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Corn extrusion and enzyme addition improves digestibility of corn/soy based diets by pigs: *In vitro* and *in vivo* studies

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ABSTRACT

Three experiments were conducted to assess enzymes with potential to improve the digestibility of dietary components by pigs fed diets based on extruded (EXd) or non-extruded (nEXd) corn. In Exp. 1, effects of enzyme blends (amylase, protease and xylanase) at different dose rates [high (H) *vs.* medium (M) *vs.* low (L)] on the coefficient of apparent digestibility (CAD) were investigated *in vitro* using a two-stage enzyme incubation method. The CAD of starch and NDF were both higher ($P<0.05$) in H- and M-enzyme diets than in L-enzyme and control diets. Though the CAD of DM, GE, starch and NDF did not differ between H- and M-enzyme diets, the CP CAD was higher ($P<0.05$) in H-enzyme than in M-enzyme diets. Exp. 2 was designed to further examine the effects of corn extrusion, the addition of phytase and its combination with H- and M-enzyme on the CAD of dietary components. In this experiment M-levels of xylanase and amylase were included in both the H- and M-enzyme blends, with protease dosage being held the same in each blend as in Exp. 1. The CAD of all dietary components evaluated was higher ($P<0.05$) in EXd than in nEXd corn diets. For nEXd corn diets, the combination of phytase with M- and H-enzymes resulted in a higher ($P<0.05$) CAD of GE, CP, NDF and starch than did phytase addition alone. In Exp. 3, both *in vitro* and *in vivo* trials were conducted to evaluate responses of corn-based diets to the extrusion process and the addition of phytase, H- and M-enzyme blends. Five cannulated pigs were fed five diets according to a 5×5 Latin square design. Similar to the results observed in the *in vitro* trial of Exp. 3, the EXd corn diet had higher ($P<0.05$) CAD of DM, CP, starch, NDF and GE than nEXd corn diets. M- and H-enzyme addition both resulted in increased ($P<0.05$) DM and CP CAD of nEXd corn diets. The CAD of amino acids of nEXd corn diets with H-enzyme addition was comparable with that of the EXd corn diet, and was higher ($P<0.05$) than that of the nEXd corn control diet except for Met, Thr, Trp and Cys. These results suggest that extrusion and multiple enzyme addition are effective ways to improve the nutritional value of corn-based diets for pigs.

Abbreviations: AA, amino acids; CP, crude protein; DM, dry matter; EXd, extruded; GE, gross energy; H, high; L, low; M, medium; NDF, neutral detergent fibre; nEXd, non-extruded.

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1. Introduction

Corn, an important cereal in livestock feeding, has often been considered to be a very uniform product and easily digestible with slight variations in protein and digestible energy content. However, growing evidence reveals that the feeding value of corn can vary significantly depending on the variety, growing conditions and further processing (Amornthewaphat and Attamangkune, 2008). This consequently can result in variable animal performance (Pack and Bedford, 1997; Summers, 2001). The digestibility and availability of amino acids (AA) and starch, and the presence of poorly digested components such as fibre and phytate of a grain, determine the fraction of net energy available to an animal. Therefore, improving the digestibility or utilization of various components in corn offers opportunities for improvement of its nutritive value (Summers, 2001).

Most of the energy in corn is derived from starch, but the digestibility of corn starch can be highly variable. Starch digestibility can be influenced by various factors including grain processing and the presence of anti-nutrients (Cowieson, 2005). Thermal processing of grain (*e.g.* extrusion) can result in the formation of retrograde and resistant starch which is not readily digested by the young animal (Eerlingen et al., 1994). Anti-nutrients such as non-starch polysaccharides (NSP) and phytate can result in a further decreased nutritional value of corn (Dierick and Decuypere, 1994). It was reported that the phytate in corn could result in almost no phosphorus of plant origin in a corn/soy diet being available to the animal (Simons et al., 1990).

