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Impact of Dietary Calcium Nano-Particles on Productive Performance, Carcass Yield, Blood Parameters, Immune-Status and Tibia Structure Of Broiler Chicks

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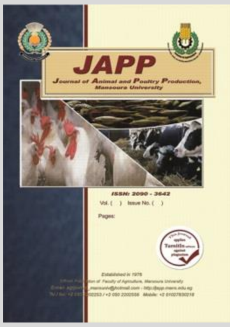

ABSTRACT

The present study was conducted to evaluate the possible effects of feeding diets enriched with calcium nanoparticles (CaNPs) on growth performance, carcass characteristics, some blood parameters and bone structure of broiler chickens. A total number of 300 unsexed day-old broiler chicks (Ross 308) were randomly and equally distributed into six groups, each of five replications. The first group was fed the basal diet and served as a control group while the 2nd, 3rd, 4th, 5th and 6th groups were fed the basal diet enriched with 0.25, 0.50, 0.75, 1.0 and 1.5g of CaNPs/kg diet, respectively. The dietary supplementation of different levels of CaNPs in the diet had a positive effect ($P \leq 0.05$) on the final LBW and total BWG of broiler chicks at 42 days of age compared to the control group, but the best results was achieved by the group fed the diet supplemented with 1.5 g/kg of the CaNPs. On the other hand, dietary supplementation of CaNPs had no significant effect on TFI and TFCR of chicks during the whole experimental period compared to control group. On the other hand, the added CaNPs at a level of 0.50 g/kg led to significantly higher levels of IgA than the other groups. While dietary supplementation with CaNPs at a level of 0.25 g/kg resulted in significantly lower level of LDL-C compared with the other groups. Thus, dietary supplementation of CaNPs can be safely used for broiler chickens without any adverse effects on their growth performance, blood parameters and bone structure.

Keywords: Broiler Chickens, Ca Nano-particles, Performance, Carcass Characteristics, Blood Parameters, Immune Status, Tibia Structure.

INTRODUCTION

The nanotechnological applications in poultry have received increasing interest because of the continuous research for alternative sources of macro- and micro-minerals that could be more efficiently used in this field. Calcium nanoparticles and their application in poultry production have also become a hot topic of research in recent years (Ganjigohari *et al*., 2018). The modern broiler chicken industry is linked with various skeletal disorders. The fast-growing birds with increased weight gain often have leg problems which leads to economic losses. The intact bone development of broilers is highly correlated with the calcium and phosphorus ratio, so providing these macro elements with diet seemed to be fundamental. The common inorganic sources of these two minerals such as limestone, mono- and di-calcium phosphates are commonly used in poultry diets nowadays. The doses used in the feed of broilers are in the range of 6–6.5 g/kg for Ca and 2–3.5 g/kg for P, depending on the supplementation of exogenous microbial phytase. However, the bioavailability of inorganic sources of .... is poorer than organic sources (Add a reference). This fact is important with regard to their impacts on the environment. Because of the continuous search for alternative sources of calcium or phosphorus, with better bioavailability due to their size, the scientific area of nanotechnology triggers increasing interest. It is well-known that nanoparticles have great potential even at very low doses. Some researches on Ca and P have demonstrated no negative effects on birds' health but improved productivity and bone quality. This provides an opportunity to use lower doses of their nano sources leading to decreasing excreta contents of Ca and P (about 50%). Thus, this aspect may be the new trend during the next years (Matuszewski *et al*., 2020).

Despite the benefits of nanotechnology, it also introduces a new order of health risks. Greater chemical reactivity and bioavailability of nano-materials might result in greater toxicity of nanoparticles compared to the same unit of mass of larger particles of the same chemical composition (Oberdorster *et al*., 2005). Though calcium phosphate nanoparticles are used as adjuvant in drug delivery, their effect on oral intake has not been evaluated. The addition of Ca and P in the form of nanoparticles is considered a strategy to lower the feed cost (Vijayakumar and Balakrishnan, 2014 a,b). Nanotechnology is gaining ground in veterinary and animal sciences by supplying minute scales that are vital for living organisms. The application of minerals in nano forms has showed an improvement in feeding efficiency, a decrease in losses from animal diseases, and conversion of animal by-products into value-added products (Chen and Yada, 2011). Nanomaterials have potential in a range of environmental applications owing to their extremely small particle size, large surface area, and high reactivity (Huang *et al*., 2015).

