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## Synergistic effect of citric acid and microbial phytase on growth performance in broiler chicken

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### Abstract

An experiment spread over a period of eight weeks was carried out to study the effect of citric acid and microbial phytase (Natuphos®-5000G) on growth performance in broiler chicken. One hundred and ninety-two, day-old broiler chicks (Vencobb) were divided into four identical groups having four replicates in each group with 12 birds in each replicate and allotted randomly into four dietary treatments viz., T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. The treatments consisted of a standard broiler ration (SBR) with 0.5 per cent available P (T<sub>1</sub>), low available P broiler ration having 0.3 per cent available P (LAPBR) and 3.0 per cent citric acid (T<sub>2</sub>), LAPBR supplemented with 700 U of phytase/kg feed (T<sub>3</sub>) and LAPBR with 1.5 per cent citric acid and 350 U of phytase/kg feed (T<sub>4</sub>). All the rations were formulated as per BIS specifications except in the level of available P. Body weight and weight gain of the experimental birds were significantly influenced by the dietary treatments. The highest weight gain and body weight being observed in T<sub>4</sub> with significant difference both at sixth week ( $p < 0.01$ ) and eighth week ( $p < 0.05$ ), indicating the synergistic effect of citric acid and microbial phytase on nutrient utilization and growth performance. The mean daily, fortnightly and cumulative feed intakes differed significantly between treatments. The birds fed on all the experimental rations (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) consumed more feed, than control (T<sub>1</sub>) group. The highest cumulative feed consumption was observed in T<sub>4</sub> at sixth week ( $p < 0.01$ ) and in T<sub>2</sub> as well as T<sub>4</sub> at eighth week ( $p < 0.01$ ). The results indicate that inclusion of citric acid or phytase improve feed intake and can cause further improvements in feed intake when these additives were added together (T<sub>4</sub>). Cumulative feed conversion ratios recorded by the birds were 2.09, 2.02, 2.07 and 2.01 at sixth week and 2.36, 2.30, 2.30 and 2.29 at eighth week for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. Though the birds maintained on different dietary treatments registered no significant difference in cumulative feed conversion ratio, an increasing trend in feed efficiency was noticed in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> over the control group (T<sub>1</sub>) in the sixth week and almost similar feed efficiency for the various groups during eighth week, indicating that citric acid or phytase favours the nutrient utilization. The percent livability was not influenced by the different dietary treatments, even though the birds maintained on low P diet in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. Over all evaluation of the present study revealed that combination of citric acid and microbial phytase in low available P diet could synergistically improve growth performance in broiler chicken.

**Keywords:** Broilers, microbial phytase (MP), citric acid (CA), phosphorus (P), growth performance

### Introduction

Phosphorus (P) is an essential mineral for broilers metabolism and skeletal development. Also, with calcium it has a main role in the formation and maintenance of bone (Underwood and Suttle, 1999)<sup>[1]</sup>. However, 60 to 70% of the provided P in typical broiler diet ingredients such as corn and soybean is bound to phytic acid (Aguilar *et al.*, 2008)<sup>[2]</sup>. Phytate-P is largely unavailable for utilization by monogastric animals, such as poultry, due to a lack of effective endogenous phytase enzyme that aids in digestion of the phytic acid complex (Waldroup *et al.*, 2000)<sup>[3]</sup>. Phytic acid can also act as an anti-nutrient due to the ability of the compound to bind with starch, proteins and minerals, such as P, Zn, Fe, Ca and Mg. Because, diets of monogastric animals are often supplemented with inorganic P sources which increase the diets cost and contribute to environmental pollution and phytase is naturally found in a number of seeds including; cereals, legume and other feedstuffs, by-products and microbial sources. Exogenous phytase supplementation of broiler liberating phytate bounded P. Exogenous phytase can improve the retention of dietary P and the addition of exogenous phytases to poultry diets improves performance parameters other than those associated with improvement in P utilization (Hajati, 2010)<sup>[4]</sup>. Recent researches have shown that the poultry gastrointestinal tract acidity is not desirable to complete hydrolyze or accepting of phytate by phytase. Given that Microbial Phytase (MP) is most active at 2.5 and 5.5 pH.

