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REGULAR ARTICLES

Effect of coated calcium feeding on growth performance, carcass traits, immunity, blood biochemistry and tibial bone morphometry in commercial broiler chicken

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Abstract

The bone abnormalities pose great concern in heavy, rapidly growing broilers, resulting in significant economic loss, and health issues in birds. To address these problems and prevent unnecessary mineral excretion into environment, it is crucial to optimise the inclusion level of dietary calcium in broiler's diet. The calcium utilisation by the bird is also affected by its source, particle size, bioavailability etc. Hence this study aimed to study the effect of partially replacing conventional dietary calcium source with coated calcium on growth performance, immune response, carcass traits, blood calcium and phosphorus, and mineral retention in broilers. About 416 broiler chicks were randomly distributed into four treatments, each with eight replicates of 13 birds and fed with iso-calcium, isocaloric, and isonitrogenous diet for 42 days. The T_0 (control) was a basal diet while the T_1, T_2, and T_3, were treatment diets in which conventional calcium source (limestone) was partially replaced with coated calcium by 10, 20, and 30% respectively. The birds of T_3 group showed higher ($P<0.05$) body weight gain, better feed conversion ratio and higher carcass yields compared to T_0, T_1, and T_2 diet fed birds. Further the treatment groups exhibited higher ($P<0.05$) cell-mediated and humoral immune responses and immune organ weights compared to control. The tibial bone weight, length and average diameter was highest ($P<0.05$) in T_3. Moreover, the T_3 diet fed group also showed higher ($P<0.05$) calcium and phosphorus content in serum and bone and lowest content of these minerals in the excreta followed by T_2, T_1 and control. These findings suggest that a 30% conventional calcium source with coated calcium may be used as a high-grade mineral source for broiler chicken feed.

Keywords Coated calcium feeding · Production performance · Blood biochemicals and tibial bone morphometry · Commercial broiler chicken

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Introduction

The bone abnormalities in heavy, rapidly growing broilers have emerged as a significant concern, contributing to the development of contact dermatitis, breast blisters, lameness, skin lesions, and poor gait scores, ultimately compromising farm profit and bird well-being. Nutrition plays a crucial role in bone quality and skeletal development, with even slight nutritional inadequacies impacting broiler health (Pohl et al. 2012). Calcium, along with Vitamin D3 and Phosphorus, is essential for healthy bone development. In the early growth phase, calcium is crucial for bone production, growth, and rapid regeneration (Veum 2010).

Broiler chickens require a balanced supply of high-grade minerals, including calcium from various sources like bone meal, oyster shell, limestone, and calcium carbonate (Vega 2012). These sources provide calcium, with inorganic sources containing 17-38.5% calcium and organic sources less than 1% (Vega 2012). Recently, Abdelqader et al. (2022) highlighted the importance of calcium sources in broiler diets to prevent skeletal disorders. Different calcium sources have been investigated, including bivalve shell, periwinkle shell, oyster shell, marble dust, and calcium carbonate (Ajakaiye et al. 2003).

Calcium carbonate, derived from limestone, is commonly used in poultry diets, but alternative sources like marine shells can also be utilized (Guinotte et al. 1991). The particle size of calcium carbonate affects its utilization, with coarse particles improving feed conversion ratio, tibia ossification, and calcium and phosphorus retention (Guinotte et al. 1991; Zohravi et al. 2001). Coated calcium, a calcium supplement treated with coating materials like fatty acids, enhances stability, solubility, and bioavailability, benefiting broiler diets by improving bioavailability, gut health, and bone development, while reducing waste.

Coated calcium, treated with coating materials like fatty acids, enhances its utilization by improving solubility, protecting it from acidic gut environments, and providing a slow release, leading to enhanced bioavailability and absorption. This results in reduced excretion and improved retention, as more calcium is absorbed and utilized by the body, rather than being wasted. However, overdosing on calcium can impede digestion and slow growth (Tamim et al. 2004). Therefore, it's essential to determine the optimal levels and durations of calcium supplementation for improved broiler health and productivity.

This study aims to investigate the effect of feeding coated calcium on growth performance, carcass traits, immune response, blood biochemical parameters, and tibial bone morphometry in commercial broiler chickens, compared to traditional calcium sources. The study seeks to provide insights into the potential benefits of coated calcium as an alternative calcium source, shedding light on its impact on broiler chicken health and productivity.

Materials and methods

Seasons and climate of the experimental location

The experiment was conducted for six weeks during November-December 2023 at the Experimental Broiler Shed located at the College of Veterinary and Animal Sciences, MAFSU, Udgir, Maharashtra, India. Udgir has a tropical wet and dry or savanna climate (Classification: Aw). The district's yearly temperature is 29.31ºC (84.76ºF) and it is 3.34% higher than India's averages. Udgir typically receives about 117.6 millimeters (4.63 inches) of precipitation and has 120.35 rainy days (32.97% of the time) annually.

Experimental birds, diets and design

A total of 416-day-old Ven Cobb 430 Y broiler chicks were randomly assigned to four different treatment groups, each consisting of eight replicates with 13 chicks per replicate, resulting in 104 broiler chicks per treatment group. The treatment groups were designated as T0 (control), T1, T2, and T3. The T0 group received a basal diet, while the T1, T2, and T3 groups received treatment diets in which conventional calcium source (limestone) was partially replaced with coated calcium by 10%, 20%, and 30%, respectively. All diets were formulated to be iso-calcium, isocaloric, and isonitrogenous and were fed to the broilers for 6 weeks. The fatty acid coated calcium was procured from Morning BIO Co., Ltd., South Korea. The coated calcium used in this study was found to be white-colored beads through physical examination. Chemical analysis revealed that it contained 52.5% calcium carbonate, corresponding to 40% calcium, and 177,300 IU/kg of vitamin D3. These analyses were conducted using Feed Standard Analysis Methods 4.1 and 6.8 for calcium carbonate and vitamin D3, respectively. During the experiment, feed and water were provided *ad-lib*. The various feed ingredients were procured from local markets to formulate a mash-type broiler diet. The ingredients and nutrients in the experimental diets formulated are presented in Tables 1 and 2. A proximate analysis of the experimental diets was performed based on AOAC recommendations (2016).

