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# Impact of different levels of calcium and phosphorus in diet of broiler chickens on performance, carcass traits and blood parameters

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**ABSTRACT**

A five week feeding study was carried out to evaluate the effects of different levels of calcium, available phosphorus (AP) on broiler performance, carcass traits and deposition of calcium and phosphorus in serum and tibia of broiler chickens. Two hundred and fifty two one day-old Ross 308 broiler chicks were used. The birds were weighed and assigned to equal nine treatment groups: three calcium to phosphorus ratios were used, 2:1, 1.75:1, and 1.5:1, there were three different values of available phosphorus for each calcium into phosphorus ratio according to the feeding stage. Each treatment had four replicates and each replicate contained seven chicks. The results showed that during starter stage, performance at available phosphorus 0.5% and 0.45% were significantly higher than that of 0.4% regardless of calcium levels for body weight, body weight gain and high feed intake, but during the finisher period, there was no significant difference between treatments. Treatment with Ca level 1.5 and AP 0.4% is the same result of treatment with Ca level 2 and AP 0.5% in all stages. Treatment with Ca level 1.75 and AP 0.35% showed the highest serum ALT, Ca and P measurements and non-significant changes in treatments with AP 0.4. AST and creatinine in treatments with AP 0.3% showed significant decreases compared to treatments with AP 0.4%. Increasing of Ca level and P availability resulted in decreased Ca levels in ash in all stages. Increasing P availability resulted in decreased P levels in all stages. Phosphorus availability 0.4% showed high significant differences from AP 0.3% in weight, eviscerated, dressing and breast. Drumstick and thigh in all treatments were the same except the high Ca level 1.5 with AP 0.35% and Ca level 2 with AP 0.3%, which showed the lowest significant difference from other treatments. It could be concluded that calcium (Ca) and phosphorus (P) utilization at low rate with low available phosphorus could be effective.

## Introduction

The poultry industry has been the most dynamic segment of the global meat market over the past ten years, with the largest expansion being reflected in the rise of global food demand (Longo *et al*., 2007). Production of broilers depends heavily on nutrition (Musilova, *et al*., 2014). Good nutrition program in poultry played an important role in achieving proper body weight gain and quality meat in the light of public health (Jha and Berrocoso, 2015). Primary constituents or diets for poultry are plant based ingredients which comes primarily from the seeds of plants as cereal grains, oil seeds meal and beans, have a relatively high content of phosphorus (Sharifi *et al*., 2012). Poultry nutrition mineral requirement can vary within flock according to species, age, sex and performance level (Rama Rao *et al*., 1999). Mineral nutrition plays a critical role in growth, bone mineralization and reproductive performance (Baoa and Choctb, 2009). Calcium and phosphorus are macro minerals needed for metabolism and mineralization of chicken bones (Charles and Afred, 2021). The two main minerals that make up bone are calcium and phosphorus. Bone contains more than 90% of the body's calcium and 80% of its phosphorus (McDonald, 2002). Phosphorus and calcium are two important minerals in poultry diets (Garcia *et al*., 2013). Normal levels of calcium to available phosphorus in growing ratio is 2:1 (Schwartz, 1996). Phosphorus is an essential nutrient for growing animals, the organism requires sufficient supply of phosphorus with feed in which animal body contains 4–7 g phosphorus/kg, depending on species and stage of growth (Mahan and Shields, 1998). Low dietary calcium improved phosphorus utilization but excess calcium may aggravate a phosphorus deficiency (Letourneau *et al*., 2008). Calcium and phosphorus play an important role in growth rate and bone mineralization (Hurwitz *et al*., 1995). Phosphorus play an important role in maintain integrity of skeletal, appetite and growth in young and older birds (Venalainen *et al*., 2006). Diet contains calcium and phosphorus induces more gain in body weight and feed conversion rate (Jadhav *et al*., 2016). Broiler received ration low in calcium and phosphorus induced reduction in average daily gain, average daily feed intake, and body weight gain (Watson *et al*., 2006). However, increased calcium to phosphorus ratio was reported to decrease weight gain and feed intake (Amerah *et al*., 2014). Moreover, insufficient supplies of calcium and phosphorus reduce and bone calcification (Hurwitz *et al*., 1995), with reduction in toe and tibia ash% (Watson *et al*. (2006)., because of the reduction in tibial and femur calcium and phosphorus (Van Straalen *et al*., 1991). Tibia ash, calcium, and P were decreased in the birds fed low-phosphorus diets (0.30% phosphorus) (Mondal *et al*. (2007). Using half of the requirements for calcium and available phosphorus in broiler diets not induce much adverse effect on carcass characteristics (dressing % and ready to cook %) (El-Faham *et al.,* 2019).

The present study was done to determine the effect of different levels of calcium, phosphorus in the diet of broiler chickens on performance, carcass traits and blood parameters of broiler chickens.

## Materials and methods

*Ethical approval*

The research was conducted in accordance with the institutional guidelines for the care and use of experimental animals approved by the Institutional Animal Care and Use Committee, Zagazig University, Egypt (Approval No. Zu- IACUC/2/F/270/2023).