Applying exogenous enzymes (Gracia et al., 2009; Cowieson and Ravindran, 2008; Yu et al., 2007) and the extrusion process (Gracia et al., 2009; Van der Poel et al., 1989, 1990) have been considered to be efficient approaches to enhance the nutritive value of corn diets for broilers. However, inconsistent results have been observed in responses of pigs fed corn-based diets to both exogenous enzymes and corn extrusion (Amornthewaphat and Attamangkune, 2008; Muley et al., 2007; Leek et al., 2007; Hongtragul et al., 1998). Therefore, *in vitro* and *in vivo* trials were conducted in this study to test the hypothesis that supplementing with appropriate enzyme preparations could improve digestibility by pigs fed diets based on corn that was either extruded (EXd) or non-extruded (nEXd). Both *in vitro* and *in vivo* trials were used to evaluate first, the feasibility of using an *in vitro* incubation method to determine dietary nutritional value, and second, to screen feed enzymes with potential to improve the digestibility of dietary components.

2. Materials and methods

2.1. Experimental procedures

2.1.1. Exp. 1: screening of dosage of enzyme blends)

The effect of dosage [high (H) *vs.* medium (M) *vs.* low (L)] of enzyme blends (amylase, protease and xylanase) on the digestibility of dietary components in corn-based diets was investigated *in vitro* using a two-stage enzyme incubation method (Fang et al., 2007b). A completely randomized design experiment with four dietary treatments (control, L-, M- and H-enzyme diets) was conducted to evaluate the responses of a nEXd corn diet to enzyme addition. The corn used was grown in the Shandong province of China and the composition of the basal diet is shown in Table 1. The amylase, protease and xylanase products were pure enzymes without side activities, and were provided by Danisco Animal Nutrition (Marlborough,

Table 1
Composition (as-fed basis) of the basal diet.

Diet composition (g/kg diet)		Calculated values (g/kg diet)	
Corn	603	Crude protein	190
Soybean meal (CP 44%)	280	Digestible energy (MJ/kg diet)	14.29
Fish meal (CP 65%)	20	Calcium	7.5
Dried whey	20	Total phosphorus	6.5
Sucrose	30	Available phosphorus	4.0
Soybean oil	10	Total lysine	13.2
Salt	2.4	Total methionine and cystine	6.7
Limestone	6.2	Total threonine	7.6
Dicalcium phosphate	14.3	Total tryptophan	2.2
Diet composition (g/kg diet)		Analyzed values (g/kg diet)	
L-Lysine (78.8%)	2.9	Dry matter	114
DL-Methionine	1.7	Crude protein	194
Threonine	1.5	Gross energy (MJ/kg diet)	16.24
Premix[a]	5	Starch	392
Titanium dioxide (TiO_2)	3	NDF	130
Total	1000		

[a] The premix provided per kilogram diet: Mn, 35 mg; Mg, 125 mg, Fe, 152.5 mg; Zn, 137.5 mg; Cu, 125 mg; I, 0.75 mg; retinol, 3525 μg; Vitamin D_3, 75 μg; d-α tocopherol, 33.5 mg; Vitamin K, 1.75 mg; Choline chloride, 750 mg; Niacin, 38 mg; calcium pantothenate, 35.75 mg; Riboflavin, 10 mg; Thiamine, 1 mg; Pyridoxine, 1 mg; Vitamin B_{12}, 27.5 mg; Biotin, 100 mg; Folic acid, 0.5 mg.

UK). Xylanase was derived from *Trichoderma longibrachiatum* and contained endo-1,4-β-xylanase (4400 U/g). Amylase was fermented from *Bacillus amyloliquefaciens* and contained 1,4-α-D-glucan glucanohydrolase (1500 U/g). Protease was derived from *Bacillus subtilis* and contained proteolytic activity (3000 U/g). Enzyme activities were determined using a colorimetric method by the supplier (Danisco Animal Nutrition). One unit of xylanase is the amount of enzyme which liberates 0.5 μmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt (a xylan-rich extraction from oat) xylan substrate at pH 5.3 and 50 °C in 1 min. One unit of amylase is the amount of enzyme which liberates 1 μmol of glucosidic linkages from a water insoluble cross-linked starch polymer substrate per minute at pH 6.5 and 37 °C. One unit of protease is the amount of enzyme which liberates 1 μmol of phenolic compound (tyrosine equivalents) from a casein substrate per minute at pH 7.5 and 40 °C. The inclusion levels of amylase, protease and xylanase were 830, 830 and 500 g/tonne, respectively, in the H-enzyme diet, 500, 500 and 500 g/tonne, respectively, in the M-enzyme diet, and 330, 330 and 60 g/tonne, respectively, in the L-enzyme diet. The inclusion levels of these enzymes were based on commercial recommendations by the supplier (Danisco Animal Nutrition). Basically the diet shown in Table 1 is a low fibre diet, so it was of interest to see if lower xylanase levels (60 g/tonne *vs.* 500 g/tonne) were sufficient to have the desired effect *in vitro* on, for example, NDF digestibility. The four diets were incubated in six replicates using the *in vitro* two-stage enzyme incubation and dialysis procedures as described in detail by Fang et al. (2007b). The diet samples and residues from the dialysis tubes were analyzed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), starch and gross energy (GE). Each sample was analyzed in duplicate and the *in vitro* digestible DM, CP, NDF, starch and energy were calculated by subtracting the amount of these components remaining in the residue from that present in the original diet. The CAD of dietary components (*e.g.* starch), was calculated from the following equation: starch CAD = digestible starch (g/kg diet)/total dietary starch (g/kg diet).

