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Effects of Isolated and Complex Dietary Fiber Matrices in Breads on Carbohydrate Digestibility and Physicochemical Properties of Ileal Effluent from Pigs

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ABSTRACT: To assess the effects of content and structure of dietary fiber (DF) on the carbohydrate digestibility and physicochemical properties of ileal digesta, five bread diets were studied in an experiment with ileum-cannulated pigs in a crossover design. The diets consisted of two experimental breads based on white wheat flour with added wheat arabinoxylan (AX) or with added isolated oat β-glucan (BG), which were compared with a low-DF commercial white wheat bread (WF) and two commercial high-DF, dark ground rye bread (GR) and rye bread with kernels (RK) as positive controls. There was no profound effect of either DF content, structure, viscosity, or water-binding capacity on the ileal digestibility of starch, which was almost completely digested in the small intestine. Arabinoxylan and β-glucan were 11 and 81% degraded in the ileum, respectively, which resulted in a significant increase and decrease of ileal extract viscosities, respectively. It is concluded that the viscosity-elevating properties of soluble DF in breads and ileal digesta are strongly dependent on the content and structure of DF and degree of resistance toward microbial enzymes.

KEYWORDS: *arabinoxylan, β-glucan, ileal digestibility, physicochemical properties, starch*

INTRODUCTION

Epidemiological studies have identified a decreased risk of obesity, type 2 diabetes, cardiovascular diseases, and some forms of cancer with dietary fiber (DF)-rich diets.[1−3] Results of a recent study indicated a reduced risk of death from cardiovascular infarction and respiratory diseases after the consumption of cereal DF as compared with fruit and vegetable DF.[4] The ability of the DF matrix polysaccharides to swell and hold water and to create viscosity has a profound influence on the physicochemical properties of digesta, which may be linked to satiety,[5,6] cholesterol levels,[7] and glycemic index.[8] Soluble and insoluble DF components have different effects on transit time, nutrient digestion, and stool output.[9,10]

The main DF polysaccharides in cereals are arabinoxylan, mixed linked (1−3)(1−4)-β-D-glucan (β-glucan), and cellulose. β-Glucan and arabinoxylan are the main constituents of the cell wall of the endosperm and aleurone layers in grains.[2] β-Glucan is a linear homopolysaccharide of D-glucopyranose arranged as blocks of consecutive (1−4)-linked β-D-glucoses (for example, oligomeric cellulose segments) separated by single (1−3)-linkages.[11] Arabinoxylan is the polymerized backbone of β-(1−4)-linked xylose coupled with α-L-arabinofuranosyl residues with additional 4-*O*-methyl-D-glucuronopyranosyl, acetyl, or feruloyl side groups attached depending on the source.[12,13] The structural features of β-glucan and arabinoxylan are important determinants of their physicochemical properties and functionality, including their physiological responses when they are considered as ingredients in food.[11] In the case of β-glucan, its structural features include a ratio of β-(1−4)/β-(1−3) linkages, a ratio of cellotriosyl/cellotetraosyl units, and the presence of long cellulose-like fragments.[14] The functional properties of arabinoxylan are highly influenced by the extent of substitution and distribution of substituents along the xylan backbone, which results in different ratios of arabinose to xylose (A:X).[15] The properties of the DF compounds in the liquid and solid phase are furthermore affected by their molecular weight, concentration, and noncovalent interactions (e.g., hydrogen linkages) and covalent bonds (e.g., dehydrodiferulic acid bridges) with other cell components and nutrients.[16] Dry or wet separation technologies enable the production of fractions for use as ingrediets in the production of foods with specific nutritional and functional properties. An example is PromOat, a white powder of soluble oat β-glucan produced from oat bran, which retains all of the natural characteristics of the β-glucan within the whole grain.[17] Another DF-rich concentrate is soluble wheat arabinoxylan fraction isolated from the soluble fraction after starch and gluten extraction[18] and with beneficial effects on the carbohydrate metabolism similar to those of β-glucan.[2,19]

The presence of DF with different structural features and composition in the diet may have variable influence on the digestibility of nutrients.[20] Depending on the interaction of the DF with other nutrients, endogenous enzymes, acids, and microbes in the gut,[21] the structural features of the DF may also change during the time from ingestion to recovery in the ileal effluent.

The main objective of the present investigation was to study the effect of breads varying in content and composition of arabinoxylan and β-glucan on the physicochemical properties of ileal digesta and on the digestibility of carbohydrates in the small intestine of pigs used as an in vivo model for human nutrition. Five bread types were studied as follows: a commercial low DF white wheat bread (WF) (negative

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control) and four iso-DF breads, two experimental breads with DF from isolated arabinoxylan and β-glucan, and two commercial whole grain rye breads without and with grain kernels (positive control), respectively.

MATERIALS AND METHODS

Ingredients for the Experimental Breads. Soluble arabinoxylan was isolated from the soluble fraction after extraction of starch and gluten, concentrated by evaporation, heat treated, further treated with α-amylase and glucoamylase, precipitated with ethanol (1:3 v/v), filtrated, and finally dried on a spray dryer (Manildra Group, Nowra, Australia). Soluble β-glucan was obtained from the subaleurone by combining wet-milling and enzymatic hydrolysis of oat (PromOat, Biovelop AB, Kimstad, Sweden). The wheat arabinoxylan fraction consisted of 46.4% nondigestible carbohydrate (NDC), 31.2% total nonstarch polysaccharides (NSP), and 23.4% arabinoxylan with an A:X ratio of 0.94. The PromOat contained 46.8% of NDC, 40.5% total NSP, and 35.2% β-glucan. The content of β-glucan in PromOat was calculated based on the NSP procedure (refer to chemical analyses section). Vitacel (WF600, J. Rettenmaier & Söhne GmbH, Rosenberg, Germany) produced from wheat straw consisted of 72.7% cellulose and 16.9% of arabinoxylan with an A:X ratio of 0.09.

