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Phytase supplementation of maize-, sorghum- and wheat-based broiler diets with identified starch pasting properties influences phytate (IP_6) and sodium jejunal and ileal digestibility

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ABSTRACT

The effects of phytase supplementation on growth performance, nutrient utilisation, starch and protein digestive dynamics in broiler chickens offered maize-, sorghum- and wheat-based diets were determined in a previous study (Liu et al., 2014). Responses to phytase were most pronounced in maize-based diets, which suggest that more phytate was degraded in these diets. Relevant retained samples of grain, diets and digesta from four small intestinal segments were retrospectively analysed for concentrations of phytate, sodium and starch pasting properties to investigate the hypothesis that phytate in maize-based diets was more completely degraded by exogenous phytase. Exogenous phytase significantly ($P<0.001$) degraded dietary phytate in the proximal jejunum, distal jejunum, proximal ileum and distal ileum and increased distal ileal phytate digestibility coefficients from 0.238 to 0.631. There were significant differences ($P<0.001$) between diets based on maize (0.515), wheat (0.449) and sorghum (0.340) for distal ileal phytate digestibility coefficients. Phytase accelerated phytate disappearance rates from all four segments and increased distal ileal phytate disappearance rates from 201 to 535 mg/bird/day. This was significantly more pronounced in maize (459 mg/bird/day) than in diets based on sorghum (301 mg/bird/day) and wheat (343 mg/bird/day). Sodium digestibility coefficients were significantly improved ($P<0.01$) by exogenous phytase in proximal jejunum, distal jejunum and proximal ileum. Exogenous phytase significantly influenced starch properties of experimental diets determined by rapid visco-analysis (RVA). There were significant negative correlations between RVA setback viscosity of starch in experimental diets and starch digestibility coefficients at the distal jejunum ($r=-0.438$; $P<0.01$) and proximal ileum ($r=-0.591$; $P<0.001$) determined

Abbreviations: AIA, acid insoluble ash; AID, apparent ileal digestibility; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; cP, centipoise; DJ, distal jejunum; DI, distal ileum; FCR, feed conversion ratio; IP_6, *myo*-inositol hexaphosphate; Na^+,K^+-ATPase, sodium-potassium adenosine triphosphate or 'sodium pump'; p*I*, isoelectric point; PJ, proximal jejunum; PI, proximal ileum; RVA, rapid visco-analysis; SGLT-1, sodium-glucose linked transporter-1.

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in the Liu et al. (2014) study. Distal ileal phytate digestibility coefficients appeared to be higher in non-supplemented, maize-based diets (0.349) than in diets based sorghum (0.128) and wheat (0.239) thus the likelihood is that phytate in maize-based diets was more readily degraded by endogenous, mucosal phytase in the small intestine. Consideration is given to the possibilities that location of phytate within grains influences phytate degradation and that the relatively low sodium concentrations in maize-based diets may have contributed to the more robust responses to exogenous phytase observed.
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1. Introduction

The effects of phytase supplementation of maize-, sorghum- and wheat-based broiler diets were previously investigated (Liu et al., 2014). A notable outcome was that phytase generated more robust responses in maize-based diets in comparison to those based on sorghum and wheat, especially for parameters of nutrient utilisation. Significant interactions were observed for apparent metabolisable energy (AME), nitrogen (N) retention and N-corrected AME (AMEn). For example, 1000 FTU/kg phytase significantly increased AMEn by 0.44 MJ/kg in maize-based diets but numerically decreased AMEn by 0.16 and 0.05 MJ/kg, in sorghum- and wheat-based diets, respectively. Leske and Coon (1999) had reported that phytate in maize was degraded to a greater extent than in wheat by an exogenous phytase in poultry. Therefore, one possible reason for the robust phytase responses observed in maize-based diets is that phytate was more readily degraded by exogenous phytase. Consequently, diets and digesta samples were retrospectively analysed for phytate (*myo*-inositol hexaphosphate; IP_6) to determine phytate degradation in four small intestinal sites. Sodium (Na) concentrations in the retained samples were also analysed because phytate and phytase have been shown to have tangible effects on Na digestibility on a total tract basis (Cowieson et al., 2004) and in the terminal ileum (Ravindran et al., 2006, 2008; Selle et al., 2009b) in broiler chickens. The four reports cited indicate that phytate triggers an egress of Na into the small intestinal lumen, which is counteracted by phytase. Consequently, it has been suggested that this transition of Na may influence absorption of nutrients, including glucose and amino acids, because increasing Na concentrations in the gut lumen may compromise 'sodium pump' (Na^+,K^+-ATPase) activity and Na^+-dependent transport systems (Selle et al., 2012).

