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Effects of phytase supplementation on growth performance, nutrient utilization and digestive dynamics of starch and protein in broiler chickens offered maize-, sorghum- and wheat-based diets

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ABSTRACT

To investigate the effects of phytase supplementation on growth performance, nutrient utilization and digestive dynamics of starch and protein, a study was conducted using 288 male Ross 308 chicks (6 treatments with 8 replicate cages of 6 birds). Birds were offered steam-pelleted diets based on maize, sorghum or wheat, without or with phytase supplementation, from 7 to 27 days post-hatch. Experimental diets were formulated to be equivalent for energy, protein/amino acids and were P-adequate. Digesta samples from proximal jejunum, distal jejunum, proximal ileum and distal ileum were collected in their entirety at day 27. Digestion rates of starch and protein were determined by fitting exponential mathematical model to apparent digestibility coefficients with mean retention times in each small intestinal segment. The growth performance of birds offered maize and sorghum were comparable but those offered wheat-based diets were inferior. Phytase improved weight gain ($P<0.001$), feed intake ($P<0.001$) and feed conversion ($P<0.05$) in maize-, sorghum- and wheat- based diets, although the most pronounced improvements tended to be for maize. There were grain type-phytase interactions ($P<0.01$) for nutrient utilization (AME, N retention, AMEn) where substantial phytase responses were observed for maize but not for sorghum- and wheat-based diets. Phytase did not influence digestion rates of starch and protein ($P>0.05$), but it significantly increased disappearance rates of starch in maize-based diets ($P<0.05$). In conclusion, phytase improved weight gain and feed conversion efficiency in maize-, sorghum- and wheat-based diets with more pronounced response in maize-based diets. Moreover, phytase also significantly enhanced nutrient utilization in maize-based diets.

Abbreviations: AME, apparent metabolizable energy; AMEn, nitrogen-corrected apparent metabolizable energy; AIA, acid insoluble ash; FCR, feed conversion ratio; FI, feed intake; MRT, mean retention time; N, nitrogen; PDN, potential digestible nitrogen; PDS, potential digestible starch.

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Table 1
Characteristics of maize, wheat, sorghum (as-is).

Item (g/kg)	Maize	Sorghum	Wheat
Dry matter	119	128	116
Protein	82.9	82.4	113
Calcium	0.034	0.069	0.309
Total phosphorus	2.33	2.9	2.37
Phytate phosphorus	1.74	2.4	1.77
Starch	636	614	593
Fat	33.1	34.0	16.3

1. Introduction

Phytate (*myo*-inositol hexaphosphate; IP_6) is a ubiquitous component of human foods and animal feedstuffs of plant origin. Dependent on context, phytate is considered to possess both positive and negative properties in human nutrition (Harland and Morris, 1995); whereas, the anti-nutritive properties of phytate are well recognized in pig and poultry nutrition (Selle and Ravindran, 2007, 2008). As a consequence, the inclusion of phytate-degrading enzymes in broiler diets is an increasingly routine practice to facilitate sustainable chicken-meat production.

Efficient feed conversion, which may be quantified by feed conversion ratios (FCR), is probably the most important parameter. The relationship between feed conversion efficiency and digestive dynamics was demonstrated by Batterham (1974) and Batterham and O'Neill (1978). Improvements in FCR in grower pigs following free lysine supplementation were more pronounced when diets were offered continuously (six times – daily) than on a restricted (once – daily) basis. Lysine HCl is rapidly absorbed in comparison to protein-bound amino acids, including lysine, because the latter requires prior digestion in the gut lumen. Continuous feeding may accommodate the potential imbalance between free and protein-bound lysine at sites of protein synthesis. Hence, increasing attention is being paid to the influence of digestive dynamics of starch and protein on growth performance (Weurding et al., 2003a; van den Borne et al., 2007; Drew et al., 2012; Kim et al., 2013; Liu et al., 2013a) in pigs and poultry because efficient muscle protein deposition requires synchronous availability of both glucose and the full complement of amino acids (Pelley and Goljan, 2011). Kim et al. (2013) showed that digestion rates of starch and protein from different dietary sources significantly influenced N retention in growing pigs. Usually, starch is digested more rapidly and completely than protein (Liu et al., 2013b). It is a challenge to synchronize the availability of glucose and amino acids at the sites of protein synthesis. Very few studies have explored the importance of small intestinal digestive dynamics of starch and protein in relation to feed conversion efficiency in broiler chickens.

Presently, exogenous phytase is routinely included in poultry diets as they effectively represent economical sources of phosphorus. The enzymatic dephosphorylation of phytate liberates phosphorus moieties, thereby increasing phosphorus digestibility. Phytate may form binary or ternary complexes with protein depending on the isoelectric point of protein and gut pH (Selle and Ravindran, 2007). Phytate may also influence starch digestion by directly binding starch or indirectly by complexing with starch granule-associated protein and/or inhibiting amylase activity (Oatway et al., 2001). Hence, the extra-phosphoric effects of phytase may influence starch and protein digestion in broiler chickens.

Maize, wheat and sorghum are the three commonly used grains in broiler diets. There may be variations in phytate concentrations in maize, sorghum and wheat (Selle et al., 2003). Moreover, phytate is located in the germ of maize but in the aleurone layers of sorghum and wheat (O'Dell et al., 1972; Doherty et al., 1982). Therefore, the response to phytase may differ in maize-, sorghum- and wheat-based broiler diets. The intention of this study was to determine the effects of phytase on efficiency of growth performance, nutrient utilization and digestive dynamics of starch and protein in maize-, sorghum- and wheat-based diets offered to broiler chickens with the hypothesis that phytase would improve these parameters irrespective of the type of grain on which the diets were based.

