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EFFECTS ON PRODUCTIVE PERFORMANCE, TIBIA CALCIUM AND PHOSPHOROUS RETENTION, AND LIVER ENZYMES ACTIVITY OF ACIDIFIED AND ALKALINIZED DIETS IN BROILER CHICKEN*

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Abstract

A 35-day experiment was carried out to study the effects of acidified and alkalinized diets on zootechnical indices, tibial calcium and phosphorous retention, bone mineralization and liver enzymes activity using 250 Ross 308 male broiler chicks. Five treatments consisting of a control diet (CD), CD acidified using 10, 20 and 30 g/kg citric acid (CA) and CD alkalinized with Ca $(OH)_2$ (8.9 g/kg in growth period and 8.6 g/kg in finisher period) were examined in 5 replicates of 10 birds each from day 7 up to day 42 of age. Inclusion of 30 g/kg CA significantly increased body weight, average daily gain (ADG), average daily feed intake (ADFI), feed efficiency, tibia ash, tibia Ca content, at day 42 of age (P<0.05). Serum alkaline phosphatase and lactate dehydrogenase activities were elevated in the birds fed with the 30 g/kg CA-treated diet at day 42 of age (P<0.05). Alkalinized diet significantly reduced ADFI, tibia ash, tibial P and Ca contents, bone breaking strength and plasma Ca concentration (P<0.05). It was concluded that the diet acidified with 30 g/kg CA promoted productive performance and tibia mineralization in broiler chicken. Alkalinized diet suppressed growth performance of the birds perhaps through disrupted mineral absorption and altered liver enzymes activity.

Key words: citric acid, broiler chicken, alkalinized diet, tibia mineral retention

Acidification of diets further activates the functionality of proteolytic enzymes (Daskiran et al., 2004), reduces ammonia and other growth-depressing microbial metabolites in broiler gut (Dibner and Buttin, 2002), favoring mineral absorption (Chowdhury et al., 2009), and lowering the incidence of subclinical infections (Chaveerach et al., 2004). Moreover, CA appears to have a positive impact on histology of the small intestine, thereby facilitating nutrient absorption and growth performance in broiler

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chicken (Adil et al., 2010). However, the effectiveness of dietary acidification may vary depending upon composition of diet and its buffering capacity in broiler gastrointestinal tract (GIT) (Islam et al., 2012).

Despite an extensive body of research findings demonstrating desired effects of acidified diets on performance, health and welfare of broiler chicken, effect of dietary alkalinisation (ALK) of diet on poultry metabolism are not fully investigated. Owen et al. (1994) reported that pH alteration of gut from the normal acid-base balance towards alkalinisation impairs health, welfare and production in broiler chicken. To the best of our knowledge, the supplemental effects of ALK diets in broilers are largely unrevealed. It was hypothesized that providing alkalinized diets to broiler chicks may benefit the bird performance through affecting certain aspects of nutrient digestion and absorption. Therefore, the present study was conducted to compare the effects of acidified or alkalinized diets on growth performance, ileal digestibility, bone mineralization, pH values of contents in the gut segments, liver enzymes activity and certain blood parameters in broiler chicken.

Material and methods

Birds and diets

Two hundred fifty 7-day-old Ross 308 male broiler chicks were distributed randomly in 25 floor pens in an environmentally controlled house up to day 42 of age. The environmental temperature was kept about 32°C during the first week and then gradually reduced by 2°C weekly to reach about 24°C during the fourth week. The chicks were fed with a typical commercial broiler starter ration providing12.66 MJ of ME/kg and 230 g CP for the first 6 days before the start of the experiment. The experimental period was divided into grower (7 to 21 d) and finisher phases (22 to 42 d). A corn soybean meal based diet (CD) was formulated for each phase to meet all nutrient recommendations published in the Ross rearing guideline (Aviagen, 2007) (Table 1). The five dietary treatments were: control diet (CD), CD + 10 g/kg citric acid (CA), CD + 20 g/kg CA, CD + 30 g/kg CA, and alkalinized diet (ALK). Alkalinized diet was prepared by addition of $Ca(OH)_2$ in the form of saturated solution of calcium hydroxide or milk of lime (8.9 g/kg for growth period and 8.6 g/kg for finisher period) in the basal diet. Milk of lime was prepared by stirring calcium hydroxide in pure water, and filtering off the excess non-dissolved $Ca(OH)_2$. Calcium hydroxide was provided at 4.8 and 4.6 g/kg Ca for growth and finisher period, respectively. For increasing the pH of the intestinal contents, the pH value for the alkaline solution was set as 12.3. Experimental diets in mash form and water were provided to the birds for *ad libitum* consumption. A light schedule of 23 h light and 1 h dark was maintained throughout the experimental period.

