



# Influence of phytase and carbohydrase enzymes on apparent ileal nutrient and standardized ileal amino acid digestibility in growing pigs fed wheat and barley-based diets<sup>☆</sup>

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## ARTICLE INFO

### Keywords:

Phytase  
Carbohydrases  
Nutrient and energy digestibility  
Growing pigs  
Endogenous losses

## ABSTRACT

Effects of phytase with or without carbohydrases on utilization of nutrients other than P are not well understood in diets adequate in P. Thus, we investigated the effects of Phyzyme XP® (PX) and carbohydrase enzymes (Porzyme®: xylanase and β-glucanase; C) on coefficients of ileal nutrient digestibility (CAID) in growing pigs fed wheat/barley-based diets. The diets were: 1) basal (B, 8% less DE than NRC, 1998, with no enzymes), 2) B + PX, 3) B + PX + 50 g C/MT (B + PX + 50C) and 4) B + PX + 100 g C/MT (B + PX + 100C). The PX was added at 100 g/MT to all phytase containing feed, and C was added at 50 and 100 g/MT to diets 3 and 4, respectively. Acid insoluble ash was used as an indigestible marker. Diets were fed to 4 barrows (BW 35.9 ± 1.4 kg) fitted with a T-cannula at the distal ileum, according to a 4 × 4 Latin square design. Experimental periods lasted 7 d and ileal digesta were collected in 12-h periods on d-6 and d-7. At the end of the 4-wk period, pigs were fed a 5% casein diet to estimate basal endogenous AA losses. Data were subjected to pre-planned contrasts. Overall, diets containing PX had higher ( $P < 0.05$ ) CAID of energy (0.60 vs. 0.58), AA (0.72 vs. 0.69) and phytate (0.56 vs. 0.33) compared with the B diet. When corrected for basal endogenous losses, PX-containing diets had higher ( $P < 0.05$ ) coefficients of standardized ileal digestibility (CSID) of Met and Thr than the B diet. In the presence of PX, the highest response to C for CAID of energy (0.59 vs. 0.62) was achieved at 50 g/MT; the AID of DM and energy increased ( $P < 0.05$ ) by 7.2 and 7.0%, respectively, with 50 g/MT of C compared to B diet. In conclusion, phytase and carbohydrase combined increased CAID of energy, and that PX-containing diets not only increased CAID of AA but resulted in lower diet-specific endogenous losses in a practical diet.

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## 1. Introduction

It is well documented that phytase enzymes have the capacity to enhance growth performance of pigs offered available P-deficient diets. However, the genesis of phytase

enhanced growth performance in pigs fed available P-adequate diets is not well understood. One explanation that has been advanced albeit contentiously is that phytase may increase AA utilization by reducing the negative effects of phytate such as increased endogenous AA flow (Adeola and Sands, 2003; Selle and Ravindran, 2008). In a practical diet, phytate is concentrated within the indigestible fibrous cell walls and inclusion of fibre degrading carbohydrases in such diets may expose phytate to phytase. Therefore, we investigated the effects of phytase and carbohydrase enzymes on apparent ileal nutrient and standardized ileal AA digestibility in growing pigs fed wheat and barley-based diets.

<sup>☆</sup> This paper is part of the special issue entitled "11th International Symposium on Digestive Physiology of Pigs".

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## 2. Materials and methods

The enzymes were phytase (Phyzyme XP®; PX) and carbohydrase (Porzyme®, a blend of xylanase and  $\beta$ -glucanase; C) (Danisco Animal Nutrition, Marlborough, Wiltshire, UK). The diets were (1) a basal diet (B, Table 1) formulated to meet NRC (1998) requirements for all nutrients except DE which was 92% of the requirement; (2) B + PX; (3) B + PX + 50 g C/MT (B + PX + 50C); (4) B + PX + 100 g C/MT (B + PX + 100C). The PX was added at 100 g/MT to all phytase containing feed, and C was added at 50 and 100 g/MT to diets 3 and 4, respectively. Diets were prepared in mash form and contained 1 g/kg celite (acid insoluble ash) as an indigestible marker (Kiarie and Nyachoti, 2007). The study used four barrows (average BW  $35.9 \pm 1.4$  kg) fitted with a T-cannula at the distal ileum (Nyachoti et al., 1997). Animal use was approved by the Animal Care Committee of the University of Manitoba and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (1993). Diets were assigned according to a  $4 \times 4$  Latin square design and were supplied in a daily amount equal to 4% BW. Drinking water was available freely. Experimental periods lasted 7 d and ileal digesta were collected in 12-h periods on d-6 and d-7. At the end of the 4-wk period, all pigs were fed a 5% casein diet for 7 d to estimate basal endogenous AA losses (Kiarie and Nyachoti, 2007). Samples of diets and digesta were analyzed for DM, CP, AA (except tryptophan), Ca and P according to AOAC (2000). Gross energy was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL) whilst NDF was determined using ANKOM<sup>200</sup> Fiber Analyzer (ANKOM Technology, Macedon, NY). Phytate content was determined using the method of Haug and Lantzschi (1983) and acid insoluble ash according to McCarthy et al. (1974). The coefficients of digestibility were calculated using standard procedures (Kiarie and Nyachoti, 2007). The data were subjected to the mixed model procedures of SAS (SAS software release 9.1; SAS Institute, Cary, NC, USA). The pig and period effects tested non-significant and thus the final model had diet as the main effect; specific contrasts were used to compare PX vs. non PX diets as well as linear and quadratic effects of C inclusion. An alpha level of  $<0.05$  was used for determination of statistical significance.

