Isolation, Partial Characterization, and Antinutritional Activity of a Factor (Pentosans) in Rye Grain

Takis Antoniou,[1] Ronald R. Marquardt,* and Peter E. Cansfield

The role of isolated pentosans as the possible antinutritional factor in rye grain was investigated by using the chick as the bioassay animal. Pentosans were fractionated into water-soluble and insoluble fractions, and the content of the two major pentoses, xylose and arabinose, were quantitated. Puma rye contained 2.1 soluble and 7.7% insoluble pentosans. When isolated pentosans supplied wheat-based diets with the same level of pentosans as that found in similarly formulated rye diets, the depression of growth was 20 and 55% for the soluble and insoluble pentosans, respectively. Both fractions depressed nutrient digestibility. At equal dietary levels, the soluble fraction was slightly more potent compared to the insoluble pentosans. The antinutritional activity of pentosans may be attributed to their ability to form highly viscous solutions which reduced the rate of digestion and/or absorption of nutrients and to the inability of the avian digestive system to digest pentosans.

Rye grain has a nutrient content that is similar to that of wheat (Miller, 1958) but is not widely used in animal feeds because of the presence of various toxic or antinutritional factors which reduce its nutritive value, particularly for monogastric animals (Wieringa, 1967; Moran et al., 1969). Ergot alkaloids were the first and most toxic growth-depressing factor identified in rye grain. However, they do not constitute a potential hazard for animals because the level of ergot bodies in rye grain can be effectively controlled (Lorenz, 1979). Wieringa (1967) reported that certain petroleum ether extractable compounds (the resorcinols) in rye bran cause severe growth depression in weanling rats and pigs. Fernandez et al. (1973), however, obtained no improvement in chick growth when resorcinol-free rye diets were consumed. These same authors and Misir and Marquardt (1978) demonstrated that water extraction of rye considerably improved chick growth while addition of the freeze-dried water extract to a wheat- or corn-based diet depressed growth and produced watery and sticky feces. This unknown water-extractable factor was also shown to reduce bone mineralization by interfering with vitamin D_3 utilization (MacAuliffe et al., 1976). More recent studies by Marquardt et al. (1979) and Antoniou et al. (1980) revealed that rye contained a nonspecific antinutritional factor (possibly a polysaccharide) which depressed the digestion and/or absorption of all nutrients, particularly saturated fats. The current study was carried out to establish the antinutritional activity of isolated water-soluble and insoluble rye pentosans when added to a wheat-based diet, to compare two methods of pentosan quantitation, and to establish the pentose composition and other characteristics of different pentosan fractions prepared from rye.

MATERIALS AND METHODS

Isolation of Pentosans. Water-soluble and insoluble pentosans were isolated according to the flow chart of Figure 1. Ground Puma rye (1-mm screen) was boiled with 3.5 volumes (w/v) of 80% ethanol for 1 h under reflux for inactivation of enzymes (including pentosanases), denaturation of protein, and removal of ethanol-soluble substances (Preece and MacKenzie, 1952). Rye was filtered and washed with 95% ethanol and air-dried at 25 °C while the filtrates (which contained 10% of the original dry matter) were pooled, concentrated at 70 °C under reduced pressure, and freeze-dried. Ethanol-boiled rye (300 g) was extracted with 4 volumes of distilled water for 15 min, followed by centrifugation at 9000*g* for 10 min. The supernatant was filtered, adjusted to pH 7.5 with NaOH, and digested for removal of protein and starch with porcine pancreatin (grade III; Sigma Chemical Co.) at 32–34 °C for 24 h with continuous stirring in the presence of 0.05% NaN_3 (w/v) for microbial inhibition. Residual NaN_3 was removed by the subsequent washings. Pancreatin was prepared as described by Simpson (1954) and was used at 1% of the weight of the boiled rye used for extraction. After 24 h of digestion, the solution was centrifuged at 9000*g* for 5 min, and the supernatant was adjusted to 80% ethanol for precipitation of pentosans (Vones et al. 1964). The precipitate was collected by filtration through Whatman No. 541 filter paper, washed with 95% ethanol, and left overnight in 95% ethanol. The pentosan fraction was then washed with acetone and ethyl ether and dried with N_2 at 30 °C under reduced pressure.

Water-insoluble pentosans were isolated by modification of the procedure used by Cole (1967) for the isolation of pentosans from wheat endosperm. After the first centrifugation of the water-extracted rye, the top layer (sludge fraction) of the precipitate was carefully scraped out and suspended in distilled water for 3–4 h while the remaining precipitate (referred to as ethanol-boiled rye after partial removal of the soluble and insoluble pentosans) was freeze-dried. The suspension was filtered through a 40-mesh sieve and the residue (bran and embryos) was discarded while the filtrate was recentrifuged at 9000*g* for 10 min. The supernatant was discarded, and the top layer of the precipitate which formed above the starch layer was either resuspended in water and boiled at 95 °C for 5 min for starch gelatinization prior to pancreatin digestion or extracted with 0.2 N NaOH for 2 h under N_2 (to prevent pentosan oxidation). In the latter case the extremely viscous solution was centrifued (1000*g* for 1 min) and the supernatant was immediately neutralized (pH 7.5) with glacial acetic acid while the precipitate was discarded. Both preparations were digested as described above with 1.4% (of the weight of ethanol-boiled rye) porcine pancreation for 30 h under nitrogen. Thereafter the insoluble pentosans were precipitated, washed, and dried by using the procedures described for the soluble pentosans.

Quantitation and Analysis of Pentosans. The quantitative estimation of rye pentosans was based on their total pentose content. Xylose and arabinose, which are

Department of Animal (T.A. and R.R.M.) and Food (P.E.C.) Sciences, University of Manitoba, Winnipeg, Canada R3T 2N2.

[1]Present address: Department of Agriculture, Nicosia, Cyprus.

