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Standard phytase inclusion in maize-based broiler diets enhances digestibility coefficients of starch, amino acids and sodium in four small intestinal segments and digestive dynamics of starch and protein

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ABSTRACT

The effects of the 500 FTU/kg inclusion of *Buttiauxella* phytase in maize-based broiler diets were investigated where each treatment consisted of eight replicates of six male Ross 308 chicks per cage. Apparent digestibility coefficients of starch, sixteen amino acids and nine minerals in four small intestinal segments were determined in broilers offered P-adequate, maize-based diets at 40 days post-hatch. The disappearance rates of starch and protein (the sum of amino acids) from the four small intestinal segments were calculated and starch:protein disappearance rate ratios deduced in order to assess the effects of phytase on digestive dynamics. Phytase increased starch digestibility coefficients in the proximal jejunum (0.681 versus 0.538; $P = 0.001$) and distal ileum (0.959 versus 0.936; $P = 0.009$) and starch disappearance rates in the proximal jejunum (58.0 versus 43.4 g/bird/day; $P = 0.004$) and proximal ileum (80.8 versus 71.4 g/bird/day; $P = 0.036$). Phytase significantly increased ($P = 0.003$ – <0.001) amino acid digestibilities in four small intestinal segments with the most pronounced responses being observed in the proximal jejunum. Average amino acid data indicated that protein digestibility coefficients and disappearance rates were significantly ($P = 0.002$ – <0.001) increased in the four small intestinal segments. The magnitude of the responses to phytase in the proximal jejunum for both protein digestibility (0.791 versus 0.481; $P < 0.001$) and protein disappearance (23.77 versus 15.06 g/bird/day; $P < 0.001$) were substantial. Digestibility coefficients of both Na and P were significantly ($P = 0.027$ – <0.001) improved in four small intestinal segments by phytase but this did not apply to Ca. Na digestibility coefficients were significantly correlated to those of starch in three small intestinal segments including the proximal jejunum ($r = 0.900$; $P < 0.001$) and Na digestibilities were significantly correlated to protein in four small intestinal segments including the proximal ileum ($r = 0.862$; $P < 0.001$). Phytase condensed starch:protein disappearance rate ratios ($P = 0.015$ – <0.001) in the three caudal segments of the small intestine. A multiple linear regression equation ($r = 0.936$; $P < 0.001$) indicated that increasing protein disappearance rates from the proximal ileum would be advantageous, whereas, increasing starch

Abbreviations: AIA, acid insoluble ash; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; Ca, calcium; Cu, copper; DI, distal ileum; DJ, distal jejunum; HCl, hydrochloric acid; iP, isoelectric point; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; P, phosphorus; PI, proximal ileum; PJ, proximal jejunum.

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disappearance rates would be disadvantageous in terms of 40-day weight gains. Consideration is given to the likelihood that the impact of phytase on Na absorption along the small intestine holds relevance to the intestinal uptakes of glucose and amino acids.

1. Introduction

Liu et al. (2015) investigated the effects of standard and elevated phytase inclusions in maize-based broiler diets with two tiers of reduced nutrient specifications. In this study, a *Buttiauxella* phytase (Axtra® PHY; Danisco Animal Nutrition, DuPont) was included at 500 and 1000 FTU/kg and has been described by Yu et al. (2014). In Liu et al. (2015) study, the finisher diet (28–40 days post-hatch) was formulated to contain 4.75 g/kg total phosphorus (P) and 2.5 g/kg non-phytate P and was considered P-adequate as phytase did not significantly increase tibial P contents. To 40 days post-hatch, 500 FTU/kg phytase improved weight gain by 11.8% (2937 versus 2627 g/bird), feed intake by 9.97% (4556 versus 4143 g/bird) and feed conversion ratios by 1.52% (1.553 versus 1.577). Interestingly, 500 FTU/kg phytase significantly improved apparent nitrogen digestibility coefficients in four small intestinal segments including an increase of 11.3% (0.883 versus 0.793) in the distal ileum, which was the most conservative response recorded. However, in the proximal jejunum the response was more pronounced with an increase of 79.9% (0.682 versus 0.379).

Consequently, retained samples of diets and digesta from four small intestinal segments were analysed for concentrations of sixteen amino acids, starch, and nine minerals to determine the impact of phytase on their apparent digestibility coefficients along the small intestine. Thus the primary purpose of this companion paper is to report on these outcomes. Also, the intention was to investigate relationships between Na digestibilities with those of starch and amino acids and the digestive dynamics of starch and protein.

Slight, but significant, increases in ileal starch digestibility to phytases in maize-based diets have been reported in broilers (Camden et al., 2001) but there are few reported investigations of the effects of phytase on starch digestibility. There is the possibility that starch digestibility determinations in more anterior segments of the small intestine, where the majority of starch digestion takes place and digestion is less complete, are more likely to detect responses to phytase and should prove more instructive. For example, Truong et al. (2015) reported that 500 FTU/kg phytase increased proximal jejunal starch digestibility by 17.6% (0.774 versus 0.658; $P < 0.005$) in maize- and wheat-based diets. Moreover, starch disappearance rates from the proximal jejunum were increased by 23.7% (65.3 versus 52.8 g/bird/day; $P < 0.001$).