2.1.2. Exp. 2: effects of corn extrusion and combination use of phytase with enzyme blends screened in Exp. 1)

An *in vitro* trial with twelve dietary treatments arranged as a 2 × 6 factorial design was conducted to evaluate the effect of corn extrusion and enzyme addition (single phytase, H-enzyme, M-enzyme, and their combinations) on the digestibility of dietary components in corn-based diets. The inclusion levels of amylase, protease and xylanase were 500, 830 and 500 g/tonne, respectively, in H-enzyme diet, and 500, 500 and 500 g/tonne, respectively, in M-enzyme diet. The composition of the basal diet was the same as that in Exp. 1 (Table 1). The nEXd corn was divided into two parts: one was used for the formulation of nEXd corn diets and the other one was subjected to extrusion and then used for the formulation of EXd corn diets. As in previous research in our lab (Wang, 2005), the extrusion processing parameters were as follows: extruder, single screw extruder (Yanggong Machine Factory, Beijing, China); extruder sleeve temperature, 120 °C; screw rotating speed, 250 r/min; feeding speed, 300 r/min; corn moisture, 180 g/kg. The degree of corn starch gelatinization for this research was determined (Lund, 1981) to be 900 g/kg. The phytase was a microbial 6-phytase (Phyzyme XP; Danisco Animal Nutrition) produced in yeast, *Schizosaccharomyces pombe*, with an activity of 5000 U/g. The inclusion level was 100 g/tonne, either as a single phytase addition or in combination with H-enzyme or M-enzyme. The enzymes were directly added to the complete diet in mash form. A total of twelve diets were formulated: the control EXd corn diet, the control nEXd corn diet, and each of these plus either phytase, M-enzyme, H-enzyme, phytase + M-enzyme, or phytase + H-enzyme. The twelve diets were incubated in six replicates with the *in vitro* two-stage enzyme incubation and dialysis procedures described in Exp. 1. The residue samples from the dialysis tubes were also analyzed for DM, CP, NDF, starch and energy after being frozen and freeze-dried. The CAD of dietary components was calculated as described in Exp. 1.

2.1.3. Exp. 3: prediction in vitro and validation in vivo of effects of phytase and enzyme blends)

Diets based on EXd and nEXd corn (same as that used in Exp. 2) were used as the positive and negative control, respectively, to test the hypothesis that corn extrusion and enzyme preparation (phytase, M-enzyme and H-enzyme) could improve the digestibility of corn-based diets both *in vitro* and *in vivo*. A completely randomized design experiment with five dietary treatments was carried out. The treatments comprised a, positive control (EXd corn), negative control (nEXd corn), nEXd corn + phytase, nEXd corn + M-enzyme and nEXd corn + H-enzyme. The formulation of the five diets was the same as that in Exp. 1 (Table 1). The inclusion levels of phytase, M-enzyme and H-enzyme were the same as that used in Exp. 2. The CAD of DM, CP, NDF, starch and GE of diets was determined using the *in vitro* two-stage enzyme incubation and dialysis procedures as described in Exp. 1.