The impact of phytate and phytase on starch digestibility is an elusive topic. In the Liu et al. (2014) study, significant phytase effects on starch digestibility were confined to an increase of 2.56% (0.920 *versus* 0.897) for all three grains in the proximal ileum and an increase of 19.9% (0.804 *versus* 0.704) for wheat-based diets in the proximal jejunum. Alternatively, starch in maize-based diets was significantly more digestible in the three posterior small intestinal segments than in diets based on sorghum and wheat. For possible clarification, starch pasting properties of unprocessed grains and experimental diets were identified by rapid visco-analysis (RVA). The main objective of these retrospective analyses was to investigate the genesis of the more robust phytase responses observed in maize compared to sorghum- and wheat-based diets with a focus on phytate degradation and the digestibilities of Na and starch.

2. Materials and methods

The methodologies adopted for the underlying study have been outlined (Liu et al., 2014). This study investigated the effects of phytase supplementation on growth performance, nutrient utilisation and digestive dynamics of starch and protein. Ross 308 chicks were offered nutritionally equivalent, P-adequate, steam-pelleted diets based on 560 g/kg maize, sorghum or wheat, without or with 1000 FTU/kg phytase. The feed enzyme used was a *Buttiauxella* phytase produced in *Trichoderma reesei* (Axtra® PHY; Danisco Animal Nutrition, Marlborough, UK). The six dietary treatments were offered to 8 replicates (6 birds per cage) from 7 to 27 days post-hatch. More specifically, starch pasting properties of maize, sorghum and wheat and the diets based on these grains were determined with a Rapid-Visco-Analyzer (RVA-4. Newport Scientific Pty Ltd. Warriewood NSW, Australia) following procedures outlined by Hernandez et al. (2008). Within 13 min intervals, 28 g mixtures of diets and water (15:85 w/w on a dry basis) were prepared and held at a temperature of 50 °C for 1 min and then heated from 50 °C to 95 °C. After holding the hot paste at 95 °C for 2.5 min, the slurry was again cooled to 50 °C, and then held at that temperature for 2 min.

The phytate analysis methodology followed in the present study was similar to that reported by Carlsson et al. (2001) with minor revisions (Yu et al., 2012). For elemental analysis, sodium ions were analysed on an ICP Emission Spectrometer (iCAP 6000 Series) according to manufacturer's instructions (Thermo Electron Corporation. Waltham, M.A.). Feed and digesta samples (1 g) were ground and extracted with 20 ml HCl (0.5 N). The filtered 40 μl extract was directly injected on HPIC for IP_6 analysis while for Na analysis the extract was diluted 100 to 200 fold.5 N). Acid insoluble ash (AIA) was used as the inert dietary marker and AIA concentrations were determined by the method of Siriwan et al. (1993). The apparent digestibility coefficients of IP_6 phytate and Na at four small intestinal sites were calculated from the following equation:

$$\text{apparent digestibility coefficient} = \frac{(IP_6/Na : AIA)_{diet} - (IP_6/Na : AIA)_{digesta}}{(IP_6/Na : AIA)_{diet}}$$

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Table 1
RVA starch pasting properties of unprocessed maize, sorghum and wheat (analyses completed in duplicate).

Grain	RVA starch pasting properties (cp)					Peak time (min)	Pasting temp. (°C)
	Peak	Holding	Breakdown	Final	Setback		
Maize	1107	622	485	1056	434	4.13	75.1
Sorghum	1371	503	868	1206	703	4.04	76.3
Wheat	996	467	529	939	472	5.33	84.3

Apparent disappearance rates of phytate and Na from the jejunum and ileum were calculated from their analysed dietary concentrations, feed intakes over the final 72 h of the feeding study and expressed on an average daily basis, and apparent digestibility coefficients were calculated at each of the four segments from the following equation:

$$\text{apparent disappearance rate (mg/bird/day)} = \text{phytate/Na dietary concentration (g/kg)} \times \text{daily feed intake (g/bird)} \times \text{phytate/Na digestibility coefficient.}$$

Experimental data were analysed using the IBM® SPSS® Statistics 20 program (IBM Corporation. Somers, NY USA). Statistical procedures included univariate analyses of variance using the general linear models procedure, linear regressions and Pearson correlations. In the 3 × 2 analysis of variance the main effects of the grain on which the diets were based and of phytase supplementation were considered and least significant differences were calculated when treatment interactions were observed. A probability level of less than 5% was taken as statistically significant.

