Growth performance, carcass and meat quality, bone strength, and immune response of broilers fed low-calcium diets supplemented with marine mineral complex and phytase

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ABSTRACT Influence of marine mineral complex (CeltiCal) as a partial substitute for limestone on growth efficiency, carcass traits, meat quality, bone strength, calcium (Ca) retention, and immune response was investigated in broilers fed low-Ca diets with or without phytase (**PHY**) addition for a 35-d trial period. A total of 300 one-day-old Ross 308 straight-run broilers were randomly allocated to: T1 (positive control), recommended Ca levels + PHY; T2 (negative control), 0.2% below the recommended Ca levels + PHY; T3, 0.1% below the recommended Ca levels + 0.2% CeltiCal + PHY; T4, 0.2% below the recommended Ca levels + 0.4% CeltiCal + PHY; T5, 0.2% below the recommended Ca levels + 0.4% CeltiCal. PHY was added at 500 phytase units/kg diets. Each dietary treatment had 10 replications of 6 chicks each. Results revealed that production efficiency factor was greater for T4 compared to T2 and T5 during 22-35 d and for T1, T3, and T4 compared to T2 during 0 to 35 d ($P < 0.05$). Feed conversion ratio was lower for T3 and T4 compared to T2 and T5 during 0 to 35 d ($P < 0.05$). T4 had a greater ($P < 0.05$) dressing percentage than T2, which had a lighter ($P < 0.01$) small intestinal relative weight than all other treatments. Breast meat temperature at 15 min postmortem was highest for T1 and lowest for T3 ($P < 0.001$). Breast meat pH was greater for T1 compared to T5 at 15 min postmortem and for T3 compared to T4 at 24 h postmortem ($P < 0.05$). T5 had a lower breast meat redness than all other treatments at 15 min postmortem and then T1 and T3 at 24 h postmortem ($P < 0.01$). Tibia and femur weights were greater ($P < 0.05$) for T3, T4, and T5 compared to T2, which had the lowest tibia ash content ($P < 0.05$) and femur geometric properties ($P < 0.001$). Greater antibodies to infectious bronchitis virus ($P < 0.01$) and Ca retention ($P < 0.001$) were observed for T3 and T4 in comparison to T2. Based on the findings of this research, CeltiCal can adequately replace a considerable portion of limestone in broiler reduced-Ca diets containing PHY.

Key words: broiler, calcium, marine mineral complex, exogenous phytase, bone strength

2022 Poultry Science 101:101849
https://doi.org/10.1016/j.psj.2022.101849

INTRODUCTION

Broiler's growth and target body weight has accelerated during recent years; for example, 91 g extra body weight is expected from the Ross 308 strain in 2019 compared to the same strain in 2016 (Aviagen, 2019a). However, the nutritional guidelines did not change and the recommended levels for calcium (**Ca**) and nonphytate phosphorus (**nPP**) in the diet for this particular strain were kept at the same level regardless of the increase in body weight (Aviagen, 2019b). Leg problems during the final stages of growth and bone fractures during the processing of carcasses for fast-growing strains are regarded as main problems in the poultry industry, which can lead to financial losses (Oviedo-Rondón et al., 2006; Shim et al., 2012).

Bone ash content is an indication of bone strength and calcification (Williams et al., 2000). Therefore, some researchers suggested that diets should contain more Ca and nPP than the current recommendations of NRC (1994) to maintain skeletal integrity, especially during the finisher period for modern strains (Venäläinen et al., 2006; Abdulla et al., 2017). On the contrary, another group of researchers has suggested that a moderate reduction of dietary Ca during the

Received December 6, 2021.
Accepted February 28, 2022.

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finisher phase had no harmful influence on performance (downward to 0.73% (Ziaei et al., 2008) or 0.6% (Driver et al., 2005)) and bone ash (toward 0.75% (Singh et al., 2013)). It is well documented that more dietary Ca increases bone ash content (Abdulla et al., 2017; Gautier et al., 2017), however, increasing dietary Ca may worsen phosphorus (P) deficiency for ash criteria (Letourneau-Montminy et al., 2008). Other disadvantages are associated with high Ca levels (0.90%) such as reducing animal performance (Paiva et al., 2013), interfering with macro-mineral absorption (Lonnerdal et al., 1989), decreasing dietary energy digestibility by forming soap precipitates with free saturated fatty acids (Hamdi et al., 2018), and forming Ca −phytate complex which could lessen the action of phytase (**PHY**) (Tamim et al., 2004).

Usually, limestone is utilized as the main inorganic source of Ca in the broiler's diet. However, there are disadvantages associated with limestone, such as low solubility and thus low bioavailability through increased acid-binding capacity of the diet (Anwar et al., 2016a). According to Manangi and Coon (2007), the in vitro solubility of limestone is determined by its source and particle size. In order for Ca to be absorbed in the intestinal lumen, it has to be soluble. The pH of the small intestine and dietary phytate concentration affect Ca solubility in the intestine. When the pH of small intestine is close to neutral, mineral chelates are formed with phytate molecules, rendering these minerals unavailable for poultry (Tamim and Angel, 2003; Plumstead et al., 2008). It has been suggested that limestone decreases protein and P solubility in the gizzard, and therefore may influence protein and P digestibility (Cowieson et al., 2009; Walk et al., 2012a).

In order to solve the problem with low solubility of limestone, different options have been explored such as the use of different limestone particle size (Manangi and Coon, 2007), use of Ca sources with greater availability in the diets like calcified seaweeds (Walk et al., 2012b; Adeola and Walk, 2013), and the extensive use of PHY in the diets to maximize phytate-P utilization (Rutherfurd et al., 2012) Paiva et al. (2013). showed that broilers received diets formulated with highly soluble marine Ca source (Calcified Seaweed) had significantly higher feed efficiency compared to those received limestone as the source of Ca. Hence, it could be suggested that this improved solubility and subsequent bioavailability are beneficial for broilers.