0021-8561/81/1429-1240$01.25/0

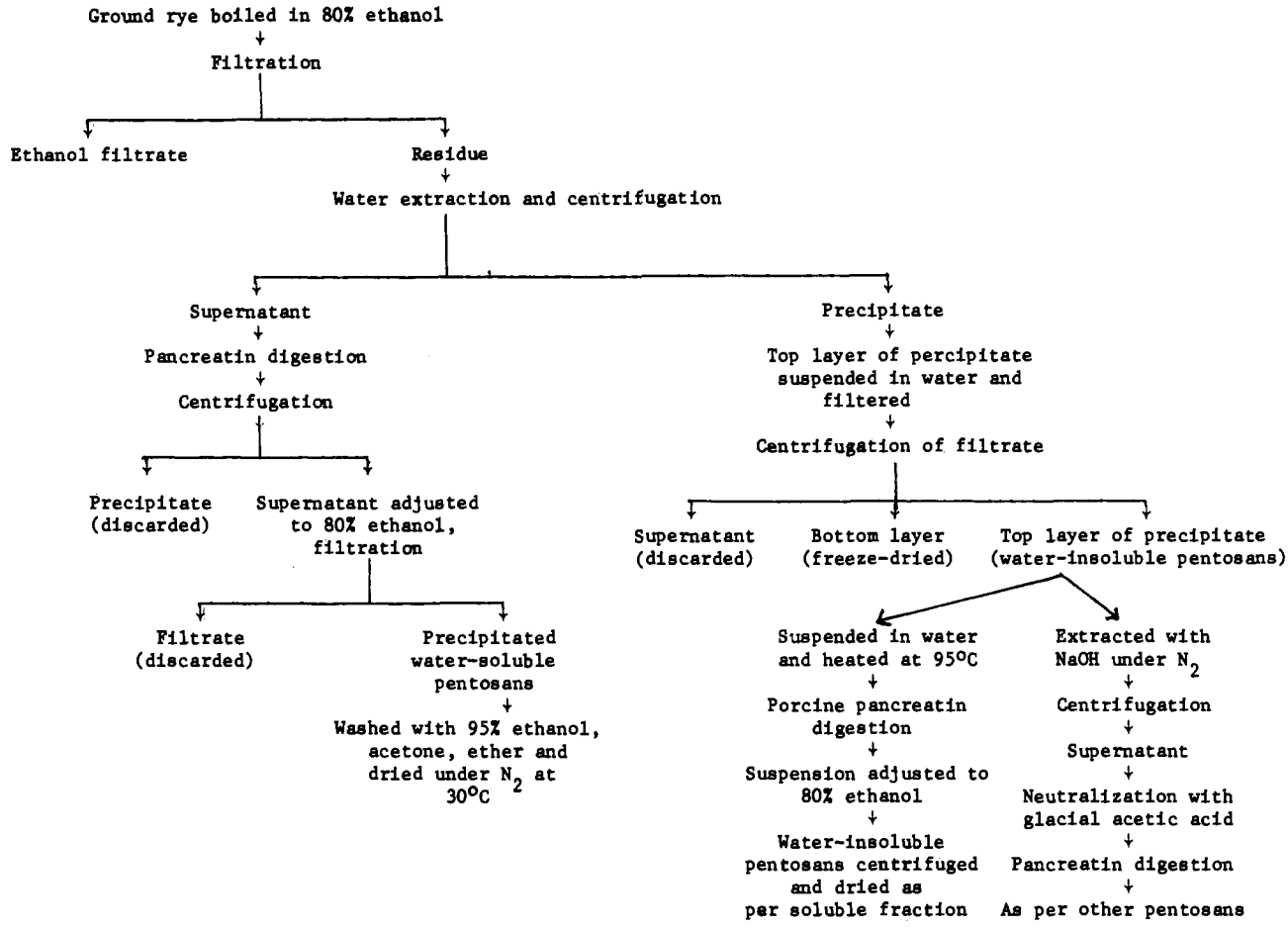

Figure 1. Flow chart for isolation of water-soluble and insoluble pentosans from rye grain.

the major components of water-soluble and insoluble pentosans of rye (Casier and Soenen, 1967), were measured by using a colorimetric (Cerning and Guilbot, 1973) or gas–liquid chromatographic (GLC) procedure.

The GLC method was based on the hydrolysis of pentosans and conversion of the resulting monosugars to the more volatile alditol acetates. Duplicate 20-mg samples with 3–4 mg of myoinositol (internal standard) and 2 mL of 2 N H_2SO_4 were placed in Pyrex tubes and hydrolyzed for 1 and 2 h at 100 °C, followed by neutralization (pH 7.5) with $BaCO_3$ and centrifugation at 10000*g* for 10 min. The precipitate was washed with 1–2 mL of distilled water and recentrifuged. This procedure was repeated once more and all three supernatants were pooled. Reduction and acetylation of monosaccharides were performed by modification, as outlined below, of the procedure of Lindberg (1972) for preparation of partially methylated alditol acetates. Reduction was allowed to occur overnight with 30 mg of $NaBH_4$, and thereafter the free $NaBH_4$ was decomposed by gradual addition of freshly washed Dowex 50 (H^+; Sigma Chemical Co.) until gas evolution ceased. Boric acid was removed by volatilization, and methanol (3 mL) was added to the alditol solution and removed by evaporation under reduced pressure at 50 °C. The procedure was carried out 5 times. After acetylation and removal of the pyridine and the excess of the acetic anhydride, the sample was dissolved in ethyl acetate. The correction factors for each sugar were calculated according to the formula of Sloneker (1972) by preparing standard solutions from pure, anhydrous alditol acetates (Supelco, Inc.) using levels proportionate to those established in preliminary pentosan analyses. Alditol acetates were separated by injecting 1 μL into a chromatograph (Varian Aerograph Series 1200) equipped with hydrogen flame ionization detector. A glass column (180 × 0.2 cm) packed with 3% Silar 10C (polysiloxan polymer with phenyl and cyanoalkyl functional groups; Applied Science Laboratories) on 100–120-mesh Chromosorb WHP (Chromatographic Specialties) was used. Column temperature was programmed between 170 and 210 °C with 2 °C/min increase and thereafter held at 210 °C until the last peak (1630 s). The injection port temperature was 215 °C and detector temperature 230 °C. Gas flow rates (mL/min) were 46 for hydrogen, 32 for nitrogen, and 150 for air. Electrometer attenuation was set at 1 with a range of 10^{-11} A/mV. Peak area was integrated by a digital integrator (Columbia Scientific Industries; Model 38). A typical chromatogram is shown in Figure 2.

