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Research Article

Application of Nano-dicalcium Phosphate in Broiler Nutrition: Performance and Excreted Calcium and Phosphorus

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Abstract

Background: The environmental issues related to the presence of phosphorus (P) in poultry excreta have led the researchers to manipulate the diet of poultry in order to decrease the P excretion without having any negative impact on the performance of birds. Presently, added minerals are used as nanoparticles in order to increase absorption and subsequent decreased presence in poultry excreta. Therefore, an experiment was conducted to study the effect of dietary nano-dicalcium phosphate (NDCP) compared to conventional dicalcium phosphate (CDCP) on performance and excreted calcium (Ca) and P in broiler chicken. **Materials and Methods:** Two hundred and eighty one day-old male broiler chicks were divided into seven treatment groups for a period of 26 days. Seven experimental diets were formulated having three levels of either CDCP or NDCP at 1.75, 1.31 and 0.88% and a lower level of NDCP at 0.44%. Thus, these diets contained 100, 75, 50 and 25% of the recommended non-phytate P i.e., 0.45%. The diet having 1.75% CDCP (100% recommended non-phytate P) served as a control diet. Every dietary treatment had 4 replicates of 10 chicks each. Broiler performance, Ca and P excretion were studied. **Results:** Birds fed different levels of NDCP gained significant more body weight ($p<0.05$) and utilized feed more efficiently than the control group (1.75% CDCP). Decreasing levels of CDCP led to decrease in body weight gain and impaired feed conversion ratio compared to the control group. Values of body weight gain and feed intake increased by about 25 and 10%, respectively, feed conversion ratio improved by about 12% for birds fed NDCP compared to those fed CDCP. Level of dietary DCP significantly ($p<0.001$) affected Ca and P excretion while source of DCP significantly ($p<0.001$) affected P excretion but had no effect on ($p>0.05$) Ca excretion. Feeding 0.44% NDCP in the diet decreased the excreted Ca and P by 50.74 and 46.24%, respectively, compared to the control. **Conclusion:** It could be concluded that using NDCP in broiler diets allow successfully to reduce the dietary DCP by 75%. Diet formulated containing only 25% of the required non phytate P in form of NDCP could be used instead of 100% CDCP. Also, using dicalcium phosphate in nanoparticle size allow to reduce the excreted Ca and P by about 50% which reduce the impact of poultry on environmental pollution.

Key words: Broilers, growth performance, nano minerals, phosphorus excretion, pollution

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Calcium (Ca) and phosphorus (P) are required in large quantities by poultry and involved in many biological processes. Calcium requirement is easily satisfied with low price sources such as limestone or oyster shell. However, diets based on plant ingredients contain large amounts of unavailable phosphorus in the form of phytates (60-80% of total P) which cannot be utilized by poultry. Thus, inorganic phosphorus, which is relatively costly, is added to meet dietary P requirements of chicken for optimal growth and production[1]. This practice results in excess amounts of P excretion that can have negative environmental effects when such litter is applied as a fertilizer to soil[2,3]. The excretion of P has direct implications for both water and air quality concerns[4].

In view of environmental concerns, attempts are being made to manipulate the poultry diets in order to decrease the concentration of P in excreta with no adverse effect on performance or feed utilization. Feeding broiler chicken with P source in accordance with nutrient recommendations can decrease P intake as well as the amount of P excreted[5,6]. Earlier, addition of phytase to improve P availability and utilization by poultry has been reported to substantially decrease P excretion[7-10].

The recent trend has been focused on use of nanoparticles. Supplementation of minerals in nano form reportedly increases the absorption and subsequent reduction in their excretion. Swain *et al.*[11] reported that nano minerals are having a great potential even at very lower doses than the conventional organic and inorganic sources. These results have been proven for nano-Se (selenium)[12,13], nano-Zn (zinc)[11,14-16] and nano-Cr (chromium)[17].

In view of beneficial effects of nano minerals, the present study was taken up with the objectives to examine the different dietary levels of nano dicalcium phosphate (NDCP) on performance and calcium and phosphorus excretion compared to conventional dicalcium phosphate (CDCP) in broiler chicken.

