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Impact of dietary dicalcium phosphate nanoparticles on productive performance of broiler chickens.

I. Rekaby[1]; Reham, A. M. Ali[2]; I. El – Wardany[3] and M., E., Ghonime[1]

1-Department of Animal and Poultry Production, Faculty of Agriculture and Natural Resources, Aswan University.
2- Department of Animal and Poultry Production, Faculty of Agriculture, Beni Sweif University.
3- Department of Poultry Production, Faculty of Agriculture, Ain Shams University.

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Abstract:

The present study was conducted to evaluate the possible effect (s) of enriching the broiler chick's diet with Di-Calcium phosphate Nanoparticles (DCaP-NPs) or vitamin D3 on growth performance of broiler chicks. A total number of 240 unsexed one-day-old broiler chicks (Ross) were used. These chicks were randomly and equally distributed into four groups of 60 chicks each, in six replicates (10 chicks each). The first group was fed the basal diet (control group), while the 2nd, 3rd and 4th groups were fed the basal diet enriched with 1-g, 2-g of DCaP-NPs / kg diet, and 1 ml Vitamin D3 / Liter of drinking water, respectively. The experimental period duration was five weeks. The criteria of response on the productive performance. Results showed that at five weeks of age, the best LBW being recorded for chicks that fed the basal diet fortified by 2g/kg (DCaP-NPs) followed by those fed the same diet but with the drinking water containing one ml Vitamin D3 / Liter. Moreover, both T2 and T3 treatment chicks significantly gained more weight than the other groups. Feed consumption was significantly reduced in T3 group than the other groups, while FCR was significantly improved for birds in the T3 group followed by those from the control treatment. After slaughtering carcass weight was significantly heavier in birds from both T2 and T3 treatments than in the control and T1 groups and then were no significant differences in the average values of carcass characteristics and meat analysis among treatments.

Key words: nano calcium particles, Vitamin D, Broiler, Bone, Production Performance

1- Introduction

In the past two decades, the consumption of poultry meat has increased globally largely due to its lower pricing and greater availability.

In fast-growing broiler chickens, the bone skeleton plays an important role in supporting their body weight (Fleming, 2008). Animal management requires knowledge of the structure and function of the skeletal system; bone morphometrics will provide this information (Applegate and Lilburn, 2002). Commercial broilers' growth rate and meat yield have improved linearly every year as a result of genetic selection, with greater input efficiency and earlier marketing age.

Corresponding author*: islamrekaby@agr.aswu.edu.eg

Broilers raised for meat production nowadays are especially prone to leg issues because of selective breeding for quick development and rapid weight gain with large breast muscle, which results in an imbalance between the body size and the weight-supporting skeletal system. (Meyer et al., 2019).

Nanotechnology is a new discipline that studies how to manipulate molecules at the atomic level to achieve favourable reactions when used as a substitute for conventional mineral sources. (Diman and Decottignies, 2018). Since this nanoparticle has shown promise as a replacement for traditional mineral sources, it has been fed. Even at relatively low concentrations compared to the traditional, organic and inorganic sources, nano minerals are showing significant promise as mineral feed supplements for animals. (Swain et al., 2015).

Because nanoscale particles exhibit innovative qualities such a large surface area, high surface activity, high catalytic efficiency, strong adsorbing capacity, and low toxicity, nano products have received a lot of attention in the recent development of nanotechnology. (Wang et al., 2007; Zhang et al., 2008). Despite being used as a medicine delivery adjuvant, calcium and phosphate nanoparticles have not been examined for their effects on oral intake. The addition of calcium and phosphorus as nanoparticles is expected to cut the price of both the feed and the supplement. (Vijayakumar and Balakrishnan, 2015).

Vitamin D3 is thought to be a pro-hormone that is produced in the skin by ultraviolet irradiation of 7-dehydrocholesterol or absorbed in the digestive system after eating. (DeLuca, 2004). It needs to be transformed into 25-hydroxyvitamin D3 in the liver and then into 1,25-dihydroxy vitamin D3 in the kidneys because it is not physiologically active in this first state (Driver et al., 2005). Numerous activities of 1,25-Dihydroxyvitamin D3, such as bone homeostasis, calcium mobilisation in bone, and calcium reabsorption in the kidney, are expressed through a nuclear receptor (Farquharso et al., 1993; Leeson et al., 2001; DeLuca, 2004).