*Birds and Management*

Two hundred and fifty two male one day old chicks (Ross 308 broiler) were purchased from a producer of commercial chicks. Chicks were housed in a conventional house using a battery system. Upon arrival, they were weighed and randomly allocated equally to nine treatment groups, each containing four replicates with seven chicks for each one. Broilers' vaccination protocols were done against Newcastle (on the 4th and 14th days) and Gumboro diseases (on the 7th and 22th day).The ingredient composition (%) of the experimental diets is shown in Table 1.

*Experimental Design and Diets*

For the experiment, isocaloric and isonitrogenous broiler diets were offered in mash form, fed according to rearing phase (Table 2), and formulated to meet the nutrient requirements set by Ross 308 Broiler Nutrition Specification (2018).Three calcium into phosphorus ratios were used at rates of 2:1, 1.75:1, and 1.5:1. There were three different values of available phosphorus for each calcium into phosphorus ratio according to the feeding stage. Available phosphorus level in starter stage (0.5, 0.45 and 0.4), grower stage (0.45, 0.4 and 0.35) and finisher stage (0.4, 0.35 and 0.3) (Table 1).

*Sampling, Procedures, and Laboratory Analysis*

The following parameters were measured: body weight, body weight gain, feed intake and feed conversion ratio for the different stages of age starter, grower, and finisher. FCR (feed conversion ratio) was calculated according to Wagner *et al.* (1983).

At the end of experiment (35 days), five birds were randomly selected from each group and slaughtered for the collection of blood samples, which were obtained from the wing vein under aseptic conditions into a sterile syringe without anticoagulants and then centrifuged at 3,000 × g for 15 min. The sera were stored at −20°C until usage to determine liver enzymes (Aspartate aminotransferase (AST) and alanine aminotransferase(ALT)) Reitman and Frankel (1957), creatinine (Husdan and Rapoport, 1968), calcium (Gindler and King, 1972) and phosphorus (Goldenberg and Fernandez, 1966).

Eviscerated carcass, dressing percentage, thigh, drumstick and breast were weighed at the end of the experimental period by selecting three birds from each group that fasted overnight, weighed, and then slaughtered by a sharp knife to complete bleeding. Subsequently, their feathers were plucked and evisceration was performed; then, the right tibia was collected for ashing. A total of three birds per treatment at days 10, 23 and 35 were euthanized and then burned in a muffle furnace at 600°C; then, Ca and P were analyzed according to the AOAC (1990) (methods927.02 and 965.17, respectively). Minerals were determined by the method of Nation and Robinson (1971).

*Statistical Analysis*

Results were reported as mean ± SEM (Standard Error of Mean). In order to assess the influence of the nine treatment groups on the different biochemical parameters, one-way analysis of variance (ANOVA)by Duncan multiple test as post hoc test were used. The value of $P < 0.05$ was used to indicate statistical significance. ALL Analyses and charts were done using Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad prism 8.0.2 (Graph Pad Software, Inc).

## Results

*Performance parameters*

In the current study, Table 3 shows that the decrease calcium (Ca) and phosphorus (P) level significantly increase body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Calcium (Ca) and phosphorus (P) utilization at a rate of 2:1, 1.75:1 and1.5:1 with available phosphorus 0.5%. Calcium (Ca) and phosphorus (P) utilization at a rate of 1.5:1 with available phosphorus 0.4% significantly increase body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) during all stages but during the finisher period, there was no significant difference between groups except BWT.

G1 with calcium level 2:1 and AP 0.5% (mostly the highest growth performance) significantly increase BWT and FI during all stage from G6 with calcium level 1.5:1 and AP0.45% (which showed the lowest result in growth performance in all stage) BWG show significant difference between them in starter period only and with comparing BWT and FI in finisher period found that non-significant between them.

G2, G3 with calcium level 1.75:1 and 1.5:1 with AP 0.5% and G9 with calcium level 1.5:1 with AP 0. 4% make the same result as in G1 calcium level 2:1 with AP 0.5% (non-significant with each other in all stage of growth performance) and with G6 with calcium level 1.5:1 and AP 0.45% in finisher stage and over all except some difference as in G2 with calcium level 1.75:1 and AP 0.5% which have a non-significant difference with all groups in FI starter stage and BWG in grower stage and show highly significant difference in BWT of finisher stage, in G3 with calcium level 1.5:1 with AP 0. 5% show non-significant difference with all groups in BWG in overall period. G9 with calcium level 1.5:1 with AP0.4% is the same result of G1 in all stage.

G4 with calcium level 2:1 with AP 0.4%show significant increase from G6 with calcium level 1.5:1 with AP 0.4%in grower stage only period. In other stages highly non-significant from each other , G5 with calcium level 1.75:1 with AP 0.45% show significant increase from G6 with calcium level 1.5:1 with AP 0.45% in grower stage and FI in overall , G7 with calcium level 2:1 with AP 0.4%show significant increase from G6 in BWT and FI in grower stage and FI in overall , G7 and G8 with level 2:1 and 1.75:1 with AP 0.4% as the same but G8 show significant increase from G6 with calcium level 1.5:1 with AP 0.45% the BWG of grower stage.