The basal diet served as control (T_0), while in the experimental diets T_1, T_2, and T_3, the conventional calcium source i.e. limestone was replaced with coated calcium at 10, 20 and 30%, respectively by keeping all diets iso-calcium, isocaloric and isonitrogenous. The present experiment was

Table 1 Ingredient composition (%) of experimental diets

Sr. No	Ingredient (Kgs)	Treatments											
		Basal broiler diets (T_0)			T_1			T_2			T_3		
		Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher	Pre-starter	Starter	Finisher
1.	Maize	54.648	55.603	59.611	54.679	55.632	59.652	54.745	55.677	59.677	54.759	55.693	59.682
2.	Soybean meal	39.205	36.895	31.83	39.205	36.895	31.83	39.19	36.893	31.83	39.19	36.89	31.83
3.	Vegetable Oil	2.474	3.92	4.958	2.462	3.908	4.942	2.44	3.892	4.932	2.435	3.886	4.932
4.	DCP#	1.78	1.83	1.90	1.78	1.83	1.90	1.78	1.83	1.90	1.78	1.83	1.90
5.	LSP##	0.89	0.87	0.85	0.801	0.783	0.765	0.712	0.696	0.68	0.623	0.609	0.595
6.	Coated Calcium	0.000	0.000	0.000	0.07	0.07	0.06	0.13	0.13	0.13	0.21	0.21	0.21
7.	Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
8.	Trace Min. Premix*	0.11	0.10	0.10	0.11	0.10	0.10	0.11	0.10	0.10	0.11	0.10	0.10
9.	Vitamin premix**	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
10.	DL-Methionine	0.148	0.131	0.106	0.148	0.131	0.106	0.148	0.131	0.106	0.148	0.131	0.106
11.	L-Lysine	0.13	0.036	0.03	0.13	0.036	0.03	0.13	0.036	0.03	0.13	0.036	0.03
12.	Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
13.	Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
14.	Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
15.	B-Complex***	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
	Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

*Trace mineral premix supplied Mg- 300, Mn- 55, I- 0.4, Fe- 56, Zn-30, and Cu- 4 mg/Kg diet. **The vitamin premixes supplied Retinol 2475 mcg, Cholecalciferol 30 mcg; Menaquinone 1 mg; Tocopherol 26.8 mg d-alpha tocopherol /Kg diet. ***B-complex provided Thiamine 2 mg, Riboflavin 4 mg, Cyanocobalamin 10mcg; Niacin 60 mg; Pantothenic acid 10 mg/Kg diet

DCP# (Dicalcium Phosphate); LSP## (Limestone Powder)

Table 2 Nutrient composition (calculated) of pre-starter, starter, and Finisher rations (% DM) basis

Sr. No.	Nutrients (%)	Pre starter	Starter	Finisher
	Basal (T_0) diet			
1.	Crude protein	23.12	22.03	19.93
2.	Calcium	1.00	1.00	1.00
3.	Available Phosphorus	0.45	0.45	0.45
4.	Lysine	1.338	1.181	1.042
5.	Methionine	0.531	0.501	0.450
6.	ME, Kcal/kg	2999.70	3100.80	3203.97
	Treatment (T_1) diet			
1.	Crude protein	23.12	22.03	19.93
2.	Calcium	1.00	1.00	1.00
3.	Available phosphorus	0.45	0.45	0.45
4.	Lysine	1.338	1.181	1.042
5.	Methionine	0.531	0.501	0.450
6.	ME, Kcal/kg	2999.70	3100.80	3203.97
	Treatment (T_2) diet			
1.	Crude protein	23.12	22.03	19.93
2.	Calcium	1.00	1.00	1.00
3.	Available Phosphorus	0.45	0.45	0.45
4.	Lysine	1.338	1.181	1.042
5.	Methionine	0.531	0.501	0.450
6.	ME, Kcal/kg	2999.70	3100.80	3200.97
	Treatment (T_3) diet			
1.	Crude protein	23.12	22.03	19.93
2.	Calcium	1.00	1.00	1.00
3.	Available Phosphorus	0.45	0.45	0.45
4.	Lysine	1.338	1.181	1.042
5.	Methionine	0.531	0.501	0.4506
6.	ME, Kcal/kg	2999.70	3100.80	3200.97

carried out with the approval of the Institutional Animal Ethics Committee (IAEC), College of veterinary and Animal Sciences, Udgir (Approval No. IV/23/Avian/IAEC/2023).

Experimental procedure

The growth performance such as body weight gain, feed intake and feed conversion ratio were recorded at weekly intervals for a period of 6 weeks. Feed intake was calculated by subtracting residual feed from offered feed, and body weights were recorded weekly using an electronic balance (0.1 g accuracy). Body weight gain and FCR were calculated from these data, with FCR determined as feed intake divided by weight gain (Leeson and Summers 2001).

Immunological response

Cell-mediated immune response

On day 22 post-hatch, cutaneous basophilic hypersensitivity was assessed in 32 broilers using PHA-P (Phytohaemagglutinin-P, a lectin from *Phaseolus vulgaris*) (Corrier and Deloach 1990). Toe web thickness of eight birds from each treatment group was measured before PHA-P administration. Then, 0.1 ml of PHA-P (1 mg/ml) was injected into the right footpad, and 0.1 ml of PBS into the left footpad as a placebo. After 24 h, swelling was measured, and the web index was calculated as: CMIR = (R2-R1) - (L2-L1), where R1 and R2 represent the thickness of the right footpad web before and after PHA-P injection, respectively, and L1 and L2 represent the thickness of the left footpad web before and after PBS injection, respectively.

