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• BASIC RESEARCH •

Effects of non-starch polysaccharides enzymes on pancreatic and small intestinal digestive enzyme activities in piglet fed diets containing high amounts of barley

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Abstract

AIM: To investigate effects of non-starch polysaccharides(NSP) enzymes on pancreatic and small intestinal digestive enzyme activities in piglet fed diets containing high amounts of barley.

METHODS: Sixty crossbred piglets averaging 13.5 kg were randomly assigned to two treatment groups with three replications (pens) based on sex and mass. Each group was fed on the diet based on barley with or without added NSP enzymes (0.15%) for a 40-d period. At the end of the experiment the pigs were weighed. Three piglets of each group were chosen and slaughtered. Pancreas, digesta from the distal end of the duodenum and jejunal mucosa were collected for determination. Activities of the digestive enzymes trypsin, chymotrypsin, amylase and lipase were determined in the small intestinal sections as well as in homogenates of pancreatic tissue. Maltase, sucrase, lactase and γ-glutamyl transpeptidase (γ-GT) activities were analyzed in jejunal mucosa.

RESULTS: Supplementation with NSP enzymes improved growth performance of piglets. It showed that NSP enzymes had no effect on digestive enzyme activities in pancreas, but decreased the activities of proteolytic enzyme, trypsin, amylase and lipase in duodenal contents by 57.56%, 76.08%, 69.03% and 40.22%(P<0.05) compared with control, and increased γ-GT activities in jejunal mucosa by 118.75%(P<0.05).

CONCLUSION: Supplementation with NSP enzymes in barley based diets could improve piglets' growth performance, decrease activities of proteolytic enzyme, trypsin, amylase and lipase in duodenal contents and increase γ-GT activities in jejunal mucosa.

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INTRODUCTION

Barley is one of the major energy sources of swine diets in many parts of the world. But anti-nutritive factors in barley limit its use in feed industry[1]. The predominant anti-nutritive factor is non-starch polysaccharides (NSP), including β-glucan ((1-3), (1-4)-β-D-glucan)[2,3] and arabinoxylan[4]. The β-glucan and pentosan content in whole barley grain was 4.2% and 6.6%, being 1.8% and 1.4% in endosperm[5]. The major nutrients in barley, starch and protein, are enclosed within endosperm cell walls, which consist mainly of mix-linked β-glucan and arabinoxylan[6]. Pigs, especially piglets, do not produce enzymes that can degrade the cell wall and NSP in barley. So β-glucan and arabinoxylan in barley may interfere with digestion and absorption of nutrients[7], even the production of digestive enzymes[8,9].

Studies have shown that mix-linked β-glucan and arabinoxylan are easily hydrolyzed by β-glucanases and xylanases respectively. Addition of cell wall degrading enzymes *in vitro* increased the release of proteins and non-starch carbohydrate in barley[10]. Supplementation of exogenous NSP enzymes to piglet diets can increase the digestibility of barley and pigs' growth[11-13]. This has been attributed mainly to the breakdown of endosperm cell wall components, resulting in more complete digestion of starch and protein in the small intestine. But there is little information on the effect of β-glucanases and xylanases supplementation on digestive enzyme activities in barley-based diets for piglets.

The aim of the present study was to investigate the effect of supplementation of NSP enzymes on pancreatic and small intestine digestive enzyme activities in piglets fed diets containing high amounts of barley.

MATERIALS AND METHODS

Animals, diets and enzyme complex

Sixty crossbred (Duroc×Landrace×Jiaxing) piglets averaging 13.5 kg were randomly assigned to two treatment groups with three replications (pens) based on sex and mass. Each group was fed on one of the two experimental diets for 40 d. As shown in Table 1, pigs received the same basal diet based on barley-soybean meal and NSP enzymes were added to the basal diet respectively at 0% and 0.15% of the diet at the expense of barley. To accustom pigs to the diets, all pigs were allowed access to the basal diet on alternate days for 7 d prior to commencement of the experiment. The diets and water were offered ad libitum throughout the experiment. Pigs were weighed individually and feed consumption per pen was measured weekly. Growth performance results as average daily gain (ADG), average daily feed intake (ADFI), feed gain ratio (FGR) were collected for all pigs for the experimental period. At the end of feeding trial, three pigs from each treatment (one pig per pen) were slaughtered under general anaesthesia. The pigs were then immediately eviscerated to collect intestinal samples. NSP enzymes complex was supplied by Primal Co. Ltd., BIOTEC, Finland, which contained 10 000 U/g β-glucanase (E.C.3.2.1.6) and 80 000 U/g xylanase (E.C.3.2.1.8).

