Asian-Aust. J. Anim. Sci.
Vol. 21, No. 2 : 270 - 276
February 2008

www.ajas.info

Effects of Enzyme Supplementation on Growth, Intestinal Content Viscosity, and Digestive Enzyme Activities in Growing Pigs Fed Rough Rice-based Diet*

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ABSTRACT : The purpose of the present study was to investigate the effects of exogenous non-starch polysaccharides (NSP) enzymes on performance, intestinal content viscosity and digestive enzyme activities of growing pigs fed a rough rice-based diet. A total of 60 crossbred barrows with an initial body weight of 35.16 kg (SD = 0.82) were blocked by body weight and randomly assigned to two treatments with three replications. Each group was fed the diet based on rice with or without exogenous NSP enzymes (2 g/kg of diet). During the 70 days of the feeding trial, all pigs were given free access to feed and water. At the end of the feeding trial, six pigs from each treatment were randomly selected and slaughtered to collect intestinal digesta, intestinal mucosa, and pancreas. The addition of NSP enzymes improved average daily gain ($p<0.05$) and feed:gain ($p<0.05$), and decreased viscosity of digesta in the jejunum ($p<0.001$) and ileum ($p<0.01$) of pigs. The supplementation of NSP enzymes increased activities of protease ($p<0.01$), trypsin ($p<0.01$) and α-amylase ($p<0.05$) in duodenal contents. However, digestive enzymes in the pancreas, jejunal and ileal mucosa were unaffected by the supplemental NSP enzymes ($p>0.10$). The results indicate that the addition of NSP enzymes to rough rice-based diets improved performance of pigs, reduced viscosity and increased digestive activity in the small intestine. (**Key Words :** Pigs, Rough Rice, NSP Enzymes, Growth, Digestion)

INTRODUCTION

Rice is an important staple for a large part of the world's population and is produced and consumed traditionally as white (polished) rice. Rice by-products are used as feedstuffs for animals. Rough rice, as early long-grain nonglutinous rice (*Oryza sativa L.*), has been planted in southern China since ancient times. As the human living standard is improved with economic development, this grain becomes almost unsalable because of its poor palatability. There are more than 50 million tons of rough rice stored in southern provinces of China, and the price of the rough rice is usually 30% lower than that of corn (M. Q. Wang, pers. comm., Zhejiang University, China). If this alternative energy source could be utilized efficiently, this would offer advantages to the animal production and feed industries.

Rough rice is rarely used as a feedstuff for livestock owing to the poor feed conversion (Ensminger et al., 1983). The relatively poor feed conversion of rough rice with hull is due to its high concentration of anti-nutritional factors including the non-starch polysaccharides (NSP, as arabinoxylan and β-glucan), and high crude fibre content, which is about 10% in rough rice with hull. The major nutrient origin in rice, starch, is enclosed within endosperm cell walls, which consist mainly of mixed-linked arabinoxylan and β-glucan (Chesson, 1993). Pigs have a limited ability to digest NSP in cereal or fibre from the hull (Omogbenigun et al., 2004). The NSP encapsulate nutrients and thus depress overall nutrient digestibility and increase endogenous nitrogen flow and bacterial fermentation in the gastrointestinal tract (Yin et al., 2004a). Arabinoxylan and β-glucan in rice may interfere with digestion and absorption of nutrients (Graham et al., 1988) and the production of digestive enzymes (Ikegami et al., 1990).

Many researchers have studied the effects of supplemental carbohydrase to various feed ingredients including barley, wheat, rye, corn, soybean meal, and de-hulled rice in weanling pigs (Inborr et al., 1993; Yin et al.,

* The work was supported by Key Scientific & Technological Research Project of Zhejiang Province, China.

