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Responses in digestibilities of macro-minerals, trace minerals and amino acids generated by exogenous phytase and xylanase in canola meal diets offered to broiler chickens

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ABSTRACT

Atypical diets based on canola meal (584 g/kg) and dextrose, supplemented with phytase and xylanase individually and in combination, were offered to 120 male Ross 308 broiler chicks with six replicates for each of the four treatments. The objective was to determine the effects of phytase and xylanase on the apparent digestibility coefficients of macro-minerals, trace minerals and amino acids along the small intestine of broiler chickens. The combination increased phosphorus digestibility by a two-fold factor (0.694 versus 0.324), calcium digestibility by a three-fold factor (0.527 versus 0.178), increased zinc from -0.141 to 0.324 and increased sodium digestibility coefficients from -1.402 to -0.359 in comparison to the control diet. Similarly, phytase and xylanase in tandem generated better responses in the apparent ileal digestibility coefficients of amino acids. The enzyme combination significantly increased the digestibility of nine essential amino acids by from 5.47% (methionine) to 35.4% (threonine), and seven non-essential amino acids by from 13.9% (glutamic acid) to 32.9% (proline). The better responses in apparent ileal digestibilities of phosphorus, calcium, sodium, zinc and five other trace minerals in broiler chickens offered the combination diet was confounded by its higher enzyme recovery activities in comparison to the individual supplemented diets and the study may suggest that the inclusions of phytase and xylanase in tandem should be considered in diets containing canola meal for broiler chickens.

1. Introduction

The polyanionic phytate molecule (*myo*-inositol hexaphosphate or IP_6), which is invariably present in food and feed ingredients of plant origin, has a powerful capacity to chelate divalent cations, particularly zinc, which has been demonstrated in poultry (Maddaiah et al., 1964). The affinity of phytate to form mineral-phytate complexes has been ranked in the following descending order: Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+} and Ca^{2+} (Vohra et al., 1965). The vulnerability of phytate to enzymic degradation in these mineral-phytate complexes is reduced and the descending order of mineral potency as inhibitors of phytate hydrolysis at neutral

Abbreviations: NSP, non-starch polysaccharide; FTU, phytase units; U, units; N, protein; AIA, acid insoluble ash; UPLC, ultra performance liquid chromatography; FI, feed intake; ADC, apparent digestibility coefficient; MRT, mean retention time; ANOVA, analysis of variance

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pH was ranked as $Zn^{2+} > > Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$ by Maenz et al. (1999). The presence of phytate in poultry diets has unequivocally negative impacts on the performance of broiler chickens (Selle and Ravindran, 2007).

As a consequence the addition of phytate-degrading feed enzymes to poultry (and pig) diets is now a routine procedure as is the addition of non-starch polysaccharide (NSP) degrading enzymes to diets based on 'viscous' feed grains, including wheat and barley. Certainly, it has been demonstrated that the combined inclusions of phytase and xylanase can generate synergistic responses in broiler chickens offered wheat-based diets (Ravindran et al., 1999a; Zyla et al., 1999). The practical application of feed enzymes in poultry and pig production has undoubtedly been advantageous. Additionally, the associated research into exogenous enzymes has clarified our understanding of the anti-nutritive properties of the relevant substrates in a reciprocal manner and this holds for phytate in particular.

Approximately 150,000 tonnes of canola meal are incorporated into diets for broiler chickens in Australia as a partial alternative source of protein to soyabean meal (Spragg, 2016). Canola meal is considered inferior to soyabean meal as it contains less digestible lysine and protein and a lower energy density (Kocher et al., 2001); however, the two meals are complementary to some extent in that canola meal contains more methionine and cysteine and soyabean meal contains more lysine. Canola meal may constitute up to 20% of starter and 30% of grower diets for broiler chickens without deleterious effects (Canola Council of Canada, 2015) but its dietary inclusion levels are quite often less than 100 g/kg in practice. The higher dietary inclusions recommended for canola meal have been permitted by reduced glucosinolate concentrations (less than 30 μmoles/gram) in canola in comparison to its precursor, rapeseed (Mailer, 2004). Canola meal contains higher concentrations of phytate or phytate-P than soyabean meal, in one local survey 16 canola meal samples contained an average of 6.69 g/kg phytate-P (76% of total P); whereas, 22 soyabean meal samples contained an average of 4.53 g/kg phytate-P (68% of total P) (Selle et al., 2003). Phytase has been shown to improve ileal amino acid digestibilities significantly in broilers where either canola (526 g/kg) or soyabean (438 g/kg) meals were the only dietary source of protein (Ravindran et al., 1999b). In the present study, the digestibility of amino acids in broiler diets containing canola meal was assessed to determine 'extra-phosphoric' effects of exogenous phytase. In Australia, canola meal is often included in wheat-based diets which often contain xylanase. Therefore, in the present study, broiler diets based on canola meal and dextrose were supplemented with phytase and xylanase, individually and in combination, to determine their effects on the apparent digestibilities of macro-minerals (Ca and P), trace minerals, protein and amino acids along the small intestine of broiler chickens.