Humoral immune responses (antibody titre against ND virus in broilers)

The chicks were vaccinated against ND (LaSota) and IBD on days 7 and 14 post-hatch, with booster doses of ND (LaSota) and IBD (Georgia intermediate strain) given on days 21 and 28, respectively. Antibody titers against New Castle disease virus were measured in 8 birds per treatment using the Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests, as described by Allan and Gough (1974). The HA test determined the highest antigen dilution showing 50% haemagglutination, while the HI test measured the reciprocal of the highest serum dilution inhibiting 50% haemagglutination.

Weight of immune organs

The weights of immune organs, specifically the bursa, spleen, and thymus, were recorded using a digital weighing balance in eight birds from each treatment group after they were humanely sacrificed at the end of the trial.

Carcass traits

At the end of the trial, 8 birds from each dietary treatment group were sacrificed to evaluate carcass traits. After an 8-hour fasting period with access to water, the birds were slaughtered and various parameters were recorded, including: fasting weight, feather-free weight, eviscerated weight, weights of cut-up parts (breast, thigh, drumstick, back, neck, wings), giblet weights (heart, liver, gizzard), and fat pad percentage of live weight.

Tibial bone morphometric analysis

After measuring cut-up parts yield, the left drumstick from each replicate was cleaned and stored at -20ºC. Fat was extracted from the bone samples using petroleum spirit (boiling point: 40–60ºC) for 24 h. The dried bone sample was then weighed and measured using a digital balance and

vernier callipers to record bone length, and proximal, middle, and distal widths to calculate average tibia diameter.

Tibial bone calcium and phosphorus estimation

The tibial bones were dried at 105ºC for 24 h and then incinerated in a muffle furnace at 550ºC for 6 h. The resulting ash was used to estimate bone calcium (Ca) and phosphorus (P) content.

The calcium content in the tibial ash was determined using the saturated ammonium oxalate method (AOAC 1990), wherein calcium present in an aliquot was precipitated as insoluble calcium oxalate in an acidic medium by adding saturated ammonium oxalate solution. The precipitate was then dissolved in dilute sulfuric acid (1:9), heated, and the released oxalic acid was titrated against standard potassium permanganate solution at 60 °C to determine the calcium content of the sample (Vogel 1961).

Similarly, the phosphorus content in the tibial ash was determined using the ammonium molybdate method (APHA 1992), wherein the ash was digested with hydrochloric and nitric acids, and phosphorus was precipitated as ammonium phosphomolybdate. The precipitate was washed until free from acid and dissolved in a known excess of standard N/10 HCl or HNO3 to arrive at the exact quantity of standard N/10 NaOH required to neutralize the excess acid, as described by Jackson (1958).

Serum calcium and phosphorus estimation

At the end of the trial, blood samples were collected from the wing vein of 8 birds per treatment group using sterile serum collection tubes (Guide for the Care and Use of Laboratory Animals, National Research Council, 1996). Following coagulation, the samples were centrifuged at 418.86 rad/s. for 10 min using a refrigerated centrifuge at a temperature of 4 °C to collect serum (XL 200 Biochemistry Analyzer User Manual, Erba Mannheim, 2019). Subsequently, the serum was stored at -20 °C until needed for estimation of calcium (Ca) and phosphorus (P) concentrations using a biochemical analyzer (Erba Mannheim, XL 200).

The calcium concentration was measured using the Arsenazo III method, employing a Calcium Erba Diagnostic kit (ERBA CALCIUM-Arzenazo Cat. No. 120226, Erba Mannheim, 2020). The color intensity was measured in the analyzer at a wavelength of 650 nm at 37 °C. The Arsenazo III method operates on the principle that it forms a colored chromophore when combined with calcium ions at pH 6.5 (Quantitative Inorganic Vogel 1961). The absorbance of this chromophore is then measured at 650 nm (650–660 nm), and the resulting value is directly proportional to the calcium concentration.

The serum phosphorus concentration was estimated using a Phosphorus Erba Diagnostic kit (ERBA PHOSPHOROUS Cat. No. 120229, Erba Mannheim, 2020). In the presence of strong acids, inorganic phosphorus reacts with ammonium molybdate to produce phosphomolybdate (Standard Methods for the Examination of Water and Wastewater, APHA 1992). The measurement of reduced phosphomolybdate is conducted at 340 nm and is directly correlated to the concentration of inorganic phosphorus in the sample (Soil Chemical Analysis, Jackson 1958).

Mineral retention – faecal calcium and phosphorus estimation

A 3-day balance study was conducted in thirty-two birds (eight birds per treatment) from 39 to 41 days of age. During this period, the net feed consumed by each bird in their respective dietary treatment group was recorded, and the droppings voided over the same period were collected quantitatively.

After completing the 38-day feeding trial, these birds were starved for 2 h (from 8 to 10 AM). During this time, preliminary preparations were carried out, including cleaning of feeders and fecal trays, provision of fresh drinking water, and weighing of the required quantity of feed into respective feeders. Exactly at 10 AM, the respective feeders and fecal trays were introduced into the cages housing the birds, and a weighed quantity of feed was offered to all birds.

The droppings from each bird in their respective dietary group were collected separately once daily and transferred into pre-weighed aluminum dishes, which were then weighed again and placed into a forced draft hot air oven at 60 ± 5 °C for all three days of collection. On the last day, the feeders were removed at 10 AM to determine the net feed intake (FI), and the fecal trays were removed to collect the excreta in aluminum dishes.

