Molecular Weight Changes in the (1→3)(1→4)-β-D-Glucan of Oats Incurred by the Digestive Processes in the Upper Gastrointestinal Tract of Pigs[†]

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Pigs were fed three diets containing different mill fractions from oats—oat flour, rolled oats, and oat bran. The molecular weight of the (1→3)(1→4)-β-D-glucan (β-glucan) in diets and from the jejunal contents of the pigs was determined by size exclusion chromatography. There was no significant difference in the molecular weight (MW) of the β-glucan in the diet, regardless of origin. MW was reduced 7-35-fold after passage through the stomach and the proximal small intestine of pigs, oat bran being least affected. Depolymerization toward a specific MW range of about 100 000 was seen in some digesta samples, indicating cleavage at randomly distributed susceptible bonds. The oat fractions themselves had some capacity to degrade β-glucan, but this was insufficient to account for the MW loss during digestion. Degradation also took place when isolated β-glucan was incubated with jejunal digesta from pigs fed an oat-free diet. Fractionation of the digesta by ultracentrifugation showed that the degrading activity was mostly associated with the supernatant. Since autoclaved digesta were without effect, the activity was presumably enzymatic, originating from microbes present in the gut. Neither isolated β-glucan nor crude extracts from oat bran were sensitive to trypsin, indicating that cleavage of peptide bonds was not responsible for the reduction in molecular weight and loss of viscosity in digesta from pigs.

INTRODUCTION

The soluble fraction of dietary fiber (DF) of oats mainly consists of (1→3)(1→4)-β-D-glucan (β-glucan) (Bach Knudsen and Hansen, 1991; Wood et al., 1989a). Recent studies have shown a highly significant inverse relationship between blood glucose and insulin levels of healthy human subjects and log[viscosity] of the β-glucan solution fed (Wood et al., 1993). Increased luminal viscosity is believed to cause delayed gastric emptying and reduced rate of absorption of nutrients in the small intestine, thereby inducing an attenuated glucemic response to an oral carbohydrate load (Jenkins et al., 1987). Studies of *in vivo* viscosity in pig jejuna, however, failed to find a direct relation to glucose absorption (Low et al., 1986; Rainbird and Low, 1986), and jejunal viscosities attained with guar gum of different molecular weights have been nonproportionally lower than the *in vitro* viscosity of a comparable solution (Roberts et al., 1990).

The viscosity of solutions of oat DF depends, *inter alia*, on molecular weight (MW), concentration, and structure of the cell wall polysaccharides (Morris, 1990; Vårum and Smidsrød, 1988; Wood et al., 1990). The behavior of the intact cell walls of oat products undergoing digestion will additionally depend on extractability of the fiber (Wood et al., 1989a, 1990; Asp, 1990). The aim of the present investigation was to study the MW of β-glucan from three differently milled fractions of oats after passage through the stomach and duodenum of pigs and to identify factors responsible for any changes observed.

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EXPERIMENTAL PROCEDURES

General. Chemicals and feed ingredients were obtained from appropriate commercial sources. Oat flour was produced by disk milling and sifting of oat groats (Bach Knudsen et al., 1993); a quantity of rolled oats was supplied by OTA A/S, Nakskov, Denmark, and oat bran by Kungsörnen AB, Järna, Sweden. Barley β-glucan was obtained from Biocon (USA) Inc., Lexington, KY. Oat gum (PPOG) was prepared (Wood et al., 1989b) at the POS Pilot Plant Corp., Saskatoon, SK, Canada. A further sample of oat gum, oat gum grade I (OGGI), was obtained from VTT Technical Research Centre of Finland, Espoo, Finland. Porcine trypsin (EC 3.4.21.4, activity 1.130 BAEE and 1.540 ATEE units/mg of solid) was obtained from Sigma Chemical Co., St. Louis, MO.

