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Research article

Response evaluation of broiler chickens to different dietary calcium-to-phosphorus ratios: Effect on growth performance, nutrients retention and intestinal morphology

Zeyad Kamal Imari

Department of Animal Production Techniques, Technical College of Al-Musaib, Al-Furat Al-Awsat Technical University, Babylon City 51001, Iraq

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Abstract

Importance of the work: Calcium (Ca) and available phosphorus (AvP) are necessary minerals that have important roles in the animals; however, increasing dietary calcium causes several health problems.

Objectives: To evaluate the effect in broiler chickens of Ca:AvP ratios on the productive performance and the retention of nutrients.

Materials & Methods: In total, 360 Ross 308 broilers aged 11d (mean initial body weight ± SD = 249.80 ± 10.45 g) were randomly allocated to six dietary treatments with six replicates for each treatment and 10 birds per replicate. The experimental diets contained six concentrations of calcium (1.4%, 1.2%, 1%, 0.8%, 0.6% or 0.4%) and one level of phosphorus (0.4%) that were used to form six calcium-to-phosphorus ratios (3.5:1, 3:1, 2.5:1, 2:1, 1.5:1 and 1:1, respectively).

Results: The Ca:AvP ratio had a significant ($p < 0.001$) effect on body weight and weight gain. The feed intake was reduced ($p = 0.002$) for high Ca:AvP ratios (3.5:1 and 3:1) compared to the standard Ca:AvP ratio (2:1) during the grower period but this effect disappeared in later periods. The feed conversion ratio was significantly ($p = 0.001$) improved in birds fed the standard Ca:AvP ratio (2:1) compared to the other ratios. The production index was the highest ($p < 0.001$) in birds fed the standard Ca:P ratio compared to those fed other unbalanced ratios. The different Ca:AvP ratios had no significant ($p < 0.05$) effect on mortality percentage. The nutrients retention and jejunum morphology were impaired with high Ca:AvP ratios (3.5:1 and 3:1) compared to several other ratios (2:1, 1.5:1 and 1:1).

Main finding: These results suggested that 2:1 was the best ratio between calcium and phosphorus to achieve optimum growth performance.

zeyad.kamal@atu.edu.iq (Z.K. Imari)

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Introduction

Calcium (Ca) and available phosphorus (AvP) are important major elements that should be adequately provided in the diet because they are involved in several vital processes (Proszkowiec-Weglarz and Angel, 2013). Similarly for animals, they are the most abundant minerals in the body and are required in large quantities compared to other minerals; therefore they are called major or macro minerals (Bao and Choct, 2009; Bougouin et al., 2014). However, increasing the calcium concentration in the diet causes physiological and digestive problems in broiler chickens. For example, Driver et al. (2005) observed high dietary calcium levels reduced the energy amount by chelating available molecules of lipids that were subsequently unavailable for absorption. Excess calcium concentration in the diet can diminish the availability of other minerals, such as phosphorus, magnesium, manganese and zinc through the formation of insoluble Ca-phytate complexes in the intestinal tract (Adamu et al., 2012).

These compounds (insoluble Ca-phytates) prevent mineral absorption in the gut and thus cause decrease mineral concentration in the blood serum, which in turn induces the release of the parathyroid hormone in the blood that works to withdraw calcium and phosphorus from the bone and transport it to the blood to maintain normal levels of calcium and phosphorus in the blood serum (de Matos, 2008). This can lead to infections in birds, such as osteoporosis and rickets, restricting normal eating and drinking; thus, these birds become a source of infection for several bacterial and viral diseases, causing great economic losses to producers (Dinev, 2012).

Zanu et al. (2020a) reported that high dietary calcium led to decreased concentrations of phosphorus, magnesium, iron, sodium, zinc and manganese in the tibia and femur in broiler chickens at ages 16 d and 29 d. For this reason, it is preferable to use a standard calcium-to-phosphorous ratio (2:1) in the diet of broiler chickens to obtain good growth performance and optimum bone mineralization. In this regard, Gautier et al. (2017) pointed out the insoluble phytate complexes in the digestive tract were the lowest and growth performance was the greatest in birds fed a diet containing a strict calcium-to-non-phytate phosphorus ratio (2:1).

Compared to a range of diets containing 8 g Ca and 3 g P/kg (2.7:1 ratio), 8 g Ca and 3.5 g P/kg (2.3:1 ratio) and 8 g Ca and 4.5 g P /kg (1.8:1 ratio), the birds fed a diet containing 8 g Ca and 4 g P /kg (2:1 ratio) had a higher tibia ash content and lower phosphorus excretion (Rao et al., 2006). Productive performance, tibia traits and nutrients retention were not affected by reduced calcium and phosphorus concentration in the diet when the Ca-to-AvP ratio was constant at 2:1 (Gautier et al., 2017). The aim of the current study was to evaluate the optimal ratio between calcium and phosphorus by investigating the response of studied parameters (growth performance, nutrients retention and intestinal morphology) to various ratios.