The collected droppings were dried for 4–5 days in the oven at 60 ± 5 °C until a constant weight was attained, representing the net dried excreta output. The dried and pooled feed and excreta samples were ground and stored in airtight containers for further analysis of calcium (Ca) and phosphorus (P) content (AOAC 1990).

Statistical analysis

Data from multiple treatment groups were analyzed using a completely randomized design and standard statistical procedures (Snedecor and Cochran 1994). Antibody titers were expressed as log2 values. Statistical analysis was performed using SPSS version 20.0, employing Analysis of Variance (ANOVA) followed by Duncan's Multiple Range

Test (DMRT) as the post-hoc test for variables with unequal observations. Results are presented as means ± standard error, with significance set at $P < 0.05$.

Results

Body weight gain, feed intake and FCR

The results of body weight gain, feed consumption and FCR for different treatment groups on phase-wise basis (g/b) is shown in Table 3. The research findings indicated that the substitution of conventional calcium source with coated calcium resulted in a significant increase in body weight gain compared to birds in the control group. The body weight gain (g/b) after 42 days for treatment groups T_0, T_1, T_2, and T_3 were 2747.6, 2784.3, 2816.1, and 2846.0, respectively. The gain in body weight during the 0–21, 0–28, 0–35, and 0–42 days was also significantly higher in the treatment groups. The study revealed that the body weight gain in treatment groups T_1, T_2, and T_3 during the 0–42 days - period was 36.7 g, 68.5 g, and 98.4 g higher than the control group.

The phase-wise feed consumption was comparable among treatment groups. By the end of the sixth week, cumulative feed intake was higher in treatment groups T_1, T_2, and T_3 compared to control group T_0. The inclusion of coated calcium supplementation in the diets resulted in lower feed consumption, but there were no significant improvements observed between the treatment groups. Further the addition of coated calcium to the diet at 10%, 20%, and 30% levels resulted in a significant ($P < 0.05$) improvement in FCR (0–28 days, 0–35 days, and 0–42 days). Birds that were fed with 30% coated calcium exhibited better FCR during the 0-28-, 0-35-, and 0–42-day periods. These findings demonstrate the positive impact of coated calcium on FCR.

Carcass traits

Moreover, it could be observed from Table 4 that the percentage of blood loss was higher and shrinkage ($P < 0.000$) and feather ($P < 0.007$) loss were lower in the treatment groups. The greatest blood loss was observed.

when a 30% replacement of the conventional Calcium source was made with coated Calcium. Importantly, the.

eviscerated yields for T_2 and T_3 were higher than those for T_0 and T_1, indicating that the replacement of conventional calcium by 20%, and 30% with coated calcium was beneficial to improve eviscerated yields. The.

yield was better when there was a 30% replacement of conventional calcium with coated calcium. Compared to basal control (T_0), the ready-to-cook yield of birds fed with coated calcium (T_1, T_2, and T_3) was significantly higher ($P < 0.000$). Notably, the supplementation of 30% coated calcium by replacing conventional calcium (T_3) resulted in a significantly higher yield compared to 10% (T_1) and 20% (T_2) replacement.

Table 3 Effect of dietary supplementation of coated calcium on BWG, FI and FCR of broilers during 42 days trial

Parameters	Treatments				Pooled SEM	*P* Value
	T_0	T_1	T_2	T_3		
BWG (grams)						
0–21 days	933.4^{a}	938.9^{a}	954.4^{b}	968.9^{c}	1.553	$P < 0.000$
21–42 days	1814.2^{a}	1845.3^{ab}	1861.6^{b}	1877.0^{b}	7.170	$P < 0.014$
0–28 days	1438.5^{a}	1447.1^{a}	1467.6^{b}	1478.4^{b}	2.745	$P < 0.000$
0–35 days	2043.1^{a}	2076.7^{b}	2094.3^{bc}	2114.1^{c}	4.041	$P < 0.000$
0–42 days	2747.6^{a}	2784.3^{ab}	2816.1^{bc}	2846.0^{c}	7.189	$P < 0.000$
FI (grams)						
0–21	1127.0	1110.3	1114.5	1116.2	5.21	$P < 0.727$
21–42	3230.8	3219.9	3209.9	3171.0	28.80	$P < 0.904$
0–28	1878.0	1852.5	1853.8	1835.7	11.68	$P < 0.665$
0–35	2908.3	2897.0	2878.8	2858.8	15.66	$P < 0.716$
0–42	4357.7	4330.2	4324.4	4287.3	30.00	$P < 0.885$
0–21	1127.0	1110.3	1114.5	1116.2	5.21	$P < 0.727$
FCR						
0–21 days	1.22^{b}	1.19^{ab}	1.17^{a}	1.15^{a}	0.007	$P < 0.016$
21–42 days	1.79	1.74	1.72	1.69	0.015	$P < 0.174$
0–28 days	1.31	1.28	1.27	1.25	0.010	$P < 0.149$
0–35 days	1.44^{b}	1.40^{ab}	1.38^{a}	1.36^{a}	0.010	$P < 0.024$
0–42 days	1.60^{b}	1.56^{ab}	1.54^{ab}	1.51^{a}	0.012	$P < 0.050$
0–21 days	1.22^{b}	1.19^{ab}	1.17^{a}	1.15^{a}	0.007	$P < 0.016$

Values bearing superscripts differed significantly ($P < 0.05$); NS = non-significant.

