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

*Shivani Katoch, Sumani Sharma, Varun Sankhyan, Daisy Wadhwa, Arun Sharma & Sanjiv Kumar

*Department of Animal Nutrition, DGCN College of Veterinary & Animal Sciences
CSK HP Agriculture University, Palampur. HP 176062 India*

ABSTRACT

A study was conducted to evaluate citric acid (CA) as an additive in poultry broiler diet with lower mineral density of calcium and phosphorus in commercial broiler poultry birds under complete randomized design. For this purpose, Vancobb-400 strain day old broiler chicks (n=320) were divided into 4 main treatment groups T₀, T₁, T₂ and T₃. Each treatment group was further divided into 8 replicates with 10 chicks in each. T₀ served as control diet and was given standard corn- soy flake based ration (Pre-starter%; CP;Ca;TP--23,1.00,0.70, Starter;22,1.10,0.72 and Finisher;20,0.99,0.70) and treatment T₁ served as standard ration with added 0.5 per cent CA with no change in chemical composition (Pre-starter %; CP;Ca;TP23,1.00,0.70, Starter; 22,1.10,0.72 and Finisher;20,0.99,0.70). Treatment T₂ was given standard corn-soy flake based ration containing 0.5 per cent CA with low calcium (Ca) and total phosphorus (TP) content (Pre-starter %; CP;Ca;TP --23,0.90,0.66, Starter; 22,0.99,0.71 and Finisher;20,0.90,0.69) whereas treatment T₃ was given standard corn- soy flake based ration containing 0.5 per cent CA with moderately low calcium (Ca) and total phosphorus (TP) content (Pre-starter %; CP;Ca;TP--23,0.80,0.65, Starter;22,0.88,0.70 and Finisher;20,0.79,0.68) as per ICAR (2013) standards. Perusal of the results revealed that broiler birds offered moderately low Ca and TP exhibited higher growth performance (P<0.05) for gain in weight, feed intake, feed conversion efficiency and carcass traits viz. dressing percentage and forequarters weight. Addition of 0.5 per cent CA in low and moderately low Ca and TP offered diets conferred protection to birds by lowering mortality in birds through reduction in total microbial count in the lower gastro intestinal tract. Results of the experiment revealed activation of homeostatic mechanisms in birds offered moderately low Ca and TP with added 0.5% CA modulating enhanced availability of calcium and phosphorus as evident in metabolic trial, blood and tibiae bone studies but the homeostatic mechanism was less pronounced when Ca and TP density in diet was low. Overall economics exhibited lowest cost of producing, per kg live weight in broiler birds offered moderately low Ca and TP with added 0.5 % CA. In conclusion, birds offered corn soya based diet with moderately low mineral viz. calcium and total phosphorus as per ICAR 2013 standard with added 0.5 per cent citric acid can enhance the growth, improve carcass characteristics and improve the FCR of broilers though favourable environment in lower intestinal tract and activation of homeostatic mechanisms of calcium and phosphorus digestion and absorption, thus economizing the cost of production.

Key words: Citric acid, Broiler chicken, FCR, Calcium, growth performance, microbial count, economic.

INTRODUCTION

One of the major factors governing the performance of birds is the health of gut and thus the economics of poultry production (Samik et al. 2007). The profile of intestinal micro flora also plays a very important role in health of gut. Antimicrobial drug resistance has alarmed the scientists all over the world especially for the one used as livestock feed additives. Feed grade antibiotics which are given in preventive doses often lead to survival of pathogenic microbes which in turn develop drug resistance. Therefore, in order to avoid drug resistance, there is a search for alternatives to feed grade antibiotics.

Organic acids (OA) are well recognised nowadays as non-antibiotic feed additives in poultry nutrition (Windisch et al. 2008). The organic acids elicit the response in poultry by lowering the pH of intestinal tract and thus favouring good microbes which in turn suppress the pathogenic microbes, reducing the use of antibiotics. Citric acid (CA) is an organic acid that increases the digestibility of protein and fiber (Attapattu and Nelligaswatta, 2005) as well as utilization of P. For this reason, it reduces the available P requirement (Boling et al., 2001). Citric acid also increases the acidity in the intestines. The acidity is responsible for increasing the solubility of calcium, and therefore the absorption of calcium is increased (Broz et al., 1994; Boling et al., 1999). Many researchers have reported that addition of citric acid @0.5 per cent increase weight

Corresponding Author: skatoch@hillagric.ac.in; bunnykatoch@hpkvplp.com

gain, feed intake, tibia ash deposition and non-specific immunity as well as feed efficiency and carcass yield. Its inclusion in diet have been reported to decrease colonization of pathogens and limit production of toxic metabolites, improve availability of protein, Ca, P, Mg and Zn as well acts as a substrate in intermediary metabolism.

