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Effects of phytase inclusions in diets containing ground wheat or 12.5% whole wheat (pre- and post-pellet) and phytase and protease additions, individually and in combination, to diets containing 12.5% pre-pellet whole wheat on the performance of broiler chickens

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ABSTRACT

Each of eight dietary treatments was offered to seven replicates (six birds per cage) of male Ross 308 chicks from 7 to 28 days post-hatch. The diets contained 741 g/kg wheat incorporated as ground (3.2 mm hammer-mill screen) wheat or 125 g/kg whole wheat included in diets, either pre- or post-pelleting. In Experiment 1 of the study, ground grain, pre-pellet and post-pellet whole grain diets were offered with and without phytase as a 3 × 2 factorial array of treatments. The effects of dietary treatments on gizzard and pancreas weights, bone mineralisation, excreta dry matter, growth performance, nutrient utilisation, digestibility coefficients and disappearance rates of starch and protein (N) in four small intestinal segments were determined. Post-pellet whole grain addition significantly increased gizzard weight by 12.5% (18.17 versus 16.15 g/kg; $P < 0.001$). Pre- and post-pellet whole grain additions improved FCR ($P < 0.10$) by 1.40% and 2.28%, respectively. Exogenous phytase significantly enhanced weight gain by 4.76% (1519 versus 1450 g/bird; $P < 0.001$) and FCR by 1.99% (1.332 versus 1.359; $P < 0.03$) irrespective of the context. Significant interactions between grain and phytase treatments were observed for energy utilisation parameters. However, pre- and post-pellet whole grain additions to non-supplemented diets significantly improved AMEn by 0.31 MJ (11.89 versus 11.58 MJ/kg; $P < 0.04$) and 0.48 MJ (12.06 versus 11.58 MJ/kg; $P < 0.001$), respectively. Post-pellet whole grain addition to non-supplemented diets significantly improved AME (13.49 versus 12.99 MJ/kg; $P < 0.001$) and ME:GE ratios (0.79 versus 0.77; $P < 0.003$). Phytase addition significantly improved AME in ground grain and pre-pellet whole grain diets by 0.43 MJ and 0.30 MJ, respectively. Phytase addition improved AMEn by 0.49 MJ in ground grain diets but this was not significant and otherwise did not influence AMEn. In Experiment 2, phytase and protease, individually and in combination, were included in diets containing 12.5% pre-pellet whole wheat as a 2 × 2 factorial treatment array. There was a significant interaction ($P < 0.015$) for weight gain following phytase and protease additions to pre-pellet whole grain diets where phytase significantly increased weight gain by 6.91% (1548 versus 1448 g/bird). Protease supplementation alone numerically increased weight gain, but in combination with phytase,

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Abbreviations: AIA, acid insoluble ash; AME, apparent metabolisable energy; FCR, feed conversion ratio; GE, gross energy; IP_6, myo-inositol hexaphosphate; ME, metabolisable energy; ME:GE, metabolisable to gross energy ratios; N, nitrogen; NSP, non-starch polysaccharide

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numerically decreased weight gain. Phytase improved FCR by 2.15% (1.319 versus 1.348; $P < 0.01$) and protease improved FCR by 1.41% (1.324 versus 1.343; $P < 0.05$), but in combination, both feed enzymes improved FCR by 3.52% (1.317 versus 1.365; $P < 0.005$) relative to the negative control. It is noteworthy that in the first experiment, whole wheat inclusions did not significantly influence starch digestibility but phytase inclusions increased distal ileal starch digestibility by 5.10% (0.948 versus 0.902; $P < 0.05$) in pre-pellet and by 3.85% (0.943 versus 0.908; $P < 0.05$) in post-pellet whole grain treatments.

1. Introduction

Whole grain feeding is an increasingly accepted practice in countries where wheat is the dominant feed grain for chicken-meat production. This includes Australia where whole grain and a balancing pelleted concentrate are usually offered as a blend and New Zealand where whole grain is incorporated into the pelleted ration for compliance reasons. In a previous study (Truong et al., 2017), post-pellet whole wheat inclusions of 4.5, 9.0 and 18.0% increased relative gizzard weights, reduced gizzard digesta pH, and improved both feed conversion ratios and energy utilisation. In this study, pre-pellet whole wheat inclusions increased relative gizzard weights by 7.70% (15.67 versus 14.55 g/kg), improved FCR by 4.45% (1.439 versus 1.506), increased AME by 0.20 MJ (12.27 versus 12.07 MJ/kg) and enhanced ME:GE ratios by 1.43% (0.710 versus 0.700). Pre-pellet whole wheat inclusions are of interest in that anecdotal evidence suggests that this approach precludes wastage from "feed flicking" that may occur when whole grain and pelleted concentrate are offered as a blend. Moreover, FCR responses to pre- and post-pellet whole grain additions were very similar in the Truong et al. (2017) study.

The inclusion of phytate degrading enzymes in poultry diets is now standard practice as is the inclusion of NSP-degrading enzymes in wheat-based diets. Tandem inclusions of phytase and xylanase in wheat-based diets has been shown to be beneficial (Ravindran et al., 1999) and it has been demonstrated that the transition from conventional diets to whole grain feeding regimes does not detract from the benefits of xylanase supplementation in wheat-based diets (Jones and Taylor, 2001; Wu and Ravindran, 2004; Wu et al., 2004). However, to the best of the authors' knowledge, evaluations of phytase in the context of whole grain feeding are limited in the study of Abdollahi et al. (2016), which focused on tandem inclusions of phytase and xylanase rather than phytase *per se*. The hallmark of whole grain feeding regimes is heavier, and presumably more functional, gizzards (Liu et al., 2014; Singh et al., 2014). The gizzard is almost certainly the primary site of phytate degradation in the avian digestive tract by bacterial phytases (Truong et al., 2016). While spec ulative, exogenous phytase may be more effective under whole grain feeding regimes because enzymic degradation of dietary phytate could be facilitated by the grinding and mixing actions of a heavier, more powerful gizzard. Interest in exogenous proteases is emerging and their efficacy in combination with phytase is obviously pertinent. Therefore, the primary objective of this study was to evaluate pre- and post-pellet whole grain feeding regimes in comparison to ground grain, control diets without and with phytase. The secondary objective was to assess phytase and protease, individually and in combination, with pre-pellet whole grain feeding.

