ajava

Asian Journal of Animal and Veterinary Advances

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2016.484.490

Research Article
Carcass Characteristics and Bone Measurements of Broilers Fed Nano Dicalcium Phosphate Containing Diets

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Abstract
Background: A broiler experiment was carried to study the effect of using nano dicalcium phosphate (NDCP) compared with the conventional dicalcium phosphate (CDCP) on carcass characteristics and bone measurements. **Materials and Methods:** Seven groups of one day-old (Ross 308) male broilers were fed on seven experimental diets. Diets were formulated to contain three levels of CDCP or NDCP being, 1.75, 1.31 and 0.88% and a less level of NDCP being 0.44%. These levels supplied 100, 75, 50 or 25% of the recommended dietary available P requirement, correspondingly. Diet of 1.75% CDCP served as a control. The Ca: P ratio was kept 2:1 in all the diets. At the 26th day of age, carcass characteristics and tibia bone parameters were measurements. **Results:** No significant differences were detected on liver, heart and gizzard weights (% of live body weight) among all treatments while carcass weight represents live body weight. Using NDCP instead of CDCP showed significant ($p<0.001$) increase in the measured bone parameters. Birds fed 0.44% dietary NDCP showed comparable values of tibia weight, length, width and breaking strength as those fed 1.75% CDCP. The NDCP increased tibia ash, Ca and P% by 4.61, 3.62 and 4.28%, respectively, compared to CDCP. The results of bone mineral density reflected the values obtained for tibia ash, Ca and P%. **Conclusion:** It could be concluded that using NDCP instead of CDCP improved all the measured bone parameters. Diets formulated containing only 25% of the required available P level in form of NDCP could be used instead of using 100% of the requirements in form of CDCP. The dietary dicalcium phosphat level could be successfully decreased from 1.75-0.44% when used in form of nano particle size. Dicalcium phosphate in nanoparticle size was of about 400% as available as the conventional dicalcium phosphate.

Key words: Nano minerals, broiler chicks, carcass characteristics, bone parameters

Received: April 19, 2016 Accepted: June 08, 2016 Published: July 15, 2016

Citation: M.A. Mohamed, H.M.A. Hassan, A. Samy, M.O. Abd-Elsamee and A.E. El-Sherbiny, 2016. Carcass characteristics and bone measurements of broilers fed nano dicalcium phosphate containing diets. Asian J. Anim. Vet. Adv., 11: 484-490.

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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

Phosphorus in feedstuffs of plant origins is largely present in the form of phytic acid and only partly available to poultry[1]. Therefore, supplementation of inorganic P is necessary for meeting bird's requirement for this element. Regarding feed cost and pollution from the excess voiding P, an adequate P supply without excess remains one of the most important issues of broiler nutrition[2]. The more precise the supply of dietary P has to be adjusted to the specific requirement of available P. Phytase enzyme was used to improve dietary P availability and decrease P supplement and can substantially decrease excreted P[3-5]. Attempts had been made in using minerals in nano form which could increase its absorption and utilization leading to reduction in the quantity of supplements and through higher bioavailability[6]. Poinern *et al.*[7] mentioned that materials in nano-size have higher specific surface area and surface roughness compared to conventional materials.

Present study on mineral nutrition focused on reducing the inclusion levels and increasing the absorption of minerals by reducing their particle size in nano forms[8,9]. Swain *et al.*[10] concluded that nano minerals even at very lower doses are having a great potential as mineral feed supplements than the conventional organic or inorganic source. Vijayakumar and Balakrishnan[9] found that supplementation of 50% of calcium phosphate nanoparticles can be used instead of the conventional practice of dicalcium phosphate content in broiler diet. More recently, Hassan *et al.*[11] reported that using NDCP in broiler diets allow successfully to reduce the dietary DCP by 75% without adverse effect upon chick performance.

One of the most critical tests for estimating dietary sufficient and bioavailability of P is bone development[12]. Tibia bone mineralization[13,14], bone breaking strength[15] and bone mineral density[16] were used as response criteria in the evaluation of P availability in different P sources or its adequacy in broilers.

Therefore, the objective of this study was to examine the effect of using different levels of nano dicalcium phosphate on carcass characteristics and different bone measurements of broiler chicks compared to the conventional dicalcium phosphate.

MATERIALS AND METHODS

A sample of nano dicalcium phosphate in particle size being 26 nm, with 100% purity was synthesized by the Sol-Gel method using deionized water as a solvent[17]. The applied method doesn't involve using or production of any toxic or environmentally hazardous solvents, surfactants or organic chemicals and be economical, time-saving and performed at room temperature.

