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The interactions of exogenous phytase with whole grain feeding and effects of barley as the whole grain component in broiler diets based on wheat, sorghum and wheat-sorghum blends

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ABSTRACT

The objectives of this experiment were two-fold; the first was to evaluate exogenous phytase in either conventional or whole grain diets as a 2 × 2 factorial treatment array. Wheat-sorghum blended rations containing 12.5% ground or whole barley were offered without and with 1000 FTU/kg exogenous phytase. The second objective was to evaluate barley as the whole grain component in diets based on wheat, sorghum and equal wheat-sorghum blends as a 3 × 2 factorial treatment array. Rations based on wheat, sorghum and wheat-sorghum blends were offered as an intact pellet containing 12.5% ground barley or offered as a mix of 12.5% whole barley and a pelleted concentrate. Each of the dietary treatments was offered to 7 replicates (6 birds per cage) of male Ross 308 chicks from 7 to 28 days post-hatch. Treatment effects on growth performance, gizzard and pancreas weights, gizzard pH, bone mineralisation, nutrient utilisation, digestibility coefficients of starch and protein (N) and starch:protein disappearance rate ratios in four small intestinal segments (proximal and distal jejunum, proximal and distal ileum), excreta dry matter and incidence of dilated proventriculi were determined. In the 2 × 2 analysis there was a significant ($P < 0.025$) treatment interaction for FCR. Phytase addition to whole barley diets improved FCR by 3.20% (1.362 versus 1.407) but phytase compromised FCR by 3.11% (1.391 versus 1.349) in ground barley diets. Similarly, treatment interactions ($P < 0.002 - < 0.001$) were also observed for energy utilisation (AME, ME:GE ratios, AMEn) where phytase generated positive responses in the context of whole grain feeding but not in conventional diets. In the 3 × 2 analysis, whole barley significantly increased relative gizzard weights by 22.5% (16.96 versus 20.77 g/kg; $P < 0.001$) and significantly reduced ($P < 0.05$) the incidence of dilated proventriculi from 4.76% to zero. However, whole barley compromised growth performance. There were significant treatment interactions ($P < 0.001$) for parameters of energy utilisation as whole barley significantly enhanced energy utilisation (AME, ME:GE ratios, AMEn) in birds offered sorghum-based diets but this was not the case with wheat or blended diets. Wheat-based diets generally supported better protein and starch digestibility coefficients with significant advantages being observed in some small intestinal segments in comparison to sorghum and blended diets. For example, wheat-based diets generated significantly higher protein digestibility in the ileum ($P < 0.001$) than birds offered sorghum or

Abbreviations: ADC, apparent digestibility coefficient; AIA, acid insoluble ash; AME, apparent metabolisable energy; AMEn, nitrogen corrected apparent metabolisable energy; BXU, beta-xylanase units; DI, distal ileum; DJ, distal jejunum; FCR, feed conversion ratio; FI, feed intake; FTU, phytase units; ME:GE ratio, metabolisable to gross energy ratio; MJ, megajoules; N, nitrogen; PI, proximal ileum; PJ, proximal jejunum; WGF, whole grain feeding

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blended diets. Likewise, wheat-based diets generated significantly higher starch digestibilities in the proximal jejunum and distal ileum ($P < 0.001$) than birds offered sorghum or blended diets. Whole barley reduced water intakes by 9.72% (325 versus 360 g/bird/day; $P < 0.01$) and significantly increased excreta dry matter in wheat-based diets from 22.1 to 25.1% ($P < 0.001$) but there was a decrease from 26.08 to 24.50% ($P < 0.05$) in sorghum-based diets. Therefore, it may be concluded that phytase is more effective in whole grain diets than conventional diets. Whole barley increased gizzard weights, reduced the incidence of dilated proventriculi and significantly improved energy utilisation in sorghum based diets.

1. Introduction

Inclusion of phytate–degrading feed enzymes in poultry diets has been reviewed by Selle and Ravindran (2007) and is now a standard procedure on a global basis. The practice of whole grain feeding (WGF) is becoming increasingly adopted in countries where wheat, rather than maize, is the dominant feed grain. Indeed, the high level of acceptance of WGF by the chicken-meat industry in relevant countries is disproportionate to the sparse number of investigations reported in the literature. The landmark response to WGF is heavier relative gizzard weights and presumably more functional gizzards in broiler chickens which are associated with improved feed conversion, increased energy utilisation and enhanced gut integrity (Liu et al., 2014; Singh et al., 2014). Despite the acceptance of both exogenous phytases and WGF very few relevant investigations have been completed in this context; Abdollahi et al. (2016) is one published but inconclusive example. The gizzard is a prime site for phytate degradation by phytase (Truong et al., 2016a) and a more functional gizzard could facilitate phytase activity via lower pH and longer retention times. Therefore, one objective of this study is to evaluate phytase inclusions under WGF regimes to counter the current lack of scientific data. It was anticipated that the efficacy of phytase might be enhanced by WGF.

Wheat is commonly the whole grain component in WGF regimes. However, Biggs and Parsons (2009) compared wheat, sorghum and barley as whole grain components in maize-based poultry diets. In this study 10% whole barley increased relative gizzard weights by 31.8% and 20% whole barley induced increases of 50.0%.These findings suggest that barley, rather than wheat or sorghum, may be the whole grain of choice for WGF regimes. Consequently, 125 g/kg whole or ground barley was evaluated in the present study.