Food Preparation. Five experimental bread-based diets were used in the study. WF, dark ground rye bread (GR), and rye bread with kernels (RK) breads were commercial breads provided by Schulstad Lantmannen A/S (Hammerholmen, Hvidovre, Denmark) under the commercial names of Hvede Toast, Mørkt Rugbrød, and Multi-kernerugbrød, respectively. The ingredients as labeled on the products were in decreasing order for WF: wheat flour, water, yeast, sugar, salt, vinegar, canola oil, emulsifier (E471, E472e), rye flour, barley malt flour, and flour treatment agent (ascorbic acid); for GR: rye whole meal, water, rye sourdough, rye bread crumbs, salt, vinegar, dried sourdough rye, canola oil, yeast, and barley flour; and for RK: rye kernels, water, rye sourdough, rye bread crumbs, wheat meal, salt, yeast, vinegar, dried sourdough rye, canola oil, rye, and barley flour. Wheat bread with added isolated oat β-glucans (BG) and wheat bread with added isolated wheat arabinoxylans (AX) were experimental breads baked at a local bakery (Konditor-Bageren, Ørum, Denmark) using the following recipes of dry ingredients before water addition. For AX, white wheat flour (71.6%), wheat arabinoxylan concentrate (Manildra Group Ltd., Nowra, Australia, 25.7%), baker's yeast (3.7%), margarine (1.9%), salt (1.8%), and sugar (1.0%). For the BG bread, the recipe was white wheat flour (75.0%), oat β-glucan concentrate (PromOat, BioVelop AB, Kimstad, Sweden, 14.0%), Vitacel WF 600 (J. Rettenmaier & Söhne GmbH, Rosenberg, Germany, 7.3%), bakers's yeast (3.7%), margarine (1.9%), salt (1.8%), wheat gluten (1.0%), and sugar (1.0%). Because high levels of PromOat in the bread would cause the dough to become very sticky, Vitacel, a cellulose product containing 16.9% of arabinoxylan, was used to balance the DF content in the BG bread relative to the other high DF breads.

After production, the experimental breads were cut into pieces, frozen at −20 °C, and subsequently minced in semifrozen state. All minced breads except AX were supplemented with whey protein concentrate (Lacprodan 87; Arla Foods Ingredients Amba, Viby J, Denmark) to adjust the protein content of the diets. In addition, two markers were added for estimatation of digestibility (chromic oxide and Celite), and a mixture of vitamins and minerals were added to diets (Table 1). The feed was portioned into plastic bags weighing on average 575 g of dry matter (DM) per meal and stored at −20 °C until consumption.

Water Extracts and in Vitro Digestion of Diets. Water and in vitro enzyme incubations were applied to the five experimental diets to estimate viscosity and water-binding capacity (WBC). Each of the diets was minced in a hand blender (Braun, Kronberg, Germany) for 1 min and weighed in triplicate into a tube to reach a DM content of 13% in the solution. This was done to mirror the approximate final DM content of digesta. For enzyme incubation, the diets were initially incubated with an 11.68 mL solution of pepsin (12 mg, 2000 FIP-U/g, EC 3.4.23.1, Merck, Darmstadt, Germany) in 0.1 M HCl. The mixture was incubated in a shaking water bath (150 strokes/min) for 2 h at 39 °C to simulate the pig's body temperature. Following, the pH was adjusted to 7.0 by addition of a 6.54 mL solution of porcine pancreatin (50 mg, 8 x USP, EC 232-468-9, Sigma-Aldrich, St. Louis, MO) in 1 M $NaHCO_3$ and incubated for further 2 h at 39 °C. Parallel to the enzymatic incubation, diets were incubated with an aqueous solution of 0.02% NaN_3 (Sigma-AldrichA) in the shaking water bath (150 strokes/min) for 4 h at 39 °C. After the incubation, all tubes were cooled in an ice−water box for immediate viscosity and WBC determinations as described below.

Table 1. Ingredients of Experimental Diets[a]

	diet				
	WF	GR	RK	AX	BG
bread	95.2	93.4	92.9	97.4	92.7
Lacprodan 87	2.1	4.4	4.8	0.0	4.8
vitamins/minerals[b]	2.1	1.7	1.8	2.0	1.9
chromic oxide	0.2	0.1	0.1	0.2	0.2
celite	0.4	0.4	0.4	0.4	0.4

[a]Values are expressed as percentages on an as-is basis. [b]Vitamins/minerals mixture containing synthetic amino acids provided (mg/kg diet DM): 369 L-lysine-HCl, 123 DL-methionine, 9221 $Ca(H_2PO_4)_2$, 3811 NaCl, 14016 $CaCO_3$, 103 $FeSO_4{\cdot}7H_2O$, 123 ZnO, 83 Mn_3O_4, 74 $CuSO_4{\cdot}5H_2O$, 8.1 KI, 8.2 $Na_2SeO_3{\cdot}5H_2O$, 10.3 retinyl acetate, 5.16 cholecalciferol, 106 α-tocopheryl acetate, 2.58 menadione, 2.58 riboflavin, 12.91 D-pantothenic acid, 0.026 cyanocobalamine (B_{12}), 2.58 thiamin (B_1), 25.82 niacin, 3.87 pyridoxine (B_6), and 0.065 biotin.