2. Materials and methods

2.1. Diet preparations

The feeding study comprised six dietary treatments with three grain varieties (wheat, sorghum and maize) without and with a *Buttiauxella* phytase produced in *Trichoderma reesei* (Axtra® PHY; Danisco Animal Nutrition) at 1000 FTU/kg. The nutritional characteristics of the three grains are documented in Table 1. The experimental diets were based 560 g/kg maize, sorghum or wheat and were formulated to be equivalent for energy density (12.54 MJ/kg) protein (205 g/kg) and amino acids. The formulated, calculated and analyzed nutrient compositions of the three diets are shown in Table 2. Acid insoluble ash (Celite™ World Minerals, Lompoc, CA, USA) was included in the diets at 20 g/kg as an inert marker to determine nutrient digestibility coefficients at four small intestinal sites. The three grains were hammer-milled through a 3.2 mm screen prior to dietary incorporation. The particle size distribution of milled grains was determined by sieving 100 g sample through 250 μm, 500 μm, 1000 μm, 1500 μm and 2000 μm screen for 5 min. The sieved material obtained, for each sieve fraction size and weighed. The patterns of particle size distribution are shown in Fig. 1. The diets were steam-pelleted through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) at a conditioning temperature of 80 °C

Table 2
Dietary formulation and calculated nutrient specifications of diets based on maize, sorghum and wheat for broiler chicken from 7 to 27 days post-hatch.

	Maize	Sorghum	Wheat
Ingredient composition (g/kg)			
Cereal	560	560	560
Soybean meal	283	309	259
Canola meal	67.0	38.0	80.0
Vegetable oil	32.0	34.0	39.0
Limestone	7.8	7.5	11.7
Dicalcium phosphate	17.7	17.8	17.5
Sodium chloride	2.1	0.9	1.7
Sodium bicarbonate	2.6	4.5	3.7
Lysine HCL	2.3	2.5	2.5
Methionine	2.5	3.0	2.3
Threonine	0.8	0.9	0.9
Tryptophan	0.2	0.0	0.0
Valine	0.1	0.2	0.0
Vitamin-mineral premix[a]	2.0	2.0	2.0
Celite™	20.0	20.0	20.0
Calculated nutrient composition (g/kg)			
Metabolizable energy (MJ/kg)	12.5	12.5	12.6
Crude protein	201	202	212
Calcium	8.0	8.0	8.0
Total P	7.0	7.2	6.9
Non phytate-P	4.5	4.5	4.4
Lysine	12.1	12.0	12.1
Methionine	5.5	5.8	5.3
Cysteine	3.4	3.2	3.7
Threonine	8.3	8.2	8.3
Tryptophan	2.6	2.5	2.7
Valine	9.7	9.8	9.8
Arginine	11.7	12.0	11.8
Sodium	1.8	1.8	1.8
Potassium	8.2	8.4	8.1
Chloride	2.1	2.1	2.1
Dietary electrolyte balance (meq/kg)	226	255	233
Analyzed nutrient composition (g/kg)			
Starch	410	411	404
Protein	234	235	241
Fat	67.1	67.6	64.2
Total P	6.92	7.16	6.88
Phytate-P	2.49	2.47	2.17
Non phytate-P	4.43	4.69	4.71
Calcium	10.4	10.2	11.2
Sodium	1.18	1.42	1.60

[a] The vitamin–mineral premix supplied per ton of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.

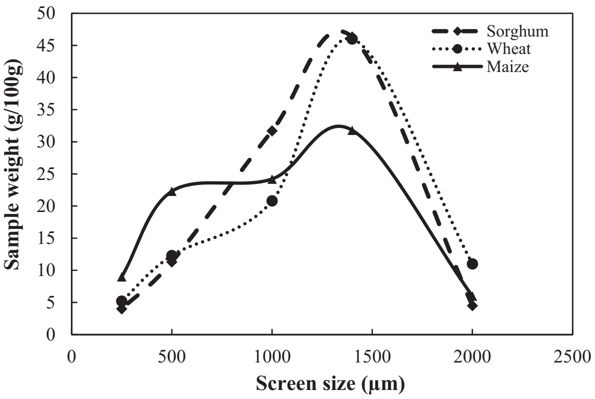

Fig. 1. Particle size distribution of hammer-milled (3.2 mm) maize, sorghum and wheat.

by the automatically controlled introduction of steam into the conditioner with a residence time of 14 s. The conditioning temperature was recorded by thermal probes at the exit of the conditioner, before the diet entered the pellet press with die dimensions of 4 mm in diameter and 45 mm in length. Finally, the diets were cooled in a vertical cooler to room temperature.

2.2. Bird management

This feeding study complied with the guidelines (N00/6-2013/1/5981) approved by the Animal Ethics Committee of the University of Sydney. A proprietary starter diet was offered to 288 male day-old chicks (Ross 308) to six days post-hatch. Then, the chickens were identified (wing-tags), individually weighed and allocated into bioassay cages on the basis of body weight in an environmentally-controlled facility. Each of the six dietary treatments was offered to eight replicate cages (6 birds per cage) 7 to 27 days post-hatch and broilers had unlimited access to water and feed under a '23-hour-on-1-hour-off' lighting regimen. An initial room temperature of 32 ± 1 °C was maintained for the first week, gradually decreased to 22 ± 1 °C by the end of the third week and maintained at the same temperature until the end of the feeding study. Initial and final body weights were determined, feed intakes were recorded from which feed conversion ratios (FCR) were calculated. The incidence of dead or culled birds was recorded daily and their body-weights used to adjust FCR calculations.

2.3. Sample collection and chemical analysis

Feed intakes were recorded and total excreta collected from days 25–27 to generate data for parameters of nutrient utilization [apparent metabolizable energy (AME; MJ/kg and MJ/day), N retention, N-corrected AME (AME_n)] on a dry matter basis. Excreta were air-forced oven dried for 24 h at 80 °C. The gross energy (GE) of diets and excreta were determined by bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL).

At day 27, all birds were euthanized by intravenous injection of sodium pentobarbitone and the gizzards and pancreas were removed, cleaned, individually weighed and relative weights were calculated from the absolute mass and their corresponding final body weight, then the cage average of relative organ weights were used in statistical analysis. Prior to emptying gizzards of digesta a pH meter was inserted into the gizzard and measurements were recorded when values stabilized. The small intestine was removed and digesta samples were collected in their entirety from the proximal jejunum, distal jejunum, proximal ileum and distal ileum, which were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction and their mid-points. Feed intake over 24 h before sampling was recorded. Samples from birds within a cage were pooled, homogenized, freeze-dried and weighed to determine mean retention time (MRT) and apparent digestibility of starch, N, fat and energy. Starch concentration in diets and digesta were determined by a procedure based on dimethyl sulfoxide, α-amylase and amyloglucosidase, as described by Mahasukhonthachat et al. (2010). Nitrogen concentrations and AIA concentrations were determined as outlined by Siriwan et al. (1993). Protein concentrations were calculated by nitrogen concentrations times the factor of 6.25. Fat content in the diet and digesta was determined by the automated Soxhlet extraction described in Luque de Castro and Priego-Capote (2010).