2. Materials and methods

2.1. Experimental design

The experiment consisted of a basal canola meal-dextrose diet as the control, to which inclusions of phytase and xylanase were added individually and in combination. Thus there were four dietary treatments in this feeding study.

2.2. Diet preparation

The expeller canola meal used in the study had a high oil content of nearly 12%, which attracts a premium over solvent extracted canola meal in Australia due to its higher energy contribution. The characteristics of the canola meal are shown in Table 1. The composition and nutrient specifications of the basal diet are provided in Table 2 and analysed values are shown in Table 3. Experimental diets were offered as mash, and Celite (Celite™ World Minerals, Lompoc, CA) was included in all diets as an inert acid

Table 1
Analysed mineral and amino acid concentrations, phytate content and near infrared spectroscopy (NIR) characteristics of canola meal.

Item	g/kg	Item	g/kg
Calcium (Ca)	6.04	Histidine	9.11
Phosphorus (P)	10.21	Serine	14.63
Sodium (Na)	0.94	Arginine	19.94
Zinc (Zn)	0.06	Glycine	17.45
Iron (Fe)	0.21	Aspartic acid	24.41
Potassium (K)	11.88	Glutamic acid	61.93
Magnesium (Mg)	4.99	Threonine	15.07
Manganese (Mn)	0.06	Alanine	15.00
Copper (Cu)	0.01	Proline	21.07
Strontium (Sr)	0.06	Lysine	17.03
		Tyrosine	7.14
Phytate	18.5	Methionine	2.68
Phytate-P	5.2	Valine	19.23
		Isoleucine	14.07
NIR (%)		Leucine	24.56
Protein	38.71	Phenylalanine	13.62
Fat	11.75	Total	296.93
Fibre	10.72		

Table 2
Composition and calculated nutrient specifications of the basal diet.

Composition	g/kg	Nutrient specifications	g/kg
Dextrose	350.0	Formulated	
Canola meal	584.17	Metabolisable energy (MJ/kg)	12.66
Soyabean oil	20.0	Protein	208.5
Sodium chloride	2.69	Fat	91.7
Sodium bicarbonate	1.47	Fibre	57.2
Limestone	4.67	Calcium	8.04
Dicalcium phosphate	12.0	Total phosphorus	7.47
Choline chloride (60%)	3.0	Available phosphorus	3.49
Vitamin-trace mineral premix[a]	2.0	Sodium	1.70
Celite[b]	20.0	Potassium	7.31
		Digestible amino acids	
		Lysine	9.16
		Methionine	3.62
		Cystine	4.05
		Threonine	6.76
		Tryptophan	2.37
		Arginine	11.06
		Isoleucine	6.62
		Valine	8.56

[a] 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.

[b] Enzymes added at the expense of Celite.

Table 3
Analysed concentration of minerals, amino acids and enzyme activities in the four experimental diets.

Mineral	g/kg	Amino acid	g/kg
Calcium (Ca)	8.14	Arginine (arg)	11.31
Phosphorus (P)	7.57	Histidine (his)	5.49
Sodium (Na)	1.89	Isoleucine (ile)	8.49
Zinc (Zn)	0.11	Leucine (leu)	14.97
Iron (Fe)	0.19	Lysine (lys)	9.20
Potassium (K)	6.69	Methionine (met)	2.61
Magnesium (Mg)	2.95	Phenylalanine (phe)	8.42
Manganese (Mn)	0.13	Threonine (thr)	9.05
Copper (Cu)	0.02	Valine (val)	10.81
Strontium (Sr)	0.04	Alanine (ala)	
		Aspartic acid (asp)	14.47
		Glutamic acid (glu)	36.34
		Glycine (gly)	10.63
		Proline (pro)	12.57
		Serine (ser)	8.83
		Tyrosine (tyr)	4.42