For quantitation of total soluble and insoluble pentosans in raw or ethanol-boiled rye, finely ground duplicate 10-g samples were extracted 3 times with 10 volumes (w/v) of distilled water for 30 min, followed each time by centrifugation at 1600*g* for 20 min. A similar procedure has been reported to extract all the water-soluble pentosans from rye (Golenkov and Traubenberg, 1966). All three supernatants were pooled, and total soluble pentosans were determined directly by using 5-mL aliquots (colorimetric method) or after freeze-drying (GLC method). Freeze-dried precipitates were also used for the determination of the total water-insoluble pentosans by both methods.

Pentosan Viscosity. The viscosities of isolated crude soluble and insoluble pentosans were measured at 30 °C in aqueous solutions by the use of a 80–100-s viscometer.

Enzymatic Determination of Starchy Glucose. So that the level of starchy (contaminant) and nonstarchy glucose in the ethanol-precipitated pentosans before and after pancreatin digestion could be established, duplicate 50-mg samples of pentosans and corn starch (as a stand-

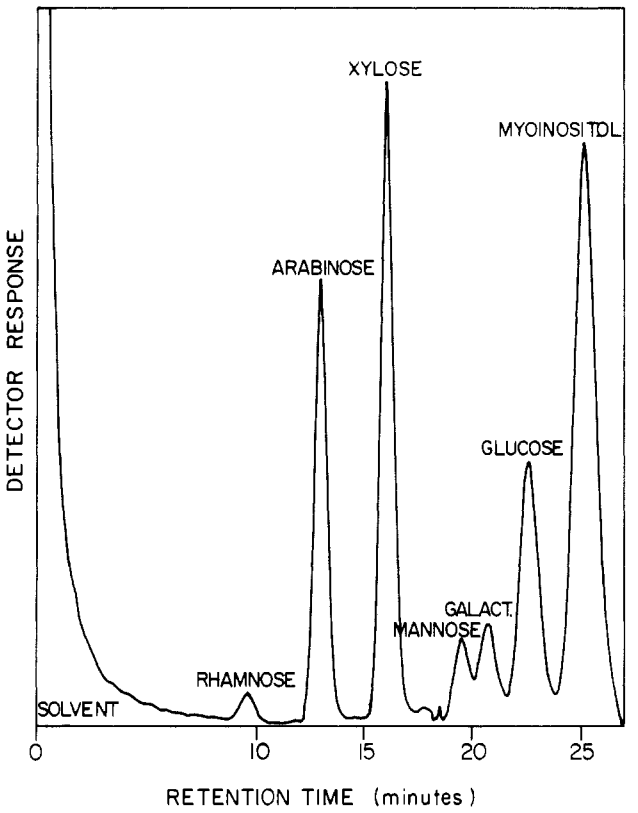

Figure 2. Gas chromatographic analysis of a 1-μL injection of derivatized sugars present in fecal samples from chicks fed a rye diet.

ard) were heated with 30 mL of distilled water at 121 °C for 2.5 h for starch gelatinization. The solutions were adjusted to 50 mL, and 2-mL aliquots were mixed with 2 mL of 0.05 N citrate buffer (pH 4.5) and 0.4 mL of 1,4-α-amyloglucosidase from *Aspergillus niger* (650 units/mL; Sigma Chemical Co.). The mixture was incubated at 40 °C for 4, 12, and 20 h. This enzyme, which can also hydrolyze 1,6-α-glucoside bonds, was inactivated by adding 10 mL of absolute ethanol, followed by cooling in an ice bath for 30 min and centrifugation at 27000*g* for 20 min for protein and pentosan precipitation. Aliquots of 0.5 mL were used from the final solution for glucose determination by the glucose oxidase and peroxidase method (Sigma Co., 1978). Total glucose was also estimated enzymatically after acid hydrolysis of sample for 3 h with 2 N H_2SO_4, neutralization with $BaCO_3$, and centrifugation.

Chick Management, Diets, and Analyses. Two experiments were carried out with 6- or 7-day-old White Leghorn cockerels. The management of chicks from day 1 to day 6 or 7 was given by Marquardt et al. (1979). Experiment 1 consisted of four dietary treatments (rye, wheat, wheat plus water-soluble pentosans, and wheat plus freeze-dried water extract). The composition of the diets were similar to those outlined in experiment 2 (Table I). Water soluble pentosans and freeze-dried extract were added to the diets at a level of 2.3 and 5.8%, respectively. The final average protein content of the diets was 19.4%, and the calculated metabolizable energy value was 2950 cal/g. A completely randomized design with two replicates of five birds each was used in this 7-day experiment. The average initial bird weight ± SE was 57.7 ± 1.0. In the second 7-day experiment, a completely randomized design was used involving nine dietary treatments with three replicates, each consisting of five chicks with an average initial weight ± SE equal to 56.7 ± 0.3 g. All nine diets (Table I) were formulated to be isocaloric and isonitrogenous according to the National Research Council (1977). The ethanol filtrate was added to wheat-based diets at the same level as that isolated from similarly formulated rye diets. The pentosan preparations and the freeze-dried water extract were added to wheat diets so that they supplied a level of soluble or insoluble pentosans equal to the corresponding levels found in similarly formulated rye-based diets. The water-soluble pentosans were also used at a level equal to that of the insoluble pentosans. The calculated levels of pentosans that were added to the diets were based on the results of the colorimetric methods as GLC values were not available when the feeding trial was designed.

Amino acid analysis (except for tryptophan, cystine, and methionine) of pentosans (prior to diet formulation) and of grain, feed, and fecal samples was performed by the method of Moore and Stein (1963) on a Beckman automatic amino acid analyzer. Fat analysis was performed as described by Antoniou et al. (1980). Other analyses were carried out according to the Association of Official Analytical Chemists (1970). Digestibility coefficients were determined according to the formula given by Church (1976). Treatment means were subjected to the Student–Newman–Keuls multiple range test (Snedecor and Cochran, 1967).

