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Effects of 500 and 1000 FTU/kg phytase supplementation of maize-based diets with two tiers of nutrient specifications on performance of broiler chickens

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ABSTRACT

Starter, grower and finisher maize-based diets (Diets A) were formulated to marginal nutrient specifications and offered to broilers from 1 to 14, 15 to 27, and 28 to 40 days post-hatch, respectively. Nutrient specifications were reduced (Diets B) and offered to broilers without and with 500 FTU/kg phytase; specifications were further reduced to create a second tier of reduced nutrient specifications (Diets C) without and with 1000 FTU/kg phytase. The study was conducted using 240 male Ross 308 chicks with each of the 5 treatments consisting of 8 replicates of 6 birds per replicate. Growth performance was monitored during each phase of the grow-out period, mineral retentions were determined in the grower phase and nutrient utilisation in the finisher phase. Apparent digestibility coefficients of nitrogen were determined in the proximal jejunum, distal jejunum, proximal ileum and distal ileum in broilers at 40 days post-hatch. In addition, N digestion rates ($K_{nitrogen}$) were determined. Over the 40-day feeding period, declining nutrient specifications decreased weight gains in broilers offered non-supplemented diets from 2721 (Diets A) to 2627 (Diets B) and 2525 g/bird (Diets C) and increased FCR from 1.551 to 1.577 and 1.605 in the corresponding diets. The differences in weight gain (7.20%) and FCR (3.48%) between Diets A and Diets C were significant ($P < 0.05$). From 1 to 40 days post-hatch, phytase improved weight gain in broilers offered diets B by 11.8% ($P < 0.001$) and those offered diets C by 13.4% ($P < 0.001$) and both treatments outperformed diets A ($P < 0.001$). Phytase enhanced FCR in chicks offered diets C by 3.80% (1.544 versus 1.605, $P < 0.05$). Phytase supplementation of diets B increased AMEn by 0.23 MJ ($P < 0.05$). Phytase supplementation of diets C increased AME by 0.41 MJ ($P < 0.001$), AME:GE ratios by 2.62% ($P < 0.001$) and N retention by 5.4 percentage units ($P < 0.001$). Phytase enhanced retentions of calcium, phosphorus, and sodium in both diets B and C ($P < 0.001$). Phytase supplementation of diets B improved ($P < 0.01$) apparent digestibility coefficients of nitrogen at four small intestinal sites with increases ranging from 79.9% (proximal jejunum) to 11.3% (distal ileum) culminating in an increase ($P < 0.01$) in N digestion rates ($K_{nitrogen}$) of 64% from 2.59 to 4.24 $\times 10^{-2}$ min^{-1}. Phytase supplementation

Abbreviations: AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; AIA, acid insoluble ash; FCR, feed conversion ratio; MRT, mean retention time; N, nitrogen; NC, negative control; P, phosphorus; PC, positive control; PDN, potential digestible nitrogen.

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of diets C increased N digestibility in the distal ileum by 7.63% ($P < 0.001$) and N digestion rates by 35% from 3.65 to 4.91 $\times 10^{-2}$ min^{-1} ($P < 0.01$). In conclusion, standard (500 FTU/kg) and elevated (1000 FTU/kg) phytase inclusions in diets with reduced nutrient specifications have the capacity to enhance performance of broilers and compensate for these reductions.

1. Introduction

Phytate and phytate-bound phosphorus (P) is invariably present in practical poultry diets which limits P bioavailability and poses ecological problems as excreted P pollutes the environment. Phytate-degrading feed enzymes or phytases have been included in broiler diets for more than 20 years primarily to liberate phytate-bound P and to harness the 'extra-phosphoric' effects as phytase has shown to improve protein and energy utilisation (Ravindran, 1995; Selle and Ravindran, 2007). The original phytase feed enzyme was of fungal origin (*Aspergillus niger*) and its capacity to reduce P excretion was reported by Simons et al. (1990); subsequently phytases of bacterial origin were introduced. The capacity of phytase to improve P (and Ca) digestibility in broiler chickens has long been recognised but the capability of phytase to enhance amino acid digestibilities and energy utilisation has been debated (Adeola and Sands, 2006; Selle and Ravindran, 2007; Selle et al., 2012). However, the remarkable surge in acceptance of phytase feed enzymes by the chicken-meat industry globally over the last 15 years reflects escalating prices for inorganic P supplements and feedstuffs in general coupled with reducing phytase inclusion costs. More recently a *Buttiauxella* sp. phytase expressed in *Trichoderma reesei* (Yu et al., 2014) has been shown to improve the digestibility of P and amino acids in broilers offered maize-based diets at an inclusion level of 1000 FTU/kg (Amerah et al., 2014); moreover, unequivocal increases in amino acid digestibilities were correlated to extents of phytate degradation over a range of dietary Ca:available P ratios.

