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Impact of nanocalcium phosphate on layers' productivity, bone traits, blood biochemical parameters, and gene expression

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Abstract

This experiment examined the effects of substituting nano calcium phosphate (NCaP) for conventional dicalcium phosphate (DCP) on laying hen productivity, health, bone traits, gene expression of intestinal and kidney P co-transporters (NaPi-IIa and NaPi-IIb), and gene expression of Ca calbindin in the duodenum and jejunum. At 27 weeks of age, 500 Lohmann Brown laying hens were divided into five equal groups and given different diets. One group received a diet that included 100% DCP (T1), while the other four groups received diets that replaced the DCP with 100, 75, 50, or 25% NCaP, respectively. The highest egg production, weight, and feed consumption were seen in birds fed 50% NCaP ($P \leq 0.05$). In contrast, adding NCaP at various amounts outperformed the control. Diet groups with NCaP at different doses exhibited thicker, heavier, and more proportional eggshells than the control group ($P \leq 0.05$). The best egg and eggshell calcium retention was in 50% NCaP-fed hens. More particularly, NCaP groups increased tibia breaking strength and weight, with 50%NCaP having the highest results. Although NCaP reduced phosphorus excretion by increasing NaPi-IIa expression in the kidney's proximal tubule epithelial cells at their most apical brush edge. The most significant ($P \leq 0.05$) gene expression of Calbindin and NaPi-IIb in the duodenum and jejunum was seen in the 50% NCaP group. The inclusion of NCaP improved egg quality and productivity. Gene expressions with calcium and phosphorus retention data show that NCaP-fed birds had higher bioavailability and lower environmental impact. The best was 50% NCaP.

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Introduction

Poultry diets include phosphorus (P), which is the third most costly component behind protein and energy; therefore, it was necessary to use alternatives that improve the availability of phosphorus (1), such as mineral nanoparticles that improved the intestine's degree of availability and absorption. Also, it helps to decrease the supplementation cost and the environmental pollution (2). Minerals in chicken feed mostly come from dicalcium phosphate (DCP), which is essential for metabolism and development (3-4). Nano minerals have a greater utilization rate compared to conventional inorganic and organic minerals (5- 6). Nano minerals have unique properties compared to macromolecules (7-10). The size of the nanominerals' particles ranges from one to one hundred nanometers (11). Due to the close connection between phosphorus (P) and calcium (Ca) minerals, imbalances in one mineral can impact the metabolism of the other (12). Factors affecting the digestibility of phosphorus in the feed include the calcium source's particle size, the limestone's solubility, and the overall calcium content in the feed (13). Recently, several

studies have focused on using nano calcium phosphate in poultry. Broiler chickens' immunological responses and functional intestinal morphology might be enhanced by applying 40% and 60% amounts of nano-dicalcium phosphate, with no detrimental effects on hematological parameters (14). Hence, the optimal dosage for the broiler chicks' digestion, absorption, and breast phosphorus content was 0.35% nano calcium phosphate (3). Furthermore, when broiler diets contain nano dicalcium phosphate, it is possible to successfully reduce the dietary dicalcium phosphate by 75% without adversely affecting broiler performance, while also reducing excreted calcium and phosphorus by 50%, thereby decreasing environmental pollution caused by poultry (15). Similarly, when used as nanoparticles, the dietary dicalcium phosphate level was successfully reduced from 1.75 to 0.44%, improving the characteristics of tibia bone (16). In addition, a small amount of NHA could be added to broiler diets as a substitute source of calcium and phosphorus (17). Additionally, calcium-phosphorus compounds have shown no adverse effects on the health of birds, improved bone quality and production performance, allowed for the use of lower dosages of nanosources, and reduced the amount of calcium and phosphorus in excreta (by around 50%) (18). The Haugh unit (HU) values of Japanese quail eggs increased ($P \leq 0.05$) by 50% NCaP in the diet (19). Supplementing laying hens' diets with nano minerals improved the quality of their eggs (20). Omara *et al.* (21) demonstrated that the dietary supplementation of broilers with nano phosphorus did not affect the abundance of D-24-hydroxylase mRNA. The Ca and P serum concentrations were influenced by dietary NCaP (22).

These findings indicated that NCaP is a promising alternative mineral source for poultry diets. Therefore, the purpose of this research was to assess the nano source compared to the traditional source of DCP on Lohmann Brown hens' performance, egg quality, tibia bone characteristics, and gene expression for the kidneys' and small intestine's P and Ca co-transporters.

Materials and methods

Ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Cairo University granted ethical permission for this work (CU/II/F/7/21).

Materials

The dicalcium phosphate (DCP) was purchased from a chemical company in Egypt. In contrast, Calcium phosphate nanopowder (NCaP)-hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) product (American Elements Co., Los Angeles, CA) contained 98.5% calcium phosphate (39.90% Ca and 18.50% P) with a Ca:P ratio of 2.15:1.00. The particle size ranged from 500 to 1,000 μm for Dical P and <100 nm for NCaP.