A broiler experiment was carried to study the effect of using NDCP on carcass characteristics and bone measurements. Seven groups of one day-old (Ross 308) male broiler chicks, forty chicks each (4 replicates X 10 birds) were fed seven experimental diets. Diets were formulated to contain three levels of either CDCP or NDCP being 1.75, 1.31 and 0.88% and a less level of NDCP being 0.44%. Thus, these diets contained 100, 75, 50 and 25% of the recommended available P requirement, correspondingly. The diet of 1.75% CDCP (100%) served as a control diet. Diets were formulated to cover all the nutrient requirements of Ross broiler chicks. The Ca:P ratio was kept 2:1 in all the diets. Dietary levels of limestone and DCP along with its content of Ca and available P are shown in Table 1.

Birds were raised in batteries at a warmed brooder house and fed the dietary treatments from 1-26 day of age. Light was provided 23 h daily and feed and water were allowed for *ad libitum* consumption.

At the 26th day of age, 6 birds per treatment with live body weight close to the group average were taken to study the carcass characteristics and bone measurements. Birds were fasted overnight, individually weighed, slaughtered, feathered and eviscerated. Weights of carcass, heart, liver and gizzard were recorded. The percentages of heart, liver and gizzard (% live body weight) for the individual bird were calculated. The right tibia was removed, cleaned of all adhering flesh, extracted with ethanol and then with diethyl ether and oven dried at 105°C for constant weight[19]. Each tibia was weighed and length was measured from its proximal to distal end. Width was recored as described by Vijayakumar and Balakrishnan[9]. The long axis width was measured at almost one cm below the proximal end of the dorsal surface

Table 1: Dietary levels of dicalcium phosphate and limestone

	CDCP (%)			NDCP (%)			
Items	100	75	50	100	75	50	25
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
Calculated composition[18]							
Calcium (%)	0.91	0.74	0.59	0.91	0.74	0.59	0.43
Available P (%)	0.45	0.37	0.29	0.45	0.37	0.29	0.21

(across the flat). The short axis width was measured at almost 1 cm below the proximal end of the lateral surface. The mean value of long and short axis width was the actual width of the tibia.

Radiographic images were taken on tibia to study the effect of different treatments on tibia bone mineral density scans using dual energy x-ray absorptiometry[20]. Tibia breaking strength was measured on apparatus digital force gauge and expressed in kilograms force necessary for bone to be broken[21]. Dried fat-free tibia was ashed and Ca and P content were measured based on the official methods of analysis[22]. Tibia ash, Ca and P were expressed as a percentage of the fat-free dry weight.

Data were statistically analyzed by analysis of variance using the general liner model of SAS[23]. One way analysis of variance was used to detect the treatment effect on carcass characteristics. Two ways analysis of variance (factorial 2×3) was used to detect main effects of source and level of the tested DCP bone measurements. Significant differences among treatment means were separated by Duncan's new multiple range test[24] with a 5% level of probability.

RESULTS

Carcass characteristics: Carcass characteristics including carcass weight and weights of liver, heart and gizzard (% of LBW) of 26 days old broilers as affected by dietary treatments are shown in Table 2. The results of carcass weight followed the same trend as live body weight. The highest carcass weight values were recorded for birds fed the different levels of NDCP while the lowest carcass weight values were recorded for birds fed 0.88% CDCP. Among levels of DCP source, decreasing dietary level of CDCP showed significant (p<0.001) and remarkable decrease on carcass weight.

Among groups fed the different levels of NDCP, slight decrease in carcass weight was observed. No significant differences were detected on liver, heart and gizzard weights (% of LBW) among all treatments.

Bone measurements at 26 days of age: Values of tibia weight (g), length (cm), width (cm) and breaking strength (Kgf) are shown in Table 3. Effect of lowering dietary DCP level varied from source to another (CDCP or NDCP). Decreasing level of dietary CDCP showed significant (p<0.05) decrease in the measured tibia parameters. On the other hand, decreasing dietary level of NDCP from 1.75-0.88 and 0.44% did not affect such parameters. Birds fed 0.44% dietary NDCP showed comparable values of tibia weight, length, width and breaking strength as those fed 1.75% CDCP.

Using NDCP instead of CDCP showed significant (p<0.001) increase in the measured tibia parameters. Tibia weight, length, width and breaking strength increased by 27.42, 9.39, 15.38 and 40.32%, respectively, in birds fed NDCP compared with those fed CDCP.

Table 4 shows that tibia ash and its content of Ca and P%. The results showed that decreasing level of dietary DCP caused significant decrease (p<0.001) on such parameters. These results varied from source of DCP to the other. Remarkable decrease was observed with decreasing level of dietary CDCP and slight decrease was observed with decreasing level of dietary NDCP. Using NDCP instead of CDCP increased tibia ash, tibia Ca% and tibia P% by 4.61, 3.62 and 4.28%, respectively.