The chicken-meat industry in Australia uses diets based on, in descending order, wheat, wheat-sorghum blends and sorghum as the feed grains. For this reason, inclusions of ground versus whole barley were compared in nutritionally equivalent diets based on wheat, sorghum and a wheat-sorghum blend. The blended diet is relevant because wheat is often included in essentially sorghum-based diets to enhance pellet quality. In the present study, barley was included in all diets as either ground (3.2 mm hammer-mill screen) or whole grain to avoid any confounding effects. Thus the second objective was to compare the responses of broiler chicks offered wheat, sorghum and wheat-sorghum blended diets to WGF regimes with 12.5% barley as the common ground or whole grain component. It was thought that birds offered diets based on an equal blend of wheat and sorghum might outperform their counterparts due to more favourable starch digestive dynamics. This possibility was based on the fact that starch in wheat is rapidly digested but sorghum starch is slowly digested (Giuberti et al., 2012), so an intermediate starch digestion rate might be more appropriate.

Table 1
Characteristics of feed grains that formed the basis of experimental diets.

Item (g/kg DM)	Barley	Wheat	Sorghum
Moisture (%)	11.33	10.61	12.68
Protein	99.4	119.8	97.7
Starch	674.4	739.3	724.0
Free sugars	16.1	12.9	6.1
Oligosaccharides	1.8	0.6	0.5
Soluble non-starch polysaccharides	2.0	< 0.1	< 0.1
Insoluble non-starch polysaccharides	9.0	9.0	9.1
Fat	25.9	20.4	42.1
Linoleic fibre (% of total fat)	56.1	59.6	45.0
Ash	16.2	12.0	13.7
Fibre	35.3	17.7	30.6
Neutral detergent fibre	174.4	130.2	125.8
Acid detergent fibre	44.3	21.6	64.6
1000 grain weight (g, as-is)	25.4	17.1	30.3
Specific weight (kg/hl, as-is)	71.5	78.9	76.5

On an 'as-is' basis, soybean meal contained 468.0 g/kg protein and 27.9 g/kg fat; canola meal contained 374.3 g/kg protein and 126.1 g/kg fat.

2. Materials and methods

2.1. Experimental design

The present study comprised eight treatments of nutritionally equivalent diets based on characterised feed grains as itemised:
Treatment 1: 475 g/kg wheat, 125 g/kg ground barley
Treatment 2: 475 g/kg wheat, 125 g/kg whole barley
Treatment 3: 475 g/kg sorghum, 125 g/kg ground barley
Treatment 4: 475 g/kg sorghum, 125 g/kg whole barley
Treatment 5: 237.5 g/kg wheat, 237.5 g/kg sorghum, 125 g/kg ground barley
Treatment 6: 237.5 g/kg wheat, 237.5 g/kg sorghum, 125 g/kg whole barley
Treatment 7: as per treatment 5 plus 1000 FTU/kg phytase
Treatment 8: as per treatment 6 plus 1000 FTU/kg phytase.

Diets containing ground barley were fed as intact pellets; whereas, 12.5% whole barley was fed in a blend with the balancing pelleted concentrate. As outlined in detail below, the experimental data was analysed as either 2 × 2 (treatments 5–8 inclusive) or 3 × 2 (treatments 1–6 inclusive) factorial arrays of dietary treatments.

2.2. Diet preparation

Characteristics of the three feed grains used in the present study are shown in Table 1 and on the basis of these analyses three basal diets were formulated to similar nutrient specifications as shown in Table 2 with their dietary compositions. As the 12.5% whole grain component, barley was either ground (3.2 mm hammer-mill screen) prior to incorporation into intact pelleted diets or the balancing concentrate was mixed with 125 g/kg whole barley post-pelleting and fed as a blend. The formulation of the pelleted concentrate may be deduced from Table 2. The steam-pelleted components of the diets were processed through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) at a conditioning temperature of 75 °C. Exogenous phytase (Axtra® PHY; Danisco Animal Nutrition) was included in both ground and whole barley diets based on the wheat-sorghum blend. The accuracy of

Table 2
Composition and nutrient specifications of experimental diets.

	Composition of Diets				Nutrient Specifications of Diets		
Item (g/kg)	Wheat-based diet[2]	Sorghum-based diet[2]	Blended diet[2,1]	Item (g/kg)	Wheat-based diet[2]	Sorghum-based diet[2]	Blended diet[2,1]
Barley (ground or whole)	125.0	125.0	125.0	Metabolisable energy (MJ/kg)	12.97	12.97	12.97
Wheat	475.0	–	237.5				
Sorghum	–	475	237.5	Protein	201.3	207.7	204.5
Soybean meal	243.1	271.4	257.2	Starch	454.5	449.7	452.1
Canola meal	35.0	35.0	35.0	Fat	88.5	72.2	80.4
Canola oil	69.1	44.4	56.7	Fibre	29.3	35.4	32.4
Dicalcium phosphate	17.3	17.3	17.3	Calcium	7.5	7.5	7.5
Limestone	5.97	5.03	5.50	Total phosphorus	7.0	7.6	7.3
Sodium chloride	1.52	1.92	1.72	Available phosphorus	3.6	3.6	3.6
Sodium bicarbonate	5.18	4.57	4.88	Sodium	2.0	2.0	2.0
Arginine	0.96	0.67	0.81	Potassium	8.2	8.2	8.2
Isoleucine	0.83	0.00	0.42	Chloride	2.2	2.2	2.2
Lysine HCl	3.49	2.95	3.22	DCAB (Meq/kg)	235	235	235
Methionine	3.12	3.25	3.18				
Threonine	1.66	1.24	1.45	Digestible amino acids			
Valine	0.74	0.11	0.43	Lysine	11.3	11.3	11.3
Choline chloride (60%)	0.08	0.20	0.14	Methionine	5.7	5.9	5.8
Vitamin-trace mineral premix[3]	2.00	2.00	2.00	Cystine	5.9	5.7	5.8
Celite[4]	10.0	10.0	10.0	Threonine	7.6	7.6	7.6
				Tryptophan	2.2	2.3	2.2
Analysed phytase activity (FTU/kg)				Arginine	12.1	12.1	12.1
Ground grain diets	90	30	0.0	Isoleucine	7.7	7.7	7.7
Whole grain diets	255	50	40	Valine	8.6	8.6	8.6
Phytase supplemented diets							
Ground grain	–	–	1250				
Whole grain	–	–	1270				