From the outset it was recommended practice to reduce P and Ca specifications in phytase-supplemented diets to take advantage of the liberation of these macro-minerals from phytate and mineral–phytate complexes by the phytate-degrading feed enzyme. Reductions in amino acid concentrations and energy densities have also been recommended (Selle and Ravindran, 2007). Recently, reductions in sodium specifications have been suggested because phytase improves ileal sodium digestibility (Ravindran et al., 2006, 2008; Selle et al., 2009). Moreover, this impact may be a mechanism whereby phytase enhances absorption of glucose and amino acids from the small intestine via Na^+-dependent transport systems and Na^+, K^+-ATPase activity. Initially, phytase was typically included at 500 FTU/kg but presently consideration is being given to higher inclusion rates (Cowieson et al., 2011). Thus, the objective of this experiment was to offer broilers diets with two tiers of reduced nutrient specifications supplemented with either 500 or 1000 FTU/kg phytase to determine the extent to which phytase is capable of compensating for reduced nutrient specifications and recovering broiler performance relative to a control diet.

2. Materials and methods

2.1. Diet preparation

The feeding study comprised five sets of starter, grower and finisher diets with two tiers of reduced nutrient specifications for phosphorus, calcium, sodium, energy and certain amino acids (lysine, methionine, methionine + cysteine, threonine, arginine), without and with a *Buttiauxella* phytase expressed in *T. reesei* (Axtra® PHY; Danisco Animal Nutrition) at 500 and 1000 FTU/kg. The dietary formulations and calculated nutrient specifications of Diets A, B and C are shown in Table 1. Each of the dietary treatments was offered to 8 replicates of 6 birds per replicate or a total of 40 cages and 240 birds. Day-old, male (feather-sexed) Ross 308 chicks were individually identified (wing-tags) and allocated into bioassay cages on the basis of bodyweight across 40 cages. The birds were offered starter diets from 1 to 14 days post-hatch, grower diets from 15 to 28 days post-hatch and finisher diets from 29 to 40 days post-hatch. The starter diets were fed as mash; whereas, the grower and finisher diets were steam-pelleted through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) at a conditioning temperature of 84 °C by the automatically controlled introduction of steam into the conditioner with a residence time of 14 s. Finally, the pelleted diets were cooled in a vertical cooler to room temperature and crumbled. Maize was hammer-milled (3.2 mm screen) prior to dietary incorporation. Phytase was supplied as liquid formulations that were either mixed into the mash diets or sprayed onto the pelleted diets post-pelleting at the appropriate rates. Analysed gross energy, protein, dry matter, fat, calcium, phosphorus, sodium concentrations and phytase activity in grower and finisher diets are shown in Table 2. Acid insoluble ash (Celite™ World Minerals, Lompoc, CA, USA) was included in finisher diets at 20 g/kg as an inert marker to determine apparent nitrogen digestibility coefficients at four small intestinal sites.

2.2. Bird management

This feeding study complied with specific guidelines approved by the Animal Ethics Committee of the University of Sydney. The birds were housed in an environmentally controlled facility with unlimited access to feed and water under a

Table 1
Dietary formulations and calculated nutrient specifications in starter (1–14 days post-hatch), grower (15–27 days post-hatch) and finisher (28–40 days post-hatch) diets.

Ingredients (g/kg)	Starter			Grower			Finisher		
	A[a]	B[a]	C[a]	A	B	C	A	B	C
Maize	592.5	606.2	618.9	630.6	642.2	654.4	645.9	686.0	703.9
Soybean meal	361.1	354.5	354.2	286.0	281.0	274.0	268.6	243.1	226.9
Vegetable-fat blend	9.4	3.7	1.0	27.6	23.0	19.5	33.5	23.2	20.1
Lysine HCl	3.0	3.0	2.0	3.4	3.5	3.5	2.4	3.2	3.4
Methionine	3.2	3.1	2.8	3.0	2.9	2.9	2.2	2.3	2.2
Threonine	1.1	1.1	0.7	1.3	1.3	1.3	0.6	0.8	0.9
Celite™	–	–	–	20.0	20.0	20.0	20.0	20.0	20.0
Sodium bicarbonate	1.4	0.3	2.8	1.2	0.8	0.4	0.4	0.1	0.0
Sodium chloride	3.0	3.0	1.0	3.0	3.0	3.0	3.0	3.0	2.8
Limestone	15.1	15.1	9.3	11.7	12.2	12.5	11.1	9.0	12.0
Dicalcium phosphate	8.2	6.2	4.5	10.1	8.1	6.6	9.2	7.3	5.8
Vitamin-mineral premix[b]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
ME (MJ/kg)	12.35	12.29	12.26	12.78	12.72	12.47	12.99	12.92	12.89
Protein	224.80	223.33	222.41	194.89	193.70	191.17	186.09	177.95	171.88
Fat	37.86	32.58	29.99	56.17	52.02	4.90	62.43	53.40	50.74
Fibre	25.12	25.23	25.65	23.25	23.33	23.33	22.99	22.91	22.72
Calcium	9.09	8.59	6.06	7.98	7.66	7.41	7.51	6.16	6.90
Total phosphorus	5.47	5.11	4.87	5.40	5.04	4.74	5.17	4.75	4.43
Phytate phosphorus	2.59	2.59	2.65	2.34	2.34	2.33	2.29	2.26	2.02
Non-phytate phosphorus	2.88	2.52	2.22	3.06	2.70	2.42	2.88	2.50	2.21
Lysine	14.61	14.52	14.15	12.73	12.64	12.47	11.47	11.36	11.14
Methionine	6.60	6.54	6.35	5.95	5.89	5.78	5.11	5.11	4.94
Methionine + cysteine	10.32	10.24	10.15	9.17	9.10	8.96	8.24	8.12	7.87
Threonine	9.67	9.59	9.52	8.56	8.49	8.36	7.54	7.44	7.25
Tryptophan	2.61	2.59	2.67	2.15	2.13	2.09	2.05	1.92	1.83
Arginine	15.70	15.55	16.05	12.98	12.87	12.65	12.43	11.64	11.12
Sodium	1.90	1.59	1.48	1.80	1.70	1.59	1.60	1.50	1.39
Potassium	9.97	9.89	10.18	8.37	8.31	8.19	8.05	7.61	7.32
Chloride	2.73	2.74	1.32	2.80	2.80	2.81	2.61	2.76	2.67

[a] Positive control (PC), negative control 1 (NC1) and negative control 2 (NC2).
[b] The vitamin-mineral premix supplied per tonne of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.