Analytical Methods. Viscosity was measured over the shear rate range 2.25-450 s^{-1} in a Brookfield DV-II cone and plate viscometer at 38 °C. Apparent viscosity (mPa·s) is reported at a shear rate of 45 s^{-1}.

MW was determined by high-performance size exclusion chromatography (HPSEC) on a Bio-Gel TSK 60-XL column at a flow rate of 1 mL/min in 0.05 M sodium *N*-(morpholino)-ethanesulfonate (MES) buffer, pH 6.5, essentially as described by Wood et al. (1991b). All solutions for determination of MW were filtered through Millipore filters (5 μm) prior to chromatography. Postcolumn addition of Calcofluor (0.005% w/v) in 0.1 M tris(hydroxymethyl)aminomethane (Tris), pH 8.0, at a flow rate of 1 mL/min was used, with fluorescence detection (excitation, 365 nm; emission, 425 nm). The column was calibrated with four standard solutions of β-glucan of previously determined molecular weights: barley β-glucan (MW 1.9×10^5), a purified oat β-glucan (8.8×10^5), POS-27 gum (1.2×10^6), and bench gum (2.2×10^6) (Wood et al., 1991b). Both crude extracts of β-glucan and isolated preparations are polydisperse, and values quoted are calculated from the retention volume of the chromatographic peak either directly measured or from the midpoint of the peak at half-height.

β-Glucan was analyzed according to the method of McCleary and Glennie-Holmes (1985) using the MegaZyme mixed-linkage β-glucan assay (Megazyme Pty. Ltd., North Rocks, NSW, Australia).

0021-8561/93/1441-2347$04.00/0 © 1993 American Chemical Society

Statistical Methods. Results were examined by a one-way analysis of variance model (Snedecor and Cochran, 1973)

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

where μ is the overall mean, α_i is the effect of diet or treatment, and ϵ_{ij} is a normally distributed residual random variable. Differences between parameters were inspected by pairwise comparison in the Student–Newman–Keuls test (Snedecor and Cochran, 1973). Prior to statistical analysis, data on viscosity were subjected to log transformation, and values are given as geometric means (Snedecor and Cochran, 1973). Standard errors on viscosity data are presented as antilog of SEM from logarithmic values. All statistical calculations were performed using a SuperANOVA program (Abacus Concepts, Berkeley, CA).

MW of β-Glucan in Jejunal Digesta of Pigs Fed Oats. Comparison of Oat Flour, Rolled Oats, and Oat Bran Diets (Experiment 1). Four crossbred barrow pigs weighing approximately 30 kg were fitted with two re-entrant cannulas in the jejunum as described by Horszczaruk et al. (1974) and Rainbird et al. (1984). The first set of re-entrant cannulas were placed approximately 1 m distal to the pylorus and the second set approximately 1.5 m distal to the first set. This provided an isolated loop of the jejunum. Data in this paper are part of a larger project and deal only with samples from the proximal cannula. The pigs were allowed 10 days to recover from surgery before they were fed the experimental diets over a 4-week period. The composition of the three experimental diets containing oat flour (OF), rolled oats (RO), and oat bran (OB) is shown in Table I. The pigs were fed each diet for 1 week in a Latin Square design in three equal meals at 7:00 a.m., 3:00 p.m., and 10:00 p.m. The total daily intake of feed was 45 g/kg of body weight and the water intake was 2.5–3 times the feeding level. Pigs were placed in metabolism crates before the morning feeding and stayed there during the 8-h study period. In the periods between collections the pigs were housed individually in 4-m^2 smooth-sided pens with a concrete floor. The pigs switched diets over a 3-day period. The first collection period took place after 2 days on the new diet and was repeated after 2 days. Cannulas were disconnected to allow collection of jejunal digesta. After feeding, digesta were collected on ice for 7.5 h. In short intervals digesta were weighed, and 10% was taken for analysis. The remaining digesta were returned to the pig through the cannula. Digesta were pooled into six periods, 0–0.5, 0.5–1, 1–2, 2–3, 3–5, and 5–7.5 h after feeding, and freeze-dried. Freeze-dried digesta (100 mg) were treated with 5 mL of aqueous ethanol (80% v/v) for 2 × 1 h at 85 °C with constant stirring and centrifuged for 10 min at 14500*g* after each extraction. The sediments were washed with absolute ethanol (99% v/v) and dried under nitrogen. The ethanol-treated samples were extracted with 3 mL of carbonate buffer (pH 10.0, ionic strength 0.2) for 2 h at 60 °C with constant stirring; another 2 mL of carbonate buffer was added, and the samples were centrifuged for 20 min at 14500*g* at 4 °C. RO samples were further diluted 1–2 times and OB samples 2–4 times with carbonate buffer prior to HPSEC of the supernatant.

