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Original research article

Effect of feeding broilers diets differing in susceptible phytate content

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ABSTRACT

Measurements of total phytate phosphorus content of diets may be deceptive as they do not indicate substrate availability for phytase; it may be that measurements of phytate susceptible to phytase effects are a more accurate measure of phosphorus (P) availability to the bird. To verify this hypothesis, an experiment was conducted to compare diets formulated to contain either high or low susceptible phytate, supplemented with either 0 or 500 FTU/kg phytase. Susceptible phytate was determined by exposing the feed samples to conditions that mimicked the average pH of the proximal gastrointestinal tract (pH 4.5) and the optimum temperature for phytase activity (37 °C) and then measuring phytate dissolved. Ross 308 birds ($n = 240$) were fed one of 4 dietary treatments in a 2 × 2 factorial design; 2 diets with high (8.54 g/kg, 57.90% of total phytate) or low (5.77 g/kg, 46.33% of total phytate) susceptible phytate, containing 0 or 500 FTU/kg phytase. Diets were fed to broilers (12 replicate pens of 5 birds per pen) from d 0 to 28 post hatch. Birds fed diets high in susceptible phytate had greater phytate hydrolysis in the gizzard ($P < 0.001$), jejunum ($P < 0.001$) and ileum ($P < 0.001$) and resulting greater body weight gain (BWG) ($P = 0.015$) and lower FCR ($P = 0.003$) than birds fed the low susceptible phytate diets, irrespective of phytase presence. Birds fed the high susceptible diets also had greater P solubility in the gizzard and Ca and P solubility in the jejunum and ileum ($P < 0.05$) and resulting greater tibia and femur Ca and P ($P < 0.05$) content than those fed the low susceptible diets. All the susceptible phytate was fully degraded in the tract in the absence of added phytase, suggesting the assay used in this study was able to successfully estimate the amount of total dietary phytate that was susceptible to the effects of phytase when used at standard levels. No interactions were observed between susceptible phytate and phytase on phytate hydrolysis. Hydrolysis of phytate was greater ($P < 0.05$) in the gizzard of birds fed the diets supplemented with phytase, regardless of the concentration of susceptible phytate in the diet. Phytase supplementation resulted in improved BWG ($P < 0.001$) and FCR ($P = 0.001$), increased P solubility ($P < 0.001$) in the gizzard, Ca and P solubility ($P < 0.001$) in the jejunum and ileum and Ca and P concentration ($P < 0.001$) and strength ($P < 0.001$) in the tibia and femur. Pepsin activity was higher in birds fed the diets supplemented with phytase ($P < 0.001$) and was greater ($P = 0.031$) in birds fed the high susceptible phytate diets compared with the low susceptible phytate diets. Findings from this study suggest that there may be a measure more meaningful to animal nutritionists than measurements of total phytate.

1. Introduction

Insoluble phytate–mineral precipitates and soluble mineral–phytate complexes may be resistant to hydrolysis by phytase (Maenz et al., 1999). In calculating the phosphorus (P) available from a diet following phytase addition, the total phytate-P concentration of a diet may be misleading because the total amount of phytate in the diet does not represent the quantity of P available for

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hydrolysis. This suggests the relative solubility and susceptibility of phytate to phytase, particularly at the pH of the gastrointestinal tract, should be accounted for when formulating with phytase and using phytate P as a replacement for inorganic P, as this may account for some of the anecdotal reports of apparent 'phytase failure' in diets.

Phytate susceptibility is determined by exposing samples to conditions that mimic the proximal gastrointestinal tract pH and optimum temperature for phytase activity and then measuring phytate P released. This is then assumed to be the proportion of total phytate that is susceptible to phytase degradation. Phytate susceptibility varies considerably between diets and is dependent upon the ingredients used, mineral concentrations, protein and solubility of the phytate. Gastrointestinal pH also has a significant impact on phytate susceptibility, because it is the addition of H^+ ions to the weak acid phosphate groups of phytate that convert it from being resistant to susceptible to the effects of phytase (Maenz et al., 1999). In this study, the measured total and determined susceptible phytate contents of the individual feed ingredients were analysed and diets were formulated based on these values. Diets were designed to examine the following hypothesis: degree of phytate susceptibility rather than total phytate will dictate level of response to phytase enzyme supplementation as measured by growth performance, gastrointestinal phytate hydrolysis, pepsin activity, mineral solubility and bone mineral concentration.