Effect of different levels of CDCP and NDCP on tibia Bone Mineral Density (BMD) at 26 days of age is shown in Fig. 1. Decreasing dietary level of CDCP showed decreased on BMD of tibia (Epiphysis and diaphysis). On the other hand, decreasing dietary level of NDCP from 1.75-0.88% did not affect BMD, but birds fed 0.44% dietary NDCP showed slight negative effect. The best BMD obtained in the birds fed 1.75 and 1.31% NDCP.

Birds fed 0.88% dietary NDCP showed comparable BMD as those fed 1.75% CDCP. Birds fed 1.31% dietary CDCP showed almost similar with slight changes of BMD as those fed 0.44% NDCP. The worst BMD was shown in birds fed 0.88% CDCP.

Table 2: Effect of dietary treatments on carcass characteristics (% live body weight) of broiler chicks at 26 days of age

Items						
DCP level (%)	DCP sources	Live body weight (g)	Carcass weight (g)	Liver (%)	Heart (%)	Gizzard (%)
1.75	CDCP	1188[c]	880[c]	2.54	0.61	1.93
1.31	CDCP	1066[d]	768[d]	2.53	0.60	1.94
0.88	CDCP	966[e]	690[e]	2.54	0.59	1.94
1.75	NDCP	1350[a]	1009[a]	2.52	0.59	1.94
1.31	NDCP	1332[a]	991[a]	2.53	0.60	1.95
0.88	NDCP	1343[ab]	979[ab]	2.51	0.59	1.93
0.44	NDCP	1257[b]	933[b]	2.53	0.60	1.95
SE of means		±25.35	±22.17	±0.01	±0.01	±0.03
Significances		***	***	NS	NS	NS

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)

Table 3: Effect of dietary treatments on tibia measurements of broiler chicks at 26 days of age

Items DCP level (%)	DCP source	Tibia weight (g)	Tibia length (cm)	Tibia width (cm)	Tibia breaking strength (Kgf)
1.75	CDCP	4.20[b]	7.33[c]	0.70[ab]	28.38[b]
1.31	CDCP	3.55[c]	6.86[d]	0.67[b]	20.79[c]
0.88	CDCP	3.09[d]	6.57[e]	0.59[c]	18.09[d]
1.75	NDCP	4.63 [a]	7.64[a]	0.75[a]	32.71[a]
1.31	NDCP	4.60[a]	7.55[ab]	0.74[a]	30.93[a]
0.88	NDCP	4.58[a]	7.51[b]	0.74[a]	30.75[a]
0.44	NDCP	4.35[ab]	7.45[b]	0.74[a]	28.51[b]
SE of means		±0.13	±0.08	±0.01	±1.16
Main effects					
Level (%)*					
1.75		4.41	7.49[a]	0.73[a]	30.55[a]
1.31		4.08	7.21[b]	0.71[ab]	25.86[b]
0.88		3.84	7.04[c]	0.67[b]	24.42[b]
Source					
CDCP		3.61[b]	6.92[b]	0.65[b]	22.42[b]
NDCP		4.60[a]	7.57[a]	0.75[a]	31.46[a]
Significant source of variation					
Level effect		NS	***	***	***
Source effect		***	***	***	***
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: Effect of dietary treatments on tibia ash, Ca and P content (%) of broiler chicks at 26 days of age

Items DCP level (%)	DCP sources	Tibia ash (%)	Ca (%)	P (%)
1.75	CDCP	51.69[c]	39.19[c]	19.29[c]
1.31	CDCP	48.72[d]	37.79[d]	17.95[d]
0.88	CDCP	46.62[e]	36.17[e]	16.61[e]
1.75	NDCP	53.85[a]	42.58[a]	24.60[a]
1.31	NDCP	53.77[a]	40.78[b]	21.22[b]
0.88	NDCP	53.26[ab]	40.66[b]	20.88[b]
0.44	NDCP	52.06[bc]	39.50[bc]	20.18[bc]
SE of means		±0.59	±0.46	±0.55
Main effects				
Level (%)*				
1.75		52.77[a]	40.89[a]	21.95[a]
1.31		51.25[b]	39.29[b]	19.58[b]
0.88		49.94[c]	38.41[b]	18.75[b]
Source				
CDCP		49.01[b]	37.72[b]	17.95[b]
NDCP		53.63 [a]	41.34[a]	22.23[a]
Significant source of variation				
Level effect		***	***	***
Source effect		***	***	***
Level×sourse		***	***	***

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

This result of BMD reflected the values obtained for tibia ash, Ca and P% since, the dietary NDCP levels recorded the highest values for tibia ash, Ca and P% compared to the other levels of dietary CDCP.

