

Effects of NSP Degrading Enzyme on *In vitro* Digestion of Barley*

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ABSTRACT : A digestion trial *in vitro* was conducted to study effects of supplementation of NSP (non-starch polysaccharides) degrading enzyme (feed grade) on cell wall degradation and digestibility of nutrients in barley. The slices of barley were soaked in distilled water with or without 0.15% non-starch polysaccharides degrading enzyme. Microscopic examination of the slices showed that the endosperm cell wall of barley was completely degraded by the non-starch polysaccharides degrading enzyme. The residues and supernatant of digesta *in vitro* were separated by filtration with 0.1 mm nylon fabric. The residues were used for measurement of crude protein, crude fat, crude fiber, and moisture. The supernatant was used for determination of viscosity, as well as amino-nitrogen and glucose content. The results showed that compared with the control, the amino-nitrogen and glucose content of the supernatant increased by 17.58% ($p < 0.05$) and 10.26% ($p < 0.05$), respectively, while viscosity did not change. Enzyme supplementation increased the digestibilities of dry matter, crude protein, nitrogen-free extract, crude fat and crude fiber of barley by 18.1% ($p < 0.05$), 20.3% ($p < 0.05$), 16.4% ($p < 0.05$), 26.9% ($p < 0.05$) and 30.0% ($p < 0.05$), respectively. The present study suggests that cell wall hydrolysis may contribute to improved nutrient digestion *in vivo* when non-starch polysaccharides degrading enzymes are fed to swine. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 1: 122-126)

Key Words : Barley, Digestibility, Non-starch Polysaccharides, NSP Degrading Enzyme

INTRODUCTION

Barley is generally considered to have a relatively high nutritional value compared with corn, but it has not been widely used as a feed ingredient in China. Barley contains high levels of non-starch polysaccharides (NSP), including β -glucan (referring to (1-3), (1-4)- β -D-glucan) (Aman and Graham, 1987) and arabinoxylan (Fleury et al., 1997), which are the major anti-nutritional factors in barley. The β -glucan and arabinoxylan content in whole grain barley are 4.2 and 6.6%, while the corresponding values for the endosperm are 1.8 and 1.4% (Henry, 1987). These NSP cannot be hydrolyzed by endogenous enzymes of the gastrointestinal tract, and interfere with digestion and absorption of nutrients (Bach Knudsen, 1997). The endosperm cell walls of barley which consist mainly of NSP may restrict access to the intracellular nutrients like starch and protein (Chesson, 1993). Furthermore, water soluble β -glucan and arabinoxylan increase digesta viscosity, decrease digestive enzyme contact with substrates, increase the thickness of the unstirred water layer in the mucosa and hence depresses nutrient absorption and performance in chickens (Johnson and Gee, 1981).

Mixed linked β -glucan and pentosan are easily hydrolyzed by β -glucanases and xylanases separately (Anderson and Stone, 1975; Wong et al., 1988). Addition of

cell wall degrading enzymes *in vitro* increased the release of proteins and non-starch carbohydrate of wheat (Tervila-Wilo et al., 1996). Some studies have demonstrated an increased digestibility of nutrients after addition of β -glucanase to barley based diets (Graham et al., 1988; Inbarr et al., 1993; Yin et al., 2001), but didn't reveal the mode of action of the exogenous enzymes. The effects could be attributed to the breakdown of endosperm cell wall components, resulting in more complete digestion of starch and protein in the small intestine (Hesselman and Aman, 1986), or alternatively a reduction in digesta viscosity as seen in broiler chickens (Bedford and Classen, 1992).

The aim of the present study was to investigate the *in vitro* effect of supplementation of NSP degrading enzymes (feed grade) on cell wall degradation, viscosity of digesta and digestibility of the nutrients in barley.

MATERIALS AND METHODS

Barley

Barley (Var. Zhenong-3) was obtained from the Experimental Farm of Zhejiang University. Barley grain milled to pass a 1.0 mm sieve was used as substrate. The chemical composition of the barley used is presented in Table 1.

NSP degrading enzyme preparation

Commercial NSP degrading enzyme preparation (from *Trichoderma reesei*) with high β -glucanase, xylanase and cellulase activity was supplied by Xinbao Biotechnology Company, Ltd., Zhejiang, China. The β -glucanase, xylanase

* Supported by National Natural Science Foundation of China (Project 30000118).