2. Material and methods

2.1. Dietary treatments

Birds were fed one of 4 dietary treatments in a 2 × 2 factorial design; 2 diets with either high or low determined susceptible phytate content (as a percentage of total phytate), supplemented with either 0 or 500 FTU/kg phytase (Quantum Blue AB Vista Feed Ingredients) (Table 1). Diets were mixed in house using a ribbon mixer and fed in mash form. Diets were formulated to be adequate in all nutrients.

Diets were analysed for gross energy by bomb calorimetry (Robbins and Firman, 2006) and for dry matter and protein content (calculated as nitrogen multiplied by 6.25) by the AOAC standard methods (930.15 and 990.03, respectively). Phosphorus and Ca contents of the diets were analysed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) following an aqua regia digestion step (AOAC 985.01, Leytem et al., 2008). Titanium dioxide was added at a rate of 0.5% to act as an inert marker for nutrient digestibility evaluation and the dietary content quantified by ICP-OES following aqua regia digestion (Morgan et al., 2014). Total phytate content was analysed by a K-Phyt assay (Megazyme, Wicklow, Ireland, UK). This assay quantitatively measured available P released from the samples. Diets were formulated based on the susceptible phytate content of the feed ingredients to ensure that the percentage of total phytate in the diets that was susceptible to phytase differed. Susceptible phytate content of the feed ingredients and trial diets was analysed by a modified version of the Megazyme K-Phyt assay described above. Fifty millilitres of warmed acetate buffer (2.5 M acetic acid and 2.5 M sodium acetate, pH 4.5, 37 °C) was added to 10 g of diet sample. This pH was chosen to mimic the average pH of the proximal gastrointestinal tract and the temperature was chosen as the optimum temperature for phytase activity. The samples were incubated at 37 °C for 5 min and then 2 mL was centrifuged at 9,500 × *g* for 10 min at room temperature. A 0.5-mL resulting supernatant was then neutralised with 0.5 mL 0.25 M NaOH and the pH was read using a spear tip piercing pH electrode (Sensorex, California, USA). A 1:3 dilution with ultrapure water was then carried out and phytic acid was measured using the K-Phyt assay. Susceptible phytate content was calculated by dividing the phytic acid content measured by the susceptible phytate assay by the phytic acid content measured by the total phytic acid assay. Supplemented phytase activity of the diets was analysed by Quantiplate Kit for Quantum Phytase (EnviroLogix, Maine, USA). Total phytase activity of the diets and

Table 1
Composition of high and low susceptible phytate diets (as fed basis).

Item	High susceptible phytate	Low susceptible phytate
Ingredients, %		
Wheat	53.13	48.56
Soybean meal 46[1]	31.28	31.88
Wheat bran	0.00	10.00
Rice bran	8.00	0.00
Soy oil	3.87	5.97
Salt	0.47	0.46
DL-methionine	0.29	0.29
Lysine HCl	0.23	0.21
Threonine	0.08	0.07
Limestone	0.54	0.49
Dicalcium phosphorus	1.78	1.84
Coccidiostat (Coban-monesin)	0.02	0.02
Vitamin premix[2]	0.40	0.40
Titanium dioxide	0.50	0.50
Calculated composition		
Protein content, g/kg DM	225.90	227.70
Total Ca content, g/kg DM	8.40	8.40
Total P content, g/kg DM	9.90	8.90
Free phosphorus, g/kg	3.50	3.50
Phytic acid content, g/kg	13.10	11.10
Susceptible phytate, g/kg	8.30	5.30
Fat, g/kg DM	64.80	70.75

[1] Soybean meal contains 46% protein.
[2] Supplied per kilogramme of diet: manganese, 100 mg; zinc, 80 mg; iron (ferrous sulphate), 20 mg; copper, 10 mg; iodine, 1 mg; molybdenum, 0.48 mg; selenium, 0.2 mg; retinol, 13.5 mg; cholecalciferol, 3 mg; tocopherol, 25 mg; menadione, 5.0 mg; thiamine, 3 mg; riboflavin, 10 mg; pantothenic acid, 15 mg; pyroxidine, 3.0 mg; niacin, 60 mg; cobalamin, 30 μg; folic acid, 1.5 mg; and biotin 125 mg.

ileal digesta was analysed according to the method of Engelen et al. (2001). Pepsin activity in the gizzard digesta was determined using 2% bovine haemoglobin as the substrate, based on the method presented by Liu and Cowieson (2011). Analysed values for each diet are shown in Table 2.

