Response of modern broiler chickens to dietary calcium and phosphorus levels below recommendations

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ABSTRACT
This study was carried out to investigate the effects of reduced dietary calcium (Ca) and non-phytate-phosphorus (NPP) levels on performance, carcase yield and bone mineralisation of modern broilers. 11,200 one-day-old chicks (Ross-308) were divided into 7 treatments and 8 replicates with standard recommended level and six different levels of Ca and NPP reduction with completely randomised block design. Treatments were divided into following diets; T1: ross recommended control, T2: 94%, 95% and 91% as percentage of T1, T3: 94%, 95% and 83% as percentage of T1, T4: 94%, 91% and 83% as percentage of T1, T5: 94%, 91% and 77% as percentage of T1, T6: 94%, 86% and 83% as percentage of T1 and T7: 94%, 86% and 77% as percentage of T1 for starter, grower and finisher periods, respectively. Results showed that, the Ca and NPP reduction (T2 – T7) through starter period did not significantly (p > .05) affect performance but when considered whole period, quadratically (p < .05) improved body weight gain (BWG), feed conversion ratio (FCR) and European Production Efficiency (EPEF). In line with, Ca and NPP intakes were decrease linearly and quadratically with Ca and NPP reducing in diet and higher intakes at T1 resulted in impaired BWG and FCR. On the other hand, carcase parameters and tibia mineralisation were not significantly affected by the reductions. In conclusion, Ca and NPP requirements of Ross 308 broilers seem to be lower than the Aviagen 2014 and 2019 recommendations and reducing almost 20% Ca and NPP intake of broilers is possible, especially after starter period, to maintain performance without any negative effect on bone development.

HIGHLIGHTS
- Excessive dietary Ca and P feeding have potential deleterious effects on broilers.
- Dietary Ca and NPP reduction improved growth performance, without impairment bone characteristics.
- Especially after starter period, Ca and NPP reduction, by keeping the dietary levels as 0.90:0.45, 0.75:0.38 and 0.60:0.30% for starter, grower and finisher phase respectively, seems enough for Ross-308.

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Introduction
Modern meat-type production growth patterns has changed fundamentally over the past few decades because of genetic improvements. In addition to changes, increasing desire to optimise resource utilisation validate the need to continuously review and revise the nutrient requirements of broiler chickens (Fallah et al. 2019). Calcium (Ca) and phosphorus (P) are essential minerals for growth and bone formation and are responsible for its rigidity and compressive strength. Therefore, accurate estimation of broiler’s Ca and P requirements is necessary to maximise productivity. However, the requirements of Ca and P have not been optimised due to changes in broiler genetic, the interaction between Ca and P and phytate presence in vegetable feeds. Modern broiler breeds are considered high yielding in breast meat in comparison with multi-purpose standard commercial strains. Besides selected fast-growing and late maturing strains have shown lower bone-ash content than slow-growing strains (Williams et al. 2000), which suggest that optimum requirement for broilers needs to be adjusted properly through years in accordance with genetic improvement. Although commercial broiler companies have changed Ca and P recommendations over the years, it seems that has not still been optimised yet. For...
example, Ca and non-phytate P (NPP), also called as available P (Pa) recommendations for Ross 308 broilers were reduced approximately 6.3% and 4.4% respectively from 2009 to 2014 and no changes were done for 2019, while Cobb company decreased a bit higher as 14.1% and 12.7%, respectively, from 2006 to 2012 and no changes observed for 2018.

Unlike P, Ca sources are generally inexpensive; therefore, until recent, less importance has been placed on determining the dietary Ca requirement of broiler chickens or the outcomes of excessive feeding (Powell et al. 2011; Fallah et al. 2019). Studies have shown that Ca supplied in excess of requirement may effect negatively the process of digestion owing to formation of insoluble salts with dietary fatty acids in the intestinal lumen, which may lead to a reduction of nutrient availability, decreasing the dietary energy utilisation and reduction in growth and feed efficiency (Simpson and Wise 1990; Sebastian et al. 1996; Tamim et al. 2004). In addition, excessive Ca has the capacity to interfere the digestion and absorption of micro minerals and interact with inorganic P in the gut, so lower dietary Ca level has potential to improve P utilisation (Hamdi et al. 2015). Moreover, as a widely used source of Ca, limestone which also has high acid-binding capacity, have been related to significant decreases in the P and protein solubility in the gizzard, and may affect P and nitrogen (N) utilisation (Hurwitz and Bar 1971; Selle et al. 2009; Walk et al. 2012).