Marine mineral complex (CeltiCal) is a natural marine mineral supplement of the red seaweed algae (*Lithothamnium Calcareum*) (Momeneh et al., 2018). This supplement is comprised mainly of 65% calcite, 23% aragonite, and 12% vaterite (Schlegel and Gutzwiller, 2017) and offers 30% of a highly bioavailable organic Ca source, 5.5% of highly bioavailable magnesium, and 74 trace minerals such as iron, manganese, copper, iodine, zinc, cobalt, selenium, etc. in animal feed (González-Vega et al., 2014; Cruywagen et al., 2015) Momeneh et al. (2018). concluded that utilizing Celtic sea minerals instead of limestone in a low-Ca diet has the potential to improve broiler feed efficiency Walk et al. (2012b). also concluded that substitution of limestone with calcified seaweed enables a reduction in dietary Ca level while maintaining growth performance and bone mineralization, particularly in combination with PHY. The degree of mineralization of bone matrix is considered as a major determinant for bone strength (Follet et al., 2004; Zhang et al., 2012a). Based on the above information, we hypothesized that formulating low-Ca diets with CeltiCal in the presence of PHY could exert favorable influences on performance and bone strength of broilers by improving the digestibility and availability of Ca. Even though many pieces of research have been performed with PHY in broiler diets, there is a lack of experimentation regarding the utilization of CeltiCal in broilers' nutrition. Therefore, the current work was conducted to assess the influences of using CeltiCal at the expense of limestone with or without PHY on growth performance, carcass and meat characteristics, bone strength, immune response, and apparent Ca digestibility in broilers fed low-Ca diets.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of King Saud University, Riyadh, Saudi Arabia (Ethical Reference No: KSU-SE-21-38).

Experimental Design

A total of 300 one-day-old straight-run broiler chicks (Ross 308) with a similar average BW of 44.62 ± 0.37 g were obtained from a commercial hatchery. The chicks were randomly assigned into 5 dietary treatments with 10 replications of 6 chicks each based on a completely randomized block design. The treatments were as follows: T1 (positive control), recommended Ca levels + 500 phytase units (**FTU**)/kg PHY; T2 (negative control), 0.2% below the recommended Ca levels + 500 FTU/kg PHY; T3, 0.1% below the recommended Ca levels + 0.2% CeltiCal + 500 FTU/kg PHY; T4, 0.2% below the recommended Ca levels + 0.4% CeltiCal + 500 FTU/kg PHY; T5, 0.2% below the recommended Ca levels + 0.4% CeltiCal. The CeltiCal (Celtic Sea Minerals LTD., Curraghbinny, Ireland) was formulated in the basal diets as a partial substitute for limestone. According to Celtic Sea Minerals (2014), CeltiCal provides per kg of diet: 300 g bioavailable Ca, 55 g Mg, 7 g K, 800 mg Fe, 500 mg P, 50 mg Mn, 30 mg I, 10 mg Cu, 10 mg Zn, 10 mg B, 0.2 mg Mo, 1.8 mg Se, and 0.1 mg Co. The PHY (Phyzyme XP 10000 TPT, Danisco Animal Nutrition, Marlborough, UK) is a 6-phytase produced by genetically modified *Schizosaccharomyces pombe* strains.

All birds were housed in battery cages at a stocking density of 30 kg BW/m^2 in an environmentally controlled room under recommended environmental, managemental, and hygienic conditions (Aviagen, 2018). The concentrations of Ca (method 968.08) and P (method 965.17) in dietary ingredients were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS, iCAP Q, Thermo Fisher Scientific Inc., Waltham, MA) as indicated by

Table 1. Ingredients and nutrient composition of the starter diets (% as fed basis, unless otherwise indicated).

Ingredients	T1	T2	T3	T4	T5
Yellow corn	50.6	50.6	50.7	50.6	50.6
Soybean meal	42.4	42.4	42.4	42.4	42.4
Wheat bran	0.00	0.55	0.23	0.48	0.00
Corn oil	3.60	3.60	3.60	3.60	3.60
Dicalcium phosphate	1.27	1.27	1.27	1.27	1.94
Limestone	1.08	0.53	0.64	0.19	0.04
Salt	0.30	0.30	0.30	0.30	0.30
Phytase	0.01	0.01	0.01	0.01	0.00
DL-Methionine	0.30	0.30	0.30	0.30	0.30
Lysine-HCL	0.08	0.08	0.08	0.08	0.08
Threonine	0.09	0.09	0.09	0.09	0.09
Choline CL 60	0.05	0.05	0.05	0.05	0.05
CeltiCal	0.00	0.00	0.20	0.40	0.40
Vitamin-mineral premix*	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
ME, kcal/kg	3000	3000	3000	3000	3000
Crude protein	23.0	23.0	23.0	23.0	23.0
Non phytate P	0.48	0.48	0.48	0.48	0.48
Calcium	0.96	0.76	0.86	0.76	0.75
Ca: P ratio	2.00	1.58	1.79	1.58	1.58
Digestible Lysine	1.28	1.28	1.28	1.28	1.28
Digestible TSAA	0.85	0.85	0.85	0.85	0.85
Digestible Threonine	0.86	0.86	0.86	0.86	0.86
Chemical analysis					
Non phytate P	0.48	0.47	0.49	0.49	0.48
Calcium	0.98	0.78	0.86	0.77	0.77
Crude protein	23.1	23.1	22.9	23.0	23.1

*Provides per kg diet: Vit. A, 12,000,000 IU; Vit. D3, 5,000,000 IU; Vit. E, 80,000 IU; Vit. K3, 3,200 mg; Vit. B1, 3,200 mg; Vit. B2, 8,600 mg; Vit. B3, 65,000 mg; Vit. B5, 20,000 mg; Vit. B6, 4,300 mg; Vit. B7, 220 mg; Vit. B9, 2,200 mg; Vit. B12, 17 mg; antioxidant (BHA+BHT), 50,000 mg; Cu, 16,000 mg; I, 1,250 mg; Fe, 20,000 mg; Mn, 120,000 mg; Se, 300 mg; Zn, 110,000 mg.