For MW determination of undigested β-glucan from OF, RO, and OB, duplicate samples of the dry diet were refluxed for 2 × 10 min in 50% (v/v) ethanol. The alcohol wetted samples were then extracted in carbonate buffer as for the digesta, and the supernatant from the extraction was analyzed by HPSEC.

Effect of Digesta on Isolated β-Glucan (Experiment 2). Five pigs were fitted with one set of re-entrant cannulas placed approximately 1.5 m from the pylorus. General surgical procedure and care after recovery were as described in experiment 1. Pigs were fed twice daily with an oat-free diet (wheat flour, 742 g/kg; wheat bran, 70 g/kg; casein, 118 g/kg; soy oil 40 g/kg; vitamin/mineral mixture, 30 g/kg) containing less than 0.3% β-glucan. Daily feed intake was 35 g/kg of body weight and the feed/water ratio 1:2.5.

Immediately after the morning feeding, approximately 200 mL of jejunal digesta was collected from the first cannula of the pigs and filtered through sterile cheesecloth. A portion (50 mL) of the filtered digesta was autoclaved and another 50-mL portion centrifuged at 20000*g* for 20 min to give supernatant and sediment. The sediment of digesta from two pigs (trial 1) was directly resuspended in anaerobic salt medium (content in g/L : NaCl, 0.090; KH_2PO_4, 0.45; $CaCl_2{\cdot}2H_2O$, 0.03; $MgCl_2{\cdot}6H_2O$, 0.02; $MnSO_4{\cdot}4H_2O$, 0.01; $CoCl_2{\cdot}6H_2O$, 0.01; $(NH4)_2SO_4$, 0.90; $K_2HPO_4{\cdot}3H_2O$, 0.45; $NaHCO_3$, 5.00; cysteine chloride, 0.25). In preparations from three other pigs (trial 2) the sediment was washed with the salt medium and recentrifuged before resuspension.

A 1% (w/v) stock solution of PPOG in distilled water was prepared by heating to about 70 °C and stirring. The PPOG solution (20 mL) was incubated with 20 mL of filtered digesta, autoclaved digesta, supernatant, and resuspended sediment, respectively. The mixture was incubated in a shaking water bath at 38 °C and sampled at 0.5, 1, 3, and 5 h. All preparations were kept sterile and anaerobic during handling and incubation. The incubation mixture was centrifuged at 6000*g* for 20 min at 4 °C immediately after sampling and the supernatant heated in a boiling water bath for 10 min. After cooling, the viscosity of the supernatant was determined at 38 °C.

Duplicate samples of 1 mL of the supernatant from each incubation were freeze-dried and treated with 5 mL of 50% ethanol (v/v) in a boiling water bath for 5 min. A further 5 mL of 50% ethanol was added, and the mixture was centrifuged at 6000*g* for 10 min. The supernatant was discarded and the residue mixed with 5 mL of 99% (v/v) ethanol. The samples were recentrifuged and the supernatant was again discarded. The alcohol-treated samples were dried in a vacuum oven at 50 °C overnight. The freeze-dried alcohol-treated samples from trial 1 were dissolved in MES buffer at 70 °C for 3.5 h and centrifuged at 14500*g* before analysis by HPSEC.