Across the minerals, both phosphorus and calcium are obviously relevant in phytase digestibility assays but perhaps sodium should hold equal interest. Phytate has been shown to increase Na excretion in broilers, which was counteracted by phytase (Cowieson et al., 2004). Moreover, phytase has improved ileal Na digestibility coefficients to substantial extents in several studies (Ravindran et al., 2006, 2008; Selle et al., 2009a; Truong et al., 2014) but assessments of phytase on Na digestibility along the small intestine have only been investigated in one of these studies. Truong et al. (2014) reported that Na digestibility coefficients were substantially more negative in the proximal jejunum than in the distal ileum. This was almost certainly a consequence of endogenous sodium bicarbonate secretions into the duodenum buffering hydrochloric acid generated by the proventriculus (Allen and Flemstrom, 2005). The subsequent recovery of Na along the small intestine was enhanced by phytase supplementation in Truong et al. (2014) study.

Consideration has been given to the probability that phytate has anti-nutritive properties in respect of protein and energy utilisation in poultry and that phytase generates matching 'extra-phosphoric' responses (Ravindran and Selle, 2010). Selle and Ravindran (2007) raised the possibility that Na may be involved in extra-phosphoric phytase responses on the basis of the early Cowieson et al. (2004) and Ravindran et al. (2006) studies. Therefore, this companion paper will consider the role of Na in relation to exogenous phytase responses and to the formulation of phytase-supplemented diets for broiler chickens.

2. Materials and methods

Liu et al. (2015) have already documented the overall methodology followed in this experiment. However, the composition and specifications of the maize-based N1 diets offered to broilers from 28 to 40 days post-hatch is recorded in Table 1 together with the analysed concentrations of starch, sixteen amino acids and nine minerals. In essence, N1 maize–soy diets, without and with 500 FTU/kg phytase, were offered to 8 replicates (6 birds per cage) Ross 308 male chicks from 1 to 40 days post-hatch to each of the two dietary treatments. The analysed phytase activities in the two finisher diets (28–40 days post-hatch) were 113 FTU/kg in the NC1 diet and 769 FTU/kg in the NC1 plus phytase diet. On average, the birds had weight gains of 2782 g/bird, feed intakes of 4350 g/bird and feed conversion ratios of 1.564 at 40 days post-hatch when small intestinal digestibility coefficients were determined.

Concentrations of nutrients in diets and digesta were determined. Digesta samples included those from the proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI) as defined by Liu et al. (2015). Starch concentrations were determined by a procedure based on dimethyl sulfoxide, α-amylase and amyloglucosidase, as described by Mahasukhonthachat et al. (2010). Amino acid concentrations of diets and digesta samples from four small intestinal segments were determined following 24 h liquid hydrolysis at 110 °C in 6 M HCl and then 16 amino acids are analysed using the

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Table 1
Dietary formulation and calculated nutrient specifications of the finisher diet (28–40 days post-hatch) and analysed concentrations of starch, amino acids and minerals.

Item	g/kg	Analysed concentration	g/kg
Formulation			
Maize	686.0		
Soybean meal	243.1	Starch	408.68
Vegetable-fat blend	23.2		
Lysine HCl	3.2	Arginine (Arg)	10.48
Methionine	2.3	Histidine (His)	4.67
Threonine	0.8	Isoleucine (Ile)	7.04
Celite™	20.0	Leucine (Leu)	15.16
Sodium bicarbonate	0.1	Lysine (Lys)	11.10
Sodium chloride	3.0	Methionine (Met)	3.18
Limestone	9.0	Phenylalanine (Phe)	8.70
Dicalcium phosphate	7.3	Threonine (Thr)	6.97
Vitamin–mineral premix[2]	2.0	Valine (Val)	8.16
Nutrient specification		Alanine (Ala)	8.07
ME (MJ/kg)	12.92	Aspartic acid (Asp)	14.92
Protein	177.95	Glutamic acid (Glu)	32.13
Fat	53.40	Glycine (Gly)	6.11
Fibre	22.91	Proline (Pro)	10.48
Calcium	6.16	Serine (Ser)	8.36
Total phosphorus	4.75	Tyrosine (Tyr)	3.42
Phytate phosphorus	2.26	Total amino acids	158.94
Non-phytate phosphorus	2.50		
Lysine	11.36	Sodium (Na)	1.43
Methionine	5.11	Potassium (K)	8.51
Methionine + cysteine	8.12	Phosphorus (P)	5.25
Threonine	7.44	Calcium (Ca)	6.20
Tryptophan	1.92	Magnesium (Mg)	5.04
Arginine	11.64	Copper (Cu) (mg/kg)	19.12
Sodium	1.50	Iron (Fe) (mg/kg)	148.89
Potassium	7.61	Manganese (Mn) (mg/kg)	104.95
Chloride	2.76	Zinc (Zn) (mg/kg)	100.67

Walters AccQTag Ultra chemistry on a Waters Acquity UPLC. Tryptophan and cystine cannot be analysed by this procedure. Similarly, diets and digesta samples were treated with $HNO_3 + H_2O_2 + HCl$ in open acid digestion and then 9 minerals were analysed by ICP-OES. Acid insoluble ash (AIA; Celite™ World Minerals, Lompoc, CA, USA) was used as the dietary marker and AIA concentrations were determined by the method of Siriwan et al. (1993).