Due to the continuous search for alternative sources of macro-minerals, which may be more efficiently used by birds due to, for example, their size, nanotechnology has aroused increasing interest. A general aim was to reduce the

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use of large amounts of selected sources of elements through the application of more highly bioavailable forms due to effective absorption (Gonzales-Eguia *et al*., 2009). The present investigation aimed to evaluate the influence of different levels of calcium nanoparticles (CaNPs) on growth performance, carcass yield, blood status, immunity status, antioxidant status and bone structure of broiler chickens.

MATERIALS AND METHODS

The current experimental work was conducted from April to May 2022 at the Poultry Production Farm, Center of Agricultural Research and Experiments, Faculty of Agriculture, Mansoura University, Egypt. The main goal of study was to determine the effects of dietary supplementation of calcium nanoparticles (CaNPs) on growth performance, carcass yield, immunity status, lipid peroxidation and bone structure of broiler chickens.

Chemical Synthesis of Calcium Nanoparticles:

The CaNPs were synthesized in an ecofriendly manner using a previously described method (Yugandhar and Savithramma, 2013), with slight modifications, then it was added to the previously prepared algae extract. An aqueous solution of 0.05 mol calcium chloride dehydrate (SD Fine-Chem Ltd., Mumbai, India) was prepared using deionized water and added slowly to the same volume of the prepared extract. The reaction mixture was stirred at 5,000 rpm for 1 h at 25°C ± 1°C and incubated at room temperature for 2 to 3 days. Then, the mixture was lyophilized to a fine powder (Abo El-Maaty *et al*., 2021).

Nanoparticle Characteristics via UV-Vis Spectroscopy:

Unadulterated Ca particle reduction and topping of the subsequent calcium nanoparticles were observed using an ATI Unicam UV-Vis Spectrophotometer Vision software V 3.20 by recognizing the UV-Vis spectra of the mixture at various wavelengths. The UV-Vis spectra of the combined metal nanoparticles were recorded around 240 to 440 nm based on a previously described method (El-Refai *et al*., 2018). The examination was practiced at 25°C using quartz cuvettes (1 cm optical path).

Nanoparticle Characteristics via Transmission Electron Microscope:

The size, shape, surface area, crystal structure, and morphological data of the obtained nanoparticles were characterized using a transmission electron microscope (TEM; JEOL TEM-2100) attached to a CCD camera at an accelerating voltage of 200 kV. Each sample of the synthesized metal nanoparticles was prepared by mounting a suspension of the sample on copper-coated carbon grids, and the solvent was allowed to evaporate slowly before recording the TEM images. The TEM measurements were recorded at the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Egypt.

Nanoparticle Characteristics via Zeta Potential:

Zeta potential examination is a method for determining the surface charge of nanoparticles in suspensions using the zeta potential software ver. 2.3 (Malvern Instruments Ltd.) at the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University (Abo El-Maaty *et al*., 2021)..

Birds, Management and Experimental Design:

Ross 308 broiler chicks (n = 300), at one day of age, had been divided into six treatment groups, with five equal replications in each group. Each experimental group was kept in a compartment of a battery cages and fed its respective diet for 6 weeks. The groups were assigned to six dietary treatments: group one (control) was fed on the basal diet, groups two, three, four, five and six were fed on the basal diet enriched with 0.25, 0.50, 0.75, 1.00 and 1.50 g/kg CaNPs, respectively. Battery cages measuring 70, 60 and 40 centimeters in length, width and height were used to raise the birds. During the first week of the experiment, the farm's daily temperature was 32°C. After that, it was decreased progressively to a range of 30 to 28°C at the start of the second week, and it was maintained at 18 to 24°C at the beginning of the third week until the end of experiment. The experiment was conducted with a photoperiod of 23hL: 1h D. The chickens were raised to 42 days of age, during which time they were fed a starter diet (3071 kcal of ME/kg of diet and 23.11% CP) during the first three weeks of life and a grower diet (3013 kcal of ME/kg of diet and 20.16% CP) from 21 to 42 days of age.(Table 1). According to NRC (1994), diets were designed to satisfy or exceed the required nutrients of broiler chicks. Water and mash feed were given *ad lib*.