Effect of Endogenous Enzymes of Diet on MW of β-Glucan. Diets were incubated with 0.2 M sodium acetate buffer, pH 4.5 (100 g of air-dry feed/L), at 40 °C with constant stirring. After 1, 3, and 8 h of incubation, aliquots of 1 mL in duplicate were freeze-dried and stored prior to ethanol treatment and extraction as described in experiment 1. Aliquots of the extracts were analyzed by HPSEC.

Effect of Trypsin on Oat β-Glucan Extracts. OB was treated with refluxing 75% ethanol/water (v/v) in a boiling water bath for 2 h with constant stirring and then centrifuged for 10 min at 6000*g* and the supernatant removed. The bran was washed with 75% ethanol followed by 99% ethanol and dried in a vacuum oven at 40 °C. A 10% (w/w) suspension of the alcohol-treated oat bran was extracted with 1 M sodium carbonate buffer, pH 10.0, at 38 °C for 1 h and centrifuged at 12000*g* for 20 min. The pH of the supernatant was adjusted to 6.5 with 0.1 M HCl.

The two oat gums, PPOG and OGGI, were dissolved in distilled water containing 5 mM sodium azide [0.5% and 1% (w/v), respectively]. Porcine trypsin (2, 1, and 0.5 mg/mL) was added to the oat bran extract, OGGI, and PPOG, respectively. Samples were maintained at 38 °C for 3 h, and viscosity was measured at short intervals after heat treatment in a boiling water bath for 5 min.

RESULTS

Molecular Weight of β-Glucan in Jejunal Digesta. The mean MW of β-glucan in the three oat-based diets was 2.2–2.6 × 10^6 (Table I). After passage through the stomach and proximal small intestine, the MW of β-glucan from the digesta collected within the first 0.5 h after feeding was reduced to 7.4 × 10^5 in OF, 9.9 × 10^5 in RO, and 1.7 × 10^6 in OB (Table II). Depolymerization continued with time of residence of the β-glucan in the gastrointestinal tract, resulting in a reduction in MW of β-glucan to 0.9 × 10^5 for RO, 1.8 × 10^5 for OF, and 3.5 × 10^5 for OB 5–7.5 h postprandial. Typically, in addition to having a longer retention time, the peaks became broader representing a wider range of MWs. Peak asymmetry increased, and there was a tendency for the leading edge to form a shoulder. In some digesta samples the shift in retention time resulted in splitting into two peaks, typically with maxima at 1.9 × 10^6 and 1.0 × 10^5 (63 000–166 000) indicating a specific degradation of high MW β-glucan to a population with a 20 times smaller mean MW.

Effect of Jejunal Digesta on Viscosity and Molecular Weight of Oat Gum. Autoclaved digesta were

Table I. Composition (g/kg) and Physical Properties of Diets Based on Oat Flour (OF), Rolled Oats (RO), and Oat Bran (OB) and Used in Experiment 1

	OF	RO	OB
oat flour	867		
rolled oats		900	
oat bran			943
casein	103	70	27
vitamin/mineral[a]	30	30	30
β-glucan content[b] (g/kg of DM)	19	36	80
β-glucan extractability[c]	0.70	0.52	0.45
β-glucan MW ($\times 10^{-5}$)	22.2	23.2	26.1

[a] Provided (in mg or IU) per kg of diet: $Ca_2(PO_4)_3$, 17 000; K_2PO_4, 5700; NaCl, 4000; $CaCO_3$, 2500; $FeSO_4{\cdot}7H_2O$, 212; ZnO, 85; MnO, 31; $CuSO_4{\cdot}5H_2O$, 68; KI, 0.2; $Na_2SeO_3{\cdot}5H_2O$, 0.6; retinyl acetate, 3400 IU; cholecalciferol, 850 IU; α-tocopherol, 43; menadione, 1.7; riboflavin, 3.4; pantothenic acid, 8.5; cobalamin, 0.02. [b] Determined by the method of McCleary and Glennie-Homes (1985). [c] Determined from HPSEC of carbonate extract (2 h, 60 °C) and expressed as a fraction of the total β-glucan content.