3. Results

The RVA starch properties of the three grains are shown in Table 1. The setback viscosity of sorghum (703 cP) was noticeably higher than in maize (434 cP) and wheat (472 cP). The RVA starch properties of the three experimental diets, without and with phytase, are shown in Table 2. Significant grain × phytase interactions (P<0.05– < 0.001) were observed for all RVA parameters. The effects of phytase on starch viscosities were more pronounced in wheat-based diets with uniformly significant reductions across the five measurements including a reduction in final viscosity of 15.0% (753 *versus* 886 cP). Phytase significantly decreased peak, breakdown and setback viscosities in maize-based diets and significantly increased peak time and pasting temperature. In contrast, phytase significantly increased peak and breakdown viscosities in sorghum-based diets. The differences between the three grain diets are of interest. Peak, breakdown, final and setback viscosities of wheat-based diets were significantly lower than in maize- and sorghum-based diets. Alternatively, peak time and pasting temperature were significantly higher in wheat-based diets in comparison to diets based on maize and sorghum.

The analysed phytate dietary concentrations in maize-, sorghum- and wheat based diets were 8.82, 8.74 and 7.70 g/kg, respectively. The corresponding analysed Na dietary concentrations were 1.18, 1.42 and 1.60 g/kg, respectively.

Table 2
Effects of grain type and phytase supplementation on RVA starch pasting properties of experimental diets (analyses completed in duplicate).

Treatment		RVA starch pasting properties (cp)					Peak time (min)	Pasting temp. (°C)
Grain	Phytase (FTU/kg)	Peak	Holding	Breakdown	Final	Setback		
Maize	0	1164d	474c	690d	967c	493c	4.04a	71.4a
	1000	997c	470c	527c	929bc	459b	4.37bc	75.0b
Sorghum	0	1144d	423b	721d	950c	528d	4.47c	77.5b
	1000	1313e	426b	888e	956c	530d	4.27b	75.6b
Wheat	0	889b	415b	474b	886b	471b	5.17d	85.9c
	1000	742a	317a	426a	753a	436a	5.24d	87.0c
SEM		22.2	11.7	12.7	12.7	5.01	0.032	0.842
Main effects: grain								
Maize		1080	472	608	948	476	4.20	73.2
Sorghum		1228	424	804	953	529	4.37	76.5
Wheat		815	366	450	819	454	5.20	86.5
Main effects: enzyme								
Nil		1065	437	628	934	497	4.56	78.3
1000		1017	404	613	879	475	4.62	79.2
Significance (P=)								
Grain (G)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Phytase (P)		0.038	0.013	0.208	0.002	0.002	0.029	0.206
G × P interaction		<0.001	0.009	<0.001	0.004	0.015	<0.001	0.049

abcde Means within columns not sharing a common suffix are significantly different (P<0.05).

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Table 3
The effects of grain type and phytase supplementation on apparent digestibility coefficients of phytate (IP_6) at four small intestinal sites in broiler chickens at 27 days post-hatch.

Treatment		Small intestinal segment			
Grain	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Maize	0	0.593	0.451	0.435b	0.349
	1000	0.839	0.768	0.681 cd	0.681
Sorghum	0	0.445	0.415	0.240a	0.128
	1000	0.747	0.643	0.609c	0.553
Wheat	0	0.507	0.403	0.261a	0.239
	1000	0.781	0.751	0.723d	0.660
SEM		0.045	0.043	0.036	0.034
Main effects: grain					
Maize		0.716b	0.609	0.558	0.515c
Sorghum		0.596a	0.529	0.425	0.340a
Wheat		0.644ab	0.577	0.492	0.449b
Phytase					
0[1]		0.515a	0.423a	0.312	0.238a
1000		0.789b	0.721b	0.671	0.631b
Significance (P=)					
Grain		0.045	0.166	0.002	<0.001
Phytase		<0.001	<0.001	<0.001	<0.001
Grain × Phytase interaction		0.826	0.338	0.013	0.263

abcd Means within columns not sharing a common suffix are significantly different (P<0.05).