The present research work was to incorporate citric acid @0.5 per cent as an additive with low mineral concentration viz. Ca and P in the diet for economical rearing of broiler poultry birds.

MATERIALS AND METHODS

Experimental design and management: The experimental plan was approved by the Institutional Animal Ethics Committee. A total of 320 day old meat type chicks (Vencobb-400) were reared in deep litter under standard management conditions in experimental poultry house and were immediately offered ad libitum feed and lukewarm water upon arrival. The standard management practices were followed for rearing the chicks. Temperature and relative humidity of room was maintained at 21 to 30°C and 40 to 60 per cent respectively. Proper ventilation and light was made available to birds throughout the trial.

Experimental Plan: At the start of the experiment, chicks were weighed individually on 0 day and were wing tagged and distributed randomly according to the experimental plan in battery brooder for the first seven days. Birds were distributed randomly into four groups T₀, T₁, T₂ and T₃. Each group was divided into 8 replicates with 10 chicks in each replicate, thus each treatment had 80 birds. Birds were shifted from brooder house to deep litter system on 7th day. T₀ served as control diet and was given standard corn-soy flake based ration (Pre-starter %; CP;Ca;TP--23,1.00,0.70, Starter;22,1.10,0.72 and Finisher;20,0.99,0.70), treatment T₁ served as standard ration with added 0.5 per cent CA (Pre-starter %; CP;Ca;TP 23,1.00,0.70, Starter; 22,1.10,0.72 and Finisher;20,0.99,0.70). Treatment T₂ was given standard corn-soy flake based ration containing 0.5 per cent CA but with low Calcium (Ca) and total phosphorus (TP) content (Pre-starter %; CP;Ca;TP --23,0.90,0.66, Starter; 22,0.99,0.71 and Finisher;20,0.90,0.69). Further treatment T₃ was given standard corn- soy flake based ration containing 0.5 per cent CA with moderately low Calcium (Ca) and total phosphorus (TP) content (Pre-starter %; CP;Ca;TP --23,0.80,0.65 , Starter;22,0.88,0.70 and Finisher;20,0.79,0.68) as per ICAR (2013) standards. The physical and chemical composition of broiler pre-starter, starter and finisher ration is presented in Table 1, 2 & 3 respectively.

Feed and feed ingredient analysis: The feed samples were analysed for proximate principles (AOAC, 2005). The feed samples were analysed for proximate principles. The proximate analysis of the samples was done in the laboratory of the Department of Animal Nutrition. The dry matter was determined by oven drying, total ash by muffle burning, fat by Soxhlet extraction with petroleum ether (by use of Pelican Socs Plus 08AS DLS instrument), protein by Kjeldahl nitrogen estimation (with the help of instruments: Pelican Kelplus KES 12L R digestion assembly, Pelican p Kelplus Ultimate duo Dist-TS E distillation assembly, SI Analytics Titro Line 7000 titrator), crude fiber by use of Pelican FIBRA Plus FES4 instrument. The calcium in samples were analysed in Atomic Absorption Spectrometer (AAS) (LABINDIA AA8000 Atomic Absorption Spectrophotometer) while phosphorus content was estimated spectrophotometrically (Spectronic 200 spectrophotometer) using the method proposed by Parks & Dunn (1963). The metabolizable energy (ME) was calculated using the equation as proposed by Lodhi *et al.* (1976).

Digestibility and metabolism studies: A digestibility cum metabolic trial based on total faecal collection method was conducted from 24th day of experiment for calculating the balance of nutrients. Two birds from

each replicate of same sex with comparable body weight were selected and housed in battery brooders. Birds were offered the same treatment ration for three days as in growth study to provide them adaptation time in the metabolic cages. All the faecal and feeding trays were cleaned properly to start the metabolic trial. After adaptation period of three days, the measured quantity of feed for next five consecutive days was offered to each replicate both during morning and evening. The residual feed was weighed on 5th day to record the actual consumption of feed for each replicate. The faeces voided by each replicate were collected daily in the morning and were checked for any presence of feathers and feed particles which were removed manually at the time of collection and then weight of faeces was recorded. Faeces collected were mixed properly and then divided into two portions: one for nitrogen estimation and other for dry matter estimation. 20 mL of 5 per cent sulphuric acid was mixed in the faeces collected for nitrogen estimation to avoid nitrogen loss and faeces 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 analysed for various proximate parameters in the laboratory of the Department Of Animal Nutrition. The digestibility of nutrients was calculated by total faecal collection method. The total faecal collection method was followed which involves measuring of feed intake and faeces voided.

