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Journal of Animal Science and Technology 2019;61(2):87-97
https://doi.org/10.5187/jast.2019.61.2.87

Journal of Animal Science and Technology
JAST
pISSN 1598-9429 eISSN 2055-0391

Response of broiler chickens to diets containing different levels of sodium with or without microbial phytase supplementation

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Abstract

Phytate induced excessive mineral excretion through poultry litter leads to poor performance and environmental pollution. Exogenous microbial phytase supplementation to poultry diets reduce the environmental excretion of nutrient and improve bird's performance. However, excessive dietary sodium (Na) level may hinder the phytase-mediated phytate hydrolysis and negate the beneficial effects of phytase. Therefore, this experiment was conducted to investigate the effects of different concentration dietary Na on phytase activity and subsequent impact on broiler performance, bone mineralisation and nutrient utilisation. In this study, six experimental diets, consisting of three different levels of Na (1.5, 2.5, or 3.5 g/kg) and two levels of microbial phytase (0 or 500 U/kg) were formulated by using 3 × 2 factorial design. The six experimental diets were offered to 360 day-old Ross 306 male chicks for 35 days, where, each experimental diet consisted of 6 replicates groups with 10 birds. Along with growth performance, nutrient utilization, intestinal enzyme activity, dry matter (DM) content of litter and mineral status in bone were analysed. Dietary Na and phytase had no effect on bode weight gain and feed intake. Birds on the low Na diet showed higher ($p < 0.05$) feed conversion ratio (FCR) than the mid-Na diets. High dietary Na adversely affected ($p < 0.001$) excreta DM content. Phytase supplementation to the high-Na diet increased ($p < 0.01$) the litter ammonia content. High dietary Na with phytase supplementation improved (Na × phytase, $p < 0.05$) the AME value and ileal digestibility of Ca and Mg. The total tract retention of Ca, P, and Mg was reduced with high Na diet, which was counteracted by phytase supplementation (Na × phytase, $p < 0.001$). The diets containing mid-level of Na improved ($p < 0.001$) the function of Na-K-ATPase and Mg-ATPase in the jejunum. The overall results indicate that high dietary Na did not affect phytase activity but influenced the nutrient utilization of birds, which was not reflected in bird overall performance.

Keywords: Broilers, Phytate, Phytase, Uric acid, Digestibility

Background

Sodium plays an important role in regulating different physiological functions in broiler chickens. Along with potassium and chlorine, the other regulators of dietary electrolyte balance (DEB), Na is of utmost importance for tissue protein synthesis, cellular

Received: Jan 31, 2019 Revised: Mar 12, 2019 Accepted: Mar 15, 2019
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homeostasis and the body's acid–base balance, to ensure optimum performance of broilers [1]. DEB may be defined by the following formula: DEB (mEq/kg) = $Na^+ + K^+ - Cl^-$ and 250 mEq/kg of DEB is suggested to be optimum for broiler growth and litter quality [2]. The intestinal uptake and absorption of different nutrients, particularly glucose and amino acids, are influenced by Na because of its involvement in Na-dependent transport systems and Na-K-ATPase activity [3,4]. Low Na can have a negative impact on broiler performance [2], whereas mortality rate and wet litter problems are increased by excess Na in diets [5]. Although, in 1994, the NRC [6] recommended 2.0 and 1.5 g/kg Na in starter and grower chicken diets, uncertainty exists about the optimum level of Na necessary for maximum performance.

Exogenous microbial phytase is commonly used in poultry diets to replace inorganic phosphates and reduce the anti-nutrient effect of phytate. The benefit of phytase supplementation on diet costs, bird performance, nutrient utilization and reduction of P loss to the environment is well recognized. Despite its beneficial effect on broiler performance, the full benefits of phytase have not yet been achieved due to different influential factors. Dietary mineral concentrations are considered to be one of the important factors that can regulate the activity of dietary phytase [7]. Previous *in vitro* study [8] showed that high Na concentration (3.5 g/kg) significantly lowered phytate hydrolysis by phytase at pH 2.5.

Phytase supplementation could reduce the phytate-induced Na hypersecretion from intestine and subsequently influence the protein/amino acid digestibility [9,10]. But, higher DEB or Na concentration could mute the anti-nutritive effect of phytate, resulting in a lower phytase response on nutrient digestibility [11,12]. Although, the influence of phytase supplementation on litter quality has not yet been established, but some field studies have indicated the occurrence of the phytase-induced increased moisture content in excreta [13].

Therefore, the objective of the present study was to investigate the possible effect of Na on phytase activity, with a focus on broiler growth performance, litter quality and nutrient utilization.

Materials and Methods

Experimental design and bird management

In the present study, a 3 × 2 factorial arrangement was used to investigate the effect of different levels of dietary Na (1.5, 2.5, and 3.5 g/kg) with or without microbial phytase supplementation on overall performance of broilers up to 35 days of age. Three hundred and sixty day-old Ross 308 male broiler chicks (40.04 ± 0.70 g) from a local commercial hatchery (Baiada Poultry Pty. Ltd., Tamworth, Australia) were randomly allocated to six treatment diets. Each diet was replicated six times, with 10 birds per replicate. All the birds were distributed randomly in an environmentally controlled house, with three banks of multi-tiered brooder cages (600 × 420 × 23 cm). The detailed description of bird management has been described in a previous study(Table 1, 2 and 3) [14].