Birds, managements, and diets

The experiment was placed at Cairo University's Poultry Farm, which is a component of the Animal Production Department inside the Faculty of Agriculture, Giza, Egypt, and lab analysis was conducted in the labs of Cairo University and the National Research Centre, Egypt. There were five groups of 27-week-old five hundred Lohmann Brown laying hens with an initial body weight of about 1854 g (control diet + 4 NCaP diets). Each of the one hundred birds/treatments was distributed into 10 pens (10 hens/pen). Birds fed a basal diet based on corn-soybean meal covered all nutrients according to the strain guide except DCP, which was added in 100% (T1, Control) and different levels of 100, 75, 50, and 25% (14, 29, 35) of NCaP in T2, T3, T4, and T5, respectively (Table 1). Hens were exposed to 16 h light and eight h dark, with temperature maintained at 20–24°C, relative humidity 55–65%, and a tunnel ventilation system was in place to ensure adequate air exchange and air quality within the hall to keep ammonia levels below 10 ppm. The experiment lasted for 16 weeks at 43 weeks of age.

Hen's productive performance

Egg number (EN) was daily recorded to calculate hen-day egg production (EP) for each replicate, where EP% % = EN/daily layer number X100. Average egg weight (EW) was recorded every period (4 weeks) to determine the egg mass (EM), where EM (g/hen/day) = {(EP X EW)/100}. Average daily feed intake (FI) was calculated every period (g/hen/day). Feed intake (FI)=feed offered− remaining feed. Feed conversion ratio (FCR) = FI (g.feed) / EM (g.egg). All egg production performance parameters were calculated according to the standard procedures outlined in the Lohmann Brown management guide (23).

Quality of eggs

We sampled 25 eggs from each treatment at the conclusion of the trial to find out how good the eggs were. The weight of the egg shell was measured in grams after the inside membrane was washed and dried overnight at temperatures ranging from 60 to 70°C. The thickness of each eggshell was measured in micrometers using a digital micrometer vernier caliper on four separate areas: the blunt end, the sharp end, the two sides of the egg's equator, and the egg itself. The egg shape index (SI) was derived by dividing the egg length by width, the percentage shell was computed using the micrometer-measured height of albumen, and the Haugh units were determined according to the method of Eisen *et al.* (24). The color of the egg yolk was found by comparing it to 15 different bands of the color spectrum and by utilizing the Roche enhanced yolk color fan. According to AOAC (25), total lipid (g/100g) and total cholesterol (mg/Kg) were measured in egg yolk.

Table 1: Ingredients and nutrient composition of experimental diets

	Traditional (DCP)	Nanocalcium phosphate (NCaP)			
Yellow corn	100% (T1)	100% (T2)	75% (T3)	50% (T4)	25% (T5)
	54	54.75	54.39	54.01	53.72
Soybean meal (44%)	29.60	29.60	29.62	29.64	29.49
Wheat bran	1.80	1.80	2.12	2.46	2.88
Vegetable oil	3.50	3.50	3.50	3.50	3.50
Limestone	8.65	7.95	8.38	8.81	9.25
Dicalcium phosphate	1.70	-----	-----	-----	-----
Nano calcium phosphate	-----	1.65	1.24	0.83	0.41
Salt (NaCl)	0.35	0.35	0.35	0.35	0.35
Vitamin and mineral premix *	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100
ME (Kcal/kg)	2800	2818	2813	2808	2803
Crude protein (%)	18.00	18.0٧	18.0٥	18.03	18.01
Calcium (%)	3.75	3.75	3.75	3.75	3.75
Available phosphorus (%)	0.45	0.45	0.37	0.30	0.22
Lysine (%)	1.02	1.02	1.02	1.02	1.02
Methionine (%)	0.40	0.40	0.40	0.40	0.40
Methionine + cysteine	0.68	0.68	0.68	0.68	0.68

* Vitamin and mineral premix at 0.3% of the diet supplies the following per Kg of the diet: Vitamin A 10000 I.U, Vitamin D_3 3000 I.U, Vitamin E 20mg, Vitamin K_3 3mg, Vitamin B_1 2mg, Vitamin B_2 6mg, Vitamin B_6 5mg, Vitamin B_{12} 20mg, Pantothenic acid 10mg, Folic acid 1mg, Biotin 5mg, niacin 66mg, Manganese 100mg, Iron 100mg, Zinc 75mg, Copper 8mg, Iodine 45mg, Selenium 10mg, Cobalt 10mg.

Tissue sampling

Upon completion of the experiment (43 weeks of age), 20 birds from each treatment were slaughtered, and the right tibia bone was removed for analysis of several features. Additionally, a small portion of the kidney and the intestinal duodenum and jejunum had been taken to analyze gene expression.