The apparent digestibility coefficients for starch, amino acids and minerals in four small intestinal sites were calculated from the following equation:

$$\text{Apparent digestibility coefficient} = \frac{(\%\,\text{nutrient}/\%\,\text{AIA})_{\text{diet}} - (\%\,\text{nutrient}/\%\,\text{AIA})_{\text{digesta}}}{(\%\,\text{nutrient}/\%\,\text{AIA})_{\text{diet}}}$$

In the finisher phase of the Liu et al. (2015) study, from 28 to 40 days post-hatch, phytase increased feed intakes from 2362 to 2499 g/bird or by 11.42 g/bird/day. Starch and protein disappearance rates (g/bird/day) from four small intestinal segments were calculated from the following equation:

$$\text{Disappearance rate} = \text{feed intake}_{(\text{g/bird/day})} \times \text{dietary nutrient}_{(\text{g/kg})} \times \text{digestibility coefficient}$$

Protein concentrations in diets were based on the sum of the 16 amino acids assessed and digestibility coefficients were calculated from the average values of the 16 amino acids quantified. The calculations used average daily feed intakes over the finisher period, analysed dietary protein or starch concentrations and relevant digestibility coefficients.

Experimental data were analysed as a one-way ANOVA of dietary treatments using the IBM® SPSS® Statistics 20 programme (IBM Corporation, Somers, NY, USA). Pearson correlations and regression equations were established for selected parameters where justified. The probability level of less than 5% was considered to be statistically significant. The feeding study was conducted so as to comply with specific guidelines approved by the Animal Ethics Committee of the University of Sydney.

3. Results

The effects of phytase on starch digestibility coefficients, starch and protein disappearance rates and starch and protein ratios are shown in Table 2. Phytase increased starch digestibility coefficients in the proximal jejunum (0.681 versus 0.538; $P = 0.001$) and distal ileum (0.959 versus 0.936; $P = 0.009$). Phytase increased starch disappearance rates in the proximal jejunum (58.0 versus 43.4 g/bird/day; $P = 0.004$) and proximal ileum (80.8 versus 71.4 g/bird/day; $P = 0.036$). The majority of

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Table 2
Effects of exogenous phytase on apparent digestibility coefficients of starch and protein, accumulative starch and protein disappearance rates and starch and protein disappearance rate ratios of four small intestinal segments in broilers offered maize-based diets at 40 days post-hatch ("Protein" is based on the sum of 16 amino acids).

	Starch		Protein		Starch:protein disappearance rate ratios
Item	Digestibility coefficient	Disappearance rate (g/bird/day)	Digestibility coefficient	Disappearance rate (g/bird/day)	
Proximal jejunum					
0	0.538	43.4	0.481	15.06	2.93
500 FTU/kg	0.681	58.0	0.719	23.77	2.44
SEM	0.0316	2.762	0.0258	0.8560	0.2090
Significance (P=)	0.001	0.004	<0.001	<0.001	0.132
Distal jejunum					
0	0.833	67.2	0.666	20.91	3.22
500 FTU/kg	0.865	73.6	0.802	26.49	2.78
SEM	0.0129	2.302	0.0183	0.5228	0.1041
Significance (P=)	0.170	0.077	<0.001	<0.001	0.013
Proximal ileum					
0	0.884	71.4	0.805	25.33	2.90
500 FTU/kg	0.948	80.8	0.878	29.01	2.79
SEM	0.0224	2.748	0.0045	0.5916	0.0129
Significance (P=)	0.073	0.036	<0.001	0.001	<0.001
Distal ileum					
0	0.936	75.5	0.843	26.49	2.85
500 FTU/kg	0.959	81.7	0.904	29.88	2.73
SEM	0.0049	1.995	0.0069	0.5872	0.0289
Significance (P=)	0.009	0.054	<0.001	0.002	0.015

starch was digested and absorbed in the proximal jejunum, where phytase increased starch digestion by 26.6% and starch disappearance rates by 33.6%. The response of phytase on protein digestibility coefficients and protein disappearance rates where all significant ($P \leq 0.002$). As was the case with starch, the most pronounced responses to phytase were in the proximal jejunum with an increase in protein digestibility coefficients of 49.5% (0.719 versus 0.481: $P < 0.001$) and an increase in protein disappearance rates of 57.8% (23.77 versus 15.06 g/bird/day; $P < 0.001$). The ratios of starch to protein disappearance rates were significantly condensed or narrowed following phytase supplementation in the distal jejunum, proximal ileum and distal ileum. The phytase response in the proximal ileum was the most significant where the starch:protein disappearance rate ratio was narrowed from 2.90 to 2.79 ($P < 0.001$).

In all four small intestinal segments, phytase significantly increased digestibility of all nine essential amino acids (Table 3). Methionine was the least, and threonine the most, responsive of the essential amino acids to phytase supplementation.

Table 3
Effects of exogenous phytase on apparent digestibility coefficients of essential amino acids in four small intestinal segments in broilers offered maize-based diets at 40 days post-hatch.