In line with Ca, P requirements of broilers also should be considered sensitively. Inorganic P is needed to include in the diet of broilers to attain optimal growth and production because of phytate P in vegetable feedstuffs. This eventually leads to nonutilisation of a high proportion of dietary P by poultry and excretion of P in faeces, which can lead to eutrophication (El-Hack et al. 2018). Besides, inorganic P sources are limited naturally sources and represents an important feed source constraining feed costs (Valable et al. 2018). Reducing P excretion and avoid overfeeding of P nutritional approaches has been evaluated for long time.

There are some studies conducted to optimise of Ca and NPP requirements for broilers in recent years. Diaz-Alonso et al. (2019) reported that, when Ca level adjusted to 2.1 Ca to NPP ratio there was no differences on performance and tibia ash (%) for broilers at level of Ca 0.46% or 0.86%. Fallah et al. (2019) showed that, at early age of broilers, above Ca level at 0.85% is deteriorate performance when NPP fixed at 0.40% and Kim et al. (2017) reported that, Ca level increase in diet from 0.60% to 1.00% is also deteriorate broiler performance when NPP fixed at 0.30%. Although there are some studies in recent years focussing on dietary Ca and P optimisation, most of them was involved for specific age period and a few of them was conducted through all feeding phase. Kiani and Taheri (2020) reported that moderate restrictions on Ca and NPP at level 0.76% and 0.38% through 1–24 days and 25–38 days showed better performance while maintaining bone development. Valable et al. (2018) reported that after standard starter period, restriction on Ca and NPP at level 0.60%–0.30% for grower and 0.48%–0.24% were enough for maintain performance, but with tibia mineralisation reducing. Another study, Singh et al. (2013) reported that, 0.75% and 0.60% Ca 0–21 days and 21–42 days, respectively, were enough for maintain performance and tibia mineralisation. Above-mentioned trials were generally operated with small numbers of broilers mostly in isolated university research centres where stocking density and other breeding challenges are generally low; however, there is very limited or no trials on the subject conducted in commercial case with high number of birds.

For this purpose, we aimed to evaluate the effects of reduced dietary levels of Ca and NPP in corn soybean based broiler diets especially after starter period on growth performance, carcass yield and bone mineralisation in a large number capacity broiler research unit.

**Materials and methods**

**Animals and housing**

All experimental procedures were reviewed and approved by the Animal Care Committee of Ankara University (207-02-28). The research was conducted in broiler research development centre of Beypiliç, one of the biggest broiler integration company located in Bolu region in Turkey. A total of 11200 one-day-old Ross 308 broiler chicks were weighed and randomly allocated to 56 floor pens (6.5 x 2 m each), with containing 200 chicks (100 male, 100 female), each equipped with a nipple drinkers and computer controlled hanging feeder. Through study, chicks were allowed ad libitum access to water and feed in a crumble form (0–10 days) and pellet form (11–41 days). Poultry house temperature was set to 33 °C for the first three days, then decreased to 23 °C step by step till 21 days, and then maintained at this level until the end of the experiment via automatic ventilating, cooling and heating systems. Continuous fluorescent
lighting was applied during the experimental period. Chickens were reared on wood shavings.