Table 1. Ingredient composition and calculated analysis of the basal starter (SD) and grower diets (GD) used in this study

Ingredients (%)	SD	GD	Calculated Analysis		
			Nutrients	SD	GD
Yellow corn	60.3	67.3			
Soybean meal 44% CP	19.3	19.00	ME, kcal/kg	3071.14	3013
Corn gluten meal60%CP	14.2	9.00	CP, %	23.11	20.16
Dicalcium Phosphate	1.8	108	EE, %	2.80	2.93
Limestone	2.0	108	CF, %	2.87	2.92
DL-methionine	0.1	0.1	Calcium, %	1.22	1.148
L-Lysine	0.5	0.4	Non-phytate P %	0.457	0.454
Sodium chloride	0.3	0.3	Methionine, %	0.54	0.473
Vit. + Min. Premix1	0.3	0.3	Meth, + Cys., %	0.934	0.818
Vegetable oil	0.1	0	Lysine, %	1.32	1.178
Total	100	100			

****Composition of vitamin and minerals premix. Each 3Kg of premix containing : 15000000 I.U.vit, A, 50 g. vit E, 3000 mg.vit.K33000 mg B1, 8000mg. Vit.B2 4000mg, vit.B6, 20mg vit. B12, 15000mg. pantothenic acid, 60000 mg. Niacin, 1500 mg. Folic acid, 200mg. Biotin, 200000 mg vitc, 700mg. choline choloride, 80gm Mn, 80g. Zn, 60gm, Iron, 10gm. Cu, 1gm. Cu, 1gm. Iodine and 0.2gm selenium, where Ca Co3 was taken as a carrier up to 3kg, the inclusion rate was 3kg premix/ton feed**

*****calculated analysis of the experimental diets were done according to (NRC, 1994).**

Performance of Broiler chickens:

During the whole period of study, the weekly measurements of live body weight (LBW), feed intake (FI), and body weight gain (BWG) were recorded for the broiler chickens. Then, the feed conversion ratio (FCR) was calculated as the proportion of feed to gain in grams. Birds in each replication were weighed in the early morning before getting any food or water at the start of the trial (day-old), and then at weekly intervals. During the whole period of study, deaths and medical conditions were visually observed and recorded daily.

Carcass Traits of Broiler chickens:

Upon completion of the study at 42 days of age, five birds from each treatment were selected at random for slaughter. Subsequently, the carcass, liver, gizzard, heart, and lymphoid organs (spleen and bursa of Fabricius) were gathered and individually weighed. The relative weights of these organs to the preslaughter LBW was then calculated.

Blood Biochemical Parameters:

During slaughtering, five blood samples were collected in non-heparinized test tubes from each treatment. The sera from the blood samples were obtained by centrifuging at 4000 rpm for 15 minutes, and they were then stored at -20°C for analysis. According to the instructions provided by the manufacturers, commercial kits were used to analyze serum contents of blood biochemical parameters calorimetrically. These blood serum parameters included: total protein (TP), Albumin (Alb), globulin (Glo), immunoglobulins (IgG, IgA and IgM), uric acid (UA), triglycerides (Tri), total cholesterol (Cho), high-density-lipoprotein cholesterol (HDL-C), low-density-lipoprotein-cholesterol (LDL-C), hemagglutination inhibition test for Newcastle disease (HIND), hemagglutination inhibition test for avian influenza (HIAI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD), thyroid hormones (T3 and T4) and corticosterone (COR).

Specimen Preparation of Tibia Bone for Scanning Electron Microscopy:

Preface: The method described here is an example for fixation, contrastation the tibia. For this reason only very small piece of tissue (~ 0.5 mm³) should be fixed if no perfusion fixation (fixative is directly infused into a larger vessel of a deeply anaesthized animal) should be possible. The preparation should be quick enough, i.e. 5 minutes after tissue does no longer receive oxygen, it began to show first signs of degeneration of ultrastructures.