Table 1. The experimental design.

| Groups | Ca: AP | Starter | Grower | Finisher |
|---|---|---|---|---|
| | | Age: 0 to10 days | Age: 11 to 23 days | Age: 24 to 35 days |
| G1 | Ca: AP (2:1) | 1% Ca + 0.50% AP | 0.90% Ca - 0. 45% AP | 0.80% Ca - 0. 40% AP |
| G2 | Ca: AP (1.75:1) | 0.88 % Ca + 0.50% AP | 0.79% Ca - 0.45% AP | 0.70% Ca - 0.40% AP |
| G3 | Ca: AP (1.5:1) | 0.75% Ca + 0.50% AP | 0.68% Ca - 0.45% AP | 0.60% Ca - 0.40% AP |
| G4 | Ca: AP (2:1) | 0.9% Ca + 0.45% AP | 0.80% Ca - 0.40% AP | 0.70% Ca - 0.35% AP |
| G5 | Ca: AP (1.75:1) | 0.79% Ca + 0.45% AP | 0.70% Ca - 0.40% AP | 0.62% Ca - 0.35% AP |
| G6 | Ca: AP (1.5:1) | 0.68% Ca + 0.45% AP | 0.60% Ca - 0.40% AP | 0.53% Ca - 0.35% AP |
| G7 | Ca: AP (2:1) | 0.80% Ca + 0.40%AP | 0.70% Ca - 0.35%AP | 0.60% Ca - 0.30% AP |
| G8 | Ca: AP (1.75:1) | 0.70% Ca + 0.40%AP | 0.62% Ca - 0.35%AP | 0.53% Ca - 0.30% AP |
| G9 | Ca: AP (1.5:1) | 0.60% Ca+ 0.40%AP | 0.53% Ca - 0.35%AP | 0.45% Ca - 0.30% AP |

Table 2. Composition of the experimental diet on day 10, 24 and 35.

| | Starter stage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 |
| Yellow corn | 50.1 | 50.8 | 51.3 | 50.8 | 51.44 | 52 | 51.5 | 52.2 | 52.7 |
| Soybean meal | 36.6 | 36.6 | 36.6 | 36.6 | 36.6 | 36.6 | 36.6 | 36.6 | 36.6 |
| Corn gluten, 60% | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Soybean oil | 3.5 | 3.2 | 3 | 3.19 | 2.92 | 2.7 | 2.9 | 2.62 | 2.4 |
| Monocalcium phosphate | 1.9 | 1.9 | 1.9 | 1.6 | 1.66 | 1.7 | 1.4 | 1.42 | 1.4 |
| Calcium carbonate | 1.4 | 1.1 | 0.7 | 1.25 | 0.98 | 0.6 | 1.1 | 0. 8 | 0.5 |
| L-lysine Hcl 98% | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| DL-Methionine, 99% | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 |
| L-threonine 98.5% | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.1 |
| Sodium bicarbonate | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.23 | 0.19 | 0.19 |
| Common salt | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.2 | 0.1 | 0.1 |
| Premix | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Calculated composition | | | | | | | | | |
| ME(Kcal/kg) | 3001.3 | 3001 | 3002 | 3000 | 3000 | 3001 | 3001 | 2999 | 3001 |
| CP | 23.64 | 23.65 | 23.72 | 23.69 | 23.7 | 23.7 | 23.73 | 23.72 | 23.78 |
| Ca | 0.10 | 0.89 | 0.75 | 0.90 | 0.81 | 0.86 | 0.81 | 0.70 | 0.60 |
| Available P | 0.50 | 0.50 | 0.50 | 0.45 | 0.45 | 0.46 | 0.40 | 0.40 | 0.39 |
| Lysine | 1.44 | 1.44 | 1.44 | 1.44 | 1.44 | 1.44 | 1.44 | 1.43 | 1.47 |
| Methionine | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 |
| Threonine | 0.10 | 1 | 1 | 0.1 | 0.10 | 0.99 | 1.00 | 0.99 | 0.10 |
| Grower stage | | | | | | | | | |
| Yellow corn | 54.55 | 55.1 | 55.6 | 55.3 | 55.8 | 56.2 | 56 | 56.3 | 56.8 |
| Soybean meal | 32.2 | 32.2 | 32.2 | 32 | 32 | 32 | 32 | 32 | 32 |
| Corn gluten, 60% | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| Soybean oil | 4.5 | 4.3 | 4.1 | 4.2 | 4 | 3.85 | 3.9 | 3.8 | 3.6 |
| Monocalcium phosphate | 1.7 | 1.7 | 1.7 | 1.45 | 1.45 | 1.45 | 1.2 | 1.2 | 1.2 |
| Calcium carbonate | 1.3 | 0.95 | 0.65 | 1.1 | 0. 80 | 0.55 | 0.95 | 0. 75 | 0.45 |
| L-lysine Hcl 98% | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 | 0.29 |
| DL-Methionine, 99% | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 |
| L-threonine 98.5% | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| Sodium bicarbonate | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Common salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Premix | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Calculated composition | | | | | | | | | |
| ME(Kcal/kg) | 3098 | 3100 | 3101 | 3099 | 3100 | 3102 | 3100 | 3102 | 3103 |
| CP | 21.53 | 21.57 | 21.61 | 21.58 | 21.62 | 21.65 | 21.64 | 21.66 | 21.69 |
| Ca | 0.91 | 0.79 | 0.69 | 0.80 | 0.70 | 0.61 | 0.71 | 0.64 | 0.53 |
| Available P | 0.45 | 0.45 | 0.45 | 0.40 | 0.40 | 0.40 | 0.35 | 0.35 | 0.35 |
| Lysine | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 |
| Methionine | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 | 0.63 |
| Threonine | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 |
| Finisher stage | | | | | | | | | |
| Yellow corn | 57.61 | 58.31 | 58.9 | 58.6 | 58.85 | 59.4 | 59.2 | 59.5 | 59.95 |
| Soybean meal | 28.6 | 28.5 | 28.5 | 28.5 | 28.5 | 28.5 | 28.5 | 28.5 | 28.5 |
| Corn gluten, 60% | 4.2 | 4.2 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 |
| Soybean oil | 5.7 | 5.4 | 5.2 | 5.3 | 5.3 | 5 | 5.1 | 5 | 48 |
| Monocalcium phosphate | 1.5 | 1.5 | 1.5 | 1.26 | 1.26 | 1.26 | 1 | 1 | 1 |
| Calcium carbonate | 1.1 | 0.8 | 0.51 | 0.94 | 0.7 | 0.45 | 0.8 | 0. 6 | 0.35 |
| L-lysine Hcl 98% | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 |
| DL-Methionine, 99% | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 |
| L-threonine 98.5% | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| Sodium bicarbonate | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Common salt | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.32 | 0.32 | 0.32 |
| Premix | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