Animals and Feeding. The animal experiment was conducted according to the license obtained by the Danish Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food Administration. Pigs used in the study were crossbreeds of Duroc × Danish Landrace × Yorkshire obtained from the swineherd at Aarhus University, Department of Animal Science, Denmark. Five female pigs with an initial average weight of 58 ± 2.8 kg were used in the experiment, which was performed according to a 5 × 5 Latin square design. Pigs were surgically fitted with a permanent simple T-cannula 15 cm anterior to the ileal−cecal junction following the procedure of Jørgensen et al.[22] After surgery, pigs were kept in individual smooth-walled (1.25 m × 2.50 m) pens to recover for 6−7 days and were then randomly assigned to the five experimental diets. In the investigation period, diets were thawed the day before feeding. Pigs were fed three times daily at 9:00, 14:00, and 19:00 with one treatment per week according to the design. The total daily feed intake was 1725 g DM/day with drinking water provided ad libitum. Animals were weighed once per week. On the last day of the experiment, animals were euthanized with an overdose of sodium pentobarbital followed by exsanguination.

Collection of Ileal Effluent. To measure ileal digestibility of diets and physicochemical properties of ileal digesta, pigs were adapted to the specific diets 5 days before ileal effluent was collected. In each of the experimental weeks, ileal effluent was collected over 2 days, starting at 9:00 when feeding the animals and finishing at 14:00 before the second meal was given. Samples were collected continuously and pooled during the periods 9:00−11:00, 11:00−13:00, and 13:00−14:00. Ileal digesta were collected in polyamide autoclave bags of 6 mm × 20 mm (Buch and Holm, Herlev, Denmark) attached to the ileum cannula with a plastic zip fastener. Two to three drops of an aqueous solution of 0.2% NaN_3 (Sigma-Aldrich) was added to each of bag to prevent microbial activity. A representative sample of material collected in the period 11:00−13:00, totalling 40 g, was processed for viscosity measurement and NSP determination, whereas the same amount of material collected from pooled and mixed effluent from 9:00 to 14:00 was used for WBC determination. The remaining material from the 2 day collection was pooled in a plastic container, weighed, and stored at −20 °C. The ileal samples were subsequently defrosted, mixed thoroughly, and freeze-dried prior to further analysis.

Chemical Analyses. Diets and ileal effluent were freeze-dried and ground to a particle size of less than 0.5 mm. All chemical analyses were performed in duplicate. DM was determined by drying to constant weight at 105 °C for 20 h, and ash was analyzed according to the AOAC method.[23] Chromic oxide was analyzed as described by Schürch et al.[24] Protein (N × 6.25) was determined by the Dumas method, [25] HCl fat according to the Stoldt procedure,[26] and starch by the enzymatic-colorimetric method as described by Bach Knudsen.[27] The dietary contents of sugars (glucose, fructose, sucrose, and maltose) were analyzed by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex, Sunnyvale, CA). Fifty milligrams of sample was mixed with 10 mL of 50% EtOH (v/v) containing 250 mg/L arabinose as an internal standard and incubated at 65 °C for 60 min with occasional mixing (three times). After centrifugation at 2000*g* for 10 min, 0.25 mL of the liquid phase was diluted 20 times with water and filtered through a 22 μm nylon filter. The carbohydrates were eluted by two gradients prepared from water (eluent A), 0.225 M NaOH (eluent B), and 0.5 M NaOAc (eluent C). A short elution program with a flow rate of 1.0 mL/min was used for the determination of fructose, sucrose, and maltose. In this procedure, eluent B changed from 27.5 to 53% at 9 min, was kept constant until 16 min, and returned to the initial condition for 9 min. Simultaneously, eluent C was changed from 2.5 to 5.5% at 7 min, increasing to 20% at 9 min, kept constant until 16 min, and then returned to the initial concentration for 9 min. For the determination of glucose, the flow was 0.7 mL/min, and eluent B was changed from 7.5 to 11.5% at 17 min, kept constant for 10 min, then increased to 25% at 27 min, and kept constant until 57 min, where it increased to 53% and was kept there until 69 min, where it was returned to the initial 7.5% and kept until 80 min. Eluent C was added at 1% at 27 min, changed to 20% at 57 min, and then aborted at 69 min. Quantification of the carbohydrates was carried out by external standards using mixtures in concentrations ranging from 2 to 20 mg/L.

β-Glucan in diets and ileal digesta was analyzed by the enzymatic-colorimetric method of McCleary and Glennie-Holmes.[28] NSP of diets, ileal digesta, and their supernatants were measured as described by Bach Knudsen[27] with the modification that the polysaccharides in starch free residue were hydrolyzed with 2 M H_2SO_4 for 1 h rather than 1 M H_2SO_4 for 2 h. The content of NDC in diets and ileal contents was determined by direct acid hydrolysis without starch removal and alcohol precipitation. The total NDC was calculated by subtraction of starch content determined by the enzymatic-colorimetric method. The content of low molecular weight non-digestible carbohydrates (LMW NDC) was calculated as:

$$\text{LMW NDC} = \text{total carbohydrates} - \text{NSP} - \text{starch}$$

The content of resistant starch (RS) in the diets was calculated as:

$$\text{RS} = \text{NSP}_{\text{glucose}} - (\text{cellulose} + \beta\text{-glucan})$$

The sum of cellulose and RS in the ileum content was calculated as:

$$\text{cellulose} + \text{RS} = \text{NSP}_{\text{glucose}} - \beta\text{-glucan}$$

In diets, Klason lignin was measured gravimetrically as the residue resistant to hydrolysis by 2 M H_2SO_4 following swelling with 12 M H_2SO_4.[27]

Determination of Physicochemical Properties. Water extracts, in vitro incubated diets, and ileal effluent (collected from 11:00 to 13:00) were centrifuged (10000*g*, 4 °C, 20 min) in 50 mL tubes, and the viscosity of the supernatants 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}. Values of viscosity at shear rate 45 s^{-1} are reported.