2. Materials and methods

This feeding study comprised a total of eight dietary treatments as listed in Table 1; each dietary treatment was offered to seven replicate cages (six birds per cage) or a total of 336 male Ross 308 chicks from 7 to 28 days post-hatch. The data generated was analysed as two factorial treatment arrays. The first 3 × 2 factorial (Experiment 1) compared a ground grain control diet, a diet with 12.5% whole grain added prior to pelleting, a diet with 12.5% whole grain added following pelleting, without and with 1000 FTU/kg phytase (Axtra® PHY, Danisco Animal Nutrition/DuPont). Relevant diets were analysed for phytase activity using the method of Engelen et al. (1994) which confirmed the accurate addition of the exogenous phytase as shown in Table 1. The second 2 × 2 factorial (Experiment 2) investigated the addition of phytase and protease, individually and in combination, to diets with 12.5%

Table 1
Schedule of eight dietary treatments and analysed phytase activity.

Treatment	Description	Phytase activity (FTU/kg)
1A[a]	Control, 100% ground grain	–
2B[a]	1A + 1000 FTU/kg phytase	1100
3C[a,b]	Pre-pellet 12.5% whole grain	360
4D[a,b]	3C + 1000 FTU/kg phytase	1030
5E[b]	3C + 300 units/g protease	–
6F[b]	3C + 1000 FTU/kg phytase + 300/g units protease	1200
7G[a]	Post-pellet 12.5% whole grain	–
8H[a]	7G + 1000 FTU/kg phytase	1210

[a] Experient 1.

[b] Experient 2.

Table 2
NIR characteristics (AusScan) of major feedstuffs .

Item (g/kg)	Wheat	Soybean meal	Canola meal
Protein	120	486	351
Total starch	789	151	< 1
Apparent ME (MJ/kgas fed)	12.6	14.7	12.9
Crude fibre	31	136	190
Acid detergent fibre	37	176	230
Neutral detergent fibre	90	217	241
Total soluble NSP	< 1	9	43
Total insoluble NSP	68	208	306
Insoluble arabinoxylans	60	350	282
B-glucans	< 1	70	48

whole grain added prior to pelleting. The protease (Cibenza® DP 100, Novus) used in this study at 300 U/kg has been previously evaluated in sorghum-based broiler diets (Liu et al., 2013; Selle et al., 2013). A NSP-degrading enzyme (Econase® XT, AB Vista) was added at 16,000 BXU/kg across all the wheat-based dietary treatments.

Following characterisation of the major feedstuffs via NIR spectroscopy as detailed in Table 2, one basal diet containing wheat, soybean meal and canola meal was formulated to meet the nutrient specifications shown in Table 3. Acid insoluble ash (AIA) was included in diets as Celite™ (World Minerals, Lompoc, CA, USA) as the solid-phase dietary maker. Three diets containing ground grain (3.2 mm hammer-mill screen) or 12.5% whole grain incorporated into the ration either pre- or post-pelleting were prepared with appropriate enzyme inclusions being made at the expense of Celite to arrive at eight dietary treatments. The complete diets or the pelleted concentrate were steam-pelleted through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) with a die diameter of 4.0 mm and a conditioning temperature of 80 °C with a 14 s residence time. Pelleted diets and concentrates were crumbled for the first seven days of the feeding period and were subsequently fed as intact pellets. In the case of post-pellet whole grain diets the whole grain and pelleted concentrate were blended in a horizontal mixer.

Initially, a proprietary starter diet not containing any feed enzymes was offered to the Ross 308 chicks. At 7 days post-hatch each bird was identified (wing tag), weighed and allocated into 56 bioassay cages on the basis of body weight in an environmentally controlled facility. The allocation process followed ensured that the mean and standard deviation of body weight in each cage were nearly identical. From 7 days post-hatch the eight dietary treatments were offered to seven replicate cages and birds had unlimited access to feed and water under a '23-h-on-1-h-off' lighting regime.An initial room temperature of 32 °C was maintained for the first week and gradually decreased to 22 °C by the end of the feeding study at 28 days post-hatch. Initial and final body weights were determined, and feed intakes were recorded from which feed conversion ratios were calculated. The body-weights of dead or culled birds, which were monitored on a daily basis, were used to adjust FCR calculations when necessary. From 25–27 days post-hatch, total excreta output was collected on a daily basis from trays under the cages and feed intakes recorded to determine apparent metabolisable energy (AME), metabolisable to gross energy (ME:GE) ratios, nitrogen (N) retention and N-corrected AME (AMEn).

Table 3
Composition and nutrient specifications of basic experimental diet.

Feed ingredient	g/kg	Nutrient specification	g/kg
Wheat (ground)[a]	615.8	Metabolisable energy (MJ/kg)	12.53
Wheat (whole)[a]	125.0	Protein	210.6
Soybean meal	249.0	Calcium	7.51
Canola meal (expeller)	50.0	Total phosphorus	6.30
Soy oil	41.6	Phytate phosphorus	2.77
Dicalcium phosphate	13.0	Non-phytate phosphorus	3.53
Limestone	8.50	Lysine	11.13
Lysine HCl	2.13	Methionine	5.12
Methionine	2.49	Threonine	7.21
Threonine	0.86	Tryptophan	2.41
Sodium chloride	1.20	Isoleucine	7.56
Sodium bicarbonate	3.42	Sodium	1.60
Vitamin-trace mineral premix[b]	2.00	Potassium	8.63
Celite[c]	10.0	Chloride	1.99
		Dietary electrolyte balance (meq/kg)	234

[a] 125 g/kg wheat was added whole, either pre- or post-pelleting, when appropriate.

[b] 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.

[c] Xylanase (all diets) phytase and protease (when appropriate) was added at the expense of Celite.

Table 4
Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on relative gizzard weights and contents, relative pancreas weights, and toe ash at 28 days post-hatch (Experiment 1).