Toe samples were obtained by severing the middle toe through the joint between the 2nd and 3rd tarsal bones from the distal end, for toe ash measurements. The left and right middle toes of the birds were pooled separately to yield two samples of toes per pen. These were averaged for the statistical analysis of the toe ash data. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 550 °C for 16 h.

2.4. Calculations

AME, N retention and AMEn values were calculated by the use of standard procedures and the following equations.

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

$$\text{N retention}\,(\%) = \frac{(\text{Feed intake} \times \mathrm{N_{diet}}) - (\text{Excreta output} \times \mathrm{N_{excreta}})}{(\text{Feed intake} \times \mathrm{N_{diet}})} \times 100$$

Apparent metabolizable energy intakes (MJ/day) were calculated from dietary energy densities and average daily feed intakes over the entire feeding period. N-corrected AME (AMEn MJ/kg) values were calculated by correcting to zero N retention, by using the factor of 36.54 kJ/g N retained in the body (Hill and Anderson, 1958).

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

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

Mean retention time was calculated using the following equation:

$$\mathrm{MRT(min)} = \frac{1440 \times \mathrm{AIA_{digesta}} \times W}{\mathrm{FI_{24\,h}} \times \mathrm{AIA_{feed}}}$$

Table 3
Effect of grain type and phytase supplementation on growth performance, relative organ weights and nutrient utilization in broiler chickens at 27 days post-hatch.

Treatment		Growth performance			Relative organ weights		Nutrient utilization			
Grain	Phytase (FTU/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Gizzard (g/kg)[1]	Pancreas (g/kg)[1]	AME (MJ/kg)[2]	AME (MJ/day)[3]	N retention (%)	AMEn (MJ/kg)[2]
Maize	Nil	1235	1871	1.518	19.61	3.07	12.235^{b}	1.145^{c}	61.97^{b}	11.026^{b}
	Plus	1387	2018	1.457	19.45	2.80	12.820^{a}	1.294^{a}	65.40^{c}	11.488^{a}
Sorghum	Nil	1316	1939	1.475	19.37	3.02	12.304^{b}	1.193^{bc}	60.99^{b}	11.001^{b}
	Plus	1359	1993	1.466	18.62	2.58	12.164^{b}	1.212^{b}	61.67^{b}	10.842^{b}
Wheat	Nil	1226	1879	1.532	15.86	2.68	12.185^{b}	1.144^{c}	60.46^{b}	10.846^{b}
	Plus	1281	1946	1.520	16.08	2.58	12.052^{b}	1.173^{bc}	57.40^{a}	10.791^{b}
SEM		24.529	28.405	0.016	0.360	0.081	0.112	0.019	0.957	0.100
Main effect: Grain										
Maize		1311^{a}	1945	1.487^{b}	19.53^{a}	2.94^{a}	12.528	1.219	63.68	11.257
Sorghum		1338^{a}	1966	1.471^{b}	18.99^{a}	2.80^{a}	12.234	1.202	61.33	10.922
Wheat		1254^{b}	1912	1.526^{a}	15.97^{b}	2.63^{b}	12.119	1.159	58.93	10.818
Main effect: Phytase										
Nil		1259^{b}	1896^{b}	1.508^{a}	18.28	2.92^{a}	12.241	1.161	61.14	10.958
Plus		1342^{a}	1986^{a}	1.481^{b}	18.05	2.65^{b}	12.345	1.226	61.49	11.040
P-value										
Grain		0.004	0.181	0.002	<0.001	0.003	0.002	0.013	<0.001	<0.001
Enzyme		<0.001	<0.001	0.034	0.440	<0.001	0.263	<0.001	0.658	0.318
Interaction		0.058	0.226	0.174	0.415	0.114	0.003	0.004	0.006	0.007

Means within columns not sharing common superscripts (a, b, c) are significantly different ($P<0.05$).

[1] g/kg body weight.

[2] MJ/kg dry matter.

[3] AME intake (MJ/day) were calculated from dietary energy densities and average daily feed intakes over the entire feeding period.

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

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

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

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

The disappearance rates of starch and nitrogen were calculated from the equation as below,

$$\text{Disapperance rate (g/h)} = \frac{FI_{24\,h} \times \text{Nutrient content}_{diet} \times AID}{24}$$

where $FI_{24\,h}$ is the 24 h feed intake before euthanisation (g/bird); $\text{Nutrient content}_{diet}$ is the starch or nitrogen concentration in the diets (g/g); AID is the apparent digestibility coefficients of starch or nitrogen at the distal ileum (g/g).

2.5. Statistical analysis

Two-way ANOVA was employed to determine the main effects (grain type and phytase) and their interaction by a general linear model procedure using SPSS® IBM Statistics 20 software program (IBM Corporation, Somers, NY, USA). The experimental units were cage means. Differences were considered significant at $P<0.05$. Multiple regression analyses and contour plot were carried out by using the JMP® 9.0.0 (SAS Institute Inc., JMP Software, Cary, NC).

3. Results

The overall mortality from 7 to 27 days post-hatch was 4.17% which was not influenced by dietary treatments ($P>0.75$). Table 3 showed the effects of grain type and phytase supplementation on growth performance, nutrient utilization and relative organ weights at 27 days post-hatch. Maize- and sorghum-based diets were associated with higher weight gain and better feed conversion efficiency than wheat-based diets ($P<0.01$). Phytase increased weight gain ($P<0.001$) and feed intake ($P<0.001$) and feed conversion ratio ($P<0.05$). There was a trend that responses in weight gain and feed conversion ratio to phytase were more pronounced in maize-based diets. Maize- and sorghum-based diets had higher relative gizzard and pancreas weight than wheat-based diets ($P<0.01$). Phytase reduced relative pancreas weight ($P<0.001$). There were

Table 4
Effect of grain type and phytase supplementation on gizzard pH, toe ash and retention time in proximal jejunum, distal jejunum, proximal ileum and distal ileum in broiler chickens at 27 days post-hatch.

