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Xylanase Supplementation Improved Digestibility and Performance of Growing Pigs Fed Chinese Double-low Rapeseed Meal Inclusion Diets: *In vitro* and *In vivo* Studies

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ABSTRACT : An *in vitro* and a feeding trial were conducted to investigate the effect of xylanase supplementation on the feeding value of growing pig diets containing high proportions of Chinese double-low rapeseed meals (DLRM). Seven diets were formulated to meet NRC (1998) nutrient requirements. Diet 1 based on corn-soybean meal was used as positive control 1, and diet 2, a practical diet which incorporated a conventional level of Chinese DLRM (60 g/kg diet), as positive control 2. Diet 3 contained a higher level of DLRM (100 g/kg diet) as the negative control. Diet 3 plus xylanase at 0.10, 0.25, 0.50 and 0.70 g/kg diet created diets 4, 5, 6 and 7, respectively. The seven diets were incubated in triplicate with the *in vitro* two-stage enzyme incubation method to predict responses of diets to xylanase in terms of digestibility of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF). *In vitro*, the negative control had the lowest CP and NDF digestibility. Both DM and CP digestibility were increased ($p<0.05$) owing to xylanase supplementation either at 0.50 or 0.70 g/kg diet, and NDF digestibility was improved following xylanase addition at all of the test levels. There was a high linear correlation ($r^2>0.90$, $p<0.05$) between the activity concentration of the enzyme when transformed into its logarithmic value and *in vitro* digestibility coefficients of DM, CP or NDF. In the feeding trial, 112 crossbred pigs were randomly assigned to seven dietary treatments with 16 replicate pens of one pig each. An obvious dose effect on growth rate was observed (r^2 = 0.79, $p<0.05$) within the inclusion levels of xylanase. Compared with the negative control, xylanase addition at 0.70 g/kg diet resulted in significantly increased ADG (878 g/d vs. 828 g/d, $p<0.05$), and a tendency towards improved growth rate (868 g/d vs. 828 g/d, $p = 0.10$) was also observed following the inclusion of xylanase at 0.50 g/kg diet. It would appear that the nutrient utilization of corn and Chinese DLRM diets by pigs could be enhanced by an appropriate amount of xylanase addition. The *in vitro* and *in vivo* results suggested that the *in vitro* incubation method is feasible for predicting responses of pigs to exogenous enzymes and identifying those preparations that possess potential for improvement of the nutritive values of feedstuffs. (**Key Words** : Chinese DLRM, Digestibility, Performance, Pigs, Xylanase)

INTRODUCTION

There has been a considerable interest in using double-low rapeseed meal (DLRM) as a replacement of soybean meal in monogastric animal diets. However, the unrestricted use of this feedstuff in rapidly growing animals is limited by low available energy resulting from high level of non-starch polysaccharides (NSP) in the cell wall (Simbaya et al., 1996). Our previous study has clearly indicated a significant decrease in weight gain when the inclusion of Chinese DLRM is higher than 100 g/kg diet in growing-finishing pig diets (Peng et al., 1995; Li et al., 1999). More recently, Chinese DLRM was considered to be inferior to Canadian canola meal owing to the higher content of neutral detergent fibre (NDF, 306.6 g/kg vs. 215.4 g/kg, Chen et al., 2006).

It was reported that the levels of starch, free sugars and soluble NSP in DLRM is about 150 g/kg, which should contribute to considerable digestible energy (Slominski and Campbell, 1990). Unfortunately, it appears that these carbohydrates are encapsulated by cell walls and that their actual contribution to digestible energy is modest (Bell, 1993). In this regard, it may be quite promising using fibre-degrading enzymes to disrupt cell walls thus release entrapped nutrients and improve nutrient utilization of

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Table 1. The composition and nutritional values of the three basal diets (g/kg)