[1] In non-phytase supplemented diets, phytate digestibility declines along the small intestine ($r = -0.384$; P<0.001).

The effects of grain type and phytase supplementation on apparent digestibility coefficients of phytate at four small intestinal sites in broiler chickens are shown in Table 3. There was a significant interaction (P<0.05) between grain type and phytase at the proximal ileum as phytate degradation was more pronounced in sorghum- and wheat- than in maize-based diets. As a main effect, phytase significantly increased (P<0.001) phytate degradation in the proximal jejunum (0.789 *versus* 0.515), distal jejunum (0.721 *versus* 0.423) and distal ileum (0.631 *versus* 0.238). There were significant differences in phytate digestibility between diets based on different grains at the proximal jejunum (P<0.05) and distal ileum (P<0.001). In the proximal jejunum the phytate digestibility coefficient was significantly greater in maize (0.716) than in sorghum (0.596) while digestibility in wheat-based diets (0.644) was statistically intermediate. Instructively, there were significant differences in phytate digestibility coefficients between each of the diets based on maize (0.515), wheat (0.449) and sorghum (0.340) at the distal ileum.

The effects of grain type and phytase supplementation on phytate disappearance rates from the jejunum and ileum are shown in Table 4 where there were no significant interactions between treatments. There were significant differences (P<0.05–< 0.001) in apparent disappearance rates of phytate between diet types in all four small intestinal segments. Phytate disappearance rates were significantly higher in diets based on maize than in sorghum- and wheat-based diets in proximal jejunum, proximal ileum and distal ileum and between maize and wheat at the distal jejunum. At the distal ileum, phytate disappearance in maize-based diets (459 mg/bird/day) was considerably higher than in sorghum (301 mg/bird/day) and wheat (343 mg/bird/day). Phytase generated significantly higher (P<0.001) phytate disappearance rates at all four small intestinal levels by factors of 1.54, 1.71 and 2.15 in the proximal and distal jejunum and proximal ileum, respectively. This culminated in an increase in phytate disappearance rates by a factor of 2.66 (535 *versus* 201 mg/bird/day) at the distal ileum.

The effects of grain type and phytase supplementation on apparent digestibility coefficients of Na are shown in Table 5. At the proximal jejunum there was a significant treatment interaction (P<0.001) because phytase significantly improved Na digestibility coefficients (−1.136 *versus* −2.942; P<0.05) in wheat but did not have a significant influence in maize- and sorghum-based diets. Phytase improved Na digestibility coefficients at the distal jejunum (−1.462 *versus* −1.120; P<0.005) and proximal ileum (−0.983 *versus* −0.609; P<0.001). At the distal ileum there was a significant effect of grain type (P<0.001) where Na digestibility in wheat-based diets (−0.499) was lower than in diets based on maize (−0.194) and sorghum (−0.098). Treatment effects on Na disappearance rates are shown in Table 6 where there were significant interactions in the three anterior small intestinal segments. These interactions stem from significant responses in Na disappearance rates induced by phytase in wheat-based diets, which was not the case with maize and sorghum. In the distal ileum there was a significant influence of grain type (P<0.001) on Na disappearance rates, which were more pronounced in wheat (−79 mg/bird/day) than in diets based on maize (−24 mg/bird/day) and sorghum (−13 mg/bird/day). Phytase did not influence Na disappearance rates (P>0.20) in the distal ileum. It is evident in Tables 5 and 6 that both Na digestibility coefficients and disappearance rates are negative at the proximal jejunum and remain negative at the distal ileum but the magnitude of this negativity diminishes along the jejunum and ileum. Across all treatments, the average Na digestibility coefficient ranged from −2.495

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Table 4
The effects of grain type and phytase supplementation on apparent disappearance rates (mg/bird/day) of phytate (IP_6) at four small intestinal sites in broiler chickens from 7 to 27 days post-hatch.

Treatment		Small intestinal segment (mg/bird/day)			
Grain	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Maize	0	509	383	371	299
	1000	755	700	621	619
Sorghum	0	393	373	218	116
	1000	658	563	533	487
Wheat	0	403	312	203	187
	1000	502	568	546	499
SEM		46.0	40.4	32.5	31.7
Main effects: grain					
Maize		632b	542b	496b	459b
Sorghum		525a	468ab	376a	301a
Wheat		498a	440a	374a	343a
Phytase					
0		435a	356a	264a	201a
1000		668b	610b	567b	535b
Significance (P=)					
Grain		0.021	0.047	0.001	<0.001
Phytase		<0.001	<0.001	<0.001	<0.001
Grain × Phytase interaction		0.705	0.310	0.350	0.600

ab Means within columns not sharing a common suffix are significantly different (P<0.05).

at the proximal jejunum to −0.264 at the distal ileum and the corresponding range in Na disappearance rates was from −344 to −39 mg/bird/day.