Treatment		Relative gizzard weight (g/kg)	Relative gizzard content (g/kg)	Relative pancreas weight (g/kg)	Toe ash (%)
Grain	Phytase (FTU/kg)				
Control	0	15.83	8.27	2.09	12.15ab
	1000	16.46	8.02	2.01	11.79a
Pre-pellet	0	16.11	7.76	2.48	12.21ab
	1000	15.86	7.91	2.23	11.80a
Post-Pellet	0	18.05	8.48	2.41	12.00ab
	1000	18.29	8.24	2.45	12.39b
SEM		0.4884	0.6527	0.1032	0.1586
Main Effects: Grain					
Ground		16.15a	8.14	2.05a	11.97
Pre-pellet		15.98a	7.84	2.36b	12.00
Post-pellet		18.17b	8.36	2.43b	12.20
Phytase					
0 FTU/kg		16.66	8.17	2.33	12.12
1000 FTU/kg		16.87	8.06	2.23	12.00
Significance (P =)					
Grain		< 0.001	0.674	0.002	0.308
Phytase		0.608	0.811	0.247	0.349
Grain x phytase interaction		0.68	0.93	0.393	0.027

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

Excreta were air-forced oven dried for 24 h at 80 °C and the gross energy (GE) of diets and excreta were determined via an adiabatic bomb calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL). Also, during the total excreta collection period water intake was monitored and dry matter content of excreta recorded.

At 28 days post-hatch the birds were weighed and euthanised (intravenous injection of sodium pentobarbitone). Toe samples were taken to determine bone mineralisation or percentage toe ash as per the method of Potter (1988). Gizzards were removed to determine the relative weight of the organ and its contents and relative pancreas weights were also determined. Phytase activity in gizzard digesta from birds offered dietary treatments 1A and 2B were determined by the Engelen et al. (1994) method. The small intestine was removed and divided into the four intestinal segments (proximal jejunum, distal jejunum, proximal ileum, distal ileum) which were demarcated by the end of the duodenal loop, Meckel's diverticulum, the ileo-caecal junction and their mid-points. Digesta was collected from all segments in their entirety, pooled on a cage basis, homogenised and freeze-dried to determine starch and protein (N) concentrations. The concentration of starch in diets and digesta was determined by methods described by Mahasukhonthachat et al. (2010). Nitrogen and dietary AIA concentrations were determined as outlined by Siriwan et al. (1993).

AME (MJ/kg on a dry matter basis) was calculated according to the following equation:

$$\mathrm{AME_{diet}} = \frac{(\text{Feed intake} \times \mathrm{GE_{diet}}) - (\text{Excreta output} \times \mathrm{GE_{excreta}})}{(\text{Feed intake})} \tag{1}$$

ME:GE ratios were calculated by dividing the ME recoded in birds by the GE of the appropriate diet. AMEn was calculated on the same basis by correcting to zero N retention, using the factor of 36.54 kJ/g (Hill and Anderson, 1958). N retention was calculated according to the following equation:

$$\text{N retention}(\%) = \frac{(\text{Feed intake} \times \mathrm{N_{diet}}) - (\text{Excretaoutput} \times \mathrm{N_{excreta}})}{(\text{Feed intake} \times \mathrm{N_{diet}})} \times 100 \tag{2}$$

Apparent digestibility coefficients of starch and protein (N) were calculated by the following equation:

$$\text{Digestibilitycoefficient} = \frac{(\text{Nutrient/AIA})_{\text{diet}} - (\text{Nutrient/AIA})_{\text{digests}}}{(\text{Nutrient/AIA})_{\text{diet}}} \tag{3}$$

Disappearance rates (g/bird/day) of starch and nitrogen were calculated from the following equation using feed intakes over the final 24 h:

$$\text{Disappearance rate} = \text{feed intake} \times \text{dietary nutrient content} \times \text{digestibility coefficient.} \tag{4}$$

Experimental data were analysed as 3 × 2 and 2 × 2 factorial arrays using the IBM® SPSS® Statistics 20 program (IBM Corporation. Somers, NY USA). Statistical procedures included univariate analyses of variance using the general linear models procedure, linear regressions and Pearson correlations. A probability level of less than 5% was considered to be statistically significant ($P \leq 0.05$). The feeding study was conducted so as to comply with specific guidelines approved by the Animal Ethics Committee of The University of Sydney.

3. Results

3.1. Experiment 1: the effects of phytase addition to pre- and post-pellet whole grain feeding regimes

The effects of whole grain additions and phytase supplementation on relative gizzard and pancreas weights and toe ash are shown in Table 4. Post-pellet whole grain addition significantly increased relative gizzard weights by 12.5% compared to birds offered ground grain diets (18.17 versus 16.15 g/kg; $P < 0.001$) but gizzard contents were not influenced by dietary treatment. Both pre- and post-pellet whole grain additions significantly ($P < 0.05$) increased relative pancreas weights by 15.1% (2.36 versus 2.05 g/kg) and 18.5% (2.43 versus 2.05 g/kg), respectively. Relative increases in gizzard and pancreas weights were significantly correlated ($r = 0.452$; $P < 0.005$). There was a significant treatment interaction ($P < 0.03$) for percentage toe ash because phytase was more effective in the post-pellet whole grain diets than on the other two dietary contexts.

The effects of whole grain additions and phytase supplementation on water and feed intakes and excreta dry matter are shown in Table 5. There was a significant treatment interaction ($P < 0.05$) for feed intake where phytase significantly increased feed intake of birds offered the pre-pellet whole grain diet but tended to depress feed intake in the remaining two comparisons. The transition from ground grain to pre-pellet whole grain and to post-pellet whole grain diets numerically increased dry matter content of excreta.

The effects of whole grain additions and phytase supplementation on growth performance parameters are shown in Table 6. Whole grain dietary additions tended ($P < 0.10$) to improve FCR and the transition from ground grain to pre-pellet whole grain and to post-pellet whole grain linearly improved FCR ($r = -0.333$; $P < 0.04$). As a main effect, phytase significantly increased weight gain by 4.76% (1519 versus 1450 g/bird; $P < 0.001$) and improved FCR by 1.99% (1.332 versus 1.359; $P < 0.03$). Phytase tended to increase feed intake by 2.69% (2023 versus 1970 g/bird; $P = 0.052$), which closely approached significance. The overall 3.98% mortality/cull rate was unrelated to treatment.

