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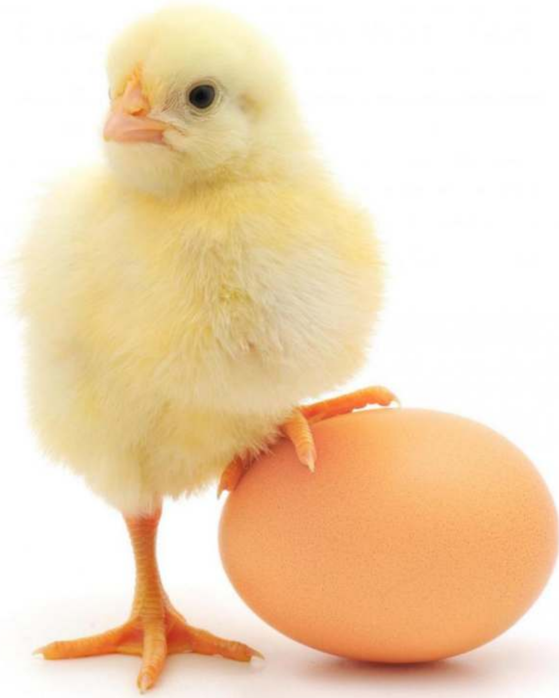

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Effect of Phytase on the Sodium Requirement of Starting Broilers 1. Sodium Bicarbonate as Primary Sodium Source

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Abstract: Recent work has indicated that phytase enzymes may influence Sodium (Na) metabolism in the chick but to date no work has conclusively demonstrated that the Na requirement of the chick for live performance is influenced by phytase supplementation. In this study male broilers were fed diets with Na levels ranging from 0.10-0.28% using sodium bicarbonate as the primary source of supplemental Na. Diets were supplemented with no phytase, 500 FTU/kg (1x) or 2,000 FTU/kg (4x) of phytase. When phytase was added the dietary Ca and Nonphytate P (NPP) were adjusted in accordance with anticipated release of these minerals. For 1x phytase the Ca and NPP were reduced 0.10% each and for 4x phytase were reduced by 0.20% each. The combination of seven Na levels and three phytase treatments resulted in 21 dietary treatments, each of which was fed to six replicate pens of five male chicks housed in electrically heated battery brooders with wire floors. Experimental diets and tap water were provided for *ad libitum* consumption from day of hatch to 18 d. At 16 d of age excreta from each pen was collected and freeze dried to determine moisture content and the excreta analyzed to determine amounts of Ca, P and Na. There were no significant differences in Body Weight (BW), Feed Conversion (FCR), Feed Intake (FI), fecal moisture (FH_2O), or mortality of broilers fed diets with different levels of phytase indicating that the dietary adjustment in NPP and Ca levels for the addition of the phytase did not adversely affect performance of the birds. The dietary Na level significantly affected BW, FCR, FI or FH_2O. There were no significant interactions of dietary Na and level of phytase supplementation. Regression analysis showed an overall estimate of 0.18±0.01% Na for BW and 0.19±0.01% Na for FCR. There was little indication that the Na requirement was affected by phytase. The level of phytase and Na in the diet had significant effects on excreta levels of P, Ca and Na. The use of lower dietary levels of NPP and Ca in association with the addition of the phytase resulted in significant reduction in excreta levels of these minerals, but had no significant effect on levels of excreted Na. Increasing the dietary Na level significantly affected the levels of all three minerals in the excreta. Although the dietary Na level had significant effects on both excreta P and Ca, these followed no consistent trends with no significant difference in Ca or P excretion between chicks fed diets with the lowest and highest Na levels. There were significant interactions between dietary Na levels and levels of phytase supplementation for all three minerals in the excreta. However, these did not appear to follow any consistent pattern. While it is apparent that phytase influences the metabolism of Na within the body, the data from the present study suggests that this has little impact on the dietary need for Na.