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Received June 20, 2003; Accepted September 20, 2003

Table 1. Chemical composition of barley samples (Dry matter (DM) content: 90.81%)

| Nutrients | % DM |
|-----------------------------|-------|
| Crude protein (CP) | 11.43 |
| Crude fat (EE) | 2.58 |
| Crude fiber (CF) | 5.23 |
| Nitrogen-free extract (NFE) | 77.98 |
| Amino acids | |
| Aspartic acid | 0.496 |
| Glutamic acid | 1.172 |
| Serine | 0.327 |
| Histidine | 0.230 |
| Glycine | 0.370 |
| Threonine | 0.355 |
| Arginine | 0.556 |
| Alanine | 0.367 |
| Tyrosine | 0.436 |
| Methionine | 0.214 |
| Valine | 0.431 |
| Phenylalanine | 0.516 |
| Isoleucine | 0.384 |
| Leucine | 0.690 |
| Lysine | 0.268 |

and cellulase activity were 6,000, 3,000 and 800 U/g, respectively (One unit is defined as the amount of enzyme that release 1 nmol reducing sugar per second).

Pepsin

Pepsin was purchased from the Applied Biochemistry Institute of Shanghai, China. The activity was 3,000U/g. The concentration of pepsin solution prepared with phosphate buffer (0.1 mol/L, pH 6.0) was 10 mg/ml.

Preparation of pancreatic digestive enzyme solution

Fifty grams of piglet pancreas were homogenized with 1,500 ml phosphate buffer (0.2 mol/L, pH 6.8), stored at 4°C for 24 h and filtered through four layers of gauze. Then, 200 ml enterokinase (SIGMA, 1 U/ml) was added to make the pancreatic digestive enzyme solution. The activity of total proteolytic enzyme, trypsin, amylase, lipase and chymotrypsin in the pancreatic digestive enzyme solution were 1.8, 36.7, 3,404.4, 15.2 and 6.0 U/ml, respectively.

In vitro digestion procedure

In vitro digestion was conducted according to the method of Boisen et al. (1997) with some modifications. The two-step digestion procedure simulates gastric and pancreatic digestion (2 and 4 h of incubation, respectively). Briefly, barley grain (var. Zhenong-3), milled to pass a 1.0 mm sieve, was used as the substrate. 60 ml pepsin solution was added to 10.0 g milled barley mixed with 0.15% NSP degrading enzyme in a 250 ml flask. The pH of the mixture was adjusted to 2.5 with 1.0 mol/L HCl. The barley was digested for 2 h in an oscillator (40°C, 50 rpm) to simulate digestion in the stomach of a pig. Then, 60 ml of pancreatic

digestive enzyme solution was added to the digestion solution, its pH adjusted to 6.8 with 1.0 mol/L NaOH, and digested for 4 h in oscillator (40°C, 50rpm) to simulate digestion in the small intestine of the pig. The digestion control was treated with pancreatin and pepsin without adding NSP degrading enzymes. Each treatment was conducted in four replicates. The residues and supernatant were separated by filtration with 0.1 mm nylon fabric. The residues were used for the measurement of crude protein, crude fat, crude fiber, moisture and amino acid, while the supernatant was used for the determination of viscosity, as well as amino-nitrogen and glucose content.

Microscopy

Barley grain was soaked for 40 h in distilled water and chipped to slices of 13 µm thickness with the slicer. A slice of barley was soaked in distilled water with or without 0.15% NSP enzyme. Four hours later, the samples were examined and the samples were photographed on a microscope (Olympus CH-2, Japan).

Chemical analysis

Viscosity was measured according to the method of Choct and Annison (1990). Glucose content was analyzed using a commercial Kit (from the Biology Production Institute of Shanghai, China). Amino-nitrogen was measured with the formaldehyde titration method described by Hu (1985). Crude protein, fat, fiber and moisture in residues were analyzed according to the methods of the AOAC (Association of Official Agricultural Chemists, 1990). Amino acids of residues were analyzed with an Amino Acid Auto-analyzer (Modular KNAUER 830, German) by the OPA method (Cooper et al. 1984).