Growth trial procedures

Considering pen as experimental unit, body weight (BW) and feed intake (FI) were recorded weekly. The data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (FCR) for 7- to 21-d, 22- to 42-d, and 7- to 42-d periods of age.

Table 1. Composition of the experimental diets in grower and finisher periods (g/kg)[1]

Ingredients (g/kg)	Grower (7 to 21 d)					Finisher (22 to 42 d)				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
1	2	3	4	5	6	7	8	9	10	11
Corn	595	569	543	518	595	628	610	590	568	628
Soybean meal	344	350	357	363	344	315	320	324	330	315
Soybean oil	20	30	39	48	20	21	24	30	36	21
DCP	9.7	9.7	9.7	9.7	9.7	11.7	11.7	11.7	11.7	11.7
$CaCO_3$	12.2	12.2	12.2	12.2	-	8.6	8.6	8.6	8.6	-
$Ca(OH)_2$	-	-	-	-	8.9	-	-	-	-	8.6
NaCl	2.0	2.0	2.0	2.0	2.0	2.3	2.3	2.3	2.3	2.3
$NaHCO_3$	2.3	2.3	2.3	2.3	2.3	2.0	2.0	2.0	2.0	2.0
DL-Methionine	3.0	3.0	3.0	3.0	3.0	2.8	2.8	2.8	2.8	2.8
L-Lysine	2.6	2.6	2.6	2.6	2.6	2.2	2.2	2.2	2.2	2.2
Threonine	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5
Phytase	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Zeolite	3.5	3.5	3.5	3.5	6.8	0.8	0.8	0.8	0.8	0.8
Citric acid	-	10	20	30	-	-	10	20	30	-
Calculated Analysis										
ME (MJ/kg)	12.35	12.35	12.35	12.35	12.35	12.56	12.56	12.56	12.56	12.56
CP (%)	20	20	20	20	20	19.0	19.0	19.0	19.0	19.0
Ca (%)	0.9	0.9	0.9	0.9	0.9	0.85	0.85	0.85	0.85	0.85

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11
NPP (g/kg)	0.45	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.42
Lys (g/kg)	1.16	1.16	1.16	1.16	1.16	1.07	1.07	1.07	1.07	1.07
Met + Cys (%)	0.88	0.88	0.88	0.88	0.88	0.84	0.84	0.84	0.84	0.84
Thr (%)	0.74	0.74	0.74	0.74	0.74	0.65	0.65	0.65	0.65	0.65
Na (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16

[1] T1 – Basal diet; T2 – basal diet + 10 g/kg citric acid (CA); T3 – basal diet + 20 g/kg citric acid (CA); T4 – basal diet + 30 g/kg citric acid (CA); T5 – alkalinized diet (ALK).

[2] Provided per kg of ration; 10 mg copper (cupric sulfate), 50 mg iron (ferrous sulfate), 100 mg manganese (manganese oxide), 85 mg zinc (zinc sulfate), 0.2 mg selenium (sodium selenite) and 1.0 mg iodine (calcium iodate); 900 IU retinol, 2000 IU cholecaciferol, 18 IU tocopherol, 2 mg menadione, 1.8 mg thiamine, 6.6 mg riboflavin, 3.0 mg pyridoxine, 0.015 mg cyanocobalamin, 30 mg niacin, 10 mg pantothenic acid, 1.25 mg folic acid, 500 mg choline and 0.1 mg biotin.