The effects of whole grain additions and phytase supplementation on parameters of nutrient utilisation are shown in Table 7. Significant treatment interactions were observed for AME ($P < 0.005$), ME:GE ($P < 0.05$) ratios and AMEn ($P < 0.005$). Phytase significantly (AME, ME:GE ratio) or numerically (AMEn) improved energy utilisation in ground grain and pre-pellet whole grain diets but depressed energy utilisation following supplementation of post-pellet whole grain diets. Taken independently, the transition from ground grain to post-pellet whole grain diets increased AME by 0.50 MJ (13.49 versus 12.99 MJ/kg), ME:GE ratios by 1.95% (0.785 versus 0.770) and AMEn by 0.48 MJ (12.06 versus 11.58 MJ/kg). Again, taken independently, phytase increased AME by 0.43 MJ (13.42 versus 12.99 MJ/kg) in ground grain diets and by 0.30 MJ (13.41 versus 13.11 MJ/kg) in pre-pellet whole grain diets. In addition, phytase improved ME:GE ratios by 2.08% (0.787 versus 0.771), individually, within pre-pellet whole grain diets. N retention was not influenced by dietary treatments.

The effects of whole grain additions and phytase supplementation on starch digestibility coefficients and disappearance rates are

Table 5
Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on water intake, feed intake and excreta dry matter during the total excreta collection period (Experiment 1).

Treatment		Water intake (g/bird/day)	Feed intake (g/bird/day)	Water to feed intake ratio	Excreta dry matter (%)
Grain	Phytase (FTU/kg)				
Control	0	262	122b	2.15	22.7
	1000	249	115ab	2.21	23.9
Pre-pellet	0	252	107a	2.36	24.9
	1000	262	123b	2.14	24.4
Post-Pellet	0	255	119ab	2.15	24.2
	1000	245	116ab	2.13	26.6
SEM		11.419	4.606	0.1200	1.1528
Main Effects: Grain					
Ground		255	118	2.18	23.3
Pre-pellet		257	115	2.25	24.6
Post-pellet		250	118	2.14	25.4
Phytase					
0 FTU/kg		256	116	2.22	23.9
1000 FTU/kg		252	118	2.16	25.0
Significance (P =)					
Grain		0.810	0.790	0.654	0.209
Phytase		0.642	0.569	0.541	0.280
Grain x phytase interaction		0.572	0.049	0.515	0.467

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

Table 6

Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on growth performance and mortality/cull rates from 7 to 28 days post-hatch (Experiment 1).

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality rate (%)
Grain	Phytase (FTU/kg)				
Control	0	1449	1982	1.368	0.00
	1000	1502	2035	1.357	9.53
Pre-pellet	0	1448	1976	1.365	4.77
	1000	1548	2045	1.321	2.39
Post-Pellet	0	1453	1952	1.343	2.39
	1000	1508	1988	1.319	4.77
SEM		24.010	31.946	0.0140	3.1022
Main effects: Grain					
Ground		1475	2008	1.362	4.76
Pre-pellet		1498	2011	1.343	3.58
Post-pellet		1481	1970	1.331	3.58
Phytase					
0 FTU/kg		1450a	1970	1.359a	2.39
1000 FTU/kg		1519b	2023	1.332b	5.56
Significance (P =)					
Grain		0.621	0.363	0.096	0.903
Phytase		0.001	0.052	0.029	0.207
Grain x phytase interaction		0.560	0.879	0.517	0.155

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

Table 7

Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on nutrient utilisation at 25–27 days post-hatch (Experiment 1).

Treatment		AME (MJ/kg DM)	ME:GE ratio (MJ/MJ)	N retention (%)	AMEn (MJ/kg DM)
Grain	Phytase (FTU/kg)				
Control	0	12.99a	0.770a	77.15	11.58a
	1000	13.42c	0.778ab	74.35	12.07b
Pre-pellet	0	13.11ab	0.771a	72.50	11.89b
	1000	13.41c	0.787b	74.39	12.03b
Post-Pellet	0	13.49c	0.785b	75.87	12.06b
	1000	13.31bc	0.780b	75.81	11.86b
SEM		0.0793	0.0046	1.5938	0.0956
Main Effects: Grain					
Ground		13.20	0.779	75.75	11.83
Pre-pellet		13.26	0.779	73.45	11.96
Post-pellet		13.40	0.782	75.84	11.96
Phytase					
0 FTU/kg		13.12	0.775	75.18	11.85
1000 FTU/kg		13.38	0.785	74.85	11.99
Significance (P =)					
Grain		0.045	0.716	0.248	0.268
Phytase		0.007	0.015	0.805	0.074
Grain x phytase interaction		0.002	0.030	0.346	0.004

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

shown in Table 8. Significant interactions were observed for all determinations with proximal ileal starch digestibility coefficients being the one exception. The transition from ground grain to post-pellet whole gain compromised starch digestibility to significant extents in both jejunal segments. Grain feeding methods did not influence starch disappearance rates along the small intestine. Phytase addition to ground grain diets did not statistically influence starch digestibility coefficients but phytase did increase distal ileal starch digestibility by 5.10% (0.948 versus 0.902; $P < 0.05$) in pre-pellet whole grain diets. In post-pellet whole grain diets, phytase increased starch digestibility by 24.5% in the proximal jejunum, 6.86% in the distal jejunum and 3.85% in the distal ileum. The effects of whole grain additions and phytase supplementation on protein (N) digestibility coefficients and disappearance rates are shown in Table 9 where significant interactions were observed for all determinations. In respect of protein (N) digestibility coefficients the transition from ground grain to pre-pellet whole grain in non-supplemented diets depressed protein digestibility to

Table 8

Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on starch digestibility coefficients and starch disappearance rates (g/bird/day) in four small intestinal segments in broiler chickens at 28 days post-hatch (Experiment 1).