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Knowing that, some intestinal sections have different pH values, the effectiveness of phytase may be enhanced, at least in theory, by combining of feeds with an organic acid. In this respect, Afsharmanesh and Pourreza (2005) [5] suggested that reduction in gastric pH occurs following organic acid feeding may increase pepsin activity. Moreover, peptides arising from pepsin proteolysis and triggers the release of hormones, including gastrin and cholecystokinin that regulate the digestion and absorption of protein. Citric Acid (CA) may change the intestinal pH and improve phytase enzyme activity, because the phytase efficiency is correlated with both acidity and concentration of other free cations. To make phytate P biologically available, CA was used as a strong chelator of Ca, making phytate P less stable and more susceptible to endogenous and exogenous phytase (Boiling *et al.*, 2000) [6]. So, it has been indicated that CA and MP may have synergistic effect. The main objective of the present study was to investigate the effect of supplementing diet with MP and CA and their combination on growth performance in broiler chicken.

### Materials and Methods

One hundred and ninety two, day-old commercial broiler chicks (Vencobb) of similar mean body weight were randomly divided into 16 groups of 12 chicks each and were allotted randomly to four dietary treatments *viz.*, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> with four replicates in each treatment. The dietary treatments consisted of a standard broiler ration as per BIS with 0.5% available P (T<sub>1</sub>), low available P broiler ration with 0.3% available P (LP) and 3.0% CA (T<sub>2</sub>), LP supplemented with 700 U of MP (Natuphos® 5000, BASF Corp., Germany)/kg diet (T<sub>3</sub>) and LP with 1.5% CA and 350U of MP/kg diet (T<sub>4</sub>). The composition and chemical analysis of different dietary treatments are presented in Table 1. The starter ration was fed up to six weeks and finisher ration from 7<sup>th</sup> to 8 weeks of age. The birds were provided *ad libitum* feed and water throughout the experimental period and were maintained under deep litter system of management.

The body weight of individual birds was recorded at fortnightly intervals from day old to study the pattern of growth rate under different dietary treatments. The fortnightly body weight gain (BWG) was obtained by calculation. Feed intake (FI) of the birds was recorded replication wise at weekly intervals. From these data, the average FI per bird per day was calculated for various treatment groups. Feed conversion ratio (FCR- kg of feed consumed/kg BWG) was calculated based on the data on BWG and FI. The chemical compositions of experimental rations were determined as per the standard procedures (AOAC, 1990) [7]. For mineral analysis, the diet samples were subjected to wet digestion, using nitric acid and perchloric acid (2:1). Ca, Mg, Zn and Mn content of the digested sample were determined using atomic absorption spectrophotometer (Perkin-Elmer Model-AAS 3110) and inorganic P by colorimetric method (AOAC, 1990) [7] using spectronic 1001<sub>plus</sub> spectrophotometer (Milton Roy Co., USA). The mortality of birds from different treatment groups was recorded and post mortem examination was conducted in each case to find out the cause of death.

Data collected on various parameters were statistically analyzed by Completely Randomised Design (CRD) method as described by Snedecor and Cochran (1989) [8]. Means were compared by Least Significant Difference (LSD) test using MSTATC. Results were expressed as mean  $\pm$  S.E. The results

were considered statistically significant if the 'p' value were 0.05 or less.

### Results and Discussion

The data on fortnightly mean body weight presented in Table 2 showed that Inclusion of CA at 3.0 per cent level in low available P diet (T<sub>2</sub>) resulted in lower fortnightly body weights at second ( $p<0.01$ ) and fourth week of age than any of the treatments and the difference could be due to the reduced FI. Sifri *et al.* (1977) [9] and Angel *et al.* (2001a) [10] also observed no effect of CA on body weight of broiler chicks during first four weeks of age and are in close agreement with the present study. After four weeks of age T<sub>2</sub> birds showed appreciable increase in body weight and attained statistically similar body weight at sixth week and significantly higher ( $p<0.01$ ) body weight at eighth week compared to control birds on standard broiler ration (T<sub>1</sub>). Boling *et al.* (2000) [6], Angel *et al.* (2001b) [11], Boling-Frankenbach *et al.* (2001) [12] and Metwally (2001) [13] also could observe significant increase in body weight on CA supplementation.