[1]Experiment 1, [2]Experiment 2, [3]The vitamin-mineral premix supplied per tonne 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, [4]Phytase added at 100 g per tonne when appropriate and xylanase was added across all diets at the expense of Celite.

the 1000 FTU/kg phytase inclusions was confirmed by analysis (Table 2) using the standard method of Engelen et al. (1994). A non-starch polysaccharide degrading enzyme (Danisco Xylanase), which is a endo-1,4-beta-xylanase produced by a genetically modified strain of *Trichoderma reesei*, was added across all diets at 16,000 U/kg. Celite (Celite™ World Minerals, Lompoc, CA, USA) was included in diets at 10 g/kg as an inert acid insoluble ash (AIA) marker in order to determine nutrient digestibility coefficients in four small intestinal sites. AIA concentrations in diets and digesta were determined by the method of Siriwan et al. (1993).

2.3. Bird management

A total of 336 male Ross 308 chicks were initially offered a proprietary starter diet. At 7 days post-hatch birds were individually identified (wing-tag), weighed and allocated into bioassay cages (6 birds per cage) on the basis of body-weights. Birds were stratified on the basis of body weights and allocated such that cage means and standard deviations were essentially identical. Each dietary treatment was offered to seven replicate cages during the 7–28 days post-hatch experimental period. Birds had unlimited access to feed and water under a '23-h-on-1-h-off' lighting regime in an environmentally controlled facility. An initial room temperature of 32 ± 1 °C was maintained for the first week, which was gradually decreased to 22 ± 1 °C by the end of the third week and maintained at this temperature for the final week.

2.4. Sample collection and chemical analysis

Initial and final body weights were determined and feed intakes recorded, from which feed conversion ratios (FCR) were calculated. Any dead or culled birds were removed on a daily basis and their body-weights recorded and used to adjust FCR calculations. Feed intakes, and excreta outputs were monitored from 25 to 27 days post-hatch in order to calculate apparent metabolisable energy (AME), metabolisable to gross energy ratio (ME:GE ratio), nitrogen (N) retention and N-corrected AME (AMEn) on a dry matter basis. Over this total excreta collection period water intakes were monitored to determine water to feed intake ratios. Excreta were air-forced oven dried for 24 h at 80∘C. The GE of diets and excreta were determined via bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL). AME was calculated by the following equation:

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

N-corrected AME values were calculated by correcting to zero N retention, using the factor of 36.54 kJ/g (Hill and Anderson, 1958).

N retention was calculated by the following equation:

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

At day 28, birds were euthanised by an intravenous injection of sodium pentobarbitone. Gizzard, gizzard contents and pancreas were then removed and weighed to determine their absolute and relative weights. The pH of digesta within the gizzard was immediately determined *in situ* with an EZ Do model 7011 pH probe. The small intestine was removed and divided into the four segments – proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI), distal ileum (DI) – which were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction and their mid-points. Digesta was collected in its entirety from each segment. Digesta samples were gently expressed from each segment, pooled by cage, homogenised, freeze dried and weighed to determine the apparent digestibility of starch and N. Concentrations of starch in diets and digesta was determined by methods as described in Mahasukhonthachat et al. (2010). Nitrogen and AIA concentrations were determined as outlined in Siriwan et al. (1993). Apparent digestibility coefficients of starch and nitrogen were calculated by the following equation:

$$\text{Digestibilitycoefficient} = \frac{(\text{Nutrient/AIA})_{\text{diet}} - (\text{Nutrient/AIA})_{\text{digests}}}{(\text{Nutrient/AIA})\,\text{diet}}$$

Starch: protein disappearance rate ratios were deduced from starch and protein disappearance rates in four small intestinal segments (data not shown) which were calculated from the following equation:

$$\text{Disappearancerate(g/bird/day)} = \text{FI} \times \text{nutrientcontent}_{\text{diet}} \times \text{ADC}.$$

FI is the 24 h feed intake immediately prior to euthanisation (g/bird), nutrient $\text{content}_{\text{diet}}$ is the dietary starch or protein concentrations (g/kg) and ADC is the apparent digestibility coefficients of the relevant nutrient.

Toe samples were collected by severing the middle toe through the joint between the 2nd and 3rd tarsal bones from the distal end. Toes from each cage were pooled and the composite samples dried to a constant weight at 100 °C and then ashed in a muffle furnace at 550 °C for 16 h for the assessment of bone mineralisation as described by Potter (1988).

2.5. Statistical analysis

Experimental data were analysed as two separate factorial arrays of treatments. The first, a 2 × 2 factorial, comprised dietary treatments 5–8 inclusive with diets based on the wheat-sorghum blend with either ground or whole barley, without and with 1000 FTU/kg phytase. The second, a 3 × 2 factorial, comprised dietary treatments 1–6 inclusive with diets of either a 12.5% ground or whole barley component based on either wheat, sorghum or a blend of both feed grains. The data was analysed by univariate, general linear models procedures and Pearson correlations using the SPSS® IBM Statistics 20 software program (IBM Corporation, Somers, NY, USA). The experimental units were cage means and differences were considered significant at the 5% level of probability. This study fully complied with the guidelines approved by the Animal Ethics Committee of The University of Sydney.