Treatment		Digestibility coefficient				Disappearance rate (g/bird/day)			
Grain	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Control	0	0.866b	0.919b	0.921	0.916ab	49.99a	53.02ab	53.11ab	52.87ab
	1000	0.850b	0.889b	0.899	0.902a	47.56ab	49.74a	50.15a	50.39a
Pre-pellet	0	0.855b	0.927b	0.901	0.902a	52.52bc	57.10b	55.26ab	55.42ab
	1000	0.857b	0.917b	0.942	0.948b	52.81bc	56.32ab	57.84b	58.25b
Post-Pellet	0	0.669a	0.846a	0.893	0.908a	43.03a	54.70ab	57.13b	58.08b
	1000	0.833b	0.904b	0.932	0.943b	59.99c	65.36c	67.30c	68.13c
SEM		0.0316	0.0145	0.0169	0.0122	2.9811	2.4903	2.3166	2.2884
Main Effects: Grain									
Ground		0.858	0.904	0.910	0.909	48.77	51.38	51.63	51.63
Pre-pellet		0.856	0.922	0.920	0.925	52.66	56.71	56.55	56.84
Post-pellet		0.751	0.879	0.913	0.926	51.51	60.03	62.22	63.10
Phytase									
0 FTU/kg		0.797	0.900	0.905	0.909	48.51	54.94	55.17	55.45
1000 FTU/kg		0.847	0.903	0.924	0.931	53.45	57.14	58.43	58.92
Significance (P =)									
Grain		0.002	0.020	0.744	0.313	0.416	0.005	< 0.001	< 0.001
Phytase		0.063	0.788	0.175	0.031	0.050	0.287	0.093	0.071
Grain x phytase interaction		0.014	0.024	0.119	0.041	0.005	0.019	0.026	0.032

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

Table 9

Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on protein (N) digestibility coefficients and protein (N) disappearance rates (g/bird/day) in four small intestinal segments in broiler chickens at 28 days post-hatch (Experiment 1).

Treatment		Digestibility coefficient					Disappearance rate (g/bird/day)		
Grain	Phytase (FTU/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
Control	0	0.700ab	0.805bc	0.808b	0.813b	23.68ab	27.27ab	27.33ab	27.52ab
	1000	0.663a	0.762a	0.775a	0.752a	21.60a	24.78a	25.18a	24.41a
Pre-pellet	0	0.680a	0.761a	0.777a	0.777a	22.91a	25.36a	25.80a	25.88a
	1000	0.747b	0.825c	0.808b	0.819bc	27.40bc	30.21b	29.57b	29.96b
Post-Pellet	0	0.703ab	0.780ab	0.771a	0.778a	30.86c	34.22c	33.90c	34.13c
	1000	0.785c	0.829c	0.839c	0.845c	35.30d	37.24c	37.70d	38.01d
SEM		0.0207	0.0120	0.0088	0.0097	1.4437	1.2238	1.1472	1.1337
Main Effects: Grain									
Ground		0.681	0.783	0.788	0.781	22.64	26.02	26.26	25.97
Pre-pellet		0.713	0.793	0.790	0.798	25.15	27.79	27.69	27.99
Post-pellet		0.744	0.804	0.805	0.812	33.08	35.73	35.80	36.07
Phytase									
0 FTU/kg		0.694	0.782	0.784	0.79	25.81	28.95	29.01	29.18
1000 FTU/kg		0.732	0.805	0.805	0.804	28.1	30.74	30.82	30.8
Significance (P =)									
Grain		0.025	0.219	0.231	0.019	< 0.001	< 0.001	< 0.001	< 0.001
Phytase		0.043	0.022	0.004	0.051	0.060	0.081	0.061	0.089
Grain x phytase interaction		0.020	< 0.001	< 0.001	< 0.001	0.043	0.014	0.018	0.004

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

significant extents in the three posterior small intestinal segments. Phytase addition to post-pellet whole grain diets increased protein digestibility but decreased protein digestibility in ground grain diets. Phytase addition to pre-pellet whole grain diets increased protein digestibility in the proximal jejunum by 9.85% (0.747 versus 0.680), distal jejunum by 8.41% (0.825 versus 0.761), proximal ileum by 3.99% (0.808 versus 0.777) and distal ileum by 5.41% (0.819 versus 0.777). The effects of whole grain additions and phytase supplementation on starch to protein (N) disappearance rate ratios are shown in Table 10 where significant interactions were observed for all determinations. In essence, ratios were narrowed by phytase supplementation in birds offered pre-pellet whole grain

Table 10
Effects of 12.5% pre- and post-pellet whole grain additions and phytase supplementation on the starch to protein (N) disappearance ratio in four small intestinal segments in broiler chickens at 28 days post-hatch (Experiment 1).

Treatment		Proximal jejunum	Proximal ileum	Distal ileum
Grain	Phytase (FTU/kg)			
Control	0	2.13cd	1.94b	1.92b
	1000	2.22cd	1.99b	2.06c
Pre-pellet	0	2.35d	2.15c	2.15c
	1000	1.93bc	1.96b	1.94b
Post-Pellet	0	1.42a	1.69a	1.71a
	1000	1.71ab	1.78a	1.75a
SEM		0.1062	0.0378	0.0338
Main Effects: Grain				
Ground		2.18	1.97	1.99
Pre-pellet		2.14	2.05	2.05
Post-pellet		1.56	1.74	1.75
Phytase				
0 FTU/kg		1.97	1.93	1.92
1000 FTU/kg		1.95	1.91	1.93
Significance ($P =$)				
Grain		< 0.001	< 0.001	< 0.001
Phytase		0.862	0.62	0.793
Grain x phytase interaction		0.006	0.001	< 0.001

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

diets but widened in birds offered ground grain and post-pellet whole grain diets.