Materials and Methods

Birds housing and dietary treatments

The chicks were purchased from a local hatchery and fed a commercial diet from age 1–10 d. At age 11 d, 360 Ross 308 broilers (initial mean body weight ± SD = 249.80 ± 10.45g) were randomly allocated to one of six dietary treatments with six replications for each treatment and 10 birds per replicate in a completely randomized design. Birds were reared in farm pens at 33 °C during the first week and then the temperature was reduced by 3 °C weekly to reach 21 °C in the last week of the study. The lighting program in the experiment consisted of a light-to-darkness ratio of 23:1 for the first week and then 20:4 for the remaining weeks.

Six dietary treatments were created using six levels of Ca (1.4%, 1.2%, 1%, 0.8%, 0.6% or 0.4%) with one level of available phosphorous (AvP; 0.4%) to form six Ca:AvP ratios (3.5:1, 3:1, 2.5:1, 2:1, 1.5:1 and 1:1, respectively). The experimental treatments were: T1) contained 1.4% Ca and 0.4% AvP (Ca:AvP = 3.5:1), T2) contained 1.2% Ca and 0.4% AvP (Ca:AvP ratio = 3:1), T3) contained 1% Ca and 0.4% AvP (Ca:AvP ratio = 2.5:1), T4) contained 0.8% Ca and 0.4% AvP (Ca:AvP ratio = 2:1), T5) contained 0.6% Ca and 0.4% AvP (Ca:AvP ratio = 1.5:1) and T6) contained 0.4% Ca and 0.4% AvP (Ca:AvP ratio = 1:1), as shown in Tables 1 and 2.

The dietary treatments were based on corn-soybean meal in mashed form. The experimental diets were formulated to supply sufficient nutrients according to Ross strain recommendations, with the exception of calcium. Regarding animal care, the protocol for this study was approved by the Scientific Committee of Department of Animal Production Techniques, Technical College of Al-Musaib Al-Furat Al-Awsat Technical University, Iraq.

Table 1 Ingredients and nutrient composition of experimental diets in finisher period (aged 11–24 d)

Treatment	T1	T2	T3	T4	T5	T6
Ca level (%)	1.4	1.2	1	0.8	0.6	0.4
AvP level (%)	0.4	0.4	0.4	0.4	0.4	0.4
Ca:AvP ratio	3.5/1	3/1	2.5/1	2/1	1.5/1	1/1
Ingredient (%)						
Corn	48	48	48	48	48	48
Soybean	39.87	39.87	39.87	39.87	39.87	39.87
Vegetable oil	6.63	6.63	6.63	6.63	6.63	6.63
Limestone	2.51	1.98	1.46	0.93	0.41	0.01
Dicalcium phosphate	1.49	1.49	1.49	1.49	1.49	1.49
NaHCO3	0.24	0.24	0.24	0.24	0.24	0.24
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.31	0.31	0.31	0.31	0.31	0.31
L-Lysine	0.12	0.12	0.12	0.12	0.12	0.12
L-Threonine	0.05	0.05	0.05	0.05	0.05	0.05
Sand	0.03	0.56	1.09	1.61	2.14	2.53
Total	100	100	100	100	100	100
Calculated nutrients						
Metabolizable energy (kcal/kg)	3100	3100	3100	3100	3100	3100
Crude protein (%)	22	22	22	22	22	22
Ca (%)	1.4	1.2	1	0.8	0.6	0.4
AvP (%)	0.4	0.4	0.4	0.4	0.4	0.4
TP	0.64	0.64	0.64	0.64	0.64	0.64
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16
Lysine	1.29	1.29	1.29	1.29	1.29	1.29
Methionine + Cysteine	0.99	0.99	0.99	0.99	0.99	0.99
Threonine	0.88	0.88	0.88	0.88	0.88	0.88

Ca = calcium; AvP = available phosphorus; TP = total phosphorus

[1] Provided per kilogram of complete diet: vitamin A, 8,000 U; vitamin D3, 2,100 U; vitamin E, 17 U; vitamin K3, 2 mg; thiamine, 1.5 mg; riboflavin, 6.8 mg; vitamin B6, 3 mg; vitamin B12, 0.03mg; biotin, 0.15 mg; pantothenic acid, 28 mg; niacin, 10 mg; choline chloride, 1,000 mg; vitamin C, 350 mg; and folic acid, 1 mg.