Growth performance: Pre-weighted 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 body weight gain (BWG), Feed intake (FI) and FCR were recorded. Body weight of broilers was measured on days 1, 7, 14, 21, 28 and 35 of the experimentation periods. After measuring the remaining feed on days 1, 7, 14, 21, 28, and 35, BWG, feed intake (FI), and FCR were calculated. Dead birds were recorded to determine the mortality (%) during the experiment.

European efficiency factor: Viability, economic factors including the European efficiency factor (EEF), and the cost of producing 1 kg of live weight (C_m) were calculated using the following formulae (Bera *et al.*, 2010; Lup *et al.*, 2012):

$$\text{Viability} = \{1 - (\text{n of dead birds} / \text{total birds})\} \times 100$$

$$\text{EEF} = \{\text{BW} \times \text{viability} \times 100 / (\text{FCR} \times \text{age})\}$$

Where EEF is the European efficiency factor and BW is the average body weight (kg). Age was measured in days. The cost of producing 1 kg of live weight was calculated as:

$C_m = \text{FCR} \times C_f$ where C_m is the cost of producing 1 kg of live weight, FCR is the feed conversion ratio, and C_f is the cost of feed per kg. Mohammad *et al.* (2018).

Carcass parameters: At the end of feeding trial, two birds from each replicate were slaughtered to record carcass weight, eviscerated weight, dressing percentage, abdominal fat and weight of heart, liver and gizzard. The birds were fasted over night to drain the intestinal content and sacrificed to assess the effect of various dietary treatments on the carcass weight, dressing percentage, muscle yield and weight of heart, liver and gizzard. At the time of slaughter, pH of the contents of duodenum, jejunum, ileum, caecum and colon were recorded with help of 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 help of scalpel blade and forceps into sterilized petri plates. The samples of caecal content were processed by preparing 1:100 dilutions. The dilutions are prepared by thorough mixing of the sample and the

diluents. 1 ml of dilution was transferred to the sterile petri dishes from the tube with highest dilution 10^{-7} . Then to each plate 15-20 ml molten agar cooled to temperature $50-55^{\circ}\text{C}$ was transferred by Plate inoculation method. Petri plates were kept inverted for incubation at 37°C for 24-48 hours. After incubation, the colonies were counted with the help of Quebec colony counter at the Department of Veterinary Public Health, COVAS (H.P). 20 different colonies from each respective treatment group were taken from the petri plates with 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 24gauge (24G) needle and collected in 10 per cent heparin coated centrifuge tubes. After collection of the whole blood, the blood was centrifuged at $2000 \times g$ for 15 minutes. The resulting supernatant, designated plasma was collected and transferred into a clean polypropylene tube using a Pasteur pipette and were analysed for blood biochemical profiles. The samples were then stored at -20°C and later analyzed in the lab by using Erba Mannheim CHEM 5 χ plasma analyzer, with the help of the kits of Agappe diagnostics in the Department of Animal Nutrition, COVAS, Palampur (Himachal Pradesh). The haematological analysis was performed immediately after collection of the blood in heparin coated tubes using fully automated BC 2800 Vet haematology analyser of Agappe diagnostics. The haematological analysis includes red blood cells (RBC), white blood cells (WBC), haematocrit (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. Bags were kept in freezer until analysis for mineral content was done. Bone processing was done as per the method given by Hall *et al.* (2003) for the estimation of minerals. The calcium in tibiae bone was analysed in Atomic Absorption Spectrometer (AAS) (LABINDIA AA8000 Atomic Absorption Spectrophotometer) while phosphorus content was estimated spectrophotometrically (Spectronic 200 spectrophotometer) using the method proposed by Parks & Dunn (1963).

Statistical Analysis: All the recorded and calculated data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test was performed for determining the significant difference using SAS software version 9.3 (Duncan 1995). Results were evaluated at 5 per cent level of significance.