Table 2
Analysed gross energy, protein, dry matter, fat, calcium, phosphorus, sodium concentrations and phytase activity in grower and finisher diets.

Diet type	Formulated phytase (FTU/kg)	Gross energy (MJ/kg)	Crude protein (g/kg)	Fat (g/kg)	Ca (g/kg)	P (g/kg)	Na (g/kg)	Analysed phytase (FTU/kg)
Grower								
A	0	18.75	226.4	63	8.59	6.06	1.74	<50
B	0	18.77	217.9	57	8.75	5.88	1.61	<50
B	500	18.40	222.0	58	9.40	5.65	1.74	559
C	0	18.61	207.1	55	8.55	5.66	1.52	<50
C	1000	18.40	211.9	54	8.40	5.54	1.38	1089
Finisher								
A	0	18.93	210.8	67	6.88	5.58	1.54	78
B	0	18.55	198.4	59	6.20	5.29	1.43	113
B	500	18.55	201.9	60	6.24	5.23	1.42	769
C	0	18.37	188.5	53	6.07	4.81	1.22	79
C	1000	18.39	199.4	52	6.50	4.97	1.32	966

light regime of 23 light:1 dark. Temperature and humidity were monitored continuously over the 40-days feeding period. The average temperature was 25.4 °C and relative humidity was 52.5%. An initial room temperature of 32 ± 1 °C was maintained for the first week, gradually decreased to 22 ± 1 °C by the end of the third week and maintained at the same temperature until the end of the feeding study. Initial and final body weights were determined for each feeding phase; feed intakes were recorded from which feed conversion ratios (FCR) were calculated. The incidence of dead or culled birds was recorded daily and their body-weights were used to adjust FCR calculations.

2.3. Sample collection and chemical analysis

Excreta samples were collected from 24–26 days post-hatch to determine mineral retentions. The concentrations of nine minerals (Ca, P, Cu, Fe, K, Mg, Mn, Na and Zn) in the diets and excreta were analysed by plasma mass spectrometry

(Truong et al., 2014). Feed intakes were recorded and total excreta collected from 37 to 39 days post-hatch to generate data for parameters of nutrient utilisation [apparent metabolisable energy (AME; MJ/kg and AME:GE ratios), N retention, N-corrected AME (AME_n)] on a dry matter basis in finisher diets. Excreta were air-forced oven dried for 24 h at 80 °C. The gross energy (GE) of diets and excreta were determined by bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL) (Liu et al., 2014). Fat retention was also determined in finisher phase by using the automated Soxhlet extraction as described by Luque de Castro and Priego-Capote (2010).

At day 40, all birds were euthanised by intravenous injection of sodium pentobarbitone, the small intestine was removed and digesta samples were collected in their entirety from the proximal jejunum, distal jejunum, proximal ileum and distal ileum. These segments were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction and their mid-points. Feed intakes over the final 24 h prior to sampling were recorded. Digesta samples from birds within each cage were pooled, homogenised, freeze-dried and weighed to determine mean retention time (MRT) and apparent digestibility of nitrogen as described by Gutierrez del Alamo et al. (2009). Nitrogen concentrations and AIA concentrations were determined as outlined by Siriwan et al. (1993). Left tibias from each bird in a pen were collected and pooled on a cage basis for determination of bone ash and mineral concentrations. The composite samples were dried to a constant weight at 100 °C and ashed in a muffle furnace at 550 °C for 16 h (Ravindran et al., 1995). The ash content was weighed and analysed for mineral concentrations by plasma mass spectrometry.

2.4. Calculations and statistical analysis

Apparent metabolisable energy was calculated using standard procedures by the following equation:

$$AME_{diet} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{(\text{Feed intake})}$$

N-corrected AME (AMEn MJ/kg) values were calculated by correcting to zero N retention by applying the factor of 36.54 kJ/g N retained in the body (Hill and Anderson, 1958).

Mineral, nitrogen and fat retentions were calculated by the following equation:

$$\text{Retention}(\%) = \frac{(\text{Feed intake} \times \text{Nutrient}_{diet}) - (\text{Excreta output} \times \text{Nutrient}_{excreta})}{(\text{Feed intake} \times \text{Nutrient}_{diet})} \times 100$$

Apparent digestibility coefficients of nitrogen were calculated by the following equation:

$$\text{Digestibility coeffienient} = \frac{(\text{Nutrient/AIA})_{diet} - (\text{Nutrient/AIA})_{digesta}}{(\text{Nutrient/AIA})_{diet}}$$

Digestible protein was calculated by the following equation:

$$\text{Digestible protein} = \text{Digestibility coefficients} \times \text{Dietary protein concentrations}$$

Mean retention time was calculated using the following equation:

$$MRT\ \ (\text{min}) = (1440 \times AIA_{digesta} \times W)/(FI_{24h} \times AIA_{feed})$$

where $AIA_{digesta}$ is the AIA concentration in the digesta (mg/g), W is the weight of dry gut content (g), FI_{24h} is the feed intake over 24 h before sampling (g), AIA_{feed} is the AIA concentration in the feed (mg/g) and 1440 equals minutes per day (Gutierrez del Alamo et al., 2009).