**Experimental design and diets**

Prior the trials, main ingredients and Ca sources were analysed for Ca, total P and the other nutrients then trial was arranged in a completely randomised block design with seven dietary treatments (1 to 7). Treatments were consisted following diets: T1: Control diet was formulated to meet suggested Ca and NPP nutrient specifications (Aviagen 2014), T2: 94% of for starter, 95% of for grower and 91% of for finisher Ca and NPP as percentage of recommended levels of Aviagen, T3: 94% of for starter, 95% of for grower and 83% of for finisher Ca and NPP as percentage of recommended levels of Aviagen, T4: 94% of for starter, 91% of for grower and 83% of for finisher Ca and NPP as percentage of recommended levels of Aviagen, T5: 94% of for starter, 91% of for grower and 77% of for finisher Ca and NPP as percentage of recommended levels of Aviagen, T6: 94% of for starter, 86% of for grower and 83% of for finisher Ca and NPP as percentage of recommended levels of Aviagen and T7: 94% of for starter, 86% of for grower and 77% of for finisher Ca and NPP as percentage of recommended levels of Aviagen. Control group (T1) had 8 replicates as there were 48 replicates for 94% reduction groups (T2–T7) for 0–10 days, and then, each treatment had eight replicates for remaining periods. Experimental diets were formulated based on corn-soybean meal based, for starter for 0–10 days, grower for 11–24 days and finisher 25–41 days phases. Experimental diets for each feeding period were formulated, as shown in Table 1, to be isonitrogenous and to meet or exceed demands of broiler chickens according to breeder guidelines (Aviagen 2014) but with varying Ca and NPP level as previously mentioned. All experimental feeds were subjected to Ca and total P and proximate analysis, and the results were shown in Table 1. Analysed results of crude ash, crude protein, Ca and total P of experimental diets were in close agreement with calculated values. Treatment feeds were produced in feed mill of Beypilic and provided in granule and pellet form during starter and after starter respectively. All mineral sources including poultry meal were weighed for each treatment batch properly in a outside scale, then directly put into 6 tons capacity mixer on the top. Rest of the ingredients were taken from the silos separated for the trial by automatically controlled dosage system, where micro ingredients were weighed by micro dosage system. The mixer was operated for 3.5 minutes after all ingredients reached and all treatment feeds then pelleted in conditioned at 75 °C.

**Measurements of growth performance**

Birds were weighed at the beginning of the experiment, at 10 and 41 days. Feed intake (FI) was recorded for each growing period: 0 to 10, 11 to 41 and 0 to 41 days on a per pen basis. Feed conversion ratio (FCR) was calculated for 0–10, 11–41 and 0–41 days by using feed intake and body weight gain (BWG). Daily mortality was recorded and was considered in the calculation of FCR. Growth performance data were given and evaluated over the whole feeding period. European Production Efficiency (EPEF) was also calculated at the end of the experiment. Ca and NPP intake were estimated from feed intake and calculated as % of BWG. Relationship between the Ca intake and FCR or BWG presented as a regression analyses.

**Measurements of carcase traits**

At the end of experiment, two chicks per pen (one male, one female), close to the average pen weight, were selected for processing. Each bird was weighed and leg-banded for identification. Feed was removed 6 h before processing. Each bird was exsanguinated by cutting the jugular vein, allowed to bleed for approximately 2 min, scalded for 30 s and defeathered. Viscera and abdominal fat were removed. Afterwards, carcase, drumsticks (with bones) and breast (with bones) were obtained and subsequently weighed. Carcase yield, drumstick and breast meat were calculated as percentage of live body weight. Left tibias from each replicate was removed for ash and P analysis and kept in −20 °C before analysis and weighed before analysis.

**Laboratory analyses**

Diets were analysed for crude ash, crude protein, Ca, and P. Crude ash was determined by placing samples in a burning oven at 550 °C for 12 h and crude protein (N × 6.25) analysis was determined by using the Kjedahl method. Total P was determined after hydrolysing the samples in a H2SO4–HNO3–water (1:1:1) solution. The resulting P was determined spectrophotometrically after reaction with ammonium molybdate and amino-naphtholsulphonic acid. The left tibias were defatted for 48 h in ethyl alcohol followed by a 48h extraction in ethyl ether. They were then dried for 12 h at 110°C and then ashed overnight at 600°C.
Table 1. Ingredient and nutrient composition of experimental diets (% as fed).

