Effect of xylanases on ileal viscosity, intestinal fiber modification, and apparent ileal fiber and nutrient digestibility of rye and wheat in growing pigs[1,2]

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ABSTRACT: Two experiments were performed to study the effect of xylanase on ileal extract viscosity, in vivo fiber solubilization and degradation, and apparent ileal digestibility (AID) of fiber constituents, OM, CP, starch, and crude fat in rye and wheat in ileal-cannulated pigs. In Exp. 1, coarse rye without (NX) or with addition of xylanase from Aspergillus niger (AN), *Bacillus subtilis* (BS), or *Trichoderma reesei* (TR) was fed to 8 ileal-cannulated barrows (initial BW 30.9 ± 0.3 kg) for 1 wk each according to a double 4 × 4 Latin square design. In Exp. 2, fine rye, fine wheat, and coarse wheat with or without a combination of xylanase from *Bacillus subtilis* and *Trichoderma reesei* were fed to 6 ileal-cannulated barrows (initial BW 33.6 ± 0.5 kg) for 1 wk according to a 6 × 6 Latin square design with a 2 × 3 factorial arrangement of enzyme and cereal matrix. Chromic oxide (0.2%) was used as an inert marker. Ileal effluent was collected for 8 h on d 5 and 7 and pooled for analysis. In Exp. 1, TR reduced intestinal viscosity of pigs fed rye from 9.3 mPa·s in the control diet (NX) to 6.0 mPa·s ($P < 0.001$), whereas AN and BS had no effect. None of the enzymes changed the concentration of total arabinoxylan, high-molecular-weight arabinoxylan (HMW-AX), or arabinoxylan oligosaccharides (AXOS) in the liquid phase of digesta. In Exp. 2, the enzyme combination reduced intestinal viscosity for all 3 cereal matrices ($P < 0.05$), but the viscosity was much higher with fine rye (7.6 mPa·s) than with fine and coarse wheat (<1.7 mPa·s). Simultaneously, the total concentration of arabinoxylan in the liquid phase of digesta increased by 82.4% in fine wheat ($P < 0.002$) and by 45.9% in coarse wheat ($P < 0.006$), and AXOS increased 16-fold with enzyme addition. Similar effects of enzyme were not seen with rye. The concentration of xylooligosaccharides in the liquid phase of digesta increased with enzyme addition, but for xylose, it was only significant for wheat, for which it increased 3.9-fold ($P < 0.001$). None of the xylanases affected AID of arabinoxylan of rye in Exp. 1. In Exp. 2, the enzyme combination increased AID of arabinoxylan by 91% to 107% ($P < 0.001$) across cereal matrices. Enzyme addition did not affect AID of nutrients in any of the experiments except for a higher starch and crude fat digestibility of fine wheat with enzyme addition ($P < 0.012$) in Exp. 2. Collectively, the results suggest that xylanase is more efficient in degrading arabinoxylan from wheat than from rye.

Key words: digestibility, pigs, rye, viscosity, wheat, xylanase

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INTRODUCTION

Because of increases in feed costs, rye has gained increased interest as a feed ingredient for pigs in Denmark because of its lower price, winter hardiness, and ability to grow on sandy soil with low fertility (Bushuk, 2001). Previously, rye has had a negative reputation because of older cultivars' susceptibility to ergot infection (Friend and Macintyr, 1969), bitter constituents (Heinio et al., 2012), and high content of fiber in the form of arabinoxylans (Bach Knudsen and Lærke, 2010), which limit unrestricted use (Jürgens et al., 2012). In particular, soluble fibers, which also increase intestinal viscosity, may reduce the digestibility of nutrients in the small intestine, a phenomenon markedly seen in broilers (Annison, 1993). Use of feed enzymes is a way to reduce intestinal viscosity, liberate entrapped nutrients, increase digestibility, and ameliorate growth retardation and digestive problems (Brufau et al., 2006). Cell wall–degrading enzymes for wheat-based diets are convincingly used in poultry, but results in pigs are inconsistent (Danicke et al., 1999). Furthermore, the efficacy of xylanases, which traditionally are developed for wheat, has not been extensively studied using rye.

In this study, we hypothesized that xylanases of different origin differed in their ability to solubilize arabinoxylan in vivo, reduce intestinal viscosity, and affect apparent ileal digestibility (**AID**) of fiber and macronutrients in growing pigs fed rye as the only carbohydrate source. The aim was to identify the most promising candidate for use in practical feeding with rye. In a second experiment, we hypothesized that adding a combination of 2 of the xylanases to finely milled rye would degrade the fiber matrix in rye as efficiently as in wheat. In this study, we also included coarsely milled wheat, which is more similar to practical feeding conditions, to study the effect of particle size in a well-known substrate.

MATERIALS AND METHODS

All experimental procedures were conducted according to protocols approved by the Danish Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Food and Veterinary Administration, Copenhagen, and in compliance with Danish laws and regulations for the Humane Care and Use of Animals in Research, Act 1306, of November 23, 2007. The animals maintained good health throughout the experimental period.

Experiment 1: Effect of Different Xylanases on Coarse Rye

Animals, Experimental Design, and Diets. Experiment 1 was designed to study the effect of adding 3 different xylanases to rye on in vivo fiber degradation and solubilization, intestinal viscosity, and AID of fiber constituents and nutrients of rye. Eight growing crossbred barrows (Duroc × [Danish Landrace × Yorkshire]) obtained from a local swineherd or the Department of Animal Science, Aarhus University, Denmark, with an initial BW of 30.9 ± 0.3 kg were surgically equipped with a simple T-cannula 15 cm anterior to the ileal-cecal junction (Jørgensen et al., 1992) and allotted to a replicated 4 × 4 Latin square design with 4 diets and 4 periods in each square. Pigs were housed individually in 3 × 2 m pens without bedding and with elevated plastic grids covering half of the pen, which allowed the pigs to rest and stay dry in an environmentally controlled room, and temperature was maintained at 18°C ± 2°C. A trough and a nipple drinker were installed in each pen. Pigs were allowed a 2-wk recovery period after the surgery, before the experiment was initiated. During the recovery period, a standard wheat–barley–soybean meal–based grower diet was provided.

Four diets containing rye milled on a roller mill with a 3.5-mm gap (coarse rye) as the only source of carbohydrates (Table 1) were supplemented with minerals and vitamins to exceed the NRC requirements (NRC, 2012) and phytase (Phyzyme XP, Danisco Animal Nutrition, Marlborough, UK) according to manufacturers' recommendations. The diets were supplemented with heat-treated wheat as an enzyme carrier with or without addition of xylanase. Chromic oxide (0.2%) was added as a digestibility marker to all diets. To minimize the risk of errors in feed allotment, the diets were supplemented with colored indigestible Microgrits (Jadis Additiva, Haarlem, Netherlands) to visually distinguish the different diets by different coloring. One diet was without xylanase addition (**NX**) and served as a control diet. The other 3 diets were supplemented with a commercially relevant dosage of xylanase from either *Aspergillus niger* (**AN**) at 1,100 xylanase units (**XU**)/kg, *Bacillus subtilis* (**BS**) at 350 XU/kg, or *Trichoderma reesei* (**TR**) at 4,000 Danisco xylanase units (**DAN xyl U**)/kg. One XU defined as the amount of enzyme that increases the optical density at 590 nm by 0.1/min using Xylazyme tablets (Megazyme International Ireland) at the conditions given by Sørensen and Sibbesen (2006). One DAN xyl U is defined as the amount of enzyme that releases 0.48 μmol of reducing sugar equivalents (as xylose) from wheat arabinoxylan per minute at pH 4.2 and 50°C by the 2,4-dinitrosalicylic acid reducing sugar method (Bailey et al., 1992). One XU is defined as the

Table 1. Ingredient composition of experimental diets, Exp. 1 and 2 (as-fed basis)

Item	Exp. 1, roller mill, coarse rye	Exp. 2, hammer mill		
		Fine rye	Fine wheat	Coarse wheat
Coarse rye	96.779	—	—	—
Fine rye	—	95.284	—	—
Fine wheat	—	—	95.284	—
Coarse wheat	—	—	—	95.284
Limestone	1.490	1.490	1.490	1.490
Monocalcium phosphate	0.774	0.774	0.774	0.774
Salt	0.402	0.402	0.402	0.402
Carrier (−/+xylanase)[1]	0.100	—	—	—
Phyzyme XP	0.005	—	—	—
Carrier (Phyzyme XP and ± xylanase)[2]	—	0.100	0.100	0.100
Micromineral-vitamin premix[3]	0.200	0.200	0.200	0.200
Chromic (III) oxide	0.200	0.200	0.200	0.200
Colored corn grits	0.050	0.050	0.050	0.050
80% Glycerol	—	1.500	1.500	1.500

[1]Enzyme carrier, heat-treated wheat, either without xylanase or with *Aspergillus niger*, *Bacillus subtilis*, or *Trichoderma reesei* xylanase.