The pattern of fractional digestibility coefficients was described by relating the digestion coefficient at each site with the digestion time (t). The digestion time (t) was calculated from the sum of MRT determined in each intestinal segment. The curve of digestion was described by the exponential model developed by Orskov and McDonald (1979):

$$D_t = D_\infty(1 - e^{-kt})$$

where D_t (g/g starch or nitrogen) is the starch or nitrogen digested at time t (min), the fraction D_∞ is the amount of potential digestible nitrogen (asymptote) (g/g), k (per unit time, min^{-1}) is defined as digestion rate constant. This mathematical model was applied with the assumptions that amino acid absorption did not take place proximal to the small intestine.

One-way ANOVA was employed to determine the dietary treatment by a general linear model procedure using JMP® 9.0.0 (SAS Institute Inc. JMP Software, Cary, NC). The experimental units were pooled cage means and differences were considered significant at $P < 0.05$ by Students' t-test.

3. Results

The mortality rates during starter, grower and finisher phases were 0.42%, 1.06% and 2.91%, respectively, which were not influenced by dietary treatment ($P > 0.20$ on the basis of a one-way analysis of variance). Growth performance parameters across the three feeding phases are shown in Table 3. Based on feed intakes in each of the three phases, weighted mean specifications of Diet A were ME 12.85 MJ/kg, protein 193 g/kg, Ca 7.83 g/kg, total P 5.28 g/kg and Na 1.78 g/kg. The corresponding

Table 3
Effects of nutrient specifications and phytase supplementation of maize-based diets on growth performance in broiler chickens from 1–14 days, 1–27 days and 1–40 days post-hatch.

Diet type	Phytase (FTU/kg)	1–14 days post-hatch			1–27 days post-hatch			1–40 days post-hatch		
		Feed intake (g/bird)	Weight gain (g/bird)	FCR (g/g)	Feed intake (g/bird)	Weight gain (g/bird)	FCR (g/g)	Feed intake (g/bird)	Weight gain (g/bird)	FCR (g/g)
A	0	421	291	1.451	1879bc	1324bc	1.420b	4220b[c]	2721b	1.551b
B	0	412	273	1.518	1781c	1249cd	1.426b	4143c	2627bc	1.577ab
B	500	454	308	1.496	2057a	1470a	1.400bc	4556a	2937a	1.553b
C	0	411	265	1.562	1782c	1213d	1.470a	4052c	2525c	1.605a
C	1000	411	272	1.522	1914b	1392ab	1.376c	4421ab	2863a	1.544b
SEM[a]		12.4494	12.6370	0.0426	43.7472	33.3126	0.0122	71.3908	42.7335	0.0148
P-value		0.088	0.130	0.470	<0.001	<0.001	<0.001	<0.001	<0.001	0.039

abcd – means within columns without common letters are significantly different at the 5% level of probability.
[a] Pooled standard error of mean.

Table 4
Effects of nutrient specifications and phytase supplementation of maize-based diets on nutrient utilisation [AME, AMEn, N and fat retention] from 37 to 39 days post-hatch.

Diet type	Phytase (FTU/kg)	AME (MJ/kg)	AME:GE[a] (MJ/MJ)	AMEn (MJ/kg)	N retention (%)	Fat retention (%)
A	0	15.22a	0.804b	13.40a	68.7b	91.4
B	0	14.74b	0.795b	13.03c	68.0b	89.1
B	500	14.90b	0.803b	13.29ab	66.9b	90.1
C	0	14.75b	0.803b	13.16bc	69.1b	89.0
C	1000	15.16a	0.824a	13.18bc	74.5a	90.3
SEM[b]		0.0719	0.0039	0.0719	1.0375	0.6907
P-value		<0.001	<0.001	0.012	<0.001	0.115

abc – means within columns without common letters are significantly different at the 5% level of probability.
[a] AME:GE = apparent metabolisable energy (MJ/kg)/gross energy in the diet (MJ/kg).
[b] Pooled standard error of mean.

figures in Diet B were ME 12.79 MJ/kg, protein 188 g/kg, Ca 7.55 g/kg, total P 4.89 g/kg and Na 1.58 g/kg and in Diet C they were ME 12.68 MJ/kg, protein 184 g/kg, Ca 6.71 g/kg, total P 4.58 g/kg and Na 1.47 g/kg. The supplementation of 500 FTU/kg phytase to diets B significantly increased feed intake and weight gain by 15.5% (1781 versus 2057 g/bird, $P < 0.001$) and 17.7% (1249 versus 1470 g/bird, $P < 0.001$), respectively, from 1–27 days post-hatch. Also, from 1 to 40 days post-hatch, 500 FTU/kg phytase significantly increased feed intake and weight gain by 10.0% (4143 versus 4556 g/bird, $P < 0.001$) and 11.8% (2937 versus 2627 g/bird, $P < 0.001$), respectively. The addition of 1000 FTU/kg phytase to diets C significantly improved feed intake, weight gain and FCR from 1 to 27 days post-hatch ($P < 0.001$). From 1 to 40 days post-hatch, 1000 FTU/kg phytase in diets C significantly increased feed intake and weight gain by 13.4% (2863 versus 2252 g/bird, $P < 0.001$) and significantly improved FCR by 3.80% (1.544 versus 1.605, $P < 0.05$).