RESULTS

Colorimetric Analysis of Rye Pentosans. The results of a colorimetric determination of rye pentosans indicated that raw rye contained (dry matter basis) approximately 2% water-soluble and 6.5% water-insoluble pentosans. The sum of these fractions did not differ from the total pentosan value (8.7%) measured directly on the raw rye. Drews and Seibel (1976) after colorimetric estimation with orcinol reported that the Canadian rye contained 7.6–8.8% total pentosans. This method was discontinued in the current study as it was nonspecific, it had different extinction coefficients for different pentoses, and the furfurals were sensitive to photodecomposition.

Chemical Composition of Rye Pentoses and Pentosan-Rich Fractions. The results of the chromatographic analyses are presented in Tables II and III and include only those values obtained after 1 h of hydrolysis as no differences were observed when samples were hydrolyzed for 1 or 2 h. According to this method, raw rye contained 10% total pentosans (xylose and arabinose) which did not differ from the sum of the soluble (2.1%) and insoluble (7.7%) pentosans (Table II). The ethanol filtrate (Table III) which represented 9.5% of the dry matter of raw rye contained mainly protein, amino acids, and glucose with negligible amounts (0.3%) of xylose plus arabinose. The preferential removal of the nonpentosan-containing compounds following ethanol extraction of rye resulted in an increased concentration of pentosans (10.4; 2.08 + 8.35 vs. 10.0% in raw rye) in the residual fraction, the ethanol-boiled rye. A similar increase in the concentration of the insoluble (8.4 vs. 7.7%) but not soluble (2.1 vs. 2.1%) pentosans from ethanol-boiled rye also occurred. In addition, the pentosan concentration of the ethanol-boiled rye after the partial removal of soluble and insoluble pentosans was greater than that of raw rye (10.9 vs. 10.0%).

Pentosan extractability was not affected by prior boiling of raw rye in ethanol, because equal quantities of pentosans were obtained when the values were expressed on the basis of yields from the original untreated sample. The recoveries of soluble pentosans (xylose and arabinose) after pentosan precipitation by ethanol were ~69% (60% for arabinose and 80% for xylose) compared to their content in the water extract. The reduced recovery is mainly at-

Table I. Formulas and Analyses of Diets (As-Fed Basis)

ingredient	% diet A	diet B	diet C	diet D	diet E	diet F	diet G	diet H	diet I
rye (9.9% protein)	58.00								
extracted rye[a]		58.00							
wheat (12.6% protein)			58.00	58.00	58.00	58.00	58.00	58.00	58.00
meat meal (49.4% protein)	27.00	29.00	25.00	22.00	22.00	25.00	24.00	22.00	22.00
basal mixture[b]	7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90
corn starch	6.06	4.61	5.52	3.88		3.56	2.31		
amino acid mixture[c]	1.04	0.49	0.58	0.22	0.37	0.22	0.25	0.32	0.20
celufil (cellulose)			3.00	3.00	0.73	3.00			
ethanol filtrate				5.00					
freeze-dried water extract					11.00				
water-soluble pentosans						2.32	7.54		
water-insoluble pentosans								11.78	
water-insoluble pentosans extracted with NaOH									11.90
chemical analyses[d]									
protein ($N \times 6.25$)	20.3 ± 0.0	19.8 ± 0.3	20.6 ± 0.0	19.9 ± 0.1	20.2 ± 0.1	20.7 ± 0.1	19.9 ± 0.0	20.1 ± 0.1	20.1 ± 0.4
fat	9.4 ± 0.2	10.1 ± 0.2	10.3 ± 0.3	11.0 ± 0.0	9.9 ± 0.4	10.1 ± 0.1	9.9 ± 0.3	10.0 ± 0.4	9.7 ± 0.3
dry matter	90.7 ± 0.4	94.0 ± 0.0	90.7 ± 0.4	90.8 ± 0.0	91.0 ± 0.1	90.7 ± 0.1	90.3 ± 0.9	90.4 ± 0.7	90.7 ± 0.8
ME[e] (kcal/kg of diet)	2980	2970	3034	2990	2960	2950	2900	2870	2870

[a] Ethanol-boiled rye was extracted with 4 volumes of water, followed by partial removal of insoluble pentosans. [b] The composition of the basal mixture (% of diet) was as follows: soybean oil, 6.00; vitamin mixture, 1.00; mineral mixture, 0.50; chromic oxide, 0.40. See Marquardt et al. (1979) for further details on mineral and vitamin mixtures. [c] All diets contained (% of diet) 0.08 DL-methionine and 0.06 L-tryptophan; diets A, B, C, E, F, G, H, and I also contained 0.90, 0.35, 0.44, 0.15, 0.08, 0.11, 0.18, and 0.06 L-glutamic acid, respectively; diets D and E contained 0.08 L-lysine hydrochloride. [d] Chemical analyses ± SE. The values for ME were calculated. [e] ME, metabolizable energy.

Table II. GLC Analysis of Carbohydrates Present in Raw Rye and Water-Extracted Ethanol-Boiled Rye and the Content of Water-Soluble and Insoluble Pentosans from Raw and Ethanol-Boiled Rye

sample analyzed	% for monosaccharide[a] arabinose	xylose	mannose	galactose	glucose	arabinose + xylose	xylose to arabinose ratio
raw rye	3.9 ± 0.1	6.1 ± 0.1	traces	traces	72.0 ± 0.2	10.0 ± 0.2	1.45
raw rye, soluble pentosans[b]	1.0 ± 0.1	1.1 ± 0.1	0.7 ± 0.2	0.1 ± 0.1	3.5 ± 0.2	2.1 ± 0.1	1.10
raw rye, insoluble pentosans[c]	3.0 ± 0.1	4.7 ± 0.3	0.1	0.1	67.8 ± 6.5	7.7 ± 0.4	1.56
ethanol-boiled rye, soluble pentosans[b]	1.0 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	2.0 ± 0.1	2.1 ± 0.1	1.19
ethanol-boiled rye, insoluble pentosans[c]	3.3 ± 0.7	5.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	72.0 ± 0.7	8.4 ± 0.1	1.57
ethanol-boiled rye after partial removal of soluble and insoluble pentosans[d]	4.3 ± 0.2	6.6 ± 0.5	traces	traces	75.8 ± 0.9	10.9 ± 0.7	1.65

[a] Mean ±SE of two samples with two injections (analyses) per sample. Results are expressed as a percent of the dry matter content of the sample. [b] Water-soluble pentosans represents values from exhaustively extracted rye or boiled rye. [c] Water-insoluble pentosans were determined on rye or ethanol-boiled rye from which the soluble pentosans had been exhaustively extracted. [d] Extracted once with 4 volumes of water, followed by partial removal of insoluble pentosans from the sludge fraction.