## 3. Results

Enzyme analyses in B, B + PX, B + PX + 50C and B + PX + 100C were: phytase (0, 700, 606 and 589 U/kg), xylanase (0, 0,

**Table 1**  
Basal diet composition as fed basis.

Ingredient	g/kg
Cereals and by-products <sup>a</sup>	725.0
Soybean meal: canola meal: peas (ratio 3:4:3, respectively)	240.0
Limestone	9.50
Dicalcium phosphorous	4.00
Salt	2.50
Vitamin–mineral premix <sup>b</sup>	10.0
Added AA (HCL-Lys, DL-Met, L-Thr and L-Try)	4.00
Acid insoluble ash (celite)	5.00

Analyzed nutrient content, g/kg DM (89.7%) basis: P, phytate, Ca, CP were 5.6, 2.6, 8.5 and 198, respectively.

<sup>a</sup> Supplied in g/kg: 100, 226.7, 208.3, 30, 100 and 60 of maize, barley, wheat, rye, corn DDGS, and wheat middlings, respectively.

<sup>b</sup> Kiarie and Nyachoti (2007).

**Table 2**

Influence of phytase and carbohydrase enzymes on coefficients of the ileal apparent energy and nutrient digestibilities in growing pigs fed wheat and barley-based diets.

Item	B <sup>a</sup>	Enzyme diets <sup>b</sup>			SEM	Model <sup>c</sup>	PX <sup>d</sup>
		B + PX	B + PX + 50C	B + PX + 100C			
DM <sup>e</sup>	0.540 <sup>b</sup>	0.548 <sup>ab</sup>	0.579 <sup>a</sup>	0.570 <sup>ab</sup>	0.008	0.025	0.035
CP	0.650	0.672	0.674	0.671	0.009	0.290	0.129
P	0.335	0.482	0.455	0.473	0.034	0.069	0.014
Phytate	0.330 <sup>b</sup>	0.519 <sup>a</sup>	0.546 <sup>a</sup>	0.622 <sup>a</sup>	0.042	0.013	0.008
GE <sup>f</sup>	0.577 <sup>b</sup>	0.588 <sup>ab</sup>	0.617 <sup>a</sup>	0.598 <sup>ab</sup>	0.008	0.016	0.013
NDF <sup>e</sup>	0.147 <sup>c</sup>	0.200 <sup>bc</sup>	0.265 <sup>ab</sup>	0.297 <sup>a</sup>	0.022	0.012	0.005
AA							
Arg	0.783	0.802	0.812	0.807	0.008	0.148	0.039
His	0.703	0.725	0.738	0.741	0.010	0.079	0.023
Iso	0.667	0.681	0.687	0.682	0.009	0.486	0.152
Leu	0.705	0.729	0.739	0.731	0.010	0.188	0.050
Lys	0.702	0.713	0.734	0.713	0.012	0.313	0.211
Met <sup>e,f</sup>	0.688 <sup>c</sup>	0.738 <sup>b</sup>	0.772 <sup>a</sup>	0.753 <sup>ab</sup>	0.011	0.001	0.0003
Phe	0.618	0.653	0.661	0.636	0.015	0.214	0.084
Thr	0.661 <sup>b</sup>	0.700 <sup>a</sup>	0.710 <sup>a</sup>	0.707 <sup>a</sup>	0.007	0.001	0.0001
Val	0.643	0.667	0.672	0.654	0.013	0.393	0.168
Mean	0.687	0.712	0.725	0.714	0.008	0.063	0.016

<sup>abc</sup>Means within a row without similar superscripts are statistically different ( $P < 0.05$ ).

<sup>a</sup> Basal diet (B); 8% less DE than NRC (1998) and no added enzymes.

<sup>b</sup> Similar ingredient and nutrient profiles as basal diet. All diets had 500 U of phytase (PX, as phyzyme XP)/kg, B + PX, B + PX + 50C and B + PX + 100C had 0, 50 and 100 g of carbohydrase (C, xylanase and  $\beta$ -glucanase blend)/MT, respectively.

