

Figures with different superscripts in a column are statistically significant (Corresponding $p$ values are depicted in the last column). $n = 80$

GIW, gain in weight; FI, feed intake; FCR, feed conversion ratio; ADWG, average daily weight gain; ADFI, average daily feed intake

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**Table 2** Overall growth performance of broiler birds (0 to 35th day)
to PC $T_0$, and treatment $T_2$ and $T_3$. Similarly, plasma phosphorus level (mg/dL) exhibited significant ($P < 0.05$) difference and varied between 6.74 and 7.43 mg/dL but were within normal range in control $T_0$, PC $T_1$, and treatments $T_2$ and $T_3$. Analysis of blood plasma (Table 4) revealed significantly ($P < 0.05$) low cholesterol in PC $T_1$ and treatments $T_2$ and $T_3$ compared to control group $T_0$. Blood hematology revealed no differences for different parameters (Table 4) thus causing no major influence at the cellular level by supplementation of CA. Tibiae ash % (Table 3) exhibited significantly ($P < 0.05$) low ash content in treatment $T_2$ compared to control $T_0$ and $T_1$ as well as treatment $T_3$. Furthermore, tibiae calcium and phosphorus content were significantly ($P < 0.05$) high in treatments $T_2$ and $T_3$. Results for the carcass characteristics revealed significantly ($P < 0.05$) higher dressing % (Table 3) in 0.5% CA-supplemented treatment compared to control $T_0$ with recorded 7.76%; 7.33%; and 4.12% higher dressing yield in $T_1$, $T_2$, and $T_3$ respectively. The average value for leg quarter (% live weight), thigh (% live weight), and drum stick (% live weight (Table 3) did not exhibit any difference among $T_1$, $T_2$, and $T_3$ but the average value for fore/breast quarter was significantly ($P < 0.05$) higher compared to control group $T_0$.

### Discussion

Results of present study are relatable with the experiment of Chowdhury et al. (2009) who reported that supplementation of CA at 0.5% in the corn-soybean-based basal diet of 161 1-day-old broiler Hubbard Classic chicks had positive effects on growth, feed intake, feed efficiency, carcass yield, bone ash, and immune status of broilers; and addition of citric acid also reduced the pH of the formulated diets, whereas Fattah et al. (2008) reported that addition of any level and source of organic acids to the basal diet of 189 1-day-old Hubbard broiler chicks increased feed digestion and absorption as a result of increasing relative pancreas weight and

### Table 3

Digestibility coefficients of proximate principles (% dry matter basis) and effect of dietary treatments on carcass characteristics, tibial bone mineral content, and microbial load in caecal contents of broiler birds

| Parameter             | Treatments                          | SEM  | $P$ value |
|-----------------------|-------------------------------------|------|-----------|
| **Digestibility**     |                                     |      |           |
| DM%                   | Control $T_0$                        | 1.84 | 0.85      |
|                       | Control + $T_1$                      | 1.98 | 0.11      |
|                       | 0.5%CA + Low Ca and TP $T_2$         | 4.29 | 0.12      |
|                       | 0.5%CA + Mod. Low Ca and TP $T_3$    | 2.56 | 0.98      |
| CP%                   |                                     |      |           |
|                       |                                     |      |           |
| CF%                   |                                     |      |           |
|                       |                                     |      |           |
| EE%                   |                                     |      |           |
|                       |                                     |      |           |
| Ca%                   |                                     |      |           |
|                       |                                     |      |           |
| P%                    |                                     |      |           |
|                       |                                     |      |           |
| **Carcass characteristics** |                                     |      |           |
| Skin (% of BW)        |                                     |      |           |
| Feet (% of BW)        |                                     |      |           |
| Head (% of BW)        |                                     |      |           |
| Wing (% of BW)        |                                     |      |           |
| Dressing %            |                                     |      |           |
| Breasts %             |                                     |      |           |
| Leg quarter (%)       |                                     |      |           |
| Thigh %               |                                     |      |           |
| Drum Stick %          |                                     |      |           |
| Tibia bone mineral content |                                     |      |           |
| Ash (%)               |                                     |      |           |
| Calcium (%)           |                                     |      |           |
| Phosphorus (%)        |                                     |      |           |
| Microbial load in caecal contents |             |      |           |
| Microbial count (cfu/g) X10^9 |             |      |           |
| Cocci (cfu) X10^7     |                                     |      |           |
| Bacilli (cfu) X10^7    |                                     |      |           |