3. Results

3.1. Phytase and WGF

The effects of WGF and phytase supplementation on growth performance, relative organ weights, gizzard pH and toe ash are shown in Table 3. WGF significantly depressed weight gain by 2.98% without influencing feed intake. However, there was a significant treatment interaction for FCR as phytase addition to whole grain diets improved FCR by 3.20% but compromised FCR by 3.11% following its inclusion in ground grain diets. WGF significantly increased relative gizzard weights by 20.7%, reduced gizzard pH from 3.01 to 2.73 and increased relative pancreas weights by 8.92%. Phytase did not influence these parameters and dietary treatments did not influence relative gizzard contents. Surprisingly, however, phytase supplementation significantly depressed toe ash.

The effects of dietary treatments on nutrient utilisation are shown in Table 4 where significant treatment interactions were observed; essentially, these interactions occurred because phytase enhanced nutrient utilisation in whole grain diets but not in ground grain diets. Phytase significantly improved AME by 0.33 MJ in whole grain diets but numerically depressed AME by 0.12 MJ in ground grain diets. Phytase significantly improved ME:GE ratios in whole grain diets but significantly depressed efficiency of energy utilisation in ground grain diets. Phytase significantly depressed N retention in ground grain diets but numerically enhanced N retention in whole grain diets. Finally, phytase significantly improved AMEn by 0.29 MJ in whole grain diets but tended to depress AMEn by 0.09 MJ in conventional diets.

The effects of WGF and phytase supplementation on protein (N) and starch digestibility in four small intestinal segments are shown in Table 5. Distal ileal protein (N) coefficients were significantly decreased by 4.03% with WGF but increased by 4.20% with phytase supplementation without a significant treatment interaction. Significant treatment effects were not observed in more anterior small intestinal segments. Phytase did not influence starch digestibilities; whereas, WGF decreased distal jejunal starch coefficients by 7.11% but increased distal ileal starch coefficients by 1.05%.

The effects of WGF and phytase supplementation on starch:protein disappearance rate ratios across four small intestinal segments are shown in Table 6. WGF increased starch:protein disappearance rate ratios in the distal jejunum, proximal ileum and distal ileum to significant extents.

Table 3
Effects of whole grain feeding and phytase supplementation on growth performance from 7 to 28 days post-hatch and on relative organ weights, gizzard pH and toe ash at 28 days post-hatch.

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Relative gizzard weight (g/kg BW)	Relative gizzard contents (g/kg BW)	Gizzard pH	Relative pancreas weight (g/kg BW)	Toe ash (%)
WGF	Phytase (FTU/kg)								
Ground barley	0	1719	2318	1.349b	17.07	9.42	3.00	2.19	12.31
	1000	1704	2366	1.391ab	16.77	8.75	3.01	2.08	12.15
Whole barley	0	1627	2288	1.407a	20.47	8.89	2.66	2.32	12.56
	1000	1694	2307	1.362ab	20.39	8.26	2.81	2.32	11.87
SEM		23.0	26.9	0.0157	0.357	0.573	0.13	0.075	0.146
Main effects: Diet type									
Ground grain		1711a	2342	1.370	16.92b	9.09	3.01a	2.13b	12.23
Whole grain		1660b	2298	1.385	20.43a	8.57	2.73b	2.32a	12.22
Phytase									
0		1673	2337	1.378	18.77	9.16	2.83	2.25	12.44a
1000 FTU/kg		1699	2336	1.376	18.58	8.51	2.91	2.20	12.01b
Significance (P =)									
WGF		0.038	0.112	0.359	< 0.001	0.379	0.047	0.019	0.918
Phytase (P)		0.266	0.230	0.918	0.598	0.268	0.558	0.474	0.008
WGF × P interaction		0.085	0.588	0.011	0.765	0.976	0.578	0.441	0.078

ab means within columns not sharing a common suffix are significantly different.

Table 4
Effects of whole grain feeding and phytase supplementation on nutrient utilisation at 25–27 days post-hatch.

Treatment		AME (MJ/kg DM)	ME:GE (MJ/MJ)	N retention (%)	AMEn(MJ/kg DM)
WGF	Phytase (FTU)				
Ground barley	0	12.60c	0.724c	64.9a	11.45c
	1000	12.48c	0.714d	62.2b	11.36c
Whole barley	0	12.74b	0.735b	63.8ab	11.65b
	1000	13.07a	0.745a	64.1ab	11.94a
SEM		0.047	0.0027	0.707	0.056
Main effects: Diet type					
Ground grain		12.54	0.719	63.5	11.41
Whole grain		12.90	0.740	64.0	11.80
Phytase (FTU)					
0		12.67	0.730	64.3	11.55
1000		12.77	0.729	63.1	11.65
Significance (P =)					
WGF		< 0.001	< 0.001	0.524	< 0.001
Phytase (P)		0.037	0.896	0.103	0.080
WGF × P interaction		< 0.001	0.002	0.043	0.002

abcd means within columns not sharing a common suffix are significantly different.

3.2. Grain type and WGF

The effects of WGF and feed grain type on growth performance and mortality/cull rates are shown in Table 7. Overall bird performance (weight gain of 1683 g/bird at an FCR of 1.380 from 7 to 28 day post-hatch) was of a high order and the 4.38% mortality rate was not related to treatment. Diets containing whole barley significantly depressed weight gain by 4.30% and feed intake by 2.05% in comparison to ground barley diets. Ground grain diets supported significantly higher weight gains and higher feed intakes than diets with whole barley by 4.50 and 2.09%, respectively. Diets with sorghum as the main feed grain constituent were consumed to significantly greater extents than diets based on wheat or the wheat-sorghum blend. FCR was significantly superior in birds offered ground grain diets as opposed to whole grain diets by 2.29%. Wheat-based diets supported significantly better FCR than sorghum-based diets by 2.71% and the wheat-sorghum blend was intermediate in terms of feed efficiency.

