Effect of sodium sources and exogenous phytase supplementation on growth performance, nutrient digestibility, and digesta pH of 21-day-old broilers

I. O. Adejumo,[*,1] B. Bryson,[*] O. C. Olojede,[*] M. R. Bedford ,[†] and S. A. Adedokun[*,2]

[*]*Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA; and* [†]*AB Vista, Marlborough, Wiltshire SN8 4AN, United Kingdom*

ABSTRACT The effect of sodium chloride (**NaCl**) and NaCl+sodium hydrogen carbonate (**$NaHCO_3$**) and supplemental phytase (0, 500, 1,000, and 2,000 FTU/kg) on performance, nutrient digestibility and utilization, and digesta pH of male broiler chickens were investigated in a 2 × 4+1 factorial arrangement of treatments in a completely randomized design with 6 replicate cages of 8 birds per replicate. Data were analyzed as a 2 × 4 factorial with contrast between the positive control and the diets containing 0 FTU phytase. Phytase supplementation linearly improved ($P < 0.05$) average body weigh gain (**BWG**) and feed intake (d 0−14 and 0−21). Apparent jejunal dry matter (**DM**) digestibility and digestible energy in birds fed diets containing only NaCl increased (linear and quadratic; $P < 0.05$) with phytase supplementation whereas quadratic ($P < 0.05$) effect was observed in birds fed diets containing a combination of NaCl and $NaHCO_3$. Phytase supplementation improved (linear and quadratic; $P < 0.05$) apparent ileal nitrogen and P digestibility. Apparent utilization of DM, nitrogen, energy, and metabolizable energy increased (linear; $P < 0.05$) with increasing level of phytase supplementation. Apparent P utilization increased (linear and quadratic; $P < 0.05$) for both sodium sources but calcium utilization only increased (linear; $P < 0.05$) with the combination of NaCl and $NaHCO_3$. Bone breaking strength (linear and quadratic) and bone ash (linear) increased ($P < 0.05$) with phytase supplementation. The combination of NaCl and $NaHCO_3$ resulted in lower ($P < 0.05$) pH of digesta in the proximal ileum whereas the pH of the digesta in the distal ileum (linear) and the average pH of ileal contents (linear and quadratic) increased ($P < 0.05$) with phytase supplementation. Results from this study showed that birds' performance and utilization of nutrients and energy by broilers in the presence of phytase was, in general, not influenced by the source of sodium in the diet. Data from this study showed that $NaHCO_3$ can replace a portion of NaCl in the diet of broilers supplemented with phytase without any significant negative effect on performance and that the 2,000 FTU phytase level resulted in better BWG and feed intake as well nutrient and energy utilization.

Key words: broiler chicken, digestibility, sodium, performance, phosphorus

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INTRODUCTION

The use of exogenous phytase in poultry diet has positively influenced poultry diet formulation and has resulted in poultry production becoming more environmentally friendly as a result of the reduction in the amount of phosphorus (**P**) that is excreted into the environment. Next to energy and protein, P is considered as the next most expensive nutrient in the diet of nonruminant animals including poultry (Létourneau−Montimy et al., 2011). Feed ingredients like cereal grains, legumes, and oilseed meals provide P in the diet but mostly in the form of phytic acid which is poorly available to poultry. In order to increase the availability of the phytate-bound P in poultry diets, exogenous phytase enzymes are supplemented to the diet. The capacity of this feed enzyme to release phytate-bound P and to reduce P excretion has been well documented (Persia and Saylor, 2006; Selle and Ravindran, 2007; Amerah and Ravindran, 2009) with benefits ranging from a reduction in feed cost to a reduction in environmental pollution as a result of a decrease in P excretion. Considering the negative charge of phytic acid at acidic, neutral, and basic pH (Maenz, 2001), it has the tendency to form complexes with positively charged dietary molecules in the gastrointestinal tract of the animals thereby reducing nutrient digestibility and enhancing endogenous secretions of nutrients into the gastrointestinal tract.

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[1]Present address: Department of Animal Science, Federal University, Gashua, Nigeria.
[2]Corresponding author: tayo.adedokun@uky.edu

Although an increase in P digestibility is the primary focus of phytase supplementation, its extra phosphoric effect has also been reported (Woyengo et al. 2012; Lu et al., 2019). One of several minerals that have benefited from phytase supplementation is sodium (**Na**) (Akter et al., 2019). Sodium and chloride (**Cl**), usually supplied by sodium chloride (**NaCl**), are important minerals in poultry diets but excess of Na in poultry diet has been associated with an increase in water consumption. In addition to this, high Na intake would lead to low feed intake, poor growth, and high moisture content in the litter (Collett, 2012). Wet litter may serve as a predisposing factor to several diseases in poultry production (Francesch and Brufau, 2004; Mushtaq et al., 2007).

Sodium hydrogen carbonate (**$NaHCO_3$**) plays an important role in acid base balance during high environmental temperatures (Hayat et al., 1999). In recent times, researchers are beginning to link the acid-binding capacity (**ABC**) of feed ingredients to their chemical compositions (Lawlor et al., 2005; Gilani et al., 2013, 2016). The ABC of a feed is the quantity of acid, in milliequivalents, needed to reduce the pH of the diet to a predetermined level (Lawlor et al., 2005; Rynsburger, 2009). The relationship between ABC and pH has been linked to the health of the gastrointestinal tract of livestock which in turn may affect nutrient digestion, absorption, and the performance of the animal.

Although several studies have been conducted to examine the effect of exogenous phytase on P digestibility, information on the use of phytase supplementation on nutrient and energy digestibility and utilization in the presence of Na solely from NaCl or a combination of both NaCl and $NaHCO_3$ is scarce. We hypothesized that diets containing a combination of NaCl and $NaHCO_3$ as sources of Na in the presence of increasing level of phyase supplementation will result in better performance and higher nutrient and energy digestibility compared with birds fed diets containing NaCl as the only source of Na. Hence, the present study evaluated the effect of 2 sources of dietary Na (NaCl and a combination of NaCl and $NaHCO_3$) and 4 levels of exogenous phytase supplementation on the concentration of nutrients in the gizzard, apparent jejunal and ileal nutrient and energy digestibility, apparent nutrient and energy utilization, digesta pH, and bone-breaking strength in 21-day-old broiler chickens.

MATERIALS AND METHODS

Experimental Approach

The management of the birds, experimental procedures, and sample collections followed the standard operating procedures for the animal facility as approved by the University of Kentucky Animal Care and Use Committee.

A total of 475 day-old broiler chicks (Cobb by-product breeder chicks) were obtained locally from a commercial hatchery at day-old (d 0 of the study) out of which 432 birds were used. On d 0 post hatch, chicks were individually weighed and allocated to treatments in a completely randomized design in such a way that the difference in the average starting body weight within each treatment and across treatments was not significantly different. Each treatment was replicated 6 times with 8 birds/cage. There were 9 treatments with 4 dietary treatments containing graded levels (0, 500, 1,000, and 2,000 FTU/kg) of exogenous phytase supplementation (Quantum Blue 5G) within each of the 2 Na sources. The phytase used in this experiment was an enhanced *E. coli* phytase (Quantum Blue) provided by AB Vista Feed Ingredients (Marlborough, UK) with an expected activity of 5,000 FTU/g. Enzyme activity determination in the diets was conducted by a laboratory (ESC, Ystrad Mynach., UK) using the reference method of analysis recommended by the supplier (Basu et al., 2007).