Enzyme activity Diet	Phytase (FTU/kg)	Xylanase (U/kg)
Phytase	657	–
Xylanase	–	1784
Phytase + Xylanase	1098	2169

insoluble ash marker in order to determine nutrient digestibility coefficients. Phytase (*Buttiauxella sp.* expressed in *Trichoderma reesei;* Axtra® PHY, Danisco Animal Nutrition, Marlborough, UK) was included at 1000 FTU/kg and xylanase (endo-1,4-β-xylanase from *Trichoderma reesei,* Danisco Animal Nutrition, Marlborough, UK) was included at 2000 U/kg, in their respective diets and their correct inclusions were confirmed by analyses (Table 3). Xylanase units defined as the amount of enzyme that releases 0.48 μmol of reducing sugar as xylose from wheat arabino xylan per minute at pH 4.2 and 50 °C.

2.3. Bird management

A total of 120 male Ross 308 chicks were offered a proprietary starter diet from hatch to 10 days. At 10 days post-hatch birds were individually identified (wing-tags), weighed and allocated into cages on the basis of body-weights so as to minimize variation within

and between cages. Each of four dietary treatments was offered to six replicate cages (five birds per cage) during the 10 to 21 days post-hatch experimental period. Birds had unrestricted access to feed and water under a '16-hours-on-8-hours-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. This study fully complied with specific guidelines (Project Number: 2016/973) approved by the Research Integrity and Ethics Administration (Animal Research Authority) of The University of Sydney.

2.4. Sample collection and chemical analysis

Feed intakes were recorded over the 11-day experimental period, from which nutrient disappearance rates were calculated. Any dead or culled birds were removed and their body-weights recorded to adjust for calculations of feed intakes (g/bird) on a cage mean basis.

At day 21, birds were euthanised by an intravenous injection of sodium pentobarbitone, the abdominal cavity opened, and pH of digesta within the gizzard was determined in situ. The gizzards were removed and gizzard pH was measured by inserting a pH meter (model: 7011, Ezdo, Taiwan) into the gizzard and measurements were recorded when values stabilised. 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 hours to assess bone mineralisation as previously described (Potter, 1988). The small intestine was removed and divided into two segments; jejunum and ileum, which were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction. Digesta was collected in its entirety by gently expressing each segment and was pooled by cage, homogenised, freeze dried and weighed to determine the apparent digestibility of protein, minerals, amino acids and mean retention times. Acid insoluble ash (AIA) and nitrogen concentrations were determined on a dry matter basis as previously outlined (Siriwan et al., 1993). Protein concentrations were calculated by multiplying nitrogen content by the factor of 6.25. Mineral concentration within diets and digesta were determined by inductively-coupled plasma mass spectrometry (Truong et al., 2014). The wavelengths used were 318 nm for Ca, 327 nm for Cu, 238 nm for Fe, 766 nm for K, 285 nm for Mg, 258 nm for Mn, 590 nm for Na, 214 nm for P, 408 nm for Sr and 206 nm for Zn. Amino acid concentrations of diets and digesta were determined via 24 h liquid hydrolysis at 110 °C in 6 M HCl followed by analysis of 16 amino acids using the Walters AccQTag Ultra chemistry on a Waters Acquity UPLC as outlined (Truong et al., 2014). Apparent digestibility coefficients were calculated by the following equation:

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

Disappearance rates of protein and amino acids in two small intestinal segments were calculated from the following equation:

$$\text{Disappearance rate (g/bird/day)} = \text{FI} \times \text{nutrient content}_{\text{diet}} \times \text{ADC}$$

Feed intake (FI) is the total feed intake during experimental period from 10 to 21 days, expressed on a daily basis, nutrient $\text{content}_{\text{diet}}$ is the dietary protein or amino acid concentrations (g/kg) and ADC is the apparent digestibility coefficients of the relevant nutrient.

Estimated mean retention time (MRT) was calculated by using the following equation (Weurding et al., 2001):

$$\text{MRT(min)} = \frac{1440 \times \text{AIAdigesta} \times \text{Weight}}{FI24\text{h} \times \text{AIAfeed}}$$

Where AIA digesta is the AIA concentration in the digesta (mg/g), Weight is the weight of dry gut content (g), FI24h is the feed intake over 24 hours before sampling (g), AIA feed is the AIA concentration in the feed (mg/g) and 1440 is the total minutes per day.