3.2. Experiment 2: addition of enzymes to pre-pellet whole grain feeding regimes

The effects of phytase and protease supplementation of pre-pellet whole grain diets on growth performance are shown in Table 11. Dietary treatments did not significantly influence feed intakes or mortality/cull rates. There was a significant interaction ($P < 0.015$) for weight gain as protease supplementation alone increased weight gain but decreased weight gain in combination with phytase. Individually, phytase significantly increased weight gain by 6.91% (1548 versus 1448 g/bird; $P < 0.005$). As main effects, phytase improved FCR by 2.15% (1.319 versus 1.348; $P < 0.01$) as did protease by 1.41% (1.324 versus 1.343; $P < 0.05$). In combination, both feed enzymes improved FCR by 3.52% (1.317 versus 1.365; $P < 0.005$) relative to the negative control on the basis of a pair-wise comparison.

The effects of phytase and protease supplementation on nutrient utilisation are shown in Table 12. As a main effect, phytase increased AME by 0.25 MJ (13.43 versus 13.18 MJ/kg; $P < 0.01$), ME:GE ratios by 1.68% (0.788 versus 0.775; $P < 0.01$) and

Table 11
Effects of phytase and protease supplementation of pre-pellet whole grain diets on growth performance and mortality/cull rates from 7 to 28 days post-hatch (Experiment 2).

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality Rate (%)
Phytase (FTU/kg)	Protease (U/kg)				
0	0	1448a	1976	1.365	4.77
0	300	1503ab	1999	1.330	2.39
1000	0	1548b	2045	1.321	2.39
1000	300	1498ab	1975	1.317	4.77
SEM		19.541	29.661	0.0094	0.0050
Main Effects: Phytase					
0 FTU/kg		1476	1988	1.348a	3.58
1000 FTU/kg		1523	2010	1.319b	3.58
Protease					
0 U/kg		1498	2011	1.343a	3.58
300 U/kg		1501	1987	1.324b	3.58
Significance ($P=$)					
Phytase		0.024	0.464	0.007	1.000
Protease		0.891	0.433	0.049	1.000
Phytase x protease interaction		0.014	0.129	0.111	0.395

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

Table 12
Effects of phytase and protease supplementation of pre-pellet whole grain diets on nutrient utilisation in broiler chickens from 25 to 27 days post-hatch (Experiment 2).

Treatment		AME (MJ/kg DM)	ME:GE ratio (MJ/MJ)	N retention (%)	AMEn (MJ/kg DM)
Phytase (FTU/kg)	Protease (U/kg)				
0	0	13.11	0.771	72.50	11.89
0	300	13.25	0.778	72.80	11.88
1000	0	13.41	0.787	74.39	12.03
1000	300	13.44	0.790	77.76	12.00
SEM		0.0819	0.0048	1.7447	0.0956
Main Effects: Phytase					
0 FTU/kg		13.18a	0.775a	72.64	11.89
1000 FTU/kg		13.43b	0.788b	76.08	12.02
Protease					
0 U/kg		13.26	0.779	73.45	11.96
300 U/kg		13.35	0.784	75.27	11.95
Significance (P =)					
Phytase		0.006	0.009	0.061	0.199
Protease		0.284	0.273	0.307	0.848
Phytase x protease interaction		0.505	0.649	0.308	0.899

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

tended to increase N retention by 3.44 percentage units (76.08 versus 72.64%; $P = 0.061$). Protease individually did not influence any nutrient utilisation parameters but protease and phytase in combination generated the numerically most favourable outcomes for AME, ME:GE ratios N retention.

The effects of phytase and protease supplementation on starch digestibility coefficients and disappearance rates are shown in Table 13. There were interactions in the proximal ($P < 0.015$) and distal ($P < 0.05$) jejunum because the protease treatment was significantly correlated with a reduction in starch digestibility coefficients by 15.4% (0.723 versus 0.855) and 3.78% (0.892 versus 0.927), respectively. In contrast, phytase increased starch digestibility by 4.60% (0.932 versus 0.891; $P < 0.02$) in the proximal ileum and by 3.43% (0.935 versus 0.904; $P < 0.005$) in the distal ileum. Phytase significantly accelerated starch disappearance rates by 12.8% (53.76 versus 47.68 g/bird/day; $P < 0.05$) in the proximal jejunum and by 9.37% (58.73 versus 53.70 g/bird/day; $P < 0.05$) in the proximal ileum.

The effects of phytase and protease supplementation on protein (N) digestibility coefficients and disappearance rates are shown in Table 14. Phytase increased protein (N) digestibility coefficients by 5.74% (0.811 versus 0.767; $P < 0.005$) in the distal jejunum, by 2.31% (0.796 versus 0.778; $P < 0.03$) in the proximal ileum and by 3.86% (0.808 versus 0.778; $P < 0.001$) in the distal ileum. In contrast, protease did not statistically influence digestibility coefficients. There were significant treatment interactions for protein (N) disappearance rates in all four small intestinal segments. Individually, both phytase and protease accelerated disappearance rates to

Table 13
Effects of phytase and protease supplementation of pre-pellet whole grain diets onstarch digestibility coefficients and starch disappearance rates (g/bird/day) in four small intestinal segments in broiler chickens at 28 days post-hatch (Experiment 2).

Treatment		Digestibility coefficient				Disappearance rate (g/bird/day)			
Phytase (FTU/kg)	Protease (U/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
0	0	0.855b	0.927b	0.901	0.902	52.52	57.10	55.26	55.42
0	300	0.723a	0.892a	0.881	0.907	42.84	52.83	52.14	53.71
1000	0	0.857b	0.917ab	0.942	0.948	52.81	56.32	57.84	58.25
1000	300	0.841b	0.923b	0.922	0.923	54.71	59.86	59.61	59.80
SEM		0.0207	0.0097	0.0169	0.0089	2.8838	2.6923	2.3837	2.5182
Main Effects: Phytase									
0 FTU/kg		0.789	0.909	0.891a	0.904a	47.68a	54.96	53.70a	54.57
1000 FTU/kg		0.849	0.920	0.932b	0.935b	53.76b	58.09	58.73b	59.03
Protease									
0 U/kg		0.856	0.922	0.921	0.925	52.66	56.71	56.55	56.84
300 U/kg		0.782	0.907	0.901	0.914	48.77	56.34	55.88	56.76
Significance (P =)									
Phytase		0.011	0.276	0.013	0.002	0.046	0.257	0.046	0.089
Protease		0.002	0.140	0.205	0.239	0.190	0.894	0.779	0.975
Phy. x protease interaction		0.014	0.048	1.000	0.099	0.056	0.160	0.316	0.524

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

Table 14
Effects of phytase and protease supplementation of pre-pellet whole grain diets on protein (N) digestibility coefficients and protein (N) disappearance rates (g/bird/day) in four small intestinal segments in broiler chickens at 28 days post-hatch (Experiment 2).