Item	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
Proximal jejunum									
0	0.609	0.481	0.455	0.474	0.684	0.700	0.509	0.394	0.403
500 FTU/kg	0.798	0.723	0.700	0.700	0.848	0.850	0.720	0.681	0.664
SEM	0.0262	0.0245	0.0287	0.0263	0.0174	0.0223	0.0247	0.0255	0.0293
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Distal jejunum									
0	0.770	0.678	0.656	0.664	0.799	0.843	0.682	0.588	0.613
500 FTU/kg	0.869	0.813	0.789	0.792	0.890	0.917	0.803	0.758	0.758
SEM	0.0132	0.0189	0.0176	0.0185	0.0100	0.0094	0.0161	0.0174	0.0216
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Proximal ileum									
0	0.872	0.824	0.797	0.817	0.867	0.920	0.820	0.733	0.774
500 FTU/kg	0.922	0.889	0.870	0.881	0.923	0.957	0.885	0.836	0.849
SEM	0.0033	0.0045	0.0049	0.0051	0.0035	0.0072	0.0048	0.0043	0.0055
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	<0.001	<0.001
Distal ileum									
0	0.901	0.862	0.839	0.856	0.881	0.948	0.860	0.767	0.813
500 FTU/kg	0.942	0.915	0.900	0.910	0.934	0.970	0.914	0.861	0.878
SEM	0.0037	0.0065	0.0074	0.0074	0.0055	0.0040	0.0063	0.0078	0.0099
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001

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Table 4
Effects of exogenous phytase on apparent digestibility coefficients of non-essential amino acids in four small intestinal segments in broilers offered maize-based diets at 40 days post-hatch.

Item	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	Tyrosine
Proximal jejunum							
0	0.465	0.429	0.554	0.390	0.415	0.455	0.277
500 FTU/kg	0.699	0.698	0.762	0.675	0.685	0.704	0.605
SEM	0.0246	0.313	0.0282	0.0290	0.0294	0.0273	0.0347
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Distal jejunum							
0	0.656	0.643	0.731	0.587	0.621	0.639	0.500
500 FTU/kg	0.788	0.787	0.844	0.756	0.778	0.783	0.712
SEM	0.0199	0.0168	0.0151	0.0193	0.0205	0.0154	0.0250
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Proximal ileum							
0	0.801	0.776	0.851	0.745	0.793	0.776	0.717
500 FTU/kg	0.870	0.861	0.907	0.839	0.869	0.861	0.830
SEM	0.0052	0.0049	0.0045	0.0041	0.0051	0.0052	0.0052
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Distal ileum							
0	0.831	0.817	0.884	0.787	0.837	0.819	0.778
500 FTU/kg	0.895	0.888	0.929	0.868	0.898	0.890	0.875
SEM	0.0099	0.0070	0.0072	0.0069	0.0069	0.0077	0.0121
Significance (P=)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Phytase increased methionine digestibility by 21.4%, 8.78%, 4.02% and 2.32% in the PJ, DJ, PI and DI, respectively. The corresponding figures for threonine were 72.8%, 28.9%, 14.1% and 12.3%. Amino acid digestibility responses to phytase were unequivocal, especially in the anterior small intestinal segments.

The effect of phytase on non-essential amino acids digestibility coefficients are shown in Table 4. Similarly, phytase significantly ($P \leq 0.001$) increased the digestibility of all seven non-essential amino acids in four small intestinal segments. Again, the most robust phytase responses were observed in anterior small intestinal segments.

The influence of phytase on apparent digestibility coefficients of nine minerals is shown in Table 5. There were no significant responses to phytase for digestibility coefficients of Ca and Zn. The effects of phytase on digestibility coefficients of Cu, K, Mg, and Mn were significant in some instances; whereas, phytase consistently improved Fe digestibility. Phytase increased P digestibility in PJ by 103%, but responses were consistently in the order of 35% in the three posterior segments. Digestibility coefficients of Na were negative in all instances; however, in the control diets the magnitude of this negativity was diminished from −3.547 in PJ to −0.889 in DI, which indicates that Na was being progressively absorbed along the small intestine. Importantly, phytase augmented Na digestion by approximately 40% in the four small intestinal segments. For

Table 5
Effects of exogenous phytase on apparent digestibility coefficients of minerals in four small intestinal segments in broilers offered maize-based diets at 40 days post-hatch.

Item	Na	P	Ca	Cu	Fe	K	Mg	Mn	Zn
Proximal jejunum									
0	−3.547	0.235	0.275	−0.394	−0.772	0.773	−0.319	0.205	0.205
500 FTU/kg	−2.008	0.477	0.380	−0.046	−0.230	0.801	−0.172	0.376	0.376
SEM	0.4211	0.0577	0.0447	0.1197	0.1147	0.0153	0.2409	0.0635	0.0632
Significance (P=)	0.027	0.015	0.138	0.067	0.008	0.219	0.324	0.087	0.087
Distal jejunum									
0	−2.352	0.398	0.460	−0.108	−0.372	0.897	−0.030	0.356	0.412
500 FTU/kg	−1.551	0.540	0.440	−0.061	−0.175	0.872	−0.147	0.399	0.437
SEM	0.2191	0.0183	0.0183	0.0224	0.0234	0.0069	0.0298	0.0288	0.0182
Significance (P=)	0.016	<0.001	0.465	0.149	<0.001	0.537	0.018	0.212	0.347
Proximal ileum									
0	−1.780	0.395	0.484	−0.251	−0.419	0.889	−0.059	0.327	0.420
500 FTU/kg	−0.930	0.534	0.485	−0.012	−0.413	0.881	−0.099	0.387	0.442
SEM	0.0837	0.0188	0.0166	0.0465	0.0218	0.0074	0.0268	0.0188	0.0147
Significance (P=)	<0.001	<0.001	0.960	0.005	<0.001	0.457	0.321	0.047	0.312
Distal ileum									
0	−0.889	0.384	0.509	−0.190	−0.370	0.846	−0.105	0.326	0.421
500 FTU/kg	−0.568	0.521	0.507	−0.032	−0.102	0.809	−0.086	0.384	0.455
SEM	0.0539	0.0234	0.0171	0.0203	0.0408	0.0093	0.0249	0.0229	0.0201
Significance (P=)	0.002	0.002	0.956	0.001	0.001	0.026	0.600	0.110	0.257

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Table 6
Pearson correlations between sodium digestibility coefficients with apparent digestibility coefficients of starch and essential amino acids and average of essential and non-essential amino acids (Av. AA) in four small intestinal segments in broilers offered maize-based diets at 40 days post-hatch.