Key words: Broilers, sodium, phytase, excretion, environment

INTRODUCTION

Phytic acid in its native state in feed ingredients is complexed with various nutrients including proteins, lipids and minerals (Cosgrove, 1966). Exogenous phytase enzymes are known to release not only phosphorus bound by the phytase molecule but also other nutrients including calcium and amino acids (Ravindran *et al*., 2006). Recent work has indicated that phytase enzymes may influence Na metabolism in the chick (Cowieson *et al*., 2004; Ravindran *et al*., 2006; Selle *et al*., 2009). While Na is not an expensive nutrient in itself, a premium is placed upon space in the diet. In addition, producers are concerned about litter moisture and excessive levels of Na in the diet are not desirable from this standpoint. As the cost of supplemental phytase has been reduced in recent years, coupled with the great increase in cost of phosphorus-rich feed ingredients such as animal proteins, there is great interest in using higher levels of phytase in broiler diets, as studies have demonstrated further improvement in P release as phytase levels increase (Shirley and Edwards, 2003). Therefore this study was conducted to evaluate the use of phytase enzyme fed at "normal" and "super" levels on the need for supplemental sodium in broiler diets.

MATERIALS AND METHODS

Dietary treatments: A corn-soybean meal diet adequate in all known nutrients (NRC, 1994) was used as the basal diet. By variation of the amounts of ground

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Table 1: Composition (g/kg) and Calculated (C) and Analyzed (A) nutrient content of basal diets with different levels of Quantum 5000 XT phytase

	No phytase		500 FTU/kg		2000 FTU/kg	
Ingredient	A	B	C	D	G	H
Yellow corn	547.43	Same for All Diets				
Soybean meal	374.36					
Poultry oil	35.16					
MHA-84[1]	1.98					
L-Lysine HCl	1.03					
Vitamin premix[2]	5.00					
Mintrex P_Se[3]	1.00					
Sodium chloride	1.67					
Limestone	7.03					
Dicalcium phosphate	5.34					
Limestone	0.57	0.57	1.07	1.07	1.57	1.57
Dicalcium phosphate	11.78	11.78	6.37	6.37	0.97	0.97
Sodium bicarbonate	7.65	0.00	7.65	0.00	7.68	0.00
Sand	0.00	7.65	4.91	12.56	9.78	17.46
Calcium % (C)	0.90	0.90	0.80	0.80	0.70	0.70
Calcium % (A)	0.88	0.92	0.82	0.77	0.72	0.68
Total P % (C)	0.76	0.76	0.66	0.66	0.56	0.56
Total P % (A)	0.78	0.74	0.66	0.63	0.51	0.56
Nonphytate P %	0.45	0.45	0.35	0.35	0.25	0.25
Sodium % (C)	0.30	0.09	0.30	0.09	0.30	0.09
Sodium % (A)	0.28	0.07	0.29	0.11	0.31	0.10
Chloride %	0.15	0.15	0.15	0.15	0.15	0.15
Crude protein % (C)	22.00	Same for All Diets				
Crude protein % (A)	22.57					
Methionine %	0.55					
Lysine %	1.30					
TSAA %	0.90					
ME kcal/lb	1400.00					

[1]Methionine hydroxy analogue calcium salt. Novus International, St. Louis MO 63141.
[2]Provides per kg of diet: vitamin A 7715 IU; cholecalciferol 5511 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.
[3]Provides per kg of diet: Mn (as manganese methionine hydroxy analogue complex) 40 mg; Zn (as zinc methionine hydroxy analogue complex) 40 mg; Cu (as copper methionine hydroxy analogue complex) 20 mg; Se (as selenium yeast) 0.3 mg. Novus International, Inc., St. Louis MO 63141