The effects of nutrient specifications and phytase supplementation on nutrient utilisation in broilers offered finisher diets are shown in Table 4. Reduced dietary nutrient specifications in diets B and C significantly compromised energy utilisation (AME and AMEn) in relation to diets A ($P < 0.05$). The addition of 500 FTU/kg phytase to diets B significantly increased AMEn by 0.26 MJ ($P < 0.05$). Addition of 1000 FTU/kg phytase to diets C significantly increased AME by 0.41 MJ ($P < 0.001$), AME:GE ratios by 2.62% (0.824 versus 0.803, $P < 0.001$) and N retention by 5.40 percentage units or 7.81% (74.5 versus 69.1%, $P < 0.001$).

The effects of phytase supplementation on mineral retention in grower diets are shown in Table 5, where Ca, P and Na are considered to be important. Reduced dietary nutrient specifications did not influence Ca and P retention but significantly increased Na retention ($P < 0.001$). The addition of 500 FTU/kg phytase to diets B significantly increased Ca retention by 12.0 percentage points (52.0 versus 64.0%, $P < 0.001$), P retention by 7.9 percentage points (58.1 versus 66.0, $P < 0.001$) and Na retention 6.9 percentage points (66.0 versus 72.0%, $P < 0.001$). The addition of 1000 FTU/kg phytase to diets C significantly

Table 5
Effects of nutrient specifications and phytase supplementation of maize-based diets on mineral retention (%) from 24 to 26 days post-hatch.

Diet type	Phytase	Ca	P	Cu	Fe	K	Mg	Mn	Na	Zn
A	0	50.1b	56.5c	10.0c	23.5d	31.3c	25.0c	8.5	60.5d	14.2b
B	0	52.0b	58.1c	23.6ab	25.1cd	34.8bc	29.5bc	17.5	66.0c	29.3a
B	500	64.0a	66.0b	30.7a	39.2ab	33.2bc	28.0bc	20.5	72.9b	31.3a
C	0	51.6b	59.4c	28.4ab	31.7bc	38.9ab	34.1ab	15.7	72.0b	17.6b
C	1000	67.7a	74.4a	20.5b	42.5a	42.7a	37.2a	15.7	80.5a	32.7a
SEM[a]		1.4684	1.2798	2.8482	2.7231	2.0163	2.3058	2.9128	1.7199	4.0309
P-value		<0.001	<0.001	<0.001	<0.001	0.002	0.005	0.080	<0.001	0.005

abcd – means within columns without common letters are significantly different at the 5% level of probability.
[a] Pooled standard error of mean.

Table 6
Effects of nutrient specifications and phytase supplementation of maize-based diets on ash content in tibia bones and mineral contents (Ca, P, Zn) in tibia ash at 40 days post-hatch.

Diet type	Phytase (FTU/kg)	Ash (%)	Ca (%)	P (%)	Zn (mg/kg)
A	0	41.4a	36.0ab	18.3b	324.4b
B	0	38.4b	35.7b	18.2b	325.2b
B	500	41.8a	35.8b	18.4ab	352.6a
C	0	38.9b	35.6b	18.2b	329.5b
C	1000	42.3a	36.3a	18.6a	355.7a
SEM[a]		0.5170	0.1486	0.0836	5.8233
P-value		<0.001	0.018	0.008	<0.001

ab – means within columns without common letters are significantly different at the 5% level of probability.
[a] Pooled standard error of mean.

Table 7
Effects of nutrient specifications and phytase supplementation of maize-based diets on mean retention time (minutes) in the proximal jejunum, distal jejunum, proximal ileum and distal ileum of broiler chickens at 40 days post-hatch.

Diet type	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total
A	0	16.5	24.3b	36.0b	39.0b	115.7b
B	0	22.8	38.5a	42.6ab	53.6a	157.5ab
B	500	29.0	40.7a	55.1a	61.6a	186.4a
C	0	24.4	39.1a	47.1ab	48.6ab	159.3ab
C	1000	27.7	27.5b	35.8b	37.8b	128.8b
SEM[a]		5.3030	3.6477	4.8242	4.8843	16.2652
P-value		0.499	0.009	0.045	0.010	0.041

ab – means within columns without common letters are significantly different at the 5% level of probability.
[a] Pooled standard error of mean.

increased Ca retention by 15.0 percentage points (51.6 versus 67.7%, $P < 0.001$), P retention by 12.0 percentage points (59.4 versus 74.4%, $P < 0.001$) and Na retention 8.5 percentage points (72.0 versus 80.5%, $P < 0.001$).