Table 4 Effect of dietary supplementation of coated calcium on carcass traits and organ weight (% of live weight) of broilers at 42 days of age

Traits	Treatments				Pooled SEM	*P* Value
	T_0	T_1	T_2	T_3		
Shrinkage	5.63^{c}	5.23^{b}	5.31^{b}	4.89^{a}	0.062	$P<0.000$
Blood loss	2.98^{a}	3.07^{b}	3.16^{c}	3.24^{d}	0.021	$P<0.000$
Feather loss	4.70^{a}	4.64^{ab}	4.54^{a}	4.55^{a}	0.019	$P<0.007$
Eviscerated yields	69.09^{a}	69.45^{a}	69.76^{ab}	70.41^{b}	0.141	$P<0.003$
Ready to cook yield	74.12^{a}	74.67^{ab}	74.99^{b}	75.79^{c}	0.157	$P<0.000$
Heart	0.78^{a}	0.79^{a}	0.79^{a}	0.82^{b}	0.005	$P<0.015$
Liver	2.23^{a}	2.32^{b}	2.34^{bc}	2.39^{c}	0.013	$P<0.000$
Gizzard	2.03^{a}	2.12^{bc}	2.10^{b}	2.17^{c}	0.014	$P<0.001$
Giblet	5.04^{a}	5.22^{b}	5.23^{b}	5.37^{c}	0.026	$P<0.000$
Breast	21.50^{a}	21.90^{b}	22.03^{b}	22.40^{c}	0.075	$P<0.000$
Back	15.63	15.53	15.57	15.57	0.043	$P<0.868$
Drumstick	10.47^{a}	10.55^{a}	10.55^{a}	10.70^{b}	0.023	$P<0.003$
Thigh	10.57^{a}	10.65^{a}	10.65^{a}	10.82^{b}	0.029	$P<0.010$
Neck	3.62	3.59	3.60	3.65	0.011	$P<0.140$
Wing	7.29	7.22	7.35	7.26	0.030	$P<0.493$
Abdominal fat pad Thickness	0.65	0.63	0.62	0.60	0.007	$P<0.058$

Values bearing superscripts differed significantly ($P<0.05$); NS = non-significant

Table 5 Effect of dietary supplementation of coated calcium on the immune response of broilers

Immune response	Treatments				Pooled SEM	*P*-Value
	T_0	T_1	T_2	T_3		
CMI PHP-P foot web index (mm)	0.24^{a}	0.27^{ab}	0.29^{bc}	0.32^{c}	0.007	0.001
Humoral-HI titer against ND (log 2)	4.38^{a}	5.38^{b}	5.63^{b}	5.88^{b}	0.193	0.024
Lymphoid Organ Weight						
Bursa (% of live wt.)	0.14^{a}	0.15^{a}	0.16^{ab}	0.17^{b}	0.003	0.008
Spleen (% of live wt.)	0.22	0.22	0.23.	0.23	0.002	0.129
Thymus (%of live wt)	0.40^{a}	0.41^{a}	0.43^{b}	0.43^{b}	0.003	0.000

Values bearing superscripts differed significantly ($P<0.05$); NS = non-significant

The relative weights of heart, liver and gizzard were significantly higher in treatment groups (T_1, T_2 and T_3) compared to control group T_0. The giblet weight was significantly ($P<0.000$) higher in treatment group T_3 compared to control group T_0. Regarding the cutup parts yields, significant effects were observed on the relative yields of the breast ($P<0.000$), drumstick ($P<0.003$), thigh ($P<0.010$), and neck ($P<0.140$). The breast yield was 21.50%, 21.90%, 22.03%, and 22.40% for the treatment groups T_0, T_1, T_2 and T_3 respectively. Compared to the other treatment groups, the breast yield was higher in T_2 and T_3 when birds were fed 20% and 30% coated calcium by replacing conventional limestone. The yield increased when birds were fed 30% coated calcium. The dietary supplementation of 30% coated calcium was significantly better than the control group diet T_0. However, no significant difference was observed in the back, wing and fat yield among the treatments. Nevertheless, the inclusion of 30% coated calcium resulted in a noteworthy decrease in fat pad thickness.

Immune response

The results of the cell-mediated and humoral immune response are presented in Table 5. As compared to basal control; the foot web index increased by a significant ($P<0.001$) amount in coated calcium-fed birds. Birds fed by replacing 30% conventional calcium with coated calcium had a higher foot web index. The serum antibody titer (expressed as log 2 values) against New castle disease was significantly higher in treatment groups (T_1, T_2, and T_3) compared to control (T_0). The relative weight of the bursa and thymus was significantly higher when the conventional calcium source was replaced with a coated calcium source either by 20 or 30%.

Tibial morphometry and mineral profile

The impact of coated calcium on the weight of dry fat-free tibia (measured in grams), and the morphometry of the tibia (measured in millimeters), are illustrated in Table 6. After 42 days of age, the tibial.

Table 6 Effect of dietary supplementation of coated calcium on tibial bone morphometry and mineralization of broilers at 42 days of age

Treatments	Tibial bone								
	Weight (g)	Length (mm)	Width (mm)			Average diameter (mm)	Ash (%)	Ca (%)	*P* %
			Proximal	Middle	Distal				
T_0	9.47^{a}	99.73^{a}	22.52^{a}	7.11^{a}	17.25^{a}	15.63^{a}	40.35^{a}	17.09^{a}	8.52^{a}
T_1	9.76^{a}	101.44^{b}	25.41^{b}	8.03^{b}	17.61^{ab}	17.02^{b}	43.84^{ab}	18.06^{ab}	9.51^{ab}
T_2	10.76^{ab}	101.59^{b}	25.44^{b}	8.60^{b}	18.53^{bc}	17.52^{b}	47.02^{ab}	18.30^{ab}	10.02^{b}
T_3	11.19^{c}	102.37^{b}	25.90^{b}	8.53^{b}	19.16^{c}	17.86^{b}	51.37^{c}	19.49^{b}	10.18^{b}
Pooled SEM	0.254	0.239	0.334^{b}	0.159^{b}	0.232	0.218	1.475	0.299	0.241
P -Value	0.043	0.000	0.000	0.001	0.008	0.000	0.046	0.034	0.50

Values bearing superscripts differed significantly ($P < 0.05$); NS = non-significant