| Ingredients                  | 100% Ca&NPP<sup>b</sup> | 94% Ca&NPP | 100% Ca&NPP | 95% Ca&NPP | 91% Ca&NPP | 86% Ca&NPP | 100% Ca&NPP | 91% Ca&NPP | 83% Ca&NPP | 77% Ca&NPP |
|------------------------------|--------------------------|------------|-------------|------------|------------|------------|-------------|------------|------------|------------|
| Corn                         | 46.34                    | 46.88      | 48.79       | 49.07      | 49.35      | 49.63      | 53.38       | 53.92      | 54.39      | 54.75      |
| Wheat                        | 5.00                     | 5.00       | 6.00        | 6.00       | 6.00       | 6.00       | 7.00        | 7.00       | 7.00       | 7.00       |
| Soybean meal                 | 19.28                    | 19.98      | 14.49       | 14.39      | 14.30      | 14.20      | 9.69        | 9.95       | 10.11      | 10.14      |
| Sunflower meal               | 3.50                     | 3.50       | 4.50        | 4.50       | 4.50       | 4.50       | 5.00        | 5.00       | 5.00       | 5.00       |
| Full-fat soybean             | 15.89                    | 14.87      | 15.22       | 15.28      | 15.34      | 15.39      | 12.40       | 11.95      | 11.64      | 11.53      |
| Maize gluten meal            | 1.50                     | 1.50       | 1.00        | 1.00       | 1.00       | 1.00       | 1.00        | 1.00       | 1.00       | 1.00       |
| Poultry meal                 | 3.00                     | 3.00       | 4.00        | 4.00       | 4.00       | 4.00       | 5.00        | 5.00       | 5.00       | 5.00       |
| Soybean oil                  | 1.40                     | 1.40       | 2.50        | 2.40       | 2.30       | 2.20       | 3.50        | 3.40       | 3.30       | 3.20       |
| Dicalcium phosphate, 23% Ca, 18% P | 1.04                     | 0.87       | 0.95        | 0.84       | 0.72       | 0.61       | 0.72        | 0.53       | 0.47       | 0.33       |
| Monocalcium phosphate, 20% Ca, 21% P | 0.70                     | 0.70       | 0.50        | 0.50       | 0.50       | 0.50       | 0.50        | 0.50       | 0.50       | 0.50       |
| Limestone                    | 0.79                     | 0.74       | 0.67        | 0.64       | 0.61       | 0.57       | 0.58        | 0.53       | 0.46       | 0.42       |
| Sodium bicarbonate           | 0.18                     | 0.18       | 0.15        | 0.15       | 0.15       | 0.15       | 0.15        | 0.14       | 0.14       | 0.14       |
| Salt                         | 0.23                     | 0.23       | 0.22        | 0.22       | 0.22       | 0.22       | 0.22        | 0.20       | 0.20       | 0.20       |
| DL-Met, 99%                  | 0.16                     | 0.16       | 0.00        | 0.00       | 0.00       | 0.00       | 0.14        | 0.14       | 0.14       | 0.14       |
| Methionine hydroxy analogue, 88% | 0.20                     | 0.20       | 0.15        | 0.15       | 0.15       | 0.15       | 0.15        | 0.10       | 0.10       | 0.10       |
| L-Lys, 99%                   | 0.39                     | 0.39       | 0.15        | 0.15       | 0.15       | 0.15       | 0.15        | 0.36       | 0.36       | 0.36       |
| Vitamin premix<sup>c</sup>   | 0.10                     | 0.10       | 0.34        | 0.34       | 0.34       | 0.34       | 0.34        | 0.10       | 0.10       | 0.10       |
| Mineral premix<sup>d</sup>   | 0.20                     | 0.20       | 0.10        | 0.10       | 0.10       | 0.10       | 0.10        | 0.20       | 0.20       | 0.20       |
| Choline chloride, 75%        | 0.05                     | 0.05       | 0.20        | 0.20       | 0.20       | 0.20       | 0.20        | 0.04       | 0.04       | 0.04       |
| Salinomisin                  | 0.05                     | 0.05       | 0.05        | 0.05       | 0.05       | 0.05       | 0.05        | 0.05       | 0.05       | 0.05       |
| Nutrient level<sup>f</sup>   |                          |            |             |             |             |             |             |             |             |             |
| ME<sub>e</sub>, kcal/kg      | 3000.00                  | 3000.00    | 3100.00     | 3100.00    | 3100.00    | 3100.00    | 3200.00     | 3200.00    | 3200.00    | 3200.00    |
| Crude protein                | 23.00 (22.89)            | 23.00 (22.99)| 21.50 (21.41)| 21.50 (21.41)| 21.50 (21.55)| 21.50 (21.21)| 19.50 (19.22)| 19.50 (19.40)| 19.50 (19.39)| 19.50 (19.33)|
| Ca                          | 6.53 (6.58)              | 6.32 (6.39)| 5.86 (5.81)| 5.86 (5.81)| 5.72 (5.70)| 5.58 (5.55)| 5.38 (5.46)| 5.14 (5.20)| 4.92 (4.87)| 4.74 (4.75)|
| Total P                     | 0.96 (0.98)              | 0.90 (0.92)| 0.87 (0.85)| 0.83 (0.83)| 0.79 (0.78)| 0.75 (0.75)| 0.78 (0.77)| 0.71 (0.71)| 0.65 (0.63)| 0.60 (0.59)|
| NPP<sup>g</sup>             | 0.48                     | 0.45       | 0.44        | 0.42       | 0.42       | 0.40       | 0.38        | 0.39       | 0.39       | 0.33       |
| Lys                         | 1.44                     | 1.44       | 1.29        | 1.29       | 1.29       | 1.29       | 1.15        | 1.15       | 1.15       | 1.15       |
| Met + Cys                   | 1.08                     | 1.08       | 0.99        | 0.99       | 0.99       | 0.99       | 0.90        | 0.90       | 0.90       | 0.90       |