The experimental diets were corn-soybean meal-based and were fed for 21 d. In order to make sure that the nutrient and energy contents of all the diets across treatments were similar, all the diets were mixed from 2 common basal diets. The 2 basal diets were similar in all respect except for the Na sources. The positive control (**PC**) diet and the four diets containing only NaCl were mixed from the same basal diet while the remaining 4 diets containing a combination of NaCl and $NaHCO_3$ were produced from the second basal diet. Treatment number 9 was a PC diet that was adequate in all nutrient and energy. The requirement for the non-phytate P was also met (4.5 g/kg) and no phytase was supplemented to the diet. The feed ingredient composition of the experimental diets and the analyzed nutrient and energy contents of the diets are reported in Tables 1 and 2, respectively. The PC diet was formulated to meet all nutrients and energy requirements for birds of this age (Na from NaCl = 2.0, Ca = 10.0, and nPP = 4.5 g/kg). The 2 Na sources used in this study were Na from NaCl (100%) and Na from a combination of NaCl (38%) and $NaHCO_3$ (62%). The basal diet for each Na source were not supplemented with phytase (0 FTU/kg). Both of these diets were formulated to meet nutrient and energy requirements except for Ca (8.5 g/kg diet), nPP (3.5 g/kg diet), and Na (1.8 g/kg diet). Phytase was added at 0, 500, 1,000, and 2,000 FTU/kg of diet to produce diets with increasing phytase level within each of the 2 Na sources. All birds had *ad libitum* access to feed and water from d 0 till the end of the study (d 21).

Measurement and Chemical Analysis

On d 14 and d 21, feed intake and body weight gain (**BWG**) were determined (performance: d 0−14, 14−21, and 0−21). All the birds were euthanized by CO_2 asphyxiation and immediately after opening up of the abdominal cavity, the pH of the contents of the crop, gizzard, jejunum (proximal, middle, and distal), ileum (proximal, middle, and distal), and cecum from one bird (bird with weight closest to the cage average) per cage

Table 1. Composition of the experimental diets, g/kg (on as-fed basis).

		NaCl				$NaHCO_3$			
Diet description[1]	PC A	NC1+0 FTU/kg B	NC1+500 FTU/kg C	NC1+1,000 FTU/kg D	NC1+2,000 FTU/kg E	NC2+0 FTU/kg F	NC2+500 FTU/kg G	NC2+1,000 FTU/kg H	NC2+2,000 FTU/kg I
Ingredients, g/kg									
Corn	484.2	452.5	452.5	452.5	452.5	451.7	451.7	451.7	451.7
Soybean meal, 48%	391.7	390.0	390.0	390.0	390.0	390.1	390.1	390.1	390.1
Soybean oil	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Limestone	12.5	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2
Dicalcium phosphate	15.7	10.4	10.4	10.4	10.4	10.4	10.4	10.4	10.4
NaCl	3.92	2.93	2.93	2.93	2.93	1.39	1.39	1.39	1.39
$NaHCO_3$	0.00	0.00	0.00	0.00	0.00	2.25	2.25	2.25	2.25
Vitamin mineral premix[2]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
DL-Methionine	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
L-Lysine HCl	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Phytase premix[3]	0.00	0.00	10.00	20.00	40.00	0.00	10.00	20.00	40.00
Ground corn	0.00	40.00	30.00	20.00	0.00	40.00	30.00	20.00	0.00
Titanium dioxide premix[4]	25	25	25	25	25	25	25	25	25
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Calculated value, g/kg									
Calcium	10.04	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60
Non-phytate P	4.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Phytase activity, FTU/kg[5]	< 50	< 50	580	1,280	1,930	< 50	416	1,060	2,800

[1]PC, positive control; NC, negative control.

[2]Vitamin-mineral premix was formulated to supply the following at 2.5 grams per kilogram of diet: 11 025 IU of vitamin A; 3,528 IU of vitamin D; 33 IU of vitamin E; 0.91 mg of vitamin K; 2.21 mg of thiamin; 7.72 mg of riboflavin; 55 mg of niacin; 18 mg of pantothenate; 5 mg of vitamin B-6; 0.22 mg d-biotin; 1.10 mg of folic acid; 478 mg of choline; 0.03 of vitamin B-12; 75 mg of Zn; 40 mg of Fe; 64 mg of Mn; 10 mg of Cu; 1.85 mg of I; and 0.30 mg of Se.

[3]Phytase (Quantum Blue 5G, AB Vista) premix was formulated to supply 500 FTU/kg of diet when added to the diet at the rate of 10 g/kg. It was added to the diets at the expense of ground corn.

[4]Prepared as 5 g of titanium dioxide mixed with 20 g of ground corn.

[5]Analyzed value.

were determined by inserting the sterile glass pH electrode probe of a portable HANNA pH meter (model HI 99163; HANNA Instruments, Woonsocket, RI) into these sections from different angles. Three independent pH readings were taken in situ in each location. Gizzard contents (not flushed) and jejunal and ileal digesta from the remaining birds (5 birds/cage) were flushed into clean prelabeled plastic containers with nanopure water and stored at −20°C until processed. All the digesta samples were freeze-dried and subsequently ground using a coffee grinder. Excreta from each pen were collected on d 19 and 20, pooled, and weighed before drying in a forced-air oven at 55°C for 5 d. Diets and dried excreta samples were ground to pass through a 0.5 mm screen using a mill grinder (Wiley Mill Standard Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA). Blood, for blood chemistry, was collected from the jugular vein of one bird/cage (bird with weight closest to the cage average). Additionally, the pH of the blood was determined immediately after collection using the same pH meter described above. From 2 birds per cage (birds with weight closest to the cage average), the left tibia bones were collected to determine bone ash and bone-breaking strength. Sampling was done between 8:00 and 12:00.

Table 2. Analyzed nutrient and energy composition of the basal diets (on as-fed basis).

Diet description[1]	Positive control	Negative control 1 (NC1) NaCl	Negative control 2 (NC2) NaCl + $NaHCO_3$
Dry matter, g/kg	972.7	975.6	976.3
Nitrogen, g/kg	38.1	37.6	37.7
Chloride, g/kg	3.00	2.25	1.00
Calcium, g/kg	10.5	9.20	9.60
Sodium, g/kg	1.80	1.30	1.45
Magnesium, g/kg	1.90	1.85	1.90
Potassium, g/kg	11.5	11.0	11.4
Phosphorus, g/kg	7.50	6.38	6.50
Iron, mg/kg	296	236	242
Copper, mg/kg	20.5	21.3	20.2
Manganese, mg/kg	91.5	95.9	83.3
Zinc, mg/kg	130	135.5	127.5
Gross energy, kcal/kg	4,293	4,346	4,344
Phytic acid[1], g/kg	10.9	10.9	10.8
DEB[2], mEq/kg	288	231	322

[1]Phytic acid was analyzed for the positive control diet and the two basal diets. All the remaining diets were made from either of the two basal (negative control) diets.

[2]Dietary electrolyte balance calculated as. Calculated from analyzed dietary sodium, potassium, and chloride concentrations (DEB, mEq/kg of diet = $Na^+ + K^+ - Cl^-$, mEq/kg of diet); calculated values.

Chemical analyses were performed on the gizzard contents, diets, excreta, jejunal, and ileal digesta samples in duplicates. Dry matter (**DM**) was determined by drying the samples in a drying oven (Precision Scientific Co., Chicago, IL) at 105°C for 16 h (Method 934.01; AOAC International 2006). Diets were analyzed for DM, titanium, nitrogen (**N**), Ca, P, magnesium (**Mg**), Na, Cl, K, copper (**Cu**), manganese (**Mn**), iron (**Fe**), zinc (**Zn**), and gross energy (**GE**). The same set of analyses, as for the diets, were conducted on the gizzard contents except for titanium and Cu. Jejunal and ileal digesta and excreta samples were analyzed for DM, titanium, N, Ca, P, K, and GE. The GE of the samples (diets, jejunal and ileal digesta, and excreta) were determined using the bomb calorimeter (Parr 6200, Parr Instruments Co., Moline, IL) with benzoic acid as a standard. Samples were digested as described by Myers et al. (2004), after which titanium concentration was determined by flame atomic absorption spectroscopy. Nitrogen was determined by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; method 990.03; AOAC International 2000) with EDTA serving as the internal standard. Mineral contents of diets, excreta, and digesta were determined at the University of Missouri Experiment Station Chemical Laboratory. Sodium, K, and Cl (titration method) concentrations were also determined (method 976.25; AOAC International 2000). Phosphorus was determined following nitric and perchloric acid wet-ash digestion by spectrophotometry (method 946.06, AOAC International 2000), and the absorbance value read using a Dynex plate reader (Dynex Technologies Inc., Chantilly, VA). The concentration of Ca and other minerals was determined from the same digest using flame atomic absorption spectroscopy method (Varian Spectr. AA 220FS, Varian Australia Pty Ltd., Mulgrave, Australia).

