

## Research Article



# Requirements of Dietary Calcium for Broiler Chickens from 11 to 42 Days of Age

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**Abstract** | This study aimed to explore the optimal level of dietary calcium for 11 to 42 day old broilers. Three hundred and sixty commercial broiler chickens were used in this study. The broiler chickens were randomly placed in 36 pens with 6 groups, each group contained 6 pens with 10 birds for each pen. Six experimental diets with different levels of calcium (Ca) were formulated to be as follows: 1.4% Ca, 1.2% Ca, 1% Ca, 0.8% Ca, 0.6% Ca and 0.4% Ca. The polynomial regression equations were used to estimate nutritional requirements of calcium. Results show the different levels of dietary calcium had quadratic impact on calcium, phosphorus, and ALP (Alkaline Phosphatase) levels in the serum ( $p=0.027$ ;  $p<0.0001$  and  $p=0.0006$  respectively). The ash, calcium and phosphorus content of tibia were showed non-linear responses ( $p<0.0001$ ) when the dietary calcium reduced from 1.4% to 0.4%. Lower calcium in the diet non-linearly increases ( $p<0.05$ ) dressing percentage and relative weight of the breast and leg. The results also showed that predicted dietary calcium requirements for dressing percentage and relative weight of breast and leg were 0.86, 0.83, and 0.81% respectively. While predicted dietary calcium requirements for ash, calcium, and phosphorus content in the tibia were 0.85, 0.86, and 0.73% respectively.

**Keywords** | Requirements, Broiler chickens, Calcium, Tibia, Carcass

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## INTRODUCTION

The main goal of broiler production is to produce animal protein with high nutritional value at an affordable price. However, the expansion of poultry industry has various detrimental effects on the environment (Rodić et al., 2011). Poultry manure contains large amounts of minerals, especially phosphorus that is one of the most important pollutants of the environment. Therefore, the increased secretion of phosphorus formed risk on the environment and the economy. Accumulation of phosphorus in water stimulates excessive growth of green algae and aquatic plants (Chislock et al., 2013). Excessive growth of green algae causes enormous problems for humans and animals

by depleting dissolved oxygen and decreasing pH, which in turn leads to the death of fish and other aquatic life and thus reduces tourism and leisure (Riza et al., 2023). Many studies referred that increasing dietary calcium increases phosphorus excretion in the manure of broilers. Increasing dietary calcium bind to dietary phosphorus and formed insoluble tricalcium phosphate in small intestine (Heaney et al., 2002). Rao et al. (2006) pointed out that the phosphorus excretion was increased when the dietary calcium increased. While, Shafey (1993) observed that excretion of trace minerals was significantly increased with increased levels of calcium in the diets. Decreasing calcium availability and increasing excretion of magnesium, zinc, manganese and phosphorus are often associated with increased

calcium in the diet (Wilkinson et al., 2013; Gautier et al., 2017). Increasing dietary calcium causes impair digestion and absorption by formation of insoluble elements in the intestinal tract, thus decrease nutrient utilization such as phosphorus (Tamim et al., 2004). Therefore, estimation of dietary calcium requirements have vital importance to reduce phosphorus excretion in the manure of poultry. Thus this study aimed to estimate the optimal level of dietary calcium by studying the responses of carcass traits, tibia traits, and some blood parameters to different levels of dietary calcium.

## MATERIAL AND METHODS

### HOUSING AND EXPERIMENTAL DIETS

Three hundred and sixty commercial broiler chickens were used in this study. The broiler chickens were randomly placed in 36 pen with 6 groups, each group contained 6 pen with 10 birds for each pen. Ingredient, nutrient composition and experimental diets were showed in the Table 1. Experimental diets were formulated to meet or exceed the requirements of birds for all nutrients expect calcium (Aviagen, 2014). Six experimental diets with different levels of calcium were formulated to be as follows: 1.4% Ca, 1.2% Ca, 1% Ca, 0.8% Ca, 0.6% Ca and 0.4% Ca. The temperature was adjusted at 35 °C in the first day and gradually reduced by 3 °C every week. The light program consists of 23 hours' light and 1 hour's dark in the whole day. The birds were raised in the deep litter system, and feed and water were provided freely.