Method:

1- 2.5 % buffered leave tissue overnight at 4° C
- glutaraldehyde + 2 % paraformaldehyde
- in 0.1 M sodium phosphate buffer pH 7.4
- wash 3 x 15 minutes (min.) in 0.1 M sodium phosphate buffer + 0.1 M Sucrose
- postfix 90 min. in 2 % sodium phosphate buffered osmium tetroxide pH 7.4
- wash 3 x 15 min in 0.1 M sodium phosphate buffer pH 7.4
- dehydrate 2 x 15 min: 50 % ethanol (in distilled water)
- contrast overnight using 70% acetone + 0.5% uranyl acetate + 1% phosphotungstic acid at 4°C
- 2 x 15 min. 80 % ethanol
- 2 x 15 min. 90 % ethanol
- 2 x 15 min. 96 % ethanol
- 3 x 20 min. 100 % ethanol
- Put the specimens were coated with gold-palladium membranes and observed in a Jeol JSM-6510 L.V SEM, The microscope was operated at 30 KV at EM Unit, Mansoura University, Egypt.

Statistical Analysis:

The data obtained underwent statistical analysis through the application of one-way analysis of variance (SAS, 2006). To identify significant differences between means, the Tukey multiple range test was utilized (Tukey, 1977).

RESULTS AND DISCUSSION

Growth performance of broiler chickens:

The effects of dietary supplementation of CaNPs on the production performance of broiler chickens are given in Table 2. In the present study, supplementation of different levels of nano-calcium in the diet had a positive effect ($P \leq 0.05$) on the final LBW and total BWG of broiler chicks at 42 days of age compared to the control group, but the best treatment when the chicken fed the diet contained 1.5 g/kg from the CaNPs. On the other hand, supplementation of nano-calcium at different levels had no significant effect ($P \geq 0.05$) on total feed intake and total feed conversion ratio of chicks during the whole experiment period(0-42 days of age) compared to control groups. The results of enhanced broiler performance in the present study are in agreement with those obtained by Vijayakumar and Balakrishnan (2014) who observed that using calcium phosphate nanoparticles (hydroxyapatitein) at levels of 50% instead of the conventional dicalcium phosphate in broiler chick diets increased body weight gain compared with control.

Similarly, Sohair*et al.*, 2017results showed significant improvements in body weight gain (BWG) by 16%, 11.1% ,7.4 and 4.5% and feed intake (FI) by 11.7%, 10.15, 5.7% and 2.9 % for treatments fed 6%, 8% , 4% and 10% Nano-hydroxyapatite, respectively compared to the control group. The best values of BWG and FI were recorded for treatment fed 6% followed by that fed 8% Nano-hydroxyapatite. No significant differences in feed intake values due to fed broiler chicks on 2% Nano hydroxyapatite which recorded significance compared with the control group. Also, there were no significant effects in FCR values due to different dietary levels of Nano-hydroxyapatite. Furthermore, Mishra *et al.* (2019) observed a non-significant difference in the body weight of broiler fed either 0.42% or 0.85% of Ca-P NPs of the diet (51 nm to 200 nm) compared to the control (100% DCP). Matuszewski*et al.* (2020) stated that in the case of broiler chickens, the concentration, source, or particle size of Cahas a lesser effect on their production results. Also, it could be explained that the conventional/inorganic DCP had a low bioavailability.

Table 2. Effect of feeding CaNPs-enriched diets on broiler performance of broiler chicks from1-42days of age.

Treatments:	LBW Day old	LBW 42 Days old	TBWG 1-42 days old	TFI 1-42 days old	TFCR 1-42 days old
Ca NPs (0.00 g/kg)	41.90	2069b	2026ab	3384	1.67
Ca NPs (0.25 g/kg)	42.30	2145ab	2102ab	343.4	1.64
Ca NPs (0.50 g/kg)	41.80	2040b	1998b	3406	1.70
Ca NPs (0.75 g/kg)	41.80	2180ab	2138a	3448	1.61
Ca NPs (1.00 g/kg)	41.70	2193ab	2147ab	3479	1.62
Ca NPs (1.50 g/kg)	42.30	2244a	2197a	3505	1.59
Pooled SEM	0.20	36.50	36.40	42.14	0.02
P-value	0.19	0.004	0.005	0.35	0.13
Significance	NS	**	**	NS	NS

Means in the same column bearing different superscripts differ significantly at P ≤0.05. LBW: Live body weight, TBWG: Total body weight gain, TFI: Total feed intake and TFCR: Total feed conversion ratio. SEM: Standard error of the means. CaNPs: Calcium nanoparticles. NS: Not significant. **: Significant at P≤0.01.