RESULTS

Gain in weight (GIW), daily weight gain, feed consumption, feed consumption per day and left over record (daily basis) in each group, FCR/feed efficiency, mortality% were studied (Table:2). Results of the experiment revealed difference in growth, feed intake, weekly body weight gain, weekly and overall feed conversion ratio in birds supplemented with 0.5% CA with low Ca & TP (T_2) and moderately low, Ca & TP (T_3), in 1-14 d and 14-21d but were significant ($P<0.05$) during finisher phase i.e.21-35 days. Further, T_3 exhibited numerically 8.16 per cent higher gain in live body weight compared to control T_0 . On an average, feed intake in treatment T_1 , T_2 and T_3 was recorded to be 11.8 per cent higher than control T_0 . FCR value varied in between 1.65 to 1.81 with lowest in treatment T_3 exhibiting significant ($P<0.05$) difference compared to T_2 which recorded highest FCR of 1.81. Highest mortality (Table: 2) of 11.25 per cent was exhibited in T_0 which exceeded the normal management norms whereas treatment T_1 , T_2 and T_3 had mortality percent varying between 3.75 to 7.50 per cent acceptable as per management norms. Lowest mortality of 3.75 per cent was recorded in treatment T_3 . 0.5% CA supplemented treatments T_3 exhibited higher EEF (Table:5)

of 301 compared to 261 & 283 in control T₀ and positive control T₁ where as treatment T₂ exhibited EEF of 260 similar to control T₀. Highest value of EEF was exhibited by birds of treatment offered diet with moderately low Ca & TP supplemented 0.5 per cent CA. Cost of producing per kilogram live weight of broiler chicken during pre starter phase varied between Rs 40.9 to Rs. 44.6 and was lowest in T₃. Similarly during finisher phase, control T₀ exhibited lowest cost of feed per kg live weight gain. Overall, birds in treatment T₃ exhibited lowest cost (Rs.51.90) of feed per kg live weight gain.

Digestibility studies (Table: 3) revealed significant ($P<0.05$) difference for calcium and phosphorous retention. Treatment groups T₂ and T₃ exhibited higher calcium and phosphorus retention compared to T₀. Microbial load (Table: 3) in caeca of broiler chicks exhibited a significant ($P<0.05$) reduction in the treatment groups T₁, T₂ and T₃ compared to control T₀. Plasma calcium (mg/dL) (Table: 4) level varied between 9.94 to 11.31 mg/dL and was significantly ($P<0.05$) high in control T₀ compared to positive control T₁ and treatment T₂ and T₃. Similarly plasma phosphorus level (mg/dL) exhibited significant ($P<0.05$) difference and varied between 6.74 to 7.43 mg/dL but were within normal range in control T₀ and treatments T₁, T₂ and T₃. Analysis of blood plasma (Table:4) revealed significantly ($P<0.05$) low cholesterol in treatment T₁, T₂ and T₃ compared to control group T₀. Blood haematology revealed no differences for different parameter (Table:4) thus causing no major influence at the cellular level by supplementation of CA. Tibiae ash percent (Table:3) did not exhibit any major difference but tibiae calcium and phosphorus content was significantly ($P<0.05$) high in treatment T₂ and T₃. Results for the carcass characteristics revealed significantly ($P<0.05$) higher dressing per cent (Table-3) in 0.5% CA supplemented treatment T₁, T₂ and T₃ compared to T₀ with recorded 7.76 per cent; 7.33 per cent; and 4.12 per cent higher dressing yield. The average value for leg quarter (per cent live weight), thigh (per cent live weight) and drum stick (per cent live weight) (Table-3) did not exhibit any difference amongst treatment groups T₁, T₂ and T₃ but the average value for fore/breast quarter was significantly ($P<0.05$) higher compared to control group T₀.