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Received May 19, 2007; Accepted July 22, 2007

Table 1. Composition of ground rough rice[a] (air-dry basis)

Item	
DM (%)	87.25
GE (MJ/kg)	15.84
CP (%)	8.90
Crude fat (%)	1.92
Crude fiber (%)	9.92
Total pentose (%)	4.35
Total β-glucans (%)	0.68
Ash (%)	3.75
Ca (%)	0.08
P (%)	0.26

[a] The identity of rice is *Oryza sativa*. The sample of rough rice with hull was ground in a laboratory mill with 0.8 mm sieve. All data are analyzed values.

2000; Kim et al., 2004) and grow-finishing pigs (Thacker et al., 1991; Yin et al., 2001). The soluble NSP in the wheat and rice bran diet can be partly hydrolysed in the gastrointestinal tract of pigs by arabinoxylanase supplementation of feed (Yin et al., 2004b). However, no studies have been found investigating the effect of NSP enzymes to rough rice. Therefore, the current study investigated the effect of exogenous NSP enzymes on performance, intestinal content viscosity, and digestive enzyme activities of growing pigs fed a diet based on rough rice.

MATERIALS AND METHODS

The protocol for this study was approved by the Zhejiang University Animal Care and Use Committee. All the animal experiments were done according to the guidelines for animal experiments at the National Institute of Animal Health. The feeding trial and sample collection were done at the Xushan breeding farm, Jiaxin, China.

Experimental materials and design

Rough rice containing its hulls was provided by Anhui Grain Supply Center, Hefei, China. The composition of ground rough rice is presented in Table 1. The enzyme complex used was supplied by Feed Science Institute of Zhejiang University, Hangzhou, China, and contained 80,000 U/g xylanase (E.C.3.2.1.8), 12,000 U/g β-glucanase (E.C.3.2.1.6) and 1,200 U/g cellulase (E.C.3.2.1.4). The activities of xylanase, β-glucanase and celluase were determined according the method of Bailey and Poutanen (1989), Erfle et al. (1988), and Lowe et al. (1987), respectively. The basal diet based on rough rice met or exceeded NRC (1998) recommendations for all nutrients except for digestible energy. The diet was formulated to provide 16.6% of crude protein, 0.84% of Ca, 0.66% of P, 0.92% of lysine, and 0.38% of methionine and 12.8 MJ/kg digestible energy. The composition of basal diets is presented in Table 2.

A total of 60 barrows (Landrace×Jiaxin Black) with an average body weight of 35.16 kg (SD = 0.82) were selected from Xushan breeding farm, Jiaxin, China. The barrows were blocked by weight and randomly assigned to two treatments (0 or 2 g/kg NSP enzyme complex added to basal diet). Each treatment had three replicates with ten pigs per replicate. The pigs were penned in 3.25 m×5.25 m pens with concrete floors. During the 70 days of the feeding trial, pigs were allowed *ad libitum* access to feed and water. Body weight and feed intake were recorded at the end of trial. Body weight gain was calculated by the difference between initial body weight and final body weight. Feed:gain was calculated by dividing the amount of feed consumed with the corresponded body weight gain.

Table 2. Ingredient inclusion and chemical composition of basal diet (as-fed basis)

Item	
Ingredients (%)	
Rough rice	72.0
Soybean meal	24.0
Calcium phosphate	1.50
Limestone	1.00
Salt	0.40
Lysine HCl	0.10
Mineral premix[a]	0.80
Vitamin premix[b]	0.20
Chemical composition (analyzed, %)[c]	
Digestible energy (MJ/kg)	12.8
Dry matter	88.05
Crude protein	16.60
Lysine	0.92
Methionine	0.38
Crude fat	3.57
Crude fibre	5.78
Calcium	0.84
Phosphorus	0.66

[a] Contained per kg diet: Cu, 10 mg from $CuSO_4 5H_2O$; Zn, 100 mg from $ZnSO_4 7H_2O$; Fe, 140 mg from $FeSO_4 H_2O$; Mn, 40 mg from $MnSO_4 5H_2O$; Se, 0.1 mg from $Na_2SeO_3 \cdot 5H_2O$; I, 0.3 mg from KI.