Blood biochemistry

In order to get serum, blood samples were centrifuged at 2000 rpm after being drawn from the wing vein in plain tubes at the end of the experiment. Sample serum was used to determine the Ca, P, sodium, potassium, chloride, alkaline phosphatase, aspartate aminotransferase, glucose, cholesterol, total protein, albumin, and uric acid using commercial diagnostic kits (Biomed Diagnostics, Germany) by spectrophotometer (JEN WAY 3600). In contrast, parathyroid hormone (PTH) levels were determined by the ELISA technique using the MyBioSource chicken PTH, E-EL-H0092 ELISA kit.

Tibia bone characteristics

The right tibia has been taken out and is ready for the various measurements. Diethyl ether was used to remove the adherent flesh from the tibia bone. The tibia bones' weight was determined, and they were oven-dried for 3 hours at 105°C (17). According to Mašić *et al.* (26), the Digital Force Gauge device was utilized to assess the tibia bones' breaking strength. To determine the ash percentage, the bones of the tibia were subjected to a six-hour ashing in a muffle furnace set at 600°C. The composition of calcium and phosphorus in tibia ash was determined using a spectrophotometer (JEN WAY 3600). using a colorimetric method with BioVision kits© K380-200 and K410-500, respectively .

Gene expression

The kidney and the intestines (duodenum and jejunum) were examined for RNA using the RNA extraction Kit (QIAGEN Canada Inc., Mississauga, ON, Canada). The reverse transcription was performed using a CDNA synthesis kit from Invitrogen Canada Inc. in Burlington, ON, Canada. In order to conduct the following q-PCR, the CybrGreen technique was utilized. The purpose was to evaluate the expression levels of the cotransporter sodium/phosphate type 2 isoforms a (NaPi-IIa) and b (NaPi-IIb), as well as the Ca-binding protein (Calbindin), as shown in Table 2 (27-29).

Data analysis with statistics

A one-way analysis of variance (ANOVA) was performed on all of the data. This data set was processed using SAS's GLM method (30). The means were separated using Duncan's multiple range tests (31), with significance being recognized at a level of $P \leq 0.05$.

Table 2: The forward and reverse primers for different genes

	Forward primer Sequence (5' ->3')	Reverse primer sequence (5'->3')
β-actin	TCCTCCGTCTGGATCTGGCT	CTCTCGGCTGTGGTGGTGAA
NaPi-IIa/SLC34A1	GAAGCCAGGTGCCTCTGATG	AGAGGATGGCGTTGTCCTTG
NaPi-Iib/SLC34A2	TGGCTTTGTCCCTGCTTGTT	CCAGCCAGCCAAGTAAAAGG
Calbindin	GGCAATGGGTACATGGATGGG	AGTGGCCTTGCCATACTGGTC

NaPi-Iia: sodium/phosphate type Iia; NaPi-Iib: sodium/phosphate type Iib; Calbindin: calcium binding protein.

Results

Hen's productive performance

The effect of NcaP on laying hens' performance is presented in Table 3. Various amounts of NcaP considerably improved Hen's performance metrics (P≤0.05) compared to the control group (DCP). Most optimal (P≤0.05) egg yield, egg mass, and egg weight were recorded for birds that had diets containing 50% NcaP. There were significant differences (P<0.05) in feed intake and FCR between NcaP groups vs. the DCP group. Moreover, raising NcaP levels resulted in a reduction (P≤0.05) in feed consumption. The FCR improved (P<0.05) by adding 50% NcaP level compared to other tested groups. At the same time, the addition of NcaP at different levels gave superior results compared to the control group.

Egg quality

The results herein indicated that supplementary NCaP enhanced egg quality and egg composition compared to DCP (Table 4). The addition of NCaP gave the superior (P≤0.05) eggshell thickness, weight, and percentage compared to the control group. In general, birds receiving NCaP at different substitution levels resulted in a higher (P≤0.05) eggshell thickness by 36.23- 40.11% than those receiving the DCP group. Regarding the NCaP effect, yolk-albumin weight was markedly greater (P≤0.05) across all NCaP groups, except the 25% NCaP group, which gave similar results to the control group. The highest (P≤0.05) value of yolk-albumin weight (68.26g) was recorded for birds fed a diet containing 50% NCaP, while 25% NCaP and control groups recorded the lowest values (64.18 and 63.34g, respectively). Inclusion of dietary NCaP had no discernible change (P>0.05) in Haugh units, egg shape index, egg total lipids, total cholesterol, egg yolk color, egg albumin index, and total cholesterol.