Table 2. Ingredients and nutrient composition of the grower-finisher diets (% as fed basis, unless otherwise indicated).

Ingredients	T1	T2	T3	T4	T5
Yellow corn	65.4	65.4	65.2	65.5	65.3
Soybean meal	20.7	20.6	20.7	20.7	20.9
Corn Gluten meal	7.00	7.00	7.00	7.00	7.00
Wheat bran	0.60	1.23	1.00	1.00	0.49
Corn oil	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.03	1.02	1.03	1.03	1.69
Limestone	1.04	0.49	0.60	0.15	0.00
Salt	0.30	0.30	0.30	0.30	0.30
Phytase	0.01	0.01	0.01	0.01	0.00
DL-Methionine	0.22	0.22	0.22	0.22	0.22
Lysine-HCL	0.36	0.36	0.36	0.36	0.36
Threonine	0.11	0.11	0.11	0.11	0.11
Choline CL 60	0.05	0.05	0.05	0.05	0.05
CeltiCal	0.00	0.00	0.20	0.40	0.40
Vitamin-mineral premix*	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
ME, kcal/kg	3200	3200	3200	3200	3200
Crude protein	19.5	19.5	19.5	19.5	19.5
Non phytate P	0.40	0.40	0.40	0.40	0.40
Calcium	0.81	0.61	0.71	0.61	0.61
Ca: P ratio	2.05	1.54	1.80	1.54	1.54
Digestible Lysine	1.03	1.03	1.03	1.03	1.03
Digestible TSAA	0.80	0.80	0.80	0.80	0.80
Digestible Threonine	0.69	0.69	0.69	0.69	0.69
Chemical analysis					
Non phytate P	0.40	0.39	0.39	0.39	0.40
Calcium	0.82	0.62	0.71	0.60	0.61
Crude protein	23.1	23.1	22.9	23.0	23.1

*Provides per kg diet: Vit. A, 12,000,000 IU; Vit. D3, 5,000,000 IU; Vit. E, 80,000 IU; Vit. K3, 3,200 mg; Vit. B1, 3,200 mg; Vit. B2, 8,600 mg; Vit. B3, 65,000 mg; Vit. B5, 20,000 mg; Vit. B6, 4,300 mg; Vit. B7, 220 mg; Vit. B9, 2,200 mg; Vit. B12, 17 mg; antioxidant (BHA+BHT), 50,000 mg; Cu, 16,000 mg; I, 1,250 mg; Fe, 20,000 mg; Mn, 120,000 mg; Se, 300 mg; Zn, 110,000 mg.

AOAC (2019), and the analyzed values were incorporated into the nutrient matrix for diet formulations, considering the amount of phytate-bound these 2 elements liberated by the addition of PHY. Corn-soybean meal-based diets for starter (Table 1) and grower-finisher (Table 2) phases were prepared in accordance with the strain recommendations (Aviagen, 2019b) except for Ca. The experimental diets consisted of 0.96, 0.76, 0.86, 0.76, and 0.76% Ca, respectively with the same level of nPP (0.48%) for starter (0 to 21 d) and 0.81, 0.61, 0.71, 0.61, and 0.61% Ca, respectively with the same level of nPP (0.40%) for grower-finisher (22 to 35 d). Chicks were offered unrestricted access to mashed feed and freshwater over the 35-d trial period.

Birds were immunized against Newcastle disease virus (**NDV**) and infectious bronchitis virus (**IBV**) at 5 and 28 d as well as infectious bursal disease (**IBD**) virus at 18 d with live attenuated vaccines (Zoetis Animal Health Co., Parsippany, NJ) via drinking water following the manufacturer's guidelines. Chickens and feed were weighed weekly on a replicate basis to determine body weight gain (**BWG**), feed intake (**FI**), feed conversion rate (**FCR**, FI/BWG, adjusted for mortality), and production efficiency factor (PEF, livability (%) x BW (kg) x 100 / FCR x age (d)) of broilers.

Sample Collection

One bird from each replicate was randomly chosen and marked with a wing tag for a weekly blood collection. Blood specimens were collected from the wing veins and centrifuged at 4,500 × g for 10 min, and sera were harvested and preserved at −80°C until antibody titers examination.

At the termination of the trial, 10 birds closest to the average BW for each treatment were weighed, euthanized, plucked, processed, and eviscerated. The dressing percentage was determined as the proportion of hot carcass weight to the preslaughter weight. Carcass parts (breast muscles, leg quarters, and abdominal fat pad) and visceral organs (liver, gizzard, small intestine, spleen, and bursa of Fabricius) were dissected and individually weighed. These weights were then expressed as a percentage to the preslaughter weight. The left part of the breast was then collected and used for later meat quality analysis. Subsequently, tibia and femur from the left limbs were excised and kept frozen at −20°C in air-tight plastic bags for later determination of bone traits.