Apparent ileal digestibility

At day 30 of age, titanium oxide (1 g/kg of diet) was added to all diets for five days as an indigestible marker to determine the effect of treatments on digestibility of Ca and P. At day 35 of age, three birds per replicate (15 chicks per treatment) were randomly taken, weighed, killed by cutting the carotid arteries and jugular veins, and manually processed to collect the contents of the distal part of the ileum segment. The ileum was defined as the segment of small intestine which extended from Meckel's diverticulum to 40 mm cranial to the ileocaecal junction. The ileum samples were placed into plastic containers and freeze-dried. The samples were ground to pass through a 0.5 mm sieve before chemical analysis.

Sampling procedure for pH measurement

A sample of one gram digesta from crop, proventriculus, gizzard, duodenum, jejunum, ileum, and rectum segments of each bird were immediately collected and placed into clean Falcon tubes. The samples were mixed with deionized water (1:10 wt/vol), and pH of the solution was measured using a digital pH meter (Model 827, Metrohm, Herisau, Switzerland) at room temperature.

Blood measurements

At 42 d, two randomly selected birds from each replicate (10 per group) were used for blood sample collection by puncturing the branchial vein. The blood samples were collected into heparinised tubes for mineral concentration analyses and into non-heparinised tubes for some selected metabolites and enzyme activity analyses. The blood samples in non-heparinised tubes were centrifuged at 2,500 × *g* for 10 min and the sera collected were stored at -20°C in 3-mL Eppendorf tubes for subsequent analyses. The serum samples were analyzed for urea, cholesterol, triglycerides, globulin, albumin, plasma calcium (Ca) and phosphorous (P) concentrations and the activity of alkaline phosphatase (ALP, EC 3.1.3.1), alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1) and lactate dehydrogenase (LDH, EC 1.1.1.27) using the Express Plus (Ciba-Corning Diagnostics Corp., Medfield, MA) automated clinical chemistry analyzer. This analyzer employs enzymatic procedures using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Zone Industrielle, 61500, SEES, France) in two replicates, at 25 ºC, that have been described by Elliott (1984).

Bone mineralization

The right tibia of the two killed birds was removed and stored at -18°C to assess the total ash and minerals content. Tibia ash was determined by removing the adhering tissue, drying the bone at 110°C for 12 h and extraction of fat with ether. The dry fat-free bones were ashed in a muffle furnace at 550°C for 3 h. Ash weight was calculated as a percentage of dry fat-free bone weight. Bone strength was determined by breaking the right tibia on an Instron 4301 tensiometer, using a three-point bend with supports 60 mm apart and a load applied at 50 mm/min to the midpoint of the long axis of the bone (Knowles and Brown, 1990). The parts of the tensiometer in contact with the bone were covered in soft rubber tubing to avoid point stresses. The

breaking strength was recorded as the peak load before the bone breakage. The tibia contents of Ca and P were measured using dry-ashed bone samples following the same procedure for minerals estimation in feed and digesta.

Chemical analysis

Feed and digesta P concentrations were determined colorimetrically using the molybdo-vanadate method (method 946.06, AOAC International, 2000). Calcium concentration in feed and digesta was determined by flame atomic absorption spectroscopy (method 968.08, AOAC, 2000). Titanium oxide concentration in digesta was determined according to the procedure described by Lomer et al. (2000) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Apparent ileal digestibility (AID) of nutrients was calculated using the following formula (g/100 g DM):

i

$$AID = 100 \times \frac{(Diet\ component/Ti)_d = (Diet\ component/Ti)_i}{(Diet\ component/Ti)_d}$$

where:

$(Diet\ component/Ti)_d$ is the ratio of diet component to Ti in the diet,

$(Diet\ component/Ti)_i$ is the ratio of diet component to Ti in the ileal digesta.

Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Loughborough, UK) and was standardized with benzoic acid. The apparent metabolizable energy corrected to zero nitrogen retention (AMEn) values was calculated by subtracting GE excreted (adjusted to zero N balance) from GE intake and dividing this value by DM feed intake. For correction to zero N retention, a value of 34.39 kJ g^{-1} of N retained was used (Hill and Anderson, 1958). The AME values were calculated using the following formula:

$$AME(MJ/kg) = \frac{(Feed\ intake \times GE_{diet}) - (Excreta\ output \times GE)_{excreta}}{Feed\ intake}$$

Statistical analysis

In this experiment, pen considered as experimental unit and data were analyzed using GLM procedure (SAS, 2001) in a completely randomized design. Level of significance was set at 5% and when a significant effect was indicated, treatment means were separated using Tukey-Kramer's test.

Results

During the finisher period (22 to 42 d), implementation of broilers diet with 30 g/kg CA increased ADG ($P<0.01$) and decreased FCR ($P<0.05$) (Table 2). From days 22 to 42 and days 7 to 42 of age, the ALK diet significantly decreased ADFI compared

with the CD diet (P<0.05). The overall data showed that inclusion of 30 g/kg CA increased final body weight by 9.8% at day 42 of age (P<0.01).

Table 2. Body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (FCR) of broiler chicks fed the basal diet (CD), the diets containing different levels of citric acid (CA) and alkaline diet (ALK)

Parameter	CD	Dietary acidification (g CA/ kg)			ALK	SEM	P-value
		10	20	30			
Body weight (g)							
21 d	541	532	572	580	576	14.4	0.082
42 d	1815 b	1856 b	1850 b	1993 a	1781 b	15.7	0.003
ADG (g/b/d)							
7 to 21 d	30.3	29.7	32.5	33.0	32.6	0.82	0.097
22 to 42 d	60.2 bc	62.4 b	60.3 bc	67.6 a	57.5 c	2.02	0.004
7 to 42 d	48.2 b	49.6 b	49.3 b	53.8 a	47.6 b	1.35	0.007
ADFI (g/b/d)							
7 to 21 d	51.4	52.8	52.6	56.4	52.2	1.17	0.138
22 to 42 d	118.8 ab	122.4 a	116.3 b	118.8 ab	109.2 c	2.41	0.037
7 to 42 d	91.8 ab	94.6 a	90.9 b	93.9 a	86.4 c	1.61	0.002
FCR (g/g)							
7 to 21 d	1.69 abc	1.78 a	1.62 bc	1.71 ab	1.60 c	0.02	0.027
22 to 42 d	1.97 a	1.95 a	1.93 a	1.76 b	1.91 a	0.03	0.033
7 to 42 d	1.91 a	1.90 a	1.84 ab	1.74 b	1.82 ab	0.05	0.041

a, b, c – values in rows with different letters differ significantly (P≤0.05).

Table 3. Ileal digestibility of crude protein (CP), apparent metabolizable energy corrected to zero nitrogen retention (AMEn), calcium (Ca) and phosphorus (P) of broiler chickens fed the basal diet (CD), the diets containing different levels of citric acid (CA) and alkaline diet (ALK)

Item	CD	Dietary acidification (g CA/kg)			ALK	SEM	P-value
		10	20	30			
CP (%)	80.7 c	81.6 bc	83.1 ab	83.8 a	81.7 bc	0.4	0.038
AMEn (MJ kg^{-1})	11.36 b	11.34 b	11.40 b	11.62 a	11.34 b	0.06	0.004
Ca (%)	58.5	58.5	58.9	59.0	58.6	0.3	0.129
P (%)	38.7	38.4	38.6	38.9	38.0	0.3	0.146

a, b, c – values in rows with different letters differ significantly (P≤0.05).

Ileal digestibility of Ca and P did not differ among the birds fed experimental diets (Table 3). Inclusion of 30 g/kg CA in diet significantly increased ileal digestibility of CP (P<0.05) and AMEn (P<0.01).