On the other hand, the small size (ranging from 20 to 100 nm) and the larger surface area of NHA probably increased bioavailability and gave the same results as the control group. Nanometric forms of minerals have a high potential to support growth at lower doses compared to conventional organic or inorganic sources of minerals, including CaSwain *et al.*, 2015. The beneficial effects of feeding Ca NPs on broiler chicken's performance were also observed byMakola*et al*.,2021found that the final weight gain (kg) and feed conversion ratio of birds were affected by the treatments. Birds fed 40% nano$CaHPO_4$ had the highest final weight, which was greater than those not supplemented with nanoCaHPO4. The birds fed the other diets had final weights that were intermediate between these extremes. Birds fed 40% nanoCaHPO4 werethe most efficient, whereas those fed 80% nanoCaHPO4 had the greatest feed conversion ratio.The current study's findings on live body weight and body weight gain throughout the finisher phases contrast with those ofSobhi*et al*., 2020showed that the final body weight and BWG were lower (P≤0.05) in chickens fed diet (1% inorganic dicalcium phosphate (DCP) plus 0.1% nano-hydroxyapatite (NHA)) or diet (0.5% DCP plus 0.1 %NHA) but were similar (P>0.05) to those fed the diet (0% DCP) compared to those fed the control diet. The feed consumption was similar (P>0.05) among all treatments, but the FCR was higher (P≤0.05) in chickens fed diet (0.5% DCP plus 0.1 %NHA than those fed the other treatments.

Carcass characteristics of broiler chickens:

Table 3 shows the effects of dietary supplementation of nano-calcium at different levels on the carcass characteristics and percentage of lymphoid organs in broiler chicks (42 days old). The percentage of the carcass, head, livergizzard, spleen,heart, and giblets of broilers fed different levels of nano-calcium (0.0, 0.25, 0.50, 0.75, 1.0 and 1.5 g/kg) were not significant between-group nano-calcium. Showed no significant differences in the average values of differences detected on liver, heart and gizzard weights (% of LBW) among all treatments. These results are consistent with Sohair*et al.* (2017), Vijayakumar and Balakrishnan (2014), and Hassan *et al.* (2016). Likely, Sobhi*et al.*, 2020 found no significant differences in the carcass traits of birds fed diets supplemented with nano-hydroxyapatiteas a source of calcium and phosphorous (P) or alternative to inorganic dicalcium phosphate.The non-significant difference in dressing percentage among treatments indicates that the intervention made in feed did not negatively influence the metabolism. Similarly, Vijayakumar and Balakrishnan (2014) reported no significant difference in dressing percentage, heart weight, and liver weight by Ca-P NPs supplementation. Also, Mishra *et al.* (2019) observed that there was no significant difference in dressing percentage, breast, thigh, and drumstick yield with nanodicalcium phosphate supplementation. On the contrary, Sohair*et al.* (2017) stated that the highest carcass weight values were recorded for birds fed 6 and 8% nano-hydroxyapatite at 6 weeks of age, compared to the control group. This variation among these studies might be due to the difference in dietary treatment and the duration of the experiments.

Table 3. Effect of feeding CaNPs-enriched diets (g/kg) on carcass characteristics of 6-wk-old broiler chickens.