According to reports, supplementing with high doses of vitamin D3 decreased the prevalence of limb issues including TD and calcium rickets in broilers. (Whitehead et al., 2004; Atencio et al., 2005) (Driver et al., 2006). According to a study, liver illness and poor liver function can both lead to reduced hydroxylation of cholecalciferol and decreased production of 25(OH)2 D3 and 1,25(OH)2 D3, which can disrupt Ca and P homeostasis. (Abe et al., 1982; Whitehead et al., 1996). Therefore, consuming enough 25(OH) D3 in the diet may help to maintain Ca homeostasis and the characteristics and functionality of the skeletal system. The best-known function of vitamin D compounds is their role in promoting intestinal Ca absorption, which helps to promote bone mineralization and improve the condition of the skeletal system. (Kim et al., 2011) suggests that broiler chickens with high vitamin D3 levels develop their bones more quickly and deposit more minerals, and (Tatara et al., 2011) observed that bone characteristics were positively impacted at an advanced stage of the reproductive cycle.

Therefore, the present study was conducted to investigate the effect of different levels of di-calcium phosphate Nano - particles (DCaP-NPs) on growth performance, carcass traits and some blood parameters of broiler chicks.

2. Materials and Methods

The present study was carried out at the Department of Animal and Poultry Production, Faculty of Agriculture in Aswan University during the period from September to October 2022. The main objective of the study was to evaluate the possible effect (s) of enriching the broiler chick's diet with Di - Calcium phosphate Nanoparticles (DCaP-NPs) or vitamin D3 on growth performance, some blood biochemical parameters and carcass characteristics of broiler chicks.

2.1.Birds and Management:

The authors declare that the procedures imposed on the birds were carried out to meet the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals and birds used for scientific purposes. Since, all treatments and birds care procedures were approved by the Institutional Animal Care and Use Committee in AU-IACUC, Aswan University, Egypt.

Water and feed were offered ad libitum. Composition and calculated analysis of the diets are presented in Table (1).

A total number of 240 unsexed one-day old broiler chicks (Ross) were used. These chicks were randomly and equally distributed into four groups of 60 chicks each, in six replicates (10 chicks each). Upon arrival, all chicks were brooded at 33^{o}C for three days, reduced to 30^{o}C at the end of the first week, and then declined by 3-4^{o}C according to the optimum managerial outlines of the farm. The lighting program was via incandescent lights and was like commercial conditions; 24 hours: L during the first week and then 21:8 L: D cycle from 2nd to the 5th week of age. All chicks were fed (*ad libitum*) a commercial starter diet from 0 to 10 days of age and broiler grower diet from 11 d to 35 days. The ingredient and nutrient composition of the experimental diet are presented in Table (1).

Particle size characterization of the dicalcium phosphate Nano-form (DCaP-NPs) in comparison to its traditional form was performed using a transmission electron microscope. Since, as shown in Figures 1 and 2, the results of Transmission Electron Microscopy (TEM) revealed that the particle size of elemental calcium was between 13.6 and 41.71 nm. Experimental design:

The basal diets were supplemented with different levels of calcium and vitamin D, from the first day to the end of the experimental period (35 days).
The four experimental treatment groups were as follows:
Treatment 1 (T1): Birds were fed the basal diet without any supplements.
Treatment 2 (T2): Birds were fed the basal diet fortified with 1-gram DCaP-NPs / kg diet.
Treatment 3 (T3): Birds were fed the basal diet fortified with 2-gram DCaNPs / kg diet.
Treatment 4 (T4): Birds were fed the basal diet with 1 ml Vitamin D3 / Liter of drinking water.

2.2.Measurements

Productive performance

The chicks were individually weighed at hatch and then biweekly till five weeks of age. Live body weight (LBW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) were recorded.

Live body weight was measured to the nearest gram. Body weight gain was calculated by subtracting the average LBW of chicks in a previous period from the given period being recorded. Feed consumption (g/bird/week) was calculated by subtracting residual feed from the offered feed.

Feed conversion ratio: Feed conversion ratio (FCR) was calculated for a given period using the following formula:

Feed conversion ratio = Average intake (g) / Body weight gain.