The ABC value of the diet and gizzard contents were determined. Each diet sample was repeated 5 times and each gizzard content was repeated 3 times and the average of these represented one replicate. The initial pH of the diets was higher than 4 (between 6.4 and 6.9) while that of the gizzard contents was lower than 4 (between 3.2 and 3.9). Hence, HCl and NaOH were used for the diets and gizzard contents titration, respectively. The ABC was determined using pH4 as the titration endpoint. The respective calculations were done as described by Jasaitis et al. (1987) and Lawlor et al. (2005). Acid binding capacity was calculated as the quantity of acid (meq) needed to lower the pH of 1 kg of sample to the desired pH (pH4). These values could either be positive or negative, depending on the starting pH. With the starting pH of less than 4 (gizzard content), the titration was performed with NaOH, resulting in a negative change in pH, hence negative ABC. However, the ABC values for the diets were positive because the starting pH was higher than the final pH (titration with HCl).

Blood samples collected were analyzed at the Rood and Riddle Equine Hospital (Lexington, KY). The blood chemistry panel (alkaline phosphatase [Alk. Phos.], creatine kinase [**CK**], lactose dehydrogenase [**LDH**], Ca, P, Mg, blood urea N [**BUN**] and glucose) and electrolytes (Na^+, K^+, HCO_3^-, and Cl^-) analyses were performed using the AU480 Chemistry Analyzer (Beckman Coulter, Inc. CA).

Calculations and Statistical Analysis

Apparent jejunal and ileal energy and nutrient digestibility (**AID**) and energy and nutrient utilization (**UTZ**) were calculated using the following equation:

$$AID\ or\ UTZ,\ \% = \left[1 - \left(\frac{Ti_I}{Ti_O}\right) \times \left(\frac{n_O}{n_I}\right)\right] \times 100$$

where Ti_I and Ti_O are the titanium concentration (in %) in the diet and jejunal or ileal digesta (for AID), or excreta (for UTZ), respectively; and n_O and n_I are the concentration (%) of nutrient or energy in jejunal, ileal, or excreta, and the diet, respectively.

Apparent digestible energy (**DE**) for the jejunal and ileal samples and apparent metabolizable energy (**AME**) for excreta samples were calculated using the following equation:

$$\text{DE or AME, kcal/kg} = \text{Determined ED} \times \text{GE of diet}$$

where determined ED is the energy digestibility or utilization as derived from the above equation for jejunal, ileal, or excreta samples, while the GE is the gross energy of the diet was determined by bomb calorimeter.

Nitrogen-corrected AME (**AMEn**) was determined by correcting for N retention by simple multiplication with 8.22 kcal per gram of N retained in the body as described by Hill and Anderson (1958).

Cage served as the experimental unit for performance, digestibility, and utilization data while one bird was the experimental unit for pH and blood response measures and 2 birds per cage for bone data measurements. Before conducting statistical analysis, all outliers (data outside mean ± 3 standard deviation) were removed from the data set. Data were analyzed using the PROC GLM procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC) appropriate for the factorial arrangement of treatments with 2 sources of Na (NaCl vs. NaCl+$NaHCO_3$) and 4 levels of phytase supplementation (0, 500, 1,000, and 2,000 FTU/kg diet). In addition to this, the PC diet and the 2 basal diets (diets without phytase supplementation) were compared using contrast. Whenever the effect of phytase supplementation was significant, linear, and quadratic effects of graded levels of phytase supplementation were determined and whenever the interaction between Na sources and phytase supplementation was significant, linear and quadratic effect of phytase supplementation within each of the two Na sources were evaluated using orthogonal polynomial contrasts. The coefficients for the contrasts were obtained from PROC IML in SAS. P-value less < 0.05 was taken to be significant and P-values between 0.05 and 0.10 were taken as showing a tendency to be different.

Table 3. Effect of sodium source and phytase supplementation on the performance of 21-day-old broilers[1].

Treatment		Average BW, g			Average BW gain, g			Feed intake/bird, g			Feed efficiency, g/kg		
		D 0	D 14	D 21	D 0–14	D 14–21	D 0–21	D 0–14	D 14–21	D 0–21	D 0–14	D 14–21	D 0–21
Positive control (PC)		43.2	441.5	994	402.7	598.2	947.7	506.0	688.5	1,188.2	795.5	873.0	798.3
Sodium	Phytase												
NaCl		43.3	411.6	946.7	370.4	573.1	894.5	478.0	667.3	1,141.2	775.0	858.4	783.1
NaCl + $NaHCO_3$		43.2	414.9	959.7	371.5	588.0	916.3	480.7	684.2	1,164.6	772.5	861.5	786.6
	0	43.3	386.4	899.6	343.3	553.2	850.3	462.3	653.3	1,112.8	741.0	847.3	763.9
	500	43.3	419.9	965.1	378.8	585.1	919.2	476.9	680.2	1,155.3	793.9	860.4	794.3
	1,000	43.1	421.7	968.5	378.5	589.1	919.3	479.9	677.0	1,154.3	789.9	870.2	796.4
	2,000	43.3	425.0	979.6	383.3	594.9	932.8	498.3	692.4	1,189.2	770.3	862.0	784.8
NaCl	0	43.3	391.3	900.0	348.5	545.3	844.8	465.3	649.3	1,109.3	748.3	840.7	761.7
NaCl	500	43.2	406.2	935.2	367.5	565.7	886.5	465.8	658.3	1,121.3	787.8	859.2	789.3
NaCl	1,000	43.2	428.0	977.8	385.0	591.0	922.8	482.8	685.5	1,163.2	799.2	861.5	793.0
NaCl	2,000	43.5	420.8	973.7	380.7	590.3	923.8	497.8	676.0	1,171.0	764.8	872.3	788.5
NaCl + $NaHCO_3$	0	43.2	381.5	899.2	338.2	561.0	855.8	459.2	657.3	1,116.3	733.7	853.8	766.2
NaCl + $NaHCO_3$	500	43.3	433.7	995.0	390.2	604.5	951.8	488.0	702.0	1,189.3	800.0	861.7	799.2
NaCl + $NaHCO_3$	1,000	43.0	415.3	959.2	372.0	587.2	915.8	477.0	668.5	1,145.3	780.7	878.8	799.8
NaCl + $NaHCO_3$	2,000	43.2	429.2	985.5	385.8	599.5	941.8	498.7	708.8	1,207.3	775.8	851.7	781.2
SD[2]		2.78	39.01	65.00	36.76	38.34	66.10	24.00	40.04	51.96	64.77	43.73	784.85
							Probability						
Contrast	PC vs. 0 Phy[3]		0.005	0.004	0.001	0.017	0.003	<0.001	0.099	0.006	0.071	0.274	0.057
Trend for main effect													
Linear	Phytase		-	0.007	0.019	0.014	0.007	<0.001	-	0.002	-	-	-
Quadratic	Phytase		-	0.068	0.078	0.121	0.072	0.819	-	0.525	-	-	-
Sodium		0.877	0.769	0.491	0.916	0.184	0.259	0.694	0.152	0.127	0.894	0.808	0.744
Phytase		0.997	0.069	0.019	0.039	0.049	0.017	0.008	0.130	0.010	0.189	0.641	0.131
Sodium × phytase		0.997	0.568	0.500	0.619	0.587	0.586	0.437	0.265	0.222	0.902	0.716	0.943

[1]Means represents 6 replicate cages.
[2]Standard deviation.
[3]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

RESULTS

The effect of the Na sources and phytase supplementation on the performance of 21-day-old broiler chickens is presented in Table 3. Sodium sources did not significantly influenced average body weight, average BWG, feed intake, and feed efficiency. However, phytase supplementation improved ($P < 0.05$) average final body weight (d 21), BWG (d 0−14, 14−21, and 0−21), and feed intake (d 0−14 and 0−21). Increasing phytase supplementation linearly increased ($P < 0.05$) the measured response variables above with the highest benefits observed with the supplementation of 2,000 FTU of phytase in the diet. Birds on the 2 basal diets (diets without phytase supplementation) had lower ($P < 0.05$) BW (d 14 and 21) and BWG (d 0−14; 14−21; 0−21) and feed intake (d 0−4 and 0−21) compared with birds on the PC diet. There was a tendency for lower feed efficiency (d 0−14, $P = 0.071$ and 0-21, $P = 0.057$) in birds fed the basal diets with no phytase supplementation compared with those on the PC diet (Table 3).