In the *in vivo* digestibility trial, all experimental procedures involving animals were approved by the Animal Care and Use Committee of the College of Animal Sciences and Technology, Huazhong Agricultural University, and were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Briefly, five barrows, average initial BW 12 ± 1.2 kg, were fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The pre- and post-operative care was carried out as described in detail by Li et al. (1994). After a 12-day recovery period, each pig was fed one of the five diets the same as that used in the *in vitro* trial in a 5 × 5 Latin square design. All diets contained 0.3% titanium dioxide (TiO_2) at the expense of corn as a dietary marker. The negative control diets based on nExd corn were mixed in one batch and then subdivided and mixed with the appropriate enzymes. Pigs were housed in individual metabolism crates (1.5 × 1.5 m) that allowed freedom of movement. Crates had plastic-coated, expanded metal floors, polyvinyl chloride walls (0.68 m high) with plexiglass windows (0.25 × 0.25 m), one single-space dry feeder located at the front of the crate, and one bowl drinker located at the back of the crate. Each experimental period consisted of 7:5 day of diet adaptation followed by 2 days of collection of digesta. The pigs were fed equal amounts twice daily, at 0800 and 1600. Total feed allowance per day was around 3% of their body weight. The feed was mixed 1:1 with water. The collection of ileal

Table 2
Effects of enzyme dosage (H-, M- and L-enzyme blends) on the *in vitro* CAD of non-extruded corn-based diets as evaluated in Exp. 1[a].

	No enzyme	L-enzyme	M-enzyme	H-enzyme	SEM	P-values
	$n=6$	$n=6$	$n=6$	$n=6$		
DM	0.378b	0.398ab	0.402a	0.403a	0.0072	*
CP	0.515c	0.535b	0.540b	0.551a	0.0025	**
GE	0.306	0.324	0.336	0.336	0.01	NS
Starch	0.182c	0.308b	0.375a	0.352a	0.0076	**
NDF	0.213b	0.215b	0.310a	0.301a	0.007	**

NS, not significant.
[a] Values within a row with no common letters differ significantly (P<0.05).
* P<0.05.
** P<0.01.

digesta was initiated at 0800 on day 6 as described in detail by Barrera et al. (2004). Digesta samples were collected into bags attached to the open cannula barrel. The bags contained diluted formic acid and were stored immediately after collection at −20 °C. At the end of the experiment, ileal digesta was thawed, pooled within pig and period, and homogenized. A subsample of each homogenate was freeze-dried and ground through a 60-mesh screen, and stored at −20 °C before analysis.

2.2. Chemical analysis

Samples of diets, ileal digesta, and residues from *in vitro* hydrolysis were analyzed for DM, CP, NDF, starch and GE. DM and CP were analyzed according to AOAC (1990). NDF content was analyzed following Van Soest et al. (1991) with samples treated with α-amylase before NDF extraction. Starch content was analyzed by spectrophotometry as described by Englyst et al. (1992). TiO_2 content was determined following the procedure of Myers et al. (2004). GE was analyzed by an adiabatic bomb calorimeter (Parr Instrument Company, Moline IL, USA) with benzoic acid as a standard. AA of ileal digesta and diets used in the *in vivo* trial were analyzed using ion-exchange chromatography after hydrolysis in 6 N HCl for 24 h at 110 °C in evacuated, sealed tubes. The Cys was determined as cysteic acid and Met as Met sulfone after preoxidation with performic acid (Fang et al., 2009). Based on the results of the chemical analyses, the *in vivo* CAD of AA, DM, CP, NDF, starch and GE was calculated using the TiO_2 concentration of feed and digesta (Adeola, 2001).

2.3. Statistical analyses

The *in vitro* CAD data in Exps. 1 and 3 were analyzed according to one-way ANOVA procedure of SAS (SAS Inst., Inc., Cary, NC, USA). For Exp. 2, differences in CAD of DM, GE, CP, NDF and starch among diets were analyzed according to the two-way ANOVA procedure of SAS (SAS Inst., Inc., Cary, NC, USA). The statistical model included the following effects: extrusion, enzyme and their interactions. The *in vivo* CAD data in Exp. 3 were analyzed according to a 5×5 Latin square design using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC, USA). Linear regression analyses (SAS REG procedure) were performed to determine and compare relationships between the *in vitro* and *in vivo* CAD data in Exp. 3. Treatment differences were considered significant at an α level of 0.05. Duncan's multiple range test was used to separate means when a significant (P<0.05) effect was detected (Duncan, 1955).