Blood biochemistry

As shown in Table 5, the experimental diet had no significant effect on tested blood parameters except for Ca and P concentrations, but still within normal range (7-11 and 1.1-3.9 mmol/L for calcium and phosphorus, respectively), which are consistent with physiological values reported for laying hens during egg production. The blood Ca concentrations recorded significantly (P≤0.05) increased with NCaP groups when compared to the DCP control group (T1). The group fed 100%NCaP had the highest (P≤0.05) Ca and P concentrations among the NCaP groups, followed by 25% NCaP. Moreover, blood Ca and P amounts decreased (P≤0.05) due to decreased NCaP levels. These significant differences (P<0.05) in blood Ca and P values may be related to the different amounts of daily feed consumed, as Ca and P intake increased with levels of NCaP decreased in the tested diets (Table 3). This relation reflects the Ca and P retained.

Table 3: Effect of nano calcium phosphate on laying hens' performance

Performance	DCP	NcaP levels				SEM	P-value
	100% (T1)	100% (T2)	75% (T3)	50% (T4)	25% (T5)		
Egg production (%)	89.62^{cd}	90.10^{cb}	91.23^{b}	92.63^{a}	88.78^{d}	0.792	0.0001*
Feed intake (g/hen/day)	121.28^{a}	112.28^{c}	113.45^{c}	114.05^{c}	118.09^{b}	1.867	0.0001*
Egg weight (g)	63.88^{c}	67.14^{b}	67.26^{b}	69.02^{a}	64.92^{c}	0.942	0.0001*
Egg mass (g)	64.12^{c}	67.75^{b}	68.73^{b}	71.60^{a}	64.55^{c}	1.022	0.0001*
FCR (g feed:g egg)	1.89^{a}	1.65^{c}	1.65^{c}	1.59^{d}	1.82^{b}	0.039	0.0001*
Initial body weight (g)	1852.50	1857.92	1852.00	1.853.33	1854.50	47.341	0.998
Final body weight (g)	2012.95	2060.02	2078.80	2098.71	2024.00	47.067	0.0001*

a, b, c,…. Etc. Means in the same row within each factor with different superscripts are significantly (P ≤ 0.05) different; *significant at P≤0.05; P-value: probability value; T1: 100%DCP; T2: 100%NcaP; T3: 75%NcaP; T4: 50%NcaP; T5: 25%NcaP; DCP: dicalcium phosphate; NcaP: nano calcium phosphate.

Table 4: Effect of nano calcium phosphate (NCaP) on egg quality and egg chemical analysis

Performance	DCP	NcaP levels				SEM	P-value
	100% (T1)	100% (T2)	75% (T3)	50% (T4)	25% (T5)		
Egg shell thickness (µm)	0.541^{b}	0.749^{a}	0.751^{a}	0.758^{a}	0.737^{a}	0.019	0.0001*
Egg shell weight (g)	6.50^{c}	7.62^{ab}	7.69^{ab}	8.15^{a}	7.21^{b}	0.341	0.0001*
Egg shell (%)	10.19^{b}	11.35^{a}	11.42^{a}	11.82^{a}	11.10^{a}	0.579	0.0147*
Yolk-albumin weight (g)	63.34^{c}	66.39^{b}	66.51^{b}	68.26^{a}	64.18^{c}	0.946	0.0001*
Haugh units	62.40	62.53	62.82	63.04	62.49	2.267	0.9937
Egg shape index	77.78	78.10	78.51	79.09	77.89	0.626	0.0579
Egg albumin index	9.08	9.27	9.32	9.79	9.16	1.090	0.9016
Egg yolk index	44.39	44.63	44.80	44.93	44.68	1.282	0.9803
Egg yolk color	8.00	8.00	7.75	7.75	7.50	0.408	0.4146
Egg total lipid (g/100g)	26.80	26.04	27.66	26.35	26.08	0.779	0.0543
Egg total cholesterol (mg/kg)	17.02	16.90	16.76	16.44	16.69	0.737	0.8359

A, b, c,…. etc. means in the same row within each factor with different superscripts are significantly ($P \leq 0.05$) different; *significant at $P \leq 0.05$; P-value: probability value; T1: 100%DCP; T2: 100%NCaP; T3: 75%NCaP; T4: 50%NCaP; T5: 25%NCaP; DCP: dicalcium phosphate; NCaP: nano calcium phosphate.