Dietary treatments

Six experimental diets were formulated with low, medium and high levels of Na (1.5, 2.5, and 3.5 g/kg, respectively) with or without exogenous microbial phytase (0 or 500 U/kg). The diets were coded as LS - low Na, MS - Medium Na, HS - High Na, LSP -

Table 1. Ingredient and nutrient specifications of starter diets (0–10 days)

Ingredient composition (g/kg)	Diets					
	LS	MS	HS	LSP	MSP	HSP
Corn	570.1	563.1	556.1	586.9	579.9	572.9
Soybean meal	338.0	338.8	339.5	336.2	337.0	337.7
Meat meal	24.6	25.0	25.4	23.6	24.0	24.5
Canola oil	26.4	28.7	31.0	21.0	23.3	25.5
Limestone	11.2	11.1	11.1	11.4	11.4	11.3
Di-calcium phosphate	15.1	15.1	15	7.3	7.2	7.1
Salt	1.5	1.5	1.6	1.5	1.5	1.5
Sodium bicarbonate	1.9	5.5	9.2	0.8	4.5	8.2
Premix[1)]	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl	0.9	0.9	0.9	0.9	0.9	0.9
L-Lysine HCl	3.0	3.0	3.0	3.0	3.0	3.0
DL-Methionine	4.1	4.1	4.1	4.1	4.1	4.1
L-Threonine	1.9	1.9	1.9	1.9	1.9	1.9
Phytase (U/kg of diet)	0	0	0	500	500	500
Calculated values (g/kg)[2)]						
Calcium	9.6	9.6	9.6	9.6	9.6	9.6
Total phosphorus	7.2	7.2	7.2	7.2	7.2	7.2
Available phosphorus	4.8	4.8	4.8	4.8	4.8	4.8
Sodium	1.5	2.5	3.5	1.5	2.5	3.5
Potassium	9.51	9.50	9.50	9.52	9.51	9.5
Chloride	2.27	2.30	2.30	2.28	2.28	2.28
Analysed values (g/kg)						
Calcium	10.1	10.0	9.8	10.3	9.9	9.7
Total phosphorus	7.1	7.0	7.2	6.3	6.2	6.1
Sodium	1.5	2.4	3.5	1.3	2.3	3.3
Phytase (U/kg)	30	38	42	550	545	550

[1)]Supplied per kg of diet (mg): 11,998.8 IU vitamin A (as all-trans retinol); 3,600 IU cholecalciferol; 65.56 IU vitamin E (as d-α-tocopherol); 2 mg vitamin K_3; 2 mg thiamine; 6 mg riboflavin; 5 mg pyridoxine hydrochloride; 0.2 mg vitamin B_{12}; 0.1 mg biotin; 50 mg niacin; 12 mg D-calcium pantothenate; 2 mg folic acid; 80 mg Mn; 60 mg Fe; 8 mg Cu; 1 mg I; 0.3 mg Co; 1 mg Mo.

[2)]All diets were formulated to contain 12.6 MJ/kg metabolizable energy; 230 g/kg crude protein; 5.1 g/kg digestible methionine; 12.8 g/kg digestible lysine; 9.5 g/kg digestible methionine + cysteine; 8.6 g/kg digestible threonine, 13.7 g/kg digestible arginine.

LS, low Na; MS, mid Na; HS, high Na; LSP, low Na with phytase; MSP, mid Na with phytase; HSP, high Na with phytase.

https://doi.org/10.5187/jast.2019.61.2.87

Table 2. Ingredient and nutrient specifications of grower diets (11–24 days)

Ingredient composition (g/kg)	Diets					
	LS	MS	HS	LSP	MSP	HSP
Corn	598.3	590.8	583.3	615.2	607.8	600.4
Soybean meal	282.0	283.4	284.7	280.2	281.4	282.7
Meat meal	50.0	50.0	50.0	49.0	49.1	49.2
Canola oil	38.7	41.1	43.5	33.2	35.6	38
Limestone	6.3	6.3	6.3	6.6	6.6	6.5
Dicalcium phosphate	7.9	7.9	7.9	0	0	0
Salt	1.5	1.5	1.5	1.5	1.5	1.5
Sodium bicarbonate	1.4	5.1	8.8	0.3	4	7.7
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0
Premix[1)]	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl	1.0	1.0	1.0	1.0	1.0	1.0
L-Lysine HCl	1.9	1.9	1.9	2.0	1.9	1.9
DL-Methionine	3.4	3.4	3.4	3.4	3.4	3.4
L-Threonine	1.4	1.4	1.4	1.4	1.4	1.4
Phytase (U/kg diet)	0	0	0	500	500	500
Calculated values (g/kg)[2)]						
Calcium	8.7	8.7	8.7	8.7	8.7	8.7
Available phosphorus	4.4	4.4	4.4	4.4	4.4	4.4
Total phosphorus	6.7	6.7	6.7	6.7	6.7	6.7
Sodium	1.5	2.5	3.5	1.5	2.5	3.5
Potassium	8.5	8.5	8.5	8.5	8.5	8.5
Chloride	2.2	2.2	2.2	2.2	2.2	2.2
Analysed values (g/kg)						
Calcium	8.9	9.0	8.9	8.9	8.8	8.6
Total phosphorus	7.1	6.9	7.0	5.6	5.6	5.8
Sodium	1.5	2.6	3.5	1.4	2.4	3.4
Phytase (U/kg)	40	38	45	550	565	559

[1)]Supplied per kg of diet (mg): 11,998.8 IU vitamin A (as all-trans retinol); 3,600 IU cholecalciferol; 65.56 IU vitamin E (as d-α-tocopherol); 2 mg vitamin K_3; 2 mg thiamine; 6 mg riboflavin; 5 mg pyridoxine hydrochloride; 0.2 mg vitamin B_{12}; 0.1 mg biotin; 50 mg niacin; 12 mg D-calcium pantothenate; 2 mg folic acid; 80 mg Mn; 60 mg Fe; 8 mg Cu; 1 mg I; 0.3 mg Co; 1 mg Mo.