### COLLECTION OF SAMPLES AND CHEMICAL ANALYSES

After 6 hours of starvation, one bird from each replicate was randomly selected, weighted, and slaughtered (the weight of the selected bird was close to the average weight of their replicate). Then the carcass, breast, leg and wings were weighted in order to calculate the dressing percentage and relative weight of breast, leg and wings. The samples of tibia bone were taken from six birds of each groups. The samples of tibia were de-fleshed and then defatted by immersing in alcohol for 48 hours and then in ether for 48 hours (Hamdi et al., 2015a), after which the length and width of tibia were measured by using digital caliper. Then the tibia was ashed in a muffle furnace at 600 °C for 12 hours (Hamdi et al., 2015b) and the ash, calcium and phosphorus of tibia were measured. The blood sample were collected from the neck of birds during slaughter. The serum was separated and put in another tubes. Then the serum sample stored at -20 °C until further analysed. The length of duodenum, jejunum and ileum were measured.

### STATISTICAL ANALYSIS

The data were subjected for analysis by using statistical analysis system (SAS). Relationship between calcium lev-

els and studied traits were measured by regression analysis. The linear and quadratic responses of the variables were determined by using orthogonal contrasts. The polynomial regression equations were used to estimate nutritional requirements of calcium.

## RESULTS AND DISCUSSION

### BLOOD AND TIBIA PARAMETERS

Linear and quadratic responses of blood serum and tibia traits were showed in the Table 2. Decreasing dietary calcium from 1.4% to 0.4% increases quadratically the level of calcium and ALP in the blood ( $p=0.027$  and  $p=0.0006$  respectively). The serum phosphorus was linearly ( $P=0.05$ ) and quadratically ( $P<0.001$ ) increased when the dietary calcium decreased. The ash, calcium and phosphorus content in the tibia quadratically increased ( $p<0.001$ ) with decreased calcium in the diets (Table 2). The length and width of tibia bone were the best in the birds fed diet containing 0.8% calcium and 0.4% phosphorus compared to birds fed diet containing 1.4% calcium and 0.4% phosphorus or these fed diet containing 1.2% calcium and 0.4% phosphorus ( $p<0.001$ ;  $p=0.021$  respectively). The results of our study agreed with results of Xie et al. (2009) that the increasing calcium in the diet decreases ash content in the tibia of white Pekin duck. Increasing dietary calcium linearly increases abnormality score of leg in the broiler chickens (Rao et al., 2006). The decrease in serum phosphorus and calcium concentrations may be due to low availability of those minerals in the small intestine, resulting from effect of excess calcium in the digestive tract, which forces the body to withdraw calcium and phosphorus from the bones and transfer them to the blood stream in order to modify the level of calcium and phosphorus in the blood. The transfer of calcium and phosphorus from the bones into the bloodstream causes a decrease in bone mineralization. Impaired bone mineralization may be due to high levels of dietary calcium which enhances binding of phosphorus with other nutrients thus reducing phosphorus absorption in the intestinal tract (Liu et al., 2013). The available phosphorus, magnesium and zinc were significantly reduced in the gut when the dietary calcium increased by formation of Ca-phytate complexes (Adamu et al., 2012). Similarly, Plumstead et al. (2008) pointed out the apparent digestibility and absorption of calcium and phosphorus that increased linearly when the levels of dietary calcium decrease from 1.16 to 0.47%. The improvement in the tibia traits of birds in group 4 may be due to getting those birds on the required level of calcium and phosphorus. as well as getting those birds on the optimal ratio between calcium and phosphorus (2:1). Rao et al. (2006) observed that birds fed a normal of calcium to phosphorus ratio (2:1) achieved the best tibia ash percentage compared with other birds.

**Table 1:** Ingredients and nutrient composition of experimental diets in the grower (11–24 days) and finisher period (25–42 days).