The WBC was measured in water extracts, in vitro incubated, and ileal digesta pooled from all times of collection (9:00 to 14:00). After centrifugation, as described above, precipitates were weighed, dried in a vacuum oven for 20 h, and reweighed.

Calculations. WBC values expressed as g water/g DM were calculated using the equation described by Serena et al.[29] as:

$$\text{WBC} = \frac{(\text{WW} - \text{DW})}{\text{DW}}$$

where WW is the wet weight and DW is the dry weight of the precipitate, respectively. For the current study, Cr_2O_3 was chosen as an inert marker, and the ileal digestibility of organic matter (OM) and carbohydrates was calculated relative to the Cr_2O_3 concentration, according to standard equation:[30]

$$\text{digestibility of X (\% of intake)} = 1 - \frac{\text{Cr}_2\text{O}_{3(\text{diet})} \times \text{X}_{(\text{ileum})}}{\text{Cr}_2\text{O}_{3(\text{ileum})} \times \text{X}_{(\text{diet})}} \times 100$$

where X is the concentration of specific nutrient in the diet and the ileal material. The starch digestibility was calculated assuming that any free glucose in ileal effluent derived from starch.

Statistical Analysis. Prior to statistical analysis, all data on viscosity were subjected to logarithmic transformation to stabilize variance between groups, and the results were reported as geometric means with 95% confidence intervals.[31] Viscosity and WBC of water and enzyme-treated diet extracts were analyzed by using a one-way analysis of variance model:

$$X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where X_{ij} is the dependent variable, μ is the overall mean, α_i is the effect of the diet, and ε_{ij} is a normally distributed random variable.

Comparison of dietary and ileal A:X ratios was performed by calculating the difference in A:X ratio of the ileal content for each individual subject and the matching diet. The difference was then subjected to statistical analysis as described below.

Physicochemical properties of ileal digesta, changes in the A:X ratio relative to the diet, and the digestibility of OM and carbohydrates were analyzed using a mixed model:

$$X_{ijk} = \mu + \alpha_i + \beta_j + P_k + \varepsilon_{ijk}$$

where X_{ijk} is the dependent variable, μ is the overall mean, α_i is the fixed effect of the diet (i = 1, ..., 5), β_j is the fixed effect of week (j = 1, ..., 5), P_k is the random effect of pig (k = 1, ..., 5), and ε_{ijk} is a normally distributed random variable.

The relation between the logarithmic values of viscosity and the concentration of NSP in ileal supernatant was described by a regression model:

$$Y_i = \alpha + \beta x_i + \varepsilon_i$$

where α is the intercept on the Y-axis, β is the slope, and ε_i is a random normally distributed variable.

All statistical analyses were performed using Statistical Analysis Software (SAS Institute Inc., Cary, NC). All data are expressed as least-squares means with differences considered statistically significant at $p < 0.05$.

RESULTS

Chemical Composition of Diets. The diets were designed to provide a practically constant intake of OM, while the content of starch and NSP varied among diets (Table 2). The concentration of starch was lower in the DF-rich diets as compared with the WF diet. Furthermore, the AX diet had the lowest content of starch and the highest content of protein. This was due to the fact that the wheat arabinoxylan fraction consisted of 40.2% protein. In the commercial breads, NDC consisted mostly of NSP and little LMW, but the two experimental breads had almost twice as much LMW NDC than the two commercial rye breads. The content of arabinoxylan was almost identical in the GR, RK, and AX diets, while a markedly elevated content of LMW arabinose and xylose was detected in the AX diet only. The content of β-glucan was more than twice as high in the BG diet as compared with the GR and RK diets.

Table 2. Chemical Composition of Experimental Diets[a]

	diet				
	WF	GR	RK	AX	BG
DM (g/100g as is)	65.1	54.8	58.3	66.3	60.7
energy (kJ/g DM)	17.9	17.7	17.7	17.9	18.1
ash	5.9	6.6	6.3	7.1	6.1
protein (N × 6.25)	14.8	14.2	14.0	19.8	16.8
fat	2.8	2.3	2.4	3.1	3.2
starch	67.4	54.9	55.7	50.3	54.9
total sugars	6.0	2.7	2.3	3.0	5.3
glucose	0.8	0.5	0.3	1.2	0.1
fructose	1.5	0.9	0.9	0.9	0.6
sucrose	0.1	0.3	0.3	0.0	0.1
maltose	3.1	0.7	0.4	0.9	4.2
total NDC	8.4	17.9	19.1	19.0	23.8
A + X	1.8	7.1	7.1	9.5	3.3
glucose	5.3	8.3	9.6	6.4	19.0
total NSP	3.5	12.7	13.3	11.0	14.2
arabinoxylan	1.7	7.1	7.4	7.2	2.9
β-glucan[b]	0.4	2.0	2.1	0.5	4.6
cellulose	0.6	1.6	1.4	0.7	4.5
RS	0.3	0.9	1.2	0.6	1.4
soluble NSP	1.7	4.5	5.0	8.3	4.4
insoluble NSP	1.7	8.1	8.3	2.7	9.8
total LMW NDC	4.9	5.2	5.8	8.0	9.6
LMW A + X	0.1	0.0	−0.3	2.3	0.4
LMW glucose	4.0	3.8	4.9	4.7	8.4
Klason lignin	0.5	1.3	1.3	0.3	1.3
DF[c]	8.9	19.2	20.4	19.3	25.1

[a]Abbreviations: A + X, sum of arabinose and xylose. [b]Determined by enzymatic assay. [c]DF counted as the sum of NDC and Klason lignin. Values are expressed as percentages of DM.

