Effects of xylanase supplementation on digestibility and performance of growing-finishing 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-finishing pig diets containing high proportions of Chinese double-low rapeseed meal (DLRM). Seven diets were formulated to meet NRC (1998) nutrient requirements. Diet 1 based on corn and soybean meal was used as positive control 1, and diet 2, a practical diet incorporated with conventional level of Chinese DLRM (6% and 10% of diet for the growing and finishing phase, respectively), as positive control 2. Diet 3 contained higher level of DLRM (10% and 15% of diet for the growing and finishing phase, respectively) 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 for the growing phase with triplicate each were incubated by the in vitro two-stage incubation method and digestibility of DM, CP and NDF was determined. In vitro, the negative control had a lower CP ($P < 0.05$) and NDF ($P = 0.06$) digestibility than positive control 1. Both DM and CP digestibility were increased ($P < 0.05$) by xylanase supplementation either at 0.50 or 0.70 g/kg diet, and NDF digestibility was improved following any test levels of xylanase addition. There was a high linear correlation ($r2 > 0.90$, $P < 0.05$) between the activity of the enzyme when transformed into its logarithmic value and in vitro digestibility 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. Within the inclusion levels of xylanase an obvious dose effect on growth rate was observed ($r2 = 0.79$, $P < 0.05$) and xylanase addition at 0.70 g/kg diet resulted in significantly increased ADG over the negative control (878 g/d vs. 828 g/d, $P < 0.05$) during the growing phase, whereas the similar inclusion effect was not observed during the finishing phase. It would appear that nutritional values of corn and Chinese DLRM diet by pigs could be enhanced and therefore it was feasible to improve dietary inclusion levels of Chinese DLRM by appropriate amount of xylanase addition.

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 component (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). 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 a 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 that using fibre-degrading enzymes to disrupt cell walls thus release entrapped nutrients and improve nutrient utilization of Chinese DLRM-containing diets. Previous evidence has demonstrated the effectiveness of xylanase supplement in improving the growth performance of broilers (Bedford and Morgan, 1995) and growing pigs (Fang et al., 2006) 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-finishing 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 it was effective to improve the Chinese DLRM inclusion levels in pig diets by xylanase supplementation. At the same time, attempts were made to find an appropriate dosage 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).

Materials and methods

Basal diets and treatments: Three basal diets were formulated to meet NRC (1998) nutrient requirements containing the

same level of calcium, phosphorus, amino acids and other necessary micro-components. 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 (up to 60 and 100 g/kg diet for the growing and finishing phase, respectively), 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 (up to 100 and 150 g/kg diet for the growing and finishing phase, respectively) 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 Finnfeeds International Pte Ltd and contained endo-1,4 beta-xylanase (No EC 3.2.1.8.) 8,000 U/g, fermented from *Trichoderma Longibrachiatum*. All diets were in mash form. The basal diet formulations were presented in Table 1.

Table 1. The formulation of the three two-phase basal diets (g/kg)[a]

Ingredients	Growing phase			Finishing phase		
	1	2	3	1	2	3
Corn	690	635	595	730	640	590
Chinese double-low rapeseed meal	-	60	100	-	100	150
Wheat bran	-	60	100	-	90	145
Fishmeal	20	20	20	10	-	-
Soybean meal	250	185	145	220	130	75
Premix[a,b]	40	40	40	40	40	40
Enzyme A	-	-	-	-	-	-
Enzyme B	-	-	-	-	-	-
Nutrients as calculation						
DE (MJ/kg)	14.09	13.63	13.33	14.09	13.54	13.17
Crude Protein	180.0	179.0	180.0	163.0	164.0	163.0
Calcium	8.1	8.2	8.2	7.0	7.8	7.9
Total phosphorus	6.5	7.2	7.5	5.6	6.3	6.7
Available Phosphorus	3.3	3.3	3.3	2.6	2.6	2.6
Lysine	10.9	11.3	11.9	8.6	8.0	8.2
Apparent digestible Lysine	8.2	8.4	8.8	6.5	6.1	6.1
Apparent digestible methionine +cystine	4.7	4.9	5.1	4.5	5.0	5.2

[a]Provided per kg of diet for the growing and finishing (in brackets) phase : Vitamin A, 7200 (4950) IU; Vitamin D3, 1600 (1100) IU; Vitamin E, 12.8 (8.8) mg; menadione, 1.6 (1.1) mg; thiamine, 1.6 (1.1) mg; riboflavin, 4 (2.75) mg; niacin, 16 (11) mg; d-panthothenic acid, 8 (5.5) mg; Vitamin B6, 1.6 (1.1) mg; Vitamin B12, 12 (8.25) µg; d-biotin, 64 (44) µg; folic acid, 0.8 (0.55) mg. copper, 250 (125) mg; iron, 140 (140) mg; manganese, 50 (40) mg; zinc, 200 (160) mg; iodine, 0.8 (0.8) mg; selenium, 0.4 (0.3) mg. flavours, 120 (80) mg; antioxidant, 120 (120) mg.