3. Results

3.1. Exp. 1: screening of dosage of enzyme blends

Effects of enzyme addition on the *in vitro* CAD of dietary components are presented in Table 2. The CAD of DM, CP, starch and NDF were all affected (P<0.05) by enzyme addition. Both DM and CP CAD were higher (P<0.05) in enzyme-supplemented diets than in the control diet. Though the CAD of DM, GE, starch and NDF did not differ (P>0.05) between H- and M-enzyme diets, CP CAD was higher (P<0.05) in H-enzyme than in M-enzyme diets. NDF CAD of H- and M-enzyme diets was also higher (P<0.05) than that of L-enzyme and control diets. Both H- and M-enzyme diets had higher (P<0.05) starch CAD than L-enzyme and control diets, but there was no difference (P>0.05) in starch CAD between H- and M-enzyme diets.

3.2. Exp. 2: effects of corn extrusion and combination use of phytase with enzyme blends screened in Exp. 1

Effects of the supplementation of phytase with or without its combination with H- or M-enzyme on the digestibility of dietary components of EXd and nEXd corn diets are shown in Table 3. The CAD of CP, GE, starch and NDF were all affected (P<0.05) by the interaction between extrusion and enzyme. Compared to their respective controls, the supplementation of each enzyme resulted in improved (P<0.05) CAD of starch and GE in both nEXd corn and EXd corn diets. A further improvement (P<0.05) in GE CAD of nEXd corn diets was observed with the increase of protease from M (500 g/t) to H (830 g/t). The CAD of NDF either in nEXd corn or EXd corn diets was also increased (P<0.05) by the supplementation of

Table 3
Effects of corn extrusion, enzyme and extrusion × enzyme interaction on the *in vitro* CAD (interaction means) of corn-based diets as evaluated in Exp. 2[a].

	Non-extruded corn						Extruded corn						SEM	P-values		
	No enzyme	Phytase	M-enzyme	M-enzyme + phytase	H-enzyme	H-enzyme + phytase	No enzyme	Phytase	M-enzyme	M-enzyme + phytase	H-enzyme	H-enzyme + phytase		Extrusion	Enzyme	Extrusion × enzyme
	n = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6				
DM	0.402c	0.412bc	0.435bc	0.444b	0.421bc	0.422bc	0.642a	0.643a	0.651a	0.650a	0.656a	0.653a	0.0131			
CP	0.525f	0.546f	0.613e	0.635de	0.618de	0.627de	0.629cde	0.635bcde	0.639bcd	0.663a	0.656ab	0.652abc	0.0084	***	***	***
GE	0.274g	0.313f	0.330f	0.378e	0.406d	0.405d	0.559c	0.589b	0.596ab	0.600ab	0.621a	0.606ab	0.0076	***	***	***
Starch	0.187g	0.214f	0.354de	0.368d	0.346e	0.347e	0.798c	0.822b	0.839a	0.833ab	0.829ab	0.825ab	0.0056	***	***	***
NDF	0.136h	0.164h	0.228fg	0.244ef	0.218fg	0.224fg	0.199g	0.258de	0.292bc	0.284cd	0.331a	0.325ab	0.0112	**	**	*

NS, not significant.
[a] Values within a row with no common letters differ significantly (P<0.05).
* P<0.05.
** P<0.01.
*** P<0.001.

Table 4
The *in vitro* CAD of non-extruded corn-based diets with and without enzyme addition compared to extruded corn-based diets as evaluated in Exp. 3[a].

	Extruded corn	Non-extruded corn				SEM	P-values
		No enzyme	Phytase	M-enzyme	H-enzyme		
	n = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6		
DM	0.622a	0.399b	0.399b	0.416b	0.407b	0.0077	***
CP	0.662a	0.494b	0.515b	0.513b	0.530b	0.0155	***
Starch	0.752a	0.207d	0.298c	0.360b	0.317bc	0.0177	***
NDF	0.367a	0.220b	0.245b	0.247b	0.248b	0.0148	***
GE	0.584a	0.365b	0.368b	0.378b	0.388b	0.0106	***

[a] Values within a row with no common letters differ significantly (P<0.05).
*** P<0.001.

M-enzyme, H-enzyme or their respective combinations with phytase. The CAD of CP in nEXd corn diets was improved by the supplementation of M-enzyme, H-enzyme, or their respective combinations with phytase, but the CAD of CP in EXd corn diets was not affected (P>0.05) by these enzymes except for M-enzyme.