2.5. Statistical analysis

Experimental data were analysed as a one-way ANOVA, Pearson correlations were performed and pairwise comparisons were drawn using Tukey's post-hoc analysis via IBM® SPSS® Statistics 20 program (IBM Corporation. Somers, NY USA). Experimental units were cage means and a probability level of less than 5% was considered statistically significant.

3. Results

The effects of dietary treatments on feed intake, weight gain, percentage toe ash, gizzard pH and mean retention time of digesta in the jejunum and ileum are shown in Table 4. While intakes were less than objectives there were no significant differences between treatments ($P > 0.70$). Dietary treatments did not influence the mortality rate (1.67 %, $P > 0.55$), toe ash ($P > 0.60$) and gizzard pH ($P > 0.30$). There were significant ($P < 0.05$) but relatively subtle differences in mean retention times of digesta in the jejunum. However, the enzyme combination increased digesta retention time in the ileum by 64 minutes (172.1 versus 108.2 minutes; $P < 0.001$) relative to chicks offered the non-supplemented control diet. In contrast, individual enzyme additions did not influence ileal mean retention times.

Dietary treatments influenced ($P < 0.001$) the apparent ileal digestibility coefficients of nine minerals as shown in Table 5. The

Table 4
Effects of dietary treatments on feed intake and weight gain from 10 to 21 days post-hatch and toe ash, gizzard pH and mean retention time (MRT) of digesta at 21 days post-hatch.

Treatment	Feed Intake (g/bird)	Weight gain (g/bird)	Toe Ash (%)	Gizzard pH	MRT jejunum (minutes)	MRT ileum (minutes)
Control	551	285	13.80	2.61	53.2ab	108.2a
Phytase	556	279	13.99	2.85	48.0a	116.7a
Xylanase	560	306	13.76	2.71	59.1b	118.8a
Phytase + Xylanase	544	279	13.67	2.74	62.3b	172.1b
SEM	10.0	32.3	0.173	0.090	3.469	6.474
Significance (P =)	0.702	0.818	0.611	0.332	0.040	< 0.001
LSD (P < 0.05)	–	–	–	–	10.234	19.099

ab means within columns not sharing a common suffix are significantly different at the 5% level of probability. six observations per mean for Table 4–8.

Table 5
Effects of dietary treatments on apparent ileal digestibility coefficients of macro- and trace minerals.

Treatment	Digestibility coefficient									
	Ca	P	Na	Zn	Fe	K	Mg	Mn	Cu	Sr
Control	0.178a	0.324a	−1.402a	−0.141a	−0.124	0.676a	−0.136a	−0.283a	−0.459a	−0.091a
Phytase	0.302a	0.517b	−0.924a	−0.025a	−0.034	0.715ab	−0.017b	0.024b	−0.294ab	0.041a
Xylanase	0.259a	0.401a	−1.394a	−0.107a	−0.322	0.731b	−0.028b	−0.195ab	−0.185b	0.028a
Phytase + Xylanase	0.527b	0.694c	−0.359b	0.324b	0.060	0.821c	0.344c	0.347c	0.078c	0.367b
SEM	0.0423	0.0320	0.1805	0.0600	0.1470	0.0162	0.0608	0.0741	0.0724	0.0539
Significance (P =)	< 0.001	< 0.001	< 0.001	< 0.001	0.087	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
LSD (P < 0.05)	0.1248	0.0945	0.3828	0.1771	–	0.0478	0.1793	0.2185	0.2134	0.1590

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enzyme combination increased calcium digestibility by a three-fold factor (0.527 versus 0.178), while the individual enzymes only generated numerical increases. Phytase increased phosphorus digestibility by 60% (0.517 versus 0.324; P < 0.002) and the enzyme combination increased phosphorus digestibility by 114% (0.694 versus 0.324; P < 0.001). Xylanase numerically increased P digestibility by 23.8% (0.401 versus 0.324). Individually, phytase and xylanase did not influence zinc digestibility coefficients to any extent; whereas, the combination increased zinc digestibility from -0.141 to 0.324 (P < 0.001). Phytase numerically increased sodium digestibility by 34.1% (-0.924 versus -1.402; P = 0.074) but the enzyme combination increased sodium digestibility by 74.4% (-0.359 versus -1.402; P < 0.001) where the negative coefficients are indicative of sodium endogenous flows exceeding dietary levels of sodium. In contrast, xylanase had little effect on sodium digestibility (-1.394 versus -1.402). Similarly, phytase and xylanase in combination increased the digestibility of potassium (0.821 versus 0.676; P < 0.001), magnesium (0.344 versus -0.136; P < 0.001), manganese (0.347 versus -0.283; P < 0.001), copper (0.078 versus -0.459; P < 0.001) and strontium (0.367 versus -0.091; P < 0.001). Individually, phytase increased magnesium and manganese digestibility and xylanase increased the digestibility of potassium magnesium and copper. In contrast the digestibility of iron was not influenced (P > 0.08) by dietary treatment.