Table III. Composition of Ethanol Filtrate, Freeze-Dried Water Extract, and Pentosan Preparations following Carbohydrate Analyses by GLC

analyzed fraction	% for component[a]									xylose to arabinose ratio
	arabinose	xylose	mannose	galactose	glucose	protein ($N \times 6.25$)	ash	total	arabinose + xylose	
ethanol filtrate (yield = 9.5)[b]	0.2 ± 0.1	0.1 ± 0.1	7.7 ± 0.1	traces	23.2 ± 0.1	38.0			0.3 ± 0.1	0.36
water extract (yield = 4.67 ± 0.04)[c]	11.8 ± 0.3	12.0 ± 0.7	4.9	0.7	31.2	12.8 ± 0.6	12.3 ± 0.8	85.7	23.8 ± 1.0	1.01
water-soluble pentosans (yield = 1.14 ± 0.02)[d]	28.2 ± 1.2	38.7 ± 0.2	4.8 ± 0.2	2.9 ± 0.2	6.7 ± 0.1	10.3 ± 0.1	6.2 ± 0.2	97.8	66.9 ± 1.3	1.37
water-insoluble pentosans without NaOH extraction (yield = 2.02 ± 0.13)[d]	24.0 ± 0.6	26.1 ± 0.9	1.8 ± 0.1	2.9 ± 0.1	27.1 ± 0.2	13.3 ± 0.0	4.5 ± 0.7	99.6	50.1 ± 1.1	1.08
with NaOH extraction (yield = 1.03 ± 0.03)[d]	22.6 ± 1.0	25.4 ± 1.0	1.7 ± 0.1	2.4 ± 0.1	24.1 ± 0.1	14.8 ± 0.1	6.6 ± 0.3	97.6	48.0 ± 2.0	1.12

[a] Results are expressed as a percent of the dry matter content of the fraction. Mean ±SE of two samples with two injections (analyses) per sample for carbohydrates. Protein and ash were determined in duplicate. Values without SE represent single analysis. [b] Yield is expressed as a percent of raw rye that was extracted in 3.5 volumes (w/v) of boiling 80% ethanol. [c] The value in parentheses represents the percent yield of dry matter obtained following the extraction of ethanol-boiled rye with 4 volumes of water. [d] Yields (mean ± SE) of crude pentosans as a percent (dry matter basis) of the ethanol-boiled rye.

tributed to the pentosans not precipitated in 80% ethanol, particularly those having a high arabinose content. This was supported by the fact that the xylose to arabinose ratio was 1.01:1 for the water extract and 1.37:1 for the ethanol-precipitated water-soluble pentosans (Table III).

It was estimated from data given in Table II and III that only 37% (1.14 × 0.67 vs. 2.08%) of the soluble and ~12% (2.02 × 0.5 vs. 8.35%) of the insoluble pentosans (xylose plus arabinose) were obtained from rye by using isolation procedures designed for the preparation of large quantities of pentosan required for the subsequent feeding trial. Thus only ~17% (1.77 vs. 10.4%) of the total soluble and insoluble pentosans were removed from the ethanol-boiled rye, which indicates the great difficulty encountered in complete removal of pentosans from rye grain.

The xylose plus arabinose content of the carbohydrate portion of the different fractions was 91, 61, and 63% for the isolated water-soluble and insoluble pentosans with or without NaOH extraction, respectively (Table III). This indicated the preponderance of pentoses as the major components of rye pentosans. The soluble and insoluble pentosans also contained minor quantities of mannose, galactose, and glucose. Holas et al. (1972), using pentosan isolation procedures different from those employed in the current study, showed by paper chromatography that highly purified pentosans contained all these sugars except for mannose. The absence of mannose could be attributed to its coelution with xylose. Most of the glucose was derived from starch since the average values of the last two amyloglucosidase digestion periods (Table IV) indicated that its content in the soluble and insoluble pentosans extracted with or without NaOH were 5.5, 15.4, and 16.1%, respectively. Comparing these values with the total glucose content of the samples (Table IV) suggests that the soluble and insoluble pentosans with or without NaOH extraction contained respectively 0.5, 5.6, and 4.4% nonstarchy glucose (the difference between total glucose content and glucose derived from starch). This nondigestible glucose might be part of the pentosan molecule or might be derived from a β-glucan. By use of the colorimetric procedure, the content of pentoses in the soluble pentosan fraction was found to be the same (55.6%) as that reported by Holas et al. (1972), while the corresponding estimated purities of the insoluble pentosans were 34 and 52%, respectively. In the latter study, the water-insoluble pentosans were isolated by extraction of whole rye with NaOH for 1 month. In the current study, it was not possible to utilize procedures that yielded more highly purified pentosan preparations as large quantities of pentosans were required for the chick feeding trials. The failure to obtain pure pentosans may also be attributed to the glycoprotein nature of most pentosan subfractions (Holas et al., 1972) which complicates the purification and quantitation of both pentosan fractions. The current results demonstrated that extraction of the insoluble pentosans with NaOH did not improve the purity but it reduced the yield compared to those not treated with NaOH. However, extraction of the insoluble pentosans with NaOH increased their viscosity as indicated by time of flow (152 s) compared to that of those not extracted with NaOH (120 s). The viscosity of the soluble pentosans was the highest (188 s).