MATERIALS AND METHODS

Preparation of NDCP: Dicalcium phosphate was synthesized in nanoparticle size by Sol-Gel method using two different solvents (deionized water and ethanol). The synthesized NDCP was characterized by means of Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Transmission Electron Microscopy (TEM) and Energy Dispersive Analysis by X-ray (EDAX). Using ethanol solvent resulted in 81.8% NDCP and 18.2% calcium carbonate, but deionized water solvent gave 100% NDCP. Crystal sizes of NDCP synthesized using deionized water or ethanol as solvent was 26 and 34 nm, respectively. Crystal size of NDCP synthesized using deionized water was 26 nm, with 100% purity. The proposed method doesn't involve using or production of any toxic or environmentally hazardous solvents, surfactants, or organic chemicals and is economical, time-saving and performed at room temperature[18].

Animals and experiment design: A broiler chicken growth trial was designed to examine the NDCP produced using water as a solvent. For this purpose, two hundred and eighty one day-old (Ross 308) male broiler chicks were divided into seven treatment groups. Seven experimental diets were formulated having three levels of either CDCP or NDCP at 1.75, 1.31 and 0.88% and a lower level of NDCP at 0.44%. Thus, these diets contained 100, 75, 50 and 25% of the recommended non-phytate P i.e., 0.45%. The diet having 1.75% CDCP (100% recommended non-phytate P) served as a control diet. Diets were formulated to cover all the nutrient requirements of Ross broiler chicks except that of calcium and phosphorus. The Ca:P ratio was kept 2:1 in all the diets. Formulation and nutrient composition of these diets are shown in Table 1.

Every dietary treatment was fed to 4 replicates of 10 chicks each. The average initial live body weights of all replicates were nearly similar. Replicates were randomly allocated in batteries cages divided into 28 compartments (4 replicates $\times$7 dietary treatments). Birds were raised in a warmed brooder house and fed the dietary treatments from 1-26 days of age. Light was provided 23 h daily throughout the experimental period. Feed and water were provided *ad libitum*. A vaccination program against avian flu, New Castle, IB and IBD was strictly adopted throughout the experimental period.

Sample preparation and evaluation: At 15 and 26 days of age, after fasting overnight, birds were individually weighed and feed consumption was recorded per replicate. Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) were calculated accordingly. Samples of excreta from all treatments were collected at 24-26 days of age, to determine Ca and P excretion. Feathers and any scattered feed were taken out. The collected excreta were dried in an air-draft oven at 60°C for 24 h and left in room temperature afterwards to equilibrate with atmosphere moisture. The dried excreta from each replicate for the successive 3 days post collection were pooled; finely ground, well mixed and placed in a screw-top glass jar for the determination of Ca and P as per AOAC[19].

Table 1: Formulation and nutrients composition of experimental diets

Item	100% CDCP	75% CDCP	50% CDCP	100% NDCP	75% NDCP	50% NDCP	25% NDCP
Ingredients (%)							
Yellow corn	54.85	55.46	56.05	54.85	55.46	56.05	56.63
Soybean meal (48%)	38.30	38.30	38.30	38.30	38.30	38.30	38.30
Soybean oil	2.80	2.80	2.80	2.80	2.80	2.80	2.80
CDCP	1.75	1.31	0.88	-	-	-	-
NDCP	-	-	-	1.75	1.31	0.88	0.44
Limestone	1.05	0.88	0.72	1.05	0.88	0.72	0.58
Vitamin and mineral mix(1)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-lysine HCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition (%)(2)							
Crude protein (%)	23.05	23.10	23.15	23.05	23.10	23.15	23.20
ME (kcal kg^{-1})	3018.00	3039.00	3059.00	3018.00	3039.00	3059.00	3078.00
Lysine (%)	1.51	1.51	1.52	1.51	1.51	1.52	1.52
Methionine (%)	0.60	0.60	0.61	0.60	0.60	0.61	0.61
Methionine+cystine (%)	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Threonine (%)	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Calcium (%)	0.91	0.74	0.59	0.91	0.74	0.59	0.43
Non-phytate P (%)	0.45	0.37	0.29	0.45	0.37	0.29	0.21

(1)Vitamin: Mineral mixture supplied per kg of diet: Vit A: 12000 IU, Vit D_3: 2200 IU, Vit E: 10 mg, Vit K_3: 2 mg, Vit B_1: 1 mg, Vit B_2: 4 mg, Vit B_6: 1.5 mg, Vit B_{12}: 10 mg, Niacin: 20 mg, Pantothenic acid: 10 mg, Folic acid: 1 mg, Biotin: 50 mg, Copper: 10 mg, Iodine: 1 mg, Iron: 30 mg, Manganese: 55 mg, Zinc: 50 mg and Selenium: 0.1 mg. (2)According to NRC[22]

Statistical analysis: Data were statistically analyzed by analysis of variance using the General Liner Model of SAS[20]. One way analysis of variance was used to detect the treatment effect. Two ways analysis of variance (factorial 2×3) was used to detect main effects of source and level of the tested dicalcium phosphate (DCP). Significant differences among treatment means were determined by Duncan's multiple range test[21] with a 5% level of probability.