The concentration of nutrients in dried gizzard content is reported in Table 4. An interaction ($P < 0.05$) effect was observed for Na and Mn in the gizzard. The interaction effect observed for the Na content in the gizzard yielded no significant linear or quadratic effects for phytase supplementation within each Na source but phytase supplementation resulted in cubic ($P = 0.015$) effect for the diets containing the combination of NaCl and $NaHCO_3$. Furthermore, gizzard Mn content increased ($P < 0.05$) linearly with increasing phytase supplementation only when NaCl was the only source of Na. Gizzard contents of Mg (linear increase, $P = 0.004$) and chloride (linear decrease, $P = 0.002$) were influenced by phytase supplementation.

The interaction effect of the main factors (Na source and phytase level) was significant for apparent jejunal DM ($P = 0.002$) and energy digestibility ($P = 0.004$), and DE ($P = 0.005$). For each of the 3 response measures linear and quadratic effects ($P < 0.05$) were observed with only NaCl as the only source of Na in the diet (Table 5). With Na coming from a combination of NaCl and $NaHCO_3$, only quadratic effects ($P < 0.05$) of phytase supplementation was observed. Jejunal P digestibility increased linearly by 57.7% with 2,000 FTU phytase supplementation compared with that of the birds fed the basal diet (0 FTU phytase). Jejunal DM digestibility also increased (linear, $P < 0.05$) with increasing level of phytase supplementation.

There were no significant interactions between Na sources and phytase on apparent ileal nutrient and energy digestibility (Table 6). Furthermore, Na source did not have a significant effect on the apparent ileal nutrient and energy digestibility. Phytase supplementation improved (linear, quadratic, $P < 0.05$) apparent ileal N and P digestibility while there was a tendency for an increase ($P = 0.083$) in ileal energy digestibility and DE with increasing level of phytase supplementation. Birds on the PC diet had higher ($P < 0.05$) ileal N and lower ($P < 0.05$) ileal Ca digestibility compared with birds on the basal diets.

An interaction effect between the Na source and phytase level was observed for P ($P = 0.011$) and Ca ($P < 0.001$) utilization (Table 7). Within each of the Na sources, there was a linear and quadratic ($P < 0.05$) effect of inceasing level of phytase supplementation on P

Table 4. The concentration of nutrients in dried gizzard content of 21-day-old broiler chickens fed diets containing different sodium sources supplemented with graded levels of phytase (g/kg).[1,2]

Treatment		N, g/kg	P, g/kg	Ca, g/kg	Mg, g/kg	Na, g/kg	Cl, g/kg	K, g/kg	Mn, ppm	Fe, ppm	Zn, ppm
Positive control (PC)		23.5	3.9	6	0.6	2.5	9.8	2.80*	30.8	196.1	63.90*
Sodium	Phytase										
NaCl		24.06	3.30	5.25	0.77	2.66	9.63	3.26	32.73	156.25	70.82
NaCl + $NaHCO_3$		24.27	3.38	5.58	0.80	2.67	9.91	3.28	37.28	206.27	67.14
	0	24.35*	3.59	5.36	0.74	2.69	10.14	3.17	34.49	199.58	68.11
	500	24.54	3.36	5.12	0.75	2.57	10.04	3.37	34.30	154.34	61.39
	1,000	24.05	3.24	5.47	0.78	2.66	9.45	3.13	35.85	161.07	66.80
	2,000	23.73	3.15	5.74	0.86	2.73	9.42	3.40	35.37	210.06	79.61
NaCl	0	23.98	3.48	4.53	0.68	2.50*	10.00*	3.02	26.64*	143.95	69.12*
NaCl	500	24.38*	3.25*	4.76*	0.76*	2.78	9.97	3.32*	35.90*	149.76*	67.92
NaCl	1,000	23.40*	3.22*	5.92*	0.76*	2.6.0	9.26*	2.90*	33.16*	174.68	66.26*
NaCl	2,000	24.48	3.23	5.80*	0.86*	2.76*	9.24*	3.78	35.22*	156.60*	79.98*
NaCl + $NaHCO_3$	0	24.72	3.70	6.12	0.80*	2.88	10.28	3.32	42.34*	255.20	67.10*
NaCl + $NaHCO_3$	500	24.70	3.47	5.48	0.73	2.36*	10.12	3.42	32.70	158.92*	54.86*
NaCl + $NaHCO_3$	1,000	24.70*	3.27*	5.02*	0.80	2.72*	9.64*	3.35	38.54*	147.46*	67.34*
NaCl + $NaHCO_3$	2,000	22.98	3.07	5.68*	0.85	2.70	9.60	3.02*	35.52*	263.52	79.248
SD[3]		1.288	0.429	1.467	0.093	0.284	0.586	0.566	4.836	73.65	18.368
						Probability					
Contrast	PC vs. 0 Phy[4]	0.201	0.257	0.740	0.017	0.227	0.247	0.287	0.272	0.930	0.672
Trend for main effect											
Linear	Phytase	-	-	-	0.004	-	0.002	-	-	-	-
Quadratic	Phytase	-	-	-	0.498	-	0.304	-	-	-	-
Trend for simple effect											
Linear	NaCl + Phytase	-	-	-	-	0.295	-	-	0.036	-	-
Quadratic	NaCl + Phytase	-	-	-	-	0.677	-	-	0.094	-	-
Linear	$NaHCO_3$ + Phytase	-	-	-	-	0.861	-	-	0.164	-	-
Quadratic	$NaHCO_3$ + Phytase	-	-	-	-	0.109	-	-	0.199	-	-
Sodium		0.589	0.536	0.479	0.295	0.954	-	-	0.005	-	-
Phytase		0.487	0.103	0.805	0.027	0.610	0.008	0.572	0.871	0.226	0.168
Sodium × phytase		0.086	0.661	0.244	0.289	0.017[5]	0.965	0.072	0.001	0.076	0.810

[1]Means represents 6 replicate cages except for values with an asterisk (*) where n was 5 per treatment.
[2]Ca, calcium; Cl, chloride; K, potassium; Mg, magnesium; Mn, manganese; N, nitrogen; Na, sodium; Fe, iron; P, phosphorus; Zn, zinc.
[3]Standard deviation.
[4]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.
[5]Significnat cubic effect of NaCl + $NaHCO_3$ ($P = 0.015$).

Table 5. Effect of sodium sources and phytase supplementation on apparent jejunal nutrient and energy digestibility in 21-day-old broiler chickens (%).[1]

Treatment		Dry matter	Nitrogen	Phosphorus	Calcium	Potassium	Energy	Digestible energy, kcal/kg
Positive control (PC)		52.1	68.1	44.5*	41.5	84.9*	52.4	2,311
Sodium	Phytase							
NaCl		50.5	69.1	55.5	44.2	84.7	51.0	2,272
NaCl + $NaHCO_3$		50.3	68.7	53.7	40.0	85.6	50.9	2,265
	0	49.9	68.4	43.0	46.4	85.6	50.4	2,246
	500	48.8	68.1	50.7	43.3	84.9	49.7	2,211
	1,000	51.9	69.2	57.1	40.3	84.6	52.5	2,337
	2,000	50.9	70.0	67.8	38.4	85.6	51.3	2,282
NaCl	0	47.0*	67.2*	43.8*	51.2*	84.7*	47.5*	2,117*
NaCl	500	49.7*	69.1*	52.3	41.6	84.1	50.2*	2,237*
NaCl	1,000	54.1*	69.8	57.8	40.1*	84.8	54.8*	2441*
NaCl	2,000	51.2*	70.2	68.3	43.8*	85.2*	51.6*	2,297*
NaCl + $NaHCO_3$	0	52.9*	69.5*	42.1	41.5	86.5*	53.4*	2,374*
NaCl + $NaHCO_3$	500	48.0*	67.1	49.1*	45.0	85.7*	49.1	2,185
NaCl + $NaHCO_3$	1,000	49.7	68.5	56.5	40.4	84.3	50.2	2,232
NaCl + $NaHCO_3$	2,000	50.7*	69.7	67.2	33.0*	86.0	51.0*	2,267*
	SD[2]	2.39	2.88	7.02	9.25	2.64	2.52	111.28
					Probability			
Contrast	PC vs. 0 Phy[3]	0.162	0.88	0.646	0.362	0.61	0.242	0.367
Trend for main effect								
Linear	Phytase	0.119	-	<0.001	-	-	-	-
Quadratic	Phytase	0.465	-	0.434	-	-	-	-
Trend for simple effect								
Linear	NaCl + Phytase	0.006	-	-	-	-	0.011	0.012
Quadratic	NaCl +Phytase	0.001	-	-	-	-	0.001	0.001
Linear	$NaHCO_3$ + Phytase	0.519	-	-	-	-	0.369	0.365
Quadratic	$NaHCO_3$ + Phytase	0.013	-	-	-	-	0.029	0.029
Sodium		0.790	0.658	0.388	0.144	0.252	0.869	0.811
Phytase		0.036	0.420	<0.001	0.226	0.731	0.078	0.078
Sodium × Phytase		0.002	0.377	0.986	0.199	0.745	0.004	0.005