[b] Contained per kg diet: vitamin A, 6,000 IU; vitamin D_3, 700 IU; vitamin E, 88 IU; vitamin K, 4.4 mg; riboflavin, 8.8 mg; D-pantothenic acid 24.2 mg; niacin, 33 mg; choline chloride 330 mg; vitamin B_{12}, 22 μg; D-biotin, 300 μg; folic acid, 2.5 mg.

[c] All data are analyzed values except for digestible energy.

Chemical analysis of rice and basal diet

Samples of the rice and mixed basal diet were analyzed for DM after oven drying to a constant weight, for CP by a N analyzer (N×6.25), for a crude fat based on ether extraction, and for ash and crude fiber; all methods were based on standard procedures (AOAC, 1995). Calcium was analyzed with atomic absorption spectrophotometry after wet-ashing, and P was determined by a colorimetric procedure (AOAC, 1995). Amino acids were analyzed with ionexchange chromatography after acid hydrolysis, as 6 mol/L HCl and 0.1% phenol under vacuum for 24 h at 110±2°C. Methionine was oxidized to methionine sulfone

Table 3. Performance of pigs fed a diet based on rough rice with or without NSP enzymes[a]

	NSP enzymes added (g/kg)		SEM[b]	p value
	-	2		
Average daily gain (kg)	0.46	0.50	0.018	0.014
Average daily feed intake (kg)	1.65	1.65	0.040	0.909
Feed:gain	3.61	3.27	0.074	0.031

[a] Values are presented as means; n = 3 per treatment with ten pigs per pen contributing to a pen mean. [b] Standard error of the mean.

by treatment with performic acid before hydrolysis (Schram et al., 1954). Rice was also analyzed for GE (AOAC, 1995), total pentosans (Henry, 1985) and total β-glucans (Henry, 1984).

Sampling procedure

At the end of the feeding trial, six pigs from each treatment (two pigs per pen) were randomly selected and slaughtered by exsanguination after electrical stunning, and immediately eviscerated to collect pancreatic and intestinal samples. The pancreases were excised and freed of all extraneous material, and a portion (about 10 g) was collected and stored at -20°C until subsequent analysis. The intestines were removed and divided into three small intestinal segments (25 ('duodenum'), 50 ('jejunum') and 75 ('ileum') % along the small intestine) according to the method of Hopwood, et al. (2004). Digesta samples were collected from the three segments by massaging the tract from both ends, and were kept fresh for immediate measurement of viscosity. A portion of digesta sample from the duodenum was immediately stored at -20°C for later enzymatic activities analysis. Jejunal and ileal mucosa samples were immediately scraped according to the procedures of Sell et al. (1989), and stored at -20°C until subsequent analysis.

Determination of viscosity in small intestinal contents

Viscosity of small intestinal contents was determined using the method of Hopwood et al. (2004). Briefly, fresh digesta were diluted 1:1 (v/v) with distilled water, mixed and then centrifuged at 12,000 g for 8 min. The viscosity of 0.5 ml supernatant fractions was measured at 25°C, applying a shear rate of 60 per sec in a Brookfield LVDV-II$^+$ cone plate (CP40) rotational viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA).

Digestive enzyme assay in pancreas

The pancreatic samples were homogenized in ice-cold 0.2 mol/L Tris-HCl buffer containing 0.05 mol/L NaCl. The homogenate was centrifuged at 1,500 g for 15 min at 4°C and the supernatant was saved for analysis of protease, chymotrypsin, trypsin, α-amylase and lipase activities. Protease activity was analyzed using the method of Iwamori et al. (1997). Chymotrypsin was determined according to Erlanger et al. (1966) using glutaryl-1-phenylalanine-p-nitroanilid (GPNA) as substrate. Trypsin activity was determined according to Erlanger et al. (1961). Amylase activity was determined using a kit (No.700) from Sigma Chemical Company (Sigma Chemical Co., St. Louis, MO 63178-9916) and lipase by a pH-stat titration method using tributyrin as substrate according to Erlanson-Albertsson et al. (1987). Units of activity of protease, chymotrypsin, trypsin, α-amylase and lipase were expressed as 1 μmol substrate hydrolysed per mg lyophilized pancreas per min.