Supplementation of MP at a level of 700 U/kg in low available P diet (T<sub>3</sub>) tended to result in higher fortnightly body weight right from second week onwards when compared to control group (T<sub>1</sub>). This finding is in agreement with Yi *et al.* (1996) [14] who could observe linear increase in body weight in broilers on supplementation of MP at levels of 350, 700 or 1050 U/kg in a basal diet containing 0.27 per cent non-phytate P. Significant improvement in body weight among broiler chicken fed low available P diet supplemented with MP was also reported by Waldroup *et al.* (2000) [3], Broz *et al.* (1994) [15], Biehl *et al.* (1995) [16], Qian *et al.* (1997) [17], Huff *et al.* (1998) [18], Kanagaraju (1998) [19], Sohail and Roland (1999) [20], Balasubramanian (2000) [21] and Ravindran *et al.* (2001) [22]. On the contrary, Zanini and Sazzad (1998) [23] observed that supplemented MP at 500 U/kg diet had no effect on growth performance in broilers when fed with different metabolizable energy levels of 2800 and 3000 kcal ME/kg diet. Significantly superior body weights at all fortnights were noticed in the combination group (T<sub>4</sub>) containing 0.3 per cent available P with 1.5 per cent CA and 350 U MP/kg diet, when compared to control group and substantial increase when compared to all other treatments. These results are in close agreement with Boling *et al.* (2000) who stated that addition of 1,450 U of MP/kg of the diet with 6.0 per cent citrate and 0.1 per cent available P caused further improvements ( $p<0.05$ ) in body weight. On the contrary, Angel *et al.* (2001a,b) [10, 11] observed no interactions between CA and MP, when added together.

The results in the present study revealed that a reduction of 0.2 per cent available P in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was compensated and even resulted in better performance of commercial broilers by inclusion of CA and/or MP. The CA and/or MP in the feed might have acted upon the bound phytate P in the feed resulting in release of more inorganic P and other nutrients for utilization by the bird and subsequently better performance in comparison with standard broiler diet. Moreover, the effect of MP on growth performance can be synergistically improved by inclusion of CA, which enhances the MP activity.

From the results on BWG presented in Table 3, it can be seen that the weight gain recorded at second week of age were 327.83, 275.11, 342, 34 and 354.08 g for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. The combination group (T<sub>4</sub>) recorded the highest

weight gain ( $p < 0.01$ ) followed by T<sub>3</sub>, T<sub>1</sub> and T<sub>2</sub> in which T<sub>2</sub> being the lowest ( $p < 0.01$ ). In the second fortnight also a similar trend was observed in weight gain, the values being 787.05 for T<sub>4</sub> ( $p < 0.05$ ) followed by 745.09 (T<sub>3</sub>), 727.15 (T<sub>1</sub>) and 712.49 g (T<sub>2</sub>). The weight gain for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> at third fortnightly interval was 771.98, 946.92, 810.21 and 890.32 g and at fourth fortnightly interval, it was 677.83, 823.45, 724.29 and 735.03 g, respectively. The highest weight gain at sixth ( $p < 0.01$ ) and eighth ( $p < 0.05$ ) week of age was observed in T<sub>2</sub> group which was comparable with T<sub>4</sub> at sixth week and with T<sub>3</sub> and T<sub>4</sub> at eighth week. T<sub>1</sub> attained the lowest and was comparable with T<sub>3</sub> at sixth week and T<sub>3</sub> and T<sub>4</sub> at eight week of age.

The cumulative BWG recorded were 1826.96, 1934.52, 1897.64 and 2031.44 g at sixth week and 2504.79, 2757.97, 2621.92 and 2766.47 g, at eighth week for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. Similar to the data on sixth week body weight, the cumulative BWG of sixth week showed that the supplementation of MP (700 U/kg diet) to low available P diet (T<sub>3</sub>) had a trend for improvement in BWG and was statistically comparable with gain of birds offered standard broiler diet (T<sub>1</sub>). Similar to sixth week body weight, BWG was also highest in the combination group (T<sub>4</sub>), which was statistically comparable with CA group (T<sub>2</sub>). BWG of CA group (T<sub>2</sub>) at sixth week was also comparable with control (T<sub>1</sub>) and MP group (T<sub>3</sub>).