[1]Means represents 6 replicate cages except for values with an asterisk (*) where n was 5 per treatment.
[2]Standard deviation.
[3]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

Table 6. Effect of sodium sources and phytase supplementation on apparent ileal nutrient and energy digestibility in 21-day-old broiler chickens (%).[1]

Treatment		Dry matter	Nitrogen	Phosphorus	Calcium	Potassium	Energy	Digestible energy, kcal/kg
Positive control (PC)		72.0*	83.0	50.4	49.3	90.7	73.2	3,229*
Sodium	Phytase							
NaCl		72.0	83.1	64.3	53.3	89.2	73.1	3,257
NaCl + $NaHCO_3$		71.9	83.5	62.8	53.1	89.4	73.0	3,249
	0	71.1	81.2	51.5	56.7	89.7	71.6	3,188
Phytase	500	71.7	83.0	59.9	50.8	89.9	72.8	3,241
	1,000	72.5	84.1	67.2	52.3	89.0	73.9	3,289
	2,000	72.6	84.9	75.6	53.0	89.3	74.0	3,295
NaCl	0	71.1*	80.7*	52.3*	57.7*	90.3	70.9	3,158
NaCl	500	72.1	82.7*	60.4	50.1	89.1	73.1	3,255
NaCl	1,000	72.7	84.0	68.4	50.9*	88.4*	74.5*	3,319*
NaCl	2,000	72.2*	85.0*	76.2*	54.4	88.8	74.0	3,297
NaCl + $NaHCO_3$	0	71.1	81.7*	50.8	55.7	89.2*	72.3	3,219
NaCl + $NaHCO_3$	500	71.4	83.3*	59.4*	51.5	88.7	72.6	3,228
NaCl + $NaHCO_3$	1,000	72.2	84.1	65.9*	53.7*	89.7*	73.2	3,258
NaCl + $NaHCO_3$	2,000	73.0*	84.9*	75.1*	51.6	89.8*	74.0*	3,293*
SD[2]		2.05	1.10	3.99	5.78	2.13	2.43	108.3
					Probability			
Contrast	PC vs. 0 phy[3]	3,887	0.001	0.606	0.015	0.369	0.201	0.935
Trend for main effect								
Linear	Phytase	-	<0.001	<0.001	-	-	-	-
Quadratic	Phytase	-	0.004	0.010	-	-	-	-
Sodium		0.624	0.246	0.220	0.924	0.773	0.903	0.803
Phytase		0.311	<0.001	<0.001	0.115	0.812	0.083	0.083
Sodium × phytase		0.834	0.718	0.970	0.620	0.517	0.588	0.588

[1]Means represents 6 replicate cages except for values with an asterisk (*) where n was 5 per treatment.
[2]Standard deviation.
[3]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

Table 7. Effect of sodium sources and phytase supplementation on apparent nutrient and energy utilization in 21-day-old broiler chickens (%).[1]

Treatment		Dry matter	Nitrogen	Phosphorus	Calcium	Energy	AME[2], kcal/kg	AMEn[2], Kcal/kg
Positive control (PC)		75.0*	71.0*	62.0*	30.0	78.0*	3,461*	3,367*
Sodium	Phytase							
NaCl		75.6	69.1	55.0	38.2	78.4	3,492	3,395
NaCl + $NaHCO_3$		75.7	71.5	54.1	33.1	78.5	3,494	3,404
	0	75.1	68.7	48.5	29.5	78.0	3,472	3,374
	500	75.8	70.4	54.3	33.5	78.4	3,492	3,398
	1,000	75.3	70.4	56.6	37.0	78.2	3,480	3,385
	2,000	76.5	71.8	58.8	42.6	79.3	3,528	3,439
NaCl	0	74.9*	67.5	50.0	35.7	77.8	3,465*	3,363*
NaCl	500	75.5	68.2*	56.0	39.7	78.1	3,481*	3,380*
NaCl	1,000	75.1*	69.9	55.2*	33.6	78.1	3,479*	3,382*
NaCl	2,000	76.8	70.8*	58.8	43.8*	79.6	3,544	3,454
NaCl + $NaHCO_3$	0	75.3*	69.9	47.0	23.3	78.2	3,480*	3,386*
NaCl + $NaHCO_3$	500	76.0*	72.5*	52.5*	27.4*	78.7	3,503*	3,417*
NaCl + $NaHCO_3$	1,000	75.5	70.9	58.0	40.4*	78.3	3,481	3,388
NaCl + $NaHCO_3$	2,000	76.3	72.8	58.9*	41.5	79.0	3,512	3,425
	SD[3]	0.76	2.23	2.32	5.35	0.69	30.09	36.25
				Probability				
Contrast	PC vs. 0 Phy[4]	0.999	0.118	0.121	0.855	0.254	0.137	0.715
Trend for main effect								
Linear	Phytase	<0.001	0.005	<0.001	<0.001	<0.001	0.002	0.002
Quadratic	Phytase	0.365	0.607	<0.001	0.679	0.229	0.246	0.305
Trend for simple effect								
Linear	NaCl + Phytase	-	-	<0.001	0.123	-	-	-
Quadratic	NaCl + Phytase	-	-	<0.001	0.587	-	-	-
Linear	$NaHCO_3$ + Phytase	-	-	<0.001	<0.001	-	-	-
Quadratic	$NaHCO_3$ + Phytase	-	-	0.010	0.184	-	-	-
Sodium		0.467	0.001	0.204	0.003	0.458	0.849	0.424
Phytase		<0.001	0.019	<0.001	<0.001	0.001	0.001	0.001
Sodium × phytase		0.376	0.361	0.011	<0.001	0.173	0.177	0.182

[1]Means represents 6 replicate cages except for values with an asterisk (*) where n was 5 per treatment.
[2]Apparent metabolizable energy; Apparent metabolizable energy corrected for nitrogen.
[3]Standard deviation.
[4]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

Table 8. Effect of different inorganic sodium sources and increasing level of phytase supplementation on bone breaking strength and bone (tibia) ash.[1]

Treatment		Bone breaking strength, kgF	Bone ash, %
Positive control (PC)		29.68	52.59
Sodium source	Phytase		
NaCl		26.25	51.53
$NaCl + NaHCO_3$		26.63	51.53
	0	18.22	49.11
	500	27.93	51.68
	1,000	30.44	52.62
	2,000	29.18	52.73
NaCl	0	19.35	48.95
NaCl	500	28.03	51.70
NaCl	1,000	28.88	52.07
NaCl	2,000	28.75	53.42
$NaCl + NaHCO_3$	0	17.08	49.27
$NaCl + NaHCO_3$	500	27.82	51.65
$NaCl + NaHCO_3$	1,000	32.00	53.17
$NaCl + NaHCO_3$	2,000	29.62	52.03
SD[2]		5.322	1.730
		Probability	
Contrast	PC vs. 0 Phy[3]	0.006	0.001
Trend for main effect			
Linear	Phytase	0.010	<0.001
Quadratic	Phytase	0.021	0.120
Sodium		0.808	0.993
Phytase		<0.001	<0.001
Sodium × phytase		0.663	0.369

[1]Means represents 6 tibia bones per replicate cage.
[2]Standard deviation.
[3]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

utilization but the asymptote appears to have been reached at a lower dose with the NaCl compared with the NaCl + $NaHCO_3$ diets. However, Ca utilization linearly improved ($P < 0.05$) in birds fed diets containing a combination of NaCl and $NaHCO_3$. The inclusion of a combination of NaCl and $NaHCO_3$ in the diet improved ($P = 0.001$) utilization of N by 3.5% while the combination of NaCl and $NaHCO_3$ decreased ($P = 0.003$) Ca utilization by 13%. The DM, P, and energy utilization, and AME and AMEn were not affected by either of the Na sources. Phytase supplementation resulted in a linear increase ($P < 0.05$) in the utilization of DM, N, energy, and AME and AMEn. The improved utilization of nutrients and energy peaked at the 2,000 FTU phytase level.