The xylose to arabinose ratios (Tables II and III) indicate the degree of branching of the pentosan molecule, a compound mainly composed of a backbone of β-D-(1→4)-linked xylopyranose residues to which single α-L-arabinofuranose unit is attached through 1→3 linkages (Aspinall and Greenwood, 1962; Aspinall and Ross, 1963; D'Appolonia, 1973). Results from the isolated pentosans

Table IV. Colorimetric Determination of the Starch and Total Glucose Content of Pentosan Preparations Using α-Amyloglucosidase Digestion or Acid Hydrolysis[a]

	%			
	digestion period, h			
pentosan preparation	4	12	20	total glucose[b]
water-soluble pentosans	4.6 ± 0.5	5.6 ± 0.2	5.3 ± 0.2	6.0 ± 0.1
water-insoluble pentosans				
without NaOH extraction	12.5 ± 0.5	17.0[c]	15.2 ± 0.6	20.6 ± 0.4
with NaOH extraction	11.1 ± 0.8	14.3 ± 1.0	16.4 ± 0.3	21.0 ± 0.8
corn starch	100.3 ± 4.0	100.3 ± 1.0	100.9 ± 0.7	

[a] Results are expressed as a percent of the dry matter content of the pentosan fraction. Mean ± SE of two samples analyzed in duplicate. [b] Values obtained after a 3-h acid hydrolysis. [c] Value represents a single analysis.

Table V. Effect of Dietary Treatment on the Performance of Growing Chicks (Experiment 2)

	response criteria[a]						
dietary treatment	feed intake, g	weight gain, g	feed:gain	dry matter retention, %	fat digestibility, %	total amino acid[b] digestibility, %	pentosan digestibility, %
rye	85.2B	30.6B	2.79BC	58.8A	23.6a	64.2A	0
ethanol-boiled rye after partial removal of water-soluble and insoluble pentosans	89.7BC	32.4B	2.79BC	59.5A	31.4a	64.6A	0
wheat	100.1C	47.9D	2.10A	65.8B	65.5bc	78.8C	
wheat plus ethanol filtrate	99.4C	47.5D	2.09A	67.8B	67.6c	79.6C	
wheat plus water extract	96.3C	38.1BC	2.53AB	60.1A	60.8bc	73.0B	
wheat plus water-soluble pentosans	96.6C	40.9CD	2.36AB	61.6A	55.9b	72.9B	
wheat plus soluble pentosans equal to insoluble level	66.2A	19.7A	3.37C	60.4A	54.9b	69.1B	
wheat plus insoluble pentosans without NaOH extraction	66.2A	23.4A	2.90BC	59.3A	75.6d	71.6B	
wheat plus insoluble pentosans after NaOH extraction	67.8A	22.3A	2.94BC	58.5A	64.6bc	70.7B	
SE	2.20	1.68	0.12	0.68	2.54	0.87	

[a] Means within each column not sharing a common letter differ significantly at $P \leq 0.05$ (lower-case letters) or at $P \leq 0.01$ (upper-case letters). [b] Amino acid digestibility does not include cystine, methionine, and tryptophan.

(Table III) indicated that the soluble fraction (ratio 1.37:1) was less branched than the insoluble fraction (ratio 1.10:1). Similar results (1.32:1 and 1.17:1, respectively) were also reported by Holas et al. (1972). The xylose to arabinose ratio of the total insoluble pentosans from raw or ethanol-boiled rye (Table II) was much higher (1.57:1) than the ratio of the isolated water-insoluble pentosans (1.10:1). This indicated that those insoluble pentosans which contained a high level of xylose were not easily hydrated and therefore did not appear in the more highly hydrated sludge fraction.

Effect of Rye Pentosans on Chick Performance. In experiment 1 the addition of the water-soluble pentosan fraction (2.3%) to a wheat-based diet depressed ($P < 0.01$) weight gain (19%) and feed conversion efficiency (13%). The freeze-dried extract from rye was also active and depressed ($P < 0.05$) these two parameters but to a lesser degree (11 and 9%, respectively) compared to the isolated pentosan fraction. The actual amount of added pentosans in the latter diet (0.63%) was also less than that of the former diet (1.27%). In both treatments, however, feed intake was not significantly reduced compared to that of the wheat diet.

Results for experiment 2 are given in Table V. Diets containing wheat or wheat plus the ethanol filtrate were similar but higher in nutritional values, as indicated by the different criteria of performance, than all other diets. The inability of the ethanol filtrate to depress chick performance was attributed to its negligible pentosan content (0.3%). Also, the partial removal of the water-soluble and insoluble pentosans from the ethanol-boiled rye did not improve its feeding value compared to that of raw rye. This may be attributed to the fact that this fraction contained a pentosan level which was similar to that of raw rye. Fernandez et al. (1973), in contrast, reported considerable improvement in the feeding value of the raw rye after water extraction, possibly because pentosan degradation may have occurred in their preparation. This may be attributed to the failure to inactivate the endogenous (Preece and Hobkirk, 1953) and the exogenous (microbial) pentosanases prior to extraction. Further evidence for the enzymatic and microbial degradation of rye pentosans was demonstrated by Holas and Hampl (1973) during dough leavening. In the current study, this type of degradation would not occur as endogenous enzymes would have been inactivated by the initial ethanol heat treatment and microbial activity would have been inhibited by the use of the bacteriostatic agent.

When the freeze-dried extract or the isolated water-soluble pentosans supplied the wheat-based diets with the same level of soluble pentosans as that found in similar rye diets, all response criteria were depressed compared to those for the wheat diet except for feed intake which was slightly affected (Table V). However, only the feed to gain ratio and retention of dry matter of these two treatments were similar to those of the rye treatment. The

similar response of birds fed these two diets suggested that the antinutritional factor of the water extract was the soluble pentosans. In addition to observations reported in these studies, Antoniou and Marquardt (1981) have also reported that chick growth was depressed by isolated rye pentosans.