A digestibility trial was carried out by using a total collection method, with one broiler per replicate placed in a metabolic cage on d 25. After a 3-d adaptation period, excreta were collected daily for 4 successive d, pooled for each cage across collection days, mixed for homogeneity, and weighed. Feed intakes were recorded during the total collection period. Feed samples and excreta materials were oven-dried until constant weights, finely ground prior to passing through a 1 mm sieve screen, and preserved in air-tight containers till chemical analysis.

Sample Analysis

For antibody titers analysis, the levels of serum antibodies against NDV, IBV, and IBD were analyzed with commercial ELISA kits (MyBioSource Inc., San Diego, CA) following the manufacturer's protocols.

For meat quality analysis, breast core temperature at 15 min postmortem was measured with a portable digital thermocouple (EcoScan Temp JKT; Thermo Scientific, Waltham, MA). The breast meat pH was estimated at 15 min (pH_{15min}) and 24 h (pH_{24h}) postmortem by inserting a pH probe (pH 211; Hanna Instruments, Woonsocket, RI, USA) 2.0 cm beneath the muscle. Breast meat color values including L* (lightness), a* (redness), and b* (yellowness) after 15 min and 24 h postmortem were assessed utilizing Chroma Meter (CR-400; Konica Minolta, Tokyo, Japan).

For bone characteristics, tibia and femur were boiled to strip adherent tissue and the weight, length, and diameter of cleaned tibia and femur were estimated utilizing a digital caliper (Control Company Traceable, Friendswood, TX). The weights were also calculated as a percentage to the preslaughter weights. For the determination of the tibia ash contents, the tibias were cut lengthwise, defatted using a Soxhlet extractor, dried at 105 °C to constant weights, and ashed in a muffle furnace at 550 °C overnight. The contents were then expressed as a percentage of fat-free dry weights. The concentration of Ca in tibia ash was estimated according to the method 968.08 of AOAC (2019) with the ICP-MS (iCAP Q, Thermo Fisher Scientific Inc., Waltham, MA) following dissolution ash samples in 6 M HCl to release Ca. The femur geometric parameters involving cross-sectional cortical area (A) and polar moment of inertia (J) were measured following the procedure described by Abudabos (2012). Briefly, femur shafts were cut at three locations (1/4, 1/2, and 3/4 along the femur length) utilizing a rotary tool with a cut-off wheel (#409) (Dremel Manufacturing Co., Racine, WI), and bone measurements were taken using a digital caliper (Control Company Traceable, Friendswood, TX) with an accuracy of 0.01 mm. The following computations were then utilized to compute the A and J of the femur (Crespo et al., 2000): $A = \pi \times ([X_1 \times Y_1] - [X_2 \times Y_2])$, $J = (\pi/4) \times ([X_1 \times Y_1^3 - X_2 \times Y_2^3] + [Y_1 \times X_1^3 - Y_2 \times X_2^3])$, where X_1 and X_2 = the lateromedial diameters at each level and Y1 and Y2 = the craniocaudal diameters at each level.

For apparent retention, the Ca content of diets and excreta samples was analyzed according to the method 968.08 of AOAC (2019) with the ICP-MS (iCAP Q, Thermo Fisher Scientific Inc., Waltham, MA) after ashing the specimens at 550 °C and digestion with 6 M HCl. The apparent retention of Ca was estimated employing the following calculation (Imari et al., 2020): Apparent Retention = ([nutrient consumed - nutrient excreted]/nutrient consumed) x 100.

Data Analysis

The experimental unit for performance data was the cage and for other data was the sample bird. The data were subjected to the Shapiro-Wilk test for normality and Levene's test for homogeneity prior to analysis. A one-way ANOVA was implemented applying the general linear model procedure of SAS software (version 9.1, SAS Institute Inc., Cary, NC). Effects of the treatments on the antibody levels in weekly samples were analyzed as repeated measures over time using PROC MIXED of SAS 9.1. Differences at $P < 0.05$ were regarded as significant and separated employing the Tukey test, while variations at $0.05 < P < 0.10$ were regarded trends. The statistics are exhibited as least-square means with their pooled standard error of mean.

RESULTS

Growth Performance

Performance data for the starter (0 to 21 d), grower-finisher (22 to 35 d), and cumulative (0 to 35 d) phases are presented in Table 3. During the starter period, no differences among dietary treatments were observed on performance parameters ($P > 0.05$). During the grower-finisher period, the dietary treatments showed no effects on FI and BWG ($P > 0.05$). There was a trend ($P = 0.08$) for reduced FCR in T3 and T4 groups compared to other groups. However, PEF was higher for birds that received T4 compared to T2 and T5, with those received T1 and T3 being intermediate and not different from other treatments ($P < 0.05$). During the cumulative period, a trend was observed for BWG ($P = 0.062$); birds from T1 and T4 had numerically the highest BWG, and they gained 73 and 83.5 g, respectively more weight compared to T2. There was a difference ($P < 0.05$) in FCR due to the numeric differences in BWG. Birds from the T3 and T4 groups were the most efficient in converting feed into body gain in comparison with those received T2 and T5 treatments, however, it was not different from T1. The PEF followed the same trend; similar to T1, birds that received T3 and T4 had an improved PEF compared to T2 ($P < 0.05$), whereas T5 was intermediate and similar to all other treatments.

Carcass Characteristics

The carcass traits of the birds at 35 d are presented in Table 4. A higher dressing percentage was obtained from birds that received T4 (72.7%) in comparison with the T2 group (70.2%) ($P = 0.023$), whereas other treatments were intermediate and showed no statistically significant differences either from T4 or T2. Although there was a trend for a higher breast muscle yield in the T4 group in comparison with the T2 group ($P = 0.075$), no differences were noticed between the treatments in leg quarter yield, abdominal fat, liver, gizzard, spleen, or bursa of Fabricius ($P > 0.05$). Moreover, the relative weight of small intestine was lower for T2 than all other treatments ($P < 0.01$).