[2] Provided per kilogram of complete diet: trace mineral premix supplied the followings per kilogram of diet: Mn, 90 mg; Fe, 60 mg; Zn, 90 mg; Cu, 10 mg; I, 1 mg; Se, 0.15 mg.

Table 2 Ingredients and nutrient composition of experimental diets in finisher period (age 25–42 d)

Treatment	T1	T2	T3	T4	T5	T6
Ca level (%)	1.4	1.2	1	0.8	0.6	0.4
AvP level (%)	0.4	0.4	0.4	0.4	0.4	0.4
Ca:AvP ratio	3.5/1	3/1	2.5/1	2/1	1.5/1	1/1
Ingredient (%)						
Corn	52	52	52	52	52	52
Soybean	34.63	34.63	34.63	34.63	34.63	34.63
Vegetable Oil	7.59	7.59	7.59	7.59	7.59	7.59
Limestone	2.51	1.99	1.46	0.93	0.41	0.01
Dicalcium phosphate	1.55	1.55	1.55	1.55	1.55	1.55
NaHCO3	0.24	0.24	0.24	0.24	0.24	0.24
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.28	0.28	0.28	0.28	0.28	0.28
L-Lysine	0.12	0.12	0.12	0.12	0.12	0.12
L-Threonine	0.03	0.03	0.03	0.03	0.03	0.03

Table 2 Continued

Treatment	T1	T2	T3	T4	T5	T6
Sand	0.30	0.83	1.35	1.88	2.41	2.80
Total	100	100	100	100	100	100
Calculated nutrients						
Metabolizable energy (kcal/kg)	3200	3200	3200	3200	3200	3200
Crude protein (%)	20	20	20	20	20	20
Ca (%)	1.4	1.2	1	0.8	0.6	0.4
AvP (%)	0.4	0.4	0.4	0.4	0.4	0.4
TP	0.63	0.63	0.63	0.63	0.63	0.63
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16
Lysine	1.16	1.16	1.16	1.16	1.16	1.16
Methionine + Cysteine	0.91	0.91	0.91	0.91	0.91	0.91
Threonine	0.78	0.78	0.78	0.78	0.78	0.78

Ca = calcium; AvP = available phosphorus; TP = total phosphorus

[1]Provided per kilogram of complete diet: vitamin A, 8,000 U; vitamin D3, 2,100 U; vitamin E, 17 U; vitamin K3, 2 mg; thiamine, 1.5 mg; riboflavin, 6.8 mg; vitamin B6, 3 mg; vitamin B12, 0.03mg; biotin, 0.15 mg; pantothenic acid, 28 mg; niacin, 10 mg; choline chloride, 1,000 mg; vitamin C, 350 mg; folic acid, 1 mg.

[2]Provided per kilogram of complete diet: trace mineral premix supplied the followings per kilogram of diet: Mn, 90 mg; Fe, 60 mg; Zn, 90 mg; Cu, 10 mg; I, 1 mg; Se, 0.15 mg.

Growth performance

The provided feed to birds was recorded at the beginning of the grower period (11–24 d) and the finisher period (25–42 d), with any residual feed recorded at the end of each period to calculate the feed intake. Body weight was recorded at the beginning and end of each period (grower and finisher) to calculate the body weight gain; mortality was recorded and a feed conversion ratio was calculated.

Production efficiency factor

A production efficiency factor at the completion of the grower, finisher and overall periods was calculated based on Equation 1 (Pascariu et al., 2017):

$$\text{Production efficiency factor} = \frac{\text{Livability} \times \text{Live body weight}}{\text{age of birds} \times \text{feed conversion ratio}} \times 100 \quad (1)$$

Where the live body weight was measured in kilograms and the age was measured in days.

Excreta collection

The total excreta collection method was used to determine nutrient retention. At age 35 d, one chicken of each replicate (six chickens for each treatment) was randomly allotted into a metabolic cage. These birds were allowed an adaptation period of 3 d before fasting for 12 h to completely empty their intestinal contents (fasting period 1). Subsequently, the respective experimental diet was provided to each bird for 3 d, with trays placed under each cage to collect excreta. After 3 d, the feed was removed from the birds for 12 h (fasting period 2) but excreta were still collected. The excreta collected from each replicate was put in plastic zipper bags and kept at -18 °C until laboratory analysis. During this excreta collection period, the feed intake for each replicate was recorded.