3.3. Exp. 3: prediction in vitro and validation in vivo of effects of phytase and enzyme blends

Table 4 shows the *in vitro* CAD of nEXd corn diets with and without enzyme addition compared with that of the EXd corn diet. Similar to the results observed in Exp. 2, the EXd corn diet had substantially higher (P<0.05) CAD of DM, CP, starch, NDF and GE than nEXd corn diets. Compared with the control of nEXd corn diets, phytase, M- and H-enzyme supplementation all resulted in higher (P<0.05) starch CAD, but no difference (P>0.05) was observed in the CAD of the remaining dietary components evaluated.

Table 5 shows the *in vivo* CAD of nEXd corn diets with and without enzyme addition compared with that of EXd corn diet. Similar to the results observed in the *in vitro* trial of Exp. 3, the EXd corn diet had higher (P<0.05) CAD of DM, CP, starch, NDF and GE than nEXd corn diets. Compared with the control of nEXd corn diets, M- and H-enzyme supplementation both resulted in higher (P<0.05) DM and CP CAD, but the CAD of the remaining parameters evaluated was not different (P>0.05) among these groups.

Table 6 shows the CAD of AA of nEXd corn diets with and without enzyme addition compared with that of the EXd corn diet. Except for Met, Thr, Trp and Cys, the remaining AA evaluated had a higher (P<0.05) CAD in the EXd corn diet than in the nEXd corn diet with no enzyme addition. However, the CAD of all AA evaluated in the nEXd corn diet with H-enzyme supplementation was comparable (P>0.05) with that in the EXd corn diet. The CAD of Lys, Leu and Phe was lower (P<0.05) in the M-enzyme-supplemented nEXd corn diet than in the EXd corn diet, however the CAD of the other AA evaluated did not differ (P>0.05) between these two diets. Compared with the nEXd corn diet with no enzyme supplementation, phytase showed a positive effect on increase in CAD of His and Arg. In addition, although there were no differences in AA CAD between H- and M-enzyme addition diets, only the H-enzyme addition diet had higher (P<0.05) Lys, Phe, Tyr and Ala CAD than the phytase addition diet, indicating the further improvement effect caused by increasing the inclusion levels of protease.

The regression analyses for the *in vitro* and *in vivo* CAD are presented in Table 7. The *in vitro* CAD of starch, CP and GE all showed high linear correlations ($R^2 > 0.72$, P<0.05) with their respective *in vivo* CAD. In contrast, the *in vitro* CAD of DM and NDF was not correlated (P>0.05) with their *in vivo* CAD.

Table 5
The *in vivo* CAD of non-extruded corn-based diets with and without enzyme addition compared to extruded corn-based diets as evaluated by pigs in Exp. 3[a].

	Extruded corn	Non-extruded corn				SEM	P-values
		No enzyme	Phytase	M-enzyme	H-enzyme		
	n = 5	*n* = 5	*n* = 5	*n* = 5	*n* = 5		
DM	0.678a	0.594c	0.591c	0.630b	0.635b	0.0063	***
CP	0.755a	0.615c	0.616c	0.668b	0.674b	0.0096	***
Starch	0.767a	0.650b	0.643b	0.681b	0.693b	0.0175	**
NDF	0.443b	0.485ab	0.463b	0.559a	0.531ab	0.029	*
GE	0.695a	0.602b	0.600b	0.634b	0.637b	0.0131	***

[a] Values within a row with no common letetrs differ significantly (P<0.05).
* P<0.05.
** P<0.01.
*** P<0.001.

Table 6
The *in vivo* CAD of AA of non-extruded corn-based diets with and without enzyme addition compared to extruded corn-based diets as evaluated by pigs in Exp. 3[a].

	Extruded corn	Non-extruded corn				SEM	P-values
		No enzyme	Phytase	M-enzyme	H-enzyme		
	$n=5$	$n=5$	$n=5$	$n=5$	$n=5$		
Essential AA							
Arg	0.844a	0.775c	0.808b	0.826ab	0.834ab		
His	0.782a	0.706b	0.744a	0.746a	0.770a	0.0119	**
Ile	0.795a	0.719b	0.729b	0.765a	0.781a	0.0102	***
Leu	0.827a	0.762c	0.761c	0.789b	0.813ab	0.0087	***
Lys	0.845a	0.783d	0.805cd	0.820bc	0.832ab	0.0077	***
Met	0.700	0.630	0.673	0.723	0.714	0.0328	NS
Phe	0.828a	0.767c	0.763c	0.793bc	0.819ab	0.0109	**
Thr	0.780	0.738	0.740	0.743	0.739	0.0189	NS
Trp	0.729	0.671	0.703	0.739	0.730	0.0226	NS
Val	0.775a	0.703b	0.712b	0.753a	0.774a	0.0104	***
Non-essential AA							
Ala	0.796a	0.718c	0.733bc	0.759ab	0.799a	0.0125	**
Asp	0.768a	0.697b	0.685b	0.744a	0.763a	0.0150	**
Cys	0.693	0.624	0.664	0.725	0.709	0.0323	NS
Glu	0.791a	0.717b	0.754ab	0.777ab	0.813a	0.0202	*
Ser	0.744a	0.660b	0.672b	0.716a	0.732a	0.0113	***
Tyr	0.798a	0.749b	0.748b	0.780ab	0.804a	0.0103	**