The effects of dietary treatments on tibial ash contents and tibial concentrations of Ca, P and Zn are shown in Table 6. Reduced dietary nutrient specifications significantly ($P < 0.0001$) depressed tibial ash (41.4 versus 38.7%) but did not influence Ca, P and Zn tibial concentrations. The addition of 500 FTU/kg phytase to diets B significantly increased tibia ash by 3.4 percentage points (38.4 versus 41.8%, $P < 0.001$) and Zn concentrations by 27.4 percentage points (325.2 versus 352.6 mg/kg, $P < 0.01$). The addition of 1000 FTU/kg phytase to diets C significantly increased tibia ash again by 3.4 percentage points (38.9 versus 42.3%, $P < 0.001$), Ca concentrations 0.7 percentage points (35.6 versus 36.3%, $P < 0.05$), P concentrations 0.4 percentage points (18.2 versus 18.6%, $P < 0.01$) and Zn concentrations by 26.2 percentage points (329.5 versus 355.7 mg/kg, $P < 0.001$).

The influence of dietary treatments on retention times in the proximal jejunum, distal jejunum, proximal ileum and distal ileum in broiler chickens are shown in Table 7. The general thrust of reduced dietary nutrient specifications was to increase retention times. This was most evident in the distal jejunum where retention time significantly ($P < 0.01$) increased from 24.3 min in diets A to 38.5 and 39.1 min in diets B and C, respectively. The addition of 500 FTU/kg phytase to diets B significantly increased retention time in the distal jejunum by 5.71% (38.5 versus 40.7 min, $P < 0.01$) and tended to increase retention times in the other small intestinal segments. Conversely, 1000 FTU/kg phytase in diets C significantly decreased retention time in the distal jejunum by 29.7% (27.5 versus 39.1 min, $P < 0.01$) and tended to decrease PI and DI retention times.

The effects of dietary treatments on apparent digestibility coefficients of N in four small intestinal segments, potential digestible N and N digestion rates are shown in Table 8. Reduced dietary nutrient specifications did not have tangible effects

Table 8
Effects of nutrient specifications and phytase supplementation of maize-based diets on digestive kinetics of protein [PDN, potential digestible nitrogen; $K_{nitrogen}$, nitrogen digestion rate], apparent digestibility coefficients of nitrogen and accumulated digestible protein in the proximal jejunum, distal jejunum, proximal ileum and distal ileum of broiler chickens at 40 days post-hatch.

Diet type	Phytase (FTU/kg)	Apparent digestibility coefficients				Accumulated digestible protein (g/kg)					
		Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	PDN (g/g)	$K_{nitrogen}$ ($\times 10^{-2}$ min^{-1})
A	0	0.461c	0.646cd	0.784ab	0.821b	97.2bc	136.2b	165.3a	173.1ab	0.829	3.90ab
B	0	0.379c	0.643d	0.674c	0.793bc	75.2c	127.5c	133.7c	157.3c	0.785	2.59c
B	500	0.682a	0.775a	0.854a	0.883a	137.8a	156.5a	174.4a	178.4a	0.847	4.24ab
C	0	0.495bc	0.682bc	0.759bc	0.773c	93.3c	128.6c	143.0bc	145.8d	0.774	3.65bc
C	1000	0.559ab	0.714b	0.797ab	0.832b	119.5ab	142.5b	159.0ab	165.8b	0.818	4.91a
SEM[a]		0.0418	0.0125	0.0308	0.014	8.379	2.565	6.104	2.700	0.0198	0.4021
P-value		<0.001	<0.001	0.007	<0.001	<0.001	<0.001	0.001	<0.001	0.077	0.007

abcd – means within columns without common letters are significantly different at the 5% level of probability.
[a] Pooled standard error of mean.

on these parameters. The addition of 500 FTU/kg phytase to diets B significantly increased N digestibility in the proximal jejunum by 79.9% (0.379 versus 0.682, $P < 0.001$), distal jejunum by 20.5% (0.643 versus 0.775, $P < 0.001$), proximal ileum by 26.7% (0.674 versus 0.854, $P < 0.01$) and distal ileum by 11.3% (0.793 versus 0.883, $P < 0.001$). Also, phytase significantly increased N digestion rates by a factor of 1.64 (2.59 versus 4.24×10^{-2} min^{-1}, $P < 0.01$). Significant effects following the addition of 1000 FTU/kg phytase to diets C were confined to an increase of 7.63% in distal ileal N digestibility coefficients (0.773 versus 0.832, $P < 0.001$) and an increase in N digestion rates by a factor of 1.35 (4.91 versus 3.65×10^{-2} min^{-1}, $P < 0.01$).

4. Discussion

The application of matrix values to phytase feed enzymes in the least-cost formulation of broiler diets is an accepted practice. Matrix values are usually assigned to P and Ca, arguably should be applied to Na, and may extend to amino acids and energy density. The magnitude of phytase matrix values is challenging and the situation is further complicated by dietary substrate levels and the option of including phytase at more than standard inclusion levels. However, the viability of this approach is reflected in the fact that birds offered B plus phytase diets had greater 40-day weight gains than diets A by 7.94% (2937 versus 2721 g/bird, $P < 0.001$). Similarly, birds offered C plus phytase diets had 5.22% greater weight gains (2863 versus 2721 g/bird, $P < 0.001$) than their Diets A counterparts.