DISCUSSION

Overall results for the growth performance revealed that broiler birds offered feed supplemented with 0.5 per cent CA but with moderately low Ca and TP as per ICAR-2013 standards recorded higher gain in live weight, feed intake, low FCR, higher per day gain in live weight and feed consumption and lowest mortality per cent throughout the growth trial. The results for growth performance and feed efficiency in moderately low Ca and TP matched the growth performance of positive control offered 0.5% CA with optimal Ca and TP supplementation as per ICAR-2013 standards where as treatment birds offered low Ca and TP feed supplemented with 0.5% CA exhibited lower weight gain and feed efficiency. 0.5% CA supplemented treatments offered diet formulated with moderately low Ca and TP exhibited highest EEF of 301 and lowest cost of feed for every 1 kg live weight gain compared to control, positive control and treatment diet formulated with low Ca & TP. The results are not without precedence with earlier reports citing positive effect on live weight, feed intake and feed conversion efficiency by supplementation of 0.5 per cent citric acid in the diet. Islam *et al.* (2008). But these cited reports speak about the results obtained using corn soy based diet adhering to the feeding standards for all nutrients including Ca and TP. Where as in our study, addition of 0.5 per cent citric acid (CA) in formulated feed with moderately low Ca and TP enhanced the palatability of feed leading to increase in feed intake and live weight gain and positive effect on growth, feed intake, feed efficiency compared to both control- a standard corn-soy based diet as well as positive

control with added 0.5% CA. Improvement in FCR could possibly be due to better utilization of nutrients owing to congenial enteric environment of microbiota causing increased retention and absorption of nutrient activating the homeostatic mechanisms resulting in increased body weight gain in the birds offered organic acids in the diet. The results are not without precedence. (Chowdhury *et al.* 2009., Fattah *et al.* 2008). Better growth performance in treatment birds offered moderately low Ca and TP seems to be a function of better environment for digestion and higher uptake of nutrients in the gastro intestinal tract as revealed by higher weight of fore/breast quarter which signifies the availability of extra nutrients owing to acidification of the diet. Probable reason could be the shift in microbial ecology favouring beneficial microbes especially in caecum which plays an important role in creating positive environment for digestion and absorption of nutrients. Shivani *et al.* (1996) reported better growth performance and carcass yield in broiler birds with a shift in microbial population by added direct fed microbial especially acid producing bacteria. Similarly addition of citric acid to treatment diets caused acidification of digestive contents which supported the acid producing bacteria and thereby caused a shift in the bacterial population leading to reduction in pathogenic burden improving gut health parameters significantly and thereby leading to an increase in the availability of nutrients. Similar results were reported by Shambhavi *et al.* (2020) who supplemented the direct fed microbial on azolla based feed enhancing the positive effect on carcass characteristics of broilers, thereby improving the conditions for digestion and extra availability of nutrients for deposition. This micro flora has the ability to convert the retrograde uric acid (which is being carried to the caecum and coprodeum) to amino acids. Caecum is also important in amino acids absorption and has greater ability to transport amino acids causing higher deposition in fore/breast quarter. Haq *et al.* (2014) studied that dietary supplementation of various levels of citric acid in broilers exhibited a significant increase in dressing percentage (without skin) and the average improvement in dressing percentage was between 0.66 to 2.18 per cent by the use of citric acid in feed.

Interesting results were the display of lower growth, higher feed intake and FCR in treatment birds offered feed with low Ca and TP. Results obtained reveal the interaction of minerals viz. Ca and P in the gastro intestinal tract upon addition of 0.5 per cent citric acid (CA) and to create ideal conditions to support and activate the homeostatic mechanisms as evident in metabolic trial, blood and tibiae bone studies for enhance absorption at lowest density of calcium and total phosphorus (T_3) in the ration, then, when the levels are slightly low (T_2). Though, results revealed that 0.5 per cent CA addition supported calcium and phosphorus homeostasis of the birds in treatment T_2 and T_3 offered ration with low calcium and phosphorus content but the effect was more pronounced at moderately low Ca and TP in feed. Though, in our study, the plasma Ca level was significantly ($P < 0.05$) high in control T_0 but higher calcium and phosphorus level in blood plasma may not present the true picture regarding calcium status of the body since there is a constant homeostasis maintained for calcium and phosphorus levels in blood with blood calcium level maintained within very narrow limits by several hormones that control calcium 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 calcium blood levels (Taylor and Dacke., 1984 ;Schoulten *et al.* 2003). Tibia ash percent did not exhibit any major difference but analysis for calcium and phosphorus deposition in tibia bone in treatment T_2 and T_3 revealed significantly ($P < 0.05$) higher content

compared to T₀ and T₁. The results obtained reveal that CA supplementation in low mineral ration increase deposition of tibia ash, tibia Ca and P content. Similar results have been reported earlier (Brenes *et al.*, 2003; Livingston *et al.*, 2005).

Addition of CA at 0.5% level exerted hypocholistremic effect probably due to the reason 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 hydrolyse (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 faeces, 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). The results are not without precedent with Tollba (2010) reporting significant reduction in cholesterol when citric acid was used at the rate of 2 per cent in broilers feed.