Table 2. Composition of the experimental diet on day 10, 24 and 35 (Continue).

| | Finisher stage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 |
| Calculated composition | | | | | | | | | |
| ME(Kcal/kg) | 3201 | 3199 | 3199 | 3000 | 3197 | 3205 | 3201 | 3203 | 3203 |
| CP | 20.01 | 20.01 | 19.99 | 19.97 | 19.99 | 20.03 | 20.01 | 20.03 | 20.07 |
| Ca | 0.8 | 0.69 | 0.59 | 0.7 | 0.61 | 0.53 | 0.61 | 0.55 | 0.55 |
| Available P | 0.4 | 0.4 | 0.4 | 0.35 | 0.35 | 0.35 | 0.3 | 0.3 | 0.3 |
| Lysine | 1.19 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 | 1.18 |
| Methionine | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |
| Threonine | 0.8 | 0. 80 | 0.81 | 0. 81 | 0. 81 | 0.82 | 0.82 | 0.82 | 0.82 |

Mineral and vitamin premix: Each1kg diet contain Vit. A (12000 IU), vit. D3 (5000 IU), vit. E (80 IU), vit. k3 (3.2mg), vit. B1 (3.2 mg), Vit. B2 (8.6 mg), Vit. B6 (4.3mg), pantothenic acid (20 mg), Vit. B12 (0.017 mg), niacin (65 mg), folic acid (2.20 mg), biotin (0.22 mg), Fe (20mg),Mn (120 mg), Cu (16 mg), I (1.25 mg), Se (0.30 mg) and Zn(110 mg).

Table 3. The effect of different calcium and available phosphorus levels on growth performance in broilers from day 1 to 35.