Treatment		Retention time (min)					Gizzard pH	Toe ash (%)
Grain	Phytase	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total		
Maize	Nil	33.2	74.8^{a}	75.3	84.2^{a}	267.6^{a}	3.52	11.48
	Plus	30.3	58.8^{b}	70.4	61.2^{bc}	220.7^{b}	3.49	12.02
Sorghum	Nil	35.9	54.6^{b}	63.4	78.5^{a}	232.4^{ab}	3.30	11.68
	Plus	30.2	62.5^{ab}	66.8	72.1^{ab}	231.6^{ab}	3.78	12.29
Wheat	Nil	30.7	58.7^{b}	58.7	52.1^{c}	200.2^{b}	3.94	11.51
	Plus	46.2	61.4^{b}	64.4	60.5^{bc}	232.6^{ab}	4.03	11.72
	SEM	5.63	4.62	4.95	5.00	14.77	0.207	0.208
Main effect								
Grain	Maize	31.7	66.8	72.9	72.7	244.1	3.51	11.75
	Sorghum	33.1	58.6	65.1	75.3	232.0	3.54	11.99
	Wheat	38.5	60.0	61.6	56.3	216.4	3.99	11.61
Enzyme	Nil	33.3	62.7	65.8	71.6	233.4	3.59	11.56^{b}
	Plus	35.6	60.9	67.2	64.6	228.3	3.77	12.01^{a}
P-value	Grain	0.461	0.190	0.083	0.001	0.188	0.050	0.212
	Enzyme	0.628	0.650	0.737	0.099	0.680	0.291	0.013
	Interaction	0.145	0.041	0.537	0.013	0.039	0.448	0.592

Means within columns not sharing common superscripts (a, b, c) are significantly different ($P<0.05$).

interactions between grain type and phytase supplementation in AME, N retention and AMEn ($P<0.01$) and maize-based diet with phytase had the highest AME, N retention and AMEn.

The effects of dietary treatments on gizzard pH, toe ash and retention time in the proximal jejunum, distal jejunum, proximal ileum and distal ileum are shown in Table 4. Wheat-based diets tended to have higher gizzard pH ($P=0.05$). Phytase increased toe ash ($P<0.05$) but there were no statistical differences in toe ash between maize-, sorghum- and wheat-based diets. Overall, phytase decreased small intestinal retention time in maize-based diets ($P<0.05$) but not in sorghum- and wheat-based diets.

The effects of grain type and phytase supplementation on apparent starch and nitrogen digestibility at the four small intestinal sites are shown in Table 5. In wheat-based diets, phytase increased apparent starch digestibility in the proximal jejunum ($P<0.05$). Maize-based diets had higher apparent starch and nitrogen digestibility coefficients in the distal jejunum, proximal ileum and distal ileum ($P<0.001$). There were no dietary effects on apparent nitrogen digestibility coefficients in the proximal jejunum.

The effects of dietary treatments on apparent fat and energy digestibility coefficients are shown in Table 6. Phytase did not influence apparent fat digestibility in all four small intestinal sites but there was a trend ($P=0.052$) that grain type

Table 5
Effect of grain type and phytase supplementation on apparent starch and nitrogen digestibility coefficients in proximal jejunum, distal jejunum, proximal ileum and distal ileum in broiler chickens at 27 days post-hatch.

Treatment		Starch				Nitrogen			
Grain	Phytase	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Maize	Nil	0.795^{ab}	0.898	0.942	0.949	−0.153	0.722	0.820	0.847
	Plus	0.859^{a}	0.912	0.952	0.950	−0.044	0.679	0.806	0.828
Sorghum	Nil	0.830^{ab}	0.844	0.860	0.880	−0.035	0.488	0.745	0.792
	Plus	0.767^{bc}	0.810	0.875	0.873	−0.203	0.605	0.772	0.799
Wheat	Nil	0.704^{c}	0.858	0.889	0.866	−0.450	0.598	0.733	0.748
	Plus	0.844^{ab}	0.880	0.934	0.903	0.270	0.689	0.697	0.774
	SEM	0.027	0.016	0.012	0.012	0.186	0.049	0.021	0.012
Main effect									
Grain	Maize	0.827	0.905^{a}	0.947^{a}	0.950^{a}	−0.098	0.701^{a}	0.813^{a}	0.837^{a}
	Sorghum	0.798	0.827^{c}	0.867^{c}	0.877^{b}	−0.119	0.547^{b}	0.758^{b}	0.795^{b}
	Wheat	0.774	0.869^{b}	0.911^{b}	0.884^{b}	−0.090	0.643^{ab}	0.715^{b}	0.761^{c}
Enzyme	Nil	0.776	0.867	0.897^{b}	0.899	−0.213	0.603	0.766	0.795
	Plus	0.823	0.868	0.920^{a}	0.909	0.008	0.658	0.758	0.800
P-value	Grain	0.227	<0.001	<0.001	<0.001	0.988	0.012	0.001	<0.001
	Enzyme	0.067	0.947	0.022	0.308	0.161	0.181	0.681	0.637
	Interaction	0.010	0.194	0.274	0.180	0.082	0.213	0.413	0.188

Means within columns not sharing common superscripts (a, b, c) are significantly different ($P<0.05$).

Table 6
Effect of grain type and phytase supplementation on apparent fat and energy digestibility coefficients in proximal jejunum, distal jejunum, proximal ileum and distal ileum in broiler chickens at 27 days post-hatch.