[2]Enzyme carrier, heat-treated wheat, either with Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK) alone or in combination with *Bacillus subtilis* and *Trichoderma reesei* xylanase.

[3]Provided the following quantities of microminerals per kilogram of complete diet: Cu, 15.0 mg as copper sulfate; Fe, 84.0 mg as iron sulfate; I, 0.21 mg as potassium iodate; Mn, 42.0 mg as manganese sulfate; Se, 0.30 mg as sodium selenite; and Zn, 100 mg as zinc oxide. Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 4,200 IU as vitamin acetate; vitamin B_{12}, 0.021 mg; vitamin D_3, 420 IU; vitamin E, 69 IU as DL-α-tocopheryl acetate; vitamin K_3, 2.1 mg as menadione; D-pantothenic acid, 10.5 mg as calcium pantothenate; niacin, 21 mg; pyridoxine, 3.15 mg as pyridoxine hydrochloride; riboflavin, 2.1 mg; thiamin, 2.1 mg as thiamine mononitrate; biotin, 0.053 mg; and cyanocobalamin (B_{12}), 0.021 mg.

amount of enzyme that increases the optical density at 590 nm by 0.1/min using Xylazyme tablets (Megazyme International Ireland) at the conditions given by Sørensen and Sibbesen (2006). The 3 xylanases differ by their pH optimum, which is reflected in the different dosing and expression of activity. Xylanase from *Aspergillus niger* has its optimum activity at pH 3 to 4; the optimum for BS is pH 5 to 7, whereas TR has a wider pH optimum ranging from pH 4 to 7. Furthermore, the enzymes are considered to have different substrate affinities and modes of action in viscosity reduction. Xylanase from *Aspergillus niger* is derived from a nongenetically modified production organism and therefore has side activities such as endo-β-endoglucanase. Xylanase from *Bacillus subtilis* and TR are derived from genetically modified organisms, resulting in negligible side activities.

The daily feed allocation in each period, which was based on the average BW of the pigs at the start of each experimental period, was calculated to 90% of Danish recommended feeding levels for growing pigs and corresponded, on average, to 2.67 times the maintenance energy requirement of 197 kcal/kg $BW^{0.60}$ (NRC, 2012). Pigs were fed each experimental diet in meal form for 7 d in randomized order according to the replicated 4 × 4 Latin square design. The initial 4 d were an adaptation period to the diet. To ameliorate restrictions in growth by the experimental diets, all pigs had a 10% protein supplement of whey powder (Miprodan 30; Arla Food Ingredients, Viby J., Denmark) in the first 5 meals during the adaptation period. The daily ration was divided into 3 equal meals, which were offered at 0730, 1530, and 2330 h, and water was available ad libitum.

Sample Collection. On d 5 and 7, ileal content was collected continuously for 8 h. Briefly, digesta were collected from 0730 to 1530 h by using polyamide autoclave bags of 60 × 200 mm (Buch and Holm, Herlev, Denmark) attached to the open cannula barrel. Two or three drops of 0.2% NaN_3 (Sigma-Aldrich, St. Louis, MO) were added to each bag to prevent microbial activity. The bags were removed whenever they were filled with digesta or at least once every 30 min and were stored at −20°C until further analysis. At the end of the experiments, ileal samples from both collection days were thawed and mixed thoroughly within animal and diet, and a subsample was taken for immediate separation into a solid phase and a liquid phase and determination of extract viscosity. Another subsample was lyophilized and milled (<0.5 mm) before chemical analysis. The liquid phase was obtained by pouring approximately 4 × 30 g digesta into tarred 50-mL centrifuge tubes, centrifugation at 10,000 × *g* at 4°C for 20 min, pooling the resulting supernatant from the 4 tubes into a beaker, and mixing carefully for immediate viscosity determination. Subsamples of the liquid phase were kept at −20°C until analysis. Samples of the diets were collected on a weekly basis, and a pooled sample of each diet was used for chemical analyses.

Experiment 2: Effect of a Combination of Xylanases on Fine Rye, Fine Wheat, and Coarse Wheat

Animals, Experimental Design, and Diets. On the basis of the results obtained in Exp. 1, Exp. 2 was designed to compare the effect of a combination of xylanases from BS and TR on in vivo degradation and solubilization of arabinoxylan, intestinal viscosity, and AID of nutrients of 3 cereal matrices; fine rye, fine wheat, and coarse wheat. The purpose of both combining xylanases and reducing particle size was to study if increased exposure of the substrate would increase the degradation of arabinoxylan in rye when wheat with

the same particle size was used as reference. Furthermore, a coarse wheat relevant to practical feeding conditions was included. This allowed us to test the effect of particle size in a known substrate with expected effects of the enzymes. Six growing barrows surgically equipped with a simple T-cannula (initial BW 33.6 ± 0.5 kg) were fed the 3 cereal matrices with or without enzyme addition according to a 6 × 6 Latin square design with 1 diet per week in 6 wk. The cereals were hammer milled to pass a 3-mm perforated sieve (fine rye and fine wheat) or a 5-mm sieve (coarse wheat). The diets were formulated to contain the cereals as the only carbohydrate source and were supplemented with minerals and vitamins, chromic oxide, and colored Microgrits (Jadis Additiva) as described for Exp. 1 (Table 1). All diets were supplemented with heat-treated wheat as an enzyme carrier for either Phyzyme XP alone or Phyzyme XP together with a combination of xylanases from *Bacillus subtilis* at 370 XU/kg and *Trichoderma reesei* at 4000 DAN xyl U/kg. Furthermore, because of dust problems observed in Exp. 1, lukewarm 80% glycerol was added as a dust binder in Exp. 2. The daily feed allowance was determined as in Exp. 1 but corresponded in this experiment to 2.75 times the maintenance energy requirements because of the addition of glycerol. Apart from diet composition and experimental design, the feeding regime was performed as described in Exp. 1. Diet and ileal samples were collected as described for Exp. 1.

Laboratory Analyses

Particle size distribution of the diets was determined using a Retsch AS200 vibratory sieve shaker (Retsch GmbH, Haan, Germany) provided with 8 sieves ranging in mesh from 4,000 to 45 µm. A representative subsample (100 g) of each diet in Exp. 1 and duplicate samples of diets in Exp. 2 were sieved for 10 min with a sieving amplitude of 2 mm and 1-min shaking intervals essentially as outlined in ANSI/ASAE S319.4 (American Society of Agricultural and Biological Engineers, 2012). All other samples were analyzed in duplicate. Viscosity of the supernatants of digesta was measured in a Brookfield DV-II Cone/Plate Viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA) at 39°C at shear rates between 2.25 and 450 s^{-1}, and values of viscosity at a shear rate of 45 s^{-1} are reported.

Dry matter content of lyophilized milled diets and digesta (both <0.5 mm) was determined by drying to a constant weight at 103°C for 20 h, and ash was analyzed by combustion of OM at 525°C as a slight modification of AOAC method 942.05 (AOAC, 2007). Chromic oxide was analyzed as described by Schurch et al. (1950), protein (N × 6.25) was determined by the Dumas method (Hansen, 1989), crude fat was determined with acid hydrolysis before ether extraction according to the Stoldt procedure (Stoldt, 1952), and starch was analyzed by the enzymatic-colorimetric method as described by Bach Knudsen (1997). Dietary contents of glucose, fructose, and sucrose and also fructans were analyzed as described by Larsson and Bengtsson (1983). Nonstarch polysaccharides (**NSP**) of diets, ileal digesta, and ileal supernatants were measured as described previously (Bach Knudsen, 1997) with the modification that the polysaccharides in starch-free residue were hydrolyzed with 2 *M* H_2SO_4 for 1 h instead of 1 *M* H_2SO_4 for 2 h. In diets, the NSP were further classified into soluble and insoluble NSP, noncellulosic polysaccharides (**NCP**), and cellulose, and Klason lignin was determined gravimetrically as the acid-insoluble residue as previously described (Bach Knudsen, 1997). Total nondigestible carbohydrates (**NDC**) in ileal extracts were determined as for NSP, except that starch was not removed and the samples were not precipitated with 80% ethanol before acid hydrolysis. The contribution of starch to glucose in the hydrolysate was corrected for in the calculation (see below).