[2)]All diets were formulated to contain 13.2 MJ/kg metabolisable energy; 215 g/kg crude protein; 4.7 g/kg digestible methionine; 11.5 g/kg digestible lysine; 8.7 g/kg digestible methionine + cysteine; 7.7 g/kg digestible threonine, 12.3 g/kg digestible arginine.

LS, low Na; MS, mid Na; HS, high Na; LSP, low Na with phytase; MSP, mid Na with phytase; HSP, high Na with phytase.

Table 3. Ingredient and nutrient specifications of finisher diets (25–35 days)

Ingredient composition (g/kg)	Diets					
	LS	MS	HS	LSP	MSP	HSP
Corn	629.4	622.9	615.9	627.6	631.6	624.2
Soybean meal	259.7	259.8	260.5	283.6	269.1	270.4
Meat meal	48.7	49.5	50.0	31.4	41.5	41.5
Canola oil	40.1	42.2	44.5	39.6	38.9	41.3
Limestone	6.1	6.0	5.9	7.9	6.9	6.9
Dicalcium phosphate	6.7	6.5	6.5	1.9	0	0
Salt	2.0	2.0	2.0	2.0	2.0	2.0
Sodium carbonate	0.7	4.4	8.1	0	3.5	7.2
Premix[1)]	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl	0.9	0.9	0.9	0.8	0.9	0.9
L-Lysine HCl	0.9	0.9	0.9	0.6	0.8	0.8
DL-Methionine	2.7	2.8	2.8	2.6	2.7	2.7
L-Threonine	0.8	0.8	0.8	0.7	0.8	0.8
Phytase (U/kg of diet)	0	0	0	500	500	500
Calculated values (g/kg)[2)]						
Calcium	8.7	8.7	8.7	8.7	8.7	8.7
Available phosphorus	4.4	4.4	4.4	4.4	4.4	4.4
Total phosphorus	6.9	6.9	6.9	6.9	6.9	6.9
Sodium	1.5	2.5	3.5	1.5	2.5	3.5
Potassium	8.1	8.1	8.1	8.5	8.3	8.3
Chloride	2.3	2.3	2.3	2.2	2.2	2.2
Analysed values (g/kg)						
Calcium	8.6	8.7	8.9	8.4	8.8	8.6
Total phosphorus	7.2	7.4	7.0	5.8	5.8	5.4
Sodium	1.4	2.5	3.6	1.5	2.3	3.3
Phytase (U/kg)	40	45	42	550	526	538

[1)]Supplied per kg of diet (mg): 11,998.8 IU vitamin A (as all-trans retinol); 3,600 IU cholecalciferol; 65.56 IU vitamin E (as d-α-tocopherol); 2 mg vitamin K_3; 2 mg thiamine; 6 mg riboflavin; 5 mg pyridoxine hydrochloride; 0.2 mg vitamin B_{12}; 0.1 mg biotin; 50 mg niacin; 12 mg D-calcium pantothenate; 2 mg folic acid; 80 mg Mn; 60 mg Fe; 8 mg Cu; 1 mg I; 0.3 mg Co; 1 mg Mo.

[2)]All diets were formulated to contain 13.4 MJ/kg metabolizable energy; 195 g/kg crude protein; 4.3 g/kg digestible methionine; 10.3 g/kg digestible lysine; 8.0 g/kg digestible methionine + cysteine; 6.9 g/kg digestible threonine, 11.0 g/kg digestible arginine.

LS, low Na; MS, mid Na; HS, high Na; LSP, low Na with phytase; MSP, mid Na with phytase; HSP, high Na with phytase.

Low Na with phytase, MSP - Medium Na with phytase and HSP - High Na with phytase. The mineral matrix (1.5 g/kg AvP, 1.65 g/kg Ca & 0.35 g/kg Na) of the commercial microbial phytase product, derived from modified *Escherichia coli* 6- phytase expressed in *Trichoderma reesei* (Quantum Blue, AB Vista, Marlborough, UK) were applied in phytase-supplemented diets to achieve the Ca, AvP, and Na levels. The activity of the phytase was 5,000 U/g where a unit (U) is defined as the quantity of enzyme that liberates one μmol of inorganic P per minute from sodium phytate at pH 5.5 and 37℃. In all grower diets, titanium oxide (5 g/kg diet) was added as an indigestible marker, to enable assessment of nutrient digestibility. Diets were formulated to be iso-energetic and iso-nitrogenous and were pelleted at 65℃. Diets were pelleted between 3 and 4 mm diameter and used as such in the grower (11–24 d) and finisher (25–35 d) phases respectively. Diets were crumbled in the starter (0–10 d) phase. All diets were formulated to either meet or exceed the Aviagen, 2014 [15] nutrient recommendation and breed standards, except for Na.