Treatments: Added CaNPs	LBW (g)	CY (%)	LIV (%)	HEA (%)	GIZ (%)	SPL (%)	GIB (%)	Head (%)
Tr. 1: 0.00	2404	74.91	2.83	0.64	1.50	0.13	5.11	4.50
Tr. 2: 0.25	2596	74.10	2.96	0.53	1.54	0.13	5.17	4.28
Tr. 3: 0.50	2355	73.06	3.15	0.70	1.45	0.10	5.41	4.87
Tr. 4: 0.75	2516	72.21	3.07	0.62	1.57	0.11	5.39	4.64
Tr. 5: 1.00	2680	72.66	3.13	0.63	1.70	0.12	5.60	4.66
Tr. 6: 1.50	2527	74.38	2.97	0.61	1.77	0.11	5.48	4.71
Pooled SEM	107.3	0.74	0.11	0.04	0.15	0.01	0.21	0.17
P-value	0.31	0.10	0.36	0.33	0.67	0.72	0.56	0.28
Significance	NS	NS	NS	NS	NS	NS	NS	NS

Means in the same column bearing different superscripts differ significantly at P ≤0.05. LBW: Live body weight, CY: Carcass yield, LIV: Liver. HEA: Heart. GIZ: Gizzard. SPL: Spleen. GIB: Giblets. SEM: Standard error of the means. CaNPs: Calcium nanoparticles. NS: Not significant.

Blood serum parameters: -

Results of serum parameters (total protein, albumin, globulin, immunoglobin G, uric acid, triglycerides, cholesterol, HDL, LDL, HIND, HIAI, ALT, AST, alkaline phosphatase, antioxidant status, thyroid hormones, and corticosterone)for the different experimental groups of broiler chickens fed diets supplemented with different levels of Ca NPsare illustrated in Tables4 and 5.

Table 4. Effect of feeding CaNPs-enriched diets on blood serum parameters of 6-wk-old broiler chickens.

Blood criteria	Treatments: Levels of added CaNPs (g/kg)						Pooled SEM	P-Value	Sig.
	0.00	0.25	0.50	0.75	1.00	1.50			
TP, g/dL									
Alb, g/dL	2.55	2.62	2.67	2.55	2.60	2.80	0.12	0.70	NS
Glo, g/dL	2.87	2.87	2.90	3.39	2.95	2.55	0.19	0.12	NS
IgG	994.5	995.7	1005	998.0	1011	991.2	4.30	0.36	NS
IgM	252.6	260.5	251.0	239.1	254.5	252.3	9.25	0.71	NS
IgA	81.9^{ab}	80.5^{ab}	87.9^{a}	78.8^{ab}	74.3^{b}	74.3^{b}	2.15	0.001	**
UA, mg/dL	3.06^{bc}	2.96^{c}	2.72^{c}	2.68^{c}	3.61^{b}	4.60^{a}	0.14	0.0001	**
Tri, mg/dL	180.5	182.0	177.8	178.1	175.3	175.3	3.34	0.65	NS
Cho, mg/dL	207.7	206.3	209.9	214.6	211.3	211.3	3.51	0.62	NS
HDL-C, mg/dL	38.48	39.88	39.41	40.85	39.15	39.15	1.54	092	NS
LDL-C, mg/dL	90.48^{ab}	86.88^{b}	90.68^{ab}	94.97^{ab}	99.15^{a}	99.15^{a}	2.08	0.001	**
HIND	3.20	3.60	2.80	3.20	3.20	3.00	0.39	0.80	NS
HIAI	2.20^{b}	3.60^{a}	4.20^{a}	3.00^{ab}	3.00^{ab}	3.20^{ab}	0.28	0.001	**
ALT	64.26	67.97	65.81	66.68	66.43	66.60	1.16	0.38	NS
AST	58.73	54.18	57.75	59.21	56.11	55.88	1.36	0.12	NS
ALP	7.92	7.76	7.64	7.42	7.50	7.47	0.15	0.21	NS
TAC	425.96	427.42	421.66	426.38	425.82	422.44	3.07	0.72	NS
MDA	1.06	1.10	1.06	1.08	1.12	1.04	0.05	0.91	NS
SOD	3139	3121	3135	3125	3130	3124	4.90	0.09	NS
T3	0.19	0.18	0.15	0.17	0.15	0.18	0.01	0.38	NS
T4	8.08	8.08	7.30	7.78	7.62	7.83	0.18	0.04	*
COR	3.16^{b}	3.34^{ab}	3.80^{ab}	4.35^{a}	4.01^{ab}	4.16^{ab}	0.23	0.008	**

Means in the same rows bearing different superscripts differ significantly at P ≤0.05. SEM: Standard error of the means. CaNPs: Calcium nanoparticles. NS: Not significant. **: Significant at P≤0.01.