The concentrations of β-D-linked xylooligosaccharides in the liquid phase of ileal effluent of pigs fed fine rye and fine wheat with or without enzyme addition in Exp. 2 were analyzed by high-pressure anion exchange chromatography with pulsed amperometric detection (Dionex, Sunnyvale, CA). One milliliter of sample was mixed with 9 mL 50% ethanol (vol/vol) and incubated at 65°C for 60 min with occasional mixing (3 times). After centrifugation at 2,000 × *g* at ambient temperature for 10 min**,** 0.25 mL of the liquid was diluted 20 times with water, and 0.25 mL lactose solution was added as an internal standard to a final concentration of 12.5 mg/mL; the resulting solution was filtered through a 22-µm nylon filter. The carbohydrates were eluted by a gradient prepared from water (eluent A), 0.225 *M* NaOH (eluent B), and 0.5 *M* Na acetate (eluent C). An elution program starting at a flow rate of 0.6 mL/min with 92.5% eluent A and 7.5% eluent B was changed to 1.0 mL/min at 17 min, and the proportion of eluent B was also increased to 11.5%. After 10 min, eluent B was increased to 25% for 20 min, and simultaneously, 0.5% eluent C was added for 10 min and further increased to 1.5% for the following 10 min at the expense of eluent A. Thereafter, the proportions of eluents B and C were kept at 50% and 7%, respectively, for 18 min. After 5 min, eluent C increased to 11% for another 5 min. Finally, at 60 min, eluent composition was changed to 50% eluent B and 50% eluent C for 11 min, after which eluent composition was returned to the initial concentration and

a flow rate of 0.7 mL/min for 10 min. Quantification of the carbohydrates was performed by external standards of xylose (Merck KGaA, Darmstadt, Germany), xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose (Megazyme International Ireland, Wicklow, Ireland) using mixtures in concentrations ranging from 2 to 20 mg/L.

In Exp. 1, the xylanase activity of the diet containing TR was confirmed using a 5-g freeze-dried sample extracted by stirring for 10 min with 50 mL buffer (0.2 *M* sodium acetate, pH 4.2) at room temperature. After filtration, 0.1 mL filtrate was mixed with 0.4 mL 0.2 *M* sodium acetate buffer (pH 4.2). The diluted sample was equilibrated at 50°C, and a 60 mg Xylazyme tablet (Megazyme International Ireland) was added. At 60 min, 5 mL 2% Tris was added, and the slurry was thoroughly mixed. After centrifugation at ambient temperature for 10 min at 1,500 × g, the optical density at wavelength 590 nm (OD590) was determined, and the activity of TR quantified by the use of a standard curve was made by adding known amounts of TR to 5 samples of the control diets that were analyzed in parallel. The TR activity was expressed in DAN Xyl U. Xylanase activity of AN in the diet was determined as described above with the only modification being that McIlvaine (phosphate/citrate) buffer (pH 3.4) including 0.25% SDS was used as the extraction and reaction buffer, and known amounts of AN were used for quantification. Likewise, for the BS-containing diet, the activity was quantified by the use of McIlvaine buffer (pH 6) as the extraction and reaction buffer, and BS was used for quantification. The activity of AN and BS was expressed in XU. The diets containing BS and TR in Exp. 2 were analyzed using 0.2 *M* sodium acetate (pH 4.2) as the extraction and reaction buffer, and known amounts of TR were used for the standard curve. At this pH, the xylanase activity would predominately originate from TR at the used ratio between TR and BS. Therefore, the enzyme activity is described by the DAN xyl U.

To evaluate the effect of TR addition on the viscosity generated by arabinoxylan isolated from rye and from wheat, a 0.75% solution of arabinoxylan from rye (P-RAXY, ~33 mm^2/s) or wheat (P-WAXYH, ~43 mm^2/s), both at 1% (wt/vol) and 30°C (Megazyme International Ireland), was prepared in 50 m*M* sodium acetate buffer (pH 5.0). Five samples of each arabinoxylan solution were equilibrated at 40°C, mixed with increasing amounts of TR diluted in 50 m*M* sodium acetate buffer (pH 5.0), and incubated at 40°C with shaking at 350 rpm. At time 30 min, 1% 10 m*M* NaOH was added to inactivate the enzyme, and the viscosity was measured at 20°C using a Brookfield DV-I+ Cone/Plate Viscometer (Brookfield Engineering Laboratories Inc.). The analysis was done in duplicate.

Calculations and Statistical Analysis

Total dietary fiber (**T-DF**) in diets was determined as the sum of NSP, Klason lignin, and fructans. Arabinoxylan was calculated as the sum of the arabinose and xylose residues of the NSP procedure and the other fractions of NSP as previously described (Bach Knudsen, 1997). Total nondigestible carbohydrates in ileal contents were determined as the sum of anhydro sugars in the direct hydrolysis procedure and subtraction of starch (separate analysis) from the glucose values obtained. Total arabinoxylan in digesta was calculated as the sum of anhydrous arabinose and xylose in the direct hydrolysis procedure without ethanol precipitation, and the content of high-molecular-weight arabinoxylan (**HMW-AX**) was calculated as the sum after precipitation with 80% ethanol in the NSP procedure. The content of arabinoxylan oligosaccharides (**AXOS**) in ileal extract was calculated as the difference between the 2 procedures.

Apparent ileal digestibility of OM, starch, nitrogen, crude fat, and total NSP and constituent sugars (arabinose + xylose) was calculated by the index method (Adeola, 2001) using the analyzed mean dietary concentration and individual ileal concentrations of chromic oxide.

Before statistical analysis, all data on viscosity were subjected to logarithmic transformation, and the results were reported as geometric means with 95% confidence intervals. Data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC). The pig was the experimental unit for all analyses. Data were analyzed as a Latin square design in both experiments. In Exp. 1, dietary treatment was the main effect, and pig and period were random effects. In Exp. 2, which was a 2 × 3 factorial arrangement of dietary treatment, the effects of enzyme ($n = 2$; −/+), cereal matrix ($n = 3$; fine rye, fine wheat, coarse wheat), and their interaction were included in the model as sources of variation, and pig and period were random effects. Means were separated using an LSD test, and an α level of 0.05 was used to assess significant differences among means. Because of the exclusion of coarse wheat in the analysis of xylooligosaccharides, the data were analyzed as an incomplete Latin square design with a 2 × 2 factorial arrangement of enzyme ($n = 2$; −/+) and cereal matrix ($n = 2$; fine rye, fine wheat).