2.3.Carcass characteristics and some organs weight: -

At the end of the experiment (35 days of age), six chicks per treatment group were randomly taken, weighed, and slaughtered by severing the carotid arteries and jugular veins . After completing bleeding, the chicks were scalded and de-feathered. The head with neck and shanks were removed, and the carcasses were eviscerated and weighed to determine dressed carcass weight and percentage. Giblets including liver, heart, and empty gizzard were also individually weighed. The relative weights of these organs were calculated in relation to live body weight.

2.4.Statistical analysis:

The data were subjected to the analysis of variance procedure a using linear model of SAS software statistical analysis SAS USERs GUIDE, (1994) according to the following model:

$Y_{ij} = \mu + T_i + e_{ij}$, where,
Y_{ij} = any observation.
μ = the overall mean
T_i = effect of treatment (i = 1….4),
e_{ij} = random error.

The Duncan's multiple range test (Duncan, 1955) was used to compare the mean values of the control group to the mean values of each experimental group.

Table 1: Composition and calculated analysis of the basal starter and finisher diets

Ingredient	Starter diet	Finisher diet
Yellow corn	52.03	61.38
Soybean meal (44%)	29.60	22.50
Corn gluten meal (60%)	7.15	6.25
Vegetable oil	4.00	4.00
Wheat bran	2.80	2.60
Bone meal	3.30	2.15
Limestone	0.14	0.14
Vit. & mineral Premix*	0.30	0.30
NaCl (salt)	0.50	0.50
L-Lysine-HCl	0.18	0.18
Total	100	100
Calculated analysis: -		
Crude protein (%)	23.07	20.03
ME/Kcal/Kg	3100	3207
Crude Fiber (%)	3.80	3.32
Ether extract	6.50	6.73
Calcium (%)	0.92	0.90
Available P (%)	0.48	0.46
Lysine (%)	1.22	1.05
Methionine (%)	0.55	0.49
Meth+cysteine (%)	0.92	0.81

* Vit. & mineral Premix: each Kg contains: A, 11000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3 mg; Vit. B1, 2 mg; Vit. B2 6 mg; B6 3 mg; B12, 14 mcg; Nicotinic acid 60mg; Folic acid 1.75 mg, Pantothenic acid 13mg; and Biotin 120 mcg, Choline 600 mg; Copper 16 mg; Iron 40 mg; Manganese. 120 mg; Zinc 100 mg and Iodine 1.25 mg.

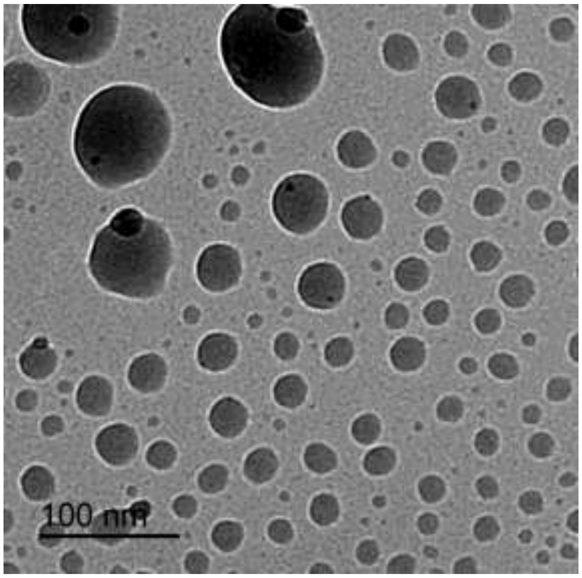

Fig.1: TEM of the traditional Di –calcium phosphate particle size.

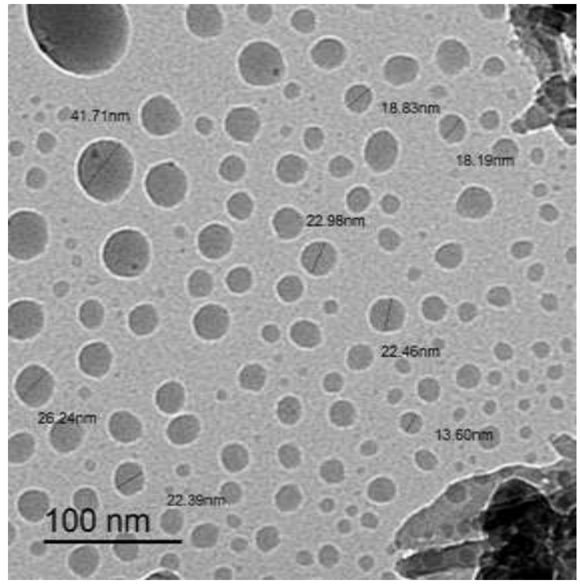

Fig.2: TEM of the Nano Di –calcium phosphate particle size.