Table 5. Effect of feeding CaNPs-enriched diets on tibia structure of 6-wk-old broiler chickens.

Tret.	Ca NPs (0.0 g/kg)		Ca NPs (0.25g/kg)		Ca NPs (0.50g/kg)		Ca NPs (0.75g/kg)		Ca NPs (1.0g/kg)		Ca NPs (1.5g/kg)	
	1	1	2	2	3	3	4	4	5	5	6	6
Elem.	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%
C K	62.06	69.86	43.80	55.31	61.12	67.92	60.83	68.34	63.81	65.83	64.81	67.38
O K	33.33	28.16	39.51	37.46	37.84	31.57	36.10	30.44	33.14	32.69	32.12	31.40
Na K	1.36	0.80	0.92	0.60	0.49	0.28	0.54	0.31	0.51	0.30	0.56	0.32
Si K	0.22	0.11	-	-	-	-	0.22	0.11	0.25	0.10	0.22	0.12
P K	1.16	0.51	6.12	3.00	0.27	0.12	0.74	0.32	0.72	0.35	0.73	0.30
S K	0.34	0.14	-	-	0.18	0.07	0.24	0.10	0.27	0.12	0.29	0.11
Ca K	0.88	0.30	9.42	3.57	0.11	0.04	0.89	0.30	0.88	0.33	0.87	0.28
Fe K	0.17	0.04	0.24	0.07	-	-	-	-	-	-	-	-
Br L	0.48	0.08	-	-	-	-	0.44	0.08	0.42	0.28	0.40	0.09
Totals	100.00	100.0	100.00	100.0	100.00	100.0	100.00	100.0	100.00	100.0	100.00	100.0

Feeding diets with differentlevels of Ca NPs had no significant effect on albumin, globulin, IgA, IgM, triglycerides, cholesterol, HDL, HIND, HIAI, ALT, AST, alkaline phosphatase, antioxidant status, thyroid hormones and corticosterone among all groups. However significantly lower serum levels oftotal protein for broilers supplementation nano-calcium at levels (0.0 and 1.5 g/kg compared with the group adding 0.75 g/kg nano-calcium but there's no significant between other groups. On the other hand, the nano-calcium at level 0.50 g/kg supplement group had significantly higher levels of IgA than the other groups. While the nano-calcium at level 0.25g/kg group had a significantly lower level of LDL compared with other groups, but the nano-calcium at level 1.5 g/kg group had a higher significance of uric acid compared with other groups. On the other side the control group (0.0) had lower significance of corticosterone compared with nano-calcium treated. The present study are in agreement with those obtained byVijayakumarand Balakrishnan, 2015 found that the supplementation of calcium phosphate nanoparticles from 50 to 100% did not have any significant negative influence on the serum glucose, total protein, albumin, cholesterol, serum urea, creatinine17, triglyceride, serum Aspartate amino Transferase (AST), Alanine amino Transferase (ALT) and

Alkaline Phosphatase (ALP).Non-significantly different alkaline phosphatase values were within the normal range of 568–8831 U/L (Meluzzi *et al.*, 1992).

Effects of nano-calcium-treated diets on the tibia structure.

One of the most crucial parameters for determining the quality of bones is bone mineral density. It is a biophysical measure of crucial experimental significance that has been applied to the assessment of because it's a dependable and non-invasive way for measuring bone quality. In order for vertebrates to grow and develop normally, their bones are important. Bone formation is the result of a complex series of interconnected processes that occur during its development in both space and time. The results of tibia mineral profile had shown in table (5). The tibia mineral profile had critical difference between the level of Ca-NPs supplementation up to 1.5g/kg diet. Through the results shown in Table 6, an improvement was found in the tibia health status of birds fed calcium –nanoparticles treated diets. The highest tibia-CaK and PK weight % were recorded for birds fed 0.25 g Ca NPs/kg diet compared with the control group. It became clear that the density of mineral elements in the tibia bone was higher in the treatments with an increase in the level of adding nano-calcium to the diet, and the best results were associated with the treatment fed with a diet containing 1.0g Ca-NPs /kg diet. From our results, the best calcification of the tibia had for treatment fed deferent levels of Ca-NPs.