Collection, processing and analysis of samples

On d 10, 24, and 35, feed intake (FI) and body weight (BW) were recorded. Mortality was recorded as it occurred. Feed conversion ratio (FCR) was calculated and corrected for mortality. From 22 to 24 d, excreta samples were collected on cage basis over three consecutive days. Daily excreta collected were pooled; mixed thoroughly and subsamples were kept in a plastic container at –20℃ until further analysis. On d 24, two birds were randomly chosen from each cage and killed by cervical dislocation, to collect the ileal digesta, left tibia and jejunal tissue samples. The procedures of collection, processing and analysis of different samples (diets, ileal digesta, excreta, tibia bone, and parts of jejunum) for nutrient digestibility, bone quality, intestinal enzyme activities, phytase activity in diet were the same as documented previously [14,16].

Excreta ammonia and urea analysis

The excreta samples collected on d 24 were analyzed to simulate the effect of the treatments on litter dry matter (DM), uric acid and ammonia concentration. In 50 mL plastic tubes, 5 g of excreta from each sample were taken and then 40 mL of Milli-Q water were added to each tube. After homogenizing for 2 mins (1084 × g), the samples were filtered through Whatman No. 1 filter paper and diluted 10 times. The filtrate was used for ammonia and uric acid measurements.

Measurement of excreta uric acid and ammonia

Uric acid content of the excreta was measured using colorimetric method as indicated [17]. Excreta ammonia was measured by following the procedure described in the ammonia assay kit (Catalogue Number AA0100, Sigma-Aldrich, and 3050 Spruce Street, Saint Louis, Missouri 63103, USA). Briefly, around 0.1 to 0.2 mL diluted excreta sample was placed into a cuvette and mixed thoroughly with 1–2 mL ammonia assay reagent, and then incubated for 5 min at 18℃–35℃. After that, absorbance was read at 340 nm against blank samples (prepared with 0.1 mL water mixed with 1.0 mL ammonia assay reagent). After this reading, 0.01 mL of L-glutamate dehydrogenase solution (Catalogue Number-G2294) was added to each cuvette and incubated at 18℃–35℃ for 5 min. The absorbance of each solution was measured again at 340 nm. The concentration of ammonia (mg/mL) was calculated from the following equations:

The ΔA340 for the reagent blank, test and standard were determined. For each:

$\Delta A_{340} = A_{initial} - A_{final}$ Δ (ΔA_{340}) Test or standard

= ΔA_{340} (Test or standard) – ΔA_{340} (Blank) mg of NH_3/mL of original sample

$$= \frac{(A)\ (TV)\ (MW\ of\ ammonia)\ (F)}{(\varepsilon)\ (d)\ (SV)\ (Conversion\ \ factor\ \ of\ \ \mu g\ \ to\ \ mg)}$$

$$= \frac{(A)\ (TV)\ (17)\ (F)}{(6.22)\ (1)\ (SV)\ (1000)}$$

$$= \frac{(A)\ (TV)\ (F) \times 0.00273}{(SV)}$$

where, A, Δ (ΔA_{340}) Test or standard; TV, Total assay volume (mL); SV, sample volume (mL); MW of ammonia, 17 g/mole; F, Dilution factor from sample preparation; Ɛ, Millimolar extinction coefficient for NADPH at 340 nm; d, Light path (1 cm).

Statistical analysis

The data were analysed as a 3 × 2 factorial ANOVA using the GLM procedure of Minitab software (Minitabe 16.0, Minitab Inc., State College, Pennsylvania, USA, 2010) for the main effects of Na and phytase, along with their interactions. Separation of means within a significant effect was conducted using Tukey's HSD test. The significance of difference between means was determined by Fisher's least significant difference at $p \leq 0.05$.

Results

Growth performance

The analysed levels of total P, Ca, Na and phytase level in the feed were in close agreement with calculated values (Tables 1, 2, and 3). Dietary Na, phytase and their interaction had no effect ($p > 0.05$) on FI and body weight gain (BWG) at any phase of rearing (Table 4). Phytase supplementation tended ($p = 0.076$) to improve BWG during 1–24 d. The FCR of birds was not affected by Na and phytase over the experimental period except from d 0 to 10. During this stage, mid-Na diets improved ($p < 0.05$) the FCR compared to low-Na diets. At all stages of rearing, the interaction between Na and phytase had no significant effect on FCR.

Ileal pH, excreta DM, ammonia and uric acid concentration

Results shown in Table 5 indicate that there was no significant effect of Na and phytase supplementation on ileal pH. None of the interaction effects was significant, except for excreta ammonia. Phytase supplementation to low and high Na diets increased (Na × phytase, $p < 0.007$) the ammonia excretion compared to phytase free diets. The ammonia excretion was not differed in phytase supplemented diets irrespective of Na levels. The high Na (3.5 g/kg) diets lowered ($p < 0.001$) the excreta DM, whereas the reverse ($p < 0.001$) was the case for uric acid concentration.

https://doi.org/10.5187/jast.2019.61.2.87

Table 4. Influence of dietary Na levels with or without microbial phytase on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers fed from day 0 to 35