Digestive enzyme assay in duodenum content

Duodenal digestive enzyme activity analyses were performed on freeze-dried material, which was extracted with 1 mmol/L HCl (50 mg of lyophilized digesta in 1 ml of 1 mmol/L HCl) for 1 h at 4°C followed by centrifugation (1,500 g). The supernatants were then collected for analysis of protease, trypsin, α-amylase and lipase activities, using methods similar to those for pancreatic enzymes described above. Units of activity of protease, trypsin, α-amylase and lipase were expressed as 1 μmol substrate hydrolysed per mg lyophilized digesta per min.

Digestive enzyme assay in jejunal and ileal mucosas

Mucosa was homogenized in 4.0 ml distilled-water and kept at 4°C followed by 10 min centrifugation (1,500 g). The supernatants were then collected for analysis of maltase, sucrase and γ-glutamyl transpeptidase (γ-GT) activities. Maltase, sucrase and γ-GT activities were analyzed using the method of Dahlqvist (1964). Units of activity of maltase, sucrase and γ-GT were defined as 10 μmol substrate hydrolysed per mg mucosa per min.

Statistical analyses

Data were analyzed for all variables using SAS software (SAS Institute, 1989). Data were subjected to *t*-test procedure to establish differences between means. Each pen was considered as an experimental unit for performance analysis, each animal for viscosity and enzyme measurements. All tests were considered significant at $p<0.05$.

RESULTS

Performance

Average daily gain of growing pigs fed the rough rice-

Table 4. Effect of exogenous NSP enzymes on intestinal viscosity of pigs fed rough rice based diet [a]

	NSP enzymes added (g/kg)		SEM[b]	p value
	-	2		
Viscosity in duodenum (mpa.s)	1.08	1.05	0.014	0.211
Viscosity in jejunum (mpa.s)	1.13	1.05	0.010	<0.001
Viscosity in ileum (mpa.s)	1.18	1.10	0.013	0.002

[a] Values are presented as means; n = 6 animals per treatment. [b] Standard error of the mean.

Table 5. Effect of NSP enzymes on digestive enzyme activities in the pancreas (μmol substrate hydrolysed per mg lyophilized pancreas per min)[a]

	NSP enzymes added (g/kg)		SEM[b]	p value
	-	2		
Protease (U)[b]	27.49	26.41	0.710	0.306
Chymotrypsin (U)	903.28	920.47	7.950	0.157
Trypsin (U)	78.98	78.45	3.755	0.779
α-amylase ($\times 10^3$ U)	29.92	28.48	1.221	0.425
Lipase (U)	53.54	52.09	1.689	0.559

[a] Values are presented as means; n = 6 animals per treatment. [b] Standard error of the mean.

Table 6. Effect of NSP enzymes on digestive enzyme activities in duodenal content (μmol substrate hydrolysed per mg lyophilized digeata per min)[a]

	NSP enzymes added (g/kg)		SEM[b]	p value
	-	2		
Protease (U)	7.53	14.99	1.208	0.007
Trypsin (U)	11.86	13.33	0.604	0.002
α-amylase ($\times 10^3$ U)	13.93	16.49	0.712	0.029
Lipase (U)	35.11	40.68	2.855	0.197

[a] Values are presented as means; n = 6 animals per treatment. [b] Standard error of the mean.

based diet supplemented with enzymes was increased by 10.1% ($p<0.05$) and feed:gain was improved by 9.4% ($p<0.05$) compared to the control group (Table 3). Feed intake of the pigs was unaffected by the enzyme supplementation.