The results of the present study are in close agreement with Boling *et al.* (2000) <sup>[6]</sup> who observed linear increase ( $p < 0.01$ ) in BWG by inclusion at 1, 2, 4 or 6 per cent CA + sodium citrate (1:1, wt: wt) mixture to low available P (0.1 per cent) diet. Improvement in weight gain by inclusion of CA in broiler diets was also recorded by Angel *et al.* (2001b) <sup>[11]</sup>, Boling-Frankenbach *et al.* (2001) <sup>[12]</sup> and Metwally (2001) <sup>[13]</sup>. Qian *et al.* (1997) <sup>[17]</sup> informed that MP supplementation at the level of 300, 600 and 900 U/kg of low P diet linearly increased BWG in broilers. Kanagaraju (1998) <sup>[19]</sup> and Balasubramanian (2000) <sup>[21]</sup> also reported improvements in weight gain due to MP supplementation. The results of the present study are in agreement with Boling *et al.* (2000) <sup>[6]</sup> who observed further improvements ( $p < 0.05$ ) in weight gain when MP (1,450 U/kg diet) and CA (6 per cent) were added together to low P (0.1 per cent available P) diet, while the findings of present study are inconsistent with the results of Angel *et al.* (2001a,b) <sup>[10, 11]</sup> who could not observe any interaction effects of CA and MP on BWG of broilers which might be due to application of shorter feeding trial (one week).

Beneficial effects in BWG by inclusion of CA and/or MP in feed for broilers were mainly due to increase in FI (Table 4). Moreover, CA and/or MP supplementation might have acted upon phytate molecule and resulted in the better utilization of P and other nutrients. In addition to its function in bone formation, P is also required in the utilization of energy and the resultant BWG. Moreover, the cumulative BWG (Table 3) of the present study indicated 5.89, 3.83 and 11.19 per cent higher weight gain ( $p < 0.01$ ) at sixth week and 10.10, 4.6 and 10.45 per cent higher weight gain ( $p < 0.05$ ) at eighth week for T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively than control group (T<sub>1</sub>). This finding shows the synergistic effect ( $p < 0.01$ ) of CA and MP combination (1.5 per cent CA and 350 U MP/kg) on BWG when included in the starter diets.

The data on fortnightly FI of birds maintained on different dietary treatments presented in Table 4 indicate that it differed

significantly between groups ( $p < 0.01$ ) in all fortnights (Table 4). The mean cumulative FI (Table 4) of birds were 3817.29, 3906.54, 3921.87 and 4075.79 g from zero to sixth week and 5904.33, 6340.86, 6035.73 and 6337.33 g from zero to eighth week for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. When the cumulative FI was considered, it can be seen that the group offered standard broiler diet (T<sub>1</sub>) consumed less feed ( $p < 0.01$ ) and a statistically similar FI was observed by T<sub>2</sub> and T<sub>3</sub> at sixth week and T<sub>3</sub> at eighth week. The combination group (T<sub>4</sub>) consumed more feed ( $p < 0.01$ ) than other groups and was statistically comparable with T<sub>3</sub> at sixth week and with T<sub>2</sub> at eighth week.

The findings of the present study are in agreement with Angel *et al.* (2001a) <sup>[10]</sup> who observed improvement ( $p < 0.05$ ) in feed consumption in broilers by supplementation of CA (1, 2 and 3 per cent) to low P diets. Metwally (2001) <sup>[13]</sup> also observed increased feed consumption in broilers by increasing levels of citrate to 4.5 per cent in low P diets. Similar results were also reported by Krause *et al.* (1994) <sup>[24]</sup>. On the contrary, Angel *et al.* (2001b) <sup>[11]</sup> observed lower ( $p < 0.05$ ) feed consumption, when CA (3 per cent) was added to low non-phytate P (0.16 per cent) diet. The results of the present study are in close agreement with Balasubramanian (2000) <sup>[21]</sup> who also observed increasing trend for FI from zero to eight weeks of age in groups fed MP supplemented diets. Increasing trend in FI by supplementation of MP in low available P diet obtained in the present trial also agrees with the findings of Yi *et al.* (1996) <sup>[14]</sup>, Broz *et al.* (1994) <sup>[15]</sup>, Qian *et al.* (1997) <sup>[17]</sup>, Kanagaraju (1998) <sup>[19]</sup>, Perney *et al.* (1993) <sup>[25]</sup>, Denbow *et al.* (1995) <sup>[26]</sup> and Aksakal and Bilal (2002) <sup>[27]</sup>.