Pearson correlations between apparent digestibility coefficients of phytate and Na at four small intestinal segments are shown in Table 6. It is noteworthy that there were significant correlations between digestibility coefficients in the distal jejunum ($r = 0.493$; P<0.005) and proximal ileum ($r = 0.578$; P<0.001). These correlations suggest that the digestibility of phytate and Na, as influenced by phytase, are linked.

The effects of grain type and phytase on apparent starch digestibility coefficients in four small intestinal segments have already been reported (Liu et al., 2014). In relation to RVA starch properties, there were significant negative correlations between setback viscosity of grains *per se* and starch digestibility coefficients at the distal jejunum ($r = -0.539$; P<0.025) and proximal ileum ($r = -0.755$; P<0.001). The regression equation in the proximal ileum is as follows: $y_{(\text{starch digestibility coefficient})} = 1.037 - 0.000256 \times \text{setback viscosity}_{(cP)}$.

Table 5
The effects of grain type and phytase supplementation on apparent digestibility coefficients of sodium at four small intestinal sites in broiler chickens at 27 days post-hatch.

Treatment		Small intestinal segment			
Grain	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Maize	0	−2.936a	−1.392	−0.843	−0.143
	1000	−2.705a	−1.270	−0.588	−0.244
Sorghum	0	−2.522a	−1.455	−0.840	−0.137
	1000	−2.729a	−1.163	−0.591	−0.059
Wheat	0	−2.942a	−1.540	−1.267	−0.616
	1000	−1.136b	−0.929	−0.647	−0.382
SEM		0.245	0.135	0.101	0.097
Main effects: grain					
Maize		−2.820	−1.331	−0.716	−0.194b
Sorghum		−2.625	−1.309	−0.716	−0.098b
Wheat		−2.039	−1.234	−0.957	−0.499a
Phytase					
0		−2.800	−1.462a	−0.983a	−0.299
1000		−2.190	−1.120b	−0.609b	−0.228
Significance (P=)					
Grain		0.009	0.758	0.054	<0.001
Phytase		0.005	0.004	<0.001	0.377
Grain × Phytase interaction		<0.001	0.202	0.172	0.239

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Table 6
The effects of grain type and phytase supplementation on apparent disappearance rates (mg/bird/day) of sodium at four small intestinal sites in broiler chickens from 7 to 27 days post-hatch.

Treatment		Small intestinal segment (mg/bird/day)			
Grain	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Maize	0	−331b	−157c	−96a	−18
	1000	−329b	−154c	−71a	−29
Sorghum	0	−367b	−208ab	−120a	−19
	1000	−388ab	−166bc	−84a	−8
Wheat	0	−470a	−246a	−202b	−98
	1000	−176b	−143c	−100a	−60
SEM		34.6	16.6	14.5	13.0
Main effects: grain					
Maize		−330	−156	−83	−24a
Sorghum		−378	−187	−102	−13a
Wheat		−323	−194	−151	−79b
Phytase					
0		−389	−204	−139	−45
1000		−298	−154	−85	−32
Significance (P=)					
Grain		0.240	0.060	<0.001	<0.001
Phytase		0.003	0.001	<0.001	0.247
Grain × phytase interaction		<0.001	0.019	0.026	0.183

abc Means within columns not sharing a common suffix are significantly different (P<0.05).

4. Discussion

In the present study phytase increased the distal ileal phytate digestibility coefficient from 0.238 to 0.631. This indicates that 63.1% of dietary phytate was degraded overall but 23.8% degradation was observed in non-supplemented diets, presumably as a consequence of endogenous mucosal phytase activity, and the balance of 39.3% dietary IP_6 was degraded by exogenous phytase. On the same basis, phytate degradation was less in the proximal jejunum (27.4%), distal jejunum (29.8%) and proximal ileum (35.9%). However, the distal ileal 39.3% net degradation of phytate compares favourably with comparable assessments of ileal phytate digestibility involving dietary markers. Using a fungal phytase at 500 FTU/kg, Camden et al. (2001) reported 29.3% phytate degradation and Tamim et al. (2004) reported 33.5% phytate degradation in a similar experiment. Subsequently, using a bacterial phytase at 1000 FTU/kg, Amerah et al. (2014) reported a mean phytate degradation of 35.1% by over a range of dietary Ca:available P ratios.