2.2. Birds and husbandry

Ross 308 broiler chicks ($n = 240$), from a 42-week-old breeder flock, were obtained from a commercial hatchery on the day of hatch. Chicks were randomised by weight, ensuring there was no significant difference in starting body weights across diets, and placed in 0.64 m^2 floor pens in groups of 5, bedded on clean wood shavings. Birds were allowed *ad libitum* access to the treatment diets and water for the duration of the trial (d 0 to 28). Each treatment was offered to 12 pens from d 0 (1 d post hatch) to 28. The room was thermostatically controlled to produce an initial temperature of 32 °C and reduced to 21 °C by d 21. The lighting regimen used was 24 h light on d 1, with darkness increasing by 1 h per day until 6 h of darkness was reached and this was maintained throughout the remainder of the study. Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the Nottingham Trent University College of Science ethical review committee.

Total weight of all the birds per pen was measured on d 0 and 28, and total feed intake of the pen was determined on d 28. Mean individual bird weight and feed intake was calculated, taking into consideration mortalities. On d 28, two birds per pen were euthanised by cervical dislocation and weighed. Gizzard, jejunum and ileum digesta content from the two euthanised birds was then collected, pooled, freeze dried and ground to a fine powder. For each pen, soluble Ca and P were determined in the gizzard, jejunum and ileum digesta by mixing the samples with ultra-pure water, centrifuging and measuring Ca and P contents of the supernatant by ICP-OES. The gizzard, jejunal and ileum digesta samples were analysed for total phytate by the K-Phyt assay and for TiO_2 content by ICP-OES as described previously. The amount of dietary phytate hydrolysed was calculated using the following equation.

$$\text{Dietary phytate hydrolysed} = \text{Dietary phytate} \times [1 - (\text{Digesta phytate} \times \text{TiO}_2\ \text{diet}) / (\text{TiO}_2\ \text{digesta} \times \text{Dietary phytate})].$$

This figure was then used to calculate the percentage of phytate remaining in each section of the tract following hydrolysis. Total phytase activity in the ileal digesta samples was analysed in triplicate according to the method of Engelen et al. (2001).

The right tibia and femur were collected from the two birds per pen and bone strength was analysed in both bones using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK) set up with a 50 kg load cell and 3 point–bend fixture (Shaw et al., 2010). The tibia and femur bones were then autoclaved for 15 min at 121 °C, defleshed and cartilage caps removed and then oven dried at 110 °C for approximately 4 d until constant weight. The dried bones were then ashed for approximately 14 h at 650 °C (Hall et al., 2003) and bone ash was calculated as ash weight as a percentage of dry bone weight. A subsample of the ashed bone samples was analysed for Ca and P contents by ICP-OES as previously described.

2.3. Statistical analysis

All data were analysed using IBM SPSS statistics version 22. After Kolmogorov–Smirnov testing to confirm normality, univariate analysis was conducted to determine interactions between measured factors and one-way ANOVA was used to determine the equality of the means. The model included phytase, phytate susceptibility and the interaction. Treatment means were separated using Duncan post hoc test where appropriate. Correlations between measured factors were analysed by bivariate correlation using Pearson product–moment correlation coefficient. Interpretations of the strength of the relationships between the factors were based on guidelines by Cohen (1988); weak relationship $r = 0.10$ to 0.29, medium relationship $r = 0.30$ to 0.49 and strong relationship $r = 0.50$ to 1.0. Statistical significance was declared at $P < 0.05$.

Table 2
Proximate composition of experimental diets (FTU = the quantity of phytase which liberates 1 μmol of inorganic phosphorus per minute from an excess of sodium phytate at 37 °C and pH of 5.5).