Table 2. Analyzed composition of experimental diets

	Exp. 1[2]				Exp. 2[3]					
					Fine rye		Fine wheat		Coarse wheat	
Item[1]	NX	AN	BS	TR	−	+	−	+	−	+
GE, MJ/kg DM	16.54	16.42	16.33	16.86	17.68	17.72	18.01	18.10	18.17	18.07
Ash, % DM	5.2	4.3	4.8	5.2	5.1	5.0	4.4	4.1	3.7	3.6
CP, % DM	9.8	9.7	10.0	9.8	9.5	9.6	12.9	12.9	13.0	13.0
Crude fat, % DM	1.6	1.5	1.6	1.5	1.6	1.7	1.7	2.1	2.1	2.2
Digestible carbohydrates										
Starch, % DM	58.1	59.0	57.2	53.4	59.7	62.0	66.7	66.7	68.2	66.4
Sugars, % DM	3.1	3.2	3.1	3.1	3.3	3.4	2.0	2.0	2.0	2.1
Nondigestible carbohydrates										
Total NSP, % DM	12.5	13.0	12.8	13.7	14.7	15.6	10.2	11.6	10.9	11.0
Arabinoxylan, % DM	7.4	7.8	7.8	8.0	9.2	9.8	6.7	7.6	6.9	7.3
NCP glucose, % DM	2.7	2.7	3.0	2.8	2.8	2.8	1.2	1.3	1.2	1.2
Cellulose, % DM	1.2	1.3	0.9	1.7	1.3	1.7	1.5	1.9	1.9	1.7
S-NSP, % DM	2.8	3.8	3.8	4.8	4.2	4.9	2.1	3.5	1.1	2.3
Arabinoxylan, % DM	2.3	2.8	3.0	3.2	3.0	3.1	1.5	2.4	0.5	1.6
NCP glucose, % DM	0.2	0.6	0.4	1.1	0.8	1.3	0.4	0.7	0.3	0.4
Fructan, % DM	2.9	2.9	2.9	2.9	2.9	2.8	1.6	1.6	1.6	1.6
Klason lignin, % DM	1.4	1.6	1.2	1.1	2.0	2.1	1.4	1.5	1.4	2.1
T-DF, % DM	16.8	17.5	16.9	17.7	19.5	20.5	13.2	14.8	13.9	14.7
Arabinose:xylose ratio										
In total NSP	0.65	0.64	0.65	0.65	0.67	0.67	0.61	0.59	0.62	0.58
In soluble NSP	0.69	0.68	0.71	0.69	0.64	0.73	0.60	0.55	0.65	0.57
Particle size										
Geometric mean, µm	1,060	1,399	1,317	1,145	676	672	722	850	1,190	1,286
<1,000 µm, %	44	31	34	42	76	77	78	70	50	45
1,000–2,000 µm, %	24	31	34	42	24	22	21	29	39	43
Enzyme activity	—	1,553[4]	334[4]	4,285[5]	—	3,884[5]	—	4,238[5]	—	3,283[5]

[1]NSP, nonstarch polysaccharides; NCP, noncellulosic polysaccharides; S-NSP, soluble nonstarch polysaccharides; T-DF, total dietary fiber determined as the sum of NSP, Klason lignin, and fructan.

[2]Diets of coarse rye without enzyme (NX) or supplemented with *Aspergillus niger* (AN), *Bacillus subtilis* (BS), or *Trichoderma reesei* (TR) xylanase.

[3]Diets of rye and wheat without enzyme (−) or supplemented with a combination of *Bacillus subtilis* and *Trichoderma reesei* xylanase (+).

[4]Xylanase unit[s]/kg, as-fed basis.

[5]Danisco xylanase units/kg, as-fed basis.

RESULTS

Diet Composition and Enzyme Activity

The rye diets contained less protein, slightly more ash, and slightly less crude fat than the wheat diets (Table 2). Carbohydrates (starch + sugars + T-DF) accounted for, on average, 77% of DM in Exp. 1 and 84% in Exp. 2, and the total carbohydrate content was almost identical for the wheat and rye diets in Exp. 2. However, there was a difference in the carbohydrate composition; the starch content was greater but the NSP content was lower in the wheat diets than in the rye diets. This difference was caused by a more than twice as high content of NCP glucose (primarily β-glucan) in rye than in wheat, whereas their cellulose contents were almost identical. The arabinoxylan contents of the rye diets were in the range 7.4% to 8.0% DM in Exp. 1 and 9.2% to 9.8% DM in Exp. 2, whereas the arabinoxylan contents of the wheat diets were 6.7% to 7.3% DM. Furthermore, the rye diets contained almost twice as much fructan as the wheat diets, which also added to the fiber content of the diets. The arabinose:xylose ratio in the wheat diets was slightly lower than in the rye diets. The geometric mean particle size of diets based on coarse rye in Exp. 1 was, on average, 1,230 ± 155 µm with 37.8% ± 6.4% being less than 1,000 µm. The particle size of the fine rye diets in Exp. 2 had a geometric mean of 674 ± 7 µm with 76.2% ± 0.8% below 1,000 µm. Using the same screen size for milling the wheat, the geometric mean of the fine wheat diets was 786 ± 74 µm with 74.2% ± 4.5% less than 1,000 µm. The coarse wheat diet had an average geometric mean size of 1,238 ± 67 µm and had 47.3% ± 3.8% particles smaller than 1,000 µm and in this respect was almost similar to the coarse

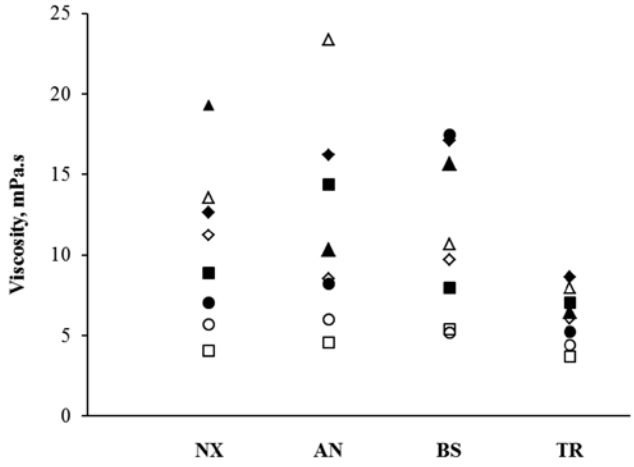

Figure 1. Individual intestinal viscosity values (mPa·s) of pigs fed coarse rye without (NX) or with *Aspergillus niger* (AN), *Bacillus subtilis* (BS), or *Trichoderma reesei* (TR) xylanase, Exp. 1. Each symbol represents 1 pig.

rye in Exp. 1. The xylanase activities measured for the diets were within an acceptable range (Table 2).

Effect of Xylanases on Ileal Viscosity

In Exp. 1, ileal extract viscosity of the pigs fed coarse rye was quite variable both without and with the addition of AN and BS, whereas TR tended to reduce the individual variation (Fig. 1). Pigs fed the control diet (NX) without enzyme had a geometric mean viscosity of 9.3 mPa·s (Fig. 2). Xylanase from *Aspergillus niger* and BS did not modify either the geometric mean or individual variability, whereas TR reduced viscosity to 6.0 mPa·s ($P < 0.001$), with a tendency for slightly less variation between pigs (Fig. 1 and 2). In Exp. 2, ileal viscosity in the pigs fed fine rye without enzyme was similar (9.7 mPa·s) to the level in Exp. 1, and addition of the xylanase cocktail reduced the viscosity by 21.6% ($P = 0.041$). The viscosity in the unsupplemented wheat diets was much lower than in the rye diets. Still, the xylanase cocktail reduced the intestinal viscosity by 28.6% and 46.9% in the fine and coarse wheat diets, respectively ($P < 0.001$). Hence, overall both the cereal matrix and enzyme supplementation had strong effects ($P < 0.001$) in Exp. 2.

Solutions of purified arabinoxylan from rye had, in concordance with suppliers' specifications, an initially lower viscosity at concentrations similar to those of wheat arabinoxylan. Addition of TR at increasing concentrations led to a slightly greater viscosity reduction for the wheat arabinoxylan at low concentrations of TR, whereas solution viscosities were similar for wheat and rye arabinoxylans at the higher xylanase levels (data not shown).