The effects of dietary treatments on relative gizzard and pancreas weights, gizzard contents, pH and incidence of dilated

Table 5
Effects of whole grain feeding and phytase supplementation on protein (N) and starch digestibility coefficients across four small intestinal segments at 28 days post-hatch.

Treatment		Protein (N) Digestibility				Starch Digestibility			
WGF	Phytase (FTU)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Ground barley	0	0.384	0.575	0.676	0.734	0.643	0.839	0.885	0.948
	1000	0.380	0.571	0.681	0.754	0.596	0.822	0.876	0.957
Whole barley	0	0.420	0.554	0.646	0.693	0.629	0.772	0.898	0.964
	1000	0.417	0.560	0.674	0.735	0.563	0.769	0.889	0.962
SEM		0.0344	0.0169	0.0113	0.0095	0.0396	0.0120	0.0169	0.0050
Main effects: Diet type									
Ground grain		0.382	0.573	0.679	0.744a	0.620	0.830a	0.880	0.953b
Whole grain		0.419	0.557	0.660	0.714b	0.596	0.771b	0.893	0.963a
Phytase (FTU)									
0		0.402	0.564	0.661	0.714b	0.636	0.805	0.891	0.956
1000		0.399	0.566	0.678	0.744a	0.580	0.795	0.883	0.960
Significance (P =)									
WGF		0.302	0.360	0.108	0.005	0.565	< 0.001	0.394	0.047
Phytase (P)		0.922	0.936	0.155	0.004	0.175	0.487	0.176	0.469
WGF × P interaction		0.994	0.765	0.323	0.267	0.810	0.619	0.995	0.259

ab means within columns not sharing a common suffix are significantly different.

Table 6
Effects of whole grain feeding and feed grain on starch:protein disappearance rate ratios[1] across four small intestinal segments at 28 days post-hatch.

Treatment		Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum
WGF	Phytase (FTU)				
Ground barley	0	2.17	1.69	1.51	1.50
	1000	3.70	1.80	1.61	1.59
Whole barley	0	2.46	2.23	2.21	2.21
	1000	2.02	2.07	1.98	1.98
SEM		0.913	0.134	0.129	0.135
Main effects: Diet type					
Ground grain		2.94	1.74b	1.56b	1.54b
Whole grain		2.24	2.15a	2.10a	2.09a
Phytase (FTU)					
0		2.32	1.96	1.86	1.85
1000		2.86	1.93	1.8	1.79
Significance (P =)					
WGF		0.454	0.006	< 0.001	< 0.001
Phytase (P)		0.559	0.865	0.610	0.662
WGF × P interaction		0.292	0.318	0.221	0.235

ab means within columns not sharing a common suffix are significantly different.
[1] ratios calculated from dividing starch disappearance rates by protein (N) disappearance rates (g/bird/day).

proventriculi are shown in Table 8. Whole barley significantly increased relative gizzard weights by 22.5% and decreased relative gizzard contents by 15.9% compared to ground barley. WGF significantly reduced the incidence of dilated proventriculi from 4.76% to zero. Dietary treatments did not influence gizzard pH or relative pancreas weight.

The effects of WGF and feed grain type on nutrient utilisation are shown in Table 9. There were significant treatment interactions for parameters of energy utilisation as WGF significantly enhanced energy utilisation in birds offered sorghum-based diets, which was not the case with wheat-based or blended treatments. WGF with sorghum-based diets significantly enhanced AME by 0.62 MJ, ME:GE by 4.59% and AMEn by 0.7 MJ. WGF did not influence N retention; however, wheat-based diets (66.1%) supported significantly higher N retention than blended diets (64.3%) which, in turn, were significantly higher than sorghum-based diets (62.1%).

The effects of dietary treatments on protein (N) and starch digestibility coefficients across four small intestinal segments are

Table 7
Effects of whole grain feeding and grain type on growth performance and mortality/cull rates from 7 to 28 days post-hatch.

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality/cull rate (%)
WGF	Grain type				
Ground barley	Wheat	1710	2306	1.350	4.77
	Sorghum	1731	2412	1.394	4.77
	Blend	1719	2318	1.349	2.39
Whole barley	Wheat	1639	2251	1.374	4.77
	Sorghum	1674	2350	1.406	7.14
	Blend	1627	2288	1.407	2.39
SEM		24.2	27.8	0.0133	3.276
Main effects: Diet type					
Ground grain		1720a	2345a	1.364b	3.98
Whole grain		1646b	2297b	1.396a	4.78
Feed grain					
Wheat		1674	2279b	1.362b	4.77
Sorghum		1702	2381a	1.400a	5.96
Blend		1673	2303b	1.378ab	2.39
Significance (P =)					
WGF		0.001	0.038	0.007	0.769
Grain type (GT)		0.394	0.002	0.026	0.545
WGF × GT interaction		0.774	0.829	0.215	0.917

ab means within columns not sharing a common suffix are significantly different.

Table 8
Effects of whole grain feeding and grain type on relative gizzard and pancreas weights, gizzard contents, pH and incidence of dilated proventriculi at 28 days post-hatch.