In vitro two-stage enzyme incubation trial: The seven diets were incubated in triplicate with the *in vitro* two-stage enzyme incubation and dialysis procedure as described in detail by Peng (2000). The residues from the dialysis tubes were then frozen, freeze-dried and analyzed for DM and 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: A total of 112 healthy crossbred pigs (average initial BW of 22.5 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 and the total experimental period involving the growing phase (2 ~ 4 Months of age) and the finishing phase (4 ~ 5 Months of age) lasted 80 days. Pigs were fed thrice and twice per day during the growing and finishing phase, respectively. Pigs were fed *at libitum* and had free access to water. Pigs were weighed individually at the beginning and the end of each phase, 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). 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.

Results and discussion

Effects of DLRM inclusion levels and enzyme addition on in vitro digestibility

Effects of enzyme addition on in vitro digestibility of DM, CP and NDF of diets containing Chinese DLRM were presented in Table 2. The negative control had a lower CP ($P < 0.05$) and NDF ($P = 0.06$) digestibility than positive control 1, indicating the remarkable negative effect of the higher fibre level resulting from Chinese DLRM inclusion on nutrient utilization. Both DM and CP digestibility were increased ($P < 0.05$) by xylanase supplementation either at 0.50 or 0.70 g/kg diet, and NDF digestibility was improved following any test levels of xylanase addition. The improved digestibility indicated 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 (Slominski and Campbell, 1990; Fang et al., 2006).

Table 2. Effects of enzyme addition on in vitro DM, CP and NDF digestibility (%)

Parameter	Treatments[e]							S.E.M.
	1	2	3	4	5	6	7	
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]	0.12c	0.11c	0.09c	0.18b	0.18b	0.22a	0.20ab	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 enzyme B, respectively, at 0.10, 0.25, 0.50 and 0.70 g/kg diet, and the resulted xylanase activity were 0, 800, 2000, 4000, and 5600 U/kg diet, respectively.

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

The regression analysis results for in vitro digestibility of DM, CP and NDF were shown in Fig. 1. In the regression analysis, the enzyme activity value that was used for the diet with no enzyme 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 enzyme concentration, and there was a high linear correlation ($r^2 > 0.90$, $P < 0.05$) between the activity concentration (X) of the enzyme when transformed into its logarithmic value and in vitro digestibility coefficients (Y) of DM, CP or NDF. The general equation could be presented as $Y = A + \log X$ where Y = in vitro digestibility coefficients of DM, CP or NDF, A = the intercept (y axis), B = the slope of the line, and X = the enzyme activity value (units per kg diet). The dose response suggested that the enhancement of nutrient digestibility was largely related to the active concentrations of NSP enzymes towards their specific substrate. 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).

Effects of DLRM and enzyme inclusion levels on pig performance

In the current study, the diet containing 60 and 100 g of Chinese DLRM/kg diet for the growing and finishing phase, respectively, was one that commonly used in commercial practice. Under the recommended inclusion levels of Chinese DLRM, no difference in performance was observed between the conventional CSM diet and the practical diet with the inclusion of Chinese DLRM at 60 ~ 100 g/kg diet (Table 3), although the latter had a lower DE (13.63 vs. 14.09 MJ/kg, Table 1) than the former as a result of decreased nutrient digestibility. This may be associated with that a diet with more balanced amino acid profile could 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 (Peng et al., 1995). However, the negative effect caused by high dietary fibre 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 in Month 2 ~ 4 of pigs fed diet containing 100 g of Chinese DLRM/kg diet with dietary DE (14.09 vs. 13.33 MJ/kg, Table 3) 5% lower than conventional CSM diet. The increased growth rate following xylanase supplementation in the negative control provided further evidence for the negative effect resulting from dietary fibre.