Ingredients	Diet 1	Diet 2	Diet 3
Corn	690	635	595
Wheat bran	-	60	100
Soybean meal	250	185	145
Chinese double-low rapeseed meal	-	60	100
Fishmeal	20	20	20
Premix [a]	40	40	40
Nutrients as calculation			
DE (MJ/kg)	14.09	13.63	13.33
Crude protein	180.0	179.0	180.0
Calcium	8.1	8.2	8.2
Total phosphorus	6.5	7.2	7.5
Available phosphorus	3.3	3.3	3.3
Lysine	10.9	11.3	11.9
Apparent digestible lysine	8.2	8.4	8.8
Apparent digestible methionine+cystine	4.7	4.9	5.1
Nutrients as analysis			
Dry matter	888.6	882.1	884.6
Crude protein	180.3	182.2	181.7
NDF	100.1	121.2	138.6

[a] Provided per kg of diet: Vitamin A, 7,200 IU; Vitamin D_3, 1,600 IU; Vitamin E, 12.8 mg; menadione, 1.6 mg; thiamine, 1.6 mg; riboflavin, 4 mg; niacin, 16 mg; d-panthothenic acid, 8 mg; Vitamin B6, 1.6 mg; Vitamin B12, 12 μg; d-biotin, 64 μg; folic acid, 0.8 mg, copper, 250 mg; iron, 140 mg; manganese, 50 mg; zinc, 200 mg; iodine, 0.8 mg; selenium, 0.4 mg; flavours, 120 mg; antioxidant, 120 mg.

Chinese DLRM-containing diets. Previous study showed that the major NSP components found in DLRM were pectic polysaccharides, which include rhamnogalacturonan with associated side chains consisting of arabinose, galactose, and xylan residues (Bacic et al., 1988). Further study revealed that the non-cellulose polysaccharides in DLRM consisted of arabinose (33%), xylose (13%), mannose (3%), rhamnose (2%), fucose (2%), uronic acids (30%), galactose (13%) and glucose (5%) (Slominski and Campbell, 1990). The high content of arabinose and xylose in DLRM indicated the presence of considerable amount of arabinoxylans (Slominski and Campbell, 1990). In this regard, xylan-related substrates may play a major role in negatively affecting the nutritional values of DLRM (Fang et al., 2006). Furthermore, previous evidence has demonstrated the effectiveness of xylanase supplementation in improving the growth performance of broilers (Bedford and Morgan, 1995) fed DLRM inclusion diets. To our knowledge, however, few study reports are available that investigate the feasibility of using xylanase preparation to improve the feeding value of growing pig diets containing higher proportions of DLRM, which is normally incorporated with less than 50-60 g/kg diet in the growing phase, whereas no more than 100 g/kg diet is recommended in the finishing phase (Peng et al., 1995).

In the present study, one of our aims was to examine whether pig diets containing Chinese DLRM could be improved by xylanase supplementation. At the same time, attempts were made to investigate the dosage responses of xylanase addition considering that enzyme concentrations would also be an important determinants of the extent of cell wall hydrolysis (Tervilai-Wilo et al., 1996) and growth improvement (Zhang et al., 1996; Fang et al., 2006). Another objective was to evaluate the feasibility of using *in vitro* nutrient digestibility to predict the pig responses, which may be beneficial for developing a simple, rapid and effective method to identify those preparations that possess potential for improvement of the nutritive values of feedstuffs.

MATERIALS AND METHODS

Basal diets and treatments

Three basal diets (Positive controls 1 and 2, and the negative control) with different inclusion levels of Chinese DLRM were formulated to meet NRC (1998) nutrient requirements (Table 1). Diet 1 based on corn and soybean meal (CSM) was used as positive control 1, and diet 2, a practical diet incorporated with conventional level of Chinese DLRM (60 g/kg diet), the incorporation rate of which has been justified by Peng et al. (1995), was used as positive control 2. Diet 3 contained higher level of DLRM (100 g/kg diet) as the negative control. Diet 3 plus xylanase at 0.10, 0.25, 0.50 and 0.70 g/kg diet created diets 4, 5, 6 and 7, respectively. The xylanase preparation were provided by Danisco Animal Nutrition (former Finnfeeds International Pte Ltd) and contained endo-1,4 beta-xylanase (EC 3.2.1.8.) 8,000 U/g, fermented from *Trichoderma Longibrachiatum*. Xylanase were added directly to other ingredients and the inclusion levels were chosen according to the supplier's recommendation. The costs of diets 1 to 7 were calculated to be 1,886, 1,807, 1,757, 1,762, 1,769.5, 1,782, and 1,792 yuan/ton, respectively. All diets were in mash form.