RESULTS AND DISCUSSION

Growth performance: Table 2 showed growth performance of birds fed different dietary CDCP and NDCP levels at 15 day of age. The results showed significant (p<0.001) improvements in Body Weight Gain (BWG) from 356-405 g (13.8%), from 356-396 g (11.2%) and from 356-382 g (7.3%) and Feed Conversion Ratio (FCR) from 1.39-1.24 (10.8%), from 139-1.27 (8.6%) and from 1.39-1.30 (6.5%) for treatments fed 1.75, 1.31 and 0.88% NDCP, correspondingly compared to the control group fed 1.75% CDCP. However, there was no significant difference on BWG and FCR among treatments fed 1.75, 1.31 CDCP and 0.44% NDCP. The best values of BWG and FCR were recorded for birds fed 1.75% NDCP. Among NDCP groups, as the level of NDCP was increased in the diet from 0.44-1.75 %, higher was the corresponding improvement in BWG and FCR. The lowest value for BWG and worst value for FCR were recorded for birds fed 0.88% CDCP. No significant differences in Feed Intake (FI) were observed among different groups except for the group fed 0.88% CDCP which had significantly (p<0.05) low FI compared to the control group.

Table 2: Growth performance of broiler chicken fed different dietary CDCP and NDCP levels at 15 day of age

Items				
DCP level (%)	DCP source	BWG (g)	FI (g)	FCR
1.75	CDCP	356[c]	495[a]	1.39[b]
1.31	CDCP	355[c]	495[a]	1.39[b]
0.88	CDCP	339[d]	487[b]	1.44[a]
1.75	NDCP	405[a]	501[a]	1.24[e]
1.31	NDCP	396[a]	502[a]	1.27[d]
0.88	NDCP	382[b]	498[a]	1.30[c]
0.44	NDCP	358[c]	500[a]	1.38[b]
SE of means		±4.61	±1.18	±0.01
Level (%)*				
1.75		381[a]	498	1.32[b]
1.31		376[a]	499	1.33[b]
0.88		361[b]	492	1.37[a]
Source				
CDCP		350[b]	492[b]	1.41[a]
NDCP		394[a]	500[a]	1.27[b]
Significant source of variation				
Level		***	NS	***
Source		***	**	***
Level×source		***	***	***

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. **p<0.01, ***p<0.001, NS: Not Significant (p>0.05), *level of 0.44% NDCP was not including in the two way statistical analysis

Table 3: Growth performance of broiler chicken fed different dietary CDCP and NDCP levels during 16-26 days of age

Items				
DCP level (%)	DCP source	BWG (g)	FI (g)	FCR
1.75	CDCP	786[c]	1195[a]	1.52[c]
1.31	CDCP	665[d]	1063[b]	1.60[b]
0.88	CDCP	583[e]	980[c]	1.68[a]
1.75	NDCP	904[a]	1241[a]	1.37[e]
1.31	NDCP	892[ab]	1231[a]	1.38[e]
0.88	NDCP	891[ab]	1234[a]	1.38[e]
0.44	NDCP	858[b]	1223[a]	1.43[d]
SE of means		±23.68	±19.15	±0.02
Level (%)*				
1.75		845[a]	1218[a]	1.45[c]
1.31		778[b]	1147[b]	1.49[b]
0.88		737[c]	1107[c]	1.53[a]
Source				
CDCP		678[b]	1079[b]	1.60[a]
NDCP		895[a]	1235[a]	1.38[b]
Significant source of variation				
Level		***	***	***
Source		***	***	***
Level×source		***	***	***

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability. ***p<0.001, NS: Not significant (p>0.05), *level of 0.44% NDCP was not including in the two way statistical analysis

Table 4: Growth performance of broiler chicken fed different dietary CDCP and NDCP levels for the entire period (1-26 days of age)