Economics of broiler feeding revealed highest gross return above feed cost in broiler birds offered moderately low Ca and TP with added 0.5 % CA whereas lowest profit was observed in treatment group of broiler birds offered low Ca and TP with added 0.5 % CA. Overall, treatment group of broiler birds offered moderately low Ca and TP with added 0.5 % CA exhibited highest gross profit return taking into account cost of feeding, FCR and prevailing selling prices.

In the present study, overall results revealed that broiler birds offered moderately low Ca and TP with added citric acid at a rate of 0.5 per cent exhibited higher growth performance for gain in weight, feed intake, feed conversion efficiency and also conferred protection to birds by lowering mortality in birds influencing, a reduction in total microbial count in the lower gastro intestinal tract. Similarly, moderately low Ca and TP in formulated feed with added 0.5 per cent citric acid positively influenced carcass traits viz. dressing percentage and forequarters weight. Results of the experiment revealed activation of homeostatic mechanisms in birds offered moderately low Ca and TP with added 0.5% CA modulating enhanced availability of calcium and phosphorus as evident in metabolic trial, blood and tibiae bone studies but the homeostatic mechanism was less pronounced when Ca and TP density in diet was slightly low. Overall, treatment group of broiler birds offered moderately low Ca and TP with added 0.5 % CA exhibited highest gross profit return taking into account cost of feeding, FCR and prevailing selling prices. In conclusion, birds offered corn soya based diet with moderately low mineral viz. calcium and total phosphorus as per ICAR 2013 standard with added 0.5 per cent citric acid can enhance the growth, improve carcass characteristics and improve the FCR of broilers though favourable environment in lower intestinal tract and activation of homeostatic mechanisms of calcium and phosphorus digestion and absorption, thus economizing the cost of production.

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Compliance with ethical standards Conflict of interest

The authors declare that they have no conflict of interest. There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Animal welfare statement

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.

Table 1. Ingredient and chemical composition of broiler diets

Attribute	Pre-starter (0-14 d)				T ₀	Starter (15-22 days)				T ₀	Finisher (22-35 days)		
	T ₀	T ₂	T ₃	T ₃		T ₂	T ₃	T ₃	T ₂		T ₃	T ₃	
Ingredient composition (%)													
Yellow corn-9%	52.00	52.00	52.00	52.00	57.00	57.00	57.00	57.00	58.00	58.00	58.00	58.00	
Soy flakes-45%	41.50	41.50	41.50	41.50	35.50	35.50	35.50	35.50	32.50	32.50	32.50	32.50	
Palm oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	5.0	5.0	5.0	5.0	
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	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)													
DM	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	
CP	23.00	23.00	23.00	23.00	22.00	22.00	22.00	22.00	20.00	20.00	20.00	20.00	
EE	3.10	3.10	3.10	3.10	3.51	3.51	3.51	3.51	4.10	4.10	4.10	4.10	
CF	5.40	5.40	5.4	5.4	5.20	5.20	5.20	5.20	5.00	5.00	5.00	5.00	
TA	4.05	4.05	4.05	4.05	3.80	3.80	3.80	3.80	3.50	3.50	3.50	3.50	
AIA	3.00	3.00	3.00	3.00	2.50	2.50	2.50	2.50	3.00	3.00	3.00	3.00	
Ca	1.00	1.00	0.90	0.80	1.10	1.10	0.99	0.88	0.99	0.99	0.90	0.79	
TP	0.70	0.70	0.66	0.65	0.72	0.72	0.71	0.70	0.70	0.70	0.69	0.68	
NFE	64.45	64.45	64.45	64.45	65.50	65.50	65.50	65.50	67.40	67.40	67.40	67.40	
ME (kcal/kg)	3077	3077	3077	3077	3109	3109	3109	3109	3150	3150	3150	3150	
Methionine	0.55	0.55	0.55	0.55	0.49	0.49	0.49	0.49	0.42	0.42	0.42	0.42	
Lysine	1.39	1.39	1.39	1.39	1.20	1.20	1.20	1.20	0.95	0.95	0.95	0.95	
Tryptophan	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	
ME:CP	134:1	134:1	134:1	134:1	141:1	141:1	141:1	141:1	157:1	157:1	157:1	157:1	

