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Research Article

Effect of Calcium to Phosphorus Ratio on Tibia Mineralization, Blood Metabolites and Carcass Traits of Broiler Chickens

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Keywords
Available phosphorus, broiler, ratio, calcium, edible viscera.

Abstract
The present study was performed to evaluate effect of calcium (Ca) to available phosphorus (AP) ratio on tibia mineralization, blood biochemical and carcass parameters. A total of 360 one-day-age broiler chicks Ross 308 was used. The birds were fed a basal diet from 1-10 day of age. On eleventh day of age, all birds divided to six dietary treatments where consisted of six levels of calcium (1.4, 1.2, 1, 0.8, 0.6 and 0.4%) with one level of available phosphorus (0.4%) thus were formed six ratios of Ca to AP (3.5:1, 3:1, 2.5:1, 2:1, 1.5:1, and 1:1). The results indicated that a high or low calcium to phosphorus ratio decreased the tibia ash percentage and concentration of calcium, phosphorus, and alkaline phosphatase in the blood. The tibia ash percentage was increased (P< 0.01) in the standard Ca: AP ratio group (2:1) compared with the high and low Ca: AP ratio groups (3.5:1, 3:1, 2.5:1, 1.5:1 and 1:1). The serum phosphorus level was decreased (P<0.05) in high Ca: AP ratio groups (3.5: 1 and 3: 1) compared to other ratio groups. Birds in the standard Ca: AP ratio group (2:1) had higher calcium and alkaline phosphatase levels in the blood compared to those in the high Ca: AP ratio groups (3.5: 1 and 3: 1) or those in the low Ca: AP ratio group (1:1). The length and width of tibia and the relative weight of the wing, heart, liver, and gizzard were not impacted by the different ratios of Ca: AP. while the relative weight of breast and leg quarter were higher (P<0.01) in the standard Ca: AP ratio group (2:1) when compared with other ratio groups. Our results indicate, that not only the level of dietary calcium and phosphorous had an important effect on the bird's health, but the ratio of calcium to phosphorus also had a significant impact on the productive and physiological performance of birds.

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1. Introduction

Poultry products is one of important sources of animal protein in human life (Wahyono et al., 2018). Because it not only provide high quality protein but provide also important fatty acids, minerals and vitamins. Poultry meat is also characterized by not having negative effects on people with diabetes or heart disease compared to red meat. So it is in great demand by people all over the world. But this industry faces many challenges, one of the most important is diseases that cause great economic losses to producers and countries, especially, nutritional diseases that occur due to a deficiency in some nutrients. On other hand, it was also noted that a high concentration of one of the nutrients in the diet may be reduced digestion and absorption of other nutrients, subsequently exposure to infection by various diseases. As, Shafey and McDonald (1991) observed that increase calcium level in the diet can reduce the availability of other minerals and impair absorption. Increase dietary of calcium may be reduce the phosphorus utilization, as well as phytase activity by chelation between calcium and phosphorus (Applegate et al., 2003; Tamim et al., 2004 and Liu et al., 2013). Wider Ca to NPP ratio lead create more of Ca-P complexes in the intestinal tract which in turn of reduced available of phosphorus for birds (Han et al., 2016).

In contrast, the use of the normal ratio of calcium and phosphorus at (2:1) has led to increase in the productive performance and tibia mineralization in the birds compared with other ratios (Rao et al., 2007). Han et al. (2016) reported also The best ratio between calcium and phosphorous (Ca:P) in order to obtain the highest values of growth performance and bone mineralization was between 2.21:1 to 2.46:1 at average of 2.32:1 in broilers fed 1alpha(OH)D3 or 25-Hydroxyvitamin D3. The improvement in growth performance of birds fed a narrow calcium to phosphorous ratio, may be due to an increase in the activity of the phytase enzyme, phosphorus digestibility and phosphorus retention (Qian et al., 1997). The aim of the experiments was to evaluate effect of different ratios of calcium and available phosphorus (Ca: AP) on bone and carcass traits of broiler chickens from 11 to 42 day of age.