<sup>a</sup>Finisher diets was used up to end of 37 days of age, then same diets only without anticoccidial introduced through 3 days.

<sup>b</sup>Dietary calcium and non-phytate phosphorus as percentage of recommended levels for treatment groups.

<sup>c</sup>Supplied per kg diet: 10,000 U vitamin A, 4500 U vitamin D3, 65 mg vitamin E, 2.8 mg vitamin B1, 65 mg vitamin B2, 3.2 mg vitamin B6, 0.017 mg vitamin B12, 3.5 mg vitamin K3, 18 mg pantothenic acid, 55 mg niacin, 0.18 mg biotin, 1.9 mg folic acid.

<sup>d</sup>Supplied per kg diet: 20 mg Fe, 16 mg Cu, 110 mg Zn, 120 mg Mn, 1.25 mg I, 0.9 mg Co, 0.3 mg Se.

<sup>e</sup>Values in parenthesis represent analysed contents of nutrients.

<sup>f</sup>ME: metabolisable energy.

<sup>g</sup>NPP: non-phytate-phosphorus.
in a muffle furnace and tibia ash, as percentage of tibia dry matter weight determined. The ash for each tibia then further analysed for phosphorus according to the procedures stated in AOAC (2005).

**Statistical analysis**

Data generated in the present study were subjected to statistical analysis using the GLM procedure of Minitab 18 in a randomised complete block design. There were eight replicates for all measurement except for 0–10 days performance parameters. At that period, two groups were analysed with eight replicates for T1-control- and 48 replicates for Ca reduction (same Ca reduction percentage implemented at that period for 6 groups) with T test. For remaining parameters, the linear and quadratic contrasts were used to compare effects of decreasing dietary Ca and NPP levels. Probability values of ≤.05 were considered significant and means were separated using Tukey HSD test. The mortality data were subjected to a chi-square test.

**Results**

**Growth performance**

Results of treatments on growth performance (FI, BWG, FCR, mortality and EPEF) in broilers were given in Table 2. As shown in Table 2, performance parameters of 0–10 days were not affected by treatments (p >.05). In other words, 6% reduction of dietary Ca and P did not negatively affect growth performances through 0–10 days. A significant improvement (p <.05) in BWG, FCR and FI was observed through 11–41 days. In that period, BWG and FCR improved quadratically with Ca and NPP reduction. BWG and FCR were similar among the Ca and NPP reduction treatments except for T4 and T7 (T4 was better BWG and FCR than T7). Besides, FI decreased linearly (p <.05) in that period. In line with 11–41 days period, any reduction in dietary Ca and NPP level improved BWG, FCR and EPEF in quadratically (p <.05). Besides regression analysis about Ca intake also demonstrated that high Ca intake could reduce BWG and FCR. Deleterious effects of excess Ca or imbalanced Ca to NPP ratio reported in previous studies. Rao et al. (2006) demonstrated that increasing dietary Ca level from 0.6% to 0.9% significantly reduced body weight and feed intake at 14 and 28 day old chicks. Similarly, Sebastian et al. (1996) and Kim et al. (2017) also showed that high level of Ca has reduced body weight and feed intake compared to low Ca in broiler diets. Zhang et al. (2019) stated that feeding low Ca and P diets with increasing Ca:P ratio to broilers significantly reduced final BW. In line with, Driver et al. (2005) also found that no significant difference among different dietary Ca and P levels in terms of growth performance when Ca:P ratio kept at 2.1 approximately. In the present study performance improvement reached by lower dietary levels of Ca and NPP at different periods may be related to annihilate deleterious effects of high concentration of Ca on P utilisation, energy, mineral and protein availability (Mello et al. 2012; Wilkinson et al. 2014; Kim et al. 2017). Attributively for this argument, in the present trial it