Phytate (myo-inositol hexaphosphate; IP_6) is ubiquitous in feedstuffs of plant origin where phytate-bound P constitutes more than 60% of the total P content (Oatway et al., 2001). As phytase has the capacity to liberate phytate-bound P (Simons et al., 1990), it is not surprising that phytase supplementation in the present study significantly improved P retention in grower diets and increased P concentration in tibia ash. Moreover, phytate is a potent chelator of minerals, forming complexes with cations in the following descending order: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$ (Vohra et al., 1965). Although phytate has the least propensity to bind calcium, calcium-phytate complexes have a considerable impact due to high Ca concentrations in diets and digesta in the gut lumen (Dendougui and Schwedt, 2004). Ca retention was significantly increased by 23.1% following the inclusion of 500 FTU/kg phytase in diets B and 1000 FTU/kg phytase significantly increased Ca retention by 31.2% in diets C. P and Ca are critical minerals for bone development and also influence growth performance. In the present study, P retention was significantly correlated with weight gain ($r = 0.353$, $P < 0.03$) and FCR ($r = -0.551$, $P < 0.001$) across all five treatments (Fig. 1). Similarly, Ca retention was significantly correlated with weight gain ($r = 0.455$, $P < 0.005$) and FCR ($r = -0.556$, $P < 0.001$).

Considerable attention has been paid to the 'protein effect' of phytase in broiler chickens (Selle et al., 2000, 2006; Selle and Ravindran, 2007). Initially, protein and amino acid digestibility responses to phytase were ambiguous (Adeola and Sands, 2003); however, the transition from fungal to bacterial phytase is associated with more consistent and robust responses (Selle et al., 2012). The inclusion of 500 FTU/kg phytase in diets B unequivocally increased N digestibilities in four small intestinal segments ranging from 11.3% in the distal ileum (0.883 versus 0.793) to 79.9% (0.682 versus 0.379) in the proximal jejunum. Curiously, N digestibility responses to 1000 FTU/kg phytase in diets C were less pronounced. Nevertheless, both phytase inclusions significantly accelerated N digestion rates by 63.7% in diets B and by 34.5% in diets C. It is difficult to explain why N digestibility responses from phytase supplementation of diets B were more robust but it appears related to the fact that inherent N digestibility coefficients in the non-supplemented diets were generally higher in birds offered diets C. In the distal jejunum, N digestibility coefficients were significantly higher in non-supplemented diets C than diets B by 6.07% (0.682 versus 0.643). It is less likely that the variations in phytase efficacy are related to the difference of protein concentrations in non-supplemented diets B and C (198.4 versus 188.5 g/kg protein). On the digestible protein basis (Table 8), the average improvement of 500 FTU/kg in diets B was 37.5% in the four small intestine segments with the most pronounced 83.2% improvement in the proximal jejunum (75.2 versus 137.8 g/kg); however, the average improvement of 1000 FTU/kg in diets C was 16.0% and the corresponding improvement in the proximal jejunum was 28.1% (93.3 versus 119.5 g/kg).

It is noteworthy that both phytase supplementation levels significantly increased Na retention by an average of 7.7 percentage units (76.7 versus 69.0%). Moreover, Na retention was correlated with FCR ($r = -0.434$, $P < 0.005$) and apparent N digestibility coefficients in the proximal ($r = 0.462$, $P < 0.05$) and distal jejunum ($r = 0.491$, $P < 0.01$) across all five treatments. Phytase has been shown to improve ileal Na digestibility coefficients in broilers or, in the main, reduce the extent to which these values are negative (Ravindran et al., 2006, 2008; Selle et al., 2009). More recently, Truong et al. (2014) reported highly negative Na digestibility coefficients in the proximal jejunum which became progressively less negative along the small intestine which demonstrates that phytase enhanced Na absorption. That total tract Na retention was positive in the present study suggests further recovery of Na was taking place in the large intestine. Sodium pump (Na^+, K^+-ATPase) activity in small intestinal enterocytes drives the co-absorption of Na with both glucose and amino acids via Na^+-dependent transport systems. Small intestinal uptakes of Na, glucose and amino acids may have been facilitated by phytase in the present study as phytase significantly enhanced Na retention. Cytoplasmic concentrations of Na are pivotal determinants of Na^+, K^+-ATPase activity in enterocytes (Therien and Blostein, 2000), thus sodium pump activity may have been upgraded by the positive effect of phytase on Na retention so that intestinal uptakes of nutrients were facilitated.

The overall retention time in four small intestinal segments in birds offered PC diets was 115.8 min which was increased to similar extents in both non-supplemented B (157.5 min) and C (159.2 min) diets. However, 500 FTU/kg phytase addition to diets B increased retention time by 18.3% to 186.4 min; whereas, 1000 FTU/kg phytase addition to diets C decreased retention time by 19.1% to 128.8 min. This is an intriguing dichotomy because phytase significantly increased feed intakes of both B and C diets from 1 to 40 days post-hatch. It is quite usual for exogenous phytase to increase feed intakes in pigs and poultry