2. Material and methods

2.1. Animals and dietary treatments

A total of 360 one-day-age chick of the Ross 308 breed were purchased a commercial hatchery. All birds were fed a basal diet from 1-10 day of age, and then (at eleventh day of age) the birds distributed into six experimental treatments from 11 to 42 day post hatch, the each treatments contained six replicate and ten chicks in the each replicate. The dietary treatments was formulated to contain on different levels of calcium (1.4, 1.2, 1, 0.8, 0.6 and 0.4%) with one level of available phosphorus (0.4%) to create different dietary Ca-to-AP ratios (3.5:1, 3:1, 2.5:1, 2:1, 1.5:1 and 1:1) table 1. all nutrients met or exceeded the requirement birds exception calcium (Aviagen, 2014). The farm temperature was fixed at 33°C for the first week then the temperature was gradually reduced by 2°C weekly to reach 22°C in The last week of the experiment. The relative humidity was kept at 50 to 60% through the period of the experiment. All birds were permitted to intake feed and water freely.

Table 1. Composition of experimental diets.

	Grower period (11–24 days)						Finisher period (25–42 days)					
Treatments	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
Ca levels %	1.4	1.2	1	0.8	0.6	0.4	1.4	1.2	1	0.8	0.6	0.4
AP levels%	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Ca: AP ratios	3.5:1	3:1	2.5:1	2:1	1.5:1	1:1	3.5:1	3:1	2.5:1	2:1	1.5:1	1:1
Ingredients (%)												
Corn	48	48	48	48	48	48	52	52	52	52	52	52
Soybean	39.87	39.87	39.87	39.87	39.87	39.87	34.63	34.63	34.63	34.63	34.63	34.63
Oil	6.63	6.63	6.63	6.63	6.63	6.63	7.59	7.59	7.59	7.59	7.59	7.59
Limestone	2.51	1.98	1.46	0.93	0.41	0.01	2.51	1.99	1.46	0.93	0.41	0.01
Dicalcium phosphate	1.49	1.49	1.49	1.49	1.49	1.49	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	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	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	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	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	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	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	0.03	0.03	0.03	0.03	0.03	0.03
Sand	0.03	0.56	1.09	1.61	2.14	2.53	0.30	0.83	1.35	1.88	2.41	2.80
Calculated nutrient												
(kcal/kg)	3100	3100	3100	3100	3100	3100	3200	3200	3200	3200	3200	3200
Crude protein, %	22	22	22	22	22	22	20	20	20	20	20	20
Ca, %	1.4	1.2	1	0.8	0.6	0.4	1.4	1.2	1	0.8	0.6	0.4
AP, %	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
sodium, %	0.16	0.16	0.16	0.16	0.16	0.16	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	1.16	1.16	1.16	1.16	1.16	1.16
Methionine+ Cysteine	0.99	0.99	0.99	0.99	0.99	0.99	0.91	0.91	0.91	0.91	0.91	0.91
Threonine	0.88	0.88	0.88	0.88	0.88	0.88	0.78	0.78	0.78	0.78	0.78	0.78

[1]Provided per kilogram of complete diet: vitamin A, 8000 U; vitamin D3, 2100 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, 1000 mg; vitamin C, 350 mg; and folic acid, 1 mg.
[2]Provided per kilogram of complete diet: the 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, and Se, 0.15 mg.
ME= Metabolizable energy; Ca= calcium; AP= available phosphorus.

2.2. Tibia traits

The ash, length and width of the tibia were measured according to the method reported by Zanu et al. (2020).

2.3. Serum biochemical

On day 42 of age, blood samples were taken from brachial vein from three birds per replicate then the blood serum was separated by centrifuge. The serum of calcium and phosphorus concentration were measured according for the method that pointed by Leske and Coon (2002). While the activity of alkaline phosphatase concentration (ALP) in the blood was estimated by spectrophotometrically that described by Hammad etal. (2018).

2.4. Carcass traits

At the last week of the experiment, three males of each replicate were selected then were weighted and them slaughter for evaluated carcass yield. The breast, thigh, wing, liver, heart and gizzard were weighted separately.

2.5. Statistical Analysis

Data were analyzed by 1-way analysis of variance using the GLM procedure of SAS (2012) program in design of completely randomized, the means were compared by Duncan test. A probability value of ($P < 0.05$) was depicted to be statistically significant.

3. Result and discussion

3.1. Tibia traits

Table 2. Shows effect of calcium to phosphorus ratio on tibia bone parameters. High significant superiority ($P<0001$) was observed in percentage of tibia ash for the birds of T4 when compared with other experimental treatments. The length and width tibia were not affected by calcium to phosphorus ratios ($P>0.05$). The current results agree with the findings of Amerah et al., (2014) who pointed out that increasing the calcium to phosphorus ratio leads a significant linear decrease in bone ash and phosphorus digestibility.