The addition of the water-soluble or insoluble pentosans to wheat-based diets at the same level as the insoluble pentosans of rye diets depressed ($P < 0.01$) average feed intake (35%), weight gain (55%), feed to gain ratio (50%), dry matter retention (10%), and total amino acid digestibility (11%) compared to those of the wheat diet. The pattern for individual amino acids, which is not given in this paper, was the same as that for total amino acids. Fat digestibility, in contrast, was depressed (16%) only by the high level of soluble pentosans (Table V). In comparison to birds fed raw rye, those fed the three high pentosan containing diets had similar feed to gain ratios and dry matter retentions but lower ($P < 0.01$) feed intake (20%) and weight gain (24%). Of the two insoluble pentosans, those extracted with NaOH depressed fat digestibility to a greater degree, perhaps because of their higher viscosity. The higher viscosity of the soluble compared to the insoluble pentosans could also be responsible for their higher growth-depressing effect at equal dietary levels. The type and the level of rye pentosans also affected the condition of feces. Birds fed the insoluble pentosans, particularly those not extracted with NaOH, produced less watery feces compared to birds fed rye or the water-soluble pentosans. The high level of the soluble pentosans produced feces with the highest water content.

The digestibility of pentosans (xylose and arabinose) for the two analyzed treatments (Table V) was found to be zero with an almost unchanged xylose to arabinose ratio (1.68:1), which further suggests the inability of the avian digestible system to degrade rye pentosans.

DISCUSSION

The current study demonstrated that the pentosans are the major growth depressing factor in rye and that the insoluble pentosans which are present rye at a much higher level than the soluble pentosans (7.7 vs. 2.1%) are responsible for the greatest portion of the effect. Rye pentosans reduced chick growth by depressing feed intake and the digestion and absorption of all nutrients. Similar effects of rye grain on chick performance were also reported by McNab and Shannon (1975) and Marquardt et al. (1979). The ability of rye pentosans to absorb and retain large volumes of water with concomitant swelling (Casier and Soenen, 1967) may have caused a premature satiety feeling in the birds which would have reduced nutrient intake. The depressed digestibility of all nutrients, particularly by the soluble pentosans, could be explained by their high viscosity, which interferes with enzymatic digestion of the nutrients and their subsequent absorption, and their ability to bind ions, like Ca^{2+} (Drews and Seibel, 1976). This latter proposal is supported by the high ash content of the isolated pentosans (Table III). Podrasky (1964) reported that the major viscosity factor of the soluble pentosans was a specific arabinoxylan. This fraction which represented 75% of the total water-soluble pentosans had the highest molecular weight relative to that of water-soluble pentosans isolated from other cereals. The effects of rye pentosans on chick growth were also similar to those observed with other mucilaginous polysaccharides (pectins, guar gum, and gum arabic) by Kratzer et al. (1967) and Vohra et al. (1979). The fact that the high level of added pentosans did not depress amino acid and fat digestibility to the level obtained by the rye diets could be attributed to various causes. First, none of the wheat diets contained both the soluble and insoluble fractions, which might have had a marked additive or synergistic effect on nutrient digestion. Second, the isolated pentosans might have been partially denatured through conformational changes which would cause reduced viscosity and antinutritional activity. Such changes have been reported for several other pentosans and might be induced by drying (Wilkie, 1979), temperature variation during the isolation procedure (Dea et al., 1973), and/or by the presence of minerals, such as Mg^{2+} and Ca^{2+} (Blake et al., 1970). Third, some highly active insoluble pentosans would not have been isolated from rye.

It may be concluded that the highly branched water-insoluble pentosans and, to a lesser degree, the water-soluble pentosans are the principal antinutritional factor in rye grains and that their effects are manifested by reduced feed intake and digestibility of all nutrients. The antinutritional activity of rye pentosans can be attributed to their high viscosity, water-absorbing properties, and possibly nutrient binding capacity. The highly viscous pentosans may interfere with the diffusion of digestible enzymes to the substrate and with the subsequent diffusion of hydrolyzed substrate to the absorptive sites in the gastrointestinal tract; the net effect is a reduced availability of nutrients to the host animal and a concomitant increase to the intestinal microflora. Given the complex structure and heterogenicity of pentosans, further research is required to accurately quantitate the effects of specific pentosans and to identify the physical, chemical, and biological properties of the various pentosan subfractions.

ACKNOWLEDGMENT

Technical assistance provided by S. Lam, J. Rogers, and J. McKirdy is greatly appreciated.

LITERATURE CITED

Antoniou, T.; Marquardt, R. R. *Poult. Sci.* **1981**, in press.
Antoniou, T.; Marquardt, R. R.; Misir, R. *Poult. Sci.* **1980**, *59*, 758.
Aspinall, G. O.; Greenwood, C. T. *J. Inst. Brew.* **1962**, *58*, 167.
Aspinall, G. O.; Ross, K. M. *J. Chem. Soc.* **1963**, 1681.
Association of Official Analytical Chemists "Official Methods of Analysis", 11th ed.; AOAC: Washington, DC, 1970.
Blake, J. D.; Murphy, P. T.; Richards, G. N. *J. Macromol. Sci., Chem.* **1970**, *A4*, 1247.
Casier, J. P. J.; Soenen, M. *Getreide Mehl* **1967**, *17*, 46.
Cerning, J.; Guilbot, A. *Cereal Chem.* **1973**, *50*, 176.
Church, D. C. "Digestive Physiology and Nutrition of Ruminants"; Church, D. C., Ed.; O & B Books: Corvallis, OR, 1976; Vol. 1, p 127.
Cole, E. W. *Cereal Chem.* **1967**, *44*, 411.
D'Appolonia, B. L. "Industrial Uses of Cereal Gums"; American Association of Cereal Chemists, Inc.: St. Paul, MN, 1973.
Dea, I. C. M.; Rees, O. A.; Beveridge, R. J.; Richards, G. N. *Carbohydr. Res.* **1973**, *29*, 363.
Drews, E.; Seibel, W. "Rye: Production, Chemistry and Technology"; American Associates of Cereal Chemists, Inc.: St. Paul, MN, 1976.
Fernandez, R.; Lucas, E.; McGinnis, J. *Poult. Sci.* **1973**, *52*, 2252.
Golenkov, V. F.; Traubenberg, G. D. *Prikl. Biokhim. Mikrobiol.* **1966**, *2*, 27.
Holas, J.; Hampl, J. *Sb. Vys. Sk. Chem.-Technol. Praze, Potraviny* **1973**, *E38*, 19.
Holas, J.; Hampl, J.; Picmanova, B. *Getreide, Mehl Brot* **1972**, *26*, 144.
Kratzer, F. H.; Rajaguru, R. W. A. S. B.; Vohra, P. *Poult. Sci.* **1967**, *46*, 1489.
Lindberg, B. *Methods Enzymol.* **1972**, *28*, 182.
Lorenz, K. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *14*, 311.
MacAuliffe, T.; Pietraszek, A.; McGinnis, J. *Poult. Sci.* **1976**, *55*, 2142.
Marquardt, R. R.; Ward, A. T.; Misir, R. *Poult. Sci.* **1979**, *58*, 631.