### Notes

$DM$, dry matter; $CP$, crude protein; $CF$, crude fiber; $EE$, ether extract; $Ca$, calcium; $P$, phosphorus. Figures with different superscripts in a column are statistically ($P < 0.05$) significant from each other.
small intestine density. CA is a weak acid and is dissociated at a faster rate in low pH in the upper part of the GIT (gastrointestinal tract) activating pepsinogen and other zymogens by adjusting gastric acidity closer to that required for optimal activity resulting in increased enzyme activity, improved digestion of proteins and other nutrients as well (Jongbloed et al. 2000). In our study, no significant (P > 0.05) change in pH of intestinal contents in different segments of intestine was recorded (Table 4). A report by Hume et al. (1993) on metabolism of organic acid in broiler chicks, when given by gavage to determine its chemical fate and distribution among organs and tissues concluded that dietary organic acid is metabolized and absorbed in the foregut and does not reach the intestine or ceca in appreciable amounts, since organic acids are very readily absorbed in the upper part of the GIT, explaining the reason for lack of pH reduction in the lower part of the GIT.

Moderately reducing the level of Ca in feed has influenced the growth and FCR. Higher level of Ca in diet causes increase in gastric pH as limestone, as a source of calcium carbonate (Table 1) which is considered to have high acid binding capacity (Lawlor et al. 2005) thus, affect/reduce the protein digestion by reducing the action of pepsin (Walk et al. 2012). Similarly, increasing concentrations of Ca in feed has also been attributed to influence the apparent digestibility of fats by soap formation with fatty acids enhancing its excretion (Ruvini K. et al. 2014). In the present study, digestibility studies revealed non-significant but numerically higher ether extract digestibility in broiler birds offered moderately low Ca (Table 3). Moreover, CA is a weak acid being an essential component of the citric acid cycle, releases energy for physiological functions (Wright, 1976) thus contributing to the net energy availability. Adil S. et al. (2011) also reported that addition of organic acids to the diets of broiler chicken significantly decreased (P < 0.05) the caecal viable coliform counts as compared to the unsupplemented group. Tollba (2010) observed that addition of CA in the feed of 1-week old Hubbard broiler chicks had statistical effects (P < 0.05) regarding the decrease in the counts of pathogenic intestinal bacteria and parasites in ileum, caecum, or fecal matter of birds. Furthermore, it also significantly improved body weight gain, feed consumption, feed conversion,
carcass characteristics and mortality rate, thereby leading to an improved gut health parameters and increase in the availability of nutrients. According to Adil S. et al. (2011) modulation of intestinal microbial population beneficially affects the host by positive impact on histology of intestine, thereby enhancing digestion and absorption of nutrients. Katoch et al. (1996) reported higher growth performance and carcass yield in broiler birds with a shift in microbial population by supplementation with direct fed microbial particularly acid producing bacteria. Shambhavi et al. (2020) also reported similar finding with supplementation of direct fed microbial on azolla-based feed to 1-day-old broiler chicks enhancing the positive effect on carcass characteristics of broilers, by enhancing digestion and eventually resulting in extra availability of nutrients for deposition. This microflora has the flexibility to convert the retrograde uric acid (which is being carried to the caecum and coprodeum) to amino acids. Moreover, role of caecum is also vital in amino acids absorption and thus has larger ability to move amino acids towards higher deposition in fore/breast quarter. The results were evident in the present study with significantly \( P < 0.05 \) high fore/breast weight and dressing % (Table 3) recorded in all CA supplemented treatments compared to control group. Haq et al. (2014) documented significant improvement in dressing percentage (improvement ranging between 0.66 and 2.18), significant reduction in abdominal fat weight (7.35–20.59% decrease) with increased level of citric acid in diet. Citric acid had non-significant but positive effect on hemogram, leukogram, and serum metabolite values of broilers.