In vitro two-stage enzyme incubation trial

The seven diets were incubated in triplicate with the *in vitro* two-stage enzyme incubation and dialysis procedures. The dialysis tubing (D-9402) was purchased from Sigma (St Louis, USA). Its diameter was 49 mm and the tubing would retain most of the molecular compounds of molecular weight 12,000 Da or greater in the dialyzed liquid. The dialysis tubing was cut into pieces of 22 cm. The pretreatment of dialysis tubing was performed according to the procedure described in detail by Huang et al. (2000). The pretreated dialysis tubing would be soaked in distilled water at 4°C before usage. The pepsin (EC 3.4.23.1, P-7000, 1:10,000) and pancreatin (EC 3.4.21.4, P-1750, 1:250) were also supplied by Sigma.

Table 2. Effects of xylanase addition on *in vitro* DM, CP and NDF digestibility coefficients

Treatments [e]	1	2	3	4	5	6	7	SEM
DM [f]	0.46^{ab}	0.43^{b}	0.43^{b}	0.46^{ab}	0.46^{ab}	0.48^{a}	0.48^{a}	0.013
CP [f]	0.70^{a}	0.61^{d}	0.61^{d}	0.65^{c}	0.67^{bc}	0.69^{ab}	0.69^{ab}	0.009
NDF [f,g]	0.12^{c}	0.11^{c}	0.09^{c}	0.18^{b}	0.18^{b}	0.22^{a}	0.20^{ab}	0.010

[e] Treatment 1: control diet 1 based on corn and soybean meal; Treatment 2: control diet 2 containing Chinese double-low rapeseed meal (DLRM) at 60 g per kg of the total diet; Treatment 3: negative control containing Chinese DLRM at 100 g per kg of the total diet; Treatment 4 to 7: the same as treatment 3 except supplementation with xylanase, respectively, at 0.10, 0.25, 0.50 and 0.70 g/kg diet, and the resulted xylanase activity were 0, 800, 2,000, 4,000 and 5,600 U/kg diet, respectively.

[f] Means (expressed as the mean value of 3 replicates) within the same row with no common letters differ ($p<0.05$).

[g] Contrast of treatment 1 vs. treatment 3 differ ($p = 0.06$).

In vitro enzyme hydrolysis procedures were carried out as described in detail by Peng (2000). In brief, a 5.0 g diet sample was placed in a 100 ml Erlenmeyer flask, 500 mg of pepsin and 50 ml hydrochloric acid (0.1 M, pH 2.0) containing 0.054 M sodium chloride were added, and the mixture was incubated for 1 h at 40°C. Subsequently, 2.5 ml sodium hydroxide solution (2.0 M) was added to make the pH approach 7.0, the content was transferred into the pre-soaked dialysis tubing, and 20 ml phosphate buffer solution (0.1 M, pH 7.0) containing 0.05% (w/v) sodium azide and 50 mg pancreatin was added. The mixture was incubated for 6 h at 40°C under continuous mixing in a controlled-environment shaker (SHA-B, Jiangsu, China). Then the sealed tubes were subjected to dialysis against distilled water for 72 h at 2°C on continuous stirring. The water was changed nine times at regular intervals. The residues from the dialysis tubes were then frozen, freeze-dried and analyzed for dry matter (DM) and crude protein (CP) using the technique outlined by AOAC (1990). NDF content in diets and residue was determined by the method of Goering and Van Soest (1970). Each sample was analyzed in duplicate and the *in vitro* digestible DM, CP and NDF were calculated by subtracting the amount of DM, CP and NDF remaining in the residue from the present in the original diet. The digestibility coefficients were calculated from the following equation (taken CP as an example): CP digestibility coefficient = digestible CP (g/kg diet)/total dietary CP (g/kg diet).