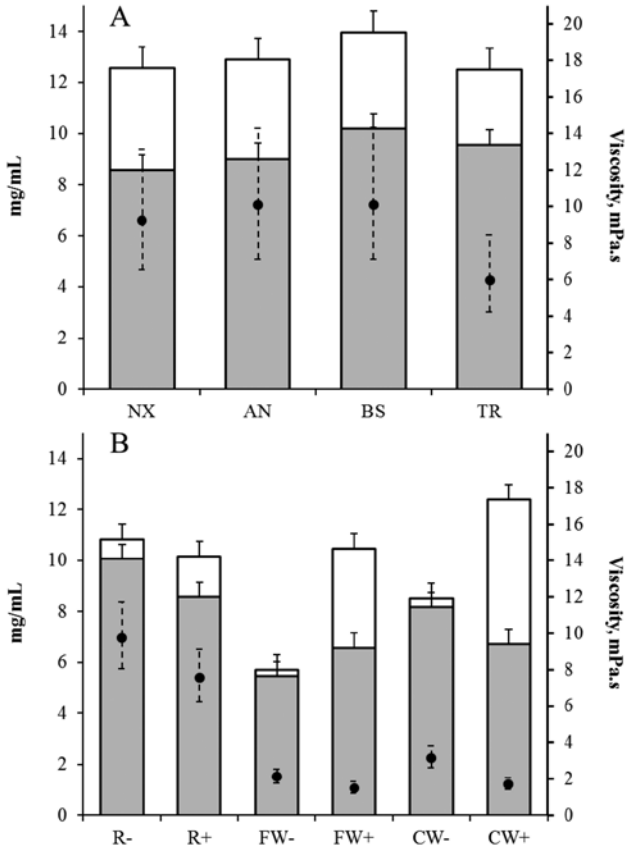

Figure 2. Concentration of high-molecular-weight arabinoxylan (gray bar) and arabinoxylan oligosaccharides (white bar) and viscosity of the liquid phase of digesta (dots) in (A) pigs fed coarse rye without (NX) or with *Aspergillus niger* (AN), *Bacillus subtilis* (BS), or *Trichoderma reesei* (TR) xylanase, Exp. 1 ($n = 8$ pigs per treatment), and (B) pigs fed fine rye (*R*), fine wheat (FW), or coarse wheat (CW) with (+) or without (−) a combination of *Bacillus subtilis* and *Trichoderma reesei* xylanase, Exp. 2 ($n = 6$ pigs per treatment). Concentrations (mg/mL) are presented as least squares means with their SEM. High-molecular-weight arabinoxylan was determined as arabinose plus xylose by precipitation in 80% ethanol in the nonstarch polysaccharides procedure. Arabinoxylan oligosaccharides were calculated as the difference in arabinose plus xylose determined with and without ethanol precipitation. Viscosity values are presented as geometric means and their 95% confidence intervals. (A) Viscosity: TR differs from NX, AN, and BS ($P < 0.001$). (B) Viscosity: main effect of cereal source (CS), $P < 0.001$, where fine rye > coarse wheat > fine wheat; main effect of enzyme (E), $P < 0.001$, where + > −; CS × E, $P = 0.097$. Total arabinoxylan: main effect of CS, $P = 0.021$; main effect of E, $P = 0.002$; CS × E, $P = 0.014$, where FW+ vs. FW- ($P = 0.002$), CW+ vs. CW- ($P = 0.006$), R- vs. FW- ($P < 0.001$), CW- vs. FW- ($P = 0.034$). High-molecular-weight arabinoxylan: Main effect of CS, $P < 0.0001$, where fine rye > coarse wheat > fine wheat; main effect of E, $P = 0.23$; CS × E, $P = 0.077$. Arabinoxylan oligosaccharides: main effect of CS, $P = 0.011$; main effect of E, $P < 0.001$; CS × E, $P = 0.002$, where FW+ vs. FW- ($P < 0.001$), CW+ vs. CW- ($P < 0.001$), R+ vs. FW+ ($P = 0.01$), CW+ vs. FW+ ($P = 0.041$).

Effect of Xylanases on Concentration of Solubilized AX in Ileal Content

Irrespective of cereal matrix, there was no effect of xylanase addition on the concentration of total arabinoxylan (13.0 mg/mL), HMW-AX (9.3 mg/mL), or AXOS (3.7 mg/mL) in the liquid phase of the ileal content of the pigs fed coarse rye in Exp. 1 (Fig. 2).

In Exp. 2, there was an effect of cereal matrix ($P < 0.001$) but no effect of enzyme addition or interaction on the concentration of HMW-AX in the liquid phase (Fig. 2). The concentration of AXOS in pigs fed diets without xylanase addition was low (<0.8 mg/mL), with no difference between cereal matrices ($P > 0.49$). The addition of the xylanase cocktail increased the concentration of AXOS in the liquid phase approximately16-fold in the pigs fed fine wheat ($P < 0.001$) and coarse wheat ($P < 0.001$) but had no significant effect on pigs fed fine rye, resulting in an overall effect of cereal matrix ($P = 0.011$), enzyme addition ($P < 0.001$), and interaction between cereal matrix and enzyme addition ($P = 0.002$). Consequently, the total concentration of solubilized arabinoxylan was similar in the unsupplemented and xylanase-supplemented fine rye, whereas enzyme supplementation increased the concentration of solubilized arabinoxylan by 82.4% in fine wheat ($P = 0.002$) and by 45.9% in coarse wheat ($P = 0.005$), leading to levels of solubilized arabinoxylan in the 2 wheat-based diets similar to those in the rye diets.

Effect of Enzymes on Content and Composition of NDC in Ileal Content

Ileal DM from pigs fed coarse rye in Exp. 1 contained, on average, 45.8% total NDC, with 23.5% being in the form of arabinoxylan with no effect of xylanase addition (Table 3). Although higher concentrations of HMW-AX was found with the AN treatment than with the control diet (NX; $P = 0.023$) and the other 2 enzyme treatments ($P < 0.013$), the corresponding lower concentration of AXOS was not significant ($P > 0.4$). Arabinoxylan oligosaccharides accounted, on average, for only 10.9% of the total arabinoxylan content. In Exp. 2, the average content of NDC in ileal content was 43.4% DM, and there was an overall increase in concentration with enzyme addition ($P = 0.011$) irrespective of cereal matrix (Table 4). A similar picture was seen for the concentration of total arabinoxylan ($P = 0.028$), with no effect of cereal matrix. The concentration of HMW-AX was not affected by either xylanase supplementation or cereal matrix, but enzyme addition increased the concentration of AXOS ($P < 0.001$), and there was an effect of cereal matrix ($P < 0.001$), with coarse wheat having a higher content than fine rye and fine wheat. Arabinoxylan oligosaccharides accounted for 13.7%, 8.4%, and 17.0% in fine rye, fine wheat, and coarse wheat, respectively, and increased by 46%, 176%, and 76% in the corresponding xylanase-supplemented diets.

Table 3. Concentration of constituents of nondigestible carbohydrates in ileal content of pigs fed coarse rye with or without different xylanases, Exp. 1[1]

	Dietary treatment[3]					
Item[2]	NX	AN	BS	TR	SEM	*P*-value
Total NDC,[4] % DM	44.8	47.3	46.7	44.4	0.92	0.100
Total arabinoxylan,[4] % DM	22.7	24.4	23.8	23.1	0.93	0.616
HMW-AX,[5] % DM	20.2[b]	23.5[a]	19.9[b]	19.3[b]	0.85	0.023
AXOS,[6] % DM	2.5	0.8	3.9	3.8	1.33	0.412

[a,b]Within a row, values with different superscripts differ ($P < 0.05$).

[1]Values are least squares means of 8 observations.

[2]NDC, nondigestible carbohydrates; HMW-AX, high-molecular-weight arabinoxylan; AXOS, arabinoxylan oligosaccharides.

[3]Diets of coarse rye without enzyme (NX) or supplemented with *Aspergillus niger* (AN), *Bacillus subtilis* (BS), or *Trichoderma reesei* (TR) xylanase.

[4]Determined by direct hydrolysis without ethanol precipitation in the nonstarch polysaccharides (NSP) procedure.

[5]Determined by precipitation in 80% ethanol in the NSP procedure.

[6]Calculated as the difference between total arabinoxylan determined by direct acid hydrolysis without ethanol precipitation and HMW-AX.

Effect of Enzymes on Release of Xylooligosaccharides

In the liquid phase of digesta, enzyme supplementation increased the concentration of all xylooligosaccharides except xylohexaose (Table 5), and for xylobiose and xylotetraose it was significantly higher in rye than in wheat. Enzyme addition led to an almost 4-fold increase ($P = 0.001$) in the concentration of xylose in the liquid phase of ileal effluent of pigs fed fine wheat (0.295 vs. 0.075 mg/mL, $P = 0.002$) but had no effect when the pigs were fed fine rye (0.319 mg/mL), resulting in an interaction between cereal matrix and enzyme ($P = 0.027$; Table 5). As a result, the total concentrations of xylooligosaccharides were more than twice as high in the xylanase-supplemented diets, with a tendency for a greater effect in wheat than in rye (interaction $P = 0.056$).