Table 5: Effect of nano calcium phosphate on blood biochemistry

Performance	DCP	NcaP levels				SEM	P-value
	100% (T1)	100% (T2)	75% (T3)	50% (T4)	25% (T5)		
Calcium (mmol/L)	7.8^{d}	10.7^{a}	10.0^{c}	9.9^{c}	10.3^{b}	0.137	0.005*
Phosphorus (mmol/L)	3.36^{a}	3.14^{b}	2.04^{c}	1.38^{d}	1.14^{e}	0.039	0.0001*
Sodium (mmol/L)	166.5	162.0	159.4	160.1	159.5	2.323	0.653
Potassium (mmol/L)	7.14	6.92	6.71	6.49	6.59	0.098	0.579
Chloride (mmol/L)	128.5	126.7	124.7	122.6	125.9	1.806	0.269
ALKP (U/L)	243.4	240.0	231.5	223.3	225.8	3.381	0.247
AST (U/L)	163.5	164.0	168.2	162.5	163.4	2.346	0.782
Glucose (mmol/L)	14.4	14.7	14.8	13.3	15.6	0.209	0.657
Cholesterol (mmol/L)	4.2	3.5	3.2	4.1	4.3	0.054	0.631
Total protein (g/L)	80.3	82.5	81.2	84.1	84.0	1.168	0.324
Albumin (g/L)	23.6	31.5	29.2	31.9	30.8	0.411	0.489
Globulin (g/L)	41.6	51.0	46.7	52.2	51.8	0.681	0.247
Albumin / Globulin ratio	0.57	0.62	0.63	0.61	0.59	0.086	0.893
Uric acid (umol/L)	411.1	367.7	346.7	361.1	391.4	5.431	0.620
PTH (pg/mL)	858.2	854.6	901.3	908.7	851.7	12.373	0.823

A, b, c,…. etc. means in same raw within each factor with different superscripts are significantly ($P \leq 0.05$) different; *significant at $P \leq 0.05$; P-value: probability value; T1: 100%DCP; T2: 100%NCaP; T3: 75%NCaP; T4: 50%NCaP; T5: 25%NCaP; DCP: dicalcium phosphate; NCaP: nano calcium phosphate; ALKP: alkaline phosphatase; AST: Aspartate transaminase; PTH: Parathyroid hormone.

Sodium, potassium, and chloride concentrations had no significant differences between the NCaP groups and the DCP control group, although decreased values of those minerals were observed in the NCaP groups. The alkaline phosphatase (ALP), aspartate aminotransferase (AST), glucose, and cholesterol values were not affected ($P>0.05$) due to the NCaP diet. In addition, there were no significant differences between all treatments ($P>0.05$) in levels of Uric acid, total protein, albumin, globulin, and albumin/globulin ratio. The value of parathyroid hormone (PTH) recorded the highest value ($P>0.05$) with 75 and 50% NCaP levels.

Figures 1 and 2 illustrate the effects of various NCaP diets on the tibia bone properties of laying hens. Compared to the control group, tibia weight was considerably increased ($P \leq 0.05$) by inclusion of NCaP levels. The birds that were fed 50% NCaP had the highest tibia weight ($P \leq 0.05$) and breaking strength of tibia ($P>0.05$). There were no changes ($P>0.05$) in the % tibia ash, calcium, or phosphorus content.

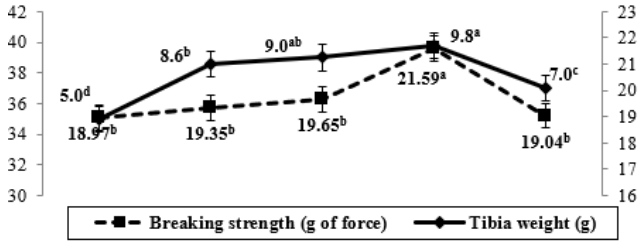

Figure 1: Tibia bone characteristics as affected by nanocalcium phosphate.

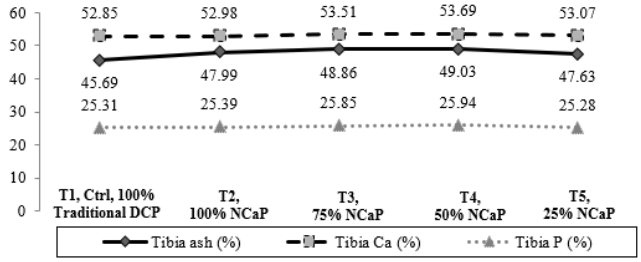

Figure 2: Tibia bone ash, calcium (Ca), and phosphorus (P) of laying hens as affected by nanocalcium phosphate.

Gene expression

The kidney's co-transporter sodium phosphate type II isoform a (NaPi-IIa) gene expression was affected (P≤0.05) by different sources of DCP (Figure 3). The addition of NCaP substantially raised (P≤0.05) the expression of the NaPi-IIa gene compared to the control group (1.12), and hens given 50% NCaP exhibited the highest expression (1.94). In the duodenum, the nano form recorded improved (P≤0.05) in NaPi-IIb; the best NaPi-IIb results (P≤0.05) were observed at 50% and 75% NCaP. In the jejunum, all groups fed NCaP enhanced substantially (P≤0.05) more in NaPi-IIb gene expression compared to the DCP control group, with the highest (P≤0.05) effect observed in the 50% NCaP group (Figure 4).