| | Starter | | | |
|---|---|---|---|---|
| Groups | BWT (g) | BWG (g) | FI (g) | FCR |
| G1 | 266±16.06$^{a}$ | 225.5±16.58$^{a}$ | 254.75±14.24$^{a}$ | 1.13±0.02 |
| G2 | 257±3.55$^{a}$ | 216.57±3.37$^{a}$ | 243.25±14.95$^{ab}$ | 1.12±0.06 |
| G3 | 265.25±7.41$^{a}$ | 224.82±7.63$^{a}$ | 256.5±3.87$^{a}$ | 1.14±0.02 |
| G4 | 244.5±17.13$^{ab}$ | 204±16.86$^{ab}$ | 231.75±16.23$^{ab}$ | 1.13±0.01 |
| G5 | 251.25±11.67$^{ab}$ | 210.62±11.67$^{ab}$ | 242.75±10.65$^{ab}$ | 1.15±0.01 |
| G6 | 228±12.56$^{b}$ | 187.55±12.96$^{b}$ | 219.25±14.17$^{b}$ | 1.16±0.02 |
| G7 | 243±10.48$^{ab}$ | 202.97±10.44$^{ab}$ | 234±6.324555$^{ab}$ | 1.15±0.02 |
| G8 | 250.75±6.13$^{ab}$ | 210.62±5.96$^{ab}$ | 242.5±4.20$^{ab}$ | 1.15±0.01 |
| G9 | 257±14.30$^{a}$ | 216.75±14.24$^{a}$ | 247.5±11.78$^{a}$ | 1.14±0.02 |
| | Grower | | | |
| G1 | 1207.5±55$^{a}$ | 941.5±49.69$^{ab}$ | 1174.5±72.89$^{a}$ | 1.24±0.05 |
| G2 | 1205±90.36$^{a}$ | 948±88.91$^{ab}$ | 1200.5±82.04$^{a}$ | 1.26±0.03 |
| G3 | 1242±33.70$^{a}$ | 976.75±28.57$^{a}$ | 1207.75±53.33$^{a}$ | 1.23±0.02 |
| G4 | 1212.5±71.35$^{a}$ | 968±60.40$^{a}$ | 1202.5±63.56$^{a}$ | 1.24±0.03 |
| G5 | 1227.5±47.87$^{a}$ | 976.25±37.5$^{a}$ | 1224±72.58$^{a}$ | 1.25±0.04 |
| G6 | 1042.5±62.38$^{b}$ | 814.5±57.52$^{b}$ | 989.25±68.03$^{b}$ | 1.21±0.01 |
| G7 | 1175±98.82$^{a}$ | 932±90.31$^{ab}$ | 1167.75±87.86$^{a}$ | 1.25±0.03 |
| G8 | 1207.5±41.12$^{a}$ | 956.75±46.23$^{a}$ | 1185.75±53.10$^{a}$ | 1.23±0.01 |
| G9 | 1185±56.86$^{a}$ | 928±60.31$^{ab}$ | 1146±87.66$^{a}$ | 1.23±0.06 |
| | Finisher | | | |
| G1 | 2212.25±64.13$^{ab}$ | 1004.75±14.93 | 1498±53.43 | 1.49±0.07 |
| G2 | 2287.5±39.58$^{a}$ | 1082.5±83.50 | 1531.25±80.31 | 1.41±0.04 |
| G3 | 2255.75±52.11$^{a}$ | 1013.75±24.54 | 1482.5±46.43 | 1.46±0.05 |
| G4 | 2206.75±127.10$^{ab}$ | 994.25±63.55 | 1438.75±50.94 | 1.44±0.06 |
| G5 | 2224.5±75.15$^{ab}$ | 997±43.85 | 1437.25±30.44 | 1.44±0.03 |
| G6 | 2011±118.67$^{b}$ | 968.5±66.20 | 1404.75±84.79 | 1.45±0.01 |
| G7 | 2201.5±178.06$^{ab}$ | 1026.5±81.45 | 1490±89.43 | 1.45±0.03 |
| G8 | 2242.75±22.15$^{ab}$ | 1035.25±38.87 | 1476.75±60.53 | 1.42±0.04 |
| G9 | 2186.5±138.83$^{ab}$ | 1001.5±92.64 | 1500.25±85.02 | 1.50±0.06 |
| | Overall | | | |
| G1 | | 2171.85±64.44$^{ab}$ | 2927.25±100.70$^{a}$ | 1.34±0.04 |
| G2 | | 2247.07±39.88$^{a}$ | 2975±77.38$^{a}$ | 1.32±0.01 |
| G3 | | 2215.32±51.86$^{ab}$ | 2946.75±94.07$^{a}$ | 1.33±0.02 |
| G4 | | 2166.25±127.31$^{ab}$ | 2873±112.86$^{b}$ | 1.32±0.04 |
| G5 | | 2183.87±75.30$^{ab}$ | 2904±82.98$^{a}$ | 1.32±0.04 |
| G6 | | 1970.55±119.11$^{b}$ | 2613.25±136.46$^{b}$ | 1.32±0.01 |
| G7 | | 2161.47±178.04$^{ab}$ | 2891.75±180.61$^{a}$ | 1.33±0.03 |
| G8 | | 2202.62±22.05$^{ab}$ | 2905±68.09$^{a}$ | 1.31±0.02 |
| G9 | | 2146.25±138.75$^{ab}$ | 2893.75±138.72$^{a}$ | 1.34±0.04 |

Table 4. The effect of different calcium and available phosphorus levels on Kidney, liver function and Ca, P in blood in broilers on 35 day.

| Groups | ALT | AST | Creatinine | Calcium | Phosphorus |
|---|---|---|---|---|---|
| G1 | 11.37±0.47[ab] | 359.5±16.62[a] | 0.34±0.01[b] | 7.66±0.06[d] | 6.66±0.09[a] |
| G2 | 10.8±0.86[abc] | 368.5±8.06[a] | 0.42±0.04[a] | 7.83±0.78[d] | 4.65±2.72[b] |
| G3 | 9.67±1.42[bcd] | 319.75±15.37[b] | 0.28±0.02[cd] | 9.84±0.28[a] | 8.11±0.37[a] |
| G4 | 11.17±0.56[abc] | 270.25±10.30[cd] | 0.27±0.01[cd] | 8.59±0.28[bcd] | 7.53±0.18[a] |
| G5 | 11.5±0.57[a] | 280±12.02[cd] | 0.24±0.02[cd] | 8.87±0.09[bc] | 8.01±0.19[a] |
| G6 | 9.67±0.47[bcd] | 280.5±12.58[cd] | 0.28±0.03[c] | 7.90±0.62[d] | 7.13±0.48[a] |
| G7 | 10.55±0.97[abc] | 282.75±6.02[cd] | 0.24±0.03[cd] | 7.88±0.26[d] | 6.51±0.19[a] |
| G8 | 8.8±0.58[d] | 297.25±13.93[bc] | 0.22±0.01[d] | 9.21±0.48[ab] | 7.71±0.19[a] |
| G9 | 9.55±0.42[cd] | 258±19.78[d] | 0.26±0.01[cd] | 8.18±0.15[cd] | 7.19±0.14[a] |

G1: Ca:AP (2:1); G2: Ca:AP (1.75:1); G3: Ca:AP (1.5:1); G4: Ca:AP (2:1) AP; G5: Ca:AP (1.75:1); G6: Ca : AP (1.5:1); G7: Ca:AP (2:1); G8: Ca:AP (1.75:1); G9: Ca:AP (1.5:1)

*Serum analysis*

At the end of the experiment (Table 4), using low level of calcium (Ca) and phosphorus (P) that made a decrease in creatinine and liver enzymes (ALT and AST) in serum of broiler chickens. ALT in G5 with calcium 1.75:1 with AV.P0.45% showed non-significant changes, followed by group G2 with calcium level 1.75:1 with AP 0.5%, G1 with calcium level 2:1 with AP 0.5% then from G4 with calcium level 2:1 with AP0.45% and G7 with calcium level 2:1 with AP0.4% and significantly from G3 then G6 , G1and G5 show highly significant difference with G9 with calcium level 1.5:1 with AP 0.4% and G8 with calcium level 1.75:1 with AP 0.4% which showed the lowest ALT activity .