Item	High[1]		Low[2]	
	0 FTU/kg	500 FTU/kg	0 FTU/kg	500 FTU/kg
Gross energy content, MJ/kg DM	20.43	20.57	21.11	21.13
Total P content, g/kg DM	9.70	9.74	8.66	8.74
Total Ca content, g/kg DM	8.31	8.22	8.20	8.18
Ash content, g/kg	67.52	68.01	68.43	67.70
Dry matter content, g/kg	863.30	866.18	887.71	869.18
Protein content, g/kg DM	223.74	224.08	229.29	229.97
Supplemented phytase activity, FTU/kg	0.00	872.62	0.00	835.39
Total phytase activity,[3] FTU/kg	455.72	955.50	323.33	860.06
Phytic acid content, g/kg	14.90	14.60	12.19	12.70
Phytate-P,[4] g/kg	4.20	4.12	3.44	3.58
Non-phytate-P,[5] g/kg	5.50	5.62	5.22	5.16
Susceptible phytate, g/kg	8.61	8.47	5.65	5.88
Susceptible phytate, % of phytic acid	57.78	58.01	46.35	46.30
Fat, g/kg DM	70.67	70.74	70.77	70.81

[1] High = diet with susceptible phytate content of approximately 8.54 g/kg (57.90% of total dietary phytic acid).
[2] Low = diet with susceptible phytate content of approximately 5.77 g/kg (46.33% of total dietary phytic acid).
[3] Total phytase activity was analysed by a colorimetric enzymatic method and calculated as (net optical density at 415 nm × dilution volume)/(slope of standard curve × mass × incubation time) (Engelen et al., 2001).
[4] Phytate-P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).
[5] Non-phytate-P was calculated as the difference between total P and phytate-P.

3. Results and discussion

The high susceptible phytate diets contained more total phytate than the low susceptible phytate diets, and the phytate was approximately 10% more susceptible to the effects of phytase (Table 2) according to the susceptible phytate assay. Diets were formulated to differ in the percentage of total phytate that was susceptible to phytase effects, and on analysis it was found there was a difference of 11.57%. There were no interactions between susceptible phytate and phytase on phytate hydrolysis or the amount of phytate remaining in the tract (Table 3). As expected, hydrolysis of phytate was the greatest ($P < 0.05$) and phytate remaining ($P < 0.05$) in the tract was the lowest in the gizzard of birds fed the diets supplemented with phytase compared with the birds fed the diets without phytase. The observed values and effects of phytate susceptibility in this study are similar to those presented by Maenz et al. (1999). Birds fed diets high in susceptible phytate had greater phytate hydrolysis in the gizzard ($P < 0.001$), jejunum ($P < 0.001$) and ileum ($P < 0.001$) than birds fed diets low in susceptible phytate regardless of phytase presence or absence, which confirms that in the high susceptible diet more phytate was available for hydrolysis. However, birds fed the high susceptible phytate diets had more phytate remaining ($P < 0.05$) in the gizzard than those fed the low susceptible phytate diets, likely due to the comparatively higher total phytate content of the high susceptible phytate diets (Table 3).

Findings from this study suggest that, in diets with high phytate content and standard phytase doses, 60% of the total phytate escaped degradation in the gizzard and was hydrolysed further along the tract (Table 3). As feed passes through the small intestine more phytate was hydrolysed in the high susceptible diet than the low susceptible diet, such that by the jejunum the difference between diets in phytate remaining has disappeared. This suggests that susceptibility of phytate in the small intestine is as essential as its susceptibility in the gizzard. This is also highlighted by the strong relationships observed between remaining susceptible phytate in the ileum and ileal phytase activity in birds fed the high susceptible diets ($r = 0.500$, $P = 0.018$ for High 0 FTU and $r = 0.694$, $P = 0.013$ for High 500 FTU) (Table 4). This suggests the assay for measuring phytate susceptibility may require amendment as involves just exposing the feed samples to pH conditions that mimic the gastric phase. The solubility of phytate complexes is dependent on a number of factors, including duration of incubation, pH, molar ratio of the mineral to phytate and presence of multiple cations (Maenz et al., 1999). The amount of phytate hydrolysed by the terminal ileum in the control diet was very similar to the susceptible phytate content of the diets (Table 3), indicating that all the susceptible phytate was fully degraded in the absence of added phytase. Phytase addition extended phytate hydrolysis even further, and this was regardless of the level of susceptible phytate, as indicated by a lack of phytate × phytase interaction.