NS, not significant.
[a] Values within a row with no common letters differ significantly (P<0.05).
* P<0.05.
** P<0.01.
*** P<0.001.

Table 7
Regression relationships between the *in vitro* and *in vivo* CAD of dietary components.

Items	Regression equation[a]	RSD	R^2	P-values
DM	$Y=0.485+0.313X$	0.0005	0.5368	NS
CP	$Y=0.244+0.777X$	0.0007	0.7234	*
Starch	$Y=0.601+0.222X$	0.0004	0.7948	**
NDF	$Y=0.613-0.439X$	0.0022	0.0795	NS
GE	$Y=0.477+0.376X$	0.0328	0.8632	**

NS, not significant.
[a] *X* and *Y* represent the *in vitro* and *in vivo* CAD, respectively.
* P<0.05.
** P<0.01.

4. Discussion

4.1. Effects of enzyme addition on digestibility of dietary components

In the present study, one of our objectives was to assess enzymes with potential to improve digestibility of corn-based diets by pigs. Our attention was focused on the evaluation of amylase, protease, xylanase and phytase, given that the digestive tract of young pigs is under development and that the digestibility and availability of starch and AA and the presence of anti-nutrients such as phytate and NSP determine the fraction of net energy available to an animal (Summers, 2001). Furthermore, considering that the active concentrations of enzymes are also an important determinant of the extent of substrate degradation (Fang et al., 2007b; Zhang et al., 1996), we designed the trials to study graded inclusion levels of enzymes. As shown in Table 2, the higher CP CAD in H-enzyme than in M-enzyme diets suggested a necessity to increase the inclusion levels of protease from M (1500 U/kg diet) to H (2500 U/kg diet). Similarly, the higher CAD of starch in H- and M-enzyme diets than in L-enzyme diet, combined with the similar starch CAD between H- and M-enzyme diet suggested that M (750 U/kg diet) dosage of amylase might be enough to effectively hydrolyze the starch in diets based on the corn evaluated. All of these results confirmed the importance of adequate enzyme activities to ensure the efficient degradation of the specific substrate. This notion was further supported by the observation that although there were no differences in AA CAD between H- and M-enzyme addition diets, only the H-enzyme addition diet had higher Lys, Phe, Tyr and Ala CAD than the phytase addition diet. These results agreed well with our previous studies (Fang et al., 2007a,b).

In addition to the appropriate activities of the specific enzymes like amylase, the degradation of substrates like starch by an enzyme is also likely to be influenced by pre-processing of corn. In support of this view, CP, GE, starch and NDF CAD were all affected by an extrusion × enzyme interaction (Table 3). Consequently, the improvement of starch and GE

CAD by enzyme supplementation was higher in nEXd corn diets than in EXd corn diets. The observation that CP CAD of EXd corn diets was increased by the addition of M-enzyme + phytase but was not affected by single phytase or M-enzyme addition, suggested an additive effect of the enzyme combination on the digestibility of dietary components. However, previous studies in broilers indicated that the digestibility of corn-based diets could be improved by the addition of either the single protease or an enzyme complex with xylanase, protease and α-amylase activity (Gracia et al., 2009; Cowieson and Ravindran, 2008; Yu et al., 2007). The increased protein and starch digestibility following phytase supplementation may be associated with the disruption of covalent bonds formed between phytate and starch or protein during the degradation of phytate (Fang et al., 2007a). Alternatively, the protein response may be related to phytate encouraging increased endogenous losses as reported by Cowieson et al. (2004). Enhanced CP digestibility may be also associated with the degradation of cell wall fibre components. It has been reported that the NSP levels in corn were approximately 90–100 g/kg with insoluble arabinoxylans comprising over 400 g/kg (Dierick and Decuypere, 1994), indicating the significance of xylanase, especially those with ability to hydrolyse insoluble arabinoxylans. The content of NDF, in which insoluble arabinoxylans are included, was therefore used to evaluate the hydrolysis of xylan-related fibres in corn-based diets. Because proteins are covalently bound to cell wall carbohydrates such as arabinoxylans occurring in both monocotyledonous and dicotyledonous plants (Dierick et al., 1989), this may have a negative effect on CP degradation. In support of this view, increasing NDF CAD by the supplementation of xylanase-based fibre-degrading enzymes improved CP CAD in corn/soy diets in previous *in vitro* and *in vivo* studies (Fang et al., 2007a, b).