Effect of Xylanases on Ileal Digestibility of NSP and Components Thereof

The AID of total NSP, arabinoxylan, and NSP glucose was not affected by any of the xylanase preparations in Exp. 1 (Table 6). Across diets, AID of total NSP was 9.2%, whereas arabinoxylan had an average AID of 7.4%. NSP glucose, which includes cellulose, β-glucan, and resistant starch, had an average AID of 22.7%. In Exp. 2, AID of NSP increased by the xylanase combination ($P = 0.004$), whereas it was not affected by cereal matrix (Table 7). Overall, the AID of arabinoxylan increased from 16.1% to 24.0% by en-

Table 4. Concentration of constituents of nondigestible carbohydrates in ileal content of pigs fed fine rye, fine wheat, or coarse wheat with (+) or without (−) a combination of from *Bacillus subtilis* and *Trichoderma reesei* xylanase, Exp. 2[1]

	CM				E			*P*-value		
Item[2]	Fine rye	Fine wheat	Coarse wheat	SEM	−	+	SEM	CM	E	CM × E
Total NDC,[3] % DM	43.7	42.1	44.4	0.9	42.0[b]	44.9[a]	0.7	0.193	0.011	0.376
Total arabinoxylan,[3] % DM	26.6	25.5	28.1	0.9	25.7[b]	27.8[a]	0.8	0.085	0.028	0.108
HMW-AX,[4] % DM	22.0	21.3	21.4	0.8	22.2	21.0	0.7	0.731	0.148	0.280
AXOS,[5] % DM	4.5[b]	4.2[b]	6.7[a]	0.5	3.5[b]	6.8[a]	0.4	<0.001	<0.001	0.054

[a,b]Within a row and main effect, values with different superscripts differ ($P < 0.05$).

[1]Values are least squares means of 6 observations. CM = cereal matrix; E = enzyme.

[2]NDC, nondigestible carbohydrates; HMW-AX, high-molecular-weight arabinoxylan; AXOS, arabinoxylan oligosaccharides.

[3]Determined by direct hydrolysis without ethanol precipitation in the nonstarch polysaccharides (NSP) procedure.

[4]Determined by precipitation in 80% ethanol in the NSP procedure.

[5]Calculated as the difference between total arabinoxylan determined by direct acid hydrolysis without ethanol precipitation and HMW-AX.

zyme supplementation ($P < 0.001$) and was lower in rye than coarse wheat ($P = 0.031$). On the other hand, rye had the highest AID of NSP glucose ($P = 0.022$). There was no consistent effect of enzyme supplementation on AID of NSP glucose ($P = 0.642$), but there was close to a significant interaction between enzyme and cereal matrix ($P = 0.051$).

Effect of Enzymes on Apparent Ileal Digestibility of Macronutrients

The AID of DM, OM, and the macronutrients (starch, protein, or crude fat) in coarse rye was not affected by any of the xylanases in Exp. 1 (Table 6). In Exp. 2, there was a consistent effect of cereal matrix on DM, OM, and macronutrient digestibility ($P < 0.006$), with rye having a lower AID than wheat (Table 7). Enzyme addition caused an overall increase in the digestibility of starch ($P = 0.031$). For crude fat, there was an interaction ($P = 0.006$) between cereal matrix and enzyme addition, with an increase in digestibility of the fine wheat from 44.2% to 60.1% ($P < 0.001$) by enzyme addition but with no effect on the fine rye and the coarse wheat. For DM, OM, and nitrogen there was no effect of enzyme supplementation ($P > 0.4$).

DISCUSSION

In the current study, enzyme supplementation with monocomponent xylanases alone or in combination had little effect on the degradation of arabinoxylan from rye compared with the effect seen with wheat. In spite of an initially much lower intestinal viscosity of the pigs fed wheat than those fed rye, combined xylanase supplementation led to a relatively greater reduction in viscosity of wheat than the more viscous rye diet. This limitation in reducing intestinal viscosity in the pigs fed rye was not seen when TR was added

Table 5. Concentration of xylooligosaccharides in the liquid phase of ileal content of pigs fed fine rye or fine wheat with (+) or without (−) a combination of *Bacillus subtilis* and *Trichoderma reesei* xylanase, Exp. 2[1]

	CM			E			*P*-value		
Item	Fine rye	Fine wheat	SEM	−	+	SEM	CM	E	CM × E
Xylose, mg/mL	0.142	0.185	0.029	0.098[b]	0.229[a]	0.029	0.243	0.004	0.027[2]
Xylobiose, mg/mL	0.050[a]	0.027[b]	0.004	0.020[b]	0.056[a]	0.004	0.011	0.001	0.820
Xylotriose, mg/mL	0.038	0.030	0.003	0.018[b]	0.050[a]	0.003	0.089	<0.001	0.883
Xylotetraose, mg/mL	0.058[a]	0.036[b]	0.006	0.041[b]	0.053[a]	0.006	0.002	0.044	0.326
Xylopentaose, mg/mL	0.023	0.018	0.002	0.014[b]	0.027[a]	0.002	0.214	0.004	0.816
Xylohexaose, mg/mL	0.009	0.008	0.002	0.006	0.011	0.002	0.897	0.127	0.407
Total, mg/mL	0.319	0.304	0.035	0.198[b]	0.425[a]	0.035	0.752	<0.001	0.056

[a,b]Within a row and main effect, values with different superscripts differ ($P < 0.05$).

[1]Values are lease squares means of 6 observations. CM = cereal matrix; E = enzyme.

[2]Untreated fine rye (0.122 mg/mL) vs. enzyme-supplemented fine rye (0.163 mg/mL), $P = 0.405$; untreated fine wheat (0.075 mg/mL) vs. enzyme-supplemented fine wheat (0.295 mg/mL), $P = 0.0015$.

Table 6. Apparent ileal digestibility of dietary constituents in pigs fed coarse rye with or without different xylanases, Exp. 1[1]

	Dietary treatment[2]					
Item	NX	AN	BS	TR	SEM	*P*-value
DM, %	65.9	66.0	64.5	65.2	1.28	0.864
OM, %	69.2	69.2	67.8	68.3	1.19	0.835
Starch, %	96.8	96.5	96.5	96.6	0.54	0.870
Nitrogen, %	45.7	47.9	43.1	45.3	2.32	0.561
Crude fat, %	23.9	26.8	22.7	20.3	3.82	0.629
NSP,[3] %	9.6	3.1	9.9	14.2	4.00	0.335
Arabinoxylan,[4] %	6.9	−2.5	9.3	15.6	5.13	0.175
NSP glucose,[5] %	23.7	24.0	20.8	22.4	5.24	0.975

[1]Values are least squares means of 8 observations.

[2]Diets of coarse rye without enzyme (NX) or supplemented with *Aspergillus niger* (AN), *Bacillus subtilis* (BS), or *Trichoderma reesei* (TR) xylanase.

[3]NSP, nonstarch polysaccharides.

[4]Determined by precipitation in 80% ethanol in the NSP procedure.

[5]NSP glucose, the part of NSP determined as glucose.

to purified sources of wheat and rye arabinoxylans, which suggests that the purified arabinoxylan had been structurally modified by extraction or that other factors such as structural integrity or interaction with other constituents of the cell wall matrix had limited the potency of the xylanases in the rye diet. Although the individually supplemented xylanases in Exp. 1 had no effect on AID of arabinoxylan in the coarse rye, there was a consistent increase in both rye and wheat when *Bacillus subtilis* and *Trichoderma reesei* xylanases were combined in Exp. 2, where the rye was finely milled. In concordance, the concentration of AXOS in digesta DM also increased in Exp. 2 but not in Exp. 1. However, the concentration of AXOS in the liquid phase of digesta increased only for wheat and not for rye when it was supplemented with endoxylanases either alone or in combination. On the other hand, on the basis of the release of xylooligosaccharides into the liquid phase of digesta, the combination of xylanases appears to have attacked the arabinoxylan in rye, but for xylose, the main degradation product, and with a trend for a similar response for the total amount of xylooligosaccharides, the efficiency was significantly lower for rye than for wheat. Arabinoxylans in wheat and rye consist of a backbone of (1→4)-β-D-linked xylopyranosyl residues mainly substituted with α-L-arabinofuranosyl residues to varying degrees at the O-2 position, the O-3 position, or both and may be further ester linked with ferulic acids to form dimeric crosslinks of the arabinoxylan chains (Vinkx and Delcour, 1996). Accordingly, complete degradation of the arabinoxylan structure to monosaccharides requires an array of different hydrolases, as the presence of 4-O-methyl-glucuronic acid and arabinofuranose side chains hinders the binding and hydrolysis of xylan (Shallom and Shoham, 2003). It must be noted that complete degradation to the constituent monosaccharides arabinose and xylose for absorption in the small intestine is not the target for enzyme addition, as these sugars are not metabolized very efficiently and will, to a large extent, be excreted in the urine (Yule and Fuller, 1992). Structurally, arabinoxylans in wheat and rye are almost alike (Vinkx and Delcour, 1996), although minor variations in the arabinose:xylose ratio were found in the current study with ratios of 0.59 to 0.62 in the wheat diets and 0.64 to 0.67 in the rye diets, but this difference can hardly explain the very different viscosity and impairment of xylanase efficiency in rye compared with wheat. Much larger differences in structure and digestibility between the different bo-