These findings corroborated those of Vijayakumar and Balakrishnan (2015, (2014 a and b)), who found that calcium phosphate nanoparticles have a 200% higher bioavailability than regular dicalcium phosphate. The amazing results obtained from the nanomaterial can be explained by the fact that its ultrafine size led to a significant improvement in the characteristics attributable to the increased reactivity and absorption induced by the higher surface area. According to Weiss et al. (2006), substances with nanoparticle sizes may improve the nutrients' and ingredients' bioavailability, reducing the amount of each ingredient required in the food product. In comparison to conventional calcium phosphate materials, Poinern *et al.* (2009) found that the advantages of synthetic nano-size calcium phosphate materials include increased specific surface area and surface roughness. The findings demonstrated that P in nanoparticle form is significantly more bioavailable to birds and used by them than P in conventional form, reducing the amount of P that must be consumed in diet. Regarding this, Chan *et al.* (2011) and Gross *et al.* (2014) clarified that, in comparison to ordinary calcium phosphate materials, nano-sized calcium phosphate materials had a larger specific surface area and surface roughness. Consequently, the contact between calcium phosphate minerals in nanoscale form and organic components is stronger.

CONCLUSION

We can conclude that, at lower levels than the typical sources, CaNPs have a great potential as feed supplements. Thus, dietary supplementation of CaNPs can be safely used for broiler chickens up to 1.5 g/kg diet with no adverse effects on productive performance, blood parameters and bone structure.

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تأثير جزيئات الكالسيوم النانوية الغذائية على الأداء الإنتاجي وخصائص الذبيحة ومؤشرات الدم والحالة المناعية والتركيب البنائي لعظمة الساق في كتاكيت اللحم

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الملخص

أجريت الدراسة الحالية لتقييم التأثيرات المحتملة لإثراء علائق كتاكيت اللحم بجزيئات الكالسيوم متناهية الصغر (Ca NPs) على أداء النمو وخصائص الذبيحة وبعض مؤشرات الدم وتركيب العظام في كتاكيت اللحم. تم استخدام عدد 300 كتكوتا غير مجنس عمر يوم (Ross 308). تم توزيع هذه الكتاكيت عشوائياً وبشكل متساوٍ على ستة مجاميع تحتوي كل منها على 50 كتكوتا، في خمس مكررات. تم تغذية المجموعة الأولى على العليقة الأساسية بينما تم تغذية المجموعات الثانية والثالثة والرابعة والخامسة والسادسة على العليقة الأساسية المدعمة بـ 0.25، 0.50، 0.75، 1.0 أو 1.5 جرام من Ca NPs / كجم علف على التوالي. كان لتدعيم الغذاء بالمستويات المختلفة من بجزيئات الكالسيوم متناهية الصغر تأثير إيجابي (P<0.05) على وزن الجسم النهائي لكتاكيت اللحم وإجمالي الزيادة المكتسبة في الوزن عند عمر 42 يوماً مقارنة بمجموعة المقارنة، وحققت المجموعة المغذاة علي العليقة المدعمة بـ 1.5 جم/كجم من Ca NPs أفضل أداء إنتاجي. بينما لم يكن لتدعيم الغذاء بالمستويات المختلفة من بجزيئات الكالسيوم متناهية الصغر تأثير معنوي على إستهلاك الغذاء الكلي ومعامل التحويل الغذائي الكلي للكتاكيت خلال فترة التجربة بأكملها مقارنة بمجموعات السيطرة. كما أظهرت النتائج أن تدعيم العلائق بجزيئات الكالسيوم متناهية الصغر بمستوى 0.50 جم/كجم معدل عالي من الجوبيولين المناعي IgA مقارنة بالمجموعات الأخرى. بينما كان أظهرت المجموعة المعاملة بالمستوى 0.25 جم/كجم تركيز أقل من الكوليستيرول الليبوبروتيني منخفض الكثافة مقارنة بالمجموعات الأخرى. وبالتالي، يمكن استخدام بجزيئات الكالسيوم متناهية الصغر بأمان دون أية تأثيرات سلبية على الأداء الإنتاجي، ومؤشرات الدم والبنية العظمية للكتاكيت اللحم.