The effects of dietary treatments on protein (N) digestibility coefficients and disappearance rates are shown in Table 6. Combined phytase and xylanase inclusions increased protein (N) digestibility coefficients by 55% (0.411 versus 0.265; P < 0.005) in the jejunum and 30% (0.785 versus 0.604; P < 0.001) in the ileum. There were corresponding increases in protein disappearance rates of 65% (9.1 versus 5.5 g/bird/day; P < 0.001) in the jejunum and 41% (17.6 versus 12.5 g/bid/day; P < 0.001) in the ileum. Individually, phytase depressed protein (N) digestibility in the jejunum by 46% (0.144 versus 0.265; P < 0.01) but enhanced ileal protein (N) digestibility by 10.1% (0.665 versus 0.604; P < 0.05) without influencing protein (N) disappearance rates. Xylanase

Table 6
Effects of dietary treatments on apparent protein (N) digestibility coefficients and disappearance rates (g/bird/day) in two small intestinal segments.

Treatment	Digestibility coefficient		Disappearance rate	
	jejunum	ileum	jejunum	ileum
Control	0.265b	0.604a	5.51b	12.51a
Phytase	0.144a	0.665b	3.14a	14.54a
Xylanase	0.241b	0.627ab	4.76ab	12.49a
Phytase + Xylanse	0.411c	0.785c	9.14c	17.57b
SEM	0.0288	0.0188	0.6353	0.7562
Significance (P =)	< 0.001	< 0.001	< 0.001	< 0.001
LSD (P < 0.05)	0.0851	0.0555	1.8743	2.2308

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

Table 7
Effects of dietary treatments on apparent amino acid ileal digestibility coefficients.

Treatment	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Control	0.797a	0.705a	0.689a	0.733a	0.610a	0.877a	0.758a	0.568a	0.665a
Phytase	0.815a	0.745a	0.720a	0.762a	0.631a	0.879a	0.781a	0.621a	0.703a
Xylanase	0.805a	0.738a	0.704a	0.748a	0.629a	0.869a	0.766a	0.597a	0.689a
Phytase + Xylanase	0.891b	0.844b	0.831b	0.856b	0.776b	0.925b	0.868b	0.769b	0.819b
SEM	0.0114	0.0149	0.0151	0.0128	0.0195	0.0065	0.0119	0.0197	0.0160
Significance (P =)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
LSD (P < 0.05)	0.0459	0.0439	0.0445	0.0377	0.0576	0.0192	0.0350	0.0582	0.0473

Treatment	Ala	Asp	Glu	Gly	Pro	Ser	Tyr	Average
Control	0.708a	0.606a	0.778a	0.627a	0.589a	0.615a	0.690a	0.688a
Phytase	0.741a	0.656a	0.810a	0.676a	0.644a	0.658a	0.722a	0.723a
Xylanase	0.728a	0.641a	0.800a	0.652a	0.622a	0.634a	0.699a	0.708a
Phytase + Xylanase	0.843b	0.790b	0.886b	0.805b	0.783b	0.793b	0.829b	0.832b
SEM	0.0141	0.0190	0.0110	0.0189	0.0198	0.0179	0.0151	0.0151
Significance (P =)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
LSD (P < 0.05)	0.0416	0.0560	0.0325	0.0558	0.0585	0.0527	0.0447	0.0445

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

Table 8
Effects of dietary treatments on ileal amino acid disappearance rates (g/bird/day).

Treatment	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Control	9.06a	3.89a	5.88a	11.03a	5.64a	2.30	6.41a	5.17a	7.23a
Phytase	9.30a	4.19a	6.17a		5.56a	2.08	6.65a	5.68a	7.67a
Xylanase	8.70a	4.08a	5.73a	11.55a	5.55a	2.03	6.19a	5.24a	7.20a
Phytase + Xylanase	11.05b	5.12b	7.68b	10.81a 13.97b	7.50b	2.21	7.98b	7.63b	9.63b
SEM	0.432	0.212	0.306	0.545	0.327	0.089	0.306	0.337	0.397
Significance (P =)	0.005	0.003	< 0.001	0.002	< 0.001	0.158	0.002	< 0.001	< 0.001
LSD (P < 0.05)	1.274	0.626	0.904	1.609	0.964	–	0.901	0.994	1.171