Treatment		Fat				Energy			
Grain	Phytase	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Maize	Nil	0.097	0.861	0.935	0.964	0.252^{ab}	0.695^{a}	0.767	0.791^{a}
	Plus	0.232	0.866	0.932	0.968	0.284^{a}	0.637^{ab}	0.750	0.753^{b}
Sorghum	Nil	0.256	0.616	0.883	0.920	0.286^{a}	0.538^{c}	0.681	0.714^{b}
	Plus	0.048	0.808	0.950	0.941	0.206^{ab}	0.586^{bc}	0.719	0.727^{b}
Wheat	Nil	−0.026	0.762	0.880	0.909	0.008^{b}	0.581^{bc}	0.664	0.670^{c}
	Plus	0.324	0.740	0.806	0.923	0.473^{a}	0.653^{a}	0.700	0.717^{b}
	SEM	0.127	0.042	0.035	0.016	0.091	0.021	0.014	0.014
Main effect									
Grain	Maize	0.164	0.864^{a}	0.934	0.966^{a}	0.268	0.666	0.758^{a}	0.772
	Sorghum	0.152	0.712^{b}	0.917	0.931^{b}	0.246	0.562	0.700^{b}	0.720
	Wheat	0.149	0.751^{b}	0.843	0.916^{b}	0.241	0.617	0.682^{b}	0.694
Enzyme	Nil	0.109	0.746	0.900	0.931	0.182	0.605	0.704	0.725
	Plus	0.201	0.805	0.896	0.944	0.321	0.625	0.723	0.733
P-value	Grain	0.992	0.010	0.051	0.023	0.950	<0.001	<0.001	<0.001
	Enzyme	0.392	0.117	0.898	0.247	0.077	0.245	0.140	0.518
	Interaction	0.126	0.052	0.168	0.875	0.020	0.011	0.136	0.016

Means within columns not sharing common superscripts (a, b, c) are significantly different ($P<0.05$).

interacted with phytase supplementation to influence apparent fat digestibility in the distal jejunum. Phytase tended to increase apparent fat digestibility in the distal jejunum in sorghum-based diets. Maize-based diets had higher apparent fat digestibility in the distal jejunum ($P=0.01$) and distal ileum ($P<0.05$). There were interactions between grain type and phytase supplementation on apparent energy digestibility coefficients in the proximal jejunum, distal jejunum and distal ileum ($P<0.05$). In wheat-based diets, phytase significantly increased apparent energy digestibility coefficients in the proximal jejunum, distal jejunum and distal ileum. Maize-based diets had higher energy digestibility in the proximal ileum than wheat and sorghum ($P<0.001$).

The predicted digestion curves of starch and protein (N) in maize-, sorghum- and wheat-based diets are shown in Fig. 2. The contour plot of feed conversion ratio with starch and protein digestion rate is shown in Fig. 3. The predicted apparent digestibility coefficients of starch and nitrogen fitted with their corresponding experimental values where average r^2=0.74, sum square of residuals was less than 0.15 in starch and average r^2=0.87, sum square of residuals was less than 0.10 in nitrogen. Starch digested much more rapidly than protein and Table 7 summaries the effect of grain type and phytase supplementation in digestion rates and potential digestible starch and protein. Sorghum had ($P<0.01$) lower potential digestible starch than maize and wheat. The effects of grain type and phytase supplementation on apparent disappearance rates of

Table 7
Effect of grain type and phytase supplementation on digestion kinetics [potential digestible starch (PDS), starch digestion rate (K_{starch}), potential digestible protein (PDP), protein digestion rate ($K_{protein}$)].

Treatment		PDS (g/g)	K_{starch} ($\times 10^{-2}$ min^{-1})	PDP (g/g)	$K_{protein}$ ($\times 10^{-2}$ min^{-1})
Grain	Phytase				
Maize	Nil	0.931	5.52	1.000	0.772
	Plus	0.939	7.15	0.940	0.754
Sorghum	Nil	0.889	7.32	0.990	0.702
	Plus	0.860	6.23	0.980	0.731
Wheat	Nil	0.883	5.16	1.000	0.652
	Plus	0.910	6.70	0.928	0.727
	SEM	0.016	0.943	0.024	0.066
Main effect					
Grain	Maize	0.935^{a}	6.34	0.970	0.763
	Sorghum	0.874^{c}	6.77	0.985	0.716
	Wheat	0.896^{b}	5.93	0.964	0.689
Enzyme	Nil	0.901	6.00	0.997^{a}	0.708
	Plus	0.903	6.70	0.950^{b}	0.737
P-value	Grain	0.007	0.696	0.681	0.508
	Enzyme	0.860	0.387	0.029	0.606
	Interaction	0.274	0.343	0.427	0.764

Means within columns not sharing common superscripts (a, b, c) are significantly different ($P<0.05$).

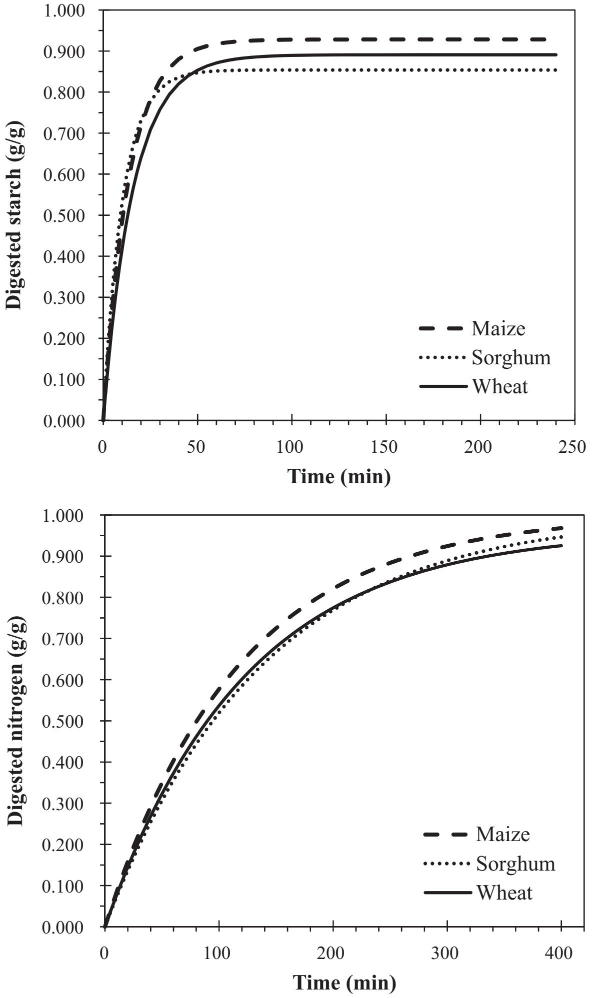

Fig. 2. Predicted digestion curve of starch and protein (nitrogen) in maize-, sorghum- and wheat-based diet.