	Experimental diets (11–24 d)						Experimental diets (25–42 d).					
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
Dietary Ca, %	1.4	1.2	1	0.8	0.6	0.4	1.4	1.2	1	0.8	0.6	0.4
Dietary P, %	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Ingredients (%)												
Yellow corn	47.8	47.8	47.8	47.8	47.8	47.8	53	53	53	53	53	53
Soybean	40	40	40	40	40	40	34	34	34	34	34	34
oil	6.8	6.8	6.8	6.8	6.8	6.8	7.4	7.4	7.4	7.4	7.4	7.4
αLimestone	2.50	2.01	1.49	0.96	0.43	0.01	2.50	2.01	1.49	0.96	0.43	0.01
DCP	1.5	1.5	1.5	1.5	1.5	1.5	1.55	1.55	1.55	1.55	1.55	1.55
NaHCO3	0.23	0.23	0.23	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24
Salt	0.21	0.21	0.21	0.21	0.21	0.21	0.2	0.2	0.2	0.2	0.2	0.2
VP <sup>1</sup>	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.22	0.22
TMP <sup>2</sup>	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.22	0.22
DL-Methionine	0.32	0.32	0.32	0.32	0.32	0.32	0.27	0.27	0.27	0.27	0.27	0.27
L-Lysine	0.13	0.13	0.13	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.11
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06	0.03	0.03	0.03	0.03	0.03	0.03
Sand	0.02	0.52	1.05	1.56	2.1	2.5	0.3	0.75	1.3	1.81	2.35	2.75
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated nutrients												
ME (kcal/kg)	3000	3000	3000	3000	3000	3000	3150	3150	3150	3150	3150	3150
CP, %	21.5	21.5	21.5	21.5	21.5	21.5	19.5	19.5	19.5	19.5	19.5	19.5
Ca, %	1.4	1.2	1	0.8	0.6	0.4	1.4	1.2	1	0.8	0.6	0.4
P, %	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine	1.28	1.28	1.28	1.28	1.28	1.28	1.14	1.14	1.14	1.14	1.14	1.14
Methionine+ Cysteine	0.98	0.98	0.98	0.98	0.98	0.98	0.90	0.90	0.90	0.90	0.90	0.90
Threonine	0.87	0.87	0.87	0.87	0.87	0.87	0.76	0.76	0.76	0.76	0.76	0.76

<sup>1</sup>Provided per kilogram of complete diet: vit. A, 8150 U; vit. D3, 2010 U; vit. E, 18 U; vit. K3, 2.1 mg; thiamine, 1.7 mg; riboflavin, 6.3 mg; vit. B6, 3.2 mg; vit. B12, 0.04mg; Biotin, 0.12 mg; Pantothenic acid, 30 mg; niacin, 11 mg; choline chloride, 1100 mg; vit.C, 345 mg; and folic acid, 1.1 mg.

<sup>2</sup>Provided per kilogram of complete diet: the trace mineral premix supplied the followings per kilogram of diet: Mn, 88 mg; Fe, 61 mg; Zn, 88 mg; Cu, 11 mg; I, 1.1 mg, and Se, 0.15 mg.

Ca= calcium; P= phosphorus; DCP= Dicalcium Phosphate; VP=vitamin premix; TMP= trace mineral premix; CP= crude protein

**Table 2:** Linear and quadratic responses of broiler chickens fed different levels of calcium

Blood serum	Dietary calcium						SD	P-value	
	G1	G2	G3	G4	G5	G6		Linear	Quadratic
	1.4	1.2	1	0.8	0.6	0.4			
Ca, mg/dl	9.95	10.24	11.28	12.48	10.56	10.37	1.40	0.42	0.027
P, mg/dl	6.32	6.21	6.86	6.90	6.85	6.56	0.43	0.05	<.0001
ALP, U/L	2232	2390	2815.6	2933.4	2628.6	2255.6	404.57	0.44	0.0006
Tibia traits									
Ash%	36.62	37.36	41.34	45.06	40.2	36.98	3.66	0.313	<.0001
Ca%	30.04	30.32	32.96	36.48	33.38	29.3	2.87	0.412	<.0001
P%	14.43	14.49	15.93	17.97	16.24	15.91	1.35	0.002	<.0001
Length, cm	9.89	9.90	10.33	10.68	10.19	10.05	0.50	0.285	<.0001

Width, cm	0.81	0.81	0.90	0.93	0.89	0.81	0.08	0.419	0.021
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G= group; Ca= calcium; P= phosphorus; ALP=Alkaline Phosphatase; mg= milligram; dl=deciliter; cm= centimeter

**Table 3:** Linear and quadratic responses of broiler carcass traits and small intestine length fed different levels of calcium

Carcass traits	Dietary calcium							P-value	
	G1	G2	G3	G4	G5	G6	SD	Linear	Quadratic
	1.4	1.2	1	0.8	0.6	0.4			
Dressing percentage %	75.21	75.91	77.74	79.11	76.62	75.64	1.56	0.345	<.0001
Breast%	23.67	24.56	26.61	28.25	27.51	24.15	2.59	0.188	0.0015
Leg%	19.75	20.49	20.69	23.19	20.81	20.60	1.66	0.219	0.034
Wings%	5.98	6.13	6.44	6.16	6.25	5.95	0.66	0.968	0.528
Small intestine									
Duodenum, cm	31.1	31.4	32	33	31.1	32.6	3.25	0.542	0.798
Jejunum, cm	86.4	83.1	91.6	87	74.4	88.4	10.19	0.596	0.870
Ileum, cm	75.1	77.2	80.2	79.8	67	79.4	10.43	0.812	0.939