Table 7. Apparent ileal digestibility of dietary constituents in pigs fed fine rye, fine wheat, or coarse wheat with (+) or without (−) a combination of *Bacillus subtilis* and *Trichoderma reesei* xylanase, Exp. 2[1]

	CM				E			*P*-value		
Item	Fine rye	Fine wheat	Coarse wheat	SEM	−	+	SEM	CM	E	CM ×E
DM, %	63.4[b]	73.2[a]	75.0[a]	1.2	70.6	70.5	1.0	<0.001	0.991	0.739
OM, %	66.6[b]	76.0[a]	77.7[a]	1.2	73.5	73.4	1.0	<0.001	0.900	0.711
Starch, %	93.6[b]	95.3[a]	96.3[a]	0.8	94.3[b]	95.8[a]	0.7	0.006	0.031	0.238
Nitrogen, %	51.0[b]	73.0[a]	71.6[a]	1.6	65.7	64.7	1.5	<0.001	0.427	0.326
Crude fat, %	34.5[c]	52.2[b]	60.0[a]	2.8	46.8	50.9	2.6	<0.001	0.096	0.006
NSP,[2] %	18.9	18.9	22.4	2.2	16.1[b]	24.0[a]	1.8	0.408	0.004	0.385
Arabinoxylan,[3] %	16.0[b]	21.6[a,b]	24.5[a]	2.1	14.6[b]	26.7[a]	1.8	0.031	<0.001	0.646
NSP glucose,[4] %	32.2[a]	22.9[b]	27.6[a,b]	2.5	27.0	28.2	2.1	0.022	0.642	0.051

[a–c]Within a row and main effect, values with different superscripts differ ($P < 0.05$).

[1]Values are least squares means of 6 observations. CM = cereal matrix; E = enzyme.

[2]NSP, nonstarch polysaccharides.

[3]Determined by precipitation in 80% ethanol in the NSP procedure.

[4]NSP glucose, the part of NSP determined as glucose.

tanical parts of the grain have previously been demonstrated for rye alone (Glitsø et al., 1998). Hence, on the basis of the structure of the arabinoxylan, the low impact on viscosity reduction in rye and differences in degradability between rye and wheat were not expected. In Exp. 1, the large particle size of the rye may have limited the accessibility and thereby the ability of the xylanases to degrade the arabinoxylan-rich fiber matrix. On the other hand, particle size appeared not to be the main reason, as the effect of xylanases on release of AXOS and xylose in the liquid phase was also lower in the fine rye than in the fine wheat in Exp. 2, with similar trends for AXOS in DM and total released xylooligosaccharides. Furthermore, a large particle size did not compromise the effect of the xylanase combination in wheat.

Cereals contain xylanase inhibitors, and the content of these varies greatly with cereal species, genotype, and environmental factors (Gebruers et al., 2010). de Vries et al. (2012) stated that the effect of enzyme addition on digestibility of the fiber fraction is 1.5 to 6 times larger when applied to heat-processed diets compared with unprocessed diets and explained this by modifications of the cell wall architecture that increase the accessibility of NSP to the enzymes. However, the xylanase inhibitors present in the grain are protease and heat sensitive (McLauchlan et al., 1999), and inactivation of the xylanase inhibitors by heating could be another explanation for the improved efficacy. In the current study, we used unheated cereal matrices, and it can be speculated that the rye had a greater content of these xylanase inhibitors than the wheat, and if so, larger effects on rye could have been obtained if the enzymes were applied to heat-treated cereal matrices.

To our knowledge, the effect of xylanases on the in vivo solubility and degradation of arabinoxylan in rye has not previously been described. Supplementation of ZY 28 providing mainly xylanase and 1,4-β-glucanase to a mixed diet consisting of barley, rye, wheat bran, and soybean meal increased the proportion of soluble AX and total NSP but did not increase the solubility of the already highly soluble β-glucan in growing pigs (Haberer et al., 1997). This was explained by an equal breakdown of insoluble and soluble β-glucan, whereas the xylanase in particular was capable of depolymerizing insoluble arabinoxylan. These results are in line with our observations in Exp. 2 of an increase in AXOS in ileal DM and in the liquid phase of digesta of the wheat diets but could not be confirmed with the coarse rye in Exp. 1, which again points toward a less efficient degradation of the insoluble arabinoxylans in rye than in wheat.

In the current study, there was no effect of xylanase on AID of nutrients in growing pigs fed either rye or wheat except for an observed overall increase in AID of starch and an increase in AID of crude fat in the pigs fed fine wheat. The results correspond well with a marginal effect of the xylanases on the rye arabinoxylans and digesta viscosity and a deviance between wheat and rye in their fiber contents, viscosities, and nutrient digestibilities. In wheat, the viscosity was very low and apparently did not interfere with ileal nutrient digestibility in the growing pig. A very limited number of studies on the effect of xylanases on pure rye diets have previously been reported in the literature. Adding xylanase to a rye-wheat-based diet was found to reduce the viscosity in the ileal digesta and increase prececal digestibilities of DM, soluble and insoluble NSP, and most essential amino acids in growing pigs (Bartelt et al., 2002). However, it was concluded that it was not the digesta viscosity per se but other properties of complex dietary fiber that affected the nutrient absorption and endogenous N flow. Increasing levels of xylanase from *Trichoderma longibrachiatum* increased the AID of major dietary constituents and amino acids along with NSP digestibility in young pigs receiving a diet based on 96% rye (Nitrayová et al., 2009). In a study by Willamil et al. (2012), adding 22,000 U xylanase and 2,000 U endoglucanase in a multienzyme complex from *Penicillium funiculosum* showed marginal, if any, effect of enzyme addition on digestibility but slight increases in ADG and ADFI on mixed cereal diets (wheat, barley, rye, 1:1:1) and even less effect on a corn-based diet. In contrast to what is seen in the current study and most previous reports, enzyme supplementation increased digesta viscosity slightly but not significantly in both the corn and the mixed cereal diets in the study of Willamil et al. (2012).

The addition of xylanase from *Trichoderma longibrachiatum* was previously shown to increase G:F for large particles (1,300 μm) but not for small particles of wheat in nursery pigs and had no effect on DM and N fecal digestibility at any particle size (Mavromichalis et al., 2000). In finishing pigs, the enzyme addition tended to increase fecal digestibility of the coarse diet but did not influence ADG or G:F (Mavromichalis et al., 2000). In the current study, we found no overall difference in AID of macronutrients between fine and coarse wheat and no difference in the effect of enzyme addition except for a larger effect on AID of crude fat for fine compared with the coarse wheat that was mainly due to an unexplained lower digestibility in the unsupplemented fine wheat. Collectively, the results from the current and previous studies indicate that matching the enzymes to the substrate seems very important and further that processing of the diets and circumstances under which they are used may influence the outcome.

Compared with barley, rye has a higher protein and energy digestibility and similar N retention (Thacker et

al., 2002) but may reduce feed intake and daily gain and prolong days until obtained slaughter weight (Friend and Macintyr, 1969; Thacker et al., 1991, 2002). By studying normal and low-viscosity rye, the addition of carboxymethyl cellulose (viscosity elevating), and xylanase (viscosity reducing), it is indicated that viscosity per se may not be a limiting factor in the use of rye for pigs (Thacker et al., 1991, 2002; Bartelt et al., 2002). This may in part explain why the addition of fiber-degrading enzymes to pig feed has given inconsistent results. It is therefore highly relevant to get a better understanding of the complexity of the rye cereal grain to increase the use of rye as a feed source for pigs.