Table 1 Formula and chemical composition of the basal diet

Ingredients (%)	Percentage
Barley	79.0
Soybean meal (dehulled, solvent)	11.0
Fishmeal	4.0
Yeast meal	2.0
Limestone	1.0
Dicalcium phosphate	1.2
Sodium chloride	0.3
L-Lysine-HCI (78%)	0.2
Vitamin-mineral premix[1]	1.3
Analyzed chemical composition (% as feed)	
Digestible energy(Kcal / kg)[2]	2960
Crude protein	18.28
Crude fat	1.70
Crude fiber	5.26
Calcium	1.11
Phosphorus	0.48

[1]The vitamin / mineral premix provided (per kg feed): 2 000 IU vitamin A, 200 IU vitamin D_3, 20 mg vitamin E, 1 mg vitamin K, l mg thiamine, 3 mg riboflavin, 10 mg d-pantothenic acid, 0.5 mg folic acid, 1 mg pyridoxine, 20 mg niacin, 10 μg cobalamin, 500 mg choline chloride, 0.1 mg biotin, 0.2 mg Se, 0.2 mg I, 80 mg Fe, 5 mg Cu, 2 mg Mn, and 80 mg Zn. [2]Digestible energy was based on calculated values.

Sampling procedure

The contents taken from the small intestine were digesta from the distal end of the duodenum to the ileo-cecal junction. Digesta samples were collected by massaging the tract from both ends. The digesta samples were stored immediately at -20 ℃ until use. Enzyme activity analyses of the samples obtained from the small intestine were performed on freeze–dried material, which was extracted with 1 mmol/L HCl (50 mg lyophilized digesta in 1 mL 1 mmol/L HCl) for 1 h at 4 ℃ followed by centrifugation (3 000 r/min). The supernatants were then collected for analysis of protease, trypsin, chymotrypsin, amylase and lipase activities.

The pancreas from slaughtered pigs was homogenized in ice-cold 0.2 mol/L Tris– HCl buffer containing 0.05 mol/L NaCl. The homogenate was centrifuged at 3 000 r/min for 15 min at 4 ℃ and the supernatant was saved. Protease, chymotrypsin, amylase and lipase activities were determined.

Jejunum mucosa was homogenized in 4.0 mL distilled-water and kept at 4 ℃ for 24 h followed by 10 min centrifugation (3 000 r/min). The supernatants were then collected for analysis of maltase, sucrase, lactase and γ-glutamyl transpeptidase (γ-GT) activities.

Digestive enzyme assay

Protease activity was analyzed using the method of Iwamori *et al*(1997)[14] Chymotrypsin (EC 3.4.21.1) was determined according to Erlanger *et al*[15] using glutaryl-1-phenylalanine-p-nitroanilid (GPNA) as substrate. Amylase (EC 3.2.1.1) activity was determined using a kit (No.700) from Sigma Chemical Company (Sigma Chemical Co., St. Louis, MO 63178-9916) and lipase (EC 3.1.1.3) by a pH-stat titration method using tributyrin as substrate according to Erlanson-Albertsson *et al*.[16]. The activities of protease, trypsin, chymotrypsin, amylase, and lipase are expressed as unit (U) which is defined as the amount of enzyme that hydrolyses 1 μmol of substrate per min. Maltase, sucrase, lactase and γ-GT activities were analyzed using the modified method of Dahlqvist[17]. The activities of maltase, sucrase, lactase and γ-GT are expressed as unit (U) which is defined as the amount of enzyme that hydrolyses 10 μmoL of substrate per min.

Statistical analysis

One way analysis of variance was performed using the General Linear Model (GLM) Procedure of SAS[18]. Differences among means were tested using Duncan's multiple range test. A significant level of 0.05 was used.

RESULTS

Growth performance

Growth performance of pigs fed NSP enzymes is presented in Table 2. As compared to control, supplementation with 1.5 g/L NSP enzymes significantly improved average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) by 6.22% (P<0.01), 2.14% (P<0.05) and 3.69% (P<0.05) respectively.

Table 2 Growth performance of piglets fed diets based on barley with and without NSP enzymes

	Dietary NSP enzymes level (%)	
	0	0.15
Initial mass(kg)	13.91±0.25	14.01±0.17
Final mass(kg)	33.95±0.38[a]	35.35±0.40[c]
Average daily gain (g)	501.16±16.18[a]	532.34±6.88[c]
Average daily feed intake (g)	1 224.00±3.62[a]	1 250.15±7.27[c]
Feed / Gain ratio	2.44±0.03[a]	2.35±0.02[c]

Values are presented as mean±SD; n=30 for average daily gain (ADG), n=3 for average daily feed intake (ADFI) and feed / gain ratio per treatment. Means in a row with different letters differ significantly, P<0.05.

Pancreatic digestive enzyme activities

The results of the effects of NSP enzymes on the digestive enzyme activities in the pancreas of piglets are shown in Table 3. Supplementation with 1.5 g/L NSP enzymes had no significant effect on the activities of protease, chymotrypsin, amylase and lipase in pancreas.

Table 3 Effects of NSP enzymes on the digestive enzyme activities (U / g pancreas) in the pancreas of piglets

	Dietary NSP enzymes level (%)	
	0	0.15
Protease	160.50±17.49	188.86±63.93
Chymotrypsin	1.09±0.28	0.99±0.19
Trypsin	32.14±21.96	27.25±6.79
Amylase	3 009.40±157.19	2 957.02±302.35
Lipase	89.08±13.86	92.15±13.86

Values are presented as mean±SD; n=3 per treatment.