Na level[1)]	Phytase[2)]	FI (g/bird)			BWG (g/bird)			FCR		
		1–10 d	1–24 d	1–35 d	1–10 d	1–24 d	1–35 d	1–10 d	1–24 d	1–35 d
Low	None	248.9	1,644.5	3,486.3	216.7	1,230.0	2,400.7	1.15	1.33	1.45
	Plus	257.9	1,688.8	3,502.3	219.7	1,256.5	2,405.5	1.17	1.34	1.46
Mid	None	250.0	1,631.9	3,432.4	222.2	1,246.4	2,367.7	1.13	1.32	1.45
	Plus	240.2	1,677.8	3,460.2	216.7	1,332.9	2,291.0	1.11	1.26	1.51
High	None	244.5	1,640.0	3,418.2	213.6	1,262.1	2,295.2	1.14	1.30	1.49
	Plus	238.3	1,662.5	3,339.8	222.9	1,303.9	2,312.0	1.12	1.27	1.45
SEM		11.5	59.58	109.27	11.05	48.67	89.58	0.02	0.06	0.04
Main effects										
Na level										
Low		253.4	1,666.6	3,494.3	218.2	1,243.2	2,403.1	1.16[a]	1.34	1.46
Mid		245.1	1,654.9	3,446.0	219.4	1,289.7	2,329.3	1.12[b]	1.29	1.48
High		241.4	1,651.3	3,379.0	213.3	1,283.0	2,303.6	1.13[ab]	1.29	1.47
Phytase										
	Phytase									
	None	247.8	1,638.8	3,445.6	217.5	1,246.1	2,354.5	1.14	1.32	1.46
	Plus	245.5	1,676.4	3,434.1	216.4	1,298.0	2,336.2	1.14	1.30	1.47
Source of variation										
Na		ns	ns	ns	ns	ns	ns	*	ns	ns
Phytase		ns	ns	ns	ns	ns	ns	ns	ns	ns
Na × phytase		ns	ns	ns	ns	ns	ns	ns	ns	ns

Means were obtained from 6 replicates (6–8) birds per cage.

[a,b]Means within a column without common superscript are statistically different ($p < 0.05$).

[1)]Low, 1.5 g Na/kg; Mid, 2.5 Na/kg; High, 3.5 g Na/kg.

[2)]None, without phytase; plus, with (500 U/kg) phytase.

[ns] $p > 0.05$; [*] $p < 0.05$.

FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio.

Apparent metabolizable energy and ileal digestibility

The interaction between Na and phytase influenced the apparent metabolizable energy (AME and ileal digestibility of Ca and Mg (Table 6). High Na diets with phytase supplementation showed improved (Na × phytase, $p < 0.001$) AME value compared to high-Na diet without phytase. The high-Na diet reduced the digestibility of Ca (Na × phytase, $p < 0.02$) which was countered by phytase supplementation. Similarly, the negative effect of low and high Na diets on Mg digestibility was alleviated (Na × phytase, $p < 0.05$) by phytase supplementation. The interaction between Na and phytase also tended ($p = 0.075$) to decrease the P digestibility, where the negative effect of high Na was compensated by phytase supplementation. The digestibility of Na was highest ($p < 0.001$) in high-Na diets and was negative in low and mid-Na diets. Dietary Na, phytase or their interaction had no effect on K digestibility.

Total tract retention of minerals

Diets containing mid and high Na reduced (Na × phytase, $p < 0.001$) the retention of N and Ca which was counterbalanced be phytase supplementation (Table 7). Phytase supplemented to low and high Na diets improved (Na × phytase, $p < 0.001$) the P retention compared to the mid-Na diets with phytase. Low Na diets with phytase supplementation showed the highest (Na × phytase, $p < 0.001$) retention of Na, K and Mg compared to mid and high Na diets with phytase.

Bone development and mineral contents

Dietary Na, phytase and their interaction had no effect ($p > 0.05$) on length, width, breaking strength, ash and mineral content of tibia bone (data not shown).

Protein content and enzyme activities in jejunum

Phytase supplementation to high-Na diets improved (Na × phytase, $p < 0.001$) the activities of AP, Ca-ATPase and Mg-ATPase compared to low or mid-Na diets with phytase (Table 8). Birds that received phytase-supplemented mid-Na diet showed the

Table 5. Influence of dietary Na level and supplemental phytase on the ileal pH, excreta DM, ammonia and uric acid concentration of 22–24-day old broilers

Na levle[1)]	Phytase[2)]	Ileal pH	DM (%)	Ammonia (mg/L)	Uric acid (g/L)
Low	None	6.24	24.8	39.8^{a}	16.0
	Plus	6.02	25.0	39.4^{a}	17.6
Mid	None	6.04	23.4	33.3^{ab}	25.1
	Plus	6.00	22.6	34.9^{ab}	29.3
High	None	6.61	20.7	30.1^{b}	32.0
	Plus	6.54	20.1	37.9^{a}	30.2
SEM		0.44	1.23	0.33	0.84
Main effects					
Na level					
Low		6.13	24.9^{a}	32.6	16.8^{b}
Mid		6.02	23.0^{a}	34.1	27.2^{a}
High		6.57	20.4^{b}	34.0	31.1^{a}
	Phytase				
	None	6.29	23.0	27.7^{b}	24.4
	Plus	6.18	22.6	37.4^{a}	25.7
Source of variation					
Na		ns	***	ns	***
Phytase		ns	ns	***	ns
Na × phytase		ns	ns	**	ns

Means were obtained from 6 replicates (2 birds per cage).

^{a,b}Means within a column without common superscript are statistically different ($p < 0.05$).

[1)]Low, 1.5 g Na/kg; Mid, 2.5 Na/kg; High, 3.5 g Na/kg.