AST in G1and G2 with calcium level 2:1 and 1.75:1 with AP 0.5% more significantly than G3 with calcium level 1.5:1 with AV.P0.5%, followed by G8 with calcium 1.75:1 with AP 0.4%, which also followed by G5 with calcium level 1.75:1 with AP 0.45% then G6 with calcium level 1.5:1 with AP 0.45% these 2 groups (G5, G6) are non-significant from G7 with calcium level 2:1 with AP 0.4%. G9 with calcium level 1.5:1 with AP 0.4% showed the lowest AST.

Concerning creatinine, G2 with calcium level 1.75:1 with AP 0.5% showed the highest level of creatinine and significantly more than that treated with all treated groups followed by G1 with calcium of 2:1 with AP 0.5%, G8 with Calcium level 1.75:1 with AP 0.4 % show lowest creatinine value which followed significantly from G6 with calcium level 1.5:1 with AP 0.45%, and non-significantly followed by other groups (when comparing with G8 (non-significant decrease), and with G6 (non-significant increase).

In phosphorus and calcium comparison founded that G2 with calcium level 2:1 with AP 0.5% showed the lowest P and Ca value when comparing with other groups, other groups are non-significant to each other according to P value, but for Ca value G3 with calcium level 1.5:1 with AP 0.5% show the highest level of Ca non-significantly followed by G8 with calcium level 1.75:1 with AP 0.4%.G2 with calcium level 2:1 with AP 0.5% was non-significantly followed by G1, G4, G6, G7 and G9.

*Minerals Deposition*

Our results showed that (Tables 5) Ca: AP ratio show the low availability with low rate, the higher ratio. During the starter, grower, and finisher stages, G9 with calcium level 1.5:1 with AP 0.4% significantly increased Ca and P deposition in the tibial ash. During the starter and finisher periods, G8 with calcium level 1.75:1 with AP 0.4% significantly decreased from G9 with calcium level 1.5:1 with AP 0.4%, but non-significant from each other in grower period and P level in all stages. These results showed that when availability of P decrease, more Ca deposition, that show in all stages as in Ca level in all stages that showed significantly decrease in value from G9 with calcium level 1.5:1 with AP 0.4% to G1 with calcium level 2:1 with AP 0.4% gradually (all significant from each other), while in P level showed the availability of P is 0.5%, the lowest P level especially when ca rate increase, the low P level (2:1 < 1.75:1 < 1.5:1) except in grower stage, when groups treated with P availability 0.4% showed the highest level non-significantly followed by G6 with calcium level 1.5:1 with AP 0.45% in starter and grower stages, G6 in finisher stage had different result for G9 with calcium level 1.5:1 with AP 0.4% that showed non-significant difference with availability 0.45 except with rate 2:1 (which show non -significant difference with availability 0.5 with all rate).

Table 5. The effect of different calcium and available phosphorus levels on Ca and AP on ashing of tibia in broilers on day 10, 24 and 35.

| Groups | Starter | | |
|---|---|---|---|
| | Ca | P | Ca : P ratio |
| G1 | 2.83±0.01[i] | 1.82±0.01[d] | 1.55±0.01[d] |
| G2 | 2.93±0.01[h] | 1.90±0.01[c] | 1.54±0.01[d] |
| G3 | 3.01±0.02[g] | 1.94±0.01[bc] | 1.55±0.01[d] |
| G4 | 3.09±0.02[f] | 1.93±0.01[bc] | 1.59±0.01[c] |
| G5 | 3.17±0.01[e] | 1.95±0.01[b] | 1.62±0.01[bc] |
| G6 | 3.22±0.01[d] | 2.00±0.03[a] | 1.61±0.02[bc] |
| G7 | 3.27±0.01[c] | 2.02±0.02[a] | 1.62±0.01[bc] |
| G8 | 3.33±0.01[b] | 2.03±0.02[a] | 1.64±0.01[b] |
| G9 | 3.44±0.01[a] | 2.02±0.03[a] | 1.70±0.02[a] |
| | Grower | | |
| G1 | 2.85±0.01[h] | 2.14±0.01[a] | 1.33±0.01[g] |
| G2 | 3.02±0.02[g] | 2.08±0.02[d] | 1.45±0.03[f] |
| G3 | 3.11±0.01[f] | 2.09±0.01[cd] | 1.48±0.01[e] |
| G4 | 3.16±0.02[e] | 2.09±0.01[cd] | 1.51±0.01[de] |
| G5 | 3.22±0.01[b] | 2.10±0.01[bc] | 1.53±0.01[d] |
| G6 | 3.31±0.03[c] | 2.12±0[abc] | 1.56±0.01[c] |
| G7 | 3.53±0.01[b] | 2.12±0[abc] | 1.66±0.01[b] |
| G8 | 3.63±0.01[a] | 2.12±0.01[ab] | 1.71±0.01[a] |
| G9 | 3.67±0.03[a] | 2.13±0.01[a] | 1.72±0.01[a] |
| | Finisher | | |
| G1 | 3.82±0.01[i] | 2.23±0.01[e] | 1.71±0.09[g] |
| G2 | 4.02±0.07[h] | 2.29±0.01[d] | 1.74±0.02[g] |
| G3 | 4.24±0.07[g] | 2.32±0.01[c] | 1.82±0.03[f] |
| G4 | 4.49±0.09[f] | 2.33±0.01[c] | 1.92±0.03[e] |
| G5 | 4.70±0.03[e] | 2.33±0.01[c] | 2.01±0.01[d] |
| G6 | 4.85±0.05[d] | 2.36±0.01[b] | 2.05±0.02[d] |
| G7 | 4.99±0.05[c] | 2.37±0.01[ab] | 2.10±0.02[c] |
| G8 | 5.16±0.03[b] | 2.37±0.01[ab] | 2.10±0.01[b] |
| G9 | 5.29±0.04[a] | 2.38±0.01[a] | 2.22±0.01[a] |