limestone, dicalcium phosphate, sodium chloride, sodium bicarbonate and washed builders sand the levels of sodium, calcium and Nonphytate P (NPP) were modified. The changes in NPP were based on the assumption that phytase would be releasing 0.10% P with 1x usage and 0.20% for 4x usage of phytase, based on previous studies from our laboratory (Karimi *et al.*, 2011). The recommended usage rate of the phytase product (Quantum Phytase 5000 XT; ABVista, Marlborough, UK) is 500 FTU per kg. One phytase unit is equal to the amount of enzyme required to release 1 μmol of inorganic phosphate from sodium phytate at 37°C and pH 5.5. Calcium levels were reduced by 0.10% with 1x phytase usage and 0.20% with 4x phytase usage levels. Diets were then prepared with one series containing a level of Na chloride sufficient to provide 0.15% chloride and the other series prepared to contain 0.30% Na with 0.15% chloride using Na bicarbonate to provide the additional Na. Sodium bicarbonate has been shown to be equal to Na chloride as a source of Na (Murakami *et al.*, 1997a). Intermediate levels of Na were obtained by blending the highest and lowest Na levels within each phytase level. The composition of the experimental diets is shown in Table 1.

The high and low diets were blended within each series to provide the following estimated levels of Na: 0.10, 0.13, 0.16, 0.19, 0.22, 0.25 and 0.28%. This resulted in a total of 21 dietary treatments. Each of these was fed to 6 replicate pens of five male chicks. The test diets and tap water were provided for *ad libitum* consumption.

Male chicks of a commercial broiler strain (Cobb 500) were obtained from a local hatchery where they had been vaccinated *in ovo* for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Five chicks were assigned to each of 126 compartments in electrically heated battery brooders with wire floors. Fluorescent lights provided 24 h of light daily. Care and management of the birds followed recommended guidelines (FASS, 2010). All procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Measurements were made of pen body weight at one and 18 d of age. Feed consumption during the period was determined. At 16 d of age excreta samples were collected from each pen on aluminum foil placed in an area with minimum contamination by spilled feed. The samples were frozen and then freeze dried for determination of moisture content. Representative samples of the excreta were ground and subjected to analysis for Na, phosphorus and calcium by a commercial laboratory specializing in these assays. The high and low diets within each series were analyzed for crude protein, calcium, total P and Na by a commercial laboratory specializing in these assays.

Pen means served as the experimental unit for statistical analysis. Data were subjected to ANOVA as a factorial arrangement of treatments using the General Linear Models procedure of the SAS Institute (1991). When significant differences among treatments were found, means were separated using repeated t-tests using the LSMEANS option of the GLM procedure. Main effects of phytase level and Na level were examined along with the interaction of the two main effects. Mortality data were transformed to $\sqrt{n+1}$ prior to analysis; data are presented as natural numbers. Statements of statistical significance are based on $p \le 0.05$, although p values up to ≤ 0.10 are discussed if the data suggests a trend. Following the ANOVA, nonlinear regression analysis was conducted using the PROC LIN procedure of SAS incorporating the SAS macro of Robbins (1986). The requirement was established as the inflection point of the one-slope regression model (Robbins *et al.*, 1979; Yu and Morris, 1999; Waldroup *et al.*, 2000). Estimates of Na requirement for body weight and feed conversion were made for each level of phytase supplementation as well as over all phytase levels.

RESULTS AND DISCUSSION

Analyzed levels of crude protein, total P, calcium and Na in the feed were in close agreement with calculated values (Table 1). There were no significant differences in body weight gain, feed conversion, feed intake, fecal moisture, or mortality of male broilers fed the diets with the different levels of phytase (Table 2). This indicates that the dietary adjustment in NPP and Ca levels from the addition of the phytase (0.10% NPP and 0.10% Ca for the 1x level and 0.20% NPP and 0.20% for the 4x levels) did not adversely affect performance of the birds. The dietary Na level significantly affected body weight gain, feed conversion, feed intake and fecal moisture (Table 2). There were no significant interactions of dietary Na and level of phytase supplementation. Regression analysis of the data (Table 3) showed an overall estimate of 0.18±0.01% Na for body weight and 0.19±0.01% Na for feed conversion. Estimates were made for Na requirements at each level of phytase supplementation, but there was little indication that it was markedly affected by the phytase supplement (Table 3).