**Carcase traits and tibia parameters**

Results of treatments on carcase traits and tibia parameters in broilers were given in Table 3. As shown Table 3, significant effects of the treatments were not found for the yield of carcase, yield of breast meat and yield of drumsticks meat (p >.05). In other words, dietary Ca reduction did not negatively affect carcase traits. Similarly, as shown Table 3, significant effects of the treatments were not found for tibia weight, tibia ash and tibia phosphorus parameters (p <.05). Consequently, with the Ca and P reduction at different level, the ash content and tibia phosphorus content were decreased numerically compared to Ross recommendation (p >.05).

**Discussion**

The results of the study demonstrated that reducing dietary Ca and NPP level during starter (0–10 days) and grower-finisher (11–41 days) periods maintained the broiler performance at any of studied levels and even gave better results (p <.05). In the whole growth period, any reduction in dietary Ca and NPP level improved BWG, FCR and EPEF in quadratically (p <.05). Besides regression analysis about Ca intake also demonstrated that high Ca intake could reduce BWG and FCR. Deleterious effects of excess Ca or imbalanced Ca to NPP ratio reported in previous studies. Rao et al. (2006) demonstrated that increasing dietary Ca level from 0.6% to 0.9% significantly reduced body weight and feed intake at 14 and 28 day old chicks. Similarly, Sebastian et al. (1996) and Kim et al. (2017) also showed that high level of Ca has reduced body weight and feed intake compared to low Ca in broiler diets. Zhang et al. (2019) stated that feeding low Ca and P diets with increasing Ca:P ratio to broilers significantly reduced final BW. In line with, Driver et al. (2005) also found that no significant difference among different dietary Ca and P levels in terms of growth performance when Ca:P ratio kept at 2.1 approximately. In the present study performance improvement reached by lower dietary levels of Ca and NPP at different periods may be related to annihilate deleterious effects of high concentration of Ca on P utilisation, energy, mineral and protein availability (Mello et al. 2012; Wilkinson et al. 2014; Kim et al. 2017). Attributively for this argument, in the present trial it
| Items     | T1 (Control) | T2 | T3 | T4 | T5 | T6 | T7 |
|-----------|--------------|----|----|----|----|----|----|
| Fl, g     | 301.09 ± 3.65 | 305.15 ± 1.42 | .258 |
| BWG, g    | 249.46 ± 3.48 | 252.64 ± 1.03 | .276 |
| FCR       | 1.21 ± 0.02   | 1.21 ± 0.01   | .922 |
| Mortality | 0.88 ± 0.16   | 0.71 ± 0.09   | .319 |
| 0–10 days |              |               |     |
| Fl, g     | 3897.89 ± 15.92 | 3902.15 ± 16.30 | .017 |
| BWG, g    | 2253.72 ± 19.13 | 2230.35 ± 9.63 | .611 |
| FCR       | 1.73 ± 0.01   | 1.68 ± 0.01   | .003 |
| Mortality | 1.38 ± 0.266  | 1.50 ± 0.32   | .569 |
| 11–41 days|              |               |     |
| Fl, g     | 4197.75 ± 17.74 | 4209.31 ± 20.61 | .040 |
| BWG, g    | 2530.18 ± 18.08 | 2560.03 ± 14.20 | .859 |
| FCR       | 1.68 ± 0.01   | 1.63 ± 0.01   | .004 |
| Mortality | 2.26 ± 0.207  | 2.37 ± 0.45   | .002 |
| EPEF      | 362.13 ± 4.08  | 380.00 ± 7.99  | .104 |
| Ca intake | 1.38 ± 0.01   | 1.25 ± 0.01   | .919 |
| NPP intake| 0.69 ± 0.14   | 0.59 ± 0.01   | .003 |

1Calcium and available phosphorus as percentage of recommended levels for starter/grower/finisher.

