The degradation of arabinoxylan-rich cell walls in digesta obtained from piglets fed wheat-based diets varies depending on digesta collection site, type of cereal, and source of exogenous xylanase

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ABSTRACT: The objective of the present study was to compare the ability of experimental and commercial xylanases to degrade, in vitro, the arabinoxyylan (AX) fraction in digesta from 28-d-old piglets fed a wheat (Triticum aestivum)-based diet (49% wheat). Pigs were euthanized at 1, 2, 3, or 4 h after feeding; stomach and ileum contents were isolated and frozen and later used for the in vitro studies. Xylan solubilization provided information regarding the ability of the enzymes to degrade AX during the harsh in vivo conditions prevailing in the gastrointestinal tract. The hydrolytic capacity of a commercial xylanase was compared with that of an experimental xylanase using stomach digesta (pH 1.8) obtained at 4 h after feeding. Relative to the control, both enzymes increased (P < 0.001) xylan solubilization 3-fold. In the ileal digesta (1 h), xylan solubilization was increased by 36% (P < 0.001). Inclusion of arabinofuranosidases (Ara f) with xylanases increased xylan solubilization in stomach samples (P = 0.007 and P = 0.030) but not in ileal samples (P = 0.873 and P = 0.997). Our results illustrate clearly the importance of using different conditions and substrates when enzyme performance is studied in vitro as a prescreening tool for setting up in vivo trials.

Key words: digesta, xylanase, xylose

INTRODUCTION

Apart from starch and protein, cereals such as wheat contain dietary fibers, mainly nonstarch polysaccharides (NSP), the major component being arabinoxyylan (AX). The reported levels of NSP in wheat are as high as 119 g/kg (Bach Knudsen, 1997). Currently there is an increased tendency to use wheat by-products as animal feed and these products vary in their arabinoxylan content (Nortey et al., 2008). Whereas starch is well used in pigs, the AX is not.

Use of enzymes has attracted growing attention as it is well documented that exogenous enzyme supplementation breaks the AX polymer chains into oligomers, releasing caged nutrients and thereby improving the nutritive value of feed ingredients (Bedford, 2002; Nortey et al., 2008). The efficacy of feed enzymes depends on their substrate specificity, activity, and stability. As the use of industrial feed enzymes continues to grow, it remains a challenge to discover and develop enzymes that can either work under or survive the harsh gastrointestinal (GI) tract conditions of monogastric species.

To evaluate enzyme efficacy, in vitro incubations in conditions simulating those of the gastrointestinal tract are often conducted (Bedford and Classen, 1993). In general, a 2-stage in vitro digestion assay, in which the feed sample with enzyme is exposed to different pH and proteolytic enzymes present in the gut and simulating the peptic and pancreatic phases, is used (Malathi and Devegowda, 2001). Correlation between in vitro and in vivo results may not always exist and in vivo results may often vary because of the conditions prevailing in the animal’s GI tract. To overcome these discrepancies, we studied enzymatic efficacies using digesta samples from stomach and ileum as substrates. The piglets had been fed a wheat-based diet and sacrificed at different time intervals after feeding. Efficacy of arabinofuranosidases (Ara f), the key debranching enzymes of AX (Saha, 2000), were studied together with the xylanases.


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MATERIALS AND METHODS

Xylanase (Ronozyme WX) and experimental enzymes (a xylanase and two Ara f) were obtained from Novozymes, A/S, Bagsværd, Denmark. The D-xylose assay kit was obtained from Megazyme, Bray, Ireland, and was used according to the manufacturer’s instructions. All other chemicals were purchased from Sigma Aldrich.

Twenty 28-d-old piglets (8.69 ± 0.87 kg BW) were deprived of feed for 12 h and then fed a bolus of feed for 15 min. Five pigs were then slaughtered at each of 1, 2, 3, or 4 h after feeding. Stomach and ileum contents were isolated, frozen, and later used for the in vitro studies in this study. Before use, portions of frozen digesta samples were thawed overnight at 4°C and centrifuged at 1200 × g for 15 min at 5°C. Pellet and supernatant were collected and stored separately. Volume of supernatant and weight and DM content of the pellet was measured. Pellet and supernatant were portioned equally into a 24-well plate. The plates with digesta samples were incubated at 40°C for 4 h with stirring at 350 rpm. The xylanases added were either commercial Ronozyme WX or an experimental xylanase, both supplemented at 10 mg enzyme protein/kg DM. Each of the experimental Ara f was added at 5 mg enzyme protein/kg of DM. After incubation, the plate was centrifuged and supernatants were collected and analyzed for release of xylose. Comparisons of means were performed by Tukey-Kramer honestly significant difference test using JMP 9.0.2 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

The stomach digesta samples had a pH of 4.8 after 1 h and a pH of 1.8 after 4 h of feeding whereas the ileum digesta sample had a pH of 8.2 after 1 h and 6.9 after 4 h of feeding. Only the 4-h stomach digesta sample and 1-h ileum digesta samples were used, because the activity of commercial and experimental enzymes could be studied at the pH extremes of 1.8 and 8.2 prevalent in the digesta.

Relative to the nonenzyme supplemented control, both enzymes increased (P < 0.001) xylose solubilization. In stomach digesta (4 h), xylose increased (P < 0.001) solubilization 3-fold compared with the control. In ileal digesta (1 h), solubilization was increased (P < 0.001) similarly for both enzymes by 36%. Notably, inclusion of Ara f in the stomach digesta further increased the capacity of the experimental (P = 0.007) and commercial enzymes (P = 0.030) to release xylose (Figure 1). In contrast, for ileal samples response to Ara f added to either of the xylanases was lacking (experimental: P = 0.873; commercial: P = 0.997). In ileal samples, the experimental enzyme tended to yield higher releases of xylose than the commercial (P = 0.053), but by addition of Ara f, the increase became solid (P = 0.017). In stomach samples, both enzymes

![Figure 1](image_url)
performed equally with \( P = 0.126 \) or without \( P = 0.345 \) Ara \( f \). The viscosity of feedstuffs was decreased when using commercial enzymes containing Ara \( f \) (Mathlouthi et al., 2002), likely due to the Ara \( f \) stripping arabinose side chains from the xylan polymer making it a better substrate for the xylanase attack. Under acidic conditions of the human stomach, Zhang et al. (2003) showed up to a 10% release of arabinose from several hemicelluloses sources. But the highly acidic conditions of the piglet stomach contents did not seem to have a detrimental effect on enzyme activity nor were enough arabinose moieties cleaved off to make the use of Ara \( f \) to improve the nutritive value of the feedstuff redundant. In conclusion, our results illustrate clearly the importance of using different conditions and substrates when enzyme performance is studied in vitro as a prescreening tool for setting up in vivo trials.

**LITERATURE CITED**


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Mathlouthi, N., L. Saulnier, B. Quemener, and M. Larbier. 2002. Xylanase and \( \beta \)-glucanase and other side enzymatic activities have greater effects on the viscosity of several feedstuffs than xylanase and \( \beta \)-glucanase used alone or in combination. J. Agric. Food Chem. 50:5121–5127.