Table 3. Growth performance of broilers fed low-Ca diets supplemented with marine mineral complex (CeltiCal) and phytase (PHY).

	Treatments[2]						
Items[1]	T1	T2	T3	T4	T5	SEM[3]	*P* Value
0 - 21 d							
FI (g/bird)	961.0	914.8	907.5	925.5	941.7	15.9	NS
BWG (g/bird)	768.1	716.3	732.4	751.5	747.2	14.0	NS
FCR (g/g)	1.25	1.28	1.24	1.23	1.26	0.01	NS
PEF	305.1	283.6	293.6	304.6	295.5	6.87	NS
22 - 35 d							
FI (g/bird)	1609.3	1581.9	1612.7	1618.3	1600.4	22.3	NS
BWG (g/bird)	1032.6	1011.3	1051.6	1059.7	1015.7	15.3	NS
FCR (g/g)	1.56	1.56	1.53	1.53	1.58	0.01	0.080
PEF	336.2[ab]	324.3[b]	336.3[ab]	344.0[a]	325.9[b]	4.79	0.035
0 - 35 d							
FI (g/bird)	2570.2	2496.7	2519.2	2543.8	2542.1	32.4	NS
BWG (g/bird)	1800.6	1727.6	1783.9	1811.1	1762.9	21.1	0.062
FCR (g/g)	1.43[ab]	1.45[a]	1.41[b]	1.40[b]	1.44[a]	0.01	0.015
PEF	367.2[a]	345.5[b]	365.4[a]	374.1[a]	356.2[ab]	6.16	0.022

[a-b]Means within the same row with different superscripts differ ($P < 0.05$). NS: not significant.
[1]FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio; PEF: production efficiency factor.
[2]T1, recommended dietary Ca + 500 FTU/kg PHY; T2, 0.2% below Ca recommendations + 500 FTU/kg PHY; T3, 0.1% below Ca recommendations + 2 g/kg CeltiCal + 500 FTU/kg PHY; T4, 0.2% below Ca recommendations + 4 g/ kg CeltiCal + 500 FTU/kg PHY; T5, 0.2% below Ca recommendations + 4g/ kg CeltiCal.
[3]SEM: pooled standard error of mean.

Meat Quality

The impact of the treatments on meat quality of broiler breast fillets at 35 d is presented in Table 5. The internal breast temperature was highest for T1 and lowest for T3 at 15 min postmortem ($P < 0.001$). The mean values of pH_{15min} and pH_{24h} differed ($P < 0.05$) between treatments. Birds that received T1 had a higher pH_{15min} compared to T5 and birds that received T3 had a higher pH_{24h} compared to T4. No differences ($P > 0.05$) were detected in L* and b* values at 15 min and 24 h postmortem. However, a* value was lower for birds that fed T5 in comparison with all other treatments at 15min postmortem and compared to T1 and T3 at 24 h postmortem ($P < 0.01$).

Table 4. Carcass dissection of broilers fed low-Ca diets supplemented with marine mineral complex (CeltiCal) and phytase (PHY).

	Treatments[2]						
Items (%)[1]	T1	T2	T3	T4	T5	SEM[3]	*P* Value
Dressing	71.2[ab]	70.2[b]	71.5[ab]	72.7[a]	71.5[ab]	0.51	0.023
Breast	22.3	21.3	22.4	23.8	23.0	0.59	0.075
Leg	21.3	22.7	23.2	24.9	22.5	0.96	NS
Fat	1.01	1.11	0.93	0.93	0.98	0.11	NS
Liver	1.70	1.78	1.69	1.66	1.67	0.06	NS
Gizzard	2.36	2.91	2.39	2.41	2.62	0.15	NS
Small intestine	3.84[a]	3.16[b]	3.88[a]	3.88[a]	3.85[a]	0.16	0.005
Spleen	0.095	0.098	0.093	0.094	0.080	0.008	NS
Bursa	0.116	0.168	0.167	0.165	0.131	0.016	NS

[a-b]Means within the same row with different superscripts differ ($P < 0.05$). NS: not significant.
[1]Weight percentages were calculated based on the preslaughter live weight.
[2]T1, recommended dietary Ca + 500 FTU/kg PHY; T2, 0.2% below Ca recommendations + 500 FTU/kg PHY; T3, 0.1% below Ca recommendations + 2 g/kg CeltiCal + 500 FTU/kg PHY; T4, 0.2% below Ca recommendations + 4 g/ kg CeltiCal + 500 FTU/kg PHY; T5, 0.2% below Ca recommendations + 4g/ kg CeltiCal.
[3]SEM: pooled standard error of mean (n = 10).

Bone Properties

The impact of the treatments on broiler bone characteristics at 35 d is presented in Table 6. The dietary treatments had no influence on the length, width, and relative weight of the tibia and femur ($P > 0.05$). However, there were differences ($P < 0.05$) among the treatments regarding the weights of tibia and femur; birds that received the T3, T4, and T5 diets had heavier tibia and femur compared to those fed on the T2 diet, with those received the T1 diet being intermediate and did not differ from other treatments. Similar to the T1 group, birds that received T3, T4, and T5 had a greater proportion of tibia ash in comparison with T2 ($P < 0.05$). The Ca concentration of the tibia was not affected ($P > 0.05$) by the treatments. Bone geometric properties (A and J) were affected by the experimental diets ($P < 0.001$); femur from the T2 group had lower A and J values compared to all other treatments.