The blood Ca level is maintained within very narrow limits by several hormones that control Ca absorption and excretion, as well as bone metabolism. Homeostasis by endocrine regulation (through the action of calcitonin and parathyroid hormone, among other hormones) could be responsible for maintaining the constant Ca and P concentrations in blood, although dietary components influence mineral balance affecting Ca blood levels (Taylor and Dacke. 1984). In the present study, tibial bone Ca and P percent was significantly \( P < 0.05 \) higher in treatment \( T_2 \) and \( T_3 \). The results obtained reveal that CA supplementation at 0.5% in low and moderately low Ca and TP ration increase deposition of Ca and P in tibial bone. Earlier report also (Swaminathan et al. 1978) suggests that efficiency of Ca absorption is enhanced in low Ca diets by a positive response of birds to Ca restriction by increased production of 1,25-dihydroxycholecalciferol in the intestine (Edelstein et al. 1975) which activates the expression of Ca binding protein (DeLuca and Schnoes, 1976). Related results have been reported earlier by Brenes et al. (2003) whereas Boling et al. (2001) observed that the CA did not significantly affect the Ca but CA increases P utilization in corn-soybean meal diets and reduces the available P requirement by approximately 0.10% of the diet.

Addition of CA at 0.5% level exerted hypcholesterolaemia effect in all the treatment groups. Probable reason for reduced serum cholesterol may be that lactic acid bacteria grow at low pH in intestine and are more resistant to organic acids as compare to E-coli. This lactic acid bacteria produce bile salt hydrolase (BSH) in the intestine which is responsible for bile salt deconjugation. Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and are more likely to be excreted in the feces, and to maintain bile salt homeostasis, more bile acids need to be synthesized and this in turn will reduce cholesterol in the body pool as cholesterol is the precursor for bile acids. (Haq et al.2014).Tollba (2010) also reported significant reduction in cholesterol when CA was used at the rate of 2% in broilers feed.

CA supplementation in diet with moderately low Ca and TP content exhibited highest EEF of 301 and lowest cost of feed for every 1-kg live weight gain. Economics of broiler feeding revealed that 0.5% CA supplementation fetched highest gross return above feed cost with moderately low Ca and TP content whereas lowest profit was observed in feed with low content of Ca and TP (Table 5).

In conclusion, addition of CA (0.5%) in broiler birds offered corn soya-based feed with low and moderately low Ca and TP content enhanced the overall growth and improved carcass characteristics. Economics of broiler feeding with moderately low Ca and TP content revealed highest profit with CA (0.5%) supplementation. Further validation is needed in the commercial set up to realize the potential gain in increased commercial scale to reap the benefits in terms of economics as well as the unnecessary dependence of antibiotics for growth, prevalent in commercial farming in the region.

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Author contribution SK: designed the experiment and the experiment was conducted under his guidance. Furthermore, the results were interpreted and discussed.

SS: conducted the experiments, collected samples, recorded the data, and wrote the manuscript.

VS and SU: analyzed the data statistically.

DR and AS: contributed by extending their services for sample analysis.

Data availability The authors confirm that the data supporting the findings of this study are available within the article. Raw data supporting the findings of this study is available from the corresponding author, upon request.
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Declarations

Ethics approval  All the animal experimentation procedures were approved by the Institutional Animal Ethics Committee (CSKHP Agriculture University), Palampur, HP, India. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent for publication  All the authors give their consent for publication of the article.

Conflict of interest  The authors declare no competing interests.

References

Adil, S., Banday, M.T., Bhat, G.A., Qureshi, S.D. and Wani, S.A. 2011. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. Livestock Research for Rural Development, 23(1): 1-8.

Ahsan-ul-Haq, M.T.C., Ahmad, F., Shafi, J. and Ashraf, M. 2014. Effect of dietary acidification with citric acid on carcass characteristics, hemogram and serum metabolite values of broiler chicken. Pakistan Journal of Life and Social Sciences, 12(1): 36-41.

AOAC, 2000. Official methods of analytical chemists (17th ed.) Washington DC: Association of Official Analytical Chemists.

AOAC, 2005. Official methods of analytical chemists (18th ed.) Washington DC: Association of Official Analytical Chemists.