Exogenous phytase appeared to generate more net degradation of phytate in sorghum (42.5%) and wheat (42.1%) at the distal ileum than in maize-based diets (33.2%), which does not support the hypothesis that phytase degraded more phytate in maize-based diets. Alternatively, the IP_6 distal ileal digestibility coefficient in maize-based diets (0.515) without and with phytase was significantly higher than diets based on wheat (0.499) and sorghum (0.340). This indicates that IP_6 in maize was more readily hydrolysed by both endogenous and exogenous phytate-degrading enzymes than in wheat and sorghum. Phytate is located mainly in the germ of maize but in aleurone layers of wheat and sorghum (O'Dell et al., 1972; Doherty et al., 1982) thus phytate in maize may be more accessible to phytate-degrading enzymes due to its location. Phytase supplementation of wheat-based diets has been shown to be more effective when combined with an NSP-degrading enzyme

Table 7
Pearson correlations (*probability values in parentheses*) between apparent digestibility coefficients of phytate (Phy) and sodium (Na) at proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI) in broiler chickens at 27 days post-hatch.

	Phy PJ	Phy DJ	Phy PI	Phy DI	Na PJ	Na DJ	Na PI	Na DI
Phy PJ	1.000							
Phy	0.686	1.000						
DJ	*(<0.001)*							
Phy	0.794	0.860	1.000					
PI	*(<0.001)*	*(<0.001)*						
Phy	0.820	0.826	0.948	1.000				
DI	*(<0.001)*	*(<0.001)*	*(<0.001)*					
Na	0.247	0.450	0.402	0.356	1.000			
PJ	*(0.173)*	*(0.006)*	*(0.015)*	*(0.033)*				
Na	0.411	0.493	0.608	0.542	0.398	1.000		
DJ	*(0.020)*	*(0.002)*	*(<0.001)*	*(0.001)*	*(0.016)*			
Na	0.376	0.449	0.578	0.560	0.161	0.648	1.000	
PI	*(0.034)*	*(0.006)*	*(<0.001)*	*(<0.001)*	*(0.349)*	*(<0.001)*		
Na	−0.009	0.106	0.146	0.066	−0.242	0.267	0.556	1.000
DI	*(0.960)*	*(0.540)*	*(0.395)*	*(0.704)*	*(0.155)*	*(0.115)*	*(<0.001)*	

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and synergistic responses have been recorded (Ravindran et al., 1999). Possibly, degradation of non-starch polysaccharides in the fibrous aleurone layer of wheat by an NSP-degrading enzyme provided phytase with greater substrate access. The proposal that phytate in maize may be more readily degraded than phytate in sorghum and wheat by phytases because of its location may be valid but does not appear to account for the more robust exogenous phytase responses observed in maize-based diets.

Apparent phytate digestibility coefficients declined along the four segments of the small intestine especially in non-phytase supplemented diets (0.515, 0.423, 0.312, 0.238) and there are two possible explanations for these observations. First, phytate may have become less susceptible to degradation by endogenous phytase as it transited the small intestine and, second, phytate may have accumulated in small intestinal digesta. In chickens, the highest activity of mucosal phytase in the small intestinal brush border membrane is in the duodenum but then progressively declines along the gut (Maenz and Classen, 1998). Thus phytate digestibility stabilises along the small intestine with declining mucosal phytase activity which may be coupled with Ca-phytate interactions which are dependent on gut pH (Selle et al., 2009a). In 10.7 g/kg Ca diets, Shafey et al. (1991) found pH values of 4.89 in the crop, 1.98 in the proventriculus and 3.14 in the gizzard but these pH values increased to 5.53 in the duodenum, 6.06 in the jejunum and 6.62 in the ileum. This elevation in pH profoundly reduces the solubility of Ca-phytate mineral complexes (Grynspan and Cheryan, 1983). With the elevation in pH along the small intestine the isoelectric point (p*I*) of proteins is exceeded and proteins become negatively charged and may be bound by phytate as ternary protein-phytate complexes *via* Ca^{2+}cationic bridges, which begins to take place in excess of pH 5 *in vitro* (Prattley et al., 1983). Relatively insoluble mineral-phytate and ternary protein-phytate complexes should reduce the vulnerability of phytate to enzymic hydrolysis by endogenous phytase in the jejunum and ileum. Secondly, Sooncharernying and Edwards (1993) reported a marked increase in the concentration of inositol hexaphosphate in the small intestine of chickens as other nutrients in the diet were digested. A progressive accumulation of increasingly less digestible phytate in small intestinal digesta as more readily digestible nutrients were being absorbed may have contributed to the declining phytate digestibility coefficients in the present study.