Treatment		Relative gizzard weight (g/kg BW)	Relative gizzard contents (g/kg BW)	Gizzard pH	Relative pancreas weight (g/kg BW)	Incidence of dilated proventriculi (%)
WGF	Grain type					
Ground barley	Wheat	16.14	8.57	3.04	2.41	4.76
	Sorghum	17.68	10.93	2.88	2.13	7.14
	Blend	17.07	9.42	3.00	2.19	2.39
Whole barley	Wheat	20.94	7.58	3.08	2.38	0.00
	Sorghum	20.91	8.48	2.82	2.50	0.00
	Blend	20.47	8.89	2.66	2.32	0.00
SEM		0.38	0.684	0.112	0.198	2.517
Main effects: Diet type						
Ground grain		16.96b	9.64a	2.97	2.25	4.76a
Whole grain		20.77a	8.32b	2.85	2.40	0.00b
Feed grain						
Wheat		18.54	8.07	3.06	2.4	2.38
Sorghum		19.29	9.70	2.85	2.32	3.57
Blend		18.77	9.16	2.83	2.25	1.19
Significance (P =)						
WGF		< 0.001	0.023	0.191	0.341	0.029
Grain type (GT)		0.146	0.066	0.081	0.771	0.655
WGF × GT interaction		0.092	0.352	0.204	0.594	0.655

ab means within columns not sharing a common suffix are significantly different.

shown in Table 10. Whole barley significantly depressed protein (N) digestibility in the three posterior segments where distal ileal protein (N) digestibility declined by 4.33%. Feed grain type significantly influenced protein (N) digestibility in both ileal segments where wheat-based diets were superior in both cases. Distal ileal protein (N) digestibility in birds offered wheat-based diets was superior to blended and sorghum-based diets by approximately 5.4%. Feed grain type significantly influenced proximal jejunal starch digestibility coefficients where birds offered wheat-based diets had significantly higher coefficients than birds offered either blended grain or sorghum-based diets. A significant interaction was observed in the distal jejunum mainly because whole grain feeding depressed starch digestibility of the blended diet by 7.99%. Significant treatment effects were not observed in the proximal ileum. In the distal ileum, WGF significantly increased starch digestibility by 1.57% and the effect of feed grain type was significant. Starch

Table 9
Effects of whole grain feeding and grain type on nutrient utilisation at 25–27 days post-hatch.

Treatment		AME (MJ/kg DM)	ME:GE (MJ/MJ)	N retention (%)	AMEn(MJ/kg DM)
WGF	Grain type				
Ground barley	Wheat	13.29a	0.757a	65.9	12.15a
	Sorghum	11.94d	0.697c	61.3	10.82c
	Blend	12.60bc	0.724b	64.9	11.45b
Whole barley	Wheat	13.31a	0.755a	66.2	12.23a
	Sorghum	12.56c	0.729c	62.9	11.52b
	Blend	12.74b	0.735c	63.8	11.65b
SEM		0.069	0.004	0.671	0.078
Main effects: Diet type					
Ground grain		12.61	0.726	64.0	11.47
Whole grain		12.87	0.740	64.3	11.8
Feed grain					
Wheat		13.30	0.756	66.1a	12.19
Sorghum		12.25	0.713	62.1c	11.17
Blend		12.67	0.730	64.3b	11.55
Significance (P =)					
WGF		< 0.001	< 0.001	0.605	< 0.001
Grain type (GT)		< 0.001	< 0.001	< 0.001	< 0.001
WGF × GT interaction		< 0.001	< 0.001	0.150	< 0.001

abcd means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 10
Effects of whole grain feeding and grain type on protein (N) and starch digestibility across four small intestinal segments at 28 days post-hatch.

Treatment		Protein (N) Digestibility				Starch Digestibility			
WGF	Grain type	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Ground barley	Wheat	0.406	0.602	0.705	0.761	0.773	0.846a	0.895	0.976
	Sorghum	0.391	0.561	0.660	0.721	0.568	0.771b	0.885	0.935
	Blend	0.384	0.575	0.676	0.734	0.643	0.839a	0.885	0.948
Whole barley	Wheat	0.309	0.553	0.688	0.749	0.713	0.841a	0.928	0.987
	Sorghum	0.259	0.511	0.635	0.709	0.614	0.809ab	0.909	0.954
	Blend	0.420	0.554	0.646	0.693	0.629	0.772b	0.898	0.964
SEM		0.0396	0.0239	0.0111	0.0093	0.0338	0.0169	0.0157	0.0040
Main effects: Diet type									
Ground grain		0.394	0.579a	0.680a	0.739a	0.661	0.818	0.888	0.953b
Whole grain		0.330	0.539b	0.656b	0.717b	0.652	0.807	0.911	0.968a
Feed grain									
Wheat		0.358	0.577	0.697a	0.755a	0.743a	0.843	0.911	0.981a
Sorghum		0.325	0.536	0.647b	0.715b	0.591b	0.790	0.897	0.945c
Blend		0.402	0.564	0.661b	0.714b	0.636b	0.805	0.891	0.956b
Significance (P =)									
WGF		0.055	0.037	0.013	0.007	0.742	0.413	0.079	< 0.001
Grain type (GT)		0.161	0.184	< 0.001	< 0.001	< 0.001	0.009	0.419	< 0.001
WGF × GT interaction		0.095	0.772	0.832	0.233	0.301	0.013	0.815	0.660

abc means within columns not sharing a common suffix are significantly different.

digestibility in wheat-based diets was superior to blended diets and sorghum-based diets where the three mean values were significantly different.

The effects of WGF and feed grain on feed intake, water intake, feed:water intake ratios and excreta dry matter during the total collection period are shown in Table 11. WGF significantly reduced water intakes by 9.72% but there were no significant treatment effects on feed intake and water:feed intake ratios. There was a significant interaction for excreta dry matter as WGF significantly increased excreta dry matter of birds offered wheat-based diets from 22.13 to 25.08% but significantly decreased excreta dry matter from 26.08 to 24.50% in birds offered sorghum-based diets.

Table 11
Effects of whole grain feeding and grain type on feed intake, water intake and excreta dry matter (DM) during the total excreta collection period.