Immune Response

Titer results of the NDV, IBV, and IBD are shown in Table 7. There was an influence of the treatments on IBV titer ($P < 0.01$), which was greater in the T1, T3, and T4 groups than the T2 group with the T5 group being intermediate. On the other hand, NDV and IBD titers were not impacted by the treatments ($P > 0.05$).

Apparent Ca Digestibility

The influence of the treatments on Ca apparent retention is summarized in Figure 1. A higher Ca retention was observed in T3 and T4 in comparison with T1 and T2 ($P < 0.001$), while the retention for birds that received T5 was comparable with T2, T3, and T4 groups.

Table 5. Breast meat quality of broilers fed low-Ca diets supplemented with marine mineral complex (CeltiCal) and phytase (PHY).

	Treatments[2]						
Items[1]	T1	T2	T3	T4	T5	SEM[3]	*P* Value
15 min post-mortem							
Temperature	29.5[a]	28.4[c]	27.4[d]	28.3[c]	29.0[b]	0.12	0.001
pH	6.35[a]	6.28[ab]	6.25[ab]	6.17[ab]	6.15[b]	0.04	0.032
L*	43.1	44.4	45.1	45.4	44.4	0.69	NS
a*	3.18[a]	3.10[a]	2.99[a]	2.88[a]	1.91[b]	0.23	0.003
b*	6.71	7.41	6.62	6.86	6.49	0.36	NS
24 h postmortem							
pH	5.99[ab]	6.03[ab]	6.08[a]	5.94[b]	6.05[ab]	0.03	0.023
L*	49.8	48.5	49.9	51.2	48.9	0.87	NS
a*	3.81[a]	3.09[ab]	3.39[a]	2.94[ab]	2.18[b]	0.28	0.005
b*	10.7	9.6	10.0	10.2	9.7	0.64	NS

[a-d]Means in the same row with different superscripts differ ($P < 0.05$). NS: not significant.
[1]L*: lightness; a*: redness; b*: yellowness.
[2]T1, recommended dietary Ca + 500 FTU/kg PHY; T2, 0.2% below Ca recommendations + 500 FTU/kg PHY; T3, 0.1% below Ca recommendations + 2 g/kg CeltiCal + 500 FTU/kg PHY; T4, 0.2% below Ca recommendations + 4 g/ kg CeltiCal + 500 FTU/kg PHY; T5, 0.2% below Ca recommendations + 4g/ kg CeltiCal.
[3]SEM: pooled standard error of mean (n = 10).

Table 6. Bone characteristics of broilers fed low-Ca diets supplemented with marine mineral complex (CeltiCal) and phytase (PHY).

	Treatments[2]						
Items[1]	T1	T2	T3	T4	T5	SEM[3]	*P* Value
Tibial measurements							
Weight (%)	0.73	0.70	0.81	0.76	0.75	0.03	NS
Weight (g)	14.1[ab]	12.6[b]	15.1[a]	15.3[a]	15.0[a]	0.67	0.044
Length (cm)	9.47	9.08	9.51	9.60	9.70	0.16	NS
Width (mm)	8.24	7.91	8.50	8.31	8.32	0.25	NS
Ash (%)	35.8[a]	32.6[b]	36.7[a]	37.2[a]	36.5[a]	0.99	0.016
Ca (%)	14.4	13.6	15.4	16.0	15.2	0.76	NS
Femoral measurements							
Weight (%)	0.50	0.47	0.57	0.54	0.54	0.25	NS
Weight (g)	9.9[ab]	9.2[b]	10.8[a]	11.2[a]	10.8[a]	0.49	0.049
Length (cm)	6.89	6.72	7.02	7.07	7.02	0.11	NS
Width (mm)	9.35	8.51	9.62	9.49	9.44	0.31	NS
A (mm^2)	57.6[ab]	52.8[c]	57.0[b]	58.3[a]	57.5[ab]	0.24	0.001
J (mm^4)	815.2[a]	760.4[b]	804.8[a]	816.0[a]	807.2[a]	3.25	0.001

[a-c]Means within the same row with different superscripts differ ($P < 0.05$). NS: not significant.
[1]Weight percentages were calculated based on preslaughter weight; A: cross-sectional cortical area; J: polar moment of inertia.
[2]T1, recommended dietary Ca + 500 FTU/kg PHY; T2, 0.2% below Ca recommendations + 500 FTU/kg PHY; T3, 0.1% below Ca recommendations + 2 g/kg CeltiCal + 500 FTU/kg PHY; T4, 0.2% below Ca recommendations + 4 g/ kg CeltiCal + 500 FTU/kg PHY; T5, 0.2% below Ca recommendations + 4g/ kg CeltiCal.
[3]SEM: pooled standard error of mean (n = 10).

Table 7. Serum antibody titers of broilers fed low-Ca diets supplemented with marine mineral complex (CeltiCal) and phytase (PHY).

	Treatments[2]						
Items[1]	T1	T2	T3	T4	T5	SEM[3]	*P* Value
NDV	3.26	3.18	3.33	3.29	3.30	0.057	NS
IBV	3.25[a]	2.95[b]	3.28[a]	3.32[a]	3.14[ab]	0.082	0.013
IBD	2.93	2.91	2.99	2.97	2.97	0.046	NS

[a-b]Means within the same row with different superscripts differ ($P < 0.05$). NS: not significant.
[1]NDV: Newcastle disease virus; IBV: infectious bronchitis virus; IBD: infectious bursal disease virus.
[2]T1, recommended dietary Ca + 500 FTU/kg PHY; T2, 0.2% below Ca recommendations + 500 FTU/kg PHY; T3, 0.1% below Ca recommendations + 2 g/kg CeltiCal + 500 FTU/kg PHY; T4, 0.2% below Ca recommendations + 4 g/ kg CeltiCal + 500 FTU/kg PHY; T5, 0.2% below Ca recommendations + 4g/ kg CeltiCal.
[3]SEM: pooled standard error of mean (n = 10).