DISCUSSION

The obtained results of Bone Mineral Density (BMD) supported all the measured bone parameters (tibia weight,

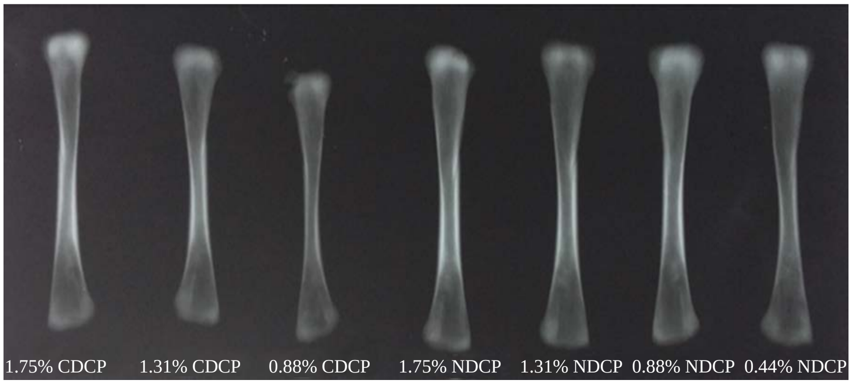

Fig. 1: Radiographic image of the effected of different levels of NDCP and CDCP on tibia bone mineral density scans using dual energy X-ray absorptiometry at 26 days of age

length, width, breaking strength, ash, Ca and P content). Therefore, it could be considered as a unique measurement that gathered the other bone parameters. Onyango *et al.*[16] found high correlation coefficients between ash and bone mineral content, bone mineral density, or breaking strength. They concluded that BMD could be used as an indicator of ash percentage in the tibia of broilers.

Different methods have been reported in assessing bone mineral density. Fleming *et al.*[25] used digitized fluoroscopy and ultrasound, the dual energy X-ray absorptiometry was applied by Hester *et al.*[20] and the quantitative computed tomography method was adopted by Korver *et al.*[26]. Bone mineral density may also be measured using bone mineral composition and breaking strength[16]. Almeida Paz *et al.*[27] described bone mineral density as a biophysical parameter with great experimental and clinical importance that might help to better understand and evaluate the process of bone mineral deposition. The technique of optical densitometry using radiographs could be used for the sequential analysis of the alterations that occur in the bone tissue and is more precise than other techniques. However, using BMD as a parameter to measure or evaluate effects on bone reduced the cost and time required for the evaluation[28]. Bone breaking strength is also a good measure for dietary Ca and P level, as tibia ash for the evaluation of dietary P in growing chicks[15].

The present study proved that, feeding broilers on diets contained NDCP improved all the measured bone parameters. Birds fed diets contained 0.88 or 0.44% NDCP did perform as those fed diet contained 1.75% CDCP with no adverse effect upon carcass characteristics and bone measurements. Formulated diets containing only 25% of the required available P level in form of NDCP could be used instead of using 100% of the requirements in form of CDCP with no significant negative effect upon carcass characteristics and bone measurements. This means that utilization of P in dicalcium phosphate of nano particle size could reach 400% as that in conventional dicalcium phosphate. These results supported those of Vijayakumar and Balakrishnan[9] who reported that the bioavailability of calcium phosphate nanoparticle is 200% compared to conventional dicalcium phosphate.

The remarkable results nano material may be explained as the nano materials is ultrafine in size led to a great improvement in the properties attributed to the higher surface area caused increase absorption and reactivity. Weiss *et al.*[6] reported that nanoparticle-sized ingredients might increase the functionality or bioavailability of ingredients and nutrients and there by minimize the concentrations needed in the food product. Poinern *et al.*[7] reported that the advantages of synthetic calcium phosphate materials in nano-size have higher specific surface area and surface roughness compared to conventional calcium phosphate materials.

The results proved that P in nano particle size is much more bioavailable and utilized for birds than the conventional form and thereby minimize the concentrations needed in the diet. On this regard, Chan *et al.*[29] and Gross *et al.*[30] explained that calcium phosphate materials in nano-size have higher specific surface area and surface roughness compared to conventional calcium phosphate materials. Therefore, nano-sized calcium phosphate materials have stronger interaction with organic materials.

The same results were reported for nano-Se[31-33], nano-zinc[10,34,35] and nano-Cr[36]. Rajendran[37] reported that supplementation of mineral (Se, Cr and Zn) in the form of nano increases bioavailability and efficiency of utilization by increasing the surface area.

CONCLUSION

It could be concluded that nano minerals are having a great potential as feed supplements at very lower doses than the conventional sources. The P in dicalcium phosphate of nano particle size could reach 400% as that in conventional dicalcium phosphate. Also, using the added minerals to poultry diets in nanoparticle size allow to reduce the excreted minerals which reduce the impact of poultry production in environmental pollution.

ACKNOWLEDGMENT

This study is a part of the Internal Research Project No.10120502 of NRC, Egypt.

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