Items				
DCP level (%)	DCP source	BWG (g)	FI (g)	FCR
1.75	CDCP	1143[c]	1690[a]	1.48[c]
1.31	CDCP	1020[d]	1559[b]	1.53[b]
0.88	CDCP	922[e]	1467[c]	1.59[a]
1.75	NDCP	1309[a]	1742[a]	1.33[f]
1.31	NDCP	1288[a]	1733[a]	1.35[ef]
0.88	NDCP	1273[a]	1732[a]	1.36[e]
0.44	NDCP	1216[b]	1723[a]	1.42[d]
SE of means		±27.55	±20.52	±0.02
Level (%)*				
1.75		1226[a]	1716[a]	1.41[b]
1.31		1154[b]	1646[b]	1.44[b]
0.88		1097[c]	1599[c]	1.47[a]
Source				
CDCP		1028[b]	1572[b]	1.53[a]
NDCP		1290[a]	1736[a]	1.35[b]
Significant source of variation				
Level		***	***	***
Source		***	***	***
Level×source		***	***	***

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, ***p<0.001, *level of 0.44% NDCP was not including in the two way statistical analysis

The main effects (level and source) showed that the highest dietary level of DCP (1.75%) improved (p<0.001) BWG and FCR by about 5.5 and 4%, respectively, compared to 0.88% DCP level. Source of DCP showed that birds fed NDCP diets gained (p<0.001) 12.6% more body weight and consumed (p<0.01) 1.6% more feed than CDCP groups. Values of FCR enhanced (p<0.001) by 10% for birds fed NDCP diets compared to those fed CDCP diets.

The effect of different dietary CDCP and NDCP levels on performance of broiler chicken from 16-26 days of age is shown in Table 3. Birds fed diets containing 1.75, 1.31, 0.88 and 0.44% NDCP showed significant (p<0.05) improvements in BWG and FCR compared to the control group. The best values of BWG (904, 892 and 891 g) and FCR (1.37, 1.38 and 1.38) were recorded for treatments fed 1.75, 1.31 and 0.88% NDCP, respectively. No significant difference in FI was detected among treatments fed different levels of NDCP and the control group. Treatments fed 1.31 or 0.88% CDCP consumed significant less feed (p<0.05) compared to the control group.

The results of the main effects indicated that both level and source of DCP significantly (p<0.001) affected the measured performance parameters (BWG, FI and FCR). Feeding low levels of CDCP or NDCP resulted in low values of BWG and FI. Also, values of FCR were negatively affected by lowering the dietary level of CDCP. The results also showed the superiority of using NDCP over CDCP. Birds fed NDCP diets gained about 32% more weight and consumed about 14% more feed compared to birds fed CDCP. This improved the values of FCR by about 14%.

The results of performance of broiler chicken fed different dietary CDCP and NDCP for the entire period (1-26 days of age) are shown in Table 4. Birds fed different levels of NDCP gained significant (p<0.05) more body weight and utilized feed more efficiently than the control group. Decreasing levels of CDCP led to decrease in BWG and impaired FCR compared to the control group. No significant differences in FI were detected among birds fed different levels of NDCP, but birds fed low CDCP consumed less feed (p<0.05) compared to the control group. Birds fed different levels of NDCP showed close range in values of BWG and FI. Values of FCR impaired with decreasing dietary level of either CDCP or NDCP. Values of BWG, FI and FCR improved by about 25, 10 and 12%, respectively, for birds fed NDCP compared to those fed CDCP. These results proved that using NDCP improved BWG, FI and FCR effectively compared to CDCP.

The results of enhanced performance observed in the present study by inclusion of NCDP in the diet are in accordance with Vijayakumar and Balakrishnan[23] who found that using 50% calcium phosphate nanoparticles (hydroxyapatite) instead of the conventional dicalcium phosphate in diet led to increased BWG of broiler chicken over the control. The same results were reported for other nano

Table 5: Effect of different dietary CDCP and NDCP levels on excreted calcium and phosphorus of broiler chicken at 26 day of age

Items					
DCP level (%)	DCP source	Excreta (Ca%)	Decrease in Ca% excretion	Excreta (P%)	Decrease in P% excretion
1.75	CDCP	2.70[a]		1.73[a]	
1.31	CDCP	2.17[b]	19.63	1.46[b]	15.61
0.88	CDCP	1.78[c]	34.07	1.26[c]	27.17
1.75	NDCP	2.68[a]	0.74	1.66[a]	4.05
1.31	NDCP	2.14[b]	20.74	1.38[b]	20.23
0.88	NDCP	1.63[c]	39.63	1.11[d]	35.84
0.44	NDCP	1.33[d]	50.74	0.93[e]	46.24
SE of means		±0.11		±0.06	
Level (%)*					
1.75		2.69 [a]		1.70[a]	
1.31		2.15 [b]	20.07	1.42[b]	16.47
0.88		1.70 [c]	36.80	1.18[c]	30.59
Source					
CDCP		2.22		1.48[a]	
NDCP		2.15	3.15	1.38[b]	6.76
Significant source of variation					
Level effect		***		***	
Source effect		NS		**	
Level×Source		***		**	