<sup>c</sup> Model effect.

<sup>d</sup> PX effect; non-phytase diet vs. average of all the other diets.

<sup>e</sup> Linear response ( $P < 0.05$ ) of carbohydrase in phytase containing diets.

<sup>f</sup> Quadratic response ( $P < 0.05$ ) of carbohydrase in phytase containing diets.

985 and 2881 U/kg) and  $\beta$ -glucanase (0, 0, 144 and 314 U/kg), respectively. Relative to the B diet, on average PX-containing diets had a higher ( $P < 0.05$ ) CIAD for phytate, energy and NDF (Table 2). Carbohydrase increased ( $P < 0.05$ ) CIAD of energy (quadratically) and NDF (linearly) in PX-containing diets. Phytase increased mean CIAD of mean indispensable AA ( $P = 0.016$ ) by 5.7% compared to B. When corrected for basal endogenous losses, PX increased mean CISED of all AA ( $P = 0.016$ ) by 3.9% compared to B. Specifically, highly significant ( $P < 0.005$ ) effects of PX were observed for CISED of Met and Thr. Linear and quadratic increases ( $P < 0.10$ ) due to C were observed for the CIAD and CISED of Met (Table 3).

## 4. Discussion

Increased NDF digestibility concomitant with energy digestibility is indicative of partial or complete removal of the negative effects of the fibrous fractions in cereal ingredients that are of lesser nutritional value for the pigs without supplemental carbohydrase. Indeed, it is noteworthy that although not statistically significant, feeding PX in combination with C at 100 g/MT increased the CIAD of phytate by 20% compared with control suggesting phytase limitation in accessing phytate in fibrous diets. In a comprehensive literature review involving 342 animal assays, Selle and Ravindran (2008) reported that phytase improved CIAD of AA by an average of 2.5% in a range of  $-2.6$  to 15.1% compared with non-phytase

**Table 3**

Influence of phytase and carbohydrase enzymes on coefficients of standardized ileal AA digestibility in growing pigs fed wheat and barley-based diets.

Item	B <sup>a</sup>	Enzyme diets <sup>b</sup>			SEM	Model <sup>c</sup>	PX <sup>d</sup>
		B + PX	B + PX + 50C	B + PX + 100C			
Arg	0.848	0.864	0.874	0.868	0.008	0.213	0.061
His	0.769	0.788	0.800	0.795	0.010	0.189	0.048
Iso	0.749	0.758	0.768	0.760	0.009	0.582	0.263
Leu	0.748	0.768	0.780	0.770	0.010	0.241	0.072
Lys	0.768	0.777	0.797	0.776	0.012	0.358	0.259
Met <sup>e</sup>	0.743 <sup>c</sup>	0.787 <sup>b</sup>	0.817 <sup>a</sup>	0.800 <sup>ab</sup>	0.011	0.004	0.002
Phe	0.659	0.693	0.700	0.676	0.015	0.228	0.094
Thr	0.739 <sup>b</sup>	0.778 <sup>a</sup>	0.785 <sup>a</sup>	0.776 <sup>a</sup>	0.007	0.001	0.001
Val	0.722	0.741	0.749	0.731	0.013	0.474	0.227
Mean indispensable	0.749	0.773	0.786	0.772	0.008	0.092	0.026

a, b, c, d, e See footnotes of Table 2.

abc Means within a row without similar superscripts are statistically different ( $P < 0.05$ ).

control. In agreement we observed a 5.7% higher CIAD of AA in phytase fed pigs compared to pigs fed the B diet. The mechanisms through which phytase improves AA utilization in swine and poultry has been a subject of discussion in a number of reviews (Adeola and Sands, 2003; Selle and Ravindran, 2008). In a practical diet as the one used in the present study, a plethora of factors may influence diet-specific endogenous AA flows, among them phytate and fibre (Nyachoti et al., 1997). Since, phytate is the target substrate for phytase; it is likely that phytase responses observed for CIAD of AA are due to counteraction of phytate. Indeed, phytase significantly increased phytate digestibility. Thus, in addition to better AA utilization, beneficial effect of phytase in P-adequate diets may be associated with decreased maintenance energetic costs emanating from reduced endogenous AA loss. In addition, perhaps the higher energy digestibility observed may be due to the better utilization of energy yielding dietary constituents (e.g. AA) in the presence of phytase. Furthermore, C increased Met digestibility indicating additional benefit from the enzyme combination. In conclusion, phytase and carbohydrase combined increased CIAD of energy, and PX-containing diets not only increased CIAD of AA but also lowered endogenous losses of AA in a practical diet.

### Conflict of interest

A. Owusu-Asiedu and P.H. Simmins are employees of Danisco Animal Nutrition.

### Acknowledgments

Funding for this project from Danisco Animal Nutrition is gratefully acknowledged.

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