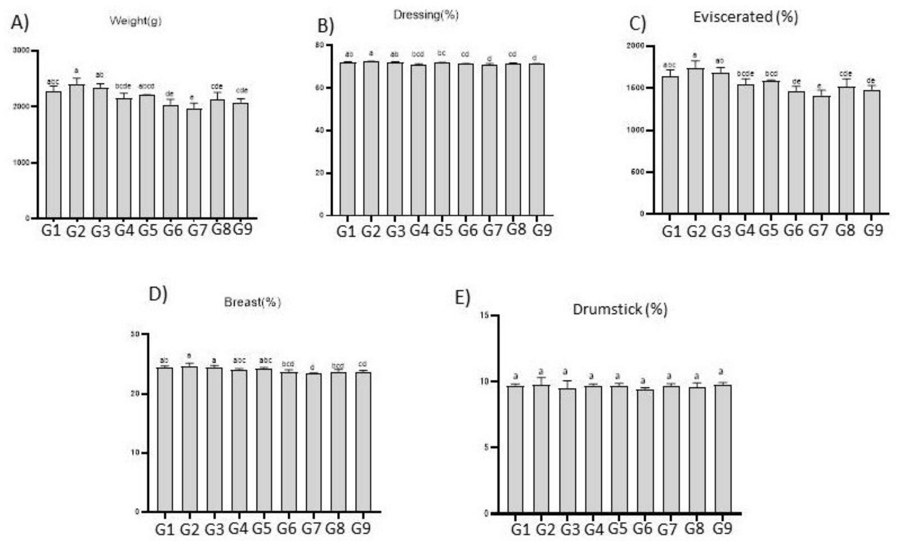


Fig. 1. The effect of different calcium and available phosphorus levels on carcass traits in broilers on day 35.

*Carcass quality traits*

Our results (Figure 1) showed that G2 with calcium level 2:1 with AP 0.5% significantly increased from all groups in all parameters, the drumstick percent which show non-significant difference between groups and, G2 show non-significant difference with AP 0.5% in all parameters and with G4 with calcium level 2:1 with AP 0.45% in thigh and breast reading , in weight reading G5 with calcium level 1.75:1 with AP 0.45% showed non-significance changes with P availability 0.5%, P availability 0.45 % showed non-significance differences compared with P availability 0.40% , eviscerated and dressing value showed the same result in weight with some exception, G5 with calcium level 1.75:1 with AP 0.45% showed significant decrease from reading G2 with calcium level 1.75:1 with AP 0.5% , in breast percentage P availability 0.5% non-significant with P availability 0.45% with calcium level more than 1.5:1, other groups were non-significant to each other, in thigh percentage all groups were non-significant to each other except in G7 with calcium level 2:1 with AP 0.4% and G6 with calcium level 1.5:1 with AP 0.45% which showed significant decrease in all groups, and non-significant from each other.

## Discussion

The present study revealed that low calcium and phosphorus levels significantly increased body weight, body weight gain, feed intake and feed conversion ratio. Calcium and phosphorus utilization at a rate of 1.5:1 with AP 0.4% significantly increased body weight, body weight gain, feed intake and feed conversion ratio during all stages but during the finisher period, there were no significant differences between groups except BWT.

Low levels of phosphorus and calcium induced loss of appetite, and reduction in growth performance meanwhile broilers ration contained enough dietary phosphorus and calcium showed good growth and body performance (Larbier and Leclercq, 1994). These results were in line with the findings of Yan *et al.* (2005) who stated that birds fed low calcium and phosphorus diet (0.30% phosphorus and 0.6% calcium) had decrease in body weighed less than those fed the control diet (0.45% phosphorus and 0.9% calcium) at 18 days of age. Same results were recorded by Watson *et al.* (2006) who mentioned that broiler received ration low in calcium and phosphorus induced reduction in average daily gain, average daily feed intake, and body weight gain. Same observation was recorded by Zeller *et al.* (2015) who stated that calcium and phosphorus in broiler ration resulted in increased body performance. In addition, Díaz *et al.* (2019) reported that broilers fed adjusted calcium: available phosphorus (2:1) ration revealed maximum body weight gain at available phosphorus levels of 0.40%, 0.52%, and 0.53% and at available phosphorus levels of 0.63%, 0.62 %, 0.59%. Using half the requirements of calcium and phosphorus in broiler ration induced much adverse effect on body performance which represented by reduction in BW, WG and increased FCR (El-Faham *et al.*, 2019). These results were in line with the findings of Fallah *et al.* (2019) who stated that normal level of calcium and phosphorus induced increase in body weight and feed efficiency in broilers. Same results were recorded by Ibrahim *et al.* (2019) who mentioned that broiler chicks fed diets containing 50% calcium and available phosphorus improved body performance. Our results agreed with the result observed by Zeyad *et al.* (2020) who reported that broiler received diet contained 30% available phosphorus induced decreases in feed intake and weight gain. Ducklings during starter and grower periods received calcium and phosphorus revealed significant increase in body weight, body weight gain and FCR (Soheir *et al.*, 2021).