Item	Starch	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Av. AA
Proximal jejunum											
Coefficient (r=)	0.900	0.519	0.618	0.552	0.599	0.592	0.537	0.583	0.616	0.588	0.582
Significance (P=)	<0.001	0.084	0.032	0.063	0.040	0.043	0.072	0.046	0.033	0.044	0.047
Distal jejunum											
Coefficient (r=)	0.489	0.683	0.714	0.728	0.715	0.772	0.701	0.726	0.743	0.725	0.721
Significance (P=)	0.106	0.014	0.009	0.007	0.009	0.008	0.011	0.007	0.006	0.008	0.008
Proximal ileum											
Coefficient (r=)	0.822	0.850	0.870	0.861	0.814	0.891	0.697	0.833	0.904	0.863	0.862
Significance (P=)	0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.012	0.001	<0.001	<0.001	<0.001
Distal ileum											
Coefficient (r=)	0.736	0.842	0.786	0.825	0.789	0.838	0.662	0.827	0.814	0.803	0.825
Significance (P=)	0.010	0.001	0.004	0.002	0.004	0.001	0.027	0.002	0.002	0.003	0.002

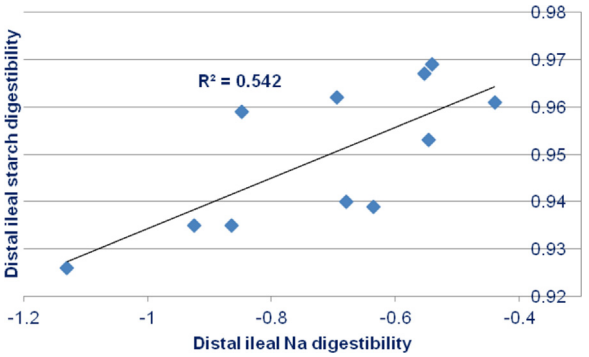

Fig. 1. Linear relationship (r=0.736; P=0.010) between apparent digestibility coefficients of Na and starch in the distal ileum.

example, in the proximal ileum phytase increased Na digestibility coefficients by 47.8% (−1.780 versus −0.930; P<0.001), which was the most robust response observed.

Pearson correlations for Na digestibility coefficients and digestibility coefficients of starch, essential amino acids and the average of all 16 amino acids are shown in Table 6. Na and starch digestibility coefficients were significantly correlated in the PJ, PI and DI where the strongest correlation was in the proximal jejunum (r=0.900; P<0.001). All essential amino acids were correlated with Na digestibility coefficients in DJ, PJ and DI and six amino acids were correlated with Na digestibility coefficients in PI. For example, Na and lysine digestibility coefficients were significantly (P=0.043 – <0.001) correlated in all four segments. The average amino acid digestibility coefficient was correlated with Na digestibility coefficients (P=0.047 – <0.001) in all four small intestinal segments where the strongest was in the proximal ileum (r=0.862; P<0.001).

The linear relationship between Na and starch digestibility coefficients in the distal ileum is shown in Fig. 1 (r=0.736; P=0.010). The value of R^2 =0.542 indicates that 54% of the variation in starch digestibility may be attributed to Na digestibility coefficients. The linear relationship between Na and protein digestibility coefficients in the distal ileum is shown in Fig. 2

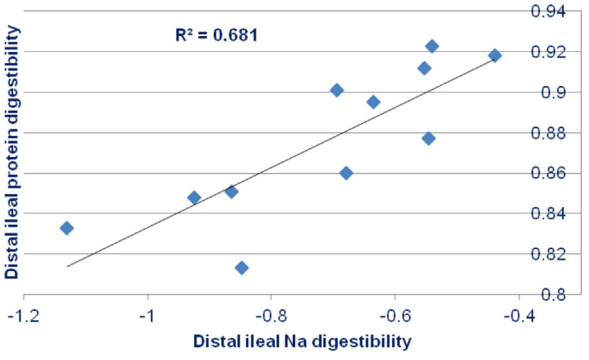

Fig. 2. Linear relationship (r=0.825; P=0.002) between digestibility coefficients of Na and protein (average of 16 amino acids) in the distal ileum.

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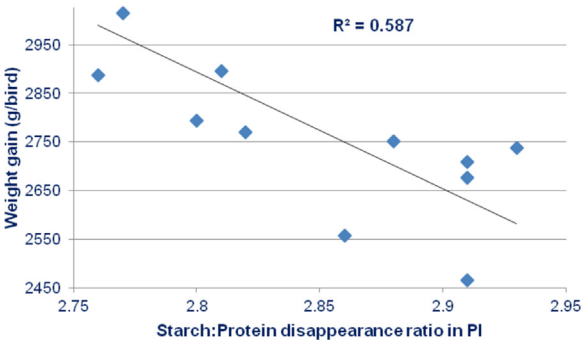

Fig. 3. Linear relationship ($r = -0.766$; $P = 0.003$) between starch:protein disappearance rate ratios in the proximal ileum and weight gain at 40 days post-hatch.