Combination of CA and MP (T<sub>4</sub>) or CA alone (T<sub>2</sub>) consumed almost equal amount of feed from zero to eighth week of age. But highest ( $p < 0.01$ ) FI in T<sub>4</sub> from zero to sixth week of age showed the interaction effects. This finding is inconsistent with the results of Angel *et al.* (2001a, b) who observed no interaction effect. This might be due to application of shorter feeding trial of one week in their study. Higher FI observed in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups could be due to increased phytate P digestibility, since phytic acid may be imposing restraint on voluntary FI.

Moreover, the cumulative mean FI (Table 4) showed 2.34, 2.74 and 6.77 per cent higher FI at sixth week and 7.39, 2.22 and 7.33 per cent higher FI at eighth week of age for T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively than control group (T<sub>1</sub>). The results of the present study indicate that inclusion of CA alone or MP alone had a favouring effect on FI, whereas when both added together in low P diets had a synergistic effect ( $p < 0.01$ ) on FI, when compared to standard diet.

The mean fortnightly feed conversion ratio (FCR) as influenced by different dietary treatments (Table 5) does not reveal any definite trend among the treatments. It ranged from 1.32 (T<sub>4</sub>) to 1.46 (T<sub>2</sub>) at the first fortnight, 1.90 (T<sub>4</sub>) to 2.05 (T<sub>1</sub>) in the second fortnight, 2.25 (T<sub>2</sub>) to 2.44 (T<sub>1</sub>) in the third fortnight and 2.92 (T<sub>3</sub>) to 3.11 (T<sub>4</sub>) in the fourth fortnight. The analysis of variance of the data on mean feed conversion ratio showed that it was not significantly influenced in all fortnights except in the first fortnight, in which superior ( $p < 0.05$ ) feed conversion was recorded in combination group (T<sub>4</sub>) which was statistically comparable with MP (T<sub>3</sub>) group and the feed efficiency was lowest with the group fed CA alone (T<sub>2</sub>), which was also statistically comparable with MP (T<sub>3</sub>) group.



The cumulative feed conversion efficiency recorded were 2.09, 2.02, 2.07 and 2.01 for zero to sixth week and 2.36, 2.30, 2.30 and 2.29 for zero to eighth week of age in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. On statistical analysis, no significant difference could be observed. However, the supplemented groups are showing an improving trend in cumulative FCR from zero to six week (Table 5).

Metwally (2001) [13] also reported improvement in feed conversion efficiency by increasing levels of dietary citrate to 4.5 per cent. Whereas, Sifri *et al.* (1977) [9] stated that the feed: gain ratio was not significantly affected, after conducting studies for 28 days on broilers fed on diets containing marginally low (0.4 per cent) and adequate (0.85 per cent) Ca along with CA (0.71 per cent). These findings correlate well with the present study. The favouring effect of MP supplementation in low available P diet on feed efficiency, in broilers observed in the present study is in agreement with Huff *et al.* (1998) [18], Sohail and Roland (1999) [20] and Perney *et al.* (1993) [25]. Whereas, Waldroup *et al.* (2000) [3], Broz *et al.* (1994) [15], Kanagaraju (1998) [19], Balasubramanian (2000) [21], Ravindran *et al.* (2001) [22] and Aksakal and Bilal (2002) [27] observed significantly improved

feed conversion efficiency by dietary supplementation of MP, when compared to unsupplemented low available P diet. The results of the present study confirm the findings of Angel *et al.* (2001a, b) [10, 11] who observed no significant interactions of CA and MP for FCR in broilers.

The inclusion of CA and/or MP might have acted upon the phytate P; with the result, P could have been released for utilization by the bird, which indicates the apparent improvement in feed conversion efficiency than control (T<sub>1</sub>) group. Increased FI in relation to increased BWG resulted in no significant difference between treatments for FCR. The cumulative feed conversion efficiency showed 3.5, 0.96 and 3.83 per cent higher feed conversion efficiency at sixth week and 2.54, 2.54 and 2.97 per cent higher feed conversion efficiency at eighth week of age for T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively than control group (T<sub>1</sub>). These results indicate that addition of MP and CA together improves feed utilization than when fed alone.

On scrutiny of the results of the present study, it could be concluded that combination of CA and MP in low available P diets (0.3%) could synergistically improve growth performance.