3.1. Performance

Body weight gain ($P = 0.015$) and FCR ($P = 0.003$) were significantly improved in birds fed the diets with high susceptible phytate compared with those fed the diets with low susceptible phytate (Table 4). This coincides with the fact there was greater phytate hydrolysis in birds fed the high susceptible diet (Table 3), although the amount of phytate remaining did not differ between diets at this point. The susceptible phytate fraction may thus indicate the "active" fraction of phytate which takes part in interfering with digestion, and the greater level of hydrolysis of this fraction in the high susceptible diet may be correlated with improved performance. Analysis of the dietary ingredients prior to diet formation revealed that approximately 47% of the phytate in the wheat bran was susceptible to the effects of phytase, whereas approximately 94% of the phytate in the rice bran was susceptible to hydrolysis by phytase. If the above hypothesis is correct, i.e., that the anti-nutritive susceptible phytate can be removed if sufficient phytase is available, then it may suggest that rice bran can be significantly improved through usage of high doses of phytase. The positive effect of phytate susceptibility on bird performance may not be just due to a direct effect on mineral and protein availability, because binding of phytate to metallic cations not only makes them unavailable as nutritional components but also has an impact on cell vesicular trafficking, DNA signalling and repair and endocytosis (Bohn et al., 2008). Phytase supplementation improved BWG ($P < 0.001$) and FCR ($P = 0.001$) regardless of the level of susceptible phytate (Table 4) and there were strong correlations between ileal phytase activity (Table 5) and BWG ($r = 0.555$, $P = 0.031$) and FCR ($r = 0.559$, $P < 0.001$) (Table 4). This may be partly due to increased P availability and improved amino acid digestion, as supplemental phytase improved access to phosphorus and amino acids from phytate complexes. Also, in the diets with phytase there was likely comparatively less precipitation of protein with phytate. It would be advantageous in future studies to investigate the phytase responses in pellet diets as opposed to mash diets as poultry producers predominantly feed steam-pelleted diets and hydrothermal treatment may change the property and solubility of phytate.

3.2. Pepsin activity

In this study, pepsin activity increased ($P < 0.001$) with phytase supplementation in the diet, regardless of the level of susceptible phytate, suggesting phytase has a direct effect on pepsin activity

Table 3
Dietary phytic acid remaining and hydrolysed after hydrolysis in the gizzard, jejunum and ileum (g/kg dry matter) in 28-d-old broilers fed diets containing either high or low susceptible phytate, supplemented with either 0 or 500 FTU/kg phytase (FTU = the quantity of phytase which liberates 1 μmol of inorganic phosphorus per minute from an excess of sodium phytate at 37 °C and pH of 5.5).[1]

Item	Phytate status	High[2]		Low[3]		SEM	Susceptibility			Phytase			*P*-value		
		0 FTU/kg	500 FTU/kg	0 FTU/kg	500 FTU/kg		High[2]	Low[3]	SEM	0 FTU/kg	500 FTU/kg	SEM	Susceptibility	Phytase	Susceptibility × phytase
Gizzard	Remaining	7.37	6.62	4.43	4.96	0.24	6.99[a]	6.24[b]	0.27	6.88[a]	6.35[b]	0.19	0.004	0.039	0.381
	Hydrolysed	5.50	6.02	4.43	4.96	0.30	5.76[a]	4.70[b]	0.38	4.96[b]	5.49[a]	0.19	<0.001	0.037	0.996
Jejunum	Remaining	6.82	5.80	6.23	5.97	0.19	6.31	6.10	0.07	6.52	5.89	0.22	0.535	0.064	0.259
	Hydrolysed	6.05	6.85	4.59	5.06	0.44	6.45[a]	4.83[b]	0.57	5.32	5.95	0.22	<0.001	0.064	0.624
Ileum	Remaining	5.40	4.61	5.22	4.91	0.15	5.01	5.07	0.02	5.31	4.76	0.20	0.853	0.101	0.468
	Hydrolysed	7.46	8.04	5.58	6.13	0.49	7.75[a]	5.86[b]	0.67	6.53	7.08	0.20	<0.001	0.100	0.639

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] Diets containing either high or low susceptible phytate supplemented with either 0 or 500 FTU/kg phytase.
[2] High = diet with susceptible phytate content of approximately 8.54 g/kg (57.90% of total dietary phytic acid).
[3] Low = diet with susceptible phytate content of approximately 5.77 g/kg (46.33% of total dietary phytic acid).