*Premix was prepared by mixing the following in 500g maize flour TRAYMIX 9g (vitamin A-82,500 IU, B2-50 mg, D3-12,000 IU, K-10 mg/g), MERIPLEX- 18g (vitamin B₁-8mg, B₆- 16mg, B₁₂- 80mcg, E- 80mg, Niacin- 120mg, Calcium D: Pantothenate- 80mg, Ca- 88 mg), E-care Se forte -10g (vitamin E- 0.5g, Se- 1mg), QUINACOX -27g (Diclazuril- 1mg) Trace minerals- 60.4g (Ferrous oxide -19.8 g, Copper sulphate -2.64g, Manganese sulphate-19.8 g, Zinc sulphate -18g, Ferrous sulphate-19.8 g, Potassium iodate-0.130 g, Magnesium sulphate-19.8g, Choline chloride: 135g), Phytase (5g/ quintal: Activity 5000FPU/g).

Table: 2. Growth performance of broiler birds (0 – 35th day)

Parameter	Days	Treatments				SEM	p Value
		Control	Control +	0.5%CA+Low	0.5%CA+		
		T ₀	T ₁	T ₂	T ₃		
GIW (g)	1-14 days	354.48	355.81	369.17	366.45	7.16	0.36
	14-21 days	377.14	420.95	391.83	381.58	14.38	0.14
	21-35 days	926.00 ^b	999.94 ^{ab}	969.10 ^{ab}	1038.41 ^a	31.92	0.0001
	1-35 days	1654.54 ^b	1789.0 ^a	1732.90 ^{ab}	1789.60 ^a	30.16	0.004
FI(g)	1-14 days	465.94 ^c	491.72 ^a	477.10 ^b	475.83 ^b	1.96	0.0001
	14-21 days	563.83 ^b	607.46 ^a	607.66 ^a	596.55 ^c	2.11	0.0001
	21-35 days	1546.88 ^d	2063.6 ^b	2587.95 ^d	1912.87 ^c	26.3	0.0001
	1-35 days	2692.66 ^b	3034.3 ^a	3111.67 ^a	3010.88 ^a	36.6	0.0001
FCR	1-14 days	1.33 ^{ab}	1.39 ^a	1.30 ^b	1.31 ^b	.03	0.14
	14-21 days	1.53	1.51	1.48	1.67	.08	0.43
	21-35 days	1.70 ^c	2.10 ^b	2.74 ^a	1.89 ^{bc}	.09	0.0001
	1-35 days	1.65 ^b	1.71 ^{ab}	1.81 ^a	1.69 ^b	.04	0.03
ADWG(g)	1-14 days	25.32	25.42	26.37	26.18	.38	0.23
	14-21 days	53.88	60.14	55.98	54.51	2.35	0.28
	21-35 days	66.14	71.42	69.22	74.17	2.28	0.08
	1-35 days	47.27	51.12	49.51	51.13	.86	0.058
ADFI (g)	1-14 days	33.28 _a	35.12 _b	34.08 _c	33.99 _{cd}	0.13	0.001
	14-21 days	80.55	86.78	85.77	86.46	1.44	0.14
	21-35 days	110.49 _a	147.40 _b	184.85 _c	136.63 _{bd}	2.77	0.001
	1-35 days	76.93 _a	86.70 _b	88.90 _b	86.03 _b	1.04	0.001
Mortality %	1-14 days	3.5	7.5	0	3.75		
	14-21 days	0	0	7.5	0		
	21-35 days	8	0	0	0		
	1-35 days	11.25	7.5	7.5	3.75		0.36

Fig. with different superscripts in a column are statistically ($P < 0.05$) significant from each other. N=80
 GIW: Gain in weight, FI: Feed intake, FCR: Feed conversion ratio, ADWG: Average daily weight gain: ADFI:
 Average daily feed intake: European efficiency factor

TABLE: 3 Digestibility coefficients (% dry matter basis), carcass characteristics and microbial load in caecal contents