Table II. Molecular Weight ($\times 10^{-5}$) of β-Glucan in Digesta Collected at Various Times after Feeding the Diets Based on Oat Flour (OF), Rolled Oats (RO), and Oat Bran (OB)[a]

digesta collection time (h)	OF	RO	OB
0–0.5	7.4[b]	9.9[b]	16.8[a]
0.5–1	8.3[b]	9.8[b]	18.4[a]
1–2	7.4[a] (2)[b]	8.1[a] (2)	13.4[a]
2–3	na[c]	2.3[b] (3)	9.3[a] (3)
3–5	2.4[b]	1.3[b]	5.9[a]
5–7.5	1.8[ab]	0.9[b]	3.5[a]

[a] Values in the same row with different letters are significanly different ($p < 0.05$). [b] Numbers in parentheses are numbers of replicates if less than 4. [c] na, not available.

Table III. Viscosity[a] (mPa·s) Change with Time of 1% Oat Gum Solution Incubated for 5 h with Fractions of Jejunal Digesta (1:1)[b]

time (h)	autoclaved	filtered	supernatant	sediment	SEM[c]
		Trial 1[d]			
0.5	35.1[aA]	12.1[bA]	9.3[bA]	26.2[aA]	1.11
1.0	39.5[aA]	5.2[cB]	4.1[cB]	24.0[bA]	1.12
3.0	39.2[aA]	2.9[cC]	2.4[cC]	15.7[bB]	1.12
5.0	35.8[aA]	2.1[cD]	1.9[cC]	10.3[bC]	1.11
SEM	1.18	1.05	1.09	1.08	
		Trial 2[d]			
0.5	42.1[aA]	13.3[cA]	10.6[cA]	24.7[bA]	1.08
1.0	44.0[aA]	4.8[cB]	4.0[cB]	18.7[bA]	1.08
3.0	48.9[aA]	2.6[cC]	2.3[cC]	7.9[bB]	1.16
5.0	46.8[aA]	2.0[bD]	2.0[bD]	3.6[bB]	1.22
SEM	1.09	1.03	1.03	1.29	

[a] As determined at 38 °C and at a shear rate of 45 s^{-1}. [b] Values in the same row (a–c) or column (A–D) with different letters were significantly different ($p < 0.05$). [c] Antilog of SEM from logarithmic data. [d] Trial 1, values are means of two pigs; trial 2, values are means of three pigs.

without effect on the oat gum solution's viscosity of approximately 40 mPa·s at 45 s^{-1}. Viscosity was reduced to 2 mPa·s after 5 h of incubation with filtered jejunal digesta (Table III). Viscosity declined most rapidly, to 4–5 mPa·s, in the first hour. Both the supernatant of the centrifuged digesta and the untreated digesta caused similar total viscosity losses at similar rates.

Resuspended sediment, which in addition to solid particles of diet also would contain most of the microorganisms from the digesta, caused a much slower reduction in viscosity than the supernatant and untreated digesta.

The regression analysis of the data from Table III, using the mean viscosity of autoclaved samples (41.8 mPa·s) for viscosity at time $t = 0$ and assuming a viscosity of 1 for water, is summarized in Table IV. The activities of filtered

Table IV. Summary of Analysis[a] of Enzyme Activity in Fractions of Jejunal Digesta

sample	enzyme activity [$d/dt\ (1/\eta_{sp}) \times 10^3$]	r^2	p value
	Trial 1		
filtered	2.9	0.995	<0.001
supernatant	3.6	0.994	<0.001
sediment	0.26	0.989	<0.001
	Trial 2		
filtered	3.3	0.995	<0.001
supernatant	3.3	0.965	0.003
sediment	0.93	0.948	0.005

[a] Data obtained from regression of $1/\eta - 1$ against time (min), where η = viscosity (mPa·s) at 45 s^{-1}.