McNab, J. M.; Shannon, D. W. F. *Br. Poult. Sci.* **1975**, *16*, 9.
Miller, D. F. National Academy of Sciences Publication 585, Washington, DC, 1958.
Misir, R.; Marquardt, R. R. *Can. J. Anim. Sci.* **1978**, *58*, 731.
Moore, S.; Stein, W. H. *Methods Enzymol.* **1963**, *6*, 819.
Moran, E. T., Jr.; Lall, S. P.; Summers, J. D. *Poult. Sci.* **1969**, *48*, 939.
National Research Council "Nutrient Requirements of Poultry", 7th revised ed.; National Academy of Sciences, National Research Council: Washington, DC, 1977.
Podrasky, V. *Chem. Ind. (London)* **1964**, *2*, 712.
Preece, I. A.; Hobkirk, R. *J. Inst. Brew.* **1953**, *59*, 385.
Preece, I. A.; MacKenzie, K. G. *J. Inst. Brew.* **1952**, *58*, 457.
Sigma Co. "The Enzymaitc Colorimetric Determination of Glucose in Whole Blood, Plasma and Serum". Technical Bulletin 510, St. Louis, MO, 1978.
Simpson, F. I. *Can. J. Microbiol.* **1954**, *1*, 131.
Sloneker, J. *Methods Carbohydr. Chem.* **1972**, *6*, 20.
Snedecor, G. W.; Cochran, W. G. "Statistical Methods", 6th ed.; Iowa State University Press: Ames, IA, 1967.
Vohra, P.; Shariff, G.; Kratzer, F. H. *Nutr. Rep. Int.* **1979**, *19*, 463.
Vones, F.; Podrasky, V.; Simova, J.; Vesely, Z. *Cereal Chem.* **1964**, *41*, 456.
Wieringa, G. W. "On the Occurrence of Growth Inhibiting Substances in Rye". Institute for Storage and Processing of Agricultural Produce Publication 156, Wageningen, The Netherlands, 1967.
Wilkie, K. C. B. *Adv. Carbohydr. Chem. Biochem.* **1979**, *36*, 215.

Received for review February 12, 1981. Revised manuscript received May 18, 1981. Accepted May 18, 1981. This investigation was supported in part by the Grains and Oilseeds Marketing Incentives Program, Department of Industry, Trade and Government of Canada.

Nutrient Composition of Millet (*Pennisetum typhoides*) Grains and Malt

Andy R. Opoku,* Sam O. Ohenhen, and Ngozi Ejiofor

Millet grains were germinated for 84 h and kilned at 45 °C to obtain a malt product. Analyses of vitamins, phytate, oxalate, tannins, total phenols, and calcium were conducted to determine the nutritional value of the grains and the malt. The levels of vitamins were higher in the malt than in the grains. Slight increases in protein and total phenol were observed in the malt, while lipid, phytate, and oxalate levels decreased during malting.

Millet (*Pennisetum typhoides*) is one of the widely cultivated crops in Nigeria and in Savannah regions immediately south of the Sahara. It is used as a cereal food and in the production of locally brewed alcoholic beverages (Nielson, 1965; Oyenuga, 1968; Oke, 1977).

There is an emphasis on the improvement of nutritive value of foods in the developing countries where the contribution of cereal protein is significant in the general protein shortage. The desire to use local cereals as malting material in the industries has enhanced the importance of millet in the economy.

The purpose of this study was to determine the nutrient composition of millet grains and malt and to assess their nutritional value. Several studies (Hiatt, 1972; Fordham, et al., 1975; Hamilton and Vanderstoep, 1979) have shown that nutritional values of seeds are increased during germination.

MATERIALS AND METHODS

Millet (*Pennisetum typhoides*) was obtained from a local market in Benin City, Nigeria. Grains were hand selected for approximately equal size. The seeds were surface sterilized with 1% sodium hypochlorite solution for 10 min at room temperature and steeped in sterile distilled water for 12 h. The hydrated grains were subsequently transferred to sterile Petri dishes lined with two circles of sterile 9-cm Whatman's No. 1 filter paper, containing 2 mL of sterilized distilled water (Aisien and Ghosh, 1978). Germination was in the dark at 25 °C for 84 h. Germinated grains were kilned at 45 °C and then ground to pass a 20-mesh screen.

Department of Biochemistry, University of Benin, Benin City, Nigeria.

Table I. Proximate Analysis of Millet Grain and Its Malt[a]

	grain	malt[b]	soaking medium[c]
moisture, %	10.2	4.3	98.7
protein, %	8.6	11.8	t
lipid, %	7.5	2.5	nd
ash, %	4.1	3.2	0.07
lignin, %	1.3	4.4	nd
fiber, %	10.4	18.6	nd
carbohydrate, %	53.9	48.5	t

[a] Analyses were made in duplicate, and results are expressed on a dry weight basis. [b] Results are absolute yields. [c] t, trace amounts (less than 0.02%); nd, not detected.

Proximate Analysis. The moisture content was determined by drying the samples at 130 °C to constant weight (AOAC, 1975). Ash was determined by weighing the residue of charred (570 °C for 6 h) sample. The micro-Kjeldahl method was used to determine nitrogen (AOAC, 1975). Crude protein was calculated by multiplying % *N* by 6.25. Fat was extracted with petroleum ether. Lignin was determined by the method of Kornshchikov (1968). Carbohydrate content was determined as starch by the method of Hassid and Neufeld (1964).

Estimation of Vitamins. Riboflavin and niacin were assayed by the chemical methods recommended by the American Association of Cereal Chemists (1962). Thiamin determination was by the chemical assay method involving

0021-8561/81/1429-1247$01.25/0