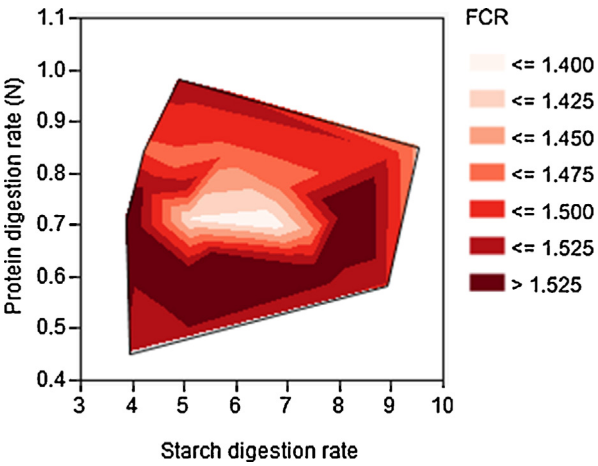

Fig. 3. Contour plot of the relationship of feed conversion ratio (FCR) with starch and protein (nitrogen) digestion rates ($\times 10^{-2}$ min^{-1}).

Table 8
Effect of grain type and phytase supplementation on apparent disappearance rates of starch and nitrogen in small intestine.

Treatment		Starch (g/h)	Nitrogen (g/h)
Grain	Phytase		
Maize	Nil	1.551^{bc}	0.127
	Plus	1.763^{a}	0.135
Sorghum	Nil	1.640^{ab}	0.127
	Plus	1.524^{bc}	0.129
Wheat	Nil	1.473^{c}	0.125
	Plus	1.556^{bc}	0.231
	SEM	0.057	0.047
Main effect			
Grain	Maize	1.657	0.131
	Sorghum	1.582	0.128
	Wheat	1.514	0.178
Enzyme	Nil	1.555	0.126
	Plus	1.614	0.165
P-value	Grain	0.060	0.503
	Enzyme	0.022	0.322
	Interaction	0.031	0.473

Means within columns not sharing common superscripts (a, b) are significantly different ($P<0.05$).

starch and nitrogen in small intestine are shown in Table 8. Interactions between grain type and phytase supplementation were observed in starch ($P<0.05$) disappearance rates.

4. Discussion

Across the three diets, phytase supplementation significantly increased weight gain, feed intake and FCR. Moreover, FCR was correlated with AME ($r=-0.439$, $P<0.01$) and N-retention ($r=-0.388$, $P<0.01$). While there were no significant interactions in growth performance, phytase addition to maize-based diets generated more pronounced responses in weight gain and FCR. Selle and Ravindran (2007) extensively reviewed the impact of exogenous phytase on broiler growth performance and concluded that feed intake and weight gain responses to phytase are usually more robust and consistent than feed efficiency responses. Therefore, the phytase-induced response in FCR in maize-based diets is noteworthy.

Phytase supplementation of maize-, sorghum- and wheat-based diets generated a number of significant interactions in nutrient utilization data, which indicates that the magnitude of responses to phytase across the three grains varied considerably. Phytase enhanced AME (MJ/kg and MJ/day), N retention and AMEn in maize-based diets, but not in sorghum- and wheat-based diets. It seems that sorghum-based broiler diets do not respond robustly to exogenous enzymes, including phytase (Selle et al., 2010). This is possibly because conventional exogenous enzymes do not address the anti-nutritive effects of one or more factors inherent in sorghum. However, the differences in phytase response patterns in maize- and wheat-based diets are more noticeable. This pattern was not consistent with the findings of Ravindran et al. (1999) where phytase supplementation increased average ileal digestibility coefficients of 14 amino acids in wheat by 9.04% (0.774 versus 0.844) versus 3.36% (0.774 versus 0.800) in maize. In contrast, Leske and Coon (1999) reported that phytase increased phytate hydrolysis by 28.2% (0.590 versus 0.308) in maize but by only 16.1% (0.468 versus 0.307) in wheat in 22-day-old broiler chickens. Phytate is located in the germ of maize but in the aleurone of wheat (O'Dell et al., 1972) and there is possibility that the more fibrous nature of the aleurone layer limited the access of phytase to its substrate thereby impeding phytate degradation in wheat.

Sorghum and maize supported superior weight gains and FCR in comparison to wheat-based diets from 7 to 27 days post-hatch. Numerically, sorghum was fractionally better than maize, which was somewhat surprising. But in the present study, sorghum-based diets supported the highest weight gain and the most efficient FCR. Sorghum has been associated with inconsistent or even sub-optimal broiler performance under Australian conditions, which may be attributable to the various anti-nutritive effects of kafirin, phytate and phenolic compounds (Selle et al., 2010). Taylor et al. (1984) found that kafirin proportions of total sorghum protein increases with protein concentrations. The sorghum used in the present study had low protein content (82.4 g/kg) and the correspondingly low kafirin proportion of protein may have contributed to the acceptable performance of birds offered sorghum-based diets.

In practice, xylanase is routinely included in wheat-based broiler diets to avoid the negative impact of soluble non-starch polysaccharides (Choct, 2006) but xylanase was not included in wheat-based diets in the present study. However, the energy densities (AME MJ/kg) of the three non-supplemented diets were statistically similar, which does not suggest that soluble NSP in wheat compromised broiler performance. On the other hand, wheat-based diets generated significantly lower relative gizzard weights than sorghum and maize and this may be attributed to the soft grain texture of wheat. The Symes particle size index (Symes, 1965), which is indicative of texture, was 6 in maize, 9 in sorghum and 12 in wheat where the highest index corresponds to the softest texture. It is also relevant that grain particle size in sorghum and wheat following hammer-milling were very similar (Fig. 1), so it is less likely particle size contributed to the differences of gizzard weight in these two diets.