[2)]None, without phytase; plus, with (500 U/kg) phytase.

^{ns} $p > 0.05$; ** $p < 0.01$; *** $p < 0.001$.

highest (Na × phytase, $p < 0.001$) protein content in jejunal mucosa than those fed phytase-free mid-Na diet. Low-Na diets reduced ($p < 0.01$) the activity of Na-K-ATPase compared to mid-Na diets. The Ca-Mg-ATPase activity was decreased ($p < 0.05$) in birds fed the mid-Na diet than high-Na diets. Supplementation of phytase improved the activities of Ca-Mg-ATPase ($p < 0.05$) and Na-K-ATPase ($p < 0.001$). The interaction between Na and phytase was not significant for Ca-Mg-ATPase and Na-K-ATPase.

Discussion

In the current study, different levels of dietary Na had no effect on BWG and FI, which is in agreement with previous studies [18,19]. Similarly, Goodgame et al. [20] reported no significant effect on the productive performance of birds by increasing Na concentration from 1.6 to 2.8 g/kg in diets. On the other hand, Na level below 1.6 g/kg [20] and above 3.5 g/kg could be detrimental to broiler performance [12]. Although, with the current experimental diets, Na (1.5 to 3.5 g/kg) concentration exceeded the recommended level of NRC [6] and Aviagen [15] (1.5–2.0 g/kg and 1.6–2.0/2.3 g/kg, respectively), the lack of any significant variation in BWG and FI due to different dietary Na levels could be an indication of the bird's capacity to tolerate a wide range of Na. There were no significant differences in BWG, FI and FCR of broilers fed diet supplemented with phytase at any stage of rearing compared to those fed non-supplemented diets. The lack of any phytase effect on performance of broilers indicates that the dietary adjustments for AvP, Ca, and Na from phytase inclusion currently used in practical diet formulation are justified. Enzyme supplementation had no effect because the mineral matrix was considered. This is a cost saving on diet formulation and is widely practiced under practical conditions [21].

Excreta DM content decreased in chickens fed high Na diets, irrespective of phytase supplementation, and this result is consistent with the previous findings [12]. Feeding diets containing Na level more than 3.0 g/kg increased the water consumption of birds, with the consequence that the excreta had higher moisture content. It has been reported that supplementation of diets with phytase increased litter moisture content [13], no such effect was observed in the present study. Assigning Na matrix value of phytase in the present study diets may be a possible reason for not observing a phytase-induced wet litter problem. High dietary Na increased the uric acid concentration of excreta, while phytase supplementation and their interaction had no effect. As most excreta N is converted into uric acid before passing out through droppings, an increased excretion of uric acid in birds on high Na diets might be an indication of excess N loss. This result is also consistent with low retention of N observed with high Na diets.

Phytase supplementation to high Na diets increased the ammonia concentration in litter but the reason for this trend is not very clear. High moisture content in the litter partly explains the increased concentration of ammonia in the aforementioned diet. Although, it has been reported that high protein diets enhanced the excretion of N and ammonia [22], which is not the case in the present study as all experimental diets contained an equal amount of crude protein. However, there could be a general increase in protein loss due to the increase in protein digestibility, in the presence of phytase. There is a need for further investigation into this area.

Phytase supplementation improved AME, especially in diets with a high level of Na (3.5 g/kg) in the diet. This result is in agreement with Ravindran et al. [12], who reported better AME in birds that received diets containing 2.0–3.5 g/kg Na (equivalent to 225 to 300 mEq/kg) supplemented with phytase. These researchers also suggested that phytase supplementation had no effect on AME when dietary Na exceeded the above-mentioned

https://doi.org/10.5187/jast.2019.61.2.87

Table 6. AME and ileal digestibility coefficient of minerals of 24-d old broilers that consumed diets with different levels of Na with or without phytase

Na level[1)]	Phytase[2)]	AME (MJ/kg)	N	Ca	P	Na	K	Mg
Low	None	14.92^{ab}	0.75	0.47^{a}	0.50	–0.56	0.85	0.12^{b}
	Plus	14.92^{ab}	0.80	0.38^{a}	0.54	–0.45	0.89	0.30^{a}
Mid	None	14.95^{ab}	0.76	0.38^{a}	0.46	0.05	0.89	0.16^{ab}
	Plus	14.88^{ab}	0.79	0.33^{a}	0.47	–0.25	0.87	0.16^{ab}
High	None	14.06^{c}	0.71	0.16^{b}	0.31	0.19	0.87	–0.12^{c}
	Plus	15.22^{a}	0.78	0.39^{a}	0.50	0.13	0.90	0.17^{ab}
SEM		0.03	0.02	0.08	0.06	0.25	0.02	0.08
Na level								
Low		14.9	0.78	0.43^{a}	0.52^{a}	–0.50^{b}	0.87	0.21^{a}
Mid		14.8	0.77	0.36^{ab}	0.47^{ab}	–0.10^{b}	0.88	0.16^{ab}
High		14.6	0.75	0.27^{b}	0.41^{b}	0.16^{a}	0.88	0.03^{b}
	Phytase							
	None	14.6^{b}	0.74^{b}	0.37	0.43^{b}	–0.11	0.87	0.05^{b}
	Plus	14.9^{a}	0.79^{a}	0.34	0.51^{a}	–0.19	0.88	0.21^{a}
Source of variation								
Na		ns	ns	*	*	***	ns	**
Phytase		*	***	ns	*	ns	ns	***
Na × phytase		***	ns	*	ns	ns	ns	*

Means were obtained from 6 replicates (2 birds per cage).