Duodenal digestive enzyme activities

Effects of NSP enzymes on duodenal digestive activities are presented in Table 4. NSP enzymes affected duodenal digestive activities significantly. Compared with the control, protease, trypsin, amylase and lipase activities were decreased by 57.56%, 76.08%, 69.03% and 40.22%(P<0.05) respectively.

Jejunal digestive enzyme activities

The activities of digestive enzyme in jejunal mucosa are shown in Table 5. Supplementation with 1.5g/LNSP enzymes had no effect on maltase, sucrase, lactase activities, but increased γ-GT activities by 118.75%(P<0.05) compared with the control.

Table 4 Effects of NSP enzymes on the digestive enzyme activities (U / mg protein) in the small intestinal contents of piglets

	Dietary NSP enzymes level (%)	
	0	0.15
Protease	60.04±12.86[a]	25.48±4.98[c]
Trypsin	37.21±11.47[a]	8.90±3.72[c]
Amylase	3 600.45±155.68[a]	1 115.16±93.32[c]
Lipase	68.68±11.93[a]	41.06±6.81[c]

Values are presented as mean±SD; n=3 per treatment. Means in a row with different letters differ significantly, P<0.05.

Table 5 Effects of NSP enzymes on the digestive enzyme activities (U / mg) in jejunal mucosa of piglets

	Dietary NSP enzymes level (%)	
	0	0.15
Maltase	28.49±6.45	29.89±10.017
Sucrase	8.13±0.62	8.92±2.16
Lactase	1.22±0.75	1.72±0.97
γ-Glutamyl transpeptidase	0.16±0.05[c]	0.35±0.13[a]

Values are presented as mean±SD; n=3 per treatment. Means in a row with different letters differ significantly, P<0.05.

DISCUSSION

Numerous researchers have reported increased growth and improved feed conversion ratio as a consequence of NSP enzymes inclusion in animal diets based on barley, especially for poultry[19-22]. Effects of exogenous enzymes on growth performance for swine have been variable. Inborr *et al*[11] reported that barley-based diets supplementation with NSP enzymes increased average daily gain and feed conversion ratio of weaned piglets significantly (P<0.05). Yin *et al* (2001)[23] showed that β-glucanases and xylanase improved growth performance and feed gain ratio when piglets were fed with barley based diets. Lindberg *et al*[24] found enzymes (including β-glucanases, xylanases and cellulase) could enhance growth performance especially body mass gain of piglets when fed with diets based on barley. However, negative results were also reported. Baas[25] found there was no effect of β-glucanases on growth performance of finishing swine. But most experiments indicated that NSP had positive effects on growth performance for young pigs. Our study verified this point. Increase of digestibility of nutrients is the main reason for this phenomenon. The inconsistent effects between the experiments may result from different stage of pigs and/or formula of diets used.

Endogenous enzyme is very important for digestibility of nutrients. Increasing gut viscosity due to viscous polysaccharides has been shown to increase the output of pancreatic juice and enzyme activities in rats[9]. However, Mosenthin *et al*[26] and Zebrowska and Low[27] observed no change in the secretion of enzymes from the exocrine pancreas when feeding pigs on diets containing different levels of dietary fibre. Makkink *et al*[28] showed that trypsin and chymotrypsin activity depended on dietary protein source. However, in the present experiment the protein source was the same as in all other diets which may explain why no differences in enzyme activities were observed. The activities of the pancreatic enzymes in the present study were not changed with the enzyme supplementation. This indicated that the synthesis of pancreatic enzymes was unaffected by these factors.

The present study also showed that NSP enzymes decreased the activities of protease, chymotrypsin, amylase and lipase significantly in digesta from the distal end of the duodenum. Almirall *et al*[29] observed that supplementation with β-glucanases increased trypsin, amylase and lipase obviously in chyme of chicken. Jensen[30] found that when pigs were fed barley based diets supplemented with NSP enzymes, chymotrypsin activity was enhanced sharply. Ikegami *et al* (1990) reported that soluble NSP could increase the activities of lipase, amylase and chymotrypsin in rat gut[9]. Our results are different from the studies mentioned above, but are consistent with the results reported by Inborr[11]. Inborr found exogenous NSP enzymes decreased the activities of endogenous enzymes and he thought NSP enzymes might provide a situation appropriate for endogenous enzyme action. But this may result from the fact that NSP enzymes degrade β-glucan and arabinoxylan in endosperm cell wall and decrease the viscosity of digesta in small intestine. Viscosity may act as a barrier to prevent contact of digestive enzymes with their substrates, thickening of the unstirred layer of mucosa and prevention of micelle formation required for absorption of lipids[31]. This process makes the endogenous enzymes to approach substrate easily and work more efficiently.

γ-GT is the key enzyme for amino acids absorption. The present study showed that supplementation with NSP enzymes increased γ-GT activities in jejunal mucosa when piglets were fed barley based diets. The results may indicate that NSP enzymes improves digestibility of the nutrients and supplies more substrates for these endogenous enzymes to act on, which then feedback on the secretion of the enzymes.

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