($r = 0.825$; $P = 0.002$). Here, the value of $R^2 = 0.681$ indicates that 68% of the variation in protein digestibility may be attributed to Na digestibility coefficients.

The linear relationship between starch:protein disappearance rate ratios and 40-day weight gains is shown in Fig. 3. The relationship is negatively correlated ($r = -0.766$; $P = 0.003$) with a value of $R^2 = 0.587$. Thus 59% of the variation in weight gain may be attributed to the ratio and, importantly, weight gains will be enhanced following an increase in the disappearance rate of protein relative to starch.

4. Discussion

The fundamental reason for including phytase in broiler diets is to liberate phytate-bound P. In this study phytase improved P digestibility by 103.0% in the proximal jejunum, 35.7% in the distal jejunum, 35.2% in the proximal ileum and by 35.7% in the distal ileum. These marked improvements were in contrast to the results for the majority of other minerals assayed, with the exception of Na. Phytase improved Na digestibility by 43.4% in the proximal jejunum, 34.1% in the distal jejunum, 47.8% in the proximal ileum and by 36.1% in the distal ileum. Thus the effects of phytase on both P and Na were of a similar order of magnitude. The implications of the pronounced Na effect of phytase are profound given the involvement of Na in the intestinal uptakes of glucose and amino acids. The significant correlations found in the present study, as illustrated in Figs. 1 and 2, between distal ileal Na digestibility and starch ($r^2 = 0.542$) and protein ($r^2 = 0.681$) digestibility do not establish causation; nevertheless, they do suggest the involvement of the positive 'Na effect' of phytase in the absorption of glucose and amino acids along the small intestine.

There are extremely few reports of the effects of exogenous phytases in conventional broiler diets on ileal starch digestibility despite the fact that phytases usually enhance energy utilisation (Selle and Ravindran, 2007). The implication is that phytase increased starch digestibility as starch is the dominant energy source in poultry diets. In the present study, phytase significantly ($P < 0.005$) improved starch digestibility by 26.6% in the proximal jejunum and by 2.46% in the distal ileum. There were corresponding improvements of 33.6% ($P < 0.005$) and 8.21% ($P < 0.06$) in starch disappearance rates from the two sites. These responses reciprocally illustrate the negative impact of phytate on starch digestion and/or glucose absorption.

A variety of mechanisms have been proposed for the phytate impeding starch digestion including direct and indirect starch–phytate interactions, inhibition of α-amylase and chelation of calcium, a pre-requisite for amylase activity (Rickard and Thompson, 1997). However, there is little evidence to support the existence of direct starch–phytate complexes although it is possible that phytate indirectly binds starch via starch granule-associated proteins (Baldwin, 2001) as either binary or ternary protein–phytate complexes involving starch and cereal proteins (Selle et al., 2012).

However, as noted by Rickard and Thompson (1997), data generated by Demjen and Thompson (1991) suggest that phytate has the capacity to retard glucose absorption, independently of any notional impact of phytate on starch digestion. Demjen and Thompson (1991) reported that phytate reduced blood glycaemic indices in humans given a test load of glucose *per se*. It has been suggested (Selle et al., 2012, Truong et al., 2014, 2015) that phytate may retard absorption of glucose by depressing the functionality of the 'sodium pump' (Na^+, -K^+, -ATPase). In essence, the co-absorption of glucose and sodium via sodium-glucose linked transporters (SGLT-1) from the gut lumen is driven by Na^+, -K^+, -ATPase, which is located in the baso-lateral membrane of enterocytes, and maintains an electrochemical gradient across enterocytes (Glynn, 1993; Wright and Loo, 2000). Moreover, there is evidence that phytate depresses sodium pump activity and reduces blood glucose levels in rats (Dilworth et al., 2005). In addition, Liu et al. (2008) found that phytase increased sodium pump and glucose concentrations in duodenal and jejunal enterocytes in chickens. The implication is that phytate depressed sodium pump activity and, in turn, intestinal uptakes of glucose.

The mechanisms whereby phytate depresses Na^+, -K^+, -ATPase have not been properly identified; however, cytoplasmic Na concentrations within enterocytes are pivotal for sodium pump function (Therein and Blostein, 2000). Therefore, if phytate was to deplete Na concentrations in enterocytes it would depress sodium pump activity and Na depletion could be a consequence of sodium bicarbonate secretion into the duodenum, perhaps largely from the pancreas (Case et al., 1969).

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Endogenous Na secretions would be ameliorated by phytase reducing the extent of binary protein–phytate complex formation, which would reduce the compensatory hypersecretion of hydrochloric acid and pepsin and less sodium bicarbonate would be required to buffer digesta in the small intestine.

The impact of phytase on digestibility of amino acids was unequivocally positive in this study as 500 FTU/kg *Buttiauxella* phytase increased average distal ileal digestibility coefficients of 16 amino acids by 7.24% (0.904 versus 0.843; $P < 0.001$). A response of this magnitude is not without precedent as Amerah et al. (2014) reported that, at a higher inclusion rate of 1000 FTU/kg, the same phytase increased average ileal digestibilities of 17 amino acids by 12.3% (0.840 versus 0.748; $P < 0.0001$) in maize-based diets with poorer inherent digestibilities. Indicatively, mean amino acid digestibility was quadratically related ($r^2 = 0.736$) to the extent of phytate degradation in the Amerah et al. (2014) study. In the present study, phytase responses were more pronounced in anterior small intestinal segments as average amino acid digestibility coefficients were increased by 9.07% in the proximal ileum, 20.4% in the distal jejunum, culminating in an increase of 49.5% (0.481 versus 0.719; $P < 0.001$) in proximal jejunal coefficients. There were corresponding increases in protein disappearance rates.