Nutrition retention

Diet and excreta samples were processed using an electric grinder. The diet and excreta samples were analyzed for dry matter, crude protein, ether extract, calcium and phosphorus contents. The dry matter in the diet and excreta samples was determined by placing each sample in a drying oven at 105 °C for 24 h after weighing, after which the samples were transferred to the desiccator to cool, then the samples were reweighed (Hamdi et al., 2015). The crude protein contents in the diet and excreta samples were determined according to method of Neijat et al. (2019). The ash content in the samples was measured following the method of Brenes et al. (2003). The calcium and phosphorus contents in the samples were measured using inductively coupled plasma optical emission spectrometry assay. Nutrients retention was calculated using Equation 2 (Li et al., 2014).

$$\text{Nutrients retention \%} = \frac{(N_D \times FI) - (N_E \times E_O)}{N_D \times FI} \times 100 \quad (2)$$

Where N_D is the concentration of nutrients in the diet, FI is

the amount of feed intake, N_E is the concentration of nutrients in the excreta and EO is the amount of excreta output.

Jejunum morphology

At age 42 d, one bird of each replicate was randomly selected and killed. A sample (approximately 2–5 cm) was taken from the jejunum and washed in saline solution before placing in 10% buffered formalin solution for 1 d at room temperature. Then, each sample was dehydrated using alcohol and immersed in xylene and then in paraffin. Section (5 µm thick) were cut from the paraffin blocks and tissue slides were stained using hematoxylin and eosin in order to measure the villus height and crypt depth (Karami et al., 2020). Five villus samples were randomly chosen on each tissue slide and measured. The villus height was measured from the tip of the villus to the crypt and the crypt depth was measured from base of the villus to the submucosa (Jayaraman et al., 2017). The villus height-to-crypt width ratio was calculated.

Statistical analysis

Data were analyzed using one-way analysis of variance in the statistical analysis system (SAS) software. The means were compared using Duncan's new multiple range test. All tests were considered significant at probability values < 0.05. Orthogonal contrasts were used to determine the linear and quadratic responses of variables.

Results and Discussion

Body weight and feed intake

The results related to the body weight and feed intake of the broiler chickens during the grower, finisher and overall periods are shown in Table 3. The body weight significantly ($p < 0.001$) increased in the T4 treatment compared with the other treatments during the grower and finisher periods. In the grower period, birds in the T4 treatment achieved higher feed intake ($p = 0.002$) than the other treatments, followed by the T5 treatment, while the feed intake was not significantly influenced by the different treatments during the finisher and overall periods. Body weight quadratically increased ($p < 0.001$) in the grower and finisher periods. There was a quadratic increase in feed intake during the grower and overall periods when the Ca:AvP ratio decreased from 3.5:1 to 1:1.

Body weight gain and feed conversion ratio

There were significant differences between treatments in the body weight gain and feed conversion ratio (Table 4). The birds in the T4 treatment had a higher weight gain compared with other treatments in the grower, finisher and overall periods ($p < 0.001$). The feed conversion ratio improved in the T4 treatment compared to the T1, T2, T3, T5 and T6 treatments in all periods ($p < 0.01$). The body weight gain and feed conversion ratio quadratically improved when the Ca:AvP ratio decreased from 3.5:1 to 1:1 ($p < 0.001$).

Table 3 Effects of calcium to phosphorus ratios on body weight and feed intake of broiler chickens during different periods of age

Ca:AvP ratio	Body weight (g/b)		Feed intake (g/b/d)		
	11–24 d (grower period)	25–42 d (finisher period)	11–24 d (grower period)	25–42 d (finisher period)	11–42 d (overall period)
3.5:1 (T1)	726.06±21.38^{c}	2182.06±84.10^{c}	60.20±1.53^{c}	147.14±6.73	110.49±4.85
3:1 (T2)	727.42±44.53^{c}	2203.40±115.3^{c}	60.82±1.56^{bc}	148.33±3.50	110.96±3.51
2.5:1 (T3)	800.51±28.12^{b}	2396.03±84.95^{b}	63.87± 2.97^{ab}	150.97±5.90	113.36±3.29
2:1 (T4)	915.61±53.13^{a}	2577.92±100.1^{a}	66.0±3.72^{a}	151.81±2.83	114.99±3.70
1.5/1 (T5)	761.5±41.84^{bc}	2284.78±39.93^{c}	61.13±3.54^{bc}	147.20±4.10	110.16±2.88
1/1 (T6)	724.29±35.35^{c}	2178.85±70.31^{c}	59.85±2.08^{c}	146.33±5.40	109.32±2.69
SE	6.474	14.30	0.453	0.823	0.593
			p value		
Model	0.001	0.001	0.002	0.314	0.077
Linear	0.441	0.478	0.912	0.708	0.621
Quadratic	0.001	0.001	0.001	0.102	0.038