Table 4
Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) in 28-day-old broilers fed diets containing either high or low susceptible phytate, supplemented with either 0 or 500 FTU/kg phytase.

Item	High[1]		Low[2]		SEM	Susceptibility			Phytase			*P*-value		
	0 FTU/kg[3]	500 FTU/kg[3]	0 FTU/kg[3]	500 FTU/kg[3]		High[1]	Low[2]	SEM	0 FTU/kg[3]	500 FTU/kg[3]	SEM	Susceptibility	Phytase	Susceptibility × phytase
FI, g	2,020	2,097	2,068	2,057	13.87	2,059	2,062	1.32	2,044	2,077	11.70	0.256	0.294	0.487
BWG, g	1,414	1,549	1,369	1,466	33.34	1,481[a]	1,417[b]	22.61	1,391[b]	1,507[a]	40.83	0.015	<0.001	0.457
r^4	0.290	0.344	0.374	0.041										
FCR	1.43	1.36	1.51	1.40	0.03	1.40[b]	1.46[a]	0.02	1.47[a]	1.38[b]	0.03	0.003	0.001	0.802
r^5	0.170	0.246	0.096	0.080										

[a, b] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] High = Diet with susceptible phytate content of approximately 8.54 g/kg (57.90% of total dietary phytic acid).
[2] Low = Diet with susceptible phytate content of approximately 5.77 g/kg (46.33% of total dietary phytic acid).
[3] FTU is the quantity of phytase which liberates 1 μmol of inorganic phosphorus per minute from an excess of sodium phytate at 37 °C and pH of 5.5.
[4] Strength of the relationship between the amount of susceptible phytate remaining after hydrolysis in the gizzard, jejunum and ileum and BWG.
[5] Strength of the relationship between the amount of susceptible phytate remaining after hydrolysis in the gizzard, jejunum and ileum and FCR.

Table 5
Pepsin activity in the gizzard and ileal phytase activity in 28-day-old broilers fed diets containing either high or low susceptible phytate, supplemented with either 0 or 500 FTU/kg phytase.

Item	High[1]		Low[2]		SEM	Susceptibility			Phytase			*P*-value		
	0 FTU/kg[3]	500 FTU/kg[3]	0 FTU/kg[3]	500 FTU/kg[3]		High[1]	Low[2]	SEM	0 FTU/kg[3]	500 FTU/kg[3]	SEM	Susceptibility	Phytase	Susceptibility × phytase
Pepsin activity, U/kg					33.12	762.75[a]	725.83[b]	13.05	682.19[b]	806.39[a]	43.91	0.031	<0.001	0.129
r^4	0.438	0.047	0.310	0.035										
Ileal phytase activity, FTU/kg	59.51[c]	664.87[a]	51.79[d]	575.05[b]	141.97	362.19	313.42	17.24				<0.001	<0.001	<0.001
r^5	0.500	0.694	0.160	0.260										

[a–d] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] High = Diet with susceptible phytate content of approximately 8.54 g/kg (57.90% of total dietary phytic acid).
[2] Low = Diet with susceptible phytate content of approximately 5.77 g/kg (46.33% of total dietary phytic acid).
[3] FTU is the quantity of phytase which liberates 1 μmol of inorganic phosphorus per minute from an excess of sodium phytate at 37°C and pH of 5.5.
[4] Strength of the relationship between the amount of susceptible phytate remaining after hydrolysis in the gizzard and pepsin activity.
[5] Strength of the relationship between the amount of susceptible phytate remaining after hydrolysis in the gizzard, jejunum and ileum and ileal phytase activity.