Inclusion of 30 g/kg of CA decreased the pH value of crop, jejunum, and rectum contents (P<0.01) (Table 4), compared with values for the control birds. In the birds

fed on diets supplemented with 20 and 30 g/kg of CA, the pH value of ileum contents significantly decreased compared to the control group (P<0.01). Except for the gizzard, a significant increase was observed in pH values for other GIT segments in the birds fed on ALK diet (P<0.01).

Table 4. Effect of the basal diet (CD), the diets containing different levels of citric acid (CA) and alkaline diet (ALK) on pH of different parts of the gastrointestinal tract at day 42 of age

Segment	CD	Dietary acidification (g CA/kg)			ALK	SEM	P-value
		10	20	30			
Crop	4.90 b	4.85 b	4.69 b	4.29 c	6.13 a	0.072	<0.0001
Proventriculus	4.37 b	4.07 b	4.14 b	4.09 b	5.50 a	0.097	<0.0001
Gizzard	3.12	3.02	2.97	2.96	3.07	0.034	0.078
Duodenum	6.24 b	6.13 b	6.20 b	6.12 b	7.53 a	0.047	<0.0001
Jejunum	6.60 b	6.56 b	6.48 b	6.33 c	6.97 a	0.030	<0.0001
Ileum	6.72 b	6.63 bc	6.51 c	6.38 c	7.28 a	0.032	<0.0001
Rectum	6.13 b	6.05 b	5.93 bc	5.85 c	7.10 a	0.046	<0.0001

a, b, c – values in rows with different letters differ significantly (P≤0.05).

Table 5. Plasma minerals concentration, metabolites and enzyme activities in the serum of 42-d-old broiler chickens fed the basal diet (CD), the diets containing different levels of citric acid (CA) and alkalinized diet (ALK)

Item	CD	Dietary acidification (g CA/kg)			ALK	SEM	P-value
		10	20	30			
Metabolite (mg dl^{-})[1]							
Globulin	1.49	1.24	1.39	1.43	1.42	0.07	0.085
Albumin	1.28	1.31	1.29	1.41	1.37	0.03	0.091
Triglycerides	43.3	44.2	44.1	43.3	44.6	0.5	0.127
Total cholesterol	136.5	138.5	132.7	136.7	135.7	1.7	0.079
Urea	2.8	3.1	3.1	3.1	2.9	0.1	0.146
Serum enzyme (UL^{-1})							
ALP	14302 a	14315 a	14321 a	14107 b	14394 a	165	0.002
ALT	1.5	1.8	1.7	1.6	1.7	0.1	0.086
AST	239	238	237	238	241	3	0.073
LDH	714 b	726 b	717 b	749 a	718 b	7	0.007
Plasma mineral							
Ca (mg dl^{-1})	11.0 a	10.8 a	11.1 a	11.4 a	8.1 b	0.3	0.004
P (mg dl^{-1})	6.2	6.5	6.1	6.0	6.1	0.2	0.137

a, b, c – values in rows with different letters differ significantly (P≤0.05).

ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; LDH – lactate dehydrogenase.

The effects of CA-containing and ALK diets on selected blood metabolites are summarized in Table 5. No change was observed in serum biochemical parameters with acidified or ALK diets. However, inclusion of 30 g/kg CA in diet significantly reduced alkaline phosphatase (ALP) activity (P<0.01) and increased LDH activity (P<0.01) compared to the birds receiving CD at day 42 of age. A significant decrease in plasma Ca (P<0.01) concentration was noted in the birds receiving ALK diet compared with the birds receiving either CD or CA-added diets at day 42 of age. The mean plasma Fe concentration was elevated due to inclusion of 30 g/kg CA in diet (P<0.01).