g/b/d = grams/body/bird; SE= standard error; T1 = 1.4% Ca and 0.4% AvP (3.5:1 ratio); T2 = 1.2% Ca and 0.4% AvP (3:1 ratio); T3 = 1% Ca and 0.4% AvP (2.5:1 ratio); T4 = 0.8% Ca and 0.4% AvP (2:1 ratio); T5 = 0.6% Ca and 0.4% AvP (1.5:1 ratio); T6 = 0.4% Ca and 0.4% AvP (3.5:1 ratio). Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 4 Effects of calcium to phosphorus ratios on weight gain and feed conversion ratio of broiler chickens during different periods of age

Ca:AvP ratio	Body weight gain (g/b/d)			Feed conversion ratio (g/g)		
	11–24 d (grower period)	25–42 d (finisher period)	11–42 d (overall period)	11–24 d (grower period)	25–42 d (finisher period)	11–42 d (overall period)
3.5:1 (T1)	33.87±1.75 ^{c}	80.88±4.44^{c}	60.31±2.65^{c}	1.77±0.07^{a}	1.83±0.10^{a}	1.81±0.09^{a}
3:1 (T2)	34.13±3.72 ^{c}	81.99±5.05^{c}	61.05±3.67^{c}	1.78±0.15^{a}	1.81±0.06^{a}	1.80±0.07^{a}
2.5:1 (T3)	39.78± 1.69^{b}	88.63±4.88^{ab}	67.26±2.78^{b}	1.60±0.07^{b}	1.70±0.06^{b}	1.67±0.05^{b}
2:1 (T4)	47.06±3.22 ^{a}	92.35±6.22^{a}	72.53±3.21^{a}	1.40±0.07^{c}	1.64±0.10^{b}	1.57±0.07^{c}
1.5/1 (T5)	36.5b ±2.94^{bc}	84.62±2.48^{bc}	63.57±1.50^{c}	1.68±0.12^{ab}	1.74±0.07^{ab}	1.72±0.06^{ab}
1/1 (T6)	34.11±3.04 ^{c}	80.80±3.80^{c}	60.37±2.25^{c}	1.76±0.11^{a}	1.81±0.08 ^{a}	1.80±0.06^{a}
SE	0.472	0.771	0.461	0.017	0.013	0.011
			p value			
Model	0.001	0.001	0.001	0.001	0.005	0.001
Linear	0.411	0.599	0.467	0.365	0.329	0.273
Quadratic	0.002	0.001	0.001	0.001	0.001	0.001

g/b/d = grams/body/bird; SE= standard error; T1 = 1.4% Ca and 0.4% AvP (3.5:1 ratio); T2 = 1.2% Ca and 0.4% AvP (3:1 ratio); T3 = 1% Ca and 0.4% AvP (2.5:1 ratio); T4 = 0.8% Ca and 0.4% AvP (2:1 ratio); T5 = 0.6% Ca and 0.4% AvP (1.5:1 ratio); T6 = 0.4% Ca and 0.4% AvP (3.5:1 ratio)
Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

These results concurred with Abdollahi et al. (2016) who reported increasing calcium levels in the diet (1.3–13.3 g/kg) with a constant phosphorus level at 3.0 g/kg that reduced the body weight gain and feed intake. Another study indicated that high calcium levels had negative effects on the weight gain, feed intake, tibia ash weight and bone ash percentage when the level of phosphorus was maintained at 0.20% (Powell et al., 2011). Li et al. (2012) noted that the body weight, weight gain and feed intake were reduced by high calcium-to-phosphorus ratio. Xu et al. (2021) observed that the levels of lameness and rickets increased in birds fed a low phosphorus diet especially when the Ca-to-AvP ratio was unbalanced (a high Ca:P ratio), whereas, birds fed a low phosphorous diet with a balanced Ca:P ratio recorded optimal growth performance and a healthy bone structure.

Imbalance in the Ca:AvP ratio in the diet may cause a change in the parathyroid hormone of bones, decrease oxygen transport activity and reduce metabolism collagen in the cartilage, which in turn led to rickets and tibial dyschondroplasia and subsequent low body weight gain (Li et al., 2012). Rickets may be observed during age 1–2 wk in birds fed a high Ca-to-P ratio compared to those fed a low Ca-to-P ratio (Shao et al., 2019) and consequently the rickets and tibial dyschondroplasia lead to decreased feed intake, body weight and impaired feed conversion ratio and an overall reduction in broiler growth performance. In addition, rickets may be a source of infection by many diseases, such as femoral head necrosis, osteomyelitis, fractures, tibia dyschondroplasia, chronic respiratory disease and ascites syndrome (Dinev, 2012).