The level of phytase supplementation and Na level of the diet had significant effects on excreta levels of phosphorus, calcium and Na (Table 4). As would be expected, the use of lower dietary levels of NPP and calcium in association with the addition of the phytase resulted in significant reduction in excreta levels of these minerals, but had no significant effect on levels of excreted Na. Increasing the dietary Na level significantly affected the levels of all three minerals in the excreta, with the increase in excreta Na following a linear trend, as would be expected (Table 4). Although the dietary Na level had significant effects on both excreta phosphorus and calcium, these followed no consistent trends with no significant difference in calcium or phosphorus excretion between chicks fed diets with the lowest (0.10%) and highest (0.28%) Na levels. There were significant interactions between dietary Na levels and levels of phytase supplementation for all three minerals in the excreta. However, these did not appear to follow any consistent pattern (Fig. 1, 2 and 3).

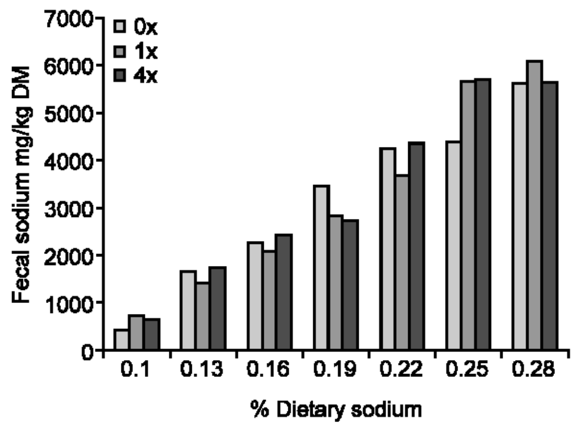

Fig. 1: Effect of dietary sodium and phytase supplementation on fecal sodium output

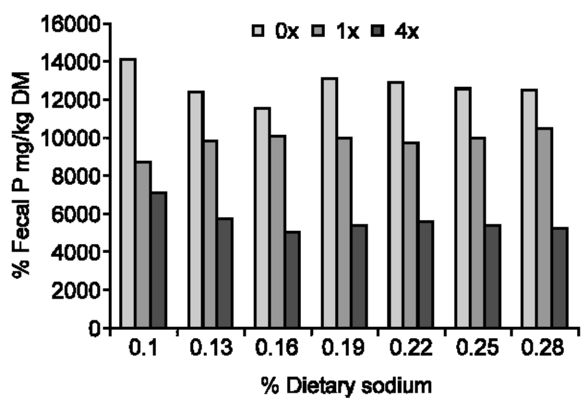

Fig. 2: Effect of dietary sodium and phytase supplementation on fecal phosphorus output

Table 2: Effects of different levels of phytase and sodium from sodium bicarbonate on live performance of male broilers when diets are adjusted in nonphytate P and calcium based on phytase supplementation levels