Table 6. Bone mineralization of broiler chickens fed the basal diet (CD), the diets containing different levels of citric acid (CA) and alkaline diet (ALK)

Item	CD	Dietary acidification (g CA/kg)			ALK	SEM	P-value
		10	20	30			
Tibia ash (%)	46.0 b	46.5 b	46.1 b	48.6 a	43.1 c	0.3	<0.0001
Ca (%)	37.0 b	38.0 b	37.8 b	38.9 a	31.6 c	0.3	<0.0001
P (%)	19.17 a	19.21 a	19.20 a	19.45 a	18.63 b	0.08	0.037

a, b, c – values in rows with different letters differ significantly (P≤0.05).

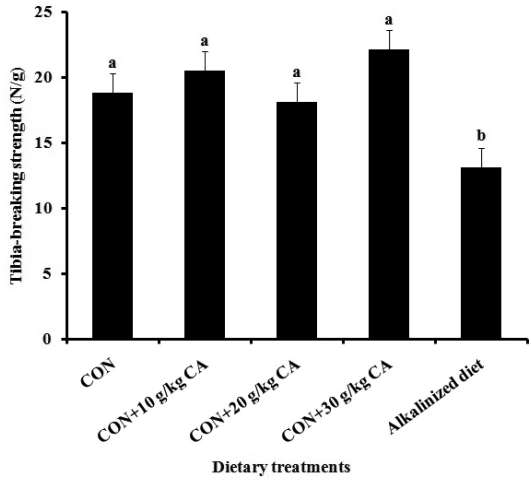

Figure 1. Tibia-breaking strength (breaking force divided by bone weight expressed as Newton per gram) by dietary inclusion of citric acid (CA) in control diet (CD) and alkalinized diet. Values are mean ± SE (n= 5). The value is significantly lower for alkalinized diet compared to the CD and acidified diets (P<0.05). SEM = 0.18

Tibia ash and Ca content were greater in the birds fed 30 g/kg CA than those receiving ALK and CD diets (P<0.05) (Table 6). Tibia ash, Ca and P contents (P<0.05) and tibia breaking strength was decreased in the birds fed on alkalinized diet (ALK) (P<0.01) (Figure 1) compared with the birds receiving the acidified and CD diets.

Discussion

There are many reports concerning effect of dietary CA on performance of broiler chickens. Many studies have reported promising effects (Boling et al., 2000; Boling-Frankenbach et al., 2001), while others demonstrated no benefits (Biggs and Parsons, 2008; Woyengo et al., 2010), and still other studies have explained negative effects for acidification of diet using CA for broilers (Brenes et al., 2003). Although most of the above mentioned researches were performed using corn- and soybean-based diets, the noticeable positive effects of dietary CA have mostly been observed for low-phosphorus corn- and soybean-based diets (Esmaeilipour et al., 2011). Results of the current study for increased ADG due to dietary CA (30 g/kg) through day 42 of age are consistent with those reported by Brenes et al. (2003) and Boling-Frankenbach et al. (2001). The later researchers observed improved growth performance in broilers fed a low-phosphorus corn-based diet (0.2% AP) supplemented with 40 g/kg CA. In the present study, all dietary treatments were isocaloric; therefore, the increased performance with 30 g/kg of CA at day 42 of age may be associated in part with the positive effect of CA on improved gut health (Pekel et al., 2009) in favor of nutrient availability (Esmaeilipour et al., 2011). It was shown that dietary acidification increases gastric proteolysis and protein/amino acid digestibility by enhancing digestive enzyme activities (Langhout, 2000). The negative response or lack of response in most of the variables to ALK diet in the current research is unknown. However, ADFI was significantly reduced for ALK diet, a phenomenon which may be reasoned by impaired plasma acid-base balance or reduced palatability of the diet. It has been well known that alkaline foods are not palatable to humans which is thought to be related to their bitter taste (Koseki et al., 2007).