Another factor reducing growth performance in birds fed high-calcium diets is the increased pH concentration in their digestive tract that stimulates the formation of complex compounds and reduces nutrient absorption in the intestinal tract. An et al. (2020) reported that a high Ca-to-P ratio decreased utilization of both calcium and phosphorus by the formation insoluble Ca–P complexes in the digestive tract, thereby making those elements less available for absorption. Likewise, the ileal digestibility of energy and protein decreased linearly with increasing calcium concentration from 0.4% to 0.9% with a maintained available phosphorus concentration of 0.32% (Walk et al., 2012).

Mortality percentage and production index

There were no significant differences among the treatments in the mortality percentage during the different periods; however, the production index was significantly influenced by different calcium-to-phosphorus ratios (Table 5). Birds fed the diet containing the normal Ca:AvP ratio (2:1) had a significantly ($p < 0.001$) increased production index compared to birds fed the other ratios of calcium-to-phosphorus in the grower, finisher and overall periods. Overall, the mortality percentage quadratically reduced ($p = 0.015$) when the Ca:AvP ratio decreased from 3.5:1 to 1:1. In addition, the results showed that increasing the Ca:AvP ratio from 1:1 to 3.5:1 had a negative effect on the production index (quadratic, $p < 0.001$).

Table 5 Effects of calcium to phosphorus ratios on mortality percentage and production index of broiler chickens during different periods of age

Ca:AvP ratio	Mortality (%)			Production index		
	11–24 d (grower period)	25–42 d (finisher period)	11–42 d (overall period)	11–24 d (grower period)	25–42 d (finisher period)	11–42 d (overall period)
3.5:1 (T1)	5.0±5.47	5.0±5.47	10.0±8.94	162.56±16.80^{c}	270.52±28.76^{d}	258.38±34.54^{c}
3:1 (T2)	5.0±5.47	3.34±5.16	8.34±4.08	164.0±33.44^{c}	280.19±24.67^{cd}	266.92±19.29^{c}
2.5:1 (T3)	1.67±4.08	1.67±4.08	3.34±5.16	205.43±18.65^{b}	330.63± 31.65^{b}	330.14±32.79^{b}
2:1 (T4)	0.0±0.0	1.67±4.08	1.67±4.08	272.51±25.09^{a}	368.23± 44.23^{a}	384.28±40.80^{a}
1.5/1 (T5)	1.67±4.08	1.67±4.08	3.34±5.16	186.77±26.0^{bc}	307.58±16.12^{bc}	305.51±25.20^{b}
1/1 (T6)	3.33±5.16	3.34±5.16	6.67±5.16	166.27±22.63^{c}	276.52±14.40^{cd}	268.98±13.86^{c}
SE	0.745	0.785	0.946	4.063	4.744	4.875
			p Value			
Model	0.323	0.773	0.107	0.001	0.001	0.001
Linear	0.201	0.398	0.108	0.326	0.326	0.218
Quadratic	0.106	0.276	0.015	0.001	0.001	0.001

SE= standard error; T1 = 1.4% Ca and 0.4% AvP (3.5:1 ratio); T2 = 1.2% Ca and 0.4% AvP (3:1 ratio); T3 = 1% Ca and 0.4% AvP (2.5:1 ratio); T4 = 0.8% Ca and 0.4% AvP (2:1 ratio); T5 = 0.6% Ca and 0.4% AvP (1.5:1 ratio); T6 = 0.4% Ca and 0.4% AvP (3.5:1 ratio).
Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

These results were supported by Coto et al. (2008) who reported that neither calcium nor phosphorus levels had an effect on the mortality percentage. In contrast, Scheideler et al. (1995) pointed out that the mortality rate increased in birds fed a high-calcium diet compared to those fed a diet containing a standard level of calcium. Similarly, birds fed a diet containing 0.9% calcium had a higher mortality rate compared to birds fed a diet containing 0.6% calcium (Paiva et al., 2014). The higher mortality rate in birds fed a high Ca:AvP ratio could be attributed to the increased activity of *Clostridium perfringens* in the intestinal tract. Zanu et al. (2020b) reported that increasing the calcium concentration in the intestinal tract enhanced necrotic enteritis by increasing the activity of *Clostridium perfringens* in the gut. Furthermore, Paiva et al. (2014) indicated high dietary calcium was likely involved in inducing necrotic enteritis.