G= group; cm= centimeter

**Table 4:** Estimation of dietary calcium requirements from 11 to 42 days of age based on quadratic broken-line

Dependent variable	Model	Equation	Calcium requirement (%)	R <sup>2</sup>
Carcass traits				
Dressing percentage%	Quadratic	Y= 69.47 +19.85X -11.47X <sup>2</sup>	0.86	0.58
Breast%	Quadratic	Y= 17.42 +24.19X -14.46X <sup>2</sup>	0.83	0.38
Leg%	Quadratic	Y=17.25 +11.01X -6.73X <sup>2</sup>	0.81	0.22
Tibia traits				
Ash%	Quadratic	Y= 24.32 +42.31X -24.62X <sup>2</sup>	0.85	0.50
Ca%	Quadratic	Y= 19.38 +34.68X -19.98X <sup>2</sup>	0.86	0.52
P%	Quadratic	Y= 13.20 +9.64X -6.52X <sup>2</sup>	0.73	0.52
Length, cm	Quadratic	Y= 90.60 +32.89X -19.89X <sup>2</sup>	0.82	0.20
Width, cm	Quadratic	Y= 6.12 +7.0X -4.10X <sup>2</sup>	0.85	0.24

R<sup>2</sup>= R-square; X= represents independent variable (calcium levels in the diets); Y= represents dependent variable (traits); cm= centimeter

### CARCASS TRAITS AND LENGTH OF SMALL INTESTINE SEGMENTS

Linear and non-linear responses of carcass traits and length of small intestine segments were showed in the Table 3. The dressing percentage nonlinearly increased (p<0.001) when the dietary calcium decreased from 1.4% to 0.4%. Lowering calcium in the diet had quadratic influences on percentage weight of breast and leg (p=0.0015 and p<0.05 respectively). Dietary calcium had no effect (p>0.05) on relative weight of wings and length of duodenum, jejunum and ileum. A similar result was reported by Abdulla et al. (2017) who noted that adding calcium to the diet by 1.5% decreases carcass weight compared to lower levels of calcium. The dressing percentage was lower in birds fed 2% calcium or those fed 3% calcium compared to birds fed 1% calcium (Talpur et al., 2012). Recently, El-Faham et al. (2019) indicated that the lowering dietary calcium from 0.9 to 0.45% significantly reduces body weight, carcass weight and dressing percentage. The decrease in the yield

percentage and relative weight of breast and thigh of the birds fed high levels of calcium may be attributed to the deterioration of their physiological status, which negatively reflected the live body weight. Hamdi et at. (2015) indicated that the body weight and bone traits were higher in birds fed 7g/kg calcium. than birds fed 9g/kg calcium. Increasing dietary Ca have a negative effect on broiler growth performance (Gautier et al., 2017).

### DIETARY CALCIUM REQUIREMENTS

Table 4. shows dietary calcium requirements for optimal performance of carcass and tibia traits. The nutritional requirements of calcium for maximum dressing percentage and relative weight of breast and leg were 0.86, 0.83, and 0.81% respectively. While the predicted requirements of calcium for maximum length, and width of tibia bone, and optimal percentages of ash, calcium, and phosphorus were 0.82, 0.85, 0.85, 0.86, and 0.73% respectively.

Our results agreed with the findings by Bai et al. (2022) who indicated that calcium requirements for maximum concentrations of calcium, phosphorus, and alkaline phosphatase in the blood and the tibia ranged from 0.8 to 1%. Driver et al. (2005) observed that the highest tibia ash percentage in birds fed a diet containing 0.7% calcium. The nutritional requirements of calcium for obtaining a maximum weight gain, concentration of blood calcium, and tibia ash content were 0.75, 0.97 and 0.98% respectively (Rao et al., 2003).

## CONCLUSION

Increasing dietary calcium above 1% during the 11 to 42 day age negatively affects the carcass traits, blood parameters and bone health of broiler chickens. Therefore, determining nutritional requirements and estimating expected nutritional requirements is the best way to obtain maximum performance, reduce production costs, and reduce environmental pollution.

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## CONFLICT OF INTEREST

There is no conflict of interests regarding the publication of this article

## NOVELTY STATEMENT

The estimation of animal nutritional requirements is an important technique for knowing the real nutritional requirements of animals. The novelty of this study is the determination of birds calcium needs from 11 to 42 d.

## AUTHOR'S CONTRIBUTION

The experiment design, experiment application, collection of data, data analysis, and article writing were performed by author Zeyad Imari.

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