Carbohydrate Composition of Ileal Digesta. The concentration of NDC and NSP in ileal digesta was significantly higher after consumption of the DF rich diets as compared with the WF diet. The total LMW NDC content was within the same level for all diets, except in digesta of the AX-fed pigs (Table 3). While glucose contributed significantly to the LMW NDC fraction in the digesta of the GR-, RK-, and BG-fed pigs, arabinose and xylose were the main contributors in the AX-fed pigs. The concentration of cellulose and RS was approximately 2.7- and 5-fold higher after consumption of the BG diet as compared with the two rye breads and the AX bread, respectively.

Arabinose:Xylose Ratio. The A:X ratio, which is a simple indicator of the arabinoxylan structure, varied among diets with the highest value for diet AX followed in descending order of diet WF, GR, RK, and BG (Figure 1). The A:X ratio increased significantly in the ileal digesta after consuming diets WF, GR, and RK and decreased significantly after consuming diet BG,

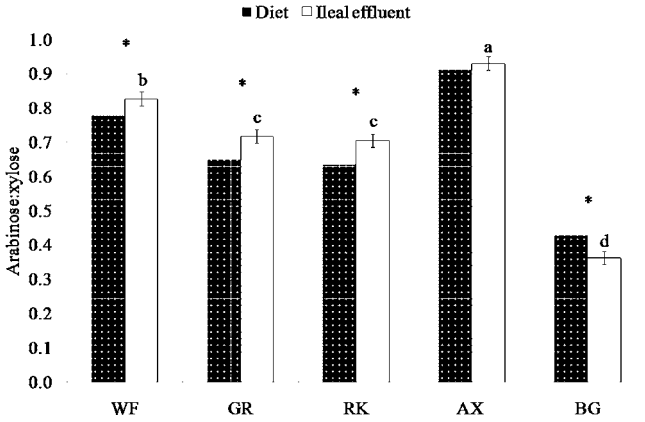

Figure 1. Arabinose:xylose ratio of NSP in diets and their ileal counterparts. Values are presented as least-squares means with standard errors. The different letters indicate significant differences at $p < 0.05$ among ileal effluents. An asterisk indicates the significance difference for the paired test comparison between diets and ileal counterparts.

Table 3. DM Concentration and Carbohydrate Composition of Ileal Digesta of Pigs[a]

	diet					
	WF	GR	RK	AX	BG	SE
DM	11.0	11.3	9.5	14.7	11.7	0.60
starch	6.1 c	5.1 d	7.1 b	2.8 a	9.8 e	0.20
total NDC	30.9 e	44.7 c	41.5 d	52.9 a	48.3 b	0.86
A + X	13.4 d	21.9 b	19.8 c	35.9 a	11.4 e	0.59
arabinose	5.6 d	8.6 b	7.8 c	15.5 a	3.2 e	0.28
xylose	7.9 d	13.3 b	12.1 c	20.4 a	8.2 d	0.33
glucose	11.1 c	14.9 b	14.8 b	6.9 d	32.6 a	0.53
total NSP	25.6 d	37.5 bc	35.4 c	41.6 ab	42.4 a	1.36
arabinoxylan	10.8 c	21.2 b	19.0 b	27.8 a	9.5 c	0.89
arabinose	4.9 d	8.8 b	7.8 c	13.4 a	2.5 e	0.32
xylose	5.9 c	12.3 b	11.2 b	14.4 a	7.0 c	0.59
β-glucan	0.3 c	2.7 b	2.4 b	0.3 c	4.1 a	0.45
sum of cellulose and RS	9.1 b	9.0 b	9.3 b	5.1 c	25.0 a	0.39
total LMW NDC	5.2 b	7.1 b	6.0 b	11.2 a	6.0 b	1.02
A + X	2.6 b	0.8 b	0.8 b	8.1 a	1.9 b	0.76
arabinose	0.7 b	−0.2 c	0.0 bc	2.2 a	0.7 b	0.34
xylose	1.9 b	1.0 b	0.8 b	6.0 a	1.2 b	0.45
glucose	1.7 b	3.1 a	3.1 a	1.5 b	3.5 a	0.48

[a]Abbreviations: A + X, sum of arabinose and xylose. Values are expressed as percentages on DM; SE, standard errors. Different letters in the same row indicate a significant difference at $p < 0.05$.

while no significant change was seen after consuming diet AX as compared to its diet counterparts. The substitution of arabinoxylan polymer present in BG diet was much lower than in the other diets and was slightly further reduced in digesta.