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, **p<0.01, ***p<0.001, NS: Not Significant (p>0.05), *Level of 0.44% NDCP was not including in the two way statistical analysis

menirals; Liao *et al.*[24], Shi *et al.*[25], Zhou and Wang[26], Cai *et al.*[27], Mohapatra *et al.*[12] and Huang *et al.*[13] concluded that nano-Se could be utilized more effectively than inorganic or organic Se. Furthermore, Cai *et al.*[28] found that the supplementation of nano-Se in broiler diets could improve meat quality, immune function, oxidation resistance and the selenium content of liver and muscles. Ahmadi *et al.*[14], Mishra *et al.*[15], Sahoo *et al.*[29], Mohammadi *et al.*[16] and Swain *et al.*[11] found that the supplementation of zinc oxide nanoparticles in broiler diets improved growth performance during the starter period. Using chromium in nanoparticle size has been reported to show similar results[17].

The improvement in the performance as a result of NCDP could be attributed to the fact that the nano materials are very minute in size, leading to a great improvement in their properties because of higher surface area and increased absorption. Chan *et al.*[30] and Gross *et al.*[31] have reported that calcium phosphate materials in nano-size have higher specific surface area and surface roughness compared to the conventional calcium phosphate. Weiss *et al.*[32] reported that nanoparticle-sized ingredients might increase the functionality or bioavailability of ingredients and nutrients, thereby minimize the concentrations needed in the food product. Calcium phosphate materials in nano-size are expected to have better bioactivity compared to conventional materials because supplementation of minerals in nano form (Se, Cr and Zn) increases the bioavailability and efficiency of utilization by increasing the surface area[33]. The usefulness of nano form in reducing the mineral quantity to half or more in the diet will reduce the cost of feeding when the actual production of calcium phosphate nano-particles is upscaled to an industrial level.

Calcium and phosphorus excretion: The effect of different dietary levels of NDCP and CDCP on calcium and phosphorus excretion of broiler chicks at 26 days of age is shown in Table 5. The Ca and P excretion significantly (p<0.001) decreased with decreasing DCP levels. Birds fed 0.88% CDCP excreted 34.07% less Ca and 27.17% less P while those fed 0.88% NDCP excreted 39.63% less Ca and 35.84% less P than those fed 1.75% CDCP diet. Level of dietary DCP significantly (p<0.001) affected Ca and P excretion while source of DCP significantly (p<0.001) affected P excretion and did not affect (p>0.05) Ca excretion. Feeding 0.44% NDCP decreased the excreted Ca and P by 50.74 and 46.24%, respectively, compared to the control diet.

Addition of dicalcium phsphate in nano form improves the absorption and subsequent reduction in their excretion. Swain *et al.*[11] reported that nano minerals are having a great potential even at very lower doses than the conventional organic and inorganic sources. Nano form of minerals supplementation increases the surface area which possibly could increase absorption and utilization leading to reduction in the quantity of supplements and ultimately reduction in mineral excretion. The growing concerns with regard to the potential contribution of phosphorus in poultry excreta on eutrophication of surface waters has led to increasing pressure to limit the amount of excess phosphorus in poultry ration and thus reduce output of phosphorus[27].

CONCLUSION

Reducing dietary CDCP had a negative effect on BWG and FCR. Meanwhile, using different levels of NDCP did enhance BWG and FCR of broiler chicks compared to CDCP. Birds fed diets contained 0.88 or 0.44% NDCP did perform as well as those fed diet contained 1.75% CDCP. Using NDCP in broiler diets allow successfully to reduce the dietary DCP by 75%. Diet formulated containing only 25% of the required P level in form of NDCP could be used instead of using 100% of the requirements in form of CDCP. Also, using dicalcium phosphate in nanoparticle size allow to reduce the excreted Ca and P by about 50%, which reduce the impact of poultry on environmental pollution.

It could be concluded that nano dicalcium phosphate is having a great potential as performance enhancer in broiler chicken production even at very lower doses than the conventional organic and inorganic sources. Further, using the added minerals to poultry diets in nano-particle size helped in reducing the excretion and subsequent possible environmental issues.

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