Treatment		Feed intake (g/bird/day)	Water intake (g/bird/day)	Water: feed ratio	Excreta DM (%)
WGF	Grain Type				
Ground barley	Wheat	161.7	362.5	2.25	22.13c
	Sorghum	165.3	356.0	2.15	26.08a
	Blend	166.3	360.1	2.17	24.60ab
Whole barley	Wheat	155.2	332.7	2.15	25.08ab
	Sorghum	151.6	328.0	2.17	24.50b
	Blend	160.4	314.9	1.79	25.30ab
SEM		5.57	14.33	0.076	0.533
Main effects: Diet type					
Ground grain		164.4	359.5a	2.19	24.27
Whole grain		155.7	325.2b	2.1	24.96
Feed grain					
Wheat		158.5	347.6	2.20	23.61
Sorghum		158.4	342.0	2.16	25.29
Blend		163.4	337.5	2.07	24.95
Significance (P =)					
WGF		0.063	0.006	0.143	0.123
Grain type (GT)		0.600	0.780	0.234	0.008
WGF × GT interaction		0.738	0.840	0.366	0.001

abc means within columns not sharing a common suffix are significantly different.

4. Discussion

4.1. Phytase and WGF

There are very few evaluations of exogenous phytase inclusions under WGF regimes. In the present study, a significant treatment interaction was observed for FCR where phytase advantaged FCR in whole-grain diets but, conversely, phytase disadvantaged FCR in conventional, ground-grain diets. Significant treatment interactions were also observed for parameters of nutrient utilisation (AME, ME:GE, N retention, AMEn). The most notable example was AME as phytase addition numerically depressed AME in ground-grain diets but phytase addition to whole grain diets significantly improved AME. Thus, in the present study, phytase was more effective in terms of feed conversion efficiency and energy utilisation when used in association with whole rather than ground barley.

It should be noted that gizzard pH was 2.91 overall, which is in close proximity to the optimal pH of 3.0 for peak activity of the phytase used in this study (Menezes-Blackburn et al., 2015). Moreover, the gizzard is the prime site of phytate degradation (Truong et al., 2016a) and the 20.7% increase in relative gizzard weight should facilitate phytase activity by more thorough grinding and increased episodes of reverse peristalsis (Svihus, 2011). Classically, WGF reduces feed intakes and the likelihood is that this stems from increased retention of digesta in the gizzard and slower passage rates along the digestive tract from reverse peristalsis (Liu et al., 2014). While these assumptions remain unconfirmed, if valid, they should advantage phytase efficacy. Longer gizzard retention times should enhance phytate degradation and slower passage rates should facilitate the absorption of nutrients released by phytase.

Additionally, phytase improved protein (N) digestibility in the distal ileum, irrespective of diet form. Surprisingly, phytase significantly reduced toe ash but there is no obvious explanation for this result which may have been biologically spurious as percentage toe ash was not correlated with any other relevant parameter.

Selected Pearson correlations from phytase and WGF data are shown in Table 12. WGF increases in relative gizzard weights which were associated ($r = -0.542$; $P < 0.005$) with reductions in gizzard pH. Also, relative gizzard weights were positively correlated with AME ($r = 0.747$; $P < 0.001$), ME:GE ($r = 0.813$; $P < 0.001$) and AMEn ($r = 0.705$; $P < 0.001$). Additionally, relative gizzard weights were associated with expanded starch:protein disappearance rate ratios in the proximal ileum ($r = 0.495$; $P < 0.01$) and distal ileum ($r = 0.504$; $P < 0.01$). Moreover, as tabulated, disappearance rate ratios in these two segments were positively correlated with AMEn to highly significant extents. This suggests that WGF and heavier gizzard weights enhance energy utilisation via favourable manipulation of starch and protein digestive dynamics in the context of phytase supplementation.

4.2. Grain type and WGF

The significant negative effect of WGF as 12.5% whole barley on FCR (Table 7) in the present study was not expected. In a recent review, Liu et al. (2014) tabulated the outcomes of 11 selected WGF studies in which the median whole grain inclusion level of 15% generated a median increase in relative gizzard weights of 25.4%. However, this was associated with a median improvement in FCR of 4.72%. In this study 12.5% whole grain increased gizzard weights by 22.5% which are very similar values but, in contrast, FCR was depressed by 2.35%. Feed wastage or 'feed-flicking' is sometimes observed when a mix of whole grain and pelleted concentrate is

Table 12
Pearson Correlations of relative gizzard weights, gizzard pH, starch: protein disappearance rate ratios in the proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI), apparent metabolisable energy (AME), metabolisable energy to gross energy ratio (ME:GE ratio) and N corrected apparent metabolisable energy (AMEn) in birds offered wheat-sorghum blended diets without and with phytase.

	Gizzard weight	Gizzard pH	S:P ratio[1] PJ	S:P ratio[1] DJ	S:P ratio[1] PI	S:P ratio[1] DI	AME	ME:GE ratio
Gizzard weight								
Gizzard pH	r = −0.542 P = 0.003							
S:P ratio[1] PJ	r = −0.181 P = 0.357	r = 0.066 P = 0.740						
S:P ratio[1] DJ	r = 0.350 P = 0.068	r = −0.184 P = 0.350	r = 0.035 P = 0.861					
S:P ratio[1] PI	r = 0.494 P = 0.007	r = −0.268 P = 0.168	r = 0.003 P = 0.989	r = 0.934 P < 0.001				
S:P ratio[1] DI	r = 0.504 P = 0.006	r = −0.317 P = 0.100	r = −0.062 P = 0.752	r = 0.924 P < 0.001	r = 0.981 P < 0.001			
AME	r = 0.747 P < 0.001	r = −0.269 P = 0.167	r = 0.010 P = 0.960	r = 0.258 P = 0.185	r = 0.326 P = 0.090	r = 0.310 P = 0.109		
ME:GE ratio	r = 0.813 P < 0.001	r = −0.330 P = 0.087	r = 0.010 P = 0.958	r = 0.307 P = 0.112	r = 0.395 P = 0.038	r = 0.376 P = 0.049	r = 0.969 P < 0.001	
AMEn	r = 0.705 P < 0.001	r = −0.317 P = 0.100	r = 0.081 P = 0.683	r = 0.469 P = 0.012	r = 0.533 P = 0.004	r = 0.522 P = 0.004	r = 0.940 P < 0.001	r = 0.919 P < 0.001

[1]Starch: protein disappearance rate ratio.

offered to birds and it is possible that this may have influenced the feed conversion outcomes.