Ileal Digestibility. The digestibility of OM decreased after consumption of the DF rich diets as compared with the WF diet (Table 4). The starch was almost completely digested in all

Table 4. Digestibility of OM and Carbohydrates at the Terminal Ileum[a]

	diet					
	WF	GR	RK	AX	BG	SE
OM	92 a	79 bc	78 c	80 bc	82 b	1.2
starch	99 a	98 b	97 c	99 a	96 c	0.2
NDC	59 a	38 b	43 b	36 b	58 a	3.2
NDC A + X	16	24	27	13	29	0.1
arabinose	26	26	28	18	31	4.5
xylose	6 b	23 a	26 a	9 b	28 a	5.0
NDC glucose	77 a	56 c	59 bc	75 a	65 b	2.6
NSP	17 bc	27 abc	29 ab	13 c	38 a	5.4
arabinoxylan	28	27	32	11	31	6.7
arabinose	26	23	28	11	39	6.3
xylose	30	30	34	12	28	7.0
NSP glucose	16 c	35 ab	34 ab	29 bc	43 a	4.7
β-glucan[b]	87 a	66 b	67 b	81 a	81 a	4.8
cellulose + RS	−5	11	10	14	14	5.4

[a]Abbreviations: A + X, sum of arabinose and xylose. [b]Determined by enzymatic assay. Values are expressed as percentages of least-squares means; SE, standard errors. Different letters in the same row indicate a significant difference at $p < 0.05$.

diets with slightly lower digestibility in GR, RK, and BG. The digestibility of NDC was significantly lower in GR, RK, and AX as compared with WF and BG. The digestibility of NSP was highest in BG, followed by RK and GR and lowest in WF and AX. The NSP digestibility was mainly attributed to a low degradation of arabinoxylan and particularly xylose in the GR, RK, and AX bread, while a high β-glucan degradation was the main cause for the high NSP digestibility of BG bread. The digestibility of the sum of cellulose and RS was low (−5 to 14%) and was not significantly different between diets.

Physicochemical Properties of in Vitro and in Vivo Digested Diets. All high-DF diets resulted in higher extract viscosities than WF with the highest viscosities found in GR and BG among all water-incubated diets (Figure 2). By inclusion of digestive enzymes in vitro, the viscosity increased in all diets but less in the AX diet than the three other high-DF diets that all had similar in vitro viscosities. Surprisingly, after consumption of the AX bread, the viscosity of the ileal supernatant was 6.4-fold higher than the viscosity measured in vitro. In contrast, the viscosity of ileal BG effluent was less than half the viscosity measured in vitro. For the other three diets, the viscosity also increased in vivo. As a result, BG led to the lowest, AX the highest, and the other three diets to intermediate in vivo viscosities. WBC of the BG and AX diets was higher than the other diets both after water extraction and after incubation with digestive enzymes (Figure 3). However, in contrast to WF and GR, the in vitro WBC of RK, BG, and AX was lower than in the water extracts, causing in vitro WBCs to be more similar among diets than after water extraction. After consumption of the diets, the in vivo WBC of the ileal digesta

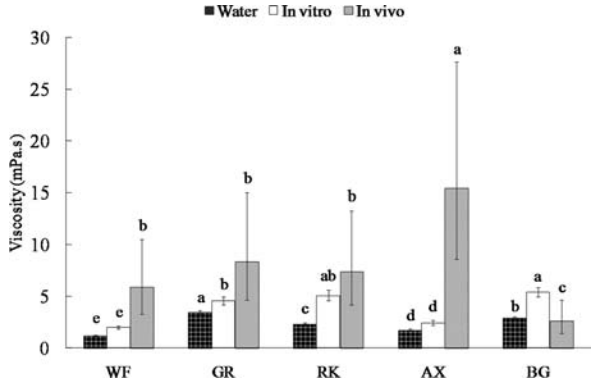

Figure 2. Viscosity of water extracts, in vitro incubated diets, and ileal digesta. Values are presented as geometric means with 95% confidence intervals. For each type of extract (water, in vitro, and in vivo), the different letters indicate significant differences at $p < 0.05$.

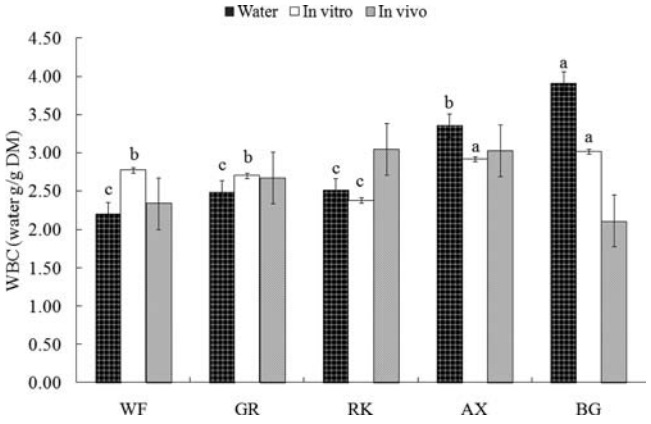

Figure 3. WBC of water extracts, in vitro incubated diets, and ileal digesta. Values are presented as least-squares means with standard errors. For each type of extract (water, in vitro, and in vivo), the different letters indicate significant differences at $p < 0.05$.

was not significantly different between dietary treatments, and the WBCs of WF, GR, and AX ileal digesta were similar to their water-extracted counterparts.

Correlation between Responses. The log-transformed ileal viscosity was positively correlated with total NSP concentration in the supernatant of ileal digesta ($r = 0.59$, $p = 0.0017$) (Figure 4). The viscosity of RK ileal supernatant showed a high variation among the samples, while low dispersion of samples was seen in the AX-fed pigs. The correlation between the viscosity of the ileal supernatant and the WBC of digesta and between the WBC of digesta and the concentration of NSP within digesta was not significant.