$^{a–c}$Means within a column without common superscript are statistically different ($p < 0.05$).

[1)]Low, 1.5 g Na/kg; Mid, 2.5 Na/kg; High, 3.5 g Na/kg.

[2)]None, without phytase; plus, with (500 U/kg) phytase.

^{ns} $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

AME, apparent metabolizable energy.

range. Although not significant, high-Na diet reduced the digestibility of N, similar to previous results [12,23]. This result suggests that N utilization is less likely to improve in high Na diets, which is consistent with excess urinary loss of uric acid.

It has been speculated that supplementation of phytase in diet substantially reduces the phytate/phytate-protein induced hypersecretion of gastric acid, digestive enzymes (pepsin and mucin) and $NaHCO_3$ [12,24]. Recent studies [10,12,25–27] reported that phytase inclusion in diet significantly improved the ileal Na digestibility and suggested possible reduction of Na inclusion level in phytase supplemented diet. This is in contrast with present study where phytase had no effect on ileal Na digestibility at different dietary Na levels. The reason of this discrepancy is not clear. The use of Na matrix value of phytase in the diet formulation of the present study may partly justify the lack of response of ileal Na digestibility to phytase. However, almost all of the aforementioned studies, including the present one; obtained negative digestibility coefficients for Na, which may be due to excessive secretion of endogenous Na, a process that would vary with the feeding status of birds. Due to complex Na and Cl flux in the intestine, it is sometimes unrealistic to make inferences on the trend of their digestibility in the small intestine [10]. However, increasing Na (3.5 g/kg) concentrations in diets resulted in significant improvements in ileal digestibility of Na but reduced total tract Na retention. This is partly consistent with the findings of Ravindran et al. [12], who suggested a possible influence of dietary Na on intestinal secretion and absorption of Na in poultry.

Phytase-mediated retention of Na was mostly observed with the diet containing 1.5 g Na/kg and there was significant interaction between Na and phytase, which indicates possibility of reduction of dietary Na need in poultry diets when supplemented with phytase. However, this may not be correct as the interaction between phytase and Na for overall performance and nutrient utilization of broilers was not consistent with total Na retention data. Besides, previous study [28] reported that that the chloride ion of salt (NaCl) electrostatically competes with phytate and reduces the phytate-protein complex formation (including digestive enzyme, trypsin, and substrate protein) and consequently attenuates the negative effect of protein-phytate complex in diets supplemented with phytase. Therefore, the association between Na and phytase in light of

Table 7. Effect of Na on total tract retention of nitrogen (N) and minerals of 24-d old broilers fed diet with or without phytase

Na level[1)]	Phytase[2)]	N	Ca	P	Mg	Na	K
Low	None	0.65^{ab}	0.60^{a}	0.56^{b}	0.25^{a}	0.64^{b}	0.35^{b}
	Plus	0.67^{a}	0.58^{a}	0.62^{a}	0.29^{a}	0.73^{a}	0.42^{a}
Mid	None	0.61^{b}	0.50^{b}	0.49^{c}	0.18^{a}	0.41^{d}	0.28^{c}
	Plus	0.65^{ab}	0.56^{a}	0.55^{b}	0.21^{a}	0.47^{c}	0.29^{bc}
High	None	0.50^{c}	0.27^{c}	0.28^{d}	–0.13^{b}	0.09^{f}	0.09^{d}
	Plus	0.67^{a}	0.60^{a}	0.63^{a}	0.26^{a}	0.35^{e}	0.35^{b}
SEM		0.05	0.03	0.02	0.06	0.02	0.03
Main effects							
Na level		0.65^{a}	0.59^{a}	0.59^{a}	0.27^{a}	0.68^{a}	0.39^{a}
Low		0.63^{a}	0.53^{b}	0.52^{b}	0.20^{a}	0.44^{b}	0.25^{b}
Mid		0.59^{b}	0.44^{c}	0.46^{c}	0.08^{b}	0.22^{c}	0.22^{c}
High							
	Phytase						
	None	0.58^{b}	0.46^{b}	0.44^{b}	0.10^{b}	0.38^{b}	0.24^{b}
	Plus	0.66^{a}	0.58^{a}	0.60^{a}	0.26^{a}	0.52^{a}	0.35^{a}
Source of variation							
Na		***	***	***	***	***	***
Phytase		***	***	***	***	***	***
Na × phytase		***	***	***	***	***	***

Means were obtained from 6 replicates (7 birds per cage).

$^{a–d}$Means within a column without common superscript are statistically different ($p < 0.05$).

[1)]Low, 1.5 g Na/kg; Mid, 2.5 Na/kg; High, 3.5 g Na/kg.

[2)]None, without phytase; plus, with (500 U/kg) phytase.

*** $p < 0.001$.

Cl effect is worthy of consideration.

Phytase supplementation improved the activity of Na-K-ATPase, which is in agreement with previous report [29]. These researchers established that phytase dephosphorylation ameliorates the negative effect of phytate on Na-K-ATPase activity in the intestine of chickens. As Na-K-ATPase maintains electrochemical gradients across the gut mucosa, it is possible that phytase also improved the absorption and intestinal uptakes of Na and other co-transported nutrients [30]. This is consistent with the phytase-related improvement of digestibility and retention of minerals in the present study. The reduction of Na-K-ATPase activity of the jejunum in birds offered low-Na diets is paltry in agreement with previous study [31]. This finding suggests that as the Na-dependent transport system and Na-K-ATPase activity in the intestine are responsible for absorption of most of the nutrients, thus, provision of sufficient Na to diets is essential to ensure the efficient activity of these enzymes and subsequent nutrient absorption as well [32].