Nutrients retention

The various calcium-to-phosphorus ratios had a significant effect on nutrients retention (Table 6). The retention levels of ash, protein, ether extract, calcium and phosphorus were influenced by different Ca:AvP ratios. The ash retention significantly ($p = 0.002$) decreased in the T1 and T2 treatments compared with the T4 and T6 treatments, whereas the T5 treatment resulted in a numerically and significantly superior ash

Table 6 Effects of calcium to phosphorus ratios on nutrients retention of broiler chickens

Ca:AvP ratio	Nutrients retention %					
	Dry matter	Ash	Protein	Ether extract	Calcium	Phosphors
3.5:1 (T1)	71.53±2.52	53.74±6.47^{c}	58.59±4.01^{b}	76.51±4.43^{b}	37.01±1.37^{c}	40.44±2.97^{b}
3:1 (T2)	70.39±1.73	54.96±2.58^{bc}	58.40±5.19^{b}	76.18±2.37^{b}	38.46±2.02^{c}	40.64±4.16^{b}
2.5:1 (T3)	72.72±1.53	57.73±3.68^{abc}	63.03±3.84^{ab}	79.72±2.44^{ab}	44.62±1.93^{b}	49.73±2.26^{a}
2:1 (T4)	72.60±1.55	62.19±1.40^{a}	66.44±3.62^{a}	82.15±2.85^{a}	48.37±3.42^{a}	51.99±3.42^{a}
1.5/1 (T5)	70.32±2.68	59.13±2.48^{ab}	65.65±3.07^{a}	81.05±2.7^{a}	49.73±2.13^{a}	51.58±3.05^{a}
1/1 (T6)	70.03±2.48	59.49±1.93^{a}	64.15±2.50^{a}	80.63±3.79^{a}	51.08±2.69^{a}	51.85±2.22^{a}
SE	0.365	0.585	0.633	0.531	0.392	0.515
			p value			
Model	0.129	0.002	0.001	0.011	0.001	0.001
Linear	0.320	0.001	0.001	0.001	0.001	0.001
Quadratic	0.096	0.001	0.001	0.002	0.001	0.001

SE= standard error; T1 = 1.4% Ca and 0.4% AvP (3.5:1 ratio); T2 = 1.2% Ca and 0.4% AvP (3:1 ratio); T3 = 1% Ca and 0.4% AvP (2.5:1 ratio); T4 = 0.8% Ca and 0.4% AvP (2:1 ratio); T5 = 0.6% Ca and 0.4% AvP (1.5:1 ratio); T6 = 0.4% Ca and 0.4% AvP (3.5:1 ratio)
Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

retention compared to the T2 and T1 treatments, respectively. Birds in the T4, T5 and T6 treatments had higher levels of protein and ether extract retention ($p < 0.001$) compared to birds in the T1and T2 treatments. Calcium and phosphorus retention significantly ($p < 0.001$) increased in birds fed diets containing lower Ca: AvP ratios (T3, T4, T5 and T6) compared to birds fed diets containing greater Ca:AvP ratios (T1 and T2). The retention levels of ash, protein, ether extract, calcium and phosphorus linearly and quadratically increased when the Ca:AvP ratio decreased from 3.5:1 to 1:1 ($p < 0.001$).

The current results were similar to those reported by Pastore et al. (2012) who indicated increasing dietary calcium or an imbalance in the calcium-to-phosphorus ratio led to a linear increase in calcium excreta and a linear decrease in calcium retention in hen layers. In broiler chickens, Hu et al. (2020) observed that a higher Ca-to-P ratio linearly decreased calcium and phosphorus digestibility. Reduced digestibility of calcium and phosphorus in birds fed a high dietary Ca:AP ratio was due to the formation of insoluble Ca-P complexes in the digestive tract (An et al., 2020). Increasing excretion of calcium and phosphorous in the feces may be attributed to the impaired availability of minerals in the intestinal tract of birds fed a high Ca-to-P ratio (Rao et al., 2006). Likewise, increasing the dietary calcium concentration from 2.5 g/kg to 10.0 g/kg with a constant level of dietary phosphorus at 2.1g/kg caused a significant decrease in the contents of dry matter, nitrogen, minerals and in amino acids digestibility (Wilkinson et al., 2013).

The reduction in nitrogen digestibility may have been due to decreased pepsin efficacy in the proventriculus and gizzard. High dietary calcium reduces protein digestion directly by increasing the gut pH, thus reducing pepsin activity and indirectly by increasing the formation of Ca-phytate complexes (Walk et al., 2012). Increasing calcium in the diet increased the pH in the crop, gizzard and ileum (Shafey et al., 1991; Walk et al., 2012). Increasing the pH led to reduced pepsin activation and function, subsequently reducing protein digestibility (Kim et al., 2005).