Table 7 Effect of dietary supplementation of coated calcium on calcium and phosphorus concentration in serum and faeces of broilers

Treatments	Serum (mg/dl)		Faeces (%)		
	Ca	*P*	Ash	Ca	*P*
T_0	9.80^{a}	4.90^{a}	15.75^{b}	2.80^{b}	1.12^{c}
T_1	10.31^{ab}	5.16^{ab}	12.10^{a}	1.86^{a}	1.05^{ab}
T_2	10.68^{c}	5.34^{bc}	10.85^{a}	1.81^{a}	1.00^{ab}
T_3	11.09^{c}	5.57^{c}	10.76^{a}	1.79^{a}	0.92^{a}
Pooled SEM	0.160	0.077	0.555	0.127	0.028
P -Value	0.022	0.009	0.001	0.005	0.050

Values bearing superscripts differed significantly ($P < 0.05$); NS = non-significant

Weight for the groups T_0, T_1, T_2, and T_3 were 9.47, 9.76, 10.76, and 11.19 g, respectively. The tibial length for groups T_0, T_1, T_2, and T_3 were 99.73 mm, 101.44 mm, 101.59 mm, and 102.37 mm, respectively. The average tibial diameter for groups T_0, T_1, T_2, and T_3 was measured as 15.63 mm, 17.02 mm, 17.52 mm, and 17.86 mm, respectively. The treatment group T_3 showed significantly higher values for tibial weight, tibial length, and average tibial diameter compared to T_0, T_1, and T_2 respectively. This indicates that replacing 30% of conventional calcium with coated calcium had a more beneficial effect on tibial morphometry compared to the control group. Further it could also be noted from Table 6 that there was a significant increase in tibial ash, calcium and phosphorus content in T_3 compared to other dietary treatments.

Blood biochemical analysis

The effect of coated calcium supplementation on serum calcium (Ca) and serum phosphorus (P) levels in mg/dl at 42nd day are shown in Table 7. There were significant differences in serum Ca ($P < 0.022$) and serum P ($P < 0.009$) concentrations among the treatment groups. Serum Ca levels were 9.80, 10.31, 10.68, and 11.09 mg/dl for T_0, T_1, T_2, and T_3, respectively. T_3 had the highest serum Ca level. Among the treatment groups T_0, T_1, T_2, and T_3, the serum P levels were 4.90, 5.16, 5.34, and 5.57 mg/dl, respectively. It is worth mentioning that T_3 exhibited the highest serum Ca and P levels. This indicates that replacing 30% of conventional calcium with coated calcium had a more beneficial effect on serum Ca and P levels compared to the control group.

Mineral retention

The analyzed levels of faecal calcium and phosphorus percentage (Table 7) show that there was significant variations in the concentrations of faecal Ca ($P < 0.005$) among the different treatment groups. The estimated faecal calcium percentage were 2.80, 1.86, 1.81, and 1.79 for T_0, T_1, T_2, and T_3 respectively. T_3 exhibited a lower faecal Ca% compared to the other treatment groups. Conversely, the recorded levels of faecal P % were 1.12, 1.05, 1.00, and 0.92 for T_0, T_1, T_2, and T_3, respectively. T_3 exhibited a lower faecal P % compared to the other treatment groups, namely T_0, T_1, and T_2. There was a significant difference in the faecal P ($P < 0.050$) between the control and treatment groups. This indicates that incorporating 30% coated calcium in the diet had a more beneficial effect on the retention of calcium and phosphorus in the birds compared to the control group.

Discussion

Body weight gain, feed intake and FCR

In the present study, the treatment groups exhibited significantly higher body weight gain during the 0-3-, 0-4-, 0–5, and 0–6-week intervals in comparison to the control. The body weight gain was highest ($P < 0.01$) in group T_3 when 30% of the conventional calcium source in the basal diet was replaced with coated calcium. The current findings are consistent with previous research conducted by Vijayakumar and Balakrishnan (2015), who found that broilers fed with nano dicalcium phosphate exhibited improved body weight gain compared to those on a diet containing regular dicalcium phosphate. Similarly, Hassan et al. (2016) revealed that broilers fed with nano dicalcium phosphate experienced a 25% gain in body weight compared to those

on a diet containing regular dicalcium phosphate. Additionally, Makola et al. (2021) discovered that birds fed with nano dicalcium phosphate exhibited the maximum weight gain compared to those fed with a diet containing regular dicalcium phosphate. Furthermore, Rekaby et al. (2023) found that at four weeks of age, broiler chicks that were fed a basal diet enriched with nano dicalcium phosphate had significantly higher gain in body weight compared to broilers fed with regular dicalcium phosphate.

However, the current results contradict the previous findings of Tabeidian and Sadeghi (2006). Their study revealed that the intake of varying levels of fat powder calcium salts of fatty acid significantly ($P < 0.05$) reduces weight gain. In another study, Fallah et al. (2018), indicated that increasing concentrations of calcium and the use of oyster shells as a source led to a greater reduction in average weight gain compared to limestone in the diet.

The weekly and phase-wise feed consumption were comparable among treatment groups in the present study. The cumulative feed intake was higher in treatment groups T_1, T_2, and T_3 compared to control group T_0. The addition of coated calcium to the diet (10%, 20%, and 30%) led to a significant improvement in FCR (0–28 days, 0–35 days, and 0–42 days). A diet in which 30% replacement of conventional Ca with coated Ca had better FCR. The current findings aligned with the study conducted by Henry and Pesti (2002), which indicated that the inclusion of calcium citrate-malate (CC-M) in the diet replaced 0.7% and 0.9% of calcium from limestone without any significant impact on the feed intake of the birds. Moreover, comparable results have been documented in studies by Hassan et al. (2016), Gado et al. (2017), and Mendonca et al. (2022).