Table V. Molecular Weight ($\times 10^{-5}$) Changes with Time of Oat Gum Incubated for 5 h with Fractions of Jejunal Digesta (1:1) (Trial 1)[a]

time (h)	autoclaved	filtered	supernatant	sediment	SEM
0.5	10.9[aA]	7.5[bA]	7.8[bA]	10.0[aA]	0.37
1.0	10.8[aA]	4.1[cB]	4.0[cB]	9.1[bAB]	0.44
3.0	10.9[aA]	2.4[cC]	2.3[cB]	8.4[bAB]	0.32
5.0	10.9[aA]	1.8[cC]	1.8[cB]	7.2[bB]	0.24
SEM	0.08	0.31	0.49	0.38	

[a] The molecular weight of unheated PPOG was 1.2×10^6. Values (means of two pigs) in the same row (a–c) or column (A–C) with different letters were significantly different ($p < 0.05$).

Table VI. Molecular Weight ($\times 10^{-5}$) of β-Glucan from Diets Based on Oat Flour (OF), Rolled Oats (RO), and Oat Bran (OB) and Extracted for 1–8 h at pH 4.5

extraction time (h)	OF	RO	OB
1	15.4	16.7	20.5
3	13.6	12.4	17.8
8	8.9	6.1	12.5

digesta and supernatant were similar in each experiment and 4–10 times the activity of the sediment.

Changes in MW corresponded to changes in viscosity (Table V). Autoclaved digesta were essentially without effect on the MW of PPOG, but the filtered digesta and supernatant from the centrifuged digesta rapidly reduced the MW from 1.2×10^6 to 1.8×10^5. MW was significantly higher, at all times of measurement, when the oat gum was incubated with sediment from centrifugation than when incubated with the supernatant or the filtered digesta. After 0.5 h, there was no significant difference between MW of β-glucan in the presence of autoclaved digesta and that in the sediment of the centrifuged digesta. However, after 1 h, MW was significantly reduced, and there was a further decline in MW to 7.2×10^5 after 5 h.

Molecular Weight Changes Related to the Diet. Diets were treated with 0.2 M acetate buffer, pH 4.5, at 38 °C for 8 h to determine whether they contained, or had the potential to produce, endogenous β-glucanase activity. Incubation of otherwise untreated diets resulted in a 2–4-fold reduction in the MW of subsequently extractable β-glucan, that from OB having an average MW one-third higher than that from OF and twice as high as to that from RO (Table VI).

Effect of Trypsin on Viscosity of Oat Bran Extract and Oat Gum. The viscosities, at 45 s^{-1}, of a 1% aqueous solution of OGGI and a 0.5% solution of PPOG were 24 and 31 mPa·s, respectively. Both remained stable for 3 h and were not affected by addition of trypsin. The β-glucan content in PPOG was 81% (db) with a MW 1.2 $\times 10^6$, whereas OGGI had a β-glucan content of 59% with a MW of 6×10^5. A neutralized and centrifuged carbonate

buffer extract of oat bran had a viscosity of 33 mPa·s which similarly was not affected by trypsin.

DISCUSSION

The currently agreed upon definition of DF as the sum of plant polysaccharides and lignin not digested by the enzymes of man (Trowell et al., 1976) leads to the expectation that there will be no digestion in the stomach and small intestine. The results of this study with pigs, however, show that the molecular weights of β-glucan in three different milling fractions of oats were significantly reduced after transit through the stomach and proximal small intestine. This is consistent with other animal studies (rats and chicks). Wood et al. (1991b) reported a 10-fold lower MW of β-glucan present in the small intestine than in the stomach in rats, and similar depolymerization has been observed in the small intestine of chicks (P. J. Wood and N. A. Cave, Centre for Food and Animal Research, unpublished data, 1991).