In conclusion, combination of 2 xylanases modified the structure of arabinoxylan in the small intestine of pigs fed wheat or rye, but the ability of the enzymes to reduce viscosity, solubilize arabinoxylan, and release arabinoxylan degradation products was lower in rye than in wheat. Hence, the lower digestibility of macronutrients and arabinoxylan in rye than in wheat was not efficiently ameliorated by enzyme supplementation. To exploit the full potential of rye as a feed ingredient, factors that impair the nutritive value of modern varieties must be investigated further, and development of xylanases that are more targeted toward degradation of the fiber matrix in rye may be required.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903–916.

American Society of Agricultural and Biological Engineers. 2012. Method of determining and expressing fineness of feed materials by sieving (2008;R2012). A. S. Engineers. Am. Soc. Agric. Biol. Eng., St. Joseph, MI.

Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. Aust. J. Agric. Res. 44:405–422. doi:10.1071/AR9930405

AOAC. 2007. W. Howitz and G. W. Latimer Jr., editors, Official methods of analysis. 18th ed., rev. 2. AOAC Int., Gaithersburg, MD.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338. doi:10.1016/S0377-8401(97)00009-6

Bach Knudsen, K. E., and H. N. Lærke. 2010. Rye arabinoxylans: Molecular structure, physicochemical properties and physiological effects in the gastrointestinal tract. Cereal Chem. 87:353–362. doi:10.1094/CCHEM-87-4-0353

Bailey, M. J., P. Biely, and K. Potanen. 1992. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol. 23:257–270. doi:10.1016/0168-1656(92)90074-J

Bartelt, J., A. Jadamus, F. Wiese, E. Swiech, L. Buraczewska, and O. Simon. 2002. Apparent precaecal digestibility of nutrients and level of endogenous nitrogen in digesta of the small intestine of growing pigs as affected by various digesta viscosities. Arch. Anim. Nutr. 56:93–107. doi:10.1080/00039420210000000658

Brufau, J., M. Francesch, and A. M. Perez-Vendrell. 2006. The use of enzymes to improve cereal diets for animal feeding. J. Sci. Food Agric. 86:1705–1713. doi:10.1002/jsfa.2557

Bushuk, W. 2001. Rye production and uses worldwide. Cereal Food World 46:70–73.

Danicke, S., G. Dusel, H. Jeroch, and H. Kluge. 1999. Factors affecting efficiency of NSP-degrading enzymes in rations for pigs and poultry. Agribiol. Res. 52:1–24.

de Vries, S., A. M. Pustjens, H. A. Schols, W. H. Hendriks, and W. J. J. Gerrits. 2012. Improving digestive utilization of fiber-rich feedstuffs in pigs and poultry by processing and enzyme technologies: A review. Anim. Feed Sci. Technol. 178:123–138. doi:10.1016/j.anifeedsci.2012.10.004

Friend, D. W., and T. M. Macintyr. 1969. Digestibility of rye and its value in pelleted rations for pigs. Can. J. Anim. Sci. 49:375–381. doi:10.4141/cjas69-049

Gebruers, K., E. Dornez, Z. Bedő, M. Rakszegi, C. M. Courtin, and J. A. Delcour. 2010. Variability in xylanase and xylanase inhibition activities in different cereals in the HEALTHGRAIN diversity screen and contribution of environment and genotype to this variability in common wheat. J. Agric. Food Chem. 58:9362–9371. doi:10.1021/jf100474m

Glitsø, L. V., G. Brunsgaard, S. Højsgaard, B. Sandstrøm, and K. E. Bach Knudsen. 1998. Intestinal degradation in pigs of rye dietary fibre with different structural characteristics. Br. J. Nutr. 80:457–468.

Haberer, B., E. Schulz, K. Aulrich, and G. Flachowsky. 1997. Effects of beta-glucanase and xylanase supplementation in pigs fed a diet rich in nonstarch polysaccharides: Composition of digesta in different prececal segments and postprandial time. J. Anim. Physiol. Anim. Nutr. 78:84–94. doi:10.1111/j.1439-0396.1997.tb00859.x

Hansen, B. 1989. Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agric. Scand. 39:113–118. doi:10.1080/00015128909438504

Heinio, R. L., E. Nordlund, K. Poutanen, and J. Buchert. 2012. Use of enzymes to elucidate the factors contributing to bitterness in rye flavour. Food Res. Int. 45:31–38. doi:10.1016/j.foodres.2011.10.006

Jørgensen, H., K. Jakobsen, and B. O. Eggum. 1992. The influence of different protein, fat and mineral levels on the digestibility of fat and fatty acids measured at the terminal ileum and in faeces of growing pigs. Acta Agric. Scand. A 42:177–184.

Jürgens, H. U., G. Jansen, and C. B. Wegener. 2012. Characterisation of several rye cultivars with respect to arabinoxylans and extract viscosity. J. Agric. Sci. 4(5):1–12. doi:10.5539/jas.v4n5p1

Larsson, K., and S. Bengtsson. 1983. Bestämning av lätttilgängeliga kolhydrater i växtmaterial. Metodrapport No. 22. Statens Lantbrukskemiska Laboratorium, Uppsala, Sweden.

Mavromichalis, I., J. D. Hancock, B. W. Senne, T. L. Gugle, G. A. Kennedy, R. H. Hines, and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. J. Anim. Sci. 78:3086–3095.

McLauchlan, W. R., M. T. Garcia-Conesa, G. Williamson, M. Roza, P. Ravestein, and J. Maat. 1999. A novel class of protein from wheat which inhibits xylanases. Biochem. J. 338:441–446. doi:10.1042/0264-6021:3380441

Nitrayová, S., J. Heger, P. Patrás, H. Kluge, and J. Broz. 2009. Effect of xylanase on apparent ileal and total tract digestibility of nutrients and energy of rye in young pigs. Arch. Anim. Nutr. 63:281–291. doi:10.1080/17450390903020455

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Schurch, A. F., L. E. Lloyd, and E. W. Crampton. 1950. The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41:629–636.

Shallom, D., and Y. Shoham. 2003. Microbial hemicellulases. Curr. Opin. Microbiol. 6:219–228. doi:10.1016/S1369-5274(03)00056-0

Stoldt, W. 1952. Vorschlag zur vereinheitlichung der fettbestimmung in lebensmitteln. Fette Seifen 54:206–207.

Sørensen, J. F., and O. Sibbesen. 2006. Mapping of residues involved in the interaction between the *Bacillus subtilis* xylanase A and proteinaceous wheat xylanase inhibitors. Protein Eng. Des. Sel. 19:205–210. doi:10.1093/protein/gzl002

Thacker, P. A., G. L. Campbell, and J. GrootWassink. 1991. The effect of enzyme supplementation on the nutritive value of rye-based diets for swine. Can. J. Anim. Sci. 71:489–496. doi:10.4141/cjas91-058

Thacker, P. A., J. G. Mcleod, and G. L. Campbell. 2002. Performance of growing-finishing pigs fed diets based on normal or low viscosity rye fed with and without enzyme supplementation. Arch. Anim. Nutr. 56:361–370. 10.1080/00039420210000042797

Vinkx, C. J. A., and J. A. Delcour. 1996. Rye (*Secale cereale* L) arabinoxylans: A critical review. J. Cereal Sci. 24:1–14. doi:10.1006/jcrs.1996.0032

Willamil, J., I. Badiola, E. Devillard, P. A. Geraert, and D. Torrallardona. 2012. Wheat-barley-rye- or corn-fed growing pigs respond differently to dietary supplementation with a carbohydrase complex. J. Anim. Sci. 90:824–832. doi:10.2527/jas.2010-3766

Yule, M. A., and M. F. Fuller. 1992. The utilization of orally administered D-xylose, L-arabinose and D-galacturonic acid in the pig. Int. J. Food Sci. Nutr. 43:31–40. doi:10.3109/09637489209027530