Treatment	Ala	Asp	Glu	Gly	Pro	Ser	Tyr	Total
Phytase	6.65a	9.71a	30.26a	7.30a	8.24a	5.88a	3.26a	8.13a
Xylanase	6.28a	9.26a	29.04a	6.71a	7.58a	5.45a	2.93a	7.67a
Phytase + Xylanase	8.21b	12.72b	35.81b	9.42b	10.84b	7.69b	3.91b	10.09b
SEM	0.3260	0.540	1.393	0.409	0.481	0.326	0.160	0.409
Significance (P =)	.001	< 0.001	0.005	< 0.001	< 0.001	< 0.001	0.001	0.001
LSD (P < 0.05)	0.962	1.594	4.110	1.206	1.418	0.960	0.471	1.205

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alone did not significantly influence these parameters.

The highly significant effects of dietary treatments on ileal digestibility coefficients of amino acids are shown in Tables 7 where the pattern of responses are remarkably consistent. On an individual basis phytase or xylanase did not alter amino acid digestibility coefficients. In contrast, however, phytase and xylanase in tandem increased the digestibility of nine essential amino acids with the magnitude of responses ranging from 5.47% for methionine (0.925 versus 0.877; P < 0.001) to 35.4% for threonine (0.769 versus 0.568; P < 0.001). Similarly, the combination increased the digestibility of seven non-essential amino acids with responses ranging from 13.9% for glutamic acid (0.886 versus 0.778; P < 0.001) to 32.9% for proline (0.783 versus 0.589; P < 0.001).

As shown in Table 8, the phytase and xylanase combination significantly increased ileal disappearance rates of eight essential amino acids in the ileum where methionine was the exception. The most pronounced response was an increase of 47.6% in the threonine disappearance rate (7.63 versus 5.17 g/bird/day; P < 0.001). Similarly, the enzyme combination significantly increased ileal disappearance rates of all seven non-essential amino acids. Disappearance rate responses ranged from 26.0% for glutamic acid (35.81 versus 28.43 g/bird/day; P < 0.01) to 45.7% for proline (10.84 versus 7.44 g/bird/day; P < 0.001).

4. Discussion

Synergistic responses to phytase and xylanase in poultry offered wheat-based diets have been previously reported and were attributed to xylanase-induced reductions in gut viscosity facilitating both access of phytase to its substrate and absorption of the released nutrients (Ravindran et al., 1999a). In the present study, phytase and xylanase in combination generated better responses in the apparent ileal digestibility coefficients of calcium phosphorus, trace minerals, including zinc and sodium, and amino acids. It is important to acknowledge that there was 67% more analysed phytase activity and 22% more analysed xylanase activity in the

combination diets compared with the individual enzyme supplemented diets. This may have contributed to the better responses observed in the combination diet. For example, phytase significantly increased P digestibility by 60% (0.517 versus 0.324) and xylanase numerically increased P by 24% (0.401 versus 0.324) on an individual basis. However, the enzyme combination increased P digestibility by 114% (0.694 versus 0.324; $P < 0.001$).

Ca is poorly digested in practical broiler diets as Amerah et al. (2014) found that ileal Ca digestibility coefficients ranged from 0.540 to 0.618 in non-supplemented maize-soy diets. The ileal digestibility coefficient of Ca in the atypical canola meal-based control diet was remarkably poor at 0.178. The majority of dietary calcium was derived from canola meal (42%) in this diet with the balance from dicalcium phosphate (37%) and limestone (21%). In standard diets, limestone represents the main source of dietary Ca. Phytase and xylanase numerically improved Ca digestibility by 70% (0.302 versus 0.178) and 46% (0.259 versus 0.178), respectively. However, in tandem both enzymes increased ileal Ca digestibility coefficients by 196% (0.527 versus 0.178), as the additive response was 116%. Ca may form complexes with either phytate or inorganic P in the gut lumen as per the following equation (Selle et al., 2009a):