Contrary to the present findings, Oso et al. (2011) conducted a study using various calcium sources such as oyster shells, snail shells, and wood ash instead of limestone as the primary source of calcium in the diet. They reported that the diet containing oyster shells had the highest feed intake compared to the one containing limestone. Moreover, the inclusion of different amounts of nano dicalcium phosphate in the diet did not have a significant impact on the average feed intake as observed by Makola et al. (2021).

The present findings are in line with the previous work by Henry and Pesti (2002), indicating that FCR was improved when 0.7% of calcium citrate malate was used in the diet by replacing limestone. Additionally, Vijayakumar and Balakrishnan (2015) reported that broilers fed nano dicalcium phosphate exhibited better FCR compared to those fed dicalcium phosphates. Moreover, Hassan et al. (2016) reported that broilers fed nano dicalcium phosphate demonstrated superior FCR compared to those fed diets containing dicalcium phosphate. Furthermore, Makola et al. (2021) observed that birds fed with 40% nano dicalcium phosphate had better FCR as compared to dicalcium phosphate. However, the results from Gultepe et al. (2021); and Sobhi et al. (2020) contradict our findings.

Carcass traits

The findings of the present study are consistent with the findings of Vijayakumar and Balakrishnan (2015), where the inclusion of nano dicalcium phosphate in the diet led to enhancements in carcass weight, and gizzard weight when compared to diets containing regular dicalcium phosphate. However, there were no notable variations in the weight of the heart, liver, and dressing percentage both in absolute terms and when expressed as a percentage of body weight. similarly, Makola et al. (2021) reported that the replacement of 40%, 60%, and 80% of conventional dicalcium phosphate with nano dicalcium phosphate had the highest slaughter, liver, gizzard, and heart weight. They further reported that the 100% replacement of conventional dicalcium phosphate results in lower breast weight.

Likewise, Mendonca et al. (2022) in a study with different organic calcium sources, including calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, and oyster shell meal revealed that these sources had no impact on the overall weight at slaughter, as well as the absolute and relative weights of the bird's carcass, cuts, and edible offal. In their research, Sobhi et al. (2020) investigated the effects of replacing inorganic dicalcium phosphate with nano-hydroxyapatite in the broiler chicken diet. The findings indicated that the dressing percentage, breast yield, and thigh weight were similar between the inorganic phosphate diet and the nano-hydroxyapatite diet. Additionally, it was observed that the carcass, breast, and drumstick weights were reduced in chickens fed with the 1% nano-hydroxyapatite diet compared to those fed with the inorganic dicalcium phosphate diet.

Mohamed et al. (2016) studied the impact of feeding broiler chickens with nano dicalcium phosphate on carcass weight. They discovered that the addition of nano dicalcium phosphate in the diet improved carcass weight compared to regular dicalcium phosphate diets. A study conducted by Rekaby et al. (2023) stated that birds that were given nano dicalcium phosphate with vitamin D exhibited notably higher dressing percentage and liver percentage. However, there was an observed increase in heart weight and gizzard weight in broilers that were fed with dicalcium phosphate alone.

Immune response

The improved growth performance seems to be linked to both the immune response mediated by cells and the relative

weights of the bursa and thymus. The broilers exhibited improved health and displayed responsiveness to their surroundings during the entire experiment. The higher relative weights of the bursa and thymus, along with increased foot weight indexes, suggest that the inclusion of 30% coated calcium in the diet by replacing conventional calcium had a more favourable impact on immunity and disease resistance compared to the basal control. Similarly, such findings have been reported by Xing et al. (2020), where different Calcium source and levels were found to influence immune organ indices.

Coated calcium improves immune response by enhancing nutrient absorption, increasing cytokine production, and activating immune cells such as T-cells and B-cells. It also supports gut-associated lymphoid tissue (GALT) function, promoting gut health and immune system function. Additionally, coated calcium may reduce inflammation by regulating calcium-mediated signaling pathways, enhance antibody production, and support immune cell signaling pathways, ensuring proper communication between immune cells to coordinate immune responses. By supporting these immune-related processes, coated calcium plays a crucial role in maintaining immune system function and overall health.

Tibial morphometry

The current findings are supported by previous findings of Henry and Pesti (2002), and Mohamed et al. (2016). Additionally, Henry and Pesti (2002), found that broiler birds fed calcium citrate malate had greater tibial weight compared to those fed limestone and dicalcium phosphate in their diet. Mohamed et al. (2016) found that broilers fed nano dicalcium phosphate had resulted in a 27.42% improvement in tibial weight compared to those fed dicalcium phosphate. Contrary to the present findings, Vijayakumar and Balakrishnan (2015) reported a comparable effect on tibial weight when birds were fed with nano dicalcium phosphate compared to those fed dicalcium phosphate. In a study conducted by Mohamed et al. (2016), also reported that broilers fed with nano dicalcium phosphate showed enhanced 9.31% tibial length in comparison to those fed with a diet containing dicalcium phosphate.

However, recent research contradicts previous studies. Vijayakumar and Balakrishnan (2015) found no significant difference in tibial length and width when broilers were fed with nano dicalcium phosphate compared to a diet containing dicalcium phosphate. Oso et al. (2011) also observed no effect on tibia bone diameter and length when broilers were fed with oyster shells, snail shells, wood ash, and limestone. Ajakaiye et al. (2003) found a comparable effect on bone length when different shell sources were used with limestone in broilers. Additionally, Guinotte et al. (1991) found that coarse particle limestone and calcium source origin had no significant effect on tibia length and mechanical parameters.

The increase in bone morphometry may be because coated calcium can be slowly released into the small intestine (thus being absorbed more evenly), which is similar to coarse limestone. maintaining the serum calcium concentration and satisfying the process of calcification and it may improve bone morphometry.