DISCUSSION

Neither the DF content, the viscosity, or the WBC had a marked influence on the digestibility of starch in the small intestine. However, a small but significant reduction in the digestibility of starch was observed after RK and BG diets consumption. With respect to the RK diet, this may be explained by the presence of intrinsic DF in partly intact grain structures protecting the starch against enzymatic digestion. For the BG diet, the most likely cause for the lower starch digestibility was the presence of β-glucan with the potential of creating a highly viscous environment in the duodenum that decreases the accessibility of starch-degrading enzymes to the

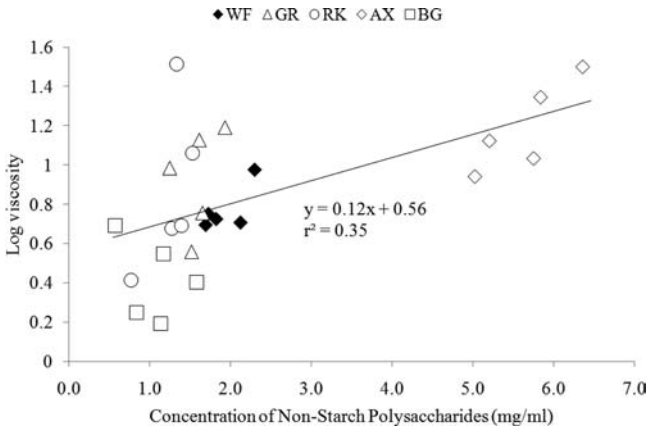

Figure 4. Relationship between viscosity and NSP of ileal supernatant from pigs fed the five experimental diets.

substrate by limiting the water availability for starch hydration as discussed by Lazaridou.[32] Although a substantial fraction of β-glucan was degraded when digesta reached the ileum and the viscosity was low, it had no concomitant influence on the absorption of starch at this site. Lia et al.[33] showed that the β-glucan in oat bran bread did not influence the digestibility of starch in the small intestine in a study of ileostomy subjects. Similarly, Hooda et al.[34] testing the addition of 5% of a low or high viscous fibers to a semisynthetic diet found no difference in ileal viscosity between a high and a low viscosity β-glucan diet and no difference in the ileal digestibility of starch between any of the diets in spite of differences in diet viscosity.

The reduction in OM digestibility after consumption of the DF-rich diets was primarily caused by the level of DF as is found in general when regressing OM digestibility to the DF level.[35] However, within the diets of equal fiber level, the DF structure influenced OM digestibility differently with a slightly smaller reduction in OM digestibility after BG diet consumption, as a consequence of the high ileal digestibility of β-glucan as compared to the other DF-rich diets. The strongest reduction in OM digestibility was found with the RK diet where the rigid nature of the intact cell walls may have physically impaired accessibility of the nutrients.[5] The digestibility of OM in the AX and GR diets was intermediate between these two extremes. Moreover, the elevating viscosity caused by the DF may potentially have hindered the emulsification of lipid and hydrolysis of protein that also contribute to a reduced OM digestion. Lastly, increased excretion of sloughed mucosal cells, mucin, and microbial biomass may also have contributed to the lower OM digestibility.[36]

The definition of DF in terms of the European Community Directive (2008) includes carbohydrates with three or more monomeric units that are neither digested nor absorbed in the human small intestine.[37] This was brought into focus by our experimental approach to determine the concentration of total NDC including the LMW NDC and NSP in the diet and digesta and digestibility of both NDC and NSP. The NDC digestibility was generally higher than the NSP digestibility in all diets. Microbial degradation of DF is a dynamic process that involves both solubilization and degradation of DF polysaccharides by microbial enzymes.[16,38] Therefore, besides NSP, total NDC digestibility also comprises the mono- and oligomers such as arabinose, xylose units, arabinoxylo-oligosaccharides, cellulosic residues of β-glucan and cellulose, and other hemicellulosic fractions that were either present in the diet or released during the passage of the stomach and small intestine.

The total number of microorganism is markedly lower in the upper gastrointestinal tract than the large intestine, and the DF provided as isolates or naturally present in the food will be depolymerized to a varying degree, depending on the chemical composition and structure of the DF.[10,21,39] In the present investigation, the purified β-glucan used in the BG diet was extensively degraded (>80%) at the terminal small intestine. A previous study with pigs[40] showed that β-glucan from oat bran in baked rolls had a small intestinal digestibility of 44%. This clearly indicates that β-glucan provided as an isolate is much more susceptible to bacterial enzymatic attack in the upper GI tract. The significantly lower digestibility of β-glucan in the GR and RK diets as compared with the BG diet was most likely due to β-glucan bound to other insoluble DF polysaccharides in the aleurone cells of rye rather than differences in the ratio of cellotriosyl/cellotetraosyl units or $\beta(1-4)/\beta(1-3)$ linkages in rye as compared with oat β-glucan.[32] Concerning arabinoxylan degradability, a previous study of Bach Knudsen and Canibe[40] showed that arabinoxylans in a roll produced from wheat flour with added wheat bran had an ileal digestibility of 8%, which is in the same order as the digestibility of 11% for diet AX in present experiment. Although there was a numeric difference in arabinoxylan digestibility between the AX and the two rye diets, there was no statistically significant effect of arabinoxylan source. However, both the relatively high concentration of LMW arabinose and xylose in the ileal content and the lower digestibility of NSP in AX-fed pigs as compared to GR and RK suggests a greater fermentability of rye arabinoxylan in the upper gut than of wheat arabinoxylan. This indicates an insufficient capacity of the microbial enzymes to degrade the highly substituted arabinoxylan present in the AX diet.