$$Ca_5KIP_6 \Leftarrow IP_6 + Ca + PO_4 \Rightarrow Ca_3(PO_4)_2$$

The likelihood is that Ca has a greater affinity for complex formation with phytate than with orthophosphates (Gosselin and Coghlan, 1953). The prior degradation of phytate by phytase is of pivotal importance to prevent the formation of such complexes. Individual phytase inclusion numerically improved ileal Na digestibility by 34% (-0.924 versus -1.402) but the phytase and xylanase combination significantly improved Na digestibility by 74% (-0.359 versus -1.402) in birds offered atypical canola meal-based diets. However, similar outcome have been reported in birds offered conventional wheat-based diets. Selle et al. (2009b) found that xylanase alone did not alter apparent ileal Na digestibility coefficients (-0.46 versus -0.52); whereas, phytase alone and phytase and xylanase in combination significantly increased Na digestibility to -0.04 and 0.04, respectively. It is our belief that this phytase-induced attenuation in endogenous flows of Na is a pivotal mechanism whereby phytase enhances digestibility of amino acids (Selle et al., 2012).

Individually, xylanase numerically increased the mean digestibility of sixteen amino acids by 2.91% (0.708 versus 0.688), and phytase by 5.09% (0.723 versus 0.688), giving a calculated additive response of 8.00%. However, phytase and xylanase in combination significantly increased the ileal digestibility of sixteen amino acids by 20.6% (0.830 versus 0.688), relative to the non-supplemented control diet. Thus, the actual mean response of 20.6% generated by the combination was greater than the 8.0% additive response.

The numerical mean improvement in amino acid digestibility in canola meal of 5.09% following phytase addition in this study can be compared favourably with the similar Ravindran et al. (1999b) study. The average amino acid digestibility in the control diet in the present study was less than amino acid digestibilities in canola meal previously reported (Ravindran et al., 1999b; Huang et al., 2006); thus, enzyme inclusions had greater scope to elicit effects. For example, at 42 days post-hatch control diets supported an average ileal amino acid digestibility coefficient of 0.778 across 14 amino acids in Ravindran et al. (1999b) and an average of 0.799 across the same 14 amino acids in Huang et al. (2006), also at 42 days post-hatch. In the present study, the average ileal amino acid digestibility coefficient in the control was 0.681 across the same 14 amino acids at 21 days post-hatch. One reason for this difference is that amino acid digestibility increases with age (Huang et al., 2006). Moreover, it is probable that Ravindran et al. (1999b) and Huang et al. (2006) used solvent extracted canola meal as opposed to expeller canola meal in the present study.

The mechanisms whereby exogenous phytase improves apparent amino acid digestibility coefficients are complex as reviewed by Selle et al. (2012). On one hand, it appears that phytase enhances protein digestion by preventing the formation of binary protein-phytate complexes which are refractory to pepsin digestion. On the other, it appears that phytase facilitates amino acid (and glucose) absorption via Na^+-dependent transport systems which are driven by the activity of the sodium pump (Na^+,K^+-ATPase). It has been proposed (Selle et al., 2012) that the pepsin-refractory nature of phytate-bound protein prompts compensatory hyper-secretions of pepsin and HCl which in turn trigger protective hyper-secretions of mucin and sodium bicarbonate. Thus the amelioration of endogenous secretions of Na as sodium bicarbonate is the likely basis for the capacity of phytase to 'retrieve' Na along the small intestine following phytase-induced reductions in protein-phytate complex formation. Certainly, the capacity of phytase effectively to retrieve Na along the small intestine has been conclusively demonstrated in three feeding studies (Truong et al., 2014; Truong et al., 2015; Truong et al., 2017). Phytase has been shown to increase glucose and Na^+,K^+-ATPase concentrations in jejunal mucosa of chickens (Liu et al., 2008). There is then the implication that phytase would enhance the absorption of glucose via the Na^+-dependent transporter SGLT-1 as Na^+,K^+-ATPase maintains electrochemical gradients across the gut mucosa (Therein and Blostein, 2000). It follows that phytase may have an analogous impact on amino acid absorption which appears to be related to Na partitioning. In this study, ileal Na digestibility coefficients were linearly related ($r = 0.817$; $P < 0.001$) to total amino acid digestibility coefficients as shown in Fig. 1. This outcome is consistent with the proposals of Truong et al. (2014,2015,2017), that phytase is enhancing amino acid absorption via Na^+-dependent transport systems.

Xylanase in its own right has been shown to improve ileal amino acid digestibilities in both pigs and poultry (Cowieson and Bedford, 2009) although in this study xylanase had only a modest impact. Nevertheless, phytase and xylanase in combination generated larger responses in amino acid digestibilities, which is consistent with the proposition advanced by Cowieson and Bedford (2009) that phytase and xylanase have complimentary modes of action. It is noteworthy that the xylanase utilised in this study has the capacity to degrade both soluble and insoluble NSP (Kiarie et al., 2014) which should be advantageous in respect of canola meal. This capacity may have contributed to the better responses observed across nearly all parameters assessed in the present study.