		0-18 d BW gain (kg)	0-18 d FCR (kg/kg)	0-18 d Feed intake (kg/bird)	Fecal Moisture (%)	Mortality (%)
Phytase[1]						
0		0.597	1.524	0.906	72.31	2.67
1x		0.599	1.527	0.905	72.91	1.52
4x		0.617	1.482	0.911	74.06	1.43
Sodium						
0.10		0.508[c]	1.669[a]	0.859[b]	70.92[c]	1.10
0.13		0.588[b]	1.554[b]	0.912[a]	71.16[bc]	0.00
0.16		0.619[ab]	1.479[c]	0.940[a]	71.55[bc]	1.30
0.19		0.630[a]	1.469[c]	0.917[a]	72.98[bc]	3.30
0.22		0.631[a]	1.447[c]	0.913[a]	74.52[ab]	2.20
0.25		0.630[a]	1.442[c]	0.919[a]	76.56[a]	1.10
0.28		0.626[a]	1.438[c]	0.893[ab]	74.23[abc]	4.00
Phytase	**% Na**					
0	0.10	0.490	1.705	0.857	70.27	3.30
0	0.13	0.566	1.613	0.922	70.83	0.00
0	0.16	0.627	1.497	0.939	72.34	0.00
0	0.19	0.617	1.491	0.920	71.98	0.00
0	0.22	0.617	1.502	0.925	73.04	0.00
0	0.25	0.622	1.447	0.917	76.59	3.30
0	0.28	0.638	1.400	0.865	71.13	12.00
1	0.10	0.511	1.662	0.858	71.08	0.00
1	0.13	0.576	1.526	0.877	68.55	0.00
1	0.16	0.588	1.473	0.949	68.93	4.00
1	0.19	0.638	1.473	0.919	72.91	6.70
1	0.22	0.650	1.418	0.961	76.29	0.00
1	0.25	0.625	1.421	0.906	76.28	0.00
1	0.28	0.607	1.429	0.865	76.32	0.00
4	0.10	0.525	1.641	0.861	71.31	0.00
4	0.13	0.619	1.522	0.940	74.05	0.00
4	0.16	0.637	1.466	0.934	72.93	0.00
4	0.19	0.633	1.442	0.911	74.04	3.30
4	0.22	0.626	1.422	0.854	74.23	6.70
4	0.25	0.643	1.458	0.935	76.81	0.00
4	0.28	0.633	1.485	0.940	75.08	0.00
P Diff						
Phytase		0.14	0.170	0.92	0.92	0.72
Sodium		0.0001	0.001	0.01	0.01	0.65
Phytase x Sodium		0.77	0.230	0.12	0.67	0.07
CV		8.18	7.910	6.84	17.18	3.08

[1]1x = 500; 4x = 2,000 FTU/kg from Quantum phytase.
[abc]Means in column with common superscripts do not differ significantly ($p \le 0.05$)

Table 3: Estimate of sodium requirement of broilers as influenced by dietary phytase level[1]

Parameter	Phytase level	Estimate % Na	SE	95% Confidence limits Lower	95% Confidence limits Upper
18 d Body weight	0	0.17	0.02	0.12	0.23
	1	0.23	0.02	0.17	0.29
	4	0.15	0.01	0.12	0.18
	All	0.18	0.01	0.16	0.20
0-18 d FCR	0	0.16	0.03	0.06	0.26
	1	0.26	0.16	-0.24	0.76
	4	0.22	0.02	0.16	0.29
	All	0.19	0.01	0.16	0.21

[1]The requirement was established as the inflection point of the one-slope regression model (Robbins *et al*., 1979; Yu and Morris, 1999; Waldroup *et al*., 2000)

Results from this study are in agreement with previous work from our laboratory on Na requirement of the young broiler chick. Watkins *et al*. (2005) reported that the requirement of Na for body weight was 0.17±0.006% with a 95% confidence interval between 0.14 and 0.19%. Studies by Murakami *et al*. (1997a; 1997b; 2000)

Table 4: Effects of different phytase and sodium levels on fecal mineral content from male broilers when diets are adjusted in nonphytate P and calcium based on phytase supplementation levels