3.Results and Discussion

3.1.Growth Performance traits:

a- Live body weight (LBW)

The effect of two Di- Calcium phosphate Nano particles (DCaP-NPs) levels on live body weight (LBW) of broiler chicks at different growth periods is presented in Table-2.

It is clear from the results that LBW of day-old broiler chicks at the beginning of the experiment was not significantly different, indicative of correct distribution of birds between treatments. This trend was also observed at the 1st, 2nd and the 3rd weeks of age, where LBW od chicks within different treatments was not significantly different, although the numerical improvements in T1 and T2 groups which fed the basal diet supplied with DCaP-NPs compared with the other groups.

At four weeks of age, broiler chicks that fed the basal diet enriched with 2g DCaP-NPs / kg of diet had significantly the heaviest LBW compared with those in the other treatments. This improvement was also observed at five weeks of age, with the best LBW being recorded for chicks from T2 followed by T3 and then the control group. The lowest LBW was recorded for chicks from T1 treatment group. It turned out that the highest weight was in T2 and the lowest weight in T1 from the control group may be due to the higher number of females in T1 compared with the other treatments, as we utilized unsexed chicks in the present experiment.

Table-2: Effect of treatments on live body weight of broiler chicks at different ages.

Trt	Day1	W1	W2	W3	W4	W5
Con	41.08	192.75	561.8	1101.6	1735.3^{b}	2385.1^{b}
T1	40.80	190.25	559.8	1114.2	1751.5^{b}	2359.9^{c}
T2	41.37	190.17	553.7	1101.0	1769.3^{a}	2407.5^{a}
T3	41.22	190.75	564.4	1107.5	1746.3^{b}	2405.6^{a}
SEM	0.404	1.782	6.120	16.182	26.343	47.664
Sig.	NS	NS	NS	NS	**	**

a,b,, Means within a column with different superscripts are significantly different ($P< 0.05$).

b- Body Weight Gain (BWG)

The effect of two Di- Calcium phosphate Nano particles (DCaP-NPs) levels on body weight gain (BWG) of broiler chicks at different growth periods is presented in Table-3.

It is clear from the results that body weight (BWG) of broiler chicks during the periods from 0 – 1 and 1 -2 weeks of age did not significantly differ between all treatments. During the period from 2-3 weeks of age, a significant increase in BWG was observed for chicks that fed the basal diet plus 1 gm (DCaP-NPs) / kg of diet (T2) followed by fed the higher level of DCaP-NPs (T2) compared to the other treatment (T3) and the control group.

From the end of 3rd week to end of 4th week, it was observed that the highest body weight gain was recorded for chicks in the T2 treatment group compared by the other groups including the control ones. However, during the last growing period (4- 5 weeks of age), the best weight gain was recoded for chicks in the T3 treatment group (vitamin D3 treatment), followed by those from T1 then T2 and the control group.

Finally, during the five-week fattening period, chicks of both T2 and T3 treatments significantly gained more weight than those of T1 and the control group, respectively. Interestingly, the data declared that chicks of the control had substantially better BWG than those from the T2 treatment group. This may be due, in part, to the apparent number of male chicks being lower in the T2 group compared with those of the control group.

Table- 3: Effect of treatments on body weight gain of broiler chicks at different growing periods

Trt	WG, 0-1	WG, 1-2	WG, 2-3	WG, 3-4	WG, 4-5	WG, 1-5
Con	151.75	369.08	539.75^{b}	633.8^{b}	649.74^{c}	2344.1^{b}
T1	149.75	369.58	554.33^{a}	637.3^{b}	608.38^{b}	2319.4^{c}
T2	148.67	363.50	547.33^{ab}	668.3^{a}	638.25^{c}	2366.0^{a}
T3	150.75	373.67	543.08^{b}	638.8^{b}	659.27^{a}	2365.6^{a}
SEM	1.545	5.138	11.375	15.725	37.633	47.63
Sig.	NS	NS	*	**	**	**

a,b,, Means within a column with different superscripts are significantly different ($P< 0.05$)

c- Feed Consumption (FC): -

The effect of DCaP-NPs levels on feed consumption (FC) of broiler chicks at different growth periods is presented in Table-4.