DISCUSSION

The relative bioavailability of Ca in limestone which is the primary inorganic Ca source in poultry feeds has traditionally been considered to be high (73 and 109%, depending on the source), but recent research has revealed that the apparent ileal Ca digestibility coefficients of limestone are not high and fluctuating from 0.51 to 0.62 (Anwar et al., 2016a, 2017). In addition, high concentrations of Ca or elevated ratios of Ca to nPP in poultry diets have been shown to lower the digestibility of Ca and P owing to augmented the precipitation of insoluble Ca-P complexes (Farhadi et al., 2017), and decrease the hydrolysis of phytate-P by PHY activities because of the generation of Ca-phytate chelates, rendering both minerals unavailable for absorption in the intestine (Amerah et al., 2014; Humer et al., 2015). Hence, dietary Ca level and source and dietary

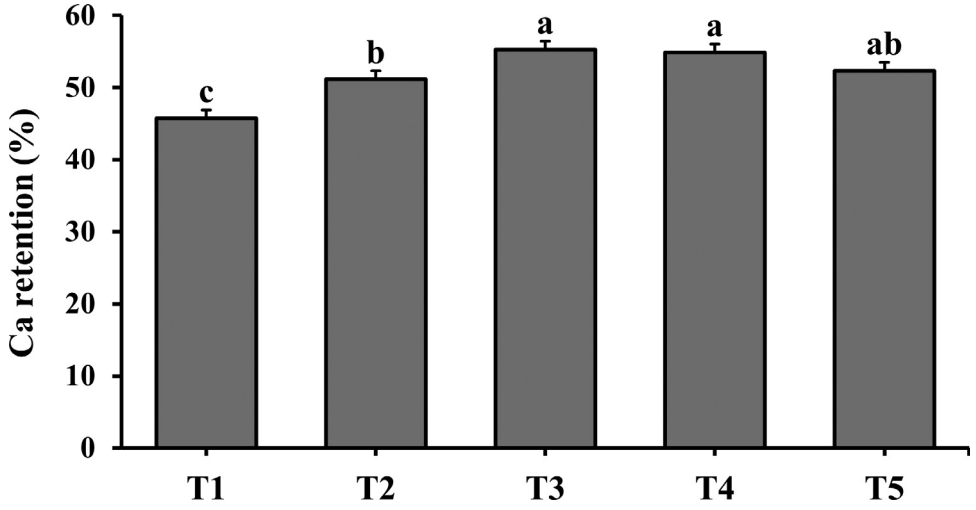

Figure 1. Apparent Ca retention of broilers fed low-Ca diets supplemented with marine mineral complex (CeltiCal) and phytase (PHY). T1, recommended dietary Ca + 500 FTU/kg PHY; T2, 0.2% below Ca recommendations + 500 FTU/kg PHY; T3, 0.1% below Ca recommendations + 2 g/kg CeltiCal + 500 FTU/kg PHY; T4, 0.2% below Ca recommendations + 4 g/ kg CeltiCal + 500 FTU/kg PHY; T5, 0.2% below Ca recommendations + 4g/ kg CeltiCal. Results are presented as mean values + pooled standard error of mean (n = 10). Bars with different superscripts differ ($P < 0.05$).

Ca: nPP ratio are vital for the absorption and utilization of these 2 elements (Wilkinson et al., 2014; Gautier et al., 2017). Accordingly, feeding a highly available Ca source such as CeltiCal (mineral supplement derived from marine algae), which provides bioavailable Ca and 74 trace minerals to the digestive system of the chicken (Momeneh et al., 2018) may improve the efficiency of Ca and P utilization with PHY-supplemented low-Ca diets Walk et al. (2012b.) showed that feeding calcified seaweed with PHY compared with limestone enabled to decrease dietary Ca level while keeping broiler performance and bone mineralization. They also concluded that P digestibility and bone P were improved in broilers given the low-Ca diet with PHY.

In the current study, formulation diets with CeltiCal as a partial replacement for limestone in the presence of PHY have been shown to enhance the efficiency of growth in broilers, as demonstrated by reduced FCR and augmented PEF during the cumulative period in birds receiving CeltiCal (0.2% for T3 and 0.4% for T4) plus 500 FTU PHY/kg, which were similar to those from T1 and were different from those of T2. The improvement with CeltiCal and PHY in the low-Ca diets might be due to the greater bioavailability of CeltiCal compared with limestone (Walk et al., 2012b; Momeneh et al., 2018), and the release of nutrients attached to phytate molecules such as proteins, lipids, carbohydrates, and minerals by the activity of PHY (Onyango et al., 2005). These results were supported by our dressing percentage data; birds fed with the T4 diet had better dressing percentage than those fed with the T2 diet, with those fed on the T1, T3, and T5 diets being intermediate and not different from T4. The performance findings are in line with Momeneh et al. (2018), who concluded that utilizing Celtic sea minerals instead of limestone in a low-Ca diet (0.6%) improved broiler feed efficiency. Our results also agree with Paiva et al. (2013), who reported that PHY supplementation in reduced-Ca diets resulted in improved BWG of broilers. Similarly, Hamdi et al. (2015) showed that a medium level of Ca (0.7%) in a diet supplemented with a high concentration of PHY is adequate for broiler performance. It has been also shown that the overall FCR was significantly lower in broilers given PHY supplemented mid-level Ca diet, concluding that high dietary Ca has a negative influence upon PHY activity (Akter et al., 2016).