4.2. Effects of corn extrusion on digestibility of dietary components

The use of EXd corn in diets to give both nutritional and palatability benefits is of interest to livestock producers (Amornthewaphat and Attamangkune, 2008). Theoretically, an extrusion process applies shear force to break down starch granules, allowing better access to digestive enzymes in the gut (Miller, 1990). This process therefore may contribute to the increase in digestibility of dietary components, particularly starch. Therefore, a second goal of the present study was to assess the effect of corn extrusion on digestibility of corn-based diets. Both the *in vitro* and the *in vivo* trial indicated a significant role of corn extrusion in improving the CAD of DM, GE, CP and starch. Similarly, previous studies show that the *in vitro* starch availability (Van der Poel et al., 1989, 1990), CAD of DM and CP (Amornthewaphat and Attamangkune, 2008; Muley et al., 2007) and the fraction of net energy available to pigs (Herkelman et al., 1990) were higher for diets with EXd corn than for those with nEXd corn. The increased CAD of EXd corn diets may be explained by the alteration of the microstructure of starch granules (Amornthewaphat and Attamangkune, 2008); for example increased starch gelatinization (Lue et al., 1991), increased enzyme susceptibility (Amornthewaphat et al., 2005), and reduced resistant starch content (Murray et al., 2001). In contrast to nEXd corn diets, enzyme addition to EXd corn diets resulted in a relatively small, although significant, increase in the CAD of dietary components. This provided further evidence for the contribution of corn extrusion to improvements in the availability of dietary components. Of particular note is that the highest improvement in the *in vitro* CAD of nEXd corn diets was observed for starch following the supplementation of enzyme (Tables 3 and 4), which suggested that the increased energy availability of nEXd corn diets was mainly a result of increases in digestible starch.

4.3. The correlation of CAD between the in vitro and in vivo trial

Overall, a large number of parameters including CP, starch and GE showed a high correlation between their *in vitro* and *in vivo* CAD. However, the difference in the *in vivo* CAD of the same components between EXd and nEXd corn diets (Table 5) was substantially smaller than that observed in the *in vitro* trial. This may be explained by the fact that animals secrete substantial amounts of endogenous enzymes such as protease and amylase to degrade protein and starch respectively, thereby reducing the differences in digestibility observed *in vitro*. In addition, compared with the control of nEXd corn diets, phytase, M- and H-enzyme supplementation all resulted in significantly higher starch CAD *in vitro* but not *in vivo*. Consistent with our results, Yu et al. (2007) observed that protease addition significantly increased CP CAD in the *in vitro* assay but did not improve the *in vivo* CAD of DM and CP of a corn-soybean diet. Cowieson and Ravindran (2008) concluded that the inclusion of an enzyme complex with xylanase, amylase and protease activity in corn-based diets was effective in improving productive performance of broilers but that the response in dietary component digestibility was very limited. It would appear that the responses of animals to exogenous enzymes may be not always reflected in the CAD of dietary components. These study results suggest that the *in vitro* incubation method may be limited in its ability to predict the responses of birds and pigs to enzyme supplements. However, the *in vitro* incubation method may be useful for screening both dosage and types of enzymes with potential to improve the digestibility of diets, as evidenced by previous studies (Fang et al., 2007a, b).

5. Conclusion

The present study indicated that extrusion could improve the digestibility of corn by pigs. The use of a combination of amylase, protease and xylanase was another efficient alternative to improve the digestibility of dietary components in diets based on nEXd corn, but it was necessary to pre-determine the appropriate enzyme dosage levels in *in vitro* trials.

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