Feeding trial

The study was approved by the College of Animal Science and Technology Animal Protocol Review Committee. Housing and care of the animals conformed to Chinese Department of Agriculture Guidelines. A total of 112 healthy crossbred pigs (Large Yorkshire×Landrace×Duroc, average initial body weight of 22.5±1.9 kg were allotted, based on weight and sex, to seven dietary treatments with 16 replicate pens of one pig each. All pigs were housed in the same piggery with two rows of crates (2 m×0.6 m) and the total experimental period lasted 55 days.

Pigs were fed thrice daily and the amount of feed was provided to ensure a little left-over after each meal consumption. Pigs had free access to water. The piggery was disinfected twice daily and kept clean. Temperature was kept at 17-21°C and relative humidity was recorded to be 65%-77%. Pigs were weighed individually at the beginning and the end, and feed intake was recorded daily for each pen. Average daily gain (ADG) and the feed to gain ratio (feed:gain) were calculated from these data.

Statistical analysis

The study was conducted in a randomized complete block design. Data from the *in vitro* and feeding trial were statistically analyzed using one-way ANOVA procedure of the SAS statistical package (SAS, 1989). Duncan's multiple range test was used to separate means when significant ($p<0.05$) effect was detected (Duncan, 1955). The means of the data from the *in vitro* trial and the performance trial where appropriate were also subjected to regression analysis using linear polynomials where the enzyme activity was transformed into its logarithmic value. The data were plotted using Sigma Plot (Kuo and Norby, 1992).

RESULTS

Effects of DLRM inclusion levels and xylanase addition on *in vitro* digestibility

Effects of xylanase addition on *in vitro* digestibility of DM, CP and NDF of diets containing Chinese DLRM were presented in Table 2. The positive control 1 (CSM diet without xylanase supplementation) had the highest digestibility of DM among the three controls. Also, the CP digestibility was significantly decreased due to Chinese DLRM inclusion in CSM based diets without xylanase supplementation (0.70 vs. 0.61, 0.61, $p<0.05$). Similarly, NDF digestibility was decreased with the inclusion of Chinese DLRM at the concentration of 100 g/kg diet without xylanase supplementation (0.12 vs. 0.09, $p = 0.06$). Compared with the positive control 2 (containing Chinese DLRM 60 g/kg diet) and the negative control (containing Chinese DLRM 100 g/kg diet), diets supplemented with xylanase either at 0.50 or 0.70 g/kg diet both had significantly ($p<0.05$) increased DM and CP digestibility, and there were no differences ($p>0.05$) in the *in vitro* digestibility of DM or CP between the positive control 1 and the diets supplemented with xylanase at 0.50 or 0.70