Sodium sources did not affect the tibia bone-breaking strength and percent bone ash (Table 8). Phytase supplementation linearly improved ($P < 0.001$) percent bone ash and bone breaking. Phytase supplementation resulted in a quadratic response ($P = 0.021$) for bone breaking strength which peaked at 1,000 FTU phytase level. Birds on the PC diet had higher ($P < 0.05$) bone-breaking strength and bone ash content compared with birds on the basal diets (Table 8). The interaction between Na source and phytase was not significant neither did Na source influenced blood and digesta pH except for the mid-ileum digesta pH where the combination of NaCl and $NaHCO_3$ resulted in lower ($P = 0.001$) pH (Table 9). Phytase supplementation resulted in a linear (distal and average ileal digesta values) and quadratic (average ileal digesta value) ($P < 0.05$) effect.

Table 9. Effect of different inorganic sodium sources and increasing level of phytase supplementation on blood and digesta pH in 21-day-old broiler chickens.[1]

					Jejunum				Ileum				
Treatment		Blood	Crop	Gizzard	Proximal	Middle	Distal	Average	Proximal	Middle	Distal	Average	Ceca
Positive control (PC)		6.29*	5.28	2.73	6.01	6.15*	6.00	6.04	5.95*	7.11*	6.89*	6.78*	6.14
Sodium	Phytase												
NaCl		7.01	5.31	2.86	6.01	6.14	6.16	6.11	6.46	7.19	7.26	7.00	6.17
$NaCl + NaHCO_3$		6.96	5.44	2.85	6.08	6.13	6.05	6.09	6.12	7.11	7.24	6.84	6.15
	0	7.05	5.30	2.56	6.01	6.08	6.05	6.05	6.15	6.87	6.89	6.55	5.97
	500	7.03	5.36	2.85	6.03	6.11	6.10	6.09	6.22	7.11	7.21	6.93	6.07
	1,000	6.87	5.36	2.97	6.08	6.18	6.11	6.13	6.36	7.28	7.42	7.08	6.31
	2,000	6.98	5.49	3.01	6.07	6.16	6.18	6.13	6.44	7.32	7.47	7.14	6.29
NaCl	0	7.05	5.21	2.50	5.94	6.09	6.10	6.05*	6.29*	6.90	6.99	6.68*	6.01
NaCl	500	7.08*	5.39*	2.94	6.03*	6.05	6.15*	6.09	6.33*	7.17	7.09	6.95*	6.07*
NaCl	1,000	6.80*	5.29*	2.97*	6.07*	6.21	6.19	6.16	6.58*	7.35*	7.42*	7.17*	6.38
NaCl	2,000	7.12	5.36	3.02	6.02*	6.20*	6.21	6.15*	6.65	7.33*	7.54*	7.22*	6.21
$NaCl + NaHCO_3$	0	7.05	5.39	2.63	6.08*	6.07	6.01	6.05	6.02	6.85	6.80*	6.41	5.92
$NaCl + NaHCO_3$	500	6.98*	5.34	2.76	6.03	6.18*	6.05	6.10*	6.10*	7.06	7.33	6.91	6.06
$NaCl + NaHCO_3$	1,000	6.94*	5.42	2.97	6.10	6.16	6.02	6.10*	6.13*	7.21*	7.42	6.99*	6.23
$NaCl + NaHCO_3$	2,000	6.85*	5.62	3.04	6.12	6.12*	6.14	6.13	6.23*	7.32*	7.41	7.05	6.38
SD[2]		0.210	0.557	0.572	0.158	0.119	0.185	0.106	0.316	0.392	0.295	0.333	0.448
Contrast	PC vs. 0 Phy[3]	0.488	0.654	0.430	0.862	0.574	0.509	0.899	0.472	0.732	0.129	0.554	0.879
Trend for main effect													
Linear	Phytase	-	-	-	-	-	-	-	-	-	<0.001	<0.001	-
Quadratic	Phytase	-	-	-	-	-	-	-	-	-	0.110	0.048	-
Sodium		0.385	0.446	0.974	0.166	0.870	0.055	0.581	0.001	0.514	0.807	0.103	0.875
Phytase		0.193	0.902	0.214	0.647	0.186	0.456	0.177	0.167	0.060	<0.001	0.001	0.197
Sodium × phytase		0.162	0.935	0.927	0.740	0.190	0.917	0.845	0.820	0.973	0.362	0.887	0.836

[1]Means represents 6 replicate cages except for values with an asterisk (*) where n was 5 per treatment.
[2]Standard deviation.
[3]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

Table 10. Effect of different inorganic sodium sources and increasing level of phytase supplementation on blood (serum) chemistry in 21-day-old broiler chickens.[1,2]

Treatment		HCO^{-}_3, mmo/L	Na, mmol/L	K, mmol/L	Ca^{2+}, mg/dL	Ca, mg/dL	P, mg/dL	Mg, mg/dL	Chloride, mmol/L	Glucose, mg/dL	AlkPho, U/L	BUN, mg/dL	CK, U/L	LDH, U/L
Positive control (PC)		26.58	149.0*	6.54*	6.12	11.15*	6.70*	2.17	104.5*	240.0*	6,902	2.00*	5,889	727
Sodium	Phytase													
NaCl		26.1	148.3	6.50	6.14	10.94	6.44	2.13	105.7	234.9	5,230	1.82	7,203	692
$NaCl + NaHCO_3$		27.4	149.0	6.39	6.23	10.94	6.53	2.18	104.9	236.3	6,003	1.83	6,590	679
	0	27.9	148.0	5.79	6.81	11.71	4.50	2.12	104.1	237.9	4,648	1.63	5,284	631
	500	26.3	148.7	6.83	5.90	10.59	6.94	2.14	105.7	236.3	7,376	2.00	5,687	723
	1,000	25.9	149.3	6.84	6.03	10.72	7.29	2.16	106.2	231.6	5,971	2.00	8,797	651
	2,000	26.8	148.7	6.32	5.99	10.74	7.21	2.21	105.2	236.5	4,470	1.67	7,818	736
NaCl	0	27.8	147.5	5.84*	6.60*	11.58	4.65	2.02*	104.8	230.0*	3,959[b]	1.60*	4,815	652*
NaCl	500	25.7*	148.2	6.80*	6.03*	10.66	6.58*	2.18	105.7*	236.0*	5,936[ab]*	2.00	6,084*	680*
NaCl	1,000	25.0*	149.0	6.85	5.98	10.93	7.30	2.18	106.6	234.4*	5,915[ab]	2.00*	8,073	658*
NaCl	2,000	25.7*	148.7	6.50	5.93*	10.60	7.23	2.15	105.7	239.0	5,110[b]*	1.67	9,838*	778*
$NaCl + NaHCO_3$	0	27.9*	148.4*	5.73	7.02*	11.84	4.35	2.22	103.3	245.8	5,338[b]*	1.67	5,753	610
$NaCl + NaHCO_3$	500	26.8*	149.3*	6.85*	5.78*	10.53*	7.30	2.10*	105.8*	236.5*	8,815[a]*	2.00*	5,290*	766*
$NaCl + NaHCO_3$	1,000	26.9	149.5	6.83	6.08*	10.50*	7.28*	2.14*	105.8	228.8*	6,028[ab]*	2.00*	9,520	645*
$NaCl + NaHCO_3$	2,000	27.9*	148.8*	6.14*	6.04*	10.88*	7.18	2.26*	104.8*	234.0	3,829[b]	1.67	5,797*	695
SD[3]		1.09	2.01	0.81	0.37	0.365	6.465	0.13	2.02	10.94	1496.2	0.385	3,867.9	181.4
								Probability						
Contrast	PC vs.0 Phy[4]	0.14	0.208	0.073	0.001	0.005	<.0001	0.591	0.616	0.873	0.011	0.081	0.783	0.282
Trend for main effect														
Linear	Phytase	0.109	0.407	0.355	0.002	<0.001	<0.001	-	-	-	0.126	0.750	-	-
Quadratic	Phytase	0.003	0.211	0.002	0.002	<0.001	<0.001	-	-	-	0.004	0.007	-	-
Trend for interaction effect														
Linear	NaCl +Phytase	-	-	-	-	-	-	-	-	-	0.412	-	-	-
Quadratic	NaCl +Phytase	-	-	-	-	-	-	-	-	-	0.028	-	-	-
Linear	$NaHCO_3$ + Phytase	-	-	-	-	-	-	-	-	-	0.004	-	-	-
Quadratic	$NaHCO_3$ + Phytase	-	-	-	-	-	-	-	-	-	0.003	-	-	-
Sodium		0.001	0.293	0.667	0.421	0.950	0.623	0.269	0.233	0.677	0.106	0.889	0.614	0.820
Phytase		0.001	0.501	0.014	<0.001	<0.001	<0.001	0.468	0.100	0.591	0.001	0.048	0.123	0.470
Sodium × phytase		0.151	0.950	0.942	0.225	0.073	0.256	0.063	0.858	0.098	0.026	0.996	0.370	0.772