The gizzard has several important functions including particle size reduction, digestion of nutrients and regulation of gut motility and the gizzard responds rapidly to changes in the texture of the diet (Svihus, 2011). In the present study, relative gizzard weights were correlated with digesta pH in the gizzard ($r=-0.455$, $P<0.01$) which suggests that relative gizzard weight is indicative of gizzard function. Moreover, relative gizzard weights were correlated with apparent distal ileal digestibility coefficients of starch ($r=0.381$, $P<0.05$), nitrogen ($r=0.545$, $P<0.001$) and fat ($r=0.418$, $P<0.05$). A functional gizzard enhances the degradation of protein to polypeptides through increased exposure to pepsin and hydrochloric acid (Svihus et al., 2004). Pepsin does not completely degrade protein to small peptides and amino acids (Crevieu-Gabriel et al., 1999) but peptide end-products of pepsin digestion stimulate pancreatic secretion of trypsin, chymotrypsin and other endogenous enzymes via the release of enteric hormones, including cholecystokinin (CCK) and gastrin (Guan and Green, 1996; Nishi et al., 2001). Also, large gizzards may increase episodes of reverse peristalsis (Ferket, 2000) which may have supported the better starch and fat digestion observed in this study. The likelihood is that the relatively light gizzards generated by wheat-based diets in this study as a result of its soft texture may have contributed to the poor growth performance of birds offered wheat-based diets.

The addition of phytase to maize-based diets generated relatively pronounced growth performance responses and even more pronounced responses in nutrient utilization. The diets were formulated to be P-adequate and the subtle increase in toe ash following phytase supplementation of maize-based diets was not significant ($P>0.09$) on the basis of a pair-wise comparison. This suggests that the phytase responses observed were “extra-phosphoric” in nature (Ravindran, 1995). However, phytase responses in digestibility coefficients of starch, N, fat and energy were equivocal and do not appear to provide the basis of the growth performance and nutrient utilization responses in maize-based diets. One remarkable difference to phytase supplementation between maize- versus sorghum- and wheat-based diets was in retention times along the small intestine. Phytase supplementation of maize-based diets significantly reduced retention times in the distal jejunum, distal ileum and all four segments; whereas, with sorghum retention times were nearly identical but were numerically increased in wheat-based diets. This is reflected in the significant grain by phytase interactions observed in the relevant small intestinal segments.

Phytate probably interferes with the digestion of protein by its capacity to form primary and ternary protein–phytate complexes in the gut (Cosgrove, 1966; Selle et al., 2000, 2012). Similarly, phytate may interfere with starch digestion by binding starch directly, via hydrogen bonds and phosphate linkages, or indirectly via starch granule-associated proteins (Selle et al., 2012). However, there is the real possibility that phytate also interferes with the absorption of amino acids and glucose, which may be even more important.

Moreover, given that the digestibility coefficients of nutrients were broadly similar, then there is the suggestion that the intestinal uptake rates of glucose and amino acids were enhanced by phytase in maize-based diets. This appears to be the case as phytase significantly increased small intestinal disappearance rates of starch and numerally increased disappearance rate of crude protein (N). Importantly, Croom et al. (1998) contended that intestinal absorption capacity is rate-limiting for the performance of broiler chickens. Thus there is the implication that phytate was impeding intestinal uptakes of glucose and amino acids, but this was counteracted by phytase addition to maize-based diets.

Cowieson et al. (2004) showed that phytate increased, and phytase attenuated, sodium excretion in broiler chickens. Moreover, Selle et al. (2009) reported profound impacts of phytase on apparent sodium ileal digestibility, which was restored to parity (−0.043) from a deficit of −0.516 by phytase. Clearly, phytate promotes the egress of Na^+ into the small-intestinal lumen and this suggests that phytate could impede absorption amino acids and glucose by compromising Na^+-dependent transport systems and the activity of Na^+-K^+-ATPase or the Na pump (Selle et al., 2012). Several studies have demonstrated the negative impact of phytate on starch digestion and glucose absorption (Rickard and Thompson, 1997); however, Demjen and Thompson (1991) found that the addition of 0.8% phytic acid to a test meal of 50 g glucose reduced blood glucose responses in humans. This suggests that phytate can influence glucose absorption *per se* in addition to impeding starch digestion. This suggests that phytate can influence absorption of amino acids in addition to impeding protein digestion. Therefore, the more pronounced phytase responses in maize-based diets may have been due to more response in digestion of starch and protein coupled with increased uptake rates of glucose and amino acids from the small intestine in the present study give the shortened retention times in maize-based diets.

The relevance of digestive dynamics of starch and protein to growth performance in broiler chickens was considered previously (Weurding et al., 2003a,b; Liu et al., 2013a). The contour plot (Fig. 3) illustrates the relationship between starch and nitrogen digestion rates and FCR in all the dietary treatments. The relationship suggests that feed conversion efficiency may be enhanced by manipulating starch and protein digestion rates. Geiger (1950) opined that the relative timing of amino acid and carbohydrate inputs can influence protein synthesis and the efficiency of feed utilization which suggests that starch and protein digestive dynamics should not be treated in isolation but in combination because a balanced provision of glucose and amino acids at sites of protein synthesis is a fundamental prerequisite for efficient growth. Apart from intestinal digestibility coefficients, the additional consideration of kinetics associated with differences in relative absorption rates of nutrients is helpful for a fuller understanding of the value of feedstuffs.

In conclusion, phytase significantly increased weight gain and feed intake in maize-, sorghum- and wheat-based diets and supported better feed conversion efficiency but phytase responses in weight gain and feed conversion efficiency tended to be more pronounced in maize-based diets. Phytase significantly increased AME, AMEn and N retention in maize-based diets but not in sorghum- and wheat-based diets. Moreover, phytase only numerically increased digestion rates of starch and protein but significantly increased starch disappearance rates in maize-based diets. The hypothesis that phytase would improve these parameters irrespective of the type of grain on which the diets were based was not established because of the more pronounced response in weight gain and feed conversion efficiency in maize-based diets.

Conflict of interest

The authors declare no conflicts of interest.

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References

Batterham, E.S., 1974. Effect of frequency of feeding on utilization of free lysine by growing pigs. Br. J. Nutr. 31, 237–242.

Batterham, E.S., O'Neill, G.H., 1978. Effect of frequency of feeding on response by growing pigs to supplements of free lysine. Br. J. Nutr. 39, 265–270.