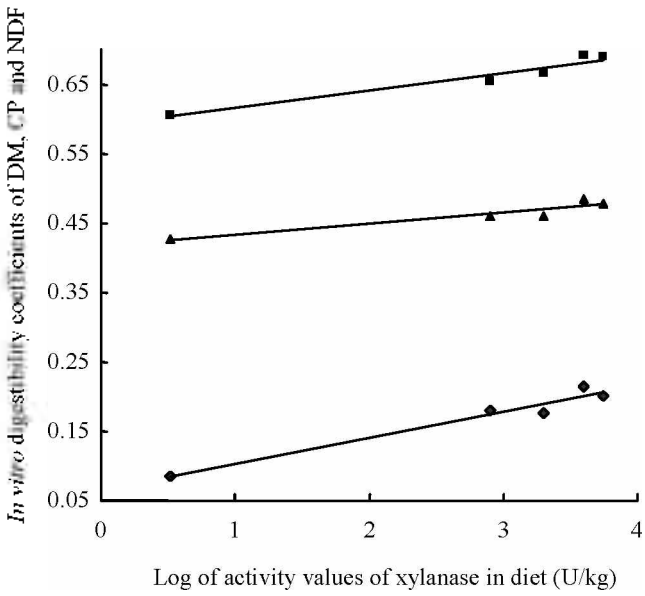

Figure 1. The linear relationship between *in vitro* digestibility coefficients and the activity values of xylanase transformed into their logarithmic values as determined from the equation Y = 0.417+0.016 log X (r^2 = 0.90, p<0.05), Y = 0.590+0.025 log X (r^2 = 0.94, p<0.01), or Y = 0.066+0.037 log X (r^2 = 0.96, p<0.01) where X = units of xylanase in the diet and Y = *in vitro* digestibility coefficients of DM, CP or NDF, respectively. Mean experiment values for *in vitro* digestibility of DM (▲), CP (■) and NDF (♦) were shown in Table 2. The activity value that was used for the diet with no xylanase supplementation was 3.27 U/kg diet. See text for the derivation of this value.

g/kg diet. Remarkably, xylanase supplementation at 0.10, 0.25, 0.50 or 0.70 g/kg all resulted in significantly enhanced (p<0.05) NDF digestibility compared with all the controls.

The regression analysis results for *in vitro* digestibility of DM, CP and NDF were shown in Figure 1. In the regression analysis, the xylanase activity value that was used for the negative control diet with no xylanase supplementation was 3.27 U/kg diet, and this value was calculated according to the method described in detail by Zhang et al. (1996). As shown in Figure 1, DM, CP and NDF digestibility were all increased with the increase of xylanase concentration, and there was a high linear correlation (r^2>0.90, p<0.05) between the activity concentration (X) of the xylanase when transformed into its logarithmic value and *in vitro* digestibility coefficients (Y) of DM, CP or NDF. The regression equations for DM, CP and NDF, respectively, were Y = 0.417+0.016 log X (r^2 = 0.90, p<0.05), Y = 0.590+0.025 log X (r^2 = 0.94, p<0.01), or Y = 0.066+0.037 log X (r^2 = 0.96, p<0.01).

Effects of DLRM inclusion levels and xylanase addition on pig performance

Effects of xylanase supplementation in Chinese DLRM-containing diets on ADG, average daily feed intake (ADFI) and feed:gain of growing pigs were presented in Table 3. Positive control 1 based on CSM and positive control 2 containing Chinese DLRM at 60 g/kg diet both had a numerically higher ADG (853 g/d, 844 g/d vs. 828 g/d, p>0.05) than the negative control containing Chinese DLRM at 100 g/kg diet. Pigs receiving xylanase at 0.70 g/kg diet had significantly higher ADG than those fed the negative control (878 g/d vs. 828 g/d, p<0.05). A tendency towards increased growth rate was also observed following xylanase addition at 0.50 g/kg diet (868 g/d vs. 828 g/d, p = 0.10). Further regression analysis showed that there was a high linear correlation (r^2 = 0.79, p<0.05) between the activity concentration of the xylanase when transformed into its logarithmic value and the ADG, and the regression equation was Y = 0.818+0.013 log X where Y = ADG (kg/d), X = the xylanase activity value (units per kg diet). In contrast, neither feed consumption nor feed efficiency were significantly affected (p>0.05) by xylanase addition.

DISCUSSION

Effects of DLRM inclusion levels and xylanase addition on *in vitro* digestibility

A significant decrease in weight gain was observed in our previous study where the inclusion of Chinese DLRM was higher than 100 g/kg diet in growing-finishing pig diets (Peng et al., 1995; Li et al., 1999). The chemical analysis conducted in our succedent study (Peng, 2000) revealed that Chinese DLRM had significantly higher levels of dietary fibre (532 g/kg vs. 345 g/kg), acid detergent fibre (ADF, 309 g/kg vs. 205 g/kg), and NDF (450 g/kg vs. 259 g/kg)