Ravindran et al. (2006) were probably the first to investigate the impacts of phytate and phytase on apparent ileal digestibility (AID) coefficients of sodium in poultry. These researchers reported that increasing dietary phytate levels from 10.0 to 13.6 g/kg significantly depressed AID of Na (−0.379 *versus* −0.237; P<0.05). Reciprocally, 1000 FTU/kg exogenous phytase significantly increased Na digestibility (−0.177 *versus* −0.515; P<0.001). These negative values indicate a net transition of Na of endogenous origin into the gut lumen at the terminal ileum. Subsequently, Selle et al. (2009b) reported that 500 FTU/kg phytase improved Na digestibility (−0.52 to −0.04; P<0.05) at the terminal ileum in broiler chickens but average total tract retention of Na was positive at 0.435. Thus the transition of Na into the small intestinal lumen was recovered by Na absorption in the large intestine.

In the present study the average ileal Na digestibility coefficient in the distal ileum was −0.229; however, remarkably lower Na digestibility coefficients were recorded in the three anterior segments of the small intestine including −2.459 in the proximal jejunum. Probably the main source of this substantial endogenous Na flow is as Na bicarbonate entering the duodenum in outputs from the pancreas, liver and stomach where the Na bicarbonate component of pancreatic juice may hold most importance (Carlsson et al., 1970). The primary purpose of Na bicarbonate is to buffer gastric secretions of hydrochloric acid (Allen and Flemstrom, 2005). It has been proposed by Selle et al. (2012) that phytate increases gastric secretions of pepsin and HCl as a compensatory mechanism because protein bound in binary protein-phytate complexes are refractory to pepsin digestion (Vaintraub and Bulmaga, 1991; Yu et al., 2012). If this is the case, phytase would be expected to attenuate the secretion of HCl and, in turn, Na bicarbonate.

It is evident that the substantial endogenous flows of Na entering the proximal jejunum are largely, if not completely, recovered by the end of the small intestine as overall Na disappearance rates were reduced from −344 mg/bird/day in the proximal jejunum to −39 mg/bird/day in the distal ileum. Thus Na is absorbed in the jejunum and ileum and this is amplified by phytase. Importantly, Na absorption in the jejunum and ileum involves co-transport of Na with glucose or amino acids from the gut lumen into mucosal enterocytes, which is mediated by several Na^+-dependent transport systems including the sodium-glucose linked transporter-1 (SGLT-1) and various amino acid transporters (Wright, 1993; Wright and Loo, 2000; Zhao and Keating, 2007; Broer, 2008). The implication is when phytase enhances jejunal and ileal Na absorption the enzyme is also promoting absorption of glucose and amino acids although the underlying mechanisms for the phytase-induced enhancement of Na absorption are not clear. Nevertheless, the role of the Na^+,K^+-ATPase, or the sodium pump, in the baso-lateral membrane of enterocytes, is almost certainly pivotal to the intestinal uptakes of Na and co-transported nutrients because the sodium pump maintains electrochemical gradients across the gut mucosa (Therein and Blostein, 2000). However, it is relevant that Liu et al. (2008) reported that 1000 FTU/kg phytase in broiler diets containing 2.2 g/kg phytate-P significantly increased concentrations of Na^+,K^+-ATPase in jejunal mucosa (13.59 *versus* 11.48 μmol/mg) with an associated increase in glucose concentrations (13.73 *versus* 9.35 μmol/mg). When data generated by Liu et al. (2014) is considered, it is noteworthy that proximal ileal Na digestibility coefficients were postively correlated to weight gain ($r = 0.465$; P<0.005) and negatively correlated to FCR ($r = -0.527$; P<0.005). Moreover, distal ileal Na digestibility coefficients were negatively correlated with FCR ($r = -0.610$; P<0.001) as shown in Fig. 1.