The results of the present study indicate that inclusion of 30 g/kg CA reduced pH values of crop, jejunum, ileum and rectum contents, whereas ALK diet increased pH values in all gut segments, with the exception of gizzard, compared to the birds fed with control diet. Prior to birth, the GIT of birds is supposed to be free of germs, and bacteria from the diet, water, excreta and environment begin to colonize the GIT almost shortly (Aydin et al., 2010). The pH value in different segments of GIT is an influencing factor in establishing a specific microbial population, digestibility and absorption of most nutrients (Islam et al., 2012). Taking into account that most of the pathogens prefer circumstances with pH close to 7 (Pope and Cherry, 2000) and that beneficial microorganisms proliferate in acidic media (5.8 to 6.2), dietary CA could bring about conditions favoring gut health. Gut health is a complicated status required for efficient nutrient absorption and improved bird performance (Chaveerach et al., 2004). It has been reported that little dietary CA reaches the lower parts of the digestive tract (Hume et al., 1993), whereas the results of the current study showed that the influence of dietary CA at 30 g/kg on decreased digesta pH extends across hindgut.

Inclusion of 30 g/kg CA increased tibia ash and Ca content in tibia ash whereas ALK diet reduced tibia ash, Ca and P content in tibia ash, and tibia-breaking strength compared with those fed with control and acidified diets. Consequently, all of the chicks fed with the ALK diet showed severe leg paralysis at day 24 of age. It

seems that the same birds were suffering from calcium tetany characterized by muscle weakness or paralysis and as a result of hypocalcemia (Martin et al., 2011). The risk factors associated with the development of calcium tetany in broilers are reported as poor flock uniformity and high calcium feed (> 1.2% Ca) (Martin et al., 2011). Calcium tetany may also develop when the normal response to a decline in blood Ca level becomes disrupted, for example by metabolic disturbances that increase blood pH and consequently cause blood Ca becoming unavailable. Birds have sensitive mechanisms to buffer H^+ ions in plasma and overcome blood pH fluctuations (Scott et al., 1982). However, under certain conditions buffering capacity may be inadequate (Glahn et al., 1988). Metabolic disturbances that increase blood pH, called respiratory or metabolic alkalosis depending upon the underlying cause, shift the plasma calcium flux from the ionized (active) form to the protein bound (inactive) form (Martin et al., 2011). In mammals, high blood pH appears to affect the responsiveness of the kidney to parathyroid hormone, which affects the mechanisms affecting mobilized calcium during milk production (Shappell et al., 1987).

It has been recognized that acidification of diets with CA improves protein and energy digestibility through reduced microbial competition with the host for nutrients, endogenous nitrogen losses and ammonia production in addition to their other beneficial effects for broilers (Islam et al., 2012). Similarly, a recent study showed that in P-deficient broiler diets, Ca and P availability increased with the addition of CA (Woyengo et al., 2010). Moreover, dietary acidification increased activity of the gastric enzymes, leading to improved digestion and absorption of protein and fibre (Abdel-Fattah et al., 2008). The organic acids have shown positive impact on histology of the small intestine, thereby facilitating nutrient absorption and growth performance in broiler chicken (Adil et al., 2010). Addition of CA in broiler diet increases the availability of minerals (Nourmohammadi et al., 2012). However, the positive response is dose dependent and may not be linear in a standard diet.

Total serum ALP measures a composite of several isoenzymes of Zn metalloenzymes by cells in a number of organs (liver, bone, muscle, small intestine, and kidney) (Zantop, 1997). The decrease in serum ALP activity associated with the diets supplemented with 30 g/kg of CA might reflect the down regulation of this enzyme caused by increased availability of phosphorus (Huff et al., 1998). In many cases, birds with severe liver damage have normal ALT activities. Moreover, there are five LDH isoenzymes in birds occurring in several tissues, including skeletal muscle, cardiac muscle, liver, kidney, bone, and red blood cells (Zantop, 1997). The increase in LDH activity that we observed in the birds fed on 30 g/kg CA may be related to normal function of liver, because this enzyme decreases sharply as a disease emerges.

In conclusion, this study demonstrated that supplementation of broiler diets with 30 g/kg of CA improved performance, minerals retention and tibial mineralization. Moreover, change of gut acidity toward alkaline by feeding an alkalinized diet resulted in retarded growth due to disrupted mineral retention and calcium tetany in broiler chickens.

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