		Total P mg/kg	Calcium mg/kg	Sodium mg/kg
Phytase[1]				
0		12,888^{a}	13,447^{a}	3,162
1x		9,846^{b}	12,935^{a}	3,227
4x		5,661^{c}	9,434^{b}	3,328
Sodium				
0.10		10,003^{a}	12,485^{ab}	605^{g}
0.13		9,345^{ab}	11,681^{b}	1,615^{f}
0.16		8,910^{b}	10,369^{c}	2,273^{e}
0.19		9,495^{ab}	11,386^{bc}	3,018^{d}
0.22		9,419^{ab}	11,984^{b}	4,113^{c}
0.25		9,330^{ab}	13,519^{a}	5,259^{b}
0.28		9,755^{a}	12,149^{b}	5,791^{a}
Phytase	**% Na**			
0	0.10	14,143^{a}	14,491^{ab}	438^{i}
0	0.13	12,427^{cd}	11,721^{def}	1,664^{h}
0	0.16	11,558^{d}	11,757^{def}	2,270^{f}
0	0.19	13,097^{ab}	14,227^{b}	3,471^{d}
0	0.22	12,909^{bc}	14,001^{bc}	4,264^{c}
0	0.25	12,604^{bcd}	13,414^{bcd}	4,392^{c}
0	0.28	12,482^{cd}	14,520^{ab}	5,637^{b}
1	0.10	8,739^{f}	12,009^{cdef}	728^{i}
1	0.13	9,858^{e}	12,807^{bcde}	1,439^{h}
1	0.16	10,100^{e}	11,938^{cdef}	2,109^{fg}
1	0.19	9,978^{e}	11,481^{def}	2,848^{e}
1	0.22	9,767^{ef}	12,640^{bcdef}	3,706^{d}
1	0.25	9,984^{e}	16,563^{a}	5,671^{ab}
1	0.28	10,497^{e}	13,111^{bcd}	6,093^{a}
4	0.10	7,126^{g}	10,955^{efg}	651^{i}
4	0.13	5,750^{h}	10,517^{fgh}	1,744^{gh}
4	0.16	5,073^{h}	7,412^{i}	2,441^{ef}
4	0.19	5,410^{h}	8,452^{hi}	2,734^{e}
4	0.22	5,583^{h}	9,312^{ghi}	4,369^{c}
4	0.25	5,401^{h}	10,579^{fgh}	5,715^{ab}
4	0.28	5,286^{h}	8,816^{ghi}	5,643^{b}
P Diff				
Phytase		0.001	0.001	0.13
Sodium		0.05	0.002	0.001
Phytase x Sodium		0.003	0.01	0.001
CV		371.7	732.9	147.3

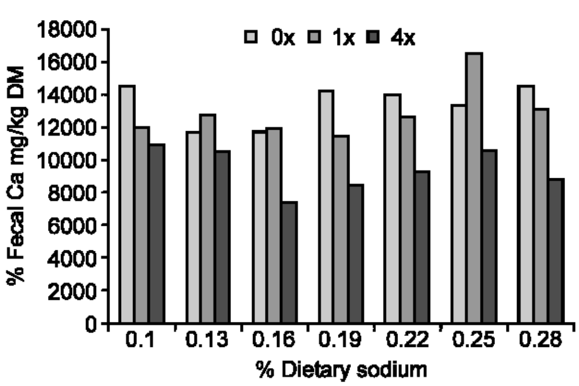

Fig. 3: Effect of dietary sodium and phytase on fecal calcium output

suggest a slightly greater need for Na during this period of 0.20-0.25%. The NRC (1994) recommends a minimum Na level of 0.20% and 0.20% Cl.

Cowieson *et al.* (2004) reported that ingestion of *myo*-inositol hexaphosphate (IP_6) by broilers increased the excretion of endogenous Na compared to birds fed only glucose, while the addition of supplemental phytase reduced the excretion of Na in birds fed IP_6. Ravindran *et al.* (2006) reported that phytase addition significantly improved the apparent ileal digestibility of Na in birds fed a corn-soybean meal diet with apparent improvement as the level of phytase increased from 500 to 1000 FTU/kg of diet. Selle *et al.* (2009) reported that phytase significantly increased the apparent ileal digestibility coefficient of Na in a wheat-based diet to -0.038 from -0.516 in the negative control diet and the combination of phytase and xylanase increase the same coefficient to 0.043. While it is apparent that phytate and phytase influence the metabolism of Na within the body, the data from the present study suggests that this has little impact on the dietary need for Na. This response may have been influenced by the use of Na bicarbonate as the Na source; while it has been found to be equivalent to Na chloride as a source of Na (Murakami *et al.*, 1997a) the buffering properties of the bicarbonate in the intestinal tract may have influenced the relationship between phytase and Na that has been demonstrated in the intestinal tract. Further studies are suggested using NaCl, the more commonly used form of Na supplement in broiler feeds.

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