In the present study, analysed concentrations for diets based on maize, sorghum and wheat were 1.18, 1.42 and 1.60 g/kg Na, respectively. These discrepancies are noteworthy and presumably are a reflection of differences in inherent Na concentrations betweeen the three grains. The relatively high Na content in wheat-based diets may have contributed to the differences observed with Na digestibility coefficients and disapperance rates relative to diets based on maize and sorghum.

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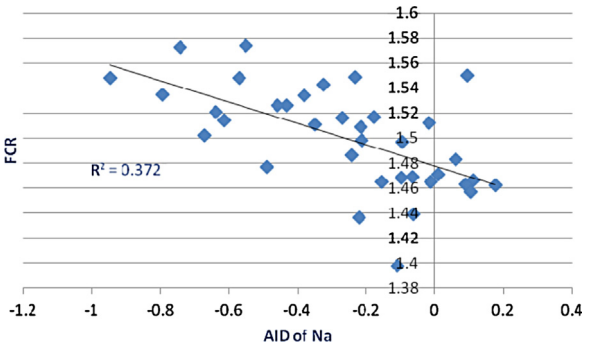

Fig. 1. Linear relationship ($r = -0.610$; P<0.001) between distal ileal Na digestibility and feed conversion ratios (FCR) of broilers from 7 to 27 days post-hatch. Linear regression equation: $y_{(FCR,\ g/g)} = 1.478 - 0.085 \times$ AID Na].

Also, the most robust phytase responses were observed on maize-based diets with the least adequate Na levels. While the treatment interaction was not significant (P=0.174), phytase improved FCR by 4.02% (1.457 *versus* 1.518) in maize-based diets but responses diminished to 0.61% (1.466 *versus* 1.475) in diets based on sorghum and to 0.78% (1.520 *versus* 1.532) in wheat-based diets. Although speculative, it appears that the relatively low Na dietary concentration in maize-based diets may have contributed to the more robust phytase responses observed. Coupled with this, the observation that phytate in maize, without and with exogenous phytase, was more readily degraded than in sorghum- and wheat-based diets remains.

Pearson correlations between apparent digestibility coefficients of phytate, sodium, starch and nitrogen at four small intestinal sites assume relevance (Table 7). These observations are consistent with the premise that the absorption of Na is linked with both glucose and amino acids.

RVA starch pasting properties are important to the human food industry but few investigations into their possible relevance to animal nutrition have been completed. However, Doucet et al. (2010) found strong negative correlations between final and peak RVA starch viscosities of diets offered to weaner pigs and starch digestibility at the mid-point of the small intestine. These researchers did not report on RVA setback viscosities of dietary starch. Interestingly, setback viscosity is indicative of starch retrogradation, which is the process whereby molecular chains of polymerised glucose in gelatinised starch reassociate as an ordered crystalline structure (Atwell et al., 1988; Shewayrga et al., 2012). In the present study there were significant negative correlations between setback viscosity of starch and coefficients of starch digestibility at the distal jejunum and proximal ileum. Additionally, there was a strong negative correlation between setback viscosity of grains *per se* and starch digestibility coefficients at proximal ileum ($r = -0.755$; P<0.001). In this segment, the digestibility of starch in maize-based diets (0.947) was superior to diets based on wheat (0.911) and, in turn, sorghum (0.867) and sorghum grain clearly had the highest setback viscosity. The *in vitro* digestibility of sorghum starch has been shown to be inferior to that of maize and wheat (Giuberti et al., 2012) and this was the case in broilers chickens in the Liu et al. (2014) study. It is possible that there is a greater propensity for starch to undergo retrogradation following steam-pelleting and cooling sorghum-based diets and that this may contribute to the relatively poor digestibility of sorghum starch in relevant contexts. It appears that *in vitro* RVA starch pasting properties may provide an indication of starch digestibility in pigs and poultry.

Interestingly, phytase influenced RVA starch properties, especially in wheat-based diets, where phytase significantly reduced all RVA viscosity parameters. This could imply that direct or indirect starch–phytate interactions occurred during the *in vitro* determinations but these were suppressed by phytase. The setback viscosity is the difference between final and holding (or trough) viscosites of starch (Batey, 2007). The setback viscosity of sorghum was noticeably higher than wheat in the present study which is consistent with the report of Ragaee and Abdel-Aal (2006) where grain sorghum had a setback viscosity of 1307 cp, which was noticeably higher than rye and barley.

Conflict of interest statement

None.

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