Increasing calcium dietary from 0.67 to 1.33% with constant level available phosphorus at 0.20% lead to decrease in weight gain, feed intake, tibia ash weight, bone ash percentage, tibia breaking strength and bone weight (Powell et al., 2011).The tibia ash percentage was the highest in birds that fed diet containing normal ratio of calcium to phosphorus at 2:1, regardless of concentration of these minerals in diet (Rao and Raju, 2006).

In contrast, Wilkinson et al. (2014) who indicated that the birds fed on 10 g of calcium/ kg diet, achieved higher percentage of tibia ash than for birds fed on 5 g of calcium/kg diet. The decrease in tibia ash in the birds of the T1 (3.5:1) and T2 (3:1) treatments may be due to the use abnormal ratios between calcium and phosphorus, which are in turn causes poor availability of these minerals in digestive tract. Hurwitz and Bar (1971) who pointed that high calcium to phosphorus ratio reduces phosphorus soluble absorption by increased precipitation of insoluble calcium and phosphorus complexes in the digestive tract. Rao and Raju (2006) who noted also that Birds that fed low levels of phosphorous at high levels of calcium had the highest incidence of leg abnormalities ($P<0.01$) It may be attributed to poor availability of these minerals in high calcium to phosphorus ratios of (2.3:1 and 3:1).

Bone mineralization and growth performance in the birds was optimal when calcium to phosphorous ratio is 2.32:1 compared with other ratios regardless 1alpha (OH)D3 or 25-hydroxyvitamin D was used as a source of vitamin D3, The best ratio between calcium and phosphorous (Ca:P) in order to obtain the highest values of growth performance and bone mineralization of broilers was between 2.21:1 to 2.46:1 at an average of 2.32:1 (Han et al., 2016).

Table 2. Effect of varying dietary calcium (Ca) to available Phosphorus (AP) ratios on Tibia Parameters and Serum biochemical at 24 days

Dietary Treatments	**Tibia Parameters**			**Serum biochemical**		
Ca: AP ratio	Ash %	Length, mm	Width, mm	Ca, mmol/L	P, mmol/L	ALP (U/L)
T1 (3.5:1)	36.62 ± 0.79^{d}	98.91 ± 3.23	8.11 ± 0.34	2.48 ± 0.09^{b}	2.04 ± 0.01^{b}	2232 ± 108.69^{c}
T2 (3:1)	37.36 ± 0.7^{cd}	99.0 ± 2.34	8.17 ± 0.35	2.56 ± 0.12^{b}	2.0 ± 0.07^{b}	2390 ± 103.0^{bc}
T3 (2.5:1)	41.34 ± 1.42^{b}	103.37 ± 2.05	9.02 ± 0.38	2.82 ± 0.17^{ab}	2.21 ± 0.01^{a}	2815.60 ± 163.82^{ab}
T4 (2:1)	45.06 ± 1.11^{a}	106.07 ± 2.07	9.30 ± 0.39	3.12 ± 0.17^{a}	2.23 ± 0.04^{a}	2933.40 ± 168.76^{a}
T5 (1.5:1)	40.20 ± 1.16^{bc}	102.62 ± 1.17	8.97 ±0.36	2.64 ± 0.10^{b}	2.21 ± 0.04^{a}	2708.60 ± 166.94^{ab}
T6 (1:1)	36.98 ± 0.46^{d}	101.33 ± 1.60	8.13 ± 0.28	2.59 ± 0.12^{b}	2.12 ± 0.08^{ab}	2255.60 ± 138.55^{c}
P-value	<.0001	0.200	0.082	0.032	0.021	0.005

[a-d]Values in the same column with different letters are significantly different ($p < 0.05$).
Ca= calcium; P= phosphorus; ALP= alkaline phosphatase.
Ca: AP ratio= calcium to available phosphorus ratio

3.2. Serum biochemical

The table 2. Shows also effect of different ratios of calcium to phosphorus on some blood traits. Calcium to phosphorus ratios did effected on serum of calcium, phosphorus and alkaline phosphates levels. The birds fed diet containing normal calcium to phosphorus ratio at 2:1 (T4) had a higher level of calcium, phosphorus and alkaline phosphatase in the blood than the T1, T2 and T6 treatments. While the T4 treatment did not record any significantly different with T3 treatment or T5 treatment in criteria mention above. These results were agreed with results of Xu et al. (2021) who reported high Ca:P ratio in the diet reduces concentration of serum phosphorus ($P<0.01$). Zhang et al. (2020) who pointed that the reduce calcium and phosphorus level in the diet causes significant reduce calcium, phosphorus and alkaline phosphates levels in serum and increase in levels of serum parathyroid hormone.