In this study, the depolymerization in the upper GI tract of pigs was not complete since there was an accumulation of β-glucan with a lower mean MW. In a study in which digesta were collected at various sites in the GI tract of pigs, no quantitative losses of β-glucan were observed in the stomach and the proximal two-thirds of the small intestine, while significant loss from microbial degradation took place in the distal small intestine (Bach Knudsen et al., 1993). Other studies have shown breakdown of barley β-glucan by the action of endoglucanases from lactobacilli in the stomach of pigs (Jonsson and Hemmingsson, 1991).

The MWs ($\approx 2.2 \times 10^6$) of β-glucan extracted from the three diets were similar, but depolymerization during digestion of the β-glucan from OF and RO was more rapid than that of the β-glucan from OB. β-Glucan extracted from digesta generally gave broader peaks with a higher mean retention time than the corresponding undigested β-glucan. Digestion, therefore, resulted in decreased MW and increased polydispersity. In some of the digesta samples—especially after extended exposure to the gastrointestinal juices—the formation of a shoulder on the leading edge of the chromatographic peak, the development of a narrow peak at a significantly higher retention volume, and the occasional development of a double peak were observed. This indicated a tendency for the β-glucan to be degraded to a distinctive MW population, in turn suggesting the presence of specifically susceptible bonds. A somewhat similar degradation pattern in β-glucan from steeped barley (Yin and MacGregor, 1988; Yin et al., 1989) was probably caused by a type of endo-1,4-β-glucanase released from common field fungi associated with the barley hulls. This enzyme's mode of action appeared to be specific cleavage of the β-glucan at sites containing four or more consecutive β-(1→4)-linkages. These structures occur at relatively low frequency in both barley (Yin and MacGregor, 1989; Woodward and Fincher, 1983) and oat (Wood et al., 1991a) β-glucan. The major product from barley β-glucan had a molecular weight of 20 000–25 000 (Yin and MacGregor, 1988), whereas the MW of oat β-glucan in the present study, following degradation, appeared to be somewhat higher (63 000–166 000). A similar mechanism of enzymatic action is possible, although from different sources. Some degradative activity was associated with the diet itself, but this was insufficient to account for the results observed during digestion and there was no evidence of specific bond cleavage. Alcohol-treated diets incubated at pH 1.5 to mimic the acidification in the stomach did not result in MW significantly different from that of diets extracted in carbonate (pH 10) or MES/azide (Johansen, 1993).

The presence of a peptide or protein component for both barley and oat β-glucan has been suggested (Forrest and Wainwright, 1977; Vårum and Smidsrød, 1988). Proteolytic action might therefore reduce a large protein–polysaccharide complex to a lower MW polysaccharide population as suggested by Forrest and Wainwright (1977). This concept, which could explain the events seen in pig digesta, is supported by evidence that thermolysin and carboxypeptidase were able to solubilize or partially degrade barley cell-wall β-glucan (Forrest and Wainwright, 1977; Baxter, 1978; Bamforth et al., 1979). Similarly, it was recently reported that trypsin depolymerized oat β-glucan (Mälkki et al., 1992). No evidence of trypsin susceptibility was found with oat gum or crude oat extracts in the present study. The crude extracts contain β-glucan of the highest MW (Wood et al., 1991b); isolated gum might already have undergone depolymerization at susceptible bonds. Previously, Wood et al. (1991b) were unable to detect depolymerization by pepsin or chymotrypsin. These results suggest that proteolytic activity in the pig digestive tract is not responsible for the depolymerization of β-glucan.

