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Original research article

Effect of feeding broilers diets differing in susceptible phytate content

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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.