Treatment		Digestibility coefficient				Disappearance rate (g/bird/day)			
Phytase (FTU/kg)	Protease (U/kg)	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
0	0	0.680	0.761	0.777	0.777	22.91a	25.36a	25.80a	25.88a
0	300	0.712	0.774	0.780	0.780	28.93b	31.46b	31.70b	31.68b
1000	0	0.747	0.825	0.808	0.819	27.40b	30.21b	29.57b	29.96b
1000	300	0.716	0.797	0.784	0.797	26.05ab	29.05ab	28.60ab	29.02ab
SEM		0.0207	0.0124	0.0075	0.0081	1.4815	1.3796	1.2669	1.2873
Main Effects: Phytase									
0 FTU/kg		0.696	0.767a	0.778a	0.778a	25.92	28.41	28.75	28.78
1000 FTU/kg		0.731	0.811b	0.796b	0.808b	26.72	29.63	29.09	29.42
Protease									
0 U/kg		0.713	0.793	0.792	0.798	25.15	27.79	27.69	27.92
300 U/kg		0.714	0.786	0.782	0.788	27.49	30.25	30.15	30.35
Significance (P =)									
Phytase		0.081	0.002	0.029	0.001	0.593	0.387	0.791	0.586
Protease		0.965	0.556	0.188	0.255	0.128	0.086	0.064	0.071
Phy. x protease interaction		0.119	0.118	0.082	0.151	0.020	0.015	0.012	0.015

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

significant extents relative to the negative control. However, in combination, the two exogenous enzymes did not accelerate rates to statistically valid extents.

4. Discussion

Overall, the weight gain of birds from 7 to 28 days post-hatch was 1485 g with an FCR of 1.345. This growth performance compares favourably with the Ross 308 objectives of 1387 g in weight gain with an FCR of 1.479.

In the present study, 12.5% post-pellet whole wheat inclusion increased relative gizzard weights by 14.0% (18.05 versus 15.83 g/kg) in non-supplemented diets, which was less than anticipated. On the one hand this is consistent with relative gizzard weight increases of 9.66 and 14.0% in response to 10 and 15% inclusions of whole wheat reported by Biggs and Parsons (2009). Alternatively, Truong et al. (2017) reported that 4.5, 9.0 and 18.0% post-pellet whole grain inclusions increased gizzard weights by 16.1, 24.7 and 37.5%, respectively. Increases in gizzard weight were modest in the present study, nevertheless, the transition from non-supplemented ground grain to 12.5% post-pellet whole grain diets increased AME by 0.50 MJ,ME:GE ratios by 1.95% and AMEn by 0.48 MJ, which were significant improvements on the basis of pair-wise comparisons.

In contrast, the transition from non-supplemented ground grain to 12.5% pre-pellet whole grain diets fractionally increased relative gizzard weights by 1.77%. Again, this was less than anticipated as pre-pellet whole grain inclusions of 4.5, 9.0 and 18.0% was previously shown to increase gizzard weights by 4.60, 5.57 and 13.0%, respectively (Truong et al., 2017). These comparisons only emphasise the variability in gizzard weight responses to whole grain inclusions as noted in the Liu et al. (2014) whole grain feeding review. Post-pellet had a bigger impact than pre-pellet whole grain additions on gizzard weights, which is consistent with several reports (Jones and Taylor, 2001; Taylor and Jones, 2004a,b; Wu et al., 2004; Truong et al., 2017) and is presumably attributable to the 'crushing' of whole grain during the pelleting process.

Despite the fractional increase in gizzard weight, 12.5% pre-pellet whole grain diets significantly increased AMEn by 0.31 MJ (11.89 versus 11.58 MJ/kg; $P < 0.03$), with no significant increase to AME, however, across all treatments, relative gizzard weights were positively correlated to AME ($r = 0.336$; $P < 0.04$). The significant response in AMEn, on the basis of a pair-wise comparison, in the absence of a tangible increase in gizzard weight, raises the question as to how indicative relative gizzard weights are and how they should be interpreted in the context of whole grain feeding.

The determination of digestibility coefficients when birds are offered a blend of whole grain plus a pelleted concentrate may be compromised by the intake ratios of the two components of the ration. Consumption of relatively more pelleted concentrate than the 'target' will effectively increase the dietary concentration of marker with decreases in digestibility coefficients. There will be a reciprocal impact of increases in digestibility coefficients with the consumption of relatively more whole grain than pelleted concentrate. Effective dietary nutrient concentrations will also vary with relative intake patterns. Thus the starch and protein (N) digestibility coefficients of birds offered a blend of 12.5% whole grain and pelleted concentrate clearly should be treated with caution. The significantly lower starch digestibility coefficients in the proximal and distal jejunum in birds offered 12.5% post-pellet whole grain diets in comparison to the pre-pellet and ground grain counterparts is a case in point (Table 8). This is probably an aberration and indicates that more than the target level of 87.5% pelleted concentrate was consumed.

The transition from non-supplemented ground grain to 12.5% pre-pellet whole grain diets depressed protein (N) digestibility coefficients to significant extents in the three posterior small intestinal segments (Table 9). However, perhaps more importantly, the corresponding protein disappearance rates were not compromised to significant extents as tabulated.