[a-b]Means with different superscripts within a row are different ($P < 0.05$).
[1]Number of replicates was 6 (1 bird/cage) except for values with asterisk (*) where n was 5 per treatment.
[2]AlkPhos, alkaline phosphatase; BUN, blood urea nitrogen; Ca, calcium; Ca^{2+}, ionized calcium; Cl, chloride; HCO_3, bicarbonate; K, potassium; LDH, lactate dehydrogenase test; Na, sodium; Mg, magnesium; P, phosphorus; PCK, creatine kinase.
[3]Standard deviation.
[4]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

The effect of the different Na sources and increasing level of phytase supplementation on blood (serum) chemistry is presented in Table 10. Sodium sources did not affect the blood chemistry except for HCO^{-}_3 where the combination of NaCl and $NaHCO_3$ resulted in higher ($P = 0.001$) pH. A quadratic ($P < 0.05$) effect of phytase supplementation was observed for HCO^{-}_3, K, ionized Ca, Ca, P, alkaline phosphatase, and blood urea N. Serum ionized Ca and Ca levels linearly ($P < 0.05$) decreased while serum P level linearly ($P < 0.05$) increased with increasing level of phytase supplementation (Table 10). There was no significant interactions between Na source and phytase supplementation on the ABC of the diet and gizzard contents and excreta DM (Table 11). The combination of NaCl and $NaHCO_3$ resulted in higher ($P = 0.001$) ABC of the diets as well as lower ($P < 0.001$) excreta DM. Increasing level of phytase supplementation showed a tendency ($P = 0.073$) to decrease the ABC of the gizzard contents.

DISCUSSION

The objective of this study was to evaluate the effect of 2 Na sources and increasing levels of exogenous phytase supplementation on performance, nutrient and energy digestibility and utilization, and tibia mineralization in 21-day-old broiler chickens. The dietary nutrient contents of the NaCl and the NaCl + $NaHCO_3$-based diets were similar and with similar trends relative to the formulated levels. The analyzed level of phytic acid in the 2 basal diets were not different (approximately 1.09%). This level is similar to what had been previously reported (Cabahug et al., 1999; Cowieson et al., 2006a). The partial replacement of NaCl with $NaHCO_3$ resulted in relatively lower chloride level in the diets containing a combination of NaCl and $NaHCO_3$ hence a higher DEB.

A total of 8 birds (1.9%) died during the study with the highest mortality (3 birds) reported for birds on the PC diet while the other five treatments had a mortality of one bird each. The performance data was adjusted for mortality. Findings from the present study revealed that the growth performance of broiler chickens fed diets containing only NaCl compared well with that of birds fed diets containing a combination of NaCl and $NaHCO_3$, which indicated that $NaHCO_3$ can replace a portion of NaCl in the diet of broiler chickens without any adverse effect on performance. The beneficial effect of phytase supplementation on the growth performance of broiler chickens reported in the current study is in line with what had been previously reported (Liu et al., 2014; Walk and Olukosi, 2019). Data from this study showed that although increasing levels of phytase supplementation did not result in significant feed efficiency effects, however, increasing levels of phytase supplementation resulted in an increase in BWG and feed intake.

Table 11. Effect of inorganic sodium sources and phytase supplementation on acid-binding capacity (ABC, mEq/kg) of the experimental diets and gizzard contents, and the dry matter contents (%) of the excreta of 21-day-old broilers.

Treatment		Diet ABC	n[1]	Gizzard ABC[2]	n[3]	Excreta DM	n[4]
Positive control (PC)		404.5		−80.3		18.7	
Sodium	Phytase						
NaCl		358.6		−65.7		19.2	
$NaCl + NaHCO_3$		395.8		−66.7		16.5	
	0	381.8		−80.3		18.2	
	500	385.7		−71.6		17.5	
	1,000	377.3		−58.9		18.4	
	2,000	364.0		−54.1		17.4	
NaCl	0	365.5	4	−92.93	6	19.6	6
NaCl	500	368.2	4	−69.65	5	18.7	6
NaCl	1,000	358.9	4	−56.37	6	19.8	6
NaCl	2,000	342.0	4	−43.98	6	18.7	5
$NaCl + NaHCO_3$	0	398.1	5	−67.66	6	16.8	6
$NaCl + NaHCO_3$	500	403.3	5	−73.63	6	16.4	6
$NaCl + NaHCO_3$	1,000	395.7	5	−61.38	6	16.9	5
$NaCl + NaHCO_3$	2,000	386.1	5	−64.19	6	16.1	5
SD[5]		24.68		26.07			
				Probability			
Contrast	PC vs. 0 Phy[6]	0.250		0.980		0.638	
Sodium		0.001		0.898		<0.001	
Phytase		0.293		0.073		0.570	
Sodium × phytase		0.965		0.206		0.979	

[1]Number of replicates for diet acid binding capacity.
[2]Negative change in pH, hence negative ABC.
[3]Number of replicates for gizzard content acid binding capacity.
[4]Number of replicates for excreta dry matter.
[5]Standard deviation.
[6]Contrast between the positive control and diets containing 0 FTU phytase/kg diet.

Although Liu et al. (2014) reported beneficial effects on feed efficiency in their study, Selle and Ravindran (2007) extensive review suggested that the impact of exogenous phytase on broiler growth performance is usually more robust and consistent in the feed intake and weight gain responses than feed efficiency responses. Superdosing has also been shown to increase intake, although results are not unequivocal (Augspurger and Baker, 2004; Cowieson et al., 2006b; Pirgozliev et al., 2008; Karadas et al., 2010; Lalpanmawia et al., 2014; de Freitas et al., 2019; Walk and Olukosi, 2019).

In the current study, the BWG of birds fed the diet without phytase and those fed diets with 2,000 FTU phytase/kg diet increased by 11.7 (d 0−14) and 9.7 (d 0 −21) percent. In line with this, feed intake increased by 7.8 (d 0−14) and 6.9 (d 0−21) percent. This finding agrees with what has been previously reported on the effect of phytase on broiler performance including its role in alleviating the negative effects of antinutritional factors such as phytic acid (Dilger et al., 2004; Woyengo et al., 2012; Olukosi et al., 2013). The higher the concentration of phytase in the NaCl-based diets, the higher the overall BWG and feed intake.