Overall, arabinoxylan both from wheat and rye is less susceptible to microbial degradation than β-glucan in the upper intestinal tract. The high resistance of arabinoxylan is caused by the complex structure that makes arabinoxylan more difficult to cleave than the β-glucan, as it requires far more enzymes to hydrolyze the xylose backbone and the different substituent groups.[41] The different anatomical parts of grain have different patterns of substitution of α-L-arabinofuranosyl groups at position C-2 and/or C-3 to the xylan backbone, which influence the extractability of the arabinoxylan.[15] In the current study, the isolated wheat arabinoxylan in diet AX with a high degree of substitution was hardly affected by the upper gut environment, as indicated by the almost similar A:X ratio in diet and digesta. However, less branched arabinoxylan as in the two rye diets increased the ratio in the ileum content. This suggests that mainly the less-substituted parts of the xylan chain were cleaved. Furthermore, the lower A:X ratio in ileal digesta after consuming the BG diet suggests that the linear arabinoxylan chain in Vitacel that is closely associated with the cellulose fiber is very resistant to disintegration. Besides microbial degradation, a study of Zhang et al.[42] has also shown that gastric acidity may induce arabinoxylan hydrolysis as indicated by up to 10% release of L-arabinose measured in vitro. Overall, cleavage of arabinose and xylose units may occur randomly and lead to a decrease of the molecular weight of the arabinoxylan.

The presence of DF in breads resulted in a marked change of viscosity in vivo, with substantial discrepancies to the viscosity of water- and in vitro-incubated diets. The low in vivo viscosity of BG diet was most likely a consequence of a high degree of

depolymerization and degradation of the β-glucan during passage of the small intestine. This is in line with a study of Åman et al.,[43] which indicated a substantial degradation of oat bran β-glucan in ileostomy subjects. Moreover, Johansen et al.[44] showed a 20-fold reduction in the molecular weight of β-glucan in oat bran during passage of the small intestine of pigs. In contrast, the elevated viscosity in AX ileal supernatant as compared with its in vitro counterpart may be due to a high degree of solubilization of arabinoxylan in the gut, which causes an increase of the arabinoxylan hydration. This allows long-chain polymers to unfold their structure and thereby increase the radius gyration of the side groups, which overall contributes to the viscous effect. In the in vitro model, the addition of digestive enzymes caused hydrolysis of starch and protein, which may have increased the water surface around the DF structure and led to an increased solubility and consequently a higher viscosity in the in vitro-incubated diets as compared with the water extracts. In contrast, Cyran and Ceglinska[45] showed that the treatment of wholemeal rye bread extract with combination of α-amylase, amyloglucosidase, and protease enzyme reduced the overall extract viscosity by 9−16% as compared with extract viscosity without enzyme addition. This shows that the way of extract preparation also influences the final viscosity. On the other hand, the lower viscosity seen in vitro in WF, GR, RK, and AX diets as compared with their in vivo counterparts may be due to insufficient imitation of the intestinal contractions and a lack of microbes. Besides depolymerization of DF, microbial enzymes may simultaneously contribute to the hydration and solubilization of DF present in isolated and intact DF cell wall and thereby elevate the viscosity.

In the in vivo study, we observed a positive but weak correlation between viscosity and NSP concentration of the ileal supernatant, confirming that NSP concentration is an important determinant of ileal viscosity. However, the viscosity in the liquid phase is only partly explained by the DF level of the diets. For example, RK resulted in a high individual variation in in vivo viscosity that may reflect variation in the extent of extraction of DF from the cell walls to the liquid phase. In contrast, GR containing disintegrated DF fragments resulted in a reduced variation in viscosity. Hence, both DF structure extractability and degradability have more influence on intestinal viscosity than merely the total content of NSP in the diet.

WBC is determined by the chemical structure of the molecules, physical integration, electrolyte concentration of the surrounding fluid, and pH.[46] Thus, theoretically, the amount of absorbed and bound water depends on the type of DF. In the current study, none of the high DF diets influenced the WBC of ileal precipitates. A marked reduction of WBC was seen with the BG diet when comparing the WBC after water extraction, in vitro incubation, and in ileal digesta in vivo. This was most likely due to different degrees of depolymerization of the β-glucan in the different models. Depolymerization of β-glucan will have a strong impact on the WBC.

In conclusion, the results reported herein imply that inclusion of different content and structure of DF into bread affects the physicochemical properties in the final product and the intestinal content differently. This has a major impact on the ileal digestibility of OM and NSP but a minor influence on the starch digestibility. Interestingly, isolated oat β-glucan, which had the most significant influence on the physicochemical properties of the breads, markedly depolymerized during passage of the small intestine, while isolated wheat arabinoxylan with a low influence on the physicochemical properties of the breads was highly resistant to microbe enzymes and provided a more pronounce effect on the ileal viscosity. In vivo viscosity is a key factor in influencing glycemic index, cholesterol, and satiety.

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ABBREVIATIONS USED

DF, dietary fiber; WF, white wheat bread; GR, dark ground rye bread; RK, rye bread with kernels; BG, wheat bread with added isolated oat β-glucans; AX, wheat bread with added isolated wheat arabinoxylans; NDC, nondigestible carbohydrates; NSP, nonstarch polysaccharides; LWM NDC, low molecular weight of nondigestible carbohydrates; RS, resistant starch; WBC, water-binding capacity; DM, dry matter; OM, organic matter

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