The digestibility of Ca and Mg was reduced in birds that consumed the high-Na (3.5 g/kg) diets, which was compensated by phytase supplementation. This partly agrees with the previous findings where increasing Na level in phytase-supplemented diets from 1.7 to 2.4 g/kg (equivalent to 234 and 266 mEq/kg of DEB, respectively) reduced the digestibility of the aforementioned minerals. In contrast, similar study [10] observed no such effect of different levels of DEB on Ca and P digestibility. Moreover, phytase improved the digestibility of P irrespective of Na levels which indicates that phytase was effective in releasing this mineral. Birds offered high Na diets showed poor retention of Ca and P, but this effect was counteracted by phytase supplementation indicating a significant Na and phytase interaction. This finding is in agreement with previous study [12] where similar effect of DEB, phytase and DEB × phytase interactions on total tract retention of Ca and P was observed.

Conclusion

The results of the present study showed that Na concentration in diets ranging from 1.5 to 3.5 g/kg had no significant effects on bird performances with or without phytase supplementation. The improved AME value in phytase-supplemented diets further confirms the extra phosphoric effect of phytase, even with a wide range of Na. Inclusion of Na at 3.5 g/kg to diets negatively affected the utilization of minerals except for Na, which implies that maintaining Na level at around 2.5 g/kg is optimal for performance and mineral utilization. A higher Na level can sometimes compromise the retention and digestibility of Ca, P, and Na, not by affecting phytate hydrolysis but by altering the absorption and reabsorption pattern of nutrients in the intestine. Despite data showing some interaction effect between Na and phytase for mineral utilization and enzyme activities, it is unclear, due to the inconsistent pattern of the data, whether Na level had any effect on phytase activity. Therefore, further investigation is warranted to explore the Na effect on phytase-induced nutrient digestibility and intestinal enzyme activities, especially when the Na content of the phytase matrix is considered.

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

This project was funded by University of New England, Australia and AB Vista, UK.

Acknowledgments

We express our sincere gratitude to the staff of Centre for Animal

https://doi.org/10.5187/jast.2019.61.2.87

Table 8. Effect of different levels of dietary Na with or without microbial phytase on total protein (mg/g) content, alkaline phosphatase (AP;μM/mg protein/mn) Ca-, Mg-, Ca-Mg-ATPase, and Na-K-ATPase (nmol/mg protein/ min) activityof jejunum mucosa

Na level[1)]	Phytase[2)]	Protein	AP	Ca-Mg ATPase	Ca-ATPase	Mg-ATPase	Na-K- ATPase
Low	None	53.53^{bc}	3.60^{a}	168.06	164.21^{b}	145.18^{c}	49.92
	Plus	53.13^{bc}	2.60^{c}	177.21	160.69^{b}	158.67^{b}	86.38
Mid	None	55.20^{ab}	2.48^{c}	153.39	159.42^{b}	190.20^{a}	73.58
	Plus	57.82^{a}	3.01^{b}	162.85	170.77^{b}	166.59^{b}	98.17
High	None	57.23^{a}	3.19^{b}	165.48	165.84^{b}	154.81^{bc}	65.13
	Plus	50.85^{c}	3.78^{a}	187.01	210.72^{a}	180.53^{a}	85.39
SEM		0.17	0.03	1.49	1.65	0.88	1.09
Main effects							
Na level							
Low		53.33^{b}	3.10^{b}	172.64^{ab}	162.45^{b}	151.92^{c}	68.15^{b}
Mid		56.51^{a}	2.75^{c}	158.12^{b}	165.10^{b}	178.40^{a}	85.88^{a}
High		54.04^{b}	3.48^{a}	176.25^{a}	188.28^{a}	167.67^{b}	75.26^{ab}
	Phytase						
	None	55.32^{a}	3.09	162.31^{b}	163.16^{b}	163.40^{a}	62.88^{b}
	Plus	53.93^{b}	3.13	175.69^{a}	180.73^{a}	168.60^{a}	89.98^{a}
Source of variation							
Na		***	***	*	***	***	**
Phytase		*	n.s.	*	**	n.s.	***
Na × Phytase		***	***	ns	***	***	n.s.

Means were obtained from 6 replicates (2 birds per cage).

$^{a-c}$Means within a column without common superscript are statistically different ($p < 0.05$).

[1)]Low, 1.5 g Na/kg; Mid, 2.5 Na/kg; High, 3.5 g Na/kg.

[2)]None, without phytase; plus, with (500 U/kg) phytase.

^{ns} $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Research and Teaching (CART), University of New England, Australia for helping with the management of chicken during the study period.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Akter M, Graham H, Iji PA.
Data curation: Akter M.
Formal analysis: Akter M.
Methodology: Akter M, Iji, PA.
Software: Akter M.
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Investigation: Akter M.
Writing - original draft: Akter M.
Writing - review & editing: Graham H, Iji PA.

Ethics approval and consent to participate

All animal experiments were in accordance with the protocol approved by Animal Ethics Committee University of New England, Australia. (Ethics approval No: AEC14-053).

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