Parameter	Treatments				SEM	P Value
<i>Digestibility</i>	Control T ₀	Control + T ₁	0.5%CA+Low Ca & TP T ₂	0.5%CA+ Mod.Low Ca & TP T ₃		
DM%	82.30	81.92	79.86	81.44	1.84	0.85
CP %	77.17	75.80	69.74	75.85	1.98	0.11
CF%	41.15	30.23	22.52	29.63	4.29	0.12
EE%	85.68	84.57	85.02	86.15	2.56	0.98
Ca%	32.48	41.57	40.64	38.81	6.12	0.78
P%	34.27	32.37	37.97	38.13	6.41	0.92
<i>Carcass Characteristics</i>						
Skin(% of BW)	6.90	6.70	7.31	6.87	0.33	0.45
Feet (% of BW)	3.78	3.53	3.85	3.70	0.33	0.35
Head (% of BW)	3.15	2.80	3.21	2.81	0.32	0.75
Wing(% of BW)	6.59	6.04	5.79	4.85	0.65	0.45
Dressing %	63.53 ^a	68.46 ^b	68.19 ^b	66.15 ^b	0.47	0.001
Breast %	19.34 ^a	22.35 ^b	23.76 ^b	23.37 ^b	0.55	0.001
Leg quarter (%)	25.43	24.89	24.89	24.88	0.74	0.98
Thigh %	14.72	15.09	14.86	14.20	0.42	0.42
Drum Stick %	9.55	10.34	10.03	10.68	0.50	0.68
<i>Tibia bone mineral content</i>						
Ash (%)	43.15 ^a	43.98 ^a	35.68 ^b	42.51 ^a	.30	0.001
Calcium (%)	10.13 ^a	13.59 ^b	18.41 ^c	18.19 ^c	.16	0.001
Phosphorus (%)	6.89 ^b	9.44 ^a	10.23 ^a	10.05 ^a	.43	0.001
<i>Microbial load in caecal contents</i>						
Microbial count (cfu/g)X10 ⁹	3.82 ^a	2.86 ^b	2.88 ^b	2.91 ^b	.05	0.001
Cocci(cfu) X10 ⁷	3.5 ^b	2.5 ^b	6.5 ^a	1.5 ^b	.5	0.001
Bacilli(cfu)X10 ⁷	16.5 ^a	17.5 ^a	13.5 ^b	18.5 ^a	.5	0.001

DM: Dry matter, CP: Crude protein, CF: Crude fibre, EE: Ether extract, Ca: Calcium, P: Phosphorus, BW: Body weight

Fig. with different superscripts in a column are statistically ($P < 0.05$) significant from each other

Table: 4 Gastrointestinal pH, plasma mineral, cholesterol content & hematology

Parameter	Treatments					
<i>Intestinal pH</i>	Control	Control +	0.5%CA+Low Ca & TP	0.5%CA+ Mod.Low Ca & TP	SEM	P Value
	T ₀	T ₁	T ₂	T ₃		
Duodenum	6.40	6.44	6.56	6.49	0.16	0.94
Jejunum	6.53	6.46	6.68	6.12	0.14	0.22
Ilium	6.54	7.01	6.9	6.27	0.15	0.056
Caecum	6.29	6.98	6.67	6.45	0.15	0.09
Colon	6.83	6.66	6.78	6.54	0.15	0.58
Blood plasma mineral & Cholesterol content						
Calcium(mg/dL)	11.31 ^a	9.94 ^b	11.15 ^{ac}	10.29 ^{bc}	0.19	0.001
Phosphorus (mg/dL)	7.11 ^b	6.74 ^b	7.43 ^a	6.78 ^{ab}	0.13	0.011
Cholesterol (mg/dL)	66.93 ^a	64.65 ^b	62.6 ^c	62.9 ^c	0.18	0.001
Complete blood count						
WBC 10 ³ /mm ³)	276.4	270.05	270.25	269.9	2.34	0.96
RBC(10 ⁶ /mm ³)	2.66	2.56	2.78	2.35	0.75	0.059
Hemoglobin (g/dL)	15.9	14.55	16	14.5	0.28	0.11
PCV (%)	48	39.5	42.5	39.5	1.62	0.06

WBC: white blood cells, RBC: red blood cells, PCV: packed cell volume

Fig. with different superscripts in a column are statistically ($P < 0.05$) significant from each other

Table 5: Economics of broiler feeding in INR

Parameter	Treatments			
<i>Economics</i>	Control	Control +	0.5%CA+Low Ca & TP	0.5%CA+ Mod. Low Ca & TP
	T ₀	T ₁	T ₂	T ₃
Viability (%)				
1-35d	88.7	92.5	92.5	96.2
EEF	261	283	260	301
C_m Rs/Kg (INR)				
1-14 d	42.6	44.6	41.2	40.9
14-21 d	49.0	48.5	46.9	52.1
21-35 d	52.7	65.3	84.6	57.1
1-35 d	52.0	54.0	56.6	51.9

INR: Indian National Rupee

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