Table 3. Effects of xylanase addition on performance of growing pigs

Treatments [c]	1	2	3	4	5	6	7	SEM
Initial weight (kg)	22.7	22.4	22.5	22.6	22.5	22.4	22.5	0.49
Final weight (kg)	69.6	68.8	68.0	69.2	69.3	70.1	70.8	1.05
ADG[d, e] (g/d)	853[ab]	844[ab]	828[b]	848[ab]	851[ab]	868[ab]	878[a]	15.0
ADFI (g/d)	2,088	2,058	2,075	2,089	2,080	2,136	2,089	45.9
Feed:gain	2.45	2.44	2.50	2.47	2.45	2.46	2.39	0.046

[c] Treatment 1: control diet 1 based on corn and soybean meal; Treatment 2: control diet 2 containing Chinese double-low rapeseed meal at 60 g per kg of the total diet; Treatment 3: negative control containing Chinese double-low rapeseed meal at 100 g per kg of the total diet; Treatment 4 to 7: the same as treatment 3 except supplementation with xylanase, respectively, at 0.10 , 0.25, 0.50 and 0.70 g/kg diet, and the resulted xylanase activity were 0, 800, 2,000, 4,000, and 5,600 U/kg diet, respectively.

[d] Means within the same row with no common letters differ (p<0.05).

[e] Contrast of treatment 6 vs. treatment 3 differ (p = 0.10).

Table 4. Chemical composition and the content of fibre and constituent sugars in non-starch polysaccharides of Chinese DLRM compared to canola meal (g/kg DM, fat free) [a]

Indexes	Chinese DLRM	Canola meal
CP	425	407
Sucrose	73	64
Oligasaccharides	20	22
Ash	81	85
Calcium	7.5	5.9
Phosphorus	10.3	11.0
Dietary fibre	532	345
NDF	450	259
ADF	309	205
Soluble NSP	83	86
Total NSP	203.48	203.45
Rhamnose	2.08	2.79
Fucose	2.22	2.34
Arabinose	47.17	49.34
Xylose	17.31	23.90
Mannose	7.55	5.93
Galactose	21.06	18.29
Glucose	65.95	66.37
Uronic acid	40.16	34.41

[a] Data adapted from "Evaluation and Improvement of Quality of Chinese Double-low Rapeseed Meal", Peng (2000).

than did canola meal, although they were similar in the content of CP, calcium, phosphorus, sucrose, oligasaccharides, neutral detergent soluble NSP, total NSP and NSP profiles (Table 4). It is therefore concluded that the increased dietary fibre resulting from the enhanced levels of Chinese DLRM may be responsible for the decreased weight gain. In the present study, to evaluate the effect of DLRM inclusion levels on *in vitro* nutrient digestibility, two positive controls and one negative control differing in DLRM inclusion levels were formulated. At the same time, considering that any hydrolysis of nutrients such as CP and NDF may be reflected in the increased DM digestibility, that it is the increased amount of CP covalently bound to fibres during overheating treatment that results in enhanced levels of NDF (Chen et al., 2006), and that the hydrolysis of NDF by xylanase addition could result in enhanced CP digestibility (Fang et al., 2006), we selected DM, CP, and NDF as indexes to predict responses of diets to xylanase addition in *in vitro* experiment. Diets containing Chinese DLRM without xylanase addition having a lower *in vitro* digestibility of DM, CP and NDF than conventional CSM based diet indicated the remarkable negative effect of the higher fibre level resulting from Chinese DLRM inclusion on nutrient utilization. The result was in agreement with previous reports (Yin, 1994; Schulze et al., 1994; Jorgensen et al., 1996; Yin et al., 2000), in which a negative relevance between dietary fibre and nutrient digestibility was demonstrated. On the other hand, the commercial Chinese DLRM used in the present study had been, to some extent, subjected to overheating treatment during its processing, which could result in increased NDF content of meals (Peng, 2001; Chen et al., 2006). Therefore, the increased NDF level caused by processing might further aggravate the decrease in nutrient digestibility of Chinese DLRM-containing diets. Notably, a positive NDF digestibility was observed in the negative control diet with no xylanase addition. This may be associated with the degradation of proteins contained in the NDF residue for that sufficient protease was provided in the diet. Dierick et al. (1989) reported that the isolated total dietary fibre fraction contained 27% protein in rapeseed meal, indicating that a large percentage of this faction of protein was bound by fibre. More recently, Chen et al. (2006) reported that the much higher NDF in Chinese DLRM than in canola meal was largely related to the increased products of Maillard reaction during overheating processing in which considerable amount of proteins (12.7%-19.1%) were covalently bound to fibres. In this regard, an enhanced *in vitro* digestibility of DM, CP and NDF following xylanase supplementation suggested the positive effect of NSP-degrading enzyme preparation in enhancing the nutritive value of Chinese DLRM-containing diets. The increased NDF digestibility revealed that the improved nutrient digestibility was associated with the degradation of dietary fibres. The results agreed well with previous reports (Slominski and Campbell, 1990; Fang et al., 2006).