Tibial mineralisation

The results of the present are consistent with the findings of Henry and Pesti (2002). They reported that the broiler bird's diet using calcium citrate malate as a calcium source has the highest percentage of tibial ash compared to the diet formulated using limestone.

The increase in bone mineralization may be because of the slow release of coated calcium into the intestine thus being absorbed more evenly, which is similar to coarse limestone. An increase in serum level of calcium can give positive feedback to the calcitonin hormone leading to more calcium deposition in a bone.

Furthermore, Oso et al. (2011) also found similar results. They reported that broilers fed oyster shells, snail shells, and limestone had higher tibial ash content, compared to broilers fed with limestone alone. Additionally, Mohamed et al. (2016) discovered that broilers fed nano dicalcium phosphate showed a 4.61% improvement in tibial ash content compared to those fed a diet containing dicalcium phosphate. The current findings do not align with the previous study conducted by Pohl et al. (2012). Their research indicated that the supplementation of calcium formate in the diet led to a lower percentage of tibial bone ash compared to limestone. Additionally, Vijayakumar and Balakrishnan (2015), also reported similar findings in their study on broilers fed with nano dicalcium phosphate.

The current findings also supported with previous work of Henry and Pesti (2002), who found that substituting limestone with calcium citrate malate as the calcium source in the diet led to an enhancement in tibial bone calcium levels. Furthermore, Mohamed et al. (2016) investigated the impact of feeding broilers with nano dicalcium phosphate, which resulted in improved 3.62% tibial calcium and phosphorus levels compared to diets containing regular dicalcium phosphate.

The research conducted by Vijayakumar and Balakrishnan (2015) reveals an inconsistency with the present findings, as they observed no significant difference in tibial calcium and phosphorus percentage between broilers fed nano dicalcium phosphate and those containing dicalcium phosphate diet.

Blood biochemical analysis

The results of the blood biochemical analysis of the present study are consistent with the research findings of Henry and Pesti (2002), where the birds fed with calcium citrate malate (CC-M) had higher levels of plasma calcium compared to those fed limestone. A similar finding was reported by Shi et al. (2020), where birds fed a 1% protected Ca diet had higher serum calcium and phosphorus concentrations compared to those fed limestone. Furthermore, Gultepe et al. (2021) conducted a similar study and found that replacing limestone in the layer diet with micro calcium carbonate particles significantly improved serum calcium levels.

The study conducted by Kowalczuk-Vasilev et al. (2023) contradicts the findings of this research and does not support these aforementioned findings. The replacement of inorganic forms of calcium (Ca), iron (Fe), copper (Cu), and zinc (Zn) with glycinates in diets for group II pheasants did not influence changes in the levels of macro elements (Calcium (Ca), potassium (K), Phosphorus (P), sodium (Na), and magnesium (Mg) in blood serum. Similarly, Gado et al. (2017) reported that the supplementation of calcium salt fatty acid with soybean oil did not lead to significant differences in serum Ca and inorganic P levels when dry fat was included in the broilers diet. Additionally, Gongruttananun et al. (2011) conducted a study where calcium from limestone was replaced with ground eggshell (50% and 100%) and observed a decrease in plasma Ca and P levels in layer birds compared to the control group.

Mineral retention

Coated calcium increases serum phosphorus levels through several mechanisms. Improved Ca absorption is achieved as the coating material enhances Ca solubility and bioavailability, leading to increased absorption in the gut, which can result in more Ca being available for absorption and utilization, and subsequently increase P absorption. Additionally, the increased Ca levels raise the Ca-P ratio in the gut and bloodstream, leading to increased P absorption and retention due to their synergistic relationship. Coated Ca may also enhance vitamin D3 activity, regulating mineral homeostasis, including Ca and P, and leading to increased P absorption and retention. Furthermore, coated Ca reduces P excretion by decreasing urinary P loss, allowing more P to be retained in the body and increasing serum P levels. Finally, coated Ca influences hormonal regulation, affecting parathyroid hormone and calcitonin, which play a role in mineral homeostasis, and changes in these hormone levels can increase serum P levels. These mechanisms may contribute to the increase in serum phosphorus levels when using coated calcium.

The current study aligns with the earlier work of Hassan et al. (2016). They reported that broilers fed with nano dicalcium phosphate had lower calcium and phosphorus excretion in droppings compared to those on a dicalcium phosphate diet. However, Sobhi et al. (2020) found inconsistent results, with no significant difference in calcium excretion but a reduction in phosphorus retention when using nano-hydroxyapatite. The coated calcium supplementation increases the surface area which probably could enhance absorption and utilization leading to a reduction in the quantity of supplements and ultimately reduction in mineral excretion.

Conclusion

The bioavailability of coated calcium is a critical factor in determining its efficacy as a dietary supplement. Coated calcium, specifically designed to enhance absorption, exhibits improved bioavailability compared to traditional calcium sources. The coating process protects the calcium from acidic environments, ensuring its stability and integrity throughout the digestive process. This results in increased solubility, dissolution, and ultimately, enhanced absorption in the gut. Furthermore, the coating material itself may also influence bioavailability, with some materials enhancing calcium absorption more effectively than others, with increased calcium levels in the blood and improved bone mineral density.

Hence the dietary replacement of 30% conventional calcium with coated calcium resulted in enhancements in growth performance, immunity, carcass traits, and mineral retention in the present study. The better growth performance and feed conversion ratios observed in birds fed coated calcium could be associated with increased calcium absorption, bioavailability of different nutrients, including minerals, as well as the minimal excretion of the essential nutrients. Hence supplementation of coated calcium improved the growth performance, immune responses and bone health of broiler chicken.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Statement of animal rights The present study was conducted in accordance with the ethical standard and with the approval of the institutional animal ethics committee.

Conflict of interest The authors declare that they have no conflict of interest.

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