The amount of feed consumed by the chick during the first four weeks was nearly similar without tangible or significant differences between all treatments, although some numerical increases in FC for T1, T2 and the control chick group was observed, but the differences lacked the significant level.

From the end of the 4th week to the end of the 5th week, the highest feed consumption was recorded for T2- chickens group that fed on the basal diet plus 2 g DCaP-NPs, followed by the group that fed on1 gm (DCaP-NPs), then the control group by similar levels each other, while the lowest feed consumption is during the fifth week in the group that the basal diet plus Vitamin D3 at 1 ml/1 liter of drinking water. This trend was also observed for the whole experimental period (0-5 weeks of age), where T3 group consumed significantly less than the other treatment groups and the control one.

Table 4: Effect of treatments on feed consumption (g/chick/week) of broiler chicks at different growing periods

Trt	FC, 0-1	FC, 1-2	FC, 2-3	FC, 3-4	FC, 4- 5	FC, 0-5
Con	167.33	423.42	861.8	888.08	1061.4^{a}	3402.0^{a}
T1	174.58	420.33	860.3	892.42	1064.8^{a}	3412.3^{a}
T2	170.58	425.00	858.5	881.33	1070.6^{a}	3406.0^{a}
T3	170.00	419.92	851.1	886.08	1036.3^{b}	3363.3^{b}
SEM	3.52	3.37	9.32	7.86	34.72	34.22
Sig.	NS	NS	NS	NS	*	*

a,b,, Means within a column with different superscripts are significantly different ($P< 0.05$).

d- Feed Conversion Ratio (FCR): -

The effect of two Di-Calcium phosphate Nanoparticle (DCaP-NPs) levels of the feed conversion ratio (FCR) of broiler chicks at different growth periods is presented in Table-5.

In the 1st week, there was no significant change in the FCR between all treatment groups. However, during the period from 1- 2 weeks of age, the best FCR was recorded for chick of the T3 group followed by those of the control group. The worst value was recorded for chicks from T2 group. Moreover, during the period from 3-4 WOA chicks from T2 treatment group had significantly the best FCR, followed by those in the T3 group compared with the other treatments.

In the last growing period (4-5WOA), FCR was significantly improved for birds in the T3 group followed by those from the control treatment, while the worst value was recorded for T1 chicks' group.

Beginning from the age of hatching until the age of marketing (35 days), the FCR of the chicks was recorded during this period, in which FCR is significantly better for both T2 and T3 broiler groups compared by the other treatments.

Table 5: Effect of treatments on feed conversion ratio (FCR) of broiler chicks at different growing periods

Trt	FCR, 0-1	FCR, 1-2	FCR, 2-3	FCR, 3-4	FCR, 4-5	FCR, 0-5
Con	1.103	1.148^{b}	1.595^{a}	1.403^{a}	1.643^{c}	1.452^{b}
T1	1.168	1.158^{ab}	1.560^{c}	1.403^{a}	1.777^{a}	1.472^{a}
T2	1.135	1.163^{a}	1.573^{b}	1.322^{c}	1.688^{b}	1.440^{bc}
T3	1.150	1.133^{c}	1.570^{bc}	1.392^{b}	1.605^{d}	1.427^{c}
SEM	0.02	0.02	0.02	0.03	0.08	0.02
Sig.	NS	*	*	**	**	*

a,b,, Means within a column with different superscripts are significantly different ($P< 0.05$).

It is clear from the previously recorded results of productive performance traits that the applied treatments have had an obvious effect on broilers growth. In this respect, both LBW and BWG were significantly improved fortification of diets with DCaP-NPs and/ or vitamin D3. This may be due, in part, to the fast remodeling of the structural bones as a result of the abundant circulating calcium supporting fast calcification of the bird's long bones. Additionally, there is evidence to suggest that calcium in the form of nanoparticles could be absorbed and used more effectively than its inorganic counterpart. This is accurate given that (Vijayakumar and Balakrishnan, 2015) claimed that adding calcium and phosphorus in the form of nanoparticles will help to minimize the cost of both the feed and the supplement.