Atapattu, N.S.B.M and Nelligaswatta, C.J. 2005. Effect of citric acid on the performance and utilization of phosphorus and crude protein in broiler chickens fed rice by products-based diets. International Journal of Poultry Science, 4: 990-993.

Bera, A.K., Bhattacharya, D., Pan, D., Dhara, A., Kumar, S., and Das, S.K. 2010. Evaluation of economic losses due to coccidiosis in poultry industry in India. Agricultural Economics Research Review, 23: 91-96.

Boling, S.D., Parsons, C.M. and Baker, D.H. 1999, August. Effect of citric acid on calcium and phosphorus utilization and requirements for chicks fed phytate-free and corn soybean meal diets. In 88th Annual Meeting of the Poultry Science Association (pp. 8–11). Poultry Science Association.

Boling, S.D., Snow, J.L., Parsons, C.M., and Baker, D.H. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. Poultry Science, 80 (6): 783-788.

Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. and Bravo, C. 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Animal Feed Science and Technology, 110 (1-4): 201-219

Broz, J., Oldale, P. and Voltz, A.P. 1994. Effect of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. British Poultry Science, 35: 273-280.

Chowdhury, R., Islam, K.M.S., Khan, M.J., Karim, M.R., Haque, M.N., Khatun, M., and Pesti G.M. 2009. Effect of citric acid, avilamycin and their combination on the performance, tibia ash and immune status of broiler chicks. Poultry Science 88(8): 1616-1622.

Das, S.K., Islam, K.M.S. and Islam, M.A. 2012. Performance and immunity of broiler due to addition of citric acid in low nutrient diet. Indian Journal of Animal Sciences, 82(6): 629-633.

DeLuca, H.F., and Schnoes H.K. 1976. Metabolism and mechanism of action of vitamin D. Annual Review of Biochemistry, 45(1): 631-666.

Diarra, M.S. and Malouni F. 2014. Antibiotics in Canadian poultry productions and anticipated alternatives. Frontiers in microbiology, 5:282.

Diarra, M.S., Silversides, F.G., Diarrassouba, F., Pritchard, J., Mason, L., Brousseau, R. 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, clostridium perfringens and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in Escherichia coli isolates. Applied and Environmental Microbiology, 73(20): 6566-6576.

Duncan, D.B., 1995. Multiple range and F tests. Biometrics, 11(1): 1–42. https://doi.org/10.2307/3001478

Edelstein, S., Harell, A., Bar, A., and Hurwitz, S. 1975. The functional metabolism of vitamin D in chicks fed low-calcium and low phosphorus diets. Biochimica et Biophysica Acta, 385(2): 438- 42.

Fattah, S.A., El-Sanhoury, M.H., EL-Medney, N.M., and Abdel-Azeem, F. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. International Journal of Poultry Science, 7: 215-222.

Forgetta, V., Rempel, H., Malouin, F., Vaillancourt, Jr., R., Topp, E., Dewar, K., and Diarra, M.S. 2012. Pathogenic and multidrug-resistant Escherichia fergusonii from broiler chicken. Poultry Science, 91(2): 512–525.

Furtula, V., Farrell, E.G., Diarrassouba, F., Rempel, H., Pritchard J. and Diarra, M.S. 2010. Veterinary pharmaceuticals and antibiotic resistance of Escherichia Coli isolates in poultry litter from commercial farms and controlled feeding trials. Poultry Science, 89(1): 180-188.

Ghadban, G.S., 2002. Probiotics in broiler production-a review. Archiv fur Geflugelkunde, 66(2): 49-58.

Ghazalah, A.A., Atta, A.M., Elkoulb, K., Moustafa, M.E.L. and Riry, F.S. 2011. Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. International Journal of Poultry Science, 10(3): 176-184.

Hall, L.E., Shirley, R.B., Bakalli, R.L., Aggrey, S.E., Pesti, G.M. and Edwards Jr, H.M. Power of two methods for the estimation of bone ash of broilers. Poultry Science, 82(3): 414-418.