(Table 5). It is known that phytate binds to pepsin directly or the peptide that activates pepsin (Yu et al., 2012), and the inhibition of pepsin was removed by hydrolysis of a portion of the phytate in the gizzard in the presence of phytase. Destruction of phytate would therefore increase the production of pepsin and thus explain this result. However, birds fed the high susceptible phytate diets had significantly higher pepsin activity ($P = 0.031$) runs counter to the above hypothesis, and suggests that pepsin secretion was stimulated by the high susceptible diet. Clearly these two observations seem irreconcilable unless the susceptible phytate assay does not predict gastric conditions correctly.

3.3. Mineral solubility and absorption

The fact that the solubility of ($P < 0.05$) P in the gizzard and the solubilities of Ca and P in the jejunum and ileum were greater in birds fed the high susceptible phytate diets (Table 6), despite the higher level of total phytate in this diet, suggests that if phytate plays a role in the solubility of these minerals, it may be dependent upon where the phytate originates. The lack of any interaction (with the exception of Ca in the gizzard) suggests the highly susceptible phytate is less likely to bind Ca and interfere with P availability, especially as pH increases in the distal part of the intestinal tract. In the gizzard, phytase improved Ca and P solubility regardless of the susceptibility of phytate in the diets, which at least confirms the tenet that phytate binds Ca and reduces its availability, but it did it to a greater extent in the low susceptible diets than the high. An interesting observation however was that the correlation between susceptible phytate remaining and solubility of Ca in all sections of the intestine was the greatest when phytase was added, suggesting that release of Ca into the aqueous phase is tied to the hydrolysis of this fraction of phytate.

3.4. Bone strength and mineralisation

The direct effect of phytase on both diets increased hydrolysis of phytate-bound Ca and P and likely reduced the anti-nutritional effects of phytate on other divalent cations. This resulted in birds fed the diets with phytase having increased ($P < 0.05$) tibia and femur Ca and P content and strength (Table 7), which is in agreement with a number of previously published studies such as Angel et al. (2006), Applegate et al. (2003) and Kocabaúli (2001). The lower tibia and femur Ca and P content in birds fed the low susceptible diet compared with the high susceptible is in line with all other observations to date relating to Ca and P solubilities, and thus reduced availability of these minerals for absorption and partitioning toward bone Ca and P.

3.5. Conclusion

The organic P component of feed ingredients fed to poultry exists in both phytase-susceptible and phytase-resistant forms, and binding of divalent cations to phytate can cause a portion of dietary phytate to be resistant to hydrolysis by phytase. It was hypothesised that formulating a diet to be rich in susceptible phytate would result in particularly poor performance and a particularly large

Table 6
Calcium and P solubility (g/kg) in the gizzard, jejunum and ileum in 28-day-old broilers fed diets containing either high or low susceptible phytate, supplemented with either 0 or 500 FTU/kg phytase.

Item	Mineral	High[1]		Low[2]		SEM	Susceptibility			Phytase			*P*-value		
		0 FTU/kg[3]	500 FTU/kg[3]	0 FTU/kg[3]	500 FTU/kg[3]		High[1]	Low[2]	SEM	0 FTU/kg[3]	500 FTU/kg[3]	SEM	Susceptibility	Phytase	Susceptibility × phytase
Gizzard	Ca	6.87[b]	7.43[a]	6.54[c]	7.36[a]	0.18	7.15	6.95	0.07	6.71	7.40	0.24	<0.001	<0.001	0.006
	P	3.67	4.34	3.55	4.22	0.17	4.01[a]	3.89[b]	0.04	3.61[b]	4.28[a]	0.24	0.007	<0.001	0.871
r[4]	Ca	0.298	0.265	0.061	0.283										
	P	0.487	0.042	0.216	0.369										
Jejunum	Ca	3.90	4.26	3.88	4.09	0.08	4.08[a]	3.99[b]	0.03	3.89[b]	4.18[a]	0.10	0.018	<0.001	0.073
	P	2.89	3.24	2.84	3.14	0.08	3.07[a]	2.99[b]	0.03	2.87[b]	3.19[a]	0.11	0.003	<0.001	0.247
r[4]	Ca	0.065	0.471	0.060	0.205										
	P	0.034	0.427	0.032	0.102										
Ileum	Ca	2.62	2.94	2.41	2.76	0.10	2.78[a]	2.58[b]	0.07	2.52[b]	2.85[a]	0.12	<0.001	<0.001	0.518
	P	1.12	1.25	1.07	1.22	0.04	1.19[a]	1.15[b]	0.01	1.10[b]	1.24[a]	0.05	0.001	<0.001	0.299
r[4]	Ca	0.113	0.651	0.225	0.171										
	P	0.108	0.115	0.375	0.115										