Viscosity of supernatant from jejunal and ileal digesta

The viscosity of the supernatant from jejunal and ileal digesta was decreased by 7.1% ($p<0.001$) and 6.8% ($p<0.01$), respectively, with the addition of NSP enzymes to an rough rice based diet (Table 4).

Activities of digestive enzymes in pancreas

Adding 2 g/kg NSP enzymes had no significant effect on the activities of protease, chymotrypsin, trypsin, α-amylase and lipase in pancreas (Table 5).

Activities of digestive enzymes in duodenal contents

Effects of exogenous NSP enzymes on duodenal digestive enzyme activities are presented in Table 6. Compared with the control group, protease, trypsin and α-amylase activities in the NSP enzyme-treated group were increased by 99.1% ($p<0.01$), 12.4% ($p<0.001$) and 18.4% ($p<0.05$), respectively. However, lipase activity in duodenal content was not influenced by dietary NSP enzyme supplementation.

Activities of digestive enzymes in jejunal and ileal mucosa

No significant effect of NSP enzyme treatment was observed on activities of maltase, sucrase, and γ-glutamyl transpeptidase in jejunal and ileal mucosa (Table 7).

DISCUSSION

Numerous experiments have been conducted to study exogenous NSP enzymes in various growth stages of swine fed cereal-based diets (Inborr et al., 1993; Yin et al., 2000, 2001; Kim et al., 2004). Effects of supplemental NSP enzymes on performance of swine receiving cereal-based diet were variable. Many researchers reported that NSP enzymes had positive effects on performance for pigs fed cereal-based diets (Inborr et al., 1993; Yin et al., 2001; Lindberg et al., 2003; Omogbenigun et al., 2004; Li et al., 2006), while others found no significant response to exogenous NSP enzymes (Baas and Thacker, 1996; Hopwood et al., 2004). The present study demonstrated the positive effect of NSP enzymes on performance of pigs fed a rough rice-based diet.

Cereals are rich in water-soluble NSP. The NSP in cereal-based diets increases the intestinal viscosity and negatively influences the digestion and absorption of nutrients (Li et al., 2004; Chiang et al., 2005). In many

Table 7. Effect of NSP enzymes on activities of maltase, sucrase and γ-glutamyl transpeptidase in jejunal and ileal mucosa (10 μmol substrate hydrolysed per mg mucosa per min)[a]

	NSP enzymes added (g/kg)		SEM[b]	p value
	-	2		
Jejunal mucosa				
Maltase (U)	0.85	0.97	0.049	0.113
Sucrase (U)	0.31	0.34	0.030	0.570
γ-glutamyl transpeptidase ($\times 10^3$ U)	7.02	8.11	0.779	0.345
Ileal mucosa				
Maltase (U)	0.68	0.70	0.027	0.636
Sucrase (U)	0.27	0.29	0.031	0.631
γ-glutamyl transpeptidase ($\times 10^3$ U)	8.81	9.24	0.974	0.760

[a] Values are presented as means; n = 6 animals per treatment. [b] Standard error of the mean.

studies testing exogenous enzyme supplementation, viscosity is measured (Choct and Annison, 1992; Inborr and Bedford, 1994; Jensen et al., 1998;), as viscosity decreases when enzymes hydrolyze the substrates. In the present study, digesta viscosity in jejunum and ileum were significantly decreased with the dietary supplementation of NSP enzymes, indicating that a significant amount of viscous NSP in digesta was degraded by the exogenous enzymes. Jensen et al. (1998) and Yin et al. (2001) reported decreased viscosities of digesta by NSP enzyme supplementation to barley-based swine diets. The viscosity level and the extent of reduction by exogenous NSP enzymes in swine studies were lower compared with studies in poultry (Inborr and Bedford, 1994). This species difference was well demonstrated by Danicke et al. (1999) and may be due to about 10 percent unit higher water content of pig digesta (Partridge, 2001). The changes of viscosity along the intestinal tract, being the lowest viscosity in the duodenum and the highest in the distal part of the small intestine, coincided with previous reports (Jensen et al., 1998; Yin et al., 2001).