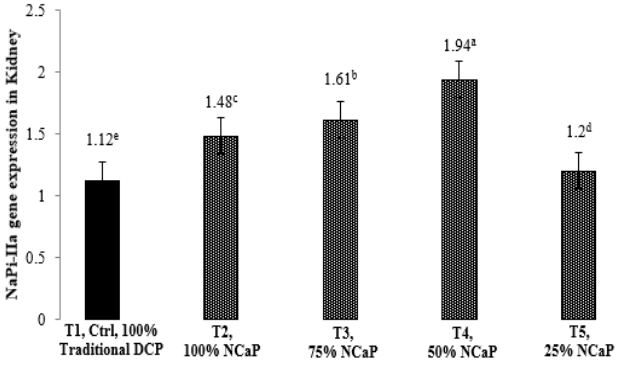

Figure 3. NaPi-IIa gene expression in the kidney of laying hens as affected by nanocalcium phosphate.

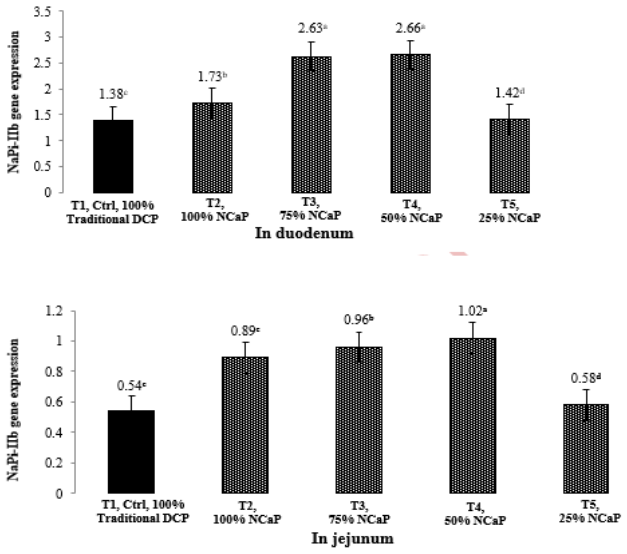

Figure 4: NaPi-IIb gene expression in the intestine of laying hens as affected by nanocalcium phosphate.

The nano-form of minerals had a positive effect (P≤0.05) on the calbindin gene expression (calcium binding protein) in the duodenum and jejunum of the gut. In the duodenum, calbindin gene expression was significantly (P≤0.05) improved with 100, 75, and 50% of NCaP, and the group provided the 50% NCaP had the highest value (P≤0.05). In the jejunum, the highest value (1.70; P≤0.05) of calbindin gene expression was shown in the 50% NCaP tested level group, whereas the DCP control group recorded the lowest value (0.94; P≤0.05) of calbindin gene expression (Figure 5).

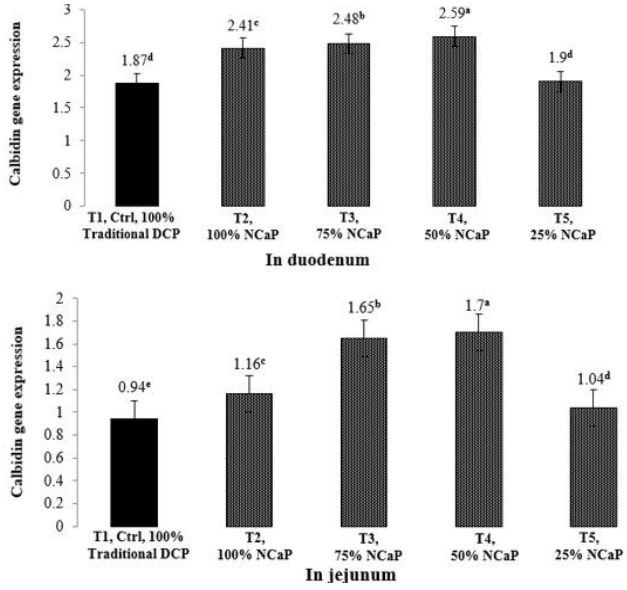

Figure 5: Calbindin gene expression in the intestine of laying hens as affected by nanocalcium phosphate.

Discussion

Hen's productive performance parameters improved (P≤0.05) with NCaP levels, which agreed with previous studies. Japanese quail' egg yield and weight were affected (P≤0.05) due to NCaP supplementation either alone or in combination with DCP, whereas birds fed a diet supplemented with 50% NCaP alone gave the best (P≤0.05) egg weight and production (19). The improvement in laying hens' performance due to the tiny particle size of the NCaP and a larger surface area than DCP, also NCaP improves the absorption through intestinal villi and increases bioavailability, which leads to enhanced productive performance (20). However, in terms of egg production, egg weight, egg mass, and FCR, no significant variations were noted in Bovans laying hens between Ca carbonate and nano-calcium carbonate (31). Generally, the groups fed 100, 75, and 50% NCaP performed better than the groups fed either control or 25% NCaP, which had almost the same performance. The findings demonstrated that NCaP levels had a noticeable (P≤0.05) and beneficial effect on the performance of laying hens.