Our results showed that apparent Ca retention was improved in the T3 and T4 groups as compared to the T1 and T2 groups, with the T5 group being intermediate and not different from either T2, T3, or T4, suggesting that the retention of Ca can be reduced by increasing dietary levels of Ca (T1 had the worst retention) and improved by CeltiCal supplementation (T2 vs. T3 and T4). The adverse effect of increased dietary Ca could be explained by increased the formation of insoluble Ca-P precipitates (Dersjant-Li et al., 2018) and the generation of insoluble Ca−phytate chelates (Selle et al., 2009). However, CeltiCal provides more bioavailable Ca compared with limestone in the intestinal lumen and can therefore help in a reduced Ca strategy (Momeneh et al., 2018). In line with the current study, Adeola and Walk (2013) observed that ileal Ca digestibility was greater in broilers fed 0.5% Ca from calcified seaweed than those fed 0.6% Ca from calcified seaweed Paiva et al. (2013). similarly concluded that broilers fed 0.6% versus 0.9% Ca plus PHY had higher P and Ca digestibility at the low Ca level Anwar et al. (2016b). also reported that rising Ca: nPP ratio (1.5, 2.0, and 2.5) reduced the true Ca digestibility of limestone (0.65, 0.57, and 0.49%, respectively).

Regarding breast meat properties, previous research has shown that low pectoralis muscle pH is a possible indicator of poor meat quality because the rapid decline in the pH postmortem can bring about protein denaturation, pale color, and low water-holding capacity (Zhang et al., 2012b). A higher pH, on the other hand,

may cause dry and dark meat (Bowker and Zhuang, 2015). Hence acidity is linked to meat quality, and the perfect range of pH at 45 min postmortem is 6.3 to 6.7 (Aljumaah et al., 2020). Our results showed that the dietary treatments kept meat pH value in the perfect range; no treatment had a pH_{15min} value below 6.15. The color of meat is another indicator of meat quality (Aljumaah et al., 2020). Breast meat color *a value in the T5 group was decreased at 15 min and 24 h postmortem, which might indicate a better color of meat.

In the current experiment, the titer of IBV-specific antibodies was improved in the T3 and T4 groups to a similar level of the T1 group when compared to the T2 group, with the T5 group being intermediate, showing that nutritional factors could influence specific immune responses Liu et al. (2008). reported that adding PHY to a phytate-rich diet increased anti-NDV antibodies at 21 and 28 d Ghosh et al. (2016). also showed that the inclusion of PHY in low-nPP broiler diets gave rise to an improvement in the immune response against the ND-LaSota vaccine on 16 and 32 d. In accordance with our results, a recent study on broilers has shown that dietary supplementation of PHY improved the anti-IBV titers at d 42, suggesting increased P availability due to PHY could support humoral immune response (Nari et al., 2020). Such improvement in immune response could be attributed to the action of PHY by increasing the availability of minerals like Zn, Cu, and Se and amino acids, which are nutrients required for an efficient and robust immune response (Khodambashi Emami et al., 2013). This improvement could also be associated with the role of dietary CeltiCal in providing highly bioavailable minerals to the digestive system of the chicken such as Zn, Mn, Se, and Cu, which are essential to promote the immune response (González-Vega et al., 2014).

It has been established that the level and source of Ca play a substantial role in bone mineralization and strength (Fallah et al., 2018). Besides, geometrical characteristics have been reported to determine the bone material properties, which give strength and hardness to the bone (Muszyński et al., 2017). In this research, weight and ash content of the tibia and weight and geometric properties (A and J) of the femur were improved by feeding CeltiCal to a similar level of the control group, suggesting that supplemental CeltiCal can maintain bone strength in modern broiler strains by delivering a highly bioavailable Ca source in the poultry intestinal tract within a broad range of pH (González-Vega et al., 2014), even with lower dietary Ca levels or without PHY addition. Although no significant difference in tibia ash Ca content was observed among the treatments, our findings also suggest that a positive correlation exists between the dietary Ca level and ash content in the tibia matrix of broilers (T1 vs. T2), indicating that Ca is a limiting factor for bone mineralization. Similarly, Rousseau et al. (2012) showed that tibia ash content improved linearly as the concentration of dietary Ca increased from 0.37 to 0.57 and 0.77% Abdulla et al. (2017). also observed a significant elevation in bone breaking strength and ash content of broilers provided a diet containing 1.25% of Ca compared with 1.00% of Ca. Moreover, Paiva et al. (2014) reported that 0.9% Ca in broiler diet supplemented with PHY resulted in a higher tibia ash percentage compared with 0.6% of Ca. However, Hamdi et al. (2015) concluded that a medium level of Ca (0.7%) and 0.38% nPP in diets comprising a high dosage of PHY are adequate for broiler bone ash.

In conclusion, the findings of the present study showed that PEF was greater for T4 compared to T2 and T5 during the grower-finisher phase and for T1, T3, and T4 compared to T2 during the cumulative phase; feed conversion ratio was lower for T3 and T4 compared to T2 and T5 during the cumulative phase; T4 had a greater dressing percentage than T2; tibia and femur weights were greater for T3, T4, and T5 compared to T2; higher tibia ash content and J value were found in T1, T3, T4, and T5 compared to T2; A value was highest in T4 and lowest in T2; greater antibodies to IBV were observed for T1, T3, and T4 compared to T2; apparent Ca retention was higher for T3 and T4 compared to T2, with T1 had the lowest Ca retention. Based on these findings, we concluded that partial replacement of limestone with CeltiCal in combination with PHY has a great potential to reduce dietary Ca level while maintaining broiler performance and bone strength.

FUNDING

This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number (15-AGR5339-02).

DISCLOSURES

The authors declares no conflicts of interest.

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