**Table 1:** Ingredient and chemical composition of starter and finisher rations %

Ingredients	Starter ration (0-6 weeks)				Finisher ration (6-8 weeks)			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Yellow maize	50.0	46.5	50.0	48.0	61.8	58.3	61.8	59.8
Rice polish	5.7	6.2	6.2	6.2	2.1	2.5	2.5	2.5
Soybean meal	36.0	36.5	36.0	36.5	28.0	28.5	28.0	28.5
Unsalted fish	5.2	5.2	5.2	5.2	5.0	5.0	5.0	5.0
Shell grit	0.8	1.5	1.5	1.5	0.8	1.5	1.5	1.5
DCP	1.5	0.3	0.3	0.3	1.6	0.5	0.5	0.5
CA	0.0	3.0	0.0	1.5	0.0	3.0	0.0	1.5
MP (U/Kg) <sup>1</sup>	0	0	700	350	0	0	700	350
<b>Chemical Composition, % DM Basis</b>								
Dry matter	91.1	91.6	91.3	91.6	90.9	90.3	90.7	90.4
Crude protein	23.6	23.1	23.6	23.4	20.2	20.4	20.2	20.4
Ether extract	4.6	4.4	4.7	4.5	4.8	4.9	5.3	4.8
Crude fibre	4.3	4.1	4.4	4.4	3.5	3.5	3.7	3.5
NFE	59.3	58.3	58.3	57.7	64.7	64.1	63.4	64.8
Total ash	8.2	10.0	9.00	10.1	6.8	7.1	7.2	6.5
AIA	2.5	2.4	2.5	2.6	1.5	1.5	1.5	1.4
Ca	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Total P	0.8	0.6	0.6	0.6	0.8	0.6	0.6	0.6
Mg	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mn (mg/Kg)	126.6	126.1	123.7	123.7	123.3	124.73	128.1	129.5
Zn (mg/kg)	81.6	84.5	88.4	83.0	80.6	80.5	82.1	83.9
<b>Calculated values</b>								
ME (Kcal/Kg)	2804.6	2814.1	2818.6	2814.1	2894.3	2901.0	2905.5	2901.0
Lysine	1.4	1.4	1.4	1.4	1.1	1.1	1.1	1.1
Methionine	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4

#### Additives added / 100 kg of feed

Vitamin AD<sub>3</sub>EK: Vitamin A – 5156250 IU, Vitamin D<sub>3</sub> – 750000 IU, Vitamin E-2500 mg, Vitamin K-625 mg.

Vitamin B complex: Vitamin B<sub>1</sub> – 250 mg, Vitamin B<sub>2</sub> – 3125 mg, Vitamin B<sub>6</sub> – 500 mg, Vitamin B<sub>12</sub> – 2500 µg

.Niacin – 3750 mg, Ca pantothenate – 2500 mg.

Trace mineral mixture (ultra TM – 130 gm) contains:

Mn – 7.02g. Zn – 6.76g. Iron – 2.6g, Iodine – 260mg, Copper – 260 mg, Cobalt – 130mg.

Toxin Binder (Alusil Premix TM): 200g

Coccidiostat: Maduramycin ammonium – 500 mg.

Common salt: 250 gram

<sup>1</sup>Each gram of MP (Natuphos @ - 5000G M/s. BASF, Germany) contained phytase activity of 5000 U (one U is the quantity of enzyme that release one µmol of inorganic P per minute from 1.5mmol/L of sodium phytate at a pH of 5.5 and temperature of 37°C).

**Table 2:** Effects of CA and MP on fortnightly mean body weight of broiler chicks, g

Treatments	Age in weeks			
	2**	4**	6**	8*
T1	368.16±5.52	1095.31 <sup>bc</sup> ±19.22	1867.29 <sup>b</sup> ±20.77	2545.12 <sup>b</sup> ±28.58
T2	315.14 <sup>c</sup> ±7.19	1027.63 <sup>c</sup> ±20.38	1974.55 <sup>ab</sup> ±23.78	2797.99 <sup>a</sup> ±37.85
T3	383.14 <sup>ab</sup> ±3.09	1128.23 <sup>ab</sup> ±18.86	1938.44 <sup>b</sup> ±39.35	2662.73 <sup>ab</sup> ±63.24
T4	394.20 <sup>a</sup> ±0.55	1181.25 <sup>a</sup> ±19.29	2071.56 <sup>a</sup> ±20.36	2806.59 <sup>a</sup> ±69.98

<sup>abc</sup> Means with different superscripts in a column differ significantly