Hume, M.E., Corrier, D.E., Ivie, G.W. and Delouch, J.R. 1993. Metabolism of [14 C] propionic acid in broiler chicks. Poultry Science, 72(5): 786-793.

ICAR, 2013. Nutrient requirements of commercial white and coloured broiler chickens. Krishi Bhavan, Dr.Rajendra Prasad Road, New Delhi-110001.

Islam, K.M.S. 2012. Use of citric acid in broiler diets. World’s Poultry Science Journal, 68(1): 104-118.

Jin, L.Z., Ho, Y.W., Abdullah, N., and Jalaludin, S. 1997. Probiotics in poultry: mode of action. World’s Poultry Science Journal, 53(4): 351-368.

Jongbloed, A.W., Mroz, Z., Van Der Weij-Jongbloed, R. and Kemme, P.A. 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livestock Production Science, 67(1-2): 113-122.

Katoch, S., Kaistha, M., Sharma, K.S., Kumari, M., Sharma, C.R. and Katoch, B.S. 1996. Effect of dietary supplementation of microbes and their combination on the performance, tibia ash and immune status of broiler chicks. Poultry Science 88(8): 1616-1622.
ingredients used in pig diets. Irish Veterinary Journal, 58: 447-452.

Lodhi, G.N., Singh, D. and Ichhponani, J.S. 1976. Variations in nutrient content of feeding stuffs rich in protein and reassessment of the chemical methods for metabolizable energy estimation for poultry. The Journal of Agricultural Science, 86(4): 293-303.

Lup, F., Drinceanu, D. and Mierlită, D. 2010. Economic efficiency and European efficiency factor in modifying of some raw materials proportion in chicken broilers feeding. Analele UniversităŃii din Oradea Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară. 569-564.

Mahmoudi, M., Azarfar, A. and Khoosavinia, H. 2018. Partial replacement of dietary methionine with betaine and choline in heat-stressed broiler chickens. The Journal of Poultry Science 55(1): 28–37. http://www.jstage.jst.go.jp/browse/jpsadoi:10.2141/jpsa.0170087

Parks, P.F. and Dunn D.E. 1963. Evaluation of the molybdenumate photometric determination of phosphorus in mixed feeds and mineral supplements. Journal Association of Official Analytical Chemists 46: 836-838.

Paul, S.K., Halder, G., Mondal, M.K. and Samanta, G. 2007. Effect of organic acid salt on the performance and gut health of broiler chicken. The Journal of Poultry Science, 44(4): 389-395.

Shambhavi., Katoch, S., Chauhan, P. and Mane, B.G. 2020. Effect of feeding Azolla pinnata in combination with direct-fed microbial on broiler performance. Tropical Animal Health and Production, 53(1):1-9. https://doi.org/10.1007/s11250-020-02437-w.(202153:5)

Srinivas, G., Swathi, B., Raju, S. and Tungani, R. 2018. Influence of dietary supplementation of organic acids, probiotics and their combinations on growth, carcass traits and serum parameters in broiler chicken. Indian Journal of Animal Nutrition, 35(2), pp.201-205.

Swaminathan, R., Care, A.D. and Wasserman, R.H. 1978. The response of different segments of the small intestine to calcium and phosphorus deprivation in chicks (Gallus domesticus). Comparative Biochemistry and Physiology Part A: Physiology, 59(4): 389-392.

Taylor, T.G. and Dacke, C.G. 1984. Calcitonin, calcitonin metabolism and its regulation. In: B. M. Freeman (ed.), Physiology and Biochemistry of the Domestic Fowl. Academic Press, Orlando FL., 125–170.

Tollba, A.H. 2010. Reduction of broilers intestinal pathogenic microflora under normal or stressed condition. Egyptian Poultry Science Journal, 30(1): 249-270.

Walk, C.L., Addo-Chidie, E.K., Bedford, M.R. and Adeola, O. 2012. Evaluation of a highly soluble calcium source and phytase in the diets of broiler chickens. Poultry Science, 91(9): 2255-2263.

Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of animal science, 86(suppl_14): 140–148.

Wright, E. and Hughes, R.E. 1976. Some effects of dietary citric acid in small animals. Food and cosmetics toxicology, 14(6): 561-564.

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