The current findings align with previous studies reporting improved eggshell quality following NCaP supplementation. For instance, inclusion of 0.02% NCaP on the shell thickness of brown LSL hen's diet increased eggshell thickness by 11.24% over the control group. This is attributed to the impact of NCaP action on carbonic anhydrase enzyme activity, which takes part in eggshell formation and affects shell thickness (32). As noticed from the present results of the NCaP effect on egg quality, NCaP significantly increased egg shell thickness, weight, and shell %. Nano Ca carbonate had improved the eggshell thickness of laying hens (31).

Recently, dietary nanocalcium phosphate significantly (P≤0.05) enhanced the egg thickness of Japanese quail (33). Moreover, eggshell weight improved due to nano-selenium (21). Eggshell quality mainly depends on the bioavailability of Ca and P, as these two are essential minerals implicated in the formation of eggs and their components (34). However, Wang *et al.* (35) concluded that the particle size of Ca sources did not affect eggshell thickness or egg shape index. There is no effect of NCaP on the Haugh unit, which is a measure of albumin quality. In the present study, NCaP significantly improved shell thickness, shell weight, and shell percentage. These improvements may result from enhanced bioavailability of calcium and phosphorus, the major minerals responsible for shell formation and strength. However, similar to previous findings, NCaP supplementation did not significantly affect the Haugh unit, egg shape index, or albumen quality (36–38).

The present findings that NCaP increased serum Ca and P concentrations are consistent with previous reports. Ganjigohari *et al.* (37) observed that plasma calcium value decreased (P≤0.05) with the decrease in the level of replacement of nanocalcium carbonate in the blood of laying hens. Makola *et al.* (14) recorded no significant differences between DCP and NCaP groups in ALP, while cholesterol concentration in broiler serum was significantly (P≤0.05) decreased. However, serum Ca and P of LSL laying hens showed the highest (P≤0.05) concentration with hens fed 400g NCaP /ton compared to the control group and 200 and 800g NCaP/ton groups (38). This confirms that nano-sized calcium and phosphorus improve mineral absorption efficiency without disturbing blood homeostasis.

These findings demonstrate that nano form is superior to traditional form due to its smaller particle size and greater surface area, which enhance absorption and bioavailability (9,39). Moreover, tibia bone characteristics (weight, length, breaking strength, and mineral content) were more improved (P<0.05) by feeding nanominerals (6-7, 16). Adding inorganic sources to poultry feeds leads to lower mineral bioavailability compared to organic sources. Increasing productive performance and bone quality were achieved by low dosages of nano Ca and P (40). However, adding nano calcium carbonate to laying hen diets increased their tibia bone weight and thickness, but did not affect tibia ash% % (41).

The NaPi-IIa was measured to observe the reabsorption of P in the kidney. The results observed that NCaP reduced P excretion by increasing the expression of NaPi-IIa in the apical brush border of the renal proximal tubule epithelial cells, which is the renal sodium phosphate cotransporter responsible for reabsorption of P from the urine to the blood, thus reducing the mineral excretion (42-43).

The present findings demonstrated that NCaP decreased P excretion. The NaPi-IIb cotransporter is an essential component for the active transport of P across intestinal epithelial cells (40). Intestinal duodenum and jejunum P absorption was improved with the addition of NCaP compared to the control. The active transport of Ca through the intestines is facilitated by calbindin, a protein that binds Ca (41). The NCaP feeding increased intestinal calcium absorption by increasing calbindin expression within the birds' duodenum and jejunum compared to those fed the traditional form. The use of small amounts of NCaP improved productivity and reduced the excretion of Ca and P by approximately half (15). A reduction in excretory Ca and P concentrations by 50% as a result of utilizing lower nano mineral dosages (18). Moreover, dietary DCP and nano P levels increased in chick diets, and the quantity of mRNA levels of NaPi-IIb decreased in the gut (14). Diets low in Ca content enhanced duodenal expression of calpindin and NaPi-IIb mRNA transporters (44-45).

Nanoparticles confer advantageous effects in poultry via several physiological, biochemical, and cellular pathways that improve food utilization, bolster antioxidant status, and boost immunity while preserving gut health (46-50).

Improved Intestinal Absorption and Bioavailability: The nanoscale dimensions of calcium phosphate particles (often <100 nm) augment their surface area and solubility within

the gastrointestinal tract. This enhances ionization and interaction with intestinal transporters (such as Ca^{2+} and PO_4^{3-} channels), resulting in increased absorption in the duodenum and jejunum. Thus, the efficiency of calcium and phosphorus utilization is superior compared to that of birds consuming standard dicalcium phosphate (DCP) (51-53).