These responses to phytase illustrate the negative impact of phytate on the extent, site and rate of protein digestion and amino acid absorption. As reviewed by Selle et al. (2012), the fundamental negative impact appears to be the capacity of phytate to bind proteins in binary protein–phytate complexes at pH less than the isoelectric point (IP) of proteins. Complexed proteins are refractory to pepsin digestion (Vaintraub and Bulmaga, 1991), which impedes the initiation of protein digestive processes and it follows that this would prompt compensatory hypersecretions of pepsin and HCl as a 'defence mechanism'. Also proteins in grains such as maize, wheat and sorghum have relatively high iP (Csonka et al., 1926), and they may remain patent along the small intestine in contrast to soy and canola protein meals. The likelihood is that phytate does interfere with protein digestion but this does not preclude the possibility that phytate also impedes the absorption of amino acids via Na-dependent transport systems as appears to be the case with glucose. The mechanisms involved in the absorption of glucose and amino acids from the small intestine are complex but active Na-dependent transport systems are probably the most important route (Stevens et al., 1984). The co-transport of sodium with either glucose or amino acids is pivotal to intestinal uptakes of these nutrients, and it has been argued that intestinal nutrient uptakes constitute the limiting factor on broiler performance (Croom et al., 1999).

The significant responses in P digestibility to phytase supplementation pursuant to the hydrolysis of dietary phytate and the liberation of phytate-bound P were anticipated outcomes (Selle and Ravindran, 2007). In contrast, phytase did not generate significant responses in Ca digestibility which was somewhat surprising. Marini et al. (1985) concluded that phytate (IP_6) could complex 4.93 Ca atoms against a theoretical maximum of 6. However, the extent of phytate binding Ca in the formation of mineral–phytate complexes is almost certainly variable and gut pH is critical to their solubility (Selle et al., 2009b). One possibility is that the extent of de novo formation of Ca–phytate complexes in broilers offered the control diet was limited in this study.

The proposal that starch and protein digestive dynamics and the post-enteral availability of glucose and amino acids at sites of protein synthesis are crucial to broiler growth performance is not new (Liu and Selle, 2014). Indeed, decades ago, the importance of dynamics of free and protein-bound amino acids, which are effectively uncoupled or unbalanced by restricted feeding regimen so as to compromise protein accretion, was demonstrated in pigs (Batterham, 1974). Therefore, it is instructive to examine the impact of phytase on the relativity of starch and protein disappearance rates. Phytase condensed or narrowed starch:protein disappearance rate ratios in all small intestinal segments and to significant extents in the three caudal segments (Table 10). As shown in Fig. 3, there is a negative, linear relationship ($r = -0.766$; $P = 0.003$) between starch:protein disappearance rate ratios in the proximal ileum and 40-day weight gains post-hatch. The multiple linear regression equation ($r = 0.936$; $P < 0.001$) is as follows:

$$y_{\text{weight gain (g/bird)}} = 857.53 + 109.12 \times \text{protein}_{\text{(g/bird/day)}} - 13.34 \times \text{starch}_{\text{(g/bird/day)}}.$$

It is evident from the equation that increases in protein disappearance rates advantage weight gain; whereas, increases in starch disappearance rates are disadvantageous. In the companion Liu et al. (2015) study phytase generated an 11.8% increase in 40-day weight gain (2937 versus 2627 g/bird). However, this was associated with non-significant differences in tibial P contents so it appears that the diets were P adequate and the 11.8% increase in gain was an 'extra-phosphoric' effect. The genesis of the weight gain response appears to stem from phytase increasing the digestion and disappearance of both protein and starch but this was amplified by the more pronounced impact on digestive dynamics of protein relative to starch.

5. Implications

This study demonstrates that phytase effectively retrieves Na along the small intestine to substantial extents. This profound Na effect of phytase has practical implications for the formulation of phytase-supplemented diets given that phytase generated 0.52 g/kg Na at the distal ileal level. As most broiler diets contain 1.8 g/kg Na, this impact of phytase illustrates the need to apply appropriate Na matrix values in ration formulation. This approach is only reinforced by the probability that high dietary Na levels may attenuate responses to phytase (Ravindran et al., 2008). This study also demonstrates that more condensed starch:protein disappearance rate ratios are advantageous. However, it would appear that phytase "narrows" these ratios because of its relatively greater impact on the disappearance (digestion–absorption) of protein than starch.

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Conflict of interest

There are no known conflicts of interest.