Calculation of digestibility

$$\text{Digestibility of the nutrient} = 1 - \frac{C \times D}{A \times B}$$

Where A is the weight of the barley sample digested, B is the nutrient percent in the barley sample digested, C is the weight of the residue sample, and D is the nutrient percent in the residues sample.

Statistical methods

Data were analyzed by analysis of variance (ANOVA) using the general linear model procedures of PC SAS (SAS, 1989). A significance level of 0.05 was used.

RESULTS AND DISCUSSION

Effect of NSP enzyme on degradation of barley endosperm cell wall

Microscopic examination showed that complete

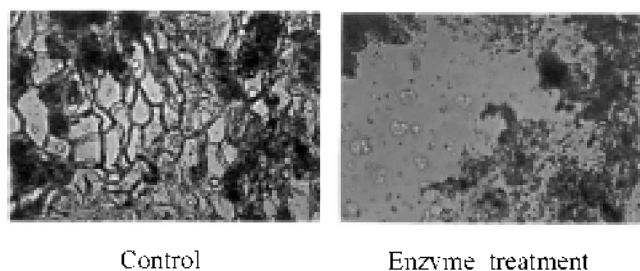


Figure 1. Degradation of hydrolysis of barley endosperm cell walls by NSP degrading enzyme treated as shown by the microscopic method (10 fold).

Table 2. Effect of NSP degrading enzymes on the concentration of glucose and amino-nitrogen as well as viscosity in supernatant¹

| Item | Control | Treatment | SEM ² |
|-----------------|---------|-----------|------------------|
| Glucose (mmol) | 1.82a | 2.14b | 0.062 |
| Amino acid (mg) | 50.31a | 55.47b | 1.285 |
| Viscosity (cP) | 1.39 | 1.38 | 0.018 |

¹ Values are presented as means; n=4 per treatment. Means in a row with different letters differ significantly ($p < 0.05$).

² Standard error of the mean.

endosperm cell wall degradation was almost achieved in the samples treated with NSP degrading enzyme. In the control sample, endosperm cells remained intact (Figure 1). This was consistent with the result obtained on wheat (Tervila-wilo et al., 1996). Digestion of wheat *in vitro* indicated that a combination of xylanase and cellulase was most effective in degrading the cell walls (Tervila-wilo et al., 1996). The endosperm cell wall of barley consists mainly of β -glucan (70-75%) and arabinoxylan (20%) (Aman and Graham, 1987). Thus, NSP degrading preparations containing β -glucanases and xylanases were very effective in degrading the endosperm cell walls of barley.

Effect of NSP degrading enzymes on the chemical composition of the supernatant

The results of the chemical analysis were consistent with those obtained by microscopy. Compared with the control, the content of amino-nitrogen and glucose in the supernatant increased by 17.58% ($p < 0.05$) and 10.26% ($p < 0.05$), respectively (Table 2). This indicated that more protein and carbohydrate were hydrolyzed to amino acids and glucose by the endogenous enzyme. This was consistent with the observation of Tervila-wilo et al. (1996). But, the viscosity of the supernatant was not different between the enzyme-treated barley and the control. This may be due to low level of enzyme preparation. Castanon et al. (1997) found that the addition of the enzyme reduced the recovery of total NSP of barley by hydrolyzing high-molecular weight NSP to low-molecular weight NSP and sugars, and the extent of hydrolysis depended on the level of enzyme. The addition of the lowest level of the enzyme preparation reduced the recovery of insoluble NSP of barley.

Table 3. Effects of NSP degrading enzymes on digestibility of nutrients in barley *in vitro* (%)