In the present study, feed efficiency was not influenced by Na sources. Jankowski et al. (2012) reported an improved body weight for turkey fed diets containing NaCl as the sole Na source compared with the performance of birds fed diets with $NaHCO_3$ and Na_2SO_4 as sources of an inorganic Na, but the effect was only sustained for the first 4 wk of feeding. However, between wk 9 and 12 of the study, $NaHCO_3$ and Na_2SO_4 resulted in a improved performance but no difference was observed at wk 13 to 19 of feeding. The study conducted by Mahmud et al. (2010) reported that 0.25% of Na from NaCl improved BWG of broiler chickens during the first phase of growth compared with 0.2% Na from $NaHCO_3$, however, this effect was not sustained to the older age. In the overall analysis (1−42 d of study), 0.2% Na from $NaHCO_3$ had better feed efficiency compared with 0.25% Na from NaCl. On the contrary, Murakami et al. (1997) reported no difference between $NaHCO_3$ and NaCl as Na sources.

In the current study, the effect of NaCl and a combination of NaCl and $NaHCO_3$ on blood pH was similar. Sodium bicarbonate has been reported to provide Na, which has a positive influence on blood pH and supplies beneficial bicarbonate (Gezen et al., 2005) which may be important during heat stress, however, others have reported no benefits (Grizzle et al., 1992).

Higher levels of phytase supplementation (1,000 and 2,000 FTU) in the current study improved apparent jejunal P digestibility. Previous studies have shown that phytase supplementation enhanced Ca and P digestibility and the improved P digestibility observed in the present study is likely associated with phytate-P hydrolysis by phytase in line with what has been previously reported (Liu et al., 1998; Lalpanmawia et al., 2014). In the current study, the interaction of phytase and Na sources in the diet resulted in both linear and quadratic responses (NaCl diet) and quadratic responses ($NaCl + NaHCO_3$) in the apparent jejunal digestibility of DM, energy, and digestible energy content. With

NaCl as the source of Na, a plateau effect was reached at 1,000 FTU phytase level while with $NaHCO_3$ inclusion, there was an initial decrease in DM and energy digestibility, and DE at 500 FTU phytase compared with 0 FTU phytase. In the jejunum, DM and energy digestibility as well as DE was higher by as much as 8.1, 8.4, and 8.6% at 1,000 FTU phytase in birds fed diets containing NaCl compared with those fed diets containing a combination of NaCl and $NaHCO_3$. Although there was no significant interaction between phytase and sodium source for ileal digestibility values, it is important to note that, unlike in the jejunal data, phytase supplementation improved ileal N and P digestibility and the effectiveness of the effect of phytase on these nutrients at 2,000 FTU exceeded that of 1,000 FTU phytase. Furthermore, a tendency for phytase to increase ileal energy digestibility and DE (3.4% or 107 kcal/kg as seen between the 0 and 2,000 FTU phytase diets) was evident. It has been suggested that energy improvements associated with the addition of phytase to the diets were brought about by the increases in amino acid absorption and enhanced starch and lipid digestibility arising from the dissociation of phytate complexes (Camden et al., 2001).

In some places, excreta from poultry is used as organic fertilizer but excessive use has been linked to negative impacts on the environment (Singh, 2008). Enhancing nutrient and energy utilization in the diet through phytase supplementation in poultry diet offers a practical and cost-effective approach to reducing excessive excretion of P into the environment, thereby reducing it's contribution to environmental pollution (McGrath et al., 2006). In the current study, P utilization between the NaCl and and a combination of NaCl and $NaHCO_3$-containing basal diets and the basal diets supplemented with 2,000 FTU phytase increased by 17.6 and 25.3%, respectively. Although the P utilization values between the 2 Na sources at 2,000 FTU phytase were similar (58.8 vs. 58.9%), the 7.7 percentage-point difference (25.3% minus 17.6%) between the 2 Na sources was because P utilization was lower for the diet containing both NaCl and $NaHCO_3$ compared with diets with only NaCl (47 vs. 50%). For Ca utilization, however, the effect of the combination of NaCl and $NaHCO_3$ was more significant. At 0 FTU phytase (NaCl-containing basal diet), Ca utilization was 35.7% (NaCl) compared with 23.3% for diets containing a combination of NaCl and $NaHCO_3$. This difference facilitated a 78% increase in Ca utilization in birds fed diets supplemented with a combination of 2,000 FTU/kg phytase and NaCl and $NaHCO_3$ compared with only 23% for birds on diets with only NaCl despite the fact that their respective Ca utilization at the higher phytase supplementation was 43.8% (NaCl) and 41.5% (NaCl + $NaHCO_3$). Increasing the level of phytase supplementation resulted in a linear increase in energy utilization, AME, and AMEn of the diets. Part of this response may relate to the ability of phytase to reduce endogenous losses of nutrients and may also improve metabolizable energy of the diet by reducing the energy required for maintenance which could result in relatively higher energy available for growth (Wu et al., 2015; Walters et al., 2019).

The ABC of feed ingredients may differ owing to variations in their composition. Cereal grains and energy source feed ingredients have been reported to have the lowest ABC followed by protein ingredients, while Ca-containing mineral ingredients possessed the highest ABC (Lawlor et al., 2005; Hajati, 2018). Jasaitis et al. (1987) reported that total ash, type of feed, and iron contents are some of the factors that influence ABC. It is therefore expected that different diets will possess different ABC as a result of differences in the type or inclusion levels of different feed ingredients. This likely explains the higher ABC of the diets containing a combination of NaCl and $NaHCO_3$.

In the present study, phytase supplementation improved bone breaking strength and tibia ash contents. High levels of phytate in the diet can result in hypocalcemia or inadequate levels of blood Ca, which may consequently result in a decrease in bone strength (Rath et al., 2000). However, data from the present study showed that phytase supplementation to the basal diets was able to restore tibia ash and tibia breaking strength to the same level as that of the birds on the PC diet even though the PC diets had 22% (formulated) and 10.5% (analyzed) more non-phytate P and Ca, respectively. This phenomenon of addressing the issue with bone strength and mineralization via exogenous phytase supplementation has previously been reported (Olukosi et al., 2013; Morgan et al., 2016).

Digesta pH has been noted as an important gastrointestinal tract factor used to assess nutrient availability and intestinal microbiota (Pang and Applegate, 2007). Any slight changes outside the normal gastrointestinal tract pH range have been documented to have a negative influence on nutrient digestin and absorption (Bristol, 2003). The average jejunum pH for each of the treatments in the current study ranged between 6.05 and 6.13 while it was between 2.50 to 3.04 for the gizzard contents, which agrees with the previously reported values (Pang and Applegate, 2007; Jiménez-Moreno et al., 2009; Walk et al., 2012; Morgan et al., 2014). The pH values obtained for the crop, gizzard, ileum, and cecum contents in the present study were relatively similar to the values reported by Ndelekwute et al. (2018) and Martínez et al. (2021). In the present study, the blood pH of the experimental diets did not differ significantly from that of the control diet, which suggests that the birds were able to tightly regulate their respective blood pH irrespective of the Na source.

Data from this study did not support our original hypothesis and this could be due to the fact that the birds in this study were not heat stressed. Furthermore, some of the observed treatment effects could be associated with the differences in the DEB values of the basal diets. In general, there were no significant interactions between Na sources and phytase supplementation on performance and ileal nutrient and energy digestibility. However, the jejunum DM digestibility and DE peaked

at 1,000 FTU phytase/kg diet while the interactions between phytase and Na from a combination of NaCl and $NaHCO_3$ resulted in higher DM and DE at 0 FTU phytase supplementation with a decreased at 500 FTU and subsequently increased from 1,000 and 2,000 FTU phytase/kg diet. The performance of birds on diets containing a combination of NaCl and $NaHCO_3$ compared well with birds on diets containing only NaCl. This shows that $NaHCO_3$ can replace a portion of NaCl in broiler chickens' diet without significant adverse effect on performance. Based on the results of this study, the 2,000 FTU phytase supplementation level was adjudged the best for performance (BWG and feed intake), apparent jejunal DM and P digestibility, apparent ileal N and P digestibility, and apparent nutrient and energy utilization whereas, the sources of Na evaluated in this study, in general, produced similar results.

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DISCLOSURES

We declare that we have no competing interest in this manuscript.

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