An obvious dose effect on *in vitro* digestibility of DM, CP and NDF among the inclusion levels of xylanase indicated that the enhancement of nutrient digestibility was largely related to the active concentrations of NSP enzymes towards their specific substrate. This was in agreement with our previous study (Fang, 2006). These results validated the hypothesis that the active concentrations of xylanase would also be an important determinant of the extent of cell wall hydrolysis (Tervila-Wilo et al., 1996). For Chinese DLRM, a total of about 15% carbohydrates including starch, free sugars and soluble NSP are encapsulated by cell walls and that their actual contribution to digestible energy is modest (Bell, 1993). Regression analysis showed that a ninefold increase in enzyme concentration in the diet (i.e., an increase to 10 times the starting amount) will result in a onefold (100%), not a ninefold, improvement in the *in vitro* digestibility. It is obvious from this relationship that relatively small amounts of enzyme can have a dramatic effect on digestibility, whereas much larger amounts are required for each additional incremental improvement (Zhang et al., 1996). The present study clearly indicated that the release of entrapped nutrients was dependent on the extent to which cell walls are disrupted and this need an adequate NSP-degrading enzyme concentration (activities) to effectively hydrolyze the cell walls.

Effects of DLRM inclusion levels and xylanase addition

on pig performance

In the current study, the diet containing Chinese DLRM at 60 g/kg diet was one that commonly used in commercial practice. Our previous study has evaluated the appropriate rate of substituting DLRM for soybean meal on equal-nitrogen basis (≤25% in the growing phase and ≤37.5% in the finishing phase) in pig diets (Peng et al., 1995). Similarly, the present study showed that there was no difference in performance between the conventional CSM diet (the positive control 1) and the practical diet with the inclusion of Chinese DLRM at 60 g/kg diet (the positive control 2), although the latter had a lower DE (13.63 vs. 14.09 MJ/kg, see Table 1) than the former as a result of decreased nutrient digestibility. This may be associated with that Chinese DLRM has a well balanced amino acid profile and, in particular, much higher content of sulfur-containing amino acids than soybean meal (Peng et al., 1995). In this regard, a diet with more balanced amino acid profile will be obtained when soybean meal and DLRM are in combination use rather than single soybean meal is used as a protein source in corn based diet. However, the negative effect caused by the high dietary fibre level may outweigh the positive effect arisen from the tendency towards more balanced amino acid profile when higher proportions of DLRM are incorporated into diet. This was supported by the decreased growth rate (853 vs. 828 g/d) of pigs fed diet containing Chinese DLRM at 100 g/kg diet with dietary DE (14.09 vs. 13.33 MJ/kg, see Table 1) 5% lower than conventional CSM diet. Similarly, a decreased weight was observed in laying quails fed diets containing canola meal which supplying 50% of CP from SBM, while the 25% canola meal level did not affect the liveweight gain (Saricicek et al., 2005). In the present study, the increased growth rate following xylanase supplementation in the negative control provided further evidence for the negative effect resulting from dietary fibre.