In comparison to the control diet, broiler chickens offered diets containing both phytase and xylanase had 17.1% higher estimated MRT in the jejunum and 59.1% higher estimated MRT in the ileum. Interestingly, Fig. 2 showed estimated retention time was correlated with apparent digestibility coefficients of protein (N) in the jejunum ($r = 0.719$, $P < 0.0001$) and ileum ($r = 0.892$,

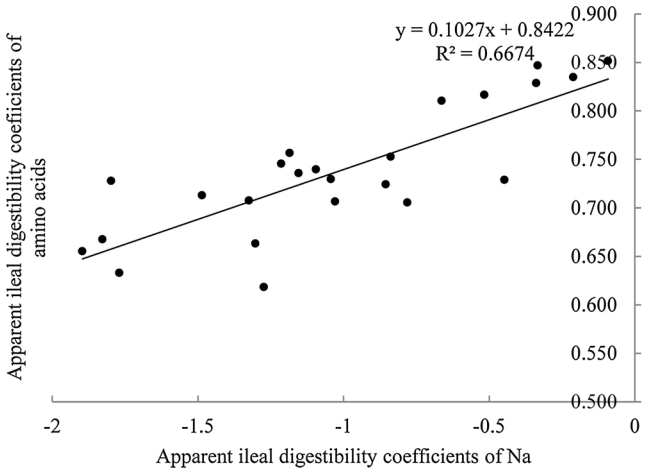

Fig. 1. Estimated retention time was correlated with apparent digestibility coefficients of protein (N) in the jejunum (r = 0.719, P < 0.0001) and ileum (r = 0.892, P < 0.0001).

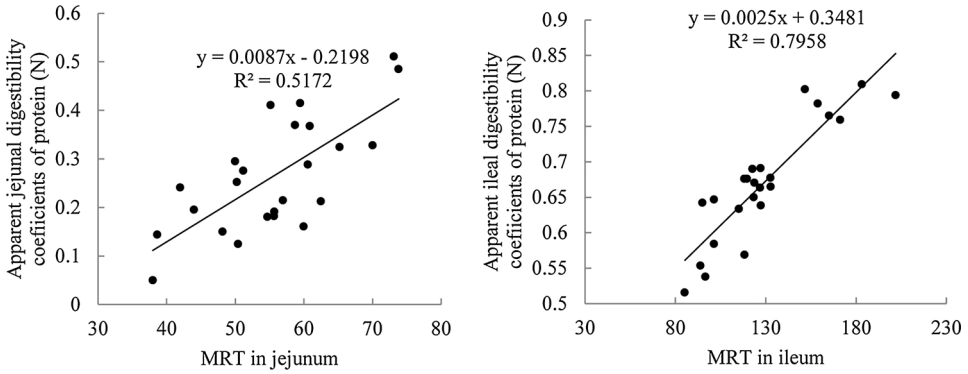

Fig. 2. Apparent digestibly coefficients of sodium were correlated with average amino acid digestibility in the ileum (r = 0.817; P < 0.001).

P < 0.0001). Comparing MRT reported in the present study with our previous publications (Liu et al., 2013) needs to be cautious because broiler chickens were offered 16 hours of lighting in the present study instead of 23 hours of lighting. This could influence feeding patterns and retention times of broiler chickens. It is not straightforward that broiler chickens offered both xylanase and phytase generated the longest retention time. Feed intakes were similar across all the dietary treatments and this suggested the total weight of digesta and the weight of jejunum and ileum may have been greater than that in broiler chickens offered the control diet. Hence, the longer retention time contributed to better nutrient utilisation in broiler chickens offered both xylanase and phytase.

In conclusion, phytase and xylanase in tandem generated better responses in atypical canola meal-based diets in the present study. The higher phytase and xylanase recovery activity in the combination diet may have contributed to this outcome. Broiler chickens are often offered wheat-based diets in Australia and xylanase is routinely included in broiler diets. Although it is not straightforward that xylanase increased digestibilities of minerals in diets based on dextrose and canola meal, the outcome of this study suggested phytase and xylanase in tandem may permit increased canola meal inclusions in diets for broiler chickens.

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Conflict of interest

All authors read and approved the final version of the manuscript. The authors declare that there are no conflicts of interest.

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