Enhanced Bone Mineralization and Skeletal Integrity: Upon absorption, NCaP provides accessible calcium and phosphorus for the synthesis of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), the principal mineral constituent of bone. The nanoscale form enhances the calcium-to-phosphorus ratio at the cellular level, facilitating improved osteoblast activity and collagen cross-linking. This leads to denser, stronger bones with enhanced tibia ash, breaking strength, and bone mineral density, hence diminishing the incidence of leg diseases and fractures in broilers and layers (54-56).

Improvement of Eggshell Quality (in Layers): NCaP offers a consistent and bioavailable source of Ca^{2+} ions essential for shell calcification in the shell gland (uterus). The enhanced calcium deposition augments shell thickness, strength, and specific gravity, hence diminishing the incidence of cracked or soft-shelled eggs. Moreover, adequate phosphorus availability inhibits calcium transport from bones, thus preserving skeletal reserves and prolonging laying persistency (57-58).

Cellular and Molecular Mechanisms: NCaP stimulates calcium-sensing receptors and improves intracellular signaling (e.g., via MAPK and PI3K/Akt pathways) via interacting with intestinal epithelial cells and osteoblast membranes. Alkaline phosphatase (ALP) and osteocalcin, two bone matrix proteins necessary for mineral deposition and bone remodeling, are upregulated as a result. By preserving redox equilibrium, NCaP also lessens oxidative stress in intestinal and bone tissues, promoting tissue integrity and cell survival (48,59-63).

Environmental and Nutritional Efficiency: Due to its elevated absorption rate, dietary NCaP permits reduced phosphorus doses while preserving performance and bone integrity. This reduces phosphorus excretion and lowers environmental pollution, hence promoting sustainable poultry production (33,54).

Conclusion

The incorporation of NCaP in laying hen diets significantly enhanced productive performance and improved egg quality. It increased the calcium and phosphorus retention, resulting in stronger eggshells and superior bone mineralization. Moreover, enhanced mineral absorption resulted in less excretion, aiding in the reduction of environmental contamination. NCaP provides an effective and sustainable approach to strengthening poultry health, productivity, and egg quality.

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Conflict of interest

No authors have disclosed any potential bias or conflict of interest.

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تأثير فوسفات الكالسيوم النانوي على إنتاجية الدجاج البياض وصفات العظام والمعايير الكيميائية الحيوية للدم والتعبير الجيني

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الخلاصة

تم في هذه التجربة دراسة تأثير استبدال فوسفات ثنائي الكالسيوم التقليدي (DCP) بفوسفات الكالسيوم النانوي (NCaP) على إنتاجية وصحة الدجاج البياض، وصفات العظام، والتعبير الجيني لناقلات الفوسفات المشتركة (NaPi-IIa وNaPi-IIb) في الأمعاء والكلى، وكذلك التعبير الجيني لبروتين كالبندين في الاثني عشر والصائم. عند عمر ٢٧ أسبوعاً، تم توزيع ٥٠٠ دجاجة بياضة من سلالة Lohmann Brown على خمس مجموعات متساوية وتغذيتها على علائق مختلفة. تلقت المجموعة الأولى عليقة تحتوي على ١٠٠% DCP (T1)، بينما استُبدل الـ DCP في المجموعات الأربع الأخرى بنسبة ١٠٠%، ٧٥%، ٥٠%، أو ٢٥% NCaP على التوالي. سُجِّل أعلى إنتاج للبيض ووزنه واستهلاك العلف في الطيور المغذاة على ٥٠% NCaP ($P\leq0.05$). وبالمقارنة بالمجموعة الضابطة، فقد تفوقت المعاملات المحتوية على NCaP بمستويات مختلفة في تحسين سمك ووزن ونسبة قشرة البيض ($P\leq0.05$). كما حققت الدجاجات المغذاة على ٥٠% NCaP أفضل نتائج في احتجاز الكالسيوم في البيض وقشرته. بالإضافة إلى ذلك، حسّنت المعاملات المحتوية على NCaP قوة كسر عظم الساق ووزنه، وكان أفضلها عند مستوى ٥٠% NCaP. عزز الـ NCaP من التعبير الجيني لناقل الفوسفات NaPi-IIa في الكلى، مما أدى إلى تقليل افراز وهدر الفوسفور. وسُجِّل أعلى مستوى معنوي ($P\leq0.05$) للتعبير الجيني لبروتين كالبندين وناقل NaPi-IIb في الاثني عشر والصائم في مجموعة ٥٠% NCaP. تلخصت الدراسة إلى أن إضافة الـ NCaP حسّنت من إنتاجية وجودة البيض. كما تشير بيانات التعبير الجيني واحتجاز الكالسيوم والفوسفور إلى أن الطيور المغذاة على NCaP أظهرت توافرًا حيوياً أعلى وانخفاضاً في الأثر البيئي، مع كون مستوى ٥٠% NCaP هو الأفضل.