The rate of change of viscosity of β-glucan solutions in the presence of digesta, and the loss of this activity on autoclaving, clearly indicates that hydrolytic enzymes are present in the digestive fluid. The pattern of degradation, in which $d/dt(1/\eta_{sp})$ is linear (Table IV), is typical of random cleavage of polymers (Bryce and Greenwood, 1957) as in acid hydrolysis but is also appropriate for specific hydrolysis of randomly distributed susceptible bonds. Although the chromatographic behavior suggests formation of a specific population of β-glucan of decreased MW, a declining response of β-glucan to Calcofluor at lower MW (Jørgensen, 1988; Manzanares et al., 1991) would prevent detection of low MW fragments.

The present results do not allow conclusions as to the origins of the observed enzyme activity. Residual activity in the sediment suggests further release of enzyme from microorganisms but could result simply from slow release of tightly particulate-bound enzyme. The purpose of washing sediment in trial 2 was to eliminate residual enzymes retained from the supernatant, but this did not reduce the rate of viscosity loss. The apparent 3-fold greater activity in the sediment from trial 2 thus seems to reflect animal variability. Overall, the evidence points to glucanolytic action from microorganisms, which are known to be responsible for loss of β-glucan in the lower small intestine (Bach Knudsen et al., 1993). Early reports by Dahlqvist (1961) suggested that small intestinal mucosa of the adult pig had β-glucosidase activity located in the microsomes, but these results seem to be an artifact of homogenate preparation with insufficient elimination of the gut microflora. The stomach, duodenum, and jejunum of pigs are more heavily colonized (10^7–10^8 viable counts/g of digesta) (Bach Knudsen et al., 1993) than the corresponding segments of humans (less than 10^4 viable bacteria/mL of gastrointestinal content) (Drasar and Hill, 1974). A similar extent of depolymerization in the human upper gastrointestinal tract thus seems to be questionable.

Luminal viscosity and intact cell-wall structure are two factors that are considered to be responsible for moderating postprandial blood glucose and insulin levels [e.g., Jenkins et al. (1987)]. The nutritional consequences of either glucanolytic or proteolytic action on the viscosity of intestinal contents are difficult to predict since the "solubilase" activity (a term used to describe both the cellulolytic and carboxypeptidase activities discussed above) has the opposing effects of increasing the concen-

tration of β-glucan in solution and hence the luminal viscosity, whereas depolymerization decreases viscosity.

The present and previous results (Bach Knudsen et al., 1990; Wood et al., 1991b; Wood and Cave, unpublished data, 1991) raise questions as to whether there might be species differences in the degradation of β-glucan in the upper gastrointestinal tract and to what extent this will affect the metabolic response to some types of soluble DF such as oat β-glucan.

CONCLUSION

High-performance size exclusion chromatography was used to show that the β-glucan from oats is depolymerized during passage through the stomach and upper jejunum of pigs. The chromatography behavior indicated that cleavage may take place at infrequently occurring, randomly distributed, susceptible bonds, tending to create a population of molecules with a MW of about 100 000. The hydrolytic activity is probably associated with microorganisms present in the gastrointestinal tract. However, these microorganisms and associated enzyme activity do not lead to a loss in β-glucan from the digesta before the lower small intestine is reached. Trypsin had no effect on viscosity of solutions of isolated β-glucan or crude extracts of oat bran. This and other evidence suggests that cleavage of peptide bonds is not responsible for the reduction in the molecular weight of β-glucan in digesta from pigs.

ACKNOWLEDGMENT

This work was supported by the Danish Agricultural and Veterinary Research Council. We thank Dr. Henry Jørgensen for carrying out the surgery, Nicole Fillion, Bodil Kristensen, and Hanne Lund Jensen for excellent technical assistance, and Drs. Bent Borg Jensen, Bjørn O. Eggum, and Brittmarie Sandström for advice and encouragement during the course of this study. H.N.J. gratefully acknowledges receipt of a Postgraduate Studentship under the Joint Financing Scholarship Programme between the Danish Research Academy and The National Institute of Animal Science and a Travel Grant donated by The Danish Research Academy.

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Received for review May 24, 1993. Accepted September 13, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1993.