Choct, M., 2006. Enzymes for the feed industry: past, present and future. World's Poult. Sci. J. 62, 5–15.

Cosgrove, D.J., 1966. Chemistry and biochemistry of inositol polyphosphates. Rev. Pure Appl. Chem. 16, 209–224.

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.

Crevieu-Gabriel, I., Gomez, J., Caffin, J.P., Carre, B., 1999. Comparison of pig and chicken pepsins for protein hydrolysis. Reprod. Nutr. Dev. 39, 443–454.

Croom, W.J., McBride, B., Bird, A.R., Fan, Y.K., Odle, J., Froetschel, M., Taylor, I.L., 1998. Regulation of intestinal glucose absorption: a new issue in animal science. Can. J. Anim. Sci. 78, 1–13.

Demjen, A.P., Thompson, L.U., 1991. Calcium and phytic acid independently lower the glycemic response to a glucose load. In: Proceedings of the 34th Canadian Federation of Biological Sciences, p. 53.

Doherty, C., Faubion, J.M., Rooney, L.W., 1982. Semiautomated determination of phytate in sorghum and sorghum products. Cereal Chem. 59, 373–378.

Drew, M.D., Schafer, T.C., Zijlstra, R.T., 2012. Glycemic index of starch affects nitrogen retention in grower pigs. J. Anim. Sci. 90, 1233–1241.

Ferket, P., 2000. Feeding whole grains to poultry improves gut health. Feedstuffs 72, 12–13.

Geiger, E., 1950. The role of the time factor in protein synthesis. Science 111, 594–599.

Guan, D.F., Green, G.M., 1996. Significance of peptic digestion in rat pancreatic secretory response to dietary protein. Am. J. Physiol. – Gastroint. Liver Physiol. 271, G42–G47.

Harland, B.F., Morris, E.R., 1995. Phytate – a good or a bad food component. Nutr. Res. 15, 733–754.

Hill, F.W., Anderson, D.L., 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64, 587–603.

Kim, J.C., Payne, H.G., Langridge, M.D., Mullan, B.P., Pluske, J.R., Edwards, A.C., Collins, C.L., Henman, D.J., 2013. Influence of nutrient asynchrony on whole body protein retention rate in growing pigs. Proc. Biennial Conf. Aust. Pig Sci. Assoc. 14, 117.

Leske, K.L., Coon, C.N., 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poult. Sci. 78, 1151–1157.

Liu, S.Y., Selle, P.H., Cowieson, A.J., 2013a. The kinetics of starch and nitrogen digestion regulate growth performance and nutrient utilisation in coarsely-ground, sorghum-based broiler diets. Anim. Prod. Sci. 53, 1033–1040.

Liu, S.Y., Selle, P.H., Cowieson, A.J., 2013b. Influence of white- and red-sorghum varieties and hydrothermal component of steam-pelleting on digestibility coefficients of amino acids and kinetics of amino acids, nitrogen and starch digestion in diets for broiler chickens. Anim. Feed Sci. Technol. 186, 53–63.

Luque de Castro, M.D.L., Priego-Capote, F., 2010. Soxhlet extraction: past and present panacea. J. Chromatogr. A 1217, 2383–2389.

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.

Nishi, T., Hara, H., Hira, T., Tomita, F., 2001. Dietary protein peptic hydrolysates stimulate cholecystokinin release via direct sensing by rat intestinal mucosal cells. Exp. Biol. Med. 226, 1031–1036.

O'Dell, B.L., Deboland, A.R., Koirtyoh Sr., 1972. Distribution of phytate and nutritionally important elements among morphological components of cereal grains. J. Agric. Food Chem. 20, 718–721.

Oatway, L., Vasanthan, T., Helm, J.H., 2001. Phytic acid. Food Rev. Int. 17, 419–431.

Orskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92, 499–503.

Pelley, J.W., Goljan, E.F., 2011. Rapid Review Biochemistry. Elsevier, Philadelphia, PA, pp. 116.

Ravindran, V., 1995. Phytases in poultry nutrition. an overview. Proc. Aust. Poult. Sci. Symp. 7, 135–139.

Ravindran, V., Cabahug, S., Ravindran, G., Bryden, W.L., 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78, 699–706.

Rickard, S.E., Thompson, L.U., 1997. Interactions and biological effects of phytic acid. In: Shahidi, F. (Ed.), Antinutrients and Phytochemicals in Food. Amer. Chemical Soc., Washington.

Selle, P.H., Ravindran, V., Caldwell, R.A., Bryden, W.L., 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13, 255–278.

Selle, P.H., Walker, A.R., Bryden, W.L., 2003. Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry. Aust. J. Exp. Agric. 43, 475–479.

Selle, P.H., Ravindran, V., 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135, 1–41.

Selle, P.H., Ravindran, V., 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113, 99–122.

Selle, P.H., Ravindran, V., Partridge, G.G., 2009. 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., Cadogan, D.J., Li, X., Bryden, W.L., 2010. Implications of sorghum in broiler chicken nutrition. Anim. Feed Sci. Technol. 156, 57–74.

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.

Svihus, B., Juvik, E., Hetland, H., Krogdahl, A., 2004. Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. Br. Poult. Sci. 45, 55–60.

Svihus, B., 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poult. Sci. J. 67, 207–223.

Symes, K.J., 1965. Inheritance of grain hardness in wheat as measured by particle size index. Aust. J. Agric. Res. 16, 113–123.

Taylor, J.R.N., Schussler, L., Vanderwalt, W.H., 1984. Fractionation of proteins from low-tannin sorghum grain. J. Agric. Food Chem. 32, 149–154.

van den Borne, J., Schrama, J.W., Heetkamp, M.J.W., Verstegen, M.W.A., Gerrits, W.J.J., 2007. Synchronising the availability of amino acids and glucose increases protein retention in pigs. Animal 1, 666–674.

Weurding, R.E., Enting, H., Verstegen, M.W.A., 2003a. The relation between starch digestion rate and amino acid level for broiler chickens. Poult. Sci. 82, 279–284.

Weurding, R.E., Enting, H., Verstegen, M.W.A., 2003b. The effect of site of starch digestion on performance of broiler chickens. Anim. Feed Sci. Technol. 110, 175–184.