As a main effect, wheat-based diets generated a superior FCR (1.362 versus 1.400) in comparison to sorghum-based diets. Sorghum supported significantly higher feed intakes, which compromised FCR. All diets were supplemented with xylanase to maintain practical relevance, but birds offered wheat-based diets are far more likely to respond to xylanase than sorghum because concentrations of soluble non-starch polysaccharides are greater in wheat. This statement has support as xylanase has been shown to improve 42-day FCR by 7.10% (1.792 versus 1.929) in broilers offered a wheat-based diets but, in comparison, generated a fractionally negative response of 0.81% (1.860 versus 1.845) in sorghum-based diets (Selle et al., 2010).

Relative gizzard weight responses generated by 12.5% whole barley were significant; however, the 22.5% increase in gizzard weight was modest in comparison to the 31.8 and 50.0% increases following whole barley inclusions of 10 and 20%, respectively, as reported in Biggs and Parsons (2009). Nevertheless, in this study, whole barley generated significantly heavier relative gizzard weights and the incidence of dilated proventriculi was reduced to zero. These responses illustrate the development of a more robust gizzard from WGF which is associated with enhanced gut integrity. Instructively, WGF has been previously shown to reduce the incidence of dilated proventriculi from 8.35 to 1.05% (Truong et al., 2016b), which is consistent with the concept that WGF promotes gut integrity.

Birds offered wheat-based diets showed better nutrient utilisation overall; however, birds offered sorghum-based diets exhibited the greatest response to whole grain as reflected in significant treatment interactions for AME, ME:GE ratios and AMEn. For example, AME of birds offered wheat-based diets was increased by only 0.02 MJ with the transition from ground to whole grain but birds offered sorghum-based diets had a significant AME response of 0.62 MJ. The pertinent point is that the energy utilisation of birds offered conventional sorghum-based diets was markedly inferior to that of their wheat counterparts. In comparison to a target energy density of 12.97 MJ/kg, wheat-based diets supported an energy density of 13.29 MJ/kg as opposed to 11.94 MJ/kg for sorghum. That sorghum responded to WGF may be attributed to its inherently poorer energy utilisation. This is supported by a review of seven WGF studies (Liu et al., 2014) where there was a negative relationship ($r = -0.513$; $P < 0.025$) between energy utilisation in birds offered control diets and their absolute responses to whole grain inclusions.

Thus, the response in energy utilisation of sorghum-based diets to whole barley illustrate the sub-standard energy utilisation in poultry offered diets based on this feed grain as reviewed by Liu et al. (2015). In this review, the poor energy utilisation of, effectively, sorghum starch was largely attributed to kafirin and phenolic compounds which are essentially unique to grain sorghum. Kafirin is the dominant protein fraction in sorghum and has been reported to compromise starch utilisation probably as a result of starch-protein interactions in sorghum endosperm (Taylor and Emmambux, 2010). It is also likely that certain 'non-tannin' phenolic compounds compromise energy utilisation by interacting with starch including polyphenols such as flavan-4-ols (Truong et al., 2016c) and phenolic acids such as ferulic acid (Khoddami et al., 2015). Thus WGF would appear to be one approach to counter the poor utilisation of starch/energy in sorghum-based broiler diets.

The reported starch and protein (N) digestibility coefficients should be considered with caution. The fundamental problem is that the dietary marker (AIA) can be included only in the pelleted concentrate of the whole barley diets, thus its dietary concentration becomes an approximation in the calculation of digestibility coefficients. Therefore, while digestibility coefficients of the ground barley diets remain valid this is not necessarily the case for the whole grain diets.

As a main effect, WGF significantly reduced water intake by 9.72% during the total excreta collection period (Table 11). However, there was a treatment interaction ($P = 0.001$) for excreta dry matter. The transition to WGF with wheat-based diets significantly increased excreta dry matter. This outcome has industry application as WGF is often associated with improved litter quality. "Wet litter" is an increasingly real problem for chicken-meat production, as reviewed by Dunlop et al. (2016), and this outcome indicates that, at least with wheat-based diets, WGF should enhance litter quality and attenuate the problems caused by footpad dermatitis.

5. Conclusions

Our suggestion that the efficacy of exogenous phytase would be enhanced by WGF was established. As confirmed by significant treatment interactions, phytase generated more robust responses in feed conversion, absolute energy utilisation (AME, AMEn), relative energy utilisation (ME:GE ratios) and N retention under a whole grain feeding regime. This is probably attributable to greater extents of phytase-induced phytate degradation in heavier, more functional gizzards facilitated by longer retention times and more conducive pH levels in this powerful grinding organ. Birds offered sorghum-based diets responded to whole barley inclusions more robustly than their wheat-based counterparts. This was attributed to the inherently poorer starch/energy utilisation of sorghum as a feed grain in poultry which was enhanced by whole grain feeding. It was also suggested that birds offered diets based on a wheat-sorghum blend might outperform their counterparts on diets based on either feed grain, individually, due to more favourable starch digestive dynamics. However, this suggestion was rejected as the performance of birds offered blended diets was intermediate. Of interest is that WGF reduced the incidence of dilated proventriculi which is consistent with the concept that WGF enhances gut integrity. This outcome has practical implications for the chicken-meat industry. Of more important relevance is that the efficacy of exogenous phytase is amplified under whole grain feeding regimes and sorghum as a feed grain will respond to whole grain inclusions.

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