In the context of whole grain feeding, phytase addition to the experimental diets increased weight gain by 4.76% and FCR by 1.99% and tended to increase feed intake by 2.69% (Table 6). Overall, phytase tended to improve energy utilisation but significant responses were confined to AME and AMEn in ground grain diets and AME and ME:GE ratios in 12.5% pre-pellet whole grain diets (Table 7). Phytase significantly increased starch digestibility coefficients in the jejunum and distal ileum and starch disappearance rates in four small intestinal segments in birds offered 12.5% post-pellet whole grain diets (Table 8). The validity of these comparisons is dependent on the assumption that relative intakes of whole grain and pelleted concentrate were similar. Under the same caveat, phytase supplementation of 12.5% post-pellet whole grain diets significantly enhances protein (N) digestibility coefficients and disappearance rates in all four small intestinal segments (Table 9). Interestingly, however, phytase supplementation of ground grain and 12.5% pre-pellet whole grain diets generated conflicting responses in protein (N) digestibility coefficients and disappearance rates. In ground grain diets phytase significantly depressed digestibility coefficients but not disappearance rates in all four segments; whereas, in 12.5% pre-pellet whole grain diets phytase significantly improved both digestibility coefficients and disappearance rates in all four segments. In summary, there is the suggestion that the positive impact of phytase on protein utilisation may be enhanced by whole grain feeding regimes and that the positive impact of phytase on growth performance was not influenced by the transition from ground grain to whole grain feeding. The bone mineralisation outcomes are of interest (Table 4) as the most positive response to phytase in percentage toe ash were recorded in post-pellet whole grain diets which suggests that this whole grain feeding approach may facilitate the enzymic liberation of phytate-bound P.

Phytase activity in the gizzard digesta of birds offered phytase–supplemented ground grain diets was 496 FTU/kg as opposed to 86 FTU/kg in their control counterparts. Wheat contains intrinsic phytase activity (Peers, 1953) which is reflected in the above comparison and phytase activities in Australian wheats ranging from 255 to 840 FTU/kg have been reported (Selle et al., 2003). The intrinsic phytase activity of wheat with post-pellet whole grain additions would not be degraded by steam-pelleting. This assumes relevance given the recent findings of Zeller et al. (2016) that wheat phytase can initiate IP_6 phytate hydrolysis in the crop and may even act synergistically with exogenous phytase.

Interestingly, relative gizzard weights were negatively correlated with starch:protein disappearance rate ratios in all four small intestinal segments to significant extents ($P < 0.005$) with correlation coefficients ranging from $r = -0.448$ (distal ileum) to $r = -0.494$ (proximal ileum) (Fig. 1). Thus, despite the modest differences in relative gizzard weights, it appears that the gizzard retained the capacity to influence the digestive dynamics of starch and protein by causing the disappearance of more starch relative to protein along the small intestine.

The tandem inclusions of phytase and NSP-degrading enzymes with predominantly xylanase activity in wheat-based poultry diets has been extensively evaluated (Woyengo and Nyachoti, 2011) and synergistic responses have been reported (Ravindran et al., 1999). However, the authors are not aware of any reported evaluations of phytase and protease in combination.

The fundamental purpose of including phytase in broiler diets is to liberate phytate-bound P. However, the 'extra-phosphoric' effects of phytase (Ravindran, 1995) are largely driven by the phytase induced diminution of protein–phytate aggregates primarily in the gizzard (Selle et al., 2012). While speculative, phytase may prevent aggregate formation by the prior degradation of IP_6 phytate and it is also possible that phytase can degrade phytate that is already involved in protein aggregations (Yu et al., 2014). It follows that the hydrolysis of proteins by an exogenous protease may reduce the extent of protein captured in protein–phytate aggregates thereby amplifying the efficacy of phytase in this context. It is then relevant that in the present study, phytase improved FCR by 2.15% and protease by 1.41%; thus the 3.52% improvement in FCR generated by the combination could be described as a fully additive response.

However, while protease numerically improved protein (N) digestibility coefficients in four small intestinal segments on an individual basis, phytase generated significant improvements in the three posterior segments. The responses to the combination were uniformly somewhat less than phytase alone (Table 14).

Protease significantly depressed starch digestibility coefficients in both jejunal segments (Table 12). This is surprising given that Selle et al. (2013) reported that the same protease significantly increased starch digestibility in the distal jejunum by 13.6% (0.770 versus 0.678) and by 4.80% (0.851 versus 0.812) in the proximal ileum in broilers offered sorghum-based diets. On the other hand,

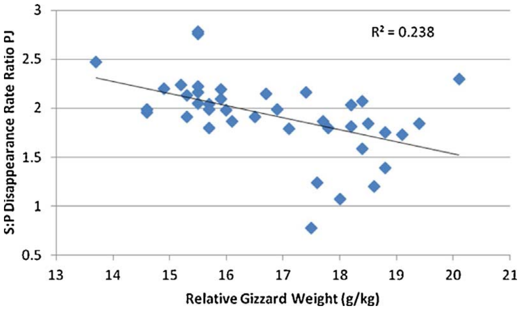

Fig. 1. Linear relationship ($r = -0.490$; $r^2 = 0.240$; $P = 0.0013$) between relative gizzard weights and starch:protein disappearance rate ratios in the proximal jejunum (Experiment 1).

phytase and protease in combination generated the most pronounced starch disappearance rates in all four small intestinal segments and supported the numerically best AME, ME:GE ratio and N retention outcomes (Table 12). It is then relevant that in Experiment 2 starch disappearance rates were significantly correlated with N retention in the three posterior small intestinal segments where the most significant correlation ($r = 0.639$; $P < 0.001$) was observed in the proximal ileum.

5. Conclusion

The transition from non-supplemented ground grain to 12.5% post-pellet whole grain diets generated a 14.0% increase in gizzard weight in association with significant improvements in AME (0.50 MJ), ME:GE (1.95%) and AMEn (0.48 MJ) and a numerical improvement of 1.83% in FCR. Phytase significantly improved weight gain and FCR in ground grain, pre-pellet and post-pellet whole grain diets, which supports the proposal phytase efficacy is not compromised, and even may be enhanced, when used under whole grain feeding regimes. Finally, tandem inclusions of phytase and protease in pre-pellet whole grain diets displayed promise in respect of FCR. Thus, further investigation into enzyme combinations for whole grain feeding may be justified.

Conflict of interest statement

The authors declare there are not any conflicts of interest.

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