T test was applied during 0–10 days because of all treatment groups were received same Ca and P level (94%) compared to control one (100%).

EPEF: \[\text{viability 0–41 days (\%)} \times \text{BW 41 days (kg)} \times 100/\text{age (d)} \times \text{FCR 0–41 days}\].

Values within a row not sharing the same superscript are significantly different (\(p < .05\)).
was found that Ca and NPP intakes decreased linearly and quadratically as level of Ca and NPP in the feeds reduced, and higher intakes at control birds resulted reduced BWG and FCR, which could be explained by possible negative effects of over feeding of Ca.

In line with our study, Delezie et al. (2015) also showed that, reducing mineral supplementation of Ca and P by 20 to 25% is feasible if done in a balanced way to obtain maximal performance results, while obtaining reduction in tibia ash. In line with this, Valable et al. (2018) reported that, balanced to NPP, Ca reduction to 0.60% and 0.48% at 11–21 days and 22–37 days respectively (without reduction 0–10 days) has no effects on performance but tibia mineralisation affected negatively. It is clear that, Ca reduction regulation for broilers is related to age of birds and phytase presence with Ca to NPP ratio for maintain bone mineralisation alongside performance. According to Applegate et al. (2003), reducing dietary Ca level from 9.0 to 4.0 g/kg resulted in increase in ileal phytate hydrolysis and Wilkinson et al. (2014) reported that, digestible P concentration was increased when dietary Ca level reduced from 0.60% and 0.48% at 11–21 days and 22–37 days respectively (without reduction 0–10 days) has no effects on performance but tibia mineralisation affected negatively. It is clear that, Ca reduction regulation for broilers is related to age of birds and phytase presence with Ca to NPP ratio for maintain bone mineralisation alongside performance. According to Applegate et al. (2003), reducing dietary Ca level from 9.0 to 4.0 g/kg resulted in increase in ileal phytate hydrolysis and Wilkinson et al. (2014) reported that, digestible P concentration was increased when dietary Ca level reduced from 1.00% to 0.25%. Likewise, increase in the dietary Ca level was associated with decreased ileal digestibility of P (from 54% to 40%) (Walk et al. 2012) and reduced Ca availability (Schoultet al. 2003). In the present study total reduction in Ca and NPP levels compared to control was reached to 19.20% in the T7, which has the lowest average dietary Ca and NPP content. The results of the present study demonstrated that dietary level of Ca and NPP could be reduced to 0.90 and 0.45, 0.75 and 0.38, 0.60 and 0.30% for starter, grower and finisher periods respectively, which can be attributed to avoiding possible negative effects of excessive dietary Ca feeding on nutrient utilisation, mainly phosphorus.

The results of the study also showed that, carcase traits and tibia weight, tibia ash and phosphorus content were not significantly affected with any level of Ca and NPP reduction (p >.05). This result indicates that reduction in Ca level has no negative effects on carcase and bone mineralisation. However that the major reduction is applied after starter period where bone development is more sensitive to the mineral deficiency must be keep in mind. In literature Rao et al. (2006) mentioned that tibia ash content in broilers fed the lowest levels of Ca and NPP (0.60% and 0.30%, respectively) were similar to those fed diets with highest level of these minerals (0.9% and 0.45% respectively) in diet. Additionally, similar growth performance and tibia ash (%) were observed at Ca:Pa levels of 0.46:0.23%, 0.66:0.33%, 0.86:0.43%, and 1.05:0.53% (Diaz-Alonso et al. 2019). At 21-day trial, Fallah et al. (2019) showed that excessive dietary Ca

**Table 3.** Effects of different dietary Ca and non-phytate P levels on relative weights (weight/BW, %) of carcase and cuts up, and tibia weight (weight/BW, %), tibia ash (% of tibia dry matter weight) and tibia phosphorus (% of tibia ash weight).