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References

Allen, A., Flemstrom, G., 2005. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am. J. Physiol. Cell Physiol. 288, C1–C19.
Amerah, A.M., Plumstead, P.W., Barnard, L.P., Kumar, A., 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93, 906–915.
Baldwin, P.M., 2001. Starch granule-associated proteins and polypeptides: a review. Starch Stärke 53, 475–503.
Batterham, E.S., 1974. The effect of frequency of feeding on the utilization of free lysine by growing pigs. Br. J. Nutr. 31, 237–242.
Camden, B.J., Morel, P.C.H., Thomas, D.V., Ravindran, V., Bedford, M.R., 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize–soya-bean meal diets for broilers. Anim. Sci. 73, 289–297.
Case, R.M., Harper, A.A., Scratcherd, T., 1969. The secretion of electrolytes and enzymes by the pancreas of the anaesthetized cat. J. Physiol. 201, 335–348.
Cowieson, A.J., Acamovic, T., Bedford, M.R., 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45, 101–108.
Croom, W.J., Brake, J., Coles, B.A., Havenstein, G.B., Christensen, V.L., McBride, B.W., Peebles, E.D., Taylor, I.L., 1999. Is intestinal absorption rate-limiting for performance in poultry? J. Appl. Poult. Res. 8, 242–252.
Csonka, F.A., Murphy, J.C., Jones, D.B., 1926. The iso-electric points of various proteins. J. Am. Chem. Soc. 48, 763–768.
Demjen, A.P., Thompson, L.U., 1991. Calcium and phytic acid independently lower the glycemic response to a glucose load. Proc. 34th Canadian Federaton of Biological Sci, 53 (Abstr).
Dilworth, L.L., Omoruyi, F.O., Simon, O., Asemota, H.N., 2005. Digestive and absorptive enzymes in rats fed phytic acid extract from sweet potato *(Ipomoea batatas)*. Diabetol. Croat. 34, 59–65.
Glynn, I.M., 1993. All hands to the sodium pump. J. Physiol. 462, 1–30.
Liu, N., Ru, Y.J., Li, F.D., Cowieson, A.J., 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohyrdase and transporter in chickens. J. Anim. Sci. 86, 3432–3439.
Liu, S.Y., Selle, P.H., 2014. A consideration of starch and protein digestive dynamics in chicken-meat production. Worlds Poult. Sci. J. (in press).
Liu, S.Y., Bold, R., Plumstead, P.W., Selle, P.H., 2015. Performance recoveries from standard and elevated phytase inclusions in maize-based broiler diets with two tiers of reduced nutrient specifications. Anim. Feed Sci. Technol. (in press).
Mahasukhonthachat, K., Sopade, P.A., Gidley, M.J., 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. J. Cereal Sci. 51, 392–401.
Marini, M.A., Evans, W.J., Morris, N.M., 1985. Calorimetric and potentiometric studies on the binding of calcium by phytic acid. J. Appl. Biochem. 7, 180–191.
Ravindran, V., Morel, P.C.H., Partridge, G.G., Hruby, M., Sands, J.S., 2006. Influence of an *E. coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85, 82–89.
Ravindran, V., Cowieson, A.J., Selle, P.H., 2008. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. Poult. Sci. 87, 677–688.
Ravindran, V., Selle, P.H., 2010. Phytate: anti-nutritive effects on energy and amino acid utilization. In: Proceedings, 46th Eastern Nutr. Conference, Anim. Nutr. Association of Canada. Ottawa, Canada, pp. 1–12.
Rickard, S.E., Thompson, L.U., 1997. Interactions and biological effects of phytic acid. In: Shahidi, F. (Ed.), Antinutrients and Phytochemicals in Food. Am. Chem. Soc., Washington, DC, pp. 294–312.
Selle, P.H., Ravindran, V., 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135, 1–41.
Selle, P.H., Partridge, G.G., Ravindran, V., 2009a. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility energy utilisation mineral retention and growth performance in wheat-based broiler diets. Anim. Feed Sci. Technol. 153, 303–313.
Selle, P.H., Cowieson, A.J., Ravindran, V., 2009b. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124, 126–141.
Selle, P.H., Cowieson, A.J., Cowieson, N.P., Ravindran, V., 2012. Protein–phytate interactions in pig and poultry nutrition; a reappraisal. Nutr. Res. Rev. 25, 1–17.
Siriwan, P., Bryden, W.L., Mollah, Y., Annison, E.F., 1993. Measurement of endogenous amino-acid losses in poultry. Br. Poult. Sci. 34, 939–949.
Stevens, B.R., Kaunitz, J.D., Wright, E.M., 1984. Intestinal transport of amino acids and sugars: advances using membrane vesicles. Annu. Rev. Physiol. 46, 417–433.
Therien, A.G., Blostein, R., 2000. Mechanisms of sodium pump regulation. Am. J. Physiol. 279, C541–C566.
Truong, H.H., Yu, S., Peron, A., Cadogan, D.J., Khoddami, A., Roberts, T.H., Liu, S.Y., Selle, P.H., 2014. Phytase supplementation of maize-, sorghum- and wheat-based broiler diets, with identified starch pasting properties, influences jejunal and ileal digestibilities of phytate (IP_6) and sodium. Anim. Feed Sci. Technol. 198, 248–256.
Truong, H.H., Liu, S.Y., Selle, P.H., 2015. Phytase influences the inherently different starch digestive dynamics of wheat- and maize-based broiler diets. Proceedings, Aust. Poult. Sci. Symp. (in press).
Vaintraub, I.A., Bulmaga, V.P., 1991. Effect of phytate on the in vitro activity of digestive proteinases. J. Agric. Food Chem. 39, 859–861.
Wright, E.M., Loo, D.D.F., 2000. Coupling between Na^+, sugar, and water transport across the intestine. Ann. N. Y. Acad. Sci. 915, 54–66.
Yu, S., Kvidtgaard, M.F., Isaksen, M.F., Dalsgaard, S., 2014. Characterization of a mutant *Buttiauxella* phytase using phytic acid and phytic acid-protein complex as substrates. Ani. Sci. Lett. 1, 18–32.

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