Low levels of calcium and phosphorus that made a decrease in creatinine and liver enzymes (ALT and AST) in serum of broiler chickens but calcium at a rate of 1.75:1 with available phosphorus 0.45% showed none significant elevation in liver enzymes (AST and ALT) meanwhile calcium at a rate of 1.75:1 with AP 0.5%, G1 with calcium level 2:1 with AP 0.5% , calcium level 2:1 with AP 0.45% and calcium level 2:1 with AP 0.4% induce significantly elevation.

Our results were similar to the result of Van Straalen *et al.* (1991) who reported that low calcium and phosphorus contents in ration reduced serum liver enzymes. Similar changes in serum liver enzymes were recorded by Zhang *et al.* (2020) who stated that broilers received ration low in calcium and phosphorus revealed reduction in serum calcium, phosphorus, AST and ALT. In addition, Broilers fed on low phosphorus levels in ration showed significant reduction in plasma ALT, AST and creatinine (Mahmoud *et al.*, 2021). Our data was confirmed by result reported by Soheir *et al.* (2021) stated that increasing dietary calcium and phosphorus improved liver enzymes and creatinine.

Our results showed that Ca: P ratio show the low availability with low rate, the higher ratio. during the starter, grower, and finisher stages, calcium level 1.5:1 with AP 0.4% significantly increased Ca and P deposition in the tibial ash meanwhile, during the starter and finisher periods, G8 with Calcium level 1.75:1 with AP 0.4% showed significant reduction from G9 with calcium level 1.5:1 with AP 0.4%, but non-significant from each other in grower period and P level in all stages. These results showed that when availability of P decrease, more Ca deposition, that show in all stages as in Ca level in all stages that showed significantly decrease in value from G9 with calcium level 1.5:1 with AP 0.4% to G1 with Calcium level 2:1 with AP 0.4% gradually (all significant from each other),while in P level showed the availability P is 0.5% the lowest P level especially when ca rate increase, the low P level (2:1 < 1.75:1 < 1.5:1) except in grower stage, when groups treated with P availability 0.4% showed the highest level non-significantly followed by G6 with calcium level 1.5:1 with AP 0.45% in starter and grower stages, G6 in finisher stage had different result for G9 with calcium level 1.5:1 with AP 0.4% that showed non-significant difference with availability 0.45 except with rate 2:1 (which show non -significant difference with availability 0.5 with all rate).

Our results were similar to the result of Van Straalen *et al.* (1991) who reported that low calcium and phosphorus contents in ration reduced serum calcium and phosphorus. Bone mineral content and ash % were linearly decreased as the level of dietary calcium (Onyango *et al.*, 2003). These results were in line with findings of Watson *et al.* (2006) mentioned that broiler received ration low in calcium and available phosphorus revealed reduction toe and tibia ash%. In Addition, Mondal *et al*. (2007) indicated that the tibia ash, calcium, and P were decreased in the birds fed low- phosphorus diets. Also, Wulandari *et al*. (2012) stated that low calcium and phosphorus in ration induce decrease in bone formation represented by low tibial ash content. Same results were recorded by Díaz, *et al*. (2019) who observed that broilers fed adjusted calcium: available phosphorus (2:1) diets revealed maximum weight of tibia ash and weight of phosphorus in tibia ash were observed at available phosphorus levels of 0.40%, 0.52%, and 0.53% and at available phosphorus levels of 0.63%, 0.62 %, 0.59% in broilers fed the variable calcium: available phosphorus diets. The above mentioned results are in agreement with those obtained by Fallah *et al*. (2019) who concluded that normal level of calcium and phosphorus induced increase in tibial calcium ash content in broilers. These results agreed with the results mentioned by Zeyad *et al*. (2020) found broilers received diet contain 30% available phosphorus showed decreased in tibia ash, calcium, and phosphorus Similar changes in serum calcium and phosphorus were recorded by Zhang *et al*. (2020) in broiler chickens received ration low calcium and phosphorus. Broilers fed on low phosphorus levels in ration showed significant decrease in plasma calcium and phosphorus (Mahmoud *et al*. (2021). Broilers fed ration low in calcium and available phosphorus revealed decrease in ash and phosphorus content of tibia (Wang *et al*. 2021).

## Conclusion

It could be concluded that usage of calcium (Ca) and Phosphorus (P) utilization at low rate with low available phosphorus could be effective and showed better results in some parameters when supplemented with calcium (Ca) and phosphorus (P) utilization at high rate with high available phosphorus ration of broiler chickens.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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