A similar trend was also observed for feed consumption and feed conversion ratio as the most important criteria for broiler chicken producers, where chicks of T2 and T3 treatment groups had better FCR than the other groups. This was expected since FCR is a function of both feed consumption and body weight.

It is of interest to observe that the final LBW and weight gain of birds that fed the low level of DCaP-NPs (T2) recorded the lowest values compared by the other treatments. This result did not reflect the expected return of the treatment, but simply due the number of females in this group compared to those in the vitamin D3 (T3) treatment. In this concern, it should be declared that our experiment has used unsexed- day old chicks, which were randomly distributed equally according to the initial body weight. As a consequence, the final LBW and weigh gain reflect the effect of sex rather than treatments on the differences in final weight. Since, the number of females to males in the treatments was 33: 26 in the control group, 35:25 in T1, 33:27 in T2, 23: 37 in T3 treatment. This means that vitamin D3 (T3) treatment group had 61.7% males vs. 38.3% females, compared to 44% vs. 56%, 41.7% vs. 58.3%, 45% vs. 55% of the control, T1 and T2 treatment groups, respectively. According to science, male chickens have bigger, thicker, and heavier bones than female chickens, just like mammal bones do (Dace et al., 2000), and this fact should be taken into account in the next study.

However, under the current experimental circumstances, the results of improved grill performance are closely in line with those found by (Vijayakumar and Balakrishnan, 2014; Ali, 2017; and Hassan et al., 2016), who found that birds fed different levels of nano-di calcium phosphate gained noticeably more body weight and utilized feed more efficiently than the control group.

e- Carcass characteristics:

Table-6 showed the impact of two levels of Di-Calcium Phosphate Nanoparticles (DCaP-NPs) and vitamin D3 on the carcass characteristics of broiler chicks at different phases of growth periods.

Data reveals that LBW at marketing age (35 days) was significantly heavier in birds from both T2 and T3 treatments compared with those of the control and T1 groups, respectively. This trend was also observed for the dressing weight of birds in the corresponding groups, where T2 and T3 are still achieving the best results. On the other hand, heart weight was significantly higher for chicks of the control group then it decreased slightly in T2, T3, and finally T1, but the percentages of heart weight to LBW were convergent in all treatments. Moreover, gizzard weight and the percentage were higher significantly for chicks of the control group, followed by those from T2 and T3, and then it decreased significantly in birds from T1 treatment. Then the weight of the liver was estimated, as it appeared that both T3 and T1, respectively, were the highest liver weight, while the liver weight decreased in the control group, followed directly by T2, and the highest percentage of liver weight attributed to LBW was in T3 and the lowest percentage in T2 attributed to LBW.

Table 6: Carcass characteristics of broiler chicks at marketing age

Trt	LBW (g)	Dressed Carcass (g)	%	Heart Weight (g)	%	Gizzard Weight (g)	%	Liver Weight (g)	%
Con	2387.5^{b}	1834.4^{b}	76.83	11.58^{a}	0.49	20.98^{a}	0.88^{a}	47.73^{b}	2.02^{b}
T1	2395.0^{b}	1862.0^{b}	77.75	10.48^{b}	0.45	16.08^{b}	0.67^{b}	52.20^{a}	2.18^{a}
T2	2445.0^{a}	1947.4^{a}	79.65	10.83^{b}	0.44	20.75^{a}	0.85^{a}	45.58^{b}	1.86^{b}
T3	2425.0^{a}	1901.3^{a}	78.40	10.78^{b}	0.44	19.70^{a}	0.81^{a}	54.48^{a}	2.25^{a}
SEM	49.95	46.23	4.865	0.976	0.03	1.317	0.12	3.413	0.231
Sig.	*	*	NS	*	NS	*	*	*	*

a,b,, Means within a column with different superscripts are significantly different ($P< 0.05$).

Conclusion:

It is concluded that fortification of broiler chick's diet with dicalcium phosphate nanoparticles at a rate of 2 g / kg or vitamin D at a rate of 1ml/L could be recommended to alleviate bone disorders resulted from genetic selection for fast growth and to support bone strength and productive performance traits and may be practically applied to improve growth performance and carcass characteristics at the marketing age.

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