In contrast, Han et al. (2016) reported that the level of plasma calcium was high at Ca/AP ratio of (2.57) compared with other ratios, while the plasma phosphorus was not impacted by different ratios of calcium to phosphorus. A high Ca/P ratio increased the concentration of serum calcium and reduced alkaline phosphatase activity in the blood compared with a normal Ca/P ratio, but serum phosphorus concentration was not affected by these ratios (Jiang et al., 2013). In order to maintain adequate serum calcium levels, the broiler reduces calcium output in the excreta and increases resorption of calcium in bone, thus reducing calcium concentration in the tibia at high levels of dietary calcium (Pelicia et al., 2011).

3.3. Carcass traits

Effects of calcium to phosphorus ratio on carcass traits were showed in table 3. The relative weight of breast and thigh were impacted by calcium to phosphorus ratio, there was a significantly increase in relative weight of breast for the T4 and T5 treatments compared to T1, T2 and T6 treatments. Birds of the T4 treatment achieved the best relative weight of thigh (P=0.014) when compared with other treatments. These data indicated that high or low calcium to phosphorus ratio decreased the meat production and muscle growth of broilers. These results were agreed with finding of Han et al. (2016) reported that the carcass and breast weight reduce significantly in birds that fed diet at low ratio of Ca/P (1.4/1) when compared with normal and high ratios of Ca/P (2/1) and (2.68/1) respectively at age 21 and 42 days. In contrast, Han et al., (2015) noted that the used different ratios of calcium to phosphorus did not effect on relative weight of carcass, breast and leg quarter. Low dietary calcium to phosphorus ratio did not affect dressing percentage and relative weight of drumstick, thigh and breast muscles (Tizziani et al., 2019). Similarly, Adamu et al. (2012) who found that birds fed high dietary calcium to phosphorus ratio (3 to1) had a higher value of breast and drum stick weight when compared with birds fed standard calcium to phosphorus ratio (2 to 1).

Table 3. Effect of varying dietary calcium (Ca) to available Phosphorus (AP) ratios on carcass traits and edible viscera of broilers chickens

Dietary Treatments	Carcass traits %			Relative weight of edible viscera %		
Ca: AP ratio	Leg quarter	Breast	Wing	Heart	Liver	Gizzard
T1 (3.5:1)	19.75 ± 0.49^{b}	23.67 ± 0.88^{c}	5.98 ± 0.19	0.44 ± 0.08	2.03 ± 0.09	1.65 ± 0.07
T2 (3:1)	20.49 ± 0.55^{b}	24.56 ± 1.10^{bc}	6.13 ± 0.20	0.36 ± 0.02	1.89 ± 0.07	1.66 ± 0.04
T3 (2.5:1)	20.69 ± 0.28^{b}	26.61 ± 1.40^{ab}	6.44 ± 0.67	0.40 ± 0.03	2.06 ± 0.09	1.52 ± 0.07
T4 (2:1)	23.19 ± 0.75^{a}	28.25 ± 0.36^{a}	6.16 ± 0.17	0.37 ± 0.04	1.97 ± 0.04	1.66 ± 0.03
T5 (1.5:1)	20.81 ± 0.78^{b}	27.51 ± 0.86^{a}	6.25 ±0.07	0.40 ±0.06	1.93 ±0.07	1.54 ± 0.08
T6 (1:1)	20.60 ± 0.69^{b}	24.15 ± 0.53^{bc}	5.95 ± 0.16	0.38 ± 0.02	2.13 ± 0.14	1.77 ± 0.06
P-value	0.014	0.006	0.888	0.902	0.509	0.117

$^{a-c}$Values in the same column with different letters are significantly different ($p< 0.05$).

Ca: AP ratio= calcium to available phosphorus ratio

3.4. Relative weight of edible viscera

The table 3. Showed effect of different ratios of calcium to phosphorus on relative weight of edible viscera. Where the results showed that no significant different between treatments for relative weight of heart, liver, and gizzard (P>0.05). Similar results were observed by Han et al., (2015) who reported that the relative weight of liver, heart and kidney were not influenced by used different ratios of calcium to phosphorus in the diet. Increasing dietary calcium did not affect average weight of liver, heart, gizzard and bursa fabricius (Demirel et al., 2007).

4. Conclusion

Results of this study showed that the different ratios of calcium and phosphorus have a significantly affect blood metabolites, ash tibia percentage and relative weight breast and thigh while these ratios did not effect on the relative weight of edible viscera. The results indicated also that the best ratio between calcium and phosphorus to obtain normal growth is 2:1.

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