Additionally, Qian et al. (1997) pointed out that additive phytase activity was linearly decreased with a high calcium-to-phosphorous ratio. Similar results by Tamim et al. (2004) showed that increasing the level of calcium in diet of broilers from 0.6% to 1.5% reduced intestinal alkaline phosphatase activity by 52% and intestinal phytase activity by 75%. On the other hand, a low Ca-to-P ratio in the diet linearly increased calcium and phosphorus digestibility (Hu et al., 2020). Plumstead et al. (2008) mentioned that the digestibility and absorption of calcium and phosphorus increased linearly with decreased dietary calcium from 1.16% to 0.47% with a stable phosphorus concentration of 0.34%.

Jejunum morphology

The Table 7 indicates that there was a significant difference between treatments in villus height, crypt depth and the villus height-to-crypt depth ratio. Birds fed a narrow Ca: AvP ratio had a higher villus height ($p = 0.001$) than birds fed a broader Ca:AvP ratio. The crypt depth significantly ($p < 0.05$) increased in the T1 and T2 treatments compared to the T4 treatment. The villus height-to-crypt depth ratio was the highest in the T4 treatment, followed by the T5 and T6 treatments, compared to the T1 and T2 treatments ($p < 0.001$). The villus height and villus height-to-crypt depth ratio increased linearly ($p < 0.001$) when the Ca:AvP ratio decreased from 3.5:1 to 1:1 while the crypt depth increased linearly with an increasing Ca:AvP ratio in the diet.

Table 7 Effects of calcium to phosphorus ratios on jejunum morphology in broiler chickens

Ca:AvP ratio	VH	CD	VH/CD ratio
3.5:1 (T1)	1032.43±68.70^{b}	227.50±13.40^{a}	4.53±0.15^{c}
3:1 (T2)	1069.50±90.68^{b}	236.75±26.84^{a}	4.56±0.58^{c}
2.5:1 (T3)	1110.83±54.62^{ab}	206.66±17.86^{ab}	5.41±0.62^{bc}
2:1 (T4)	1196.17±67.30^{a}	184.58±21.41^{b}	6.53±0.63^{a}
1.5/1 (T5)	1158.33±60.99^{a}	204.16±35.83^{ab}	5.81±0.98^{ab}
1/1 (T6)	1174.67±70.96^{a}	210.0±40.55^{ab}	5.77±1.17^{ab}
SE	11.62	4.61	0.127
p value			
Model	0.001	0.042	0.001
Linear	0.001	0.001	0.001
Quadratic	0.001	0.036	0.001

VH = villus height; CD = crypt depth; SE= standard error; T1 = 1.4% Ca and 0.4% AvP (3.5:1 ratio); T2 = 1.2% Ca and 0.4% AvP (3:1 ratio); T3 = 1% Ca and 0.4% AvP (2.5:1 ratio); T4 = 0.8% Ca and 0.4% AvP (2:1 ratio); T5 = 0.6% Ca and 0.4% AvP (1.5:1 ratio); T6 = 0.4% Ca and 0.4% AvP (3.5:1 ratio) Mean±SD in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Similar results were obtained by Paiva et al. (2014) who demonstrated that the villus height-to-crypt depth improved in birds fed a 0.6% calcium diet compared to birds fed a 0.90% calcium diet. Aptekmann et al. (2001) reported that increasing the calcium concentration in quail diets decreased the villus height and then decreased the digestion and absorption capacity. On the other hand, increasing dietary calcium had no significant effect on the villus height and crypt depth of the duodenum, jejunum and ileum as long as the calcium-to-phosphorus ratio was maintained at 2:1 (Abdulla et al., 2016; Oikeh et al., 2019). Intestinal villi play an important role in the absorption of nutrients (Fuller, 2004). Therefore, any increase in the villus height leads to an increased intestinal surface area and a subsequently increase in absorption capability (Chwen et al., 2013). High *Clostridium perfringens* activity that negatively affects intestinal morphology may be associated with increased calcium levels in the diet. Increasing dietary calcium increases the pH in the gut and in turn promotes *Clostridium perfringens* growth (Zanu et al., 2020a). Increasing pathogenic activity by bacteria, such as *Clostridium perfringens*, decreases the villus height and villus height-to-crypt depth ratio and increases the crypt depth (Zanu et al., 2020b).

In conclusion, the current results indicated that an unbalanced calcium-to-phosphorus ratio had a negative effect on production performance and nutrients retention. In addition, the results indicated that the best calcium-to-phosphorous ratio was 2:1 to obtain higher growth performance and the retention and absorption of nutrients.

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