Previous studies suggested that the active concentrations of xylanase would also be an important determinant of the extent of performance improvement (Marquardt et al., 1996; Zhang et al., 1996; Fang, 2006). In the present study, an evident dose response of pigs to enzyme supplementation further revealed that a desirable performance could be obtained on condition that the supplemented enzyme had adequate activities towards its target substrates. A similar conclusion has been obtained by Zhang et al. (1996) and Marquardt et al. (1996). They also reported that there was a higher linear correlation (r^2>0.91, p<0.05) between the concentrations of enzyme when transformed into its logarithmic values and the corresponding improvements in weight gain or the feed-to-gain ratio, and for every ten-fold increase in the amount of enzyme there was a two-fold and not ten-fold incremental improvement in animal performance. These results suggested that it be necessary for the determination of appropriate dosage of enzymes when they were used to specific animal feeds. The negative control supplemented with xylanase at 0.50 or 0.70 g/kg diet having a similar even better growth performance compared with the positive control suggested the feasibility of using appropriate concentration of xylanase to increase the Chinese DLRM inclusion levels in growing-finishing pig diets.

The feasibility of using *in vitro* nutrient digestibility to predict pig responses

Numerous studies report that for post-weaning pigs exogenous enzyme preparations increase both ileal nutrient digestibility and growth performance (Li et al., 1994; Baidoo et al., 1997; Li, 2000; Omogbenigun et al., 2004). More recently, Fang et al. (2006) investigated the effect of xylanase supplementation in Chinese DLRM inclusion diet on total-tract nutrient digestibility and growth performance of growing pigs, and observed an improved growth rate and feed efficiency as a result of enhanced overall digestibility of energy and proximate nutrients as well as anti-nutritional fibre components such as NDF and ADF. The above results suggest that the improved pig performance be largely related to the enhanced nutrient utilization as a result of disrupted nutrient encapsulation effect of dietary fibres.

In contrast to pigs, inconsistent results have been yielded about the association of increased fibre degradation and nutrient utilization with the improved bird performance. Some *in vitro* and *in vivo* studies demonstrated the positive effect of fibre-degrading enzymes in improving NSP degradation and nutrient digestibility by broilers fed DLRM diets (Slominski and Campbell, 1990; Simbaya et al., 1996). The improved nutrient utilization, however, was not reflected in growth performance of broilers receiving DLRM diets with similar enzyme preparation supplementation (Kocher et al., 2000, 2001). In contrast, recent studies observed both an improved broiler performance and an increased nutrient utilization as a result of enhanced depolymerization of cell wall polysaccharides by multicarbohydrase supplementation in DLRM diets (Meng and Slominski, 2005).

The inconsistency of the above findings may be related to the substrate specificity and animal species. The nutrient encapsulating effect of cell wall polysaccharides in canola meal diet may not be the only factor responsible for incomplete utilization by broiler chickens (Meng and Slominski, 2005), and the viscosity caused by soluble fibre may be of equal importance in determining responses of broilers to exogenous enzymes. In contrast to poultry, the naturally higher water content of pig digesta (about 10 percentage units higher than poultry on similar diets) leads to dilution effects that will negate, to some degree, this viscosity problem in the pig's gut (Danicke et al., 1999;

Partridge, 2001). Furthermore, corn and DLRM diets had lower soluble fibre levels compared with viscous cereals such as wheat and barley, and factors associated with insoluble fibre such as the packaging of nutrients inside cell wall material, together with starch structure and composition, seem to be more relevant to the discounted feeding value of these grains and meals (Slominski and Campbell, 1990; Partridge, 2001; Fang et al., 2006). The above results suggest that a relatively clear nutrient-utilization and growth-performance relationship may occur when fibre-degrading enzymes are used in pig diets based on non-viscous grains and meals such as corn and DLRM. It would appear that the *in vitro* two-stage enzyme incubation method might be feasible for predicting the responses of pigs to exogenous enzymes and thus identifying those preparations that possess potential for improvement of the nutritive values of feedstuffs.

In conclusion, the results indicated that dietary supplementation with appropriate amount of xylanase was feasible to improve the inclusion levels of Chinese DLRM in growing pig diets, and that the *in vitro* two-stage enzyme incubation method could be used to predict the responses of pigs to exogenous enzymes and hence select effective enzymes targeting specific substrates.

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