| Item | Control | Treatment | SEM ¹ |
|-----------------------------|---------|-----------|------------------|
| Dry matter (DM) | 59.08a | 69.79b | 2.131 |
| Crude protein (CP) | 71.05a | 85.45b* | 1.342 |
| Nitrogen-free extract (NFE) | 60.40a | 70.32b | 1.228 |
| Crude fat (EE) | 43.49a | 55.20b | 3.689 |
| Crude fiber (CF) | 22.05a | 28.67b | 1.430 |
| Amino acids | | | |
| Aspartic acid | 61.32a | 77.88b | 1.652 |
| Glutamic acid | 53.62a | 77.02b | 1.300 |
| Serine | 58.64a | 76.53b | 2.022 |
| Histidine | 54.00a | 87.15b | 2.144 |
| Glycine | 53.32a | 71.80b | 1.861 |
| Threonine | 60.13a | 76.66b | 1.715 |
| Arginine | 61.39a | 72.77b | 1.868 |
| Alanine | 51.24a | 70.13b | 1.758 |
| Tyrosine | 64.99a | 76.00b | 1.779 |
| Methionine | 68.26a | 84.07b | 1.822 |
| Valine | 60.79a | 77.21b | 1.617 |
| Phenylalanine | 67.53a | 78.63b | 2.001 |
| Isoleucine | 67.78a | 79.35b | 1.477 |
| Leucine | 70.85a | 79.23b | 1.267 |
| Lysine | 62.35a | 80.12b | 1.855 |

¹ Values are presented as means; n=4 per treatment. Means in a row with different letters differ significantly ($p < 0.05$). ² Standard error of the mean.

but increased the soluble NSP, which shows that some of the degraded insoluble NSP were solubilised to high-molecular weight NSP but was not further hydrolysed to low-molecular weight NSP or sugars. Rouan and Moreau (1993) also found that almost all insoluble arabinoxylans of wheat which were degraded by an enzyme preparation, were solubilised prior to their hydrolysis to low-molecular weight polysaccharides. Hydrolysis of solubilised insoluble NSP was limited by the enzyme dose. It is notable that a low level of enzyme could in fact lead to an increase in the amount of soluble NSP which would explain the high-molecular weight soluble NSP accumulation in the hind gut section reported by Bedford et al. (1992).

Effect of NSP enzyme on digestibility of barley

Enzyme supplementation increased the digestibilities of dry matter, crude protein, nitrogen-free extract, crude fat and crude fiber of barley by 18.1% ($p < 0.05$), 20.3% ($p < 0.05$), 16.4% ($p < 0.05$), 26.9% ($p < 0.05$) and 30.0% ($p < 0.05$), respectively (Table 3). The digestibilities of all amino acids increased significantly ($p < 0.05$). The results reported in the literature are highly variable with some studies reporting increased starch (Inbarr et al., 1993) and nitrogen digestibilities (Bedford et al., 1992), while other reported no effect of enzyme supplementation on the digestibility of either nitrogen or starch (Graham et al., 1986; Thacker et al., 1992; Gdala et al., 1997). Thus, the results of the present study supports the hypothesis that the availability of nutrients is improved by the breakdown of

the endosperm cell walls. The reason for the effect could be the use of a multi-enzyme preparation, as the breakdown of cell walls requires a number of different enzymes (Chesson, 1993).

There are two viewpoints on the mechanism by which exogenous enzyme supplementation to barley-based diets could increase the digestibility of nutrients. One suggestion is that a partial hydrolysis of β -glucans by a β -glucanase enzyme results in a large decrease in viscosity (Hesselman and Aman, 1986). The mechanisms by which β -glucans interfere with digestion and absorption are closely related to their physiochemical properties. This branched structure prevents compact folding of molecules and increases the water-holding capacity, resulting in characteristic viscosity and gelling properties (Fadel et al., 1987; Wang et al., 1992). A viscous environment in the small intestine could reduce absorption of lipids, protein and possibly other dietary nutrients. Viscosity may act as a barrier preventing contact of digestive enzymes with their substrates, thickening of the unstirred layer of mucosa and prevention of micelle formation required for absorption of lipids (Wang et al., 1992). Another possibility is that degradation of endosperm cell walls by NSP degrading enzymes might result in liberation of nutrients. The β -glucan and arabinoxylan, which comprise a large proportion of barley endosperm cell walls, physically limit access of digestive enzymes to the inside of the cell (Pettersson and Aman, 1988). The findings from this study have demonstrated that the primary operating mechanism by which NSP degrading enzymes increase the digestibility of nutrients is the degradation of endosperm cell walls, not a decrease in viscosity of the digesta.

CONCLUSIONS

NSP degrading enzymes are able to degrade of cell walls of endosperms of barley and liberate the nutrients therein. We postulate that cell wall hydrolysis may contribute to improved nutrient digestion *in vivo* when non-starch polysaccharides degrading enzymes are fed to swine.

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