The enzyme activities in pancreas were unaffected by exogenous NSP enzymes in the present study. Zebrowska and Low (1987) also observed no change of pancreatic enzyme concentration by feeding different levels of dietary fibre to pigs. In rat studies, the volume of pancreatic juice and total pancreatic enzyme activity was significantly increased with increased gut viscosity when animals were fed highly viscous fibres (Ikegami et al., 1990) or wheat bran (Schneeman et al., 1982). However, the biochemical composition and enzyme activities per unit mass of the pancreas was unaffected by dietary viscous fibres (Ikegami et al., 1990), which was partially in agreement with our results. This lack of response in the pancreas by NSP enzyme supplementation may be related to the relatively low magnitude of duodenal viscosity reduction (2.5%, $p>0.20$) and/or not enough dietary NSP degradation to affect the pancreas. The exogenous NSP enzymes may have increased pancreatic enzyme secretion without affecting enzyme contents in the pancreas, as the enzyme activities in pancreas may not be directly correlated with the volume of pancreatic juice secretion (Zebrowska and Low, 1987; Ikegami et al., 1990).

The result of enzyme analysis in duodenal content showed that the activities of protease, trypsin and α-amylase were significantly increased by exogenous NSP enzyme supplementation. Jensen et al. (1996) reported that dietary β-glucanse increased chymotrypsin secretion in pigs fed diets based on barley, implying that the amount of protein available for digestion was increased by exogenous enzyme supplementation. It has been suggested that the dietary contents of protein, carbohydrate, and fat proportionately change the contents and syntheses of their respective enzymes in the pancreas (Kern et al., 1987; Brannon, 1990). In addition, the digestibility of protein was improved by β-glucanse supplementation in several swine studies (Graham et al., 1988; Bedford et al., 1992; Diebold et al., 2004). In our study, therefore, the NSP enzymes are postulated to have liberated carbohydrate and protein from the endosperm cells, and these nutrients may have modulated exocrine pancreatic secretion.

Only a small quantity of amylum and protein can be decomposed to monosaccharide and free amino acid. Most of the amylum and protein are decomposed to disaccharide and polypeptide, which can be hydrolyzed by carbohydrase and peptidase in the intestinal mucosa (Shen and Wang, 1990). Digestion in the mucosa is a key step linking digestion and absorption, and is even considered as the threshold of absorption (Heitlinger, 1991). In addition, Alpers and Tedesco (1975) proposed that pancreatic enzymes may regulate the enzymes in the brush border of small intestine. Thus, the activities of enzymes in the intestinal mucosa were investigated in the current study. The results showed that activities of maltase, sucrase and γ-glutamyl transpeptidase in mucosa of jejunum and ileum were unaffected by exogenous NSP enzyme supplementation, although the enzyme activities were numerically higher in NSP enzyme added group. Partially in agreement to our results, Stock-Damge et al. (1984) reported no change of the activity of sucrase, maltase, and

aminopeptidase in the intestinal mucosa or brush border membrane by dietary fiber. However, Thomsen and Tasman-Jones (1982) observed decreased disaccharidase activities in the intestinal mucosa by dietary fibre. Further studies may be needed to elucidate the effects of exogenous NSP enzymes on the enzyme activities in the intestinal mucosa.

IMPLICATIONS

The addition of NSP degrading enzymes (including xylanase, β-glucanase and cellulase) to rough rice-based diets improved weight gain and feed efficiency of growing pigs. The results also indicated that exogenous NSP enzymes reduced viscosity of intestinal content and elevated the activities of digestive enzymes.

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