[a, b] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] High = Diet with susceptible phytate content of approximately 8.54 g/kg (57.90% of total dietary phytic acid).
[2] Low = Diet with susceptible phytate content of approximately 5.77 g/kg (46.33% of total dietary phytic acid).
[3] FTU is the quantity of phytase which liberates 1 μmol of inorganic phosphorus per minute from an excess of sodium phytate at 37°C and pH of 5.5.
[4] Strength of the relationship between the amount of susceptible phytate remaining after hydrolysis and Ca and P solubility in the section of tract.

Table 7
Tibia and femur strength, ash and Ca and P content in 28-day-old broilers fed diets containing either high or low susceptible phytate, supplemented with either 0 or 500 FTU/kg phytase.

Item		High[1]		Low[2]		SEM	Susceptibility			Phytase			*P*-value		
		0 FTU/kg[3]	500 FTU/kg[3]	0 FTU/kg[3]	500 FTU/kg[3]		High[1]	Low[2]	SEM	0 FTU/kg[3]	500 FTU/kg[3]	SEM	Susceptibility	Phytase	Susceptibility × phytase
Tibia	Strength, N	246.67	287.03	241.95	269.19	9.06	266.85	255.57	3.99	244.31[b]	278.11[a]	11.95	0.356	0.008	0.591
	Ash, %	32.81	35.20	32.75	35.11	0.59	34.01	33.93	0.03	32.78[b]	35.15[a]	0.84	0.941	0.028	0.991
	Ca, % of ash	31.42	34.09	27.96	32.14	1.11	32.75[a]	30.05[b]	0.95	29.69[b]	33.11[a]	1.21	0.002	<0.001	0.372
	P, % of ash	13.66	16.59	12.72	15.76	0.78	15.12[a]	14.24[b]	0.31	13.19[b]	16.17[a]	1.05	0.018	<0.001	0.875
Femur	Strength, N	182.86	219.02	191.56	211.44	7.30	200.94	201.50	0.20	187.21[b]	215.23[a]	9.91	0.950	0.003	0.368
	Ash, %	33.63	34.87	33.45	34.72	0.32	34.25	34.09	0.06	33.54	34.80	0.44	0.992	0.342	0.893
	Ca, % of ash	33.12	37.88	31.53	35.77	1.22	35.50[a]	33.65[b]	0.66	32.33[b]	36.82[a]	1.59	0.011	<0.001	0.615
	P, % of ash	15.23	17.62	14.85	16.45	0.54	16.42[a]	15.65[b]	0.27	15.04[c]	17.04[a]	0.71	0.021	<0.001	0.223

[a–c] Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] High = Diet with susceptible phytate content of approximately 8.54 g/kg (57.90% of total dietary phytic acid).
[2] Low = Diet with susceptible phytate content of approximately 5.77 g/kg (46.33% of total dietary phytic acid).
[3] FTU is the quantity of phytase which liberates 1 μmol of inorganic phosphorus per minute from an excess of sodium phytate at 37°C and pH of 5.5.

response to added phytase. In fact, this study showed that birds fed diets with high susceptible phytate content had significantly better cumulative BWG and FCR, P solubility and phytate degradation in the gizzard, jejunum and ileum, higher pepsin activity and femur Ca and P and tibia P content at d 28 than birds fed diets with low susceptible phytate content. In this regard, the susceptible phytate assay did show that the diets were different, but the inverse of what was expected was the result. It is possible that the low susceptible diet presented phytate which was co-ordinated with chelates which were more difficult to hydrolyse and hence the result reflected the type rather than the quantity of susceptible phytate. Indeed the assay used was really a measure of phytate solubility *in vitro* and not necessarily its availability or susceptibility *in vivo*. Consequently, further work is needed to improve the quality of the assay and to understand material differences in phytate released from different ingredients and the physico-chemical properties of the phytate beyond buffer solubility and mineral interactions, but these data do suggest that there may be a measure more meaningful to animal nutritionists than total phytate.

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