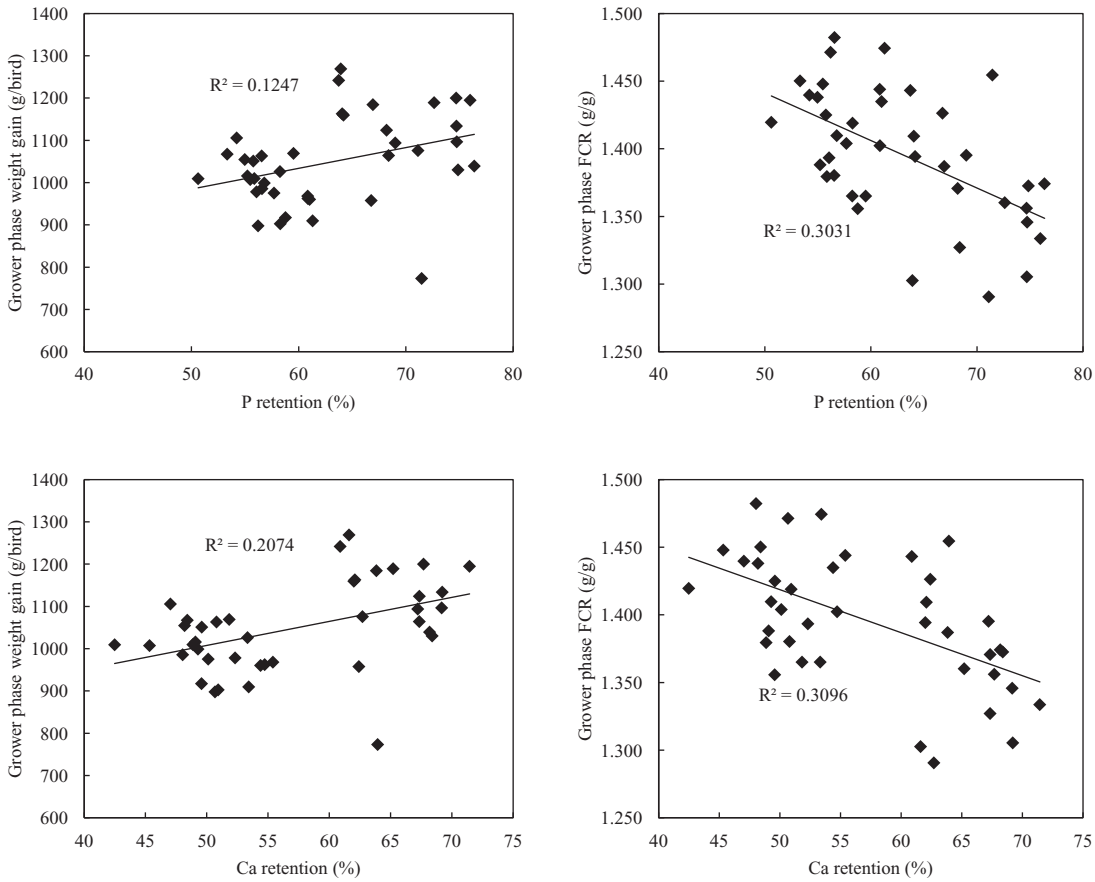

Fig. 1. The correlations between P, Ca retention and weight gain, FCR during grower phase.

which implies dietary phytate is a restraint on feed intake. Watson et al. (2006) reported that phytase increased feed intake and decreased intestinal transit time in broiler chickens. They concluded that the increase in daily weight gain in chicks offered diets containing phytase was due to an increase in feed intake. It does appear that phytate is a restraint on feed intake as increased feed intake rates is a very common response to phytase supplementation of both pig and poultry diets (Selle and Ravindran, 2007, 2008). This may be a compensatory mechanism (slowing gut transit rates) to facilitate phytate degradation by endogenous, mucosal phytase (and phosphatase). Therefore, that phytase-induced degradation of phytate at 500 FTU/kg increased feed intake but decreased small intestinal retention times is somewhat surprising. Conversely, 1000 FTU/kg decreased small intestinal retention times in association with increased feed intakes. This is consistent with the findings of Liu et al. (2014) where the same phytase at 1000 FTU/kg significantly decreased small intestinal retention times. The shortened retention times may have stemmed from amplified phytate degradation releasing more bound nutrients thereby reducing the interval required for their digestion and absorption. Interestingly, retention times were positively correlated with N digestibility coefficients ($r = 0.630$; $P < 0.001$) in the proximal jejunum across all treatments. It would appear that the extended retention times generated by 500 FTU/kg phytase in diets B are associated with the pronounced increases in N digestibility coefficients along the small intestine.

5. Conclusions

Reductions of nutrient specifications in non-supplemented diets B and C resulted in lower weight gain, less efficient feed conversion, lesser nutrient utilisation and lower tibia ash content. The impact of phytase on growth performance was more pronounced in grower and finisher diets. During grower phase, phytase significantly recovered weight gain and elevated phytase supplementation significantly improved feed conversion efficiency. Moreover, phytase significantly enhanced retentions of calcium, phosphorus and sodium. During the finisher phase, phytase significantly recovered weight gain and elevated phytase supplementation recovered AME, N retention and AME:GE ratios. Phytase significantly improved nitrogen digestion rates in both B and C diets. Therefore, phytase supplementation has the potential to recover broiler performance and nutrient specification can be reduced further in diets with increased phytase inclusions. Future studies are required to examine the capacity of phytase super-doses to recover performance in diets in association with their reduced nutrient

specifications. This would provide required information for poultry nutritionists to include higher doses of phytase in diets with appropriate nutrient specifications in the least-cost formulation of broiler rations.

Conflict of interest

The authors declare no conflicts of interest.

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