\* Significant ( $p < 0.05$ ); \*\* Significant ( $p < 0.01$ )

**Table 3:** Effects of CA and MP on fortnightly and cumulative mean BWG of broiler chicks, g

Treatments	Fortnightly mean BWG				Cumulative mean BWG	
	Age in weeks				Age in weeks	
	2**	4*	6**	8*	0-6**	0-8*
T1	327.83 <sup>b</sup> ±5.51	727.15 <sup>bc</sup> ±13.79	771.98 <sup>b</sup> ±2.60	677.83 <sup>b</sup> ±11.81	1826.96 <sup>b</sup> ±20.76	2504.79 <sup>b</sup> ±28.56
T2	275.11 <sup>c</sup> ±7.42	712.49 <sup>b</sup> ±17.22	946.92 <sup>a</sup> ±17.82	2797.99 <sup>a</sup> ±37.85	1934.52 <sup>ab</sup> ±23.98	2757.97 <sup>a</sup> ±37.91
T3	342.34 <sup>ab</sup> ±2.89	745.09 <sup>ab</sup> ±17.12	810.21 <sup>bc</sup> ±24.83	2662.73 <sup>ab</sup> ±63.24	1897.64 <sup>b</sup> ±39.40	2621.92 <sup>ab</sup> ±63.34
T4	354.08 <sup>a</sup> ±0.37	787.05 <sup>a</sup> ±19.27	890.32 <sup>ab</sup> ±27.39	2806.59 <sup>a</sup> ±69.98	2031.44 <sup>a</sup> ±20.57	2766.47 <sup>a</sup> ±70.14

<sup>abc</sup> Means with different superscripts in a column differ significantly

\* Significant ( $p < 0.05$ ); \*\* Significant ( $p < 0.01$ )

**Table 4:** Effects of CA and MP on fortnightly and cumulative mean FI of broiler chicks, g

Treatments	Fortnightly mean FI				Cumulative mean FI	
	Age in weeks				Age in weeks	
	2	4	6	8	0-6	0-8
T1	455.62 <sup>ab</sup> ±4.98	1490.63 <sup>a</sup> ±18.30	1881.05 <sup>b</sup> ±21.08	2087.04 <sup>c</sup> ±6.53	3817.29 <sup>b</sup> ±35.39	5904.33 <sup>b</sup> ±40.83
T2	401.87 <sup>b</sup> ±12.94	1377.50 <sup>b</sup> ±10.86	2127.17 <sup>a</sup> ±11.03	2434.32 <sup>a</sup> ±44.54	3906.54 <sup>b</sup> ±32.31	6340.86 <sup>a</sup> ±62.33
T3	474.79 <sup>a</sup> ±16.11	1490.62 <sup>a</sup> ±19.80	1956.46 <sup>b</sup> ±24.13	2113.86 <sup>c</sup> ±43.23	3921.87 <sup>ab</sup> ±37.39	6035.73 <sup>b</sup> ±53.41
T4	468.13 <sup>a</sup> ±3.31	1503.80 <sup>a</sup> ±21.82	2103.87 <sup>a</sup> ±29.30	2261.54 <sup>b</sup> ±23.52	4075.79 <sup>a</sup> ±47.18	6337.33 <sup>a</sup> ±62.10

<sup>abc</sup> Means with different superscripts in a column differ significantly, ( $p < 0.01$ )

**Table 5:** Effects of CA and MP on fortnightly and cumulative mean FCR of broiler chicks, g

Treatments	Fortnightly mean FCR				Cumulative mean FCR	
	Age in weeks				Age in weeks	
	2	4	6	8	0-6	0-8
T1	1.36 <sup>b</sup> ±0.03	2.05±0.05	2.44±0.02	3.08±0.06	2.09±0.03	2.36±0.03
T2	1.46 <sup>a</sup> ±0.02	1.94±0.05	2.25±0.04	2.97±0.13	2.02±0.01	2.30±0.02
T3	1.39 <sup>ab</sup> ±0.04	2.00±0.05	2.42±0.05	2.92±0.07	2.07±0.04	2.30±0.04
T4	1.32 <sup>b</sup> ±0.01	1.90±0.03	2.37±0.06	3.11±0.18	2.01±0.01	2.29±0.04

<sup>abc</sup> Means with different superscripts in a column differ significantly, ( $p < 0.05$ )

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