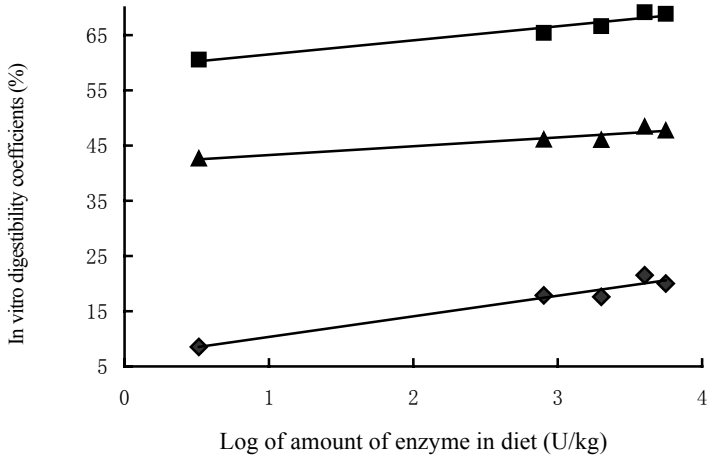

Fig.1. The linear relationship between in vitro digestibility and the amounts of enzyme transformed into their logarithmic values as determined from the equation $Y = 0.417 + 0.016 \log X$ ($r^2 = 0.90$, $P < 0.05$), $Y = 0.590 + 0.025 \log X$ ($r^2 = 0.94$, $P < 0.01$), or $Y = 0.066 + 0.037 \log X$ ($r^2 = 0.96$, $P < 0.01$) where X = units of enzyme 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 enzyme supplementation was 3.27 U/kg diet. See text for the derivation of this value.

Table 3. Effects of enzyme addition on the performance of pigs fed Chinese double-low rapeseed meal (DLRM) containing diets for the growing and finishing phase

Treatments[d]	1	2	3	4	5	6	7	S.E.M
Weight, kg								
Month 2	22.7	22.4	22.5	22.6	22.5	22.4	22.5	0.49
Month 4	69.6	68.8	68.0	69.2	69.3	70.1	70.8	1.05
Month 5	90.1	91.0	89.9	90.3	89.2	91.8	91.2	1.38
ADG[e], g								
Months 2 ~ 4	853ab	844ab	828b	848ab	851ab	868ab	878a	15.0
Months 4 ~ 5	807bc	889a	874a	844abc	787c	855ab	790c	24.3
Months 2 ~ 5	843	858	843	844	835	869	854	15.3
ADFI[e], g								
Months 2 ~ 4	2088	2058	2075	2089	2080	2136	2089	45.9
Months 4 ~ 5	2610	2580	2602	2453	2534	2661	2598	74.8
Months 2 ~ 5	2266	2221	2239	2204	2230	2306	2251	50.5
Feed : gain[e]								
Months 2 ~ 4	2.45	2.44	2.50	2.47	2.45	2.46	2.39	0.046
Months 4 ~ 5	3.25a	2.92b	2.98b	2.91b	3.23a	3.11ab	3.32a	0.074
Months 2 ~ 5	2.69	2.59	2.66	2.62	2.67	2.66	2.64	0.043

[d]Treatment 1: control diet 1 based on corn and soybean meal; Treatment 2: control diet 2 containing Chinese DLRM at 60 g (growing phase) or 100 g (finishing phase) per kg of the total diet; Treatment 3: negative control containing Chinese DLRM at 100 g (growing phase) or 150 g (finishing phase) per kg of the total diet; Treatment 4 to 7: the same as treatment 3 except supplementation with enzyme B, respectively, at 0.10, 0.25, 0.50 and 0.70 g/kg diet, and the resulted xylanase activity were 0, 800, 2000, 4000, and 5600 U/kg diet, respectively.

[e]Means within the same row with no common letters differ ($P < 0.05$).

Within the inclusion levels of xylanase an obvious dose effect on growth rate was observed ($r2 = 0.79$, $P < 0.05$) and xylanase addition at 0.70 g/kg diet resulted in significantly increased ADG over the negative control (878 g/d vs. 828 g/d, $P < 0.05$) during the growing phase. The results 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). These results indicate that it is necessary for the determination of appropriate dosage of enzymes when they are used to specific animal feeds. The negative control supplemented with xylanase at 0.5 or 0.7 g/kg diet had a similar even better growth performance compared with the positive control demonstrated the feasibility to increase the Chinese DLRM inclusion levels in swine diets by appropriate concentration of xylanase addition. Notably, the similar enyme inclusion effect was not observed during the finishing phase. The difference in response of pigs over different weight range may be associated with the difference in the development of gastrointestinal tract (Feng and Wang, 2004). In general, young animals are less mature in their digestive organ and, consequently, are more susceptible to the anti-nutritional effects of NSP present in diets. However, compared with the negative control, the similar feed efficiency but numerically higher growth rate (11 ~ 26 g/d) following xylanase addition at 0.5 or 0.70 g/kg diet still implied the positive effect of enzyme supplementation in Chinese DLRM-containing diets on pig performance during the overall phase.

Conclusion

The results indicated that it was feasible to improve the inclusion levels of Chinese DLRM in growing-finishing pig diets by appropriate amount of xylanase supplementation, and that 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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