| Items                  | T1 (Control) | T2       | T3       | T4       | T5       | T6       | T7       | p Value |
|------------------------|--------------|----------|----------|----------|----------|----------|----------|---------|
| Carcase yield          | 70.01 ± 0.49 | 70.30 ± 0.54 | 69.75 ± 0.48 | 69.40 ± 0.38 | 69.71 ± 0.54 | 70.07 ± 0.43 | 70.03 ± 0.44 | .711     |
| Breast                 | 29.71 ± 0.29 | 30.02 ± 0.38 | 30.18 ± 0.33 | 30.45 ± 0.47 | 29.28 ± 0.46 | 30.03 ± 0.34 | 29.72 ± 0.37 | .705     |
| Drumsticks             | 19.61 ± 0.34 | 19.82 ± 0.29 | 19.60 ± 0.24 | 20.00 ± 0.20 | 19.78 ± 0.19 | 19.56 ± 0.28 | 20.14 ± 0.17 | .953     |
| Tibia weight           | 0.43 ± 0.01  | 0.44 ± 0.01  | 0.43 ± 0.01  | 0.42 ± 0.01  | 0.42 ± 0.01  | 0.43 ± 0.01  | 0.44 ± 0.01  | .441     |
| Tibia ash              | 36.59 ± 0.48 | 36.13 ± 0.43 | 36.15 ± 0.52 | 36.25 ± 0.48 | 36.04 ± 0.46 | 35.07 ± 0.58 | 35.74 ± 0.54 | .102     |
| Tibia phosphorus       | 17.36 ± 0.07 | 17.44 ± 0.07 | 17.45 ± 0.08 | 17.25 ± 0.06 | 17.03 ± 0.06 | 16.93 ± 0.16 | 17.01 ± 0.07 | .211     |

*Calcium and non-phytate phosphorus as percentage of recommended levels for starter/grower/finisher.

**Figure 1.** Relationship between the Ca intake and the body weight gain (BWG) and feed conversion ratio (FCR) of broilers.
has negative effects on bone ash, Ca and P content of tibia. Another 21-day trial, Kim et al. (2017) demonstrated that bone mineralisation and bone strength of broiler chickens were not affected by reduction in Ca with phytase presence. Moreover, Rousseau et al. (2016) observed no effect of Ca reduction in the phases of 10–22 and 23–35 days of age, respectively, on the content of bone ash. It has been suggested that greater levels of Ca in diet are required to maximise bone mineralisation and bone strength compared with those to attain the optimal growth performance of broiler chickens. Driver et al. (2005) reported that Ca requirements of broilers at early age were greater for maximal tibia ash (7.2 g/kg) than for body weight gain (4.86 g/kg). Nevertheless, in this study, tibia ash and phosphorus were not affected by the Ca reduction. It is assumed that Ca and NPP levels used at treatment (0.90% for starter, 0.75% for grower and 0.60% for finisher) are enough for skeletal development and maintain bone health. Therefore, it seems that modern broilers might tolerate lower level of Ca and NPP with appropriate 2:1 ratio than recommended by breeders company without causing any adverse effect on growth and performance and bone mineralisation.

Conclusions

Because of understanding of Ca less or wrong way on bone development in the past, the level of dietary Ca in broiler diets kept higher by ignoring its reaction capacity with other nutrients and especially P. Therefore, it can be speculated that as Ca level has been kept higher, which also accordantly resulted increase in dietary NPP level in the past. In conclusion, the results of the present study indicated that the Ca and P requirements of Ross 308 broilers seem to be lower than the Aviagen (2014) and Aviagen (2019) recommendations. It can be concluded that it is possible to reduce almost 20% Ca and NPP intake of broilers, especially after starter period, by keeping the dietary levels as 0.90:0.45, 0.75:0.38 and 0.60:0.30% Ca and NPP for starter, grower and finisher phase respectively without any impairment in growth and bone development. This would also lead to reduce diet cost and keep environment more clean as less inorganic P sources supplemented into diets, and P excretion via manure reduced.

However, more works need to be continued to support the present results on Ca and NPP by especially focussing on influencing of excessive Ca on digestibility and gut health to understand the exact mechanism underlying the lower needs in modern broilers.

Disclosure statement

The authors reported no potential conflict of interest.

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