ELSEVIER

0144-8617(94)00068-9

Carbohydrate Polymers **25** (1994) 331–336

0144-8617/94/$07.00 + .00

Evaluation of oat bran as a soluble fibre source. Characterization of oat β-glucan and its effects on glycaemic response

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(Received 30 March 1994; revised version received 1 September 1994; accepted 5 September 1994)

Oat β-glucan, present in oat bran in greater concentrations than in the whole oat groat, is mainly composed of β-(1 → 3)-linked cellotriosyl and cellotetraosyl units, present at 52 and 34% by weight of the molecule, respectively. The remaining structure consists of β-(1 → 3)-linked blocks composed of four or more consecutive β-(1 → 4)-linked D-glucopyranosyl units. Size-exclusion chromatography indicated a molecular weight for oat β-glucan of 2–3 × 10^6. This was significantly reduced during digestion in the small intestine of rats and chicks. In healthy human volunteers, oat β-glucan reduced the postprandial glucose response to an oral glucose load similarly to guar gum. The effectiveness of oat β-glucan was proportional to the logarithm of the viscosity of the solution fed.

INTRODUCTION

Many studies have shown that inclusion of oat bran or rolled oats in significant amounts in the daily diet may lower serum cholesterol (Anderson *et al.*, 1990). However, a recent study by Swain *et al.* (1990) suggested that this is not a specific effect attributable to oats but simply a consequence of accompanying dietary modification, most particularly, altered fat intake.

Despite this controversy, even those who suggest that oat bran has no specific cholesterol lowering effect generally accept that soluble viscous polysaccharide supplements can reduce serum cholesterol levels significantly (Sacks, 1991). Viscous polysaccharides also decrease the postprandial rise in blood glucose and insulin levels in humans following an oral glucose load and this effect may be mechanistically related to reduction in serum cholesterol levels (Anderson *et al.*, 1990). The effectiveness of oat bran in reducing serum cholesterol levels has, therefore, been attributed to the presence of (1 → 3)(1 → 4)-β-D-glucan, or oat β-glucan, a highly viscous polysaccharide. The viscosity of the equivalent mixed linkage β-glucan of barley may cause problems in brewing and reduce the value of barley as feed.

Factors affecting the viscosity of polysaccharide solutions are measurement conditions, such as concentration, temperature and shear rate, and the fundamental molecular characteristics of structure and molecular weight. Despite the evident importance of the viscosity of barley β-glucan to the brewing and feed industry, reports in the literature have frequently only contained data on 'extract' viscosity and lacked information on concentration or shear rate. Within one study, temperature and shear rate conditions might be constant, but it is often impossible to determine whether reported differences in viscosity arose from concentration or molecular characteristics. In considering physiological response to oat products the most important variations in viscosity are likely to arise from differences in amounts of β-glucan, or in the molecular characteristics (since physiological temperature and shear rates will be constant features).

In this article, data on β-glucan concentrations in cereals are reviewed and the potential for increasing its concentration in oat bran. Methods for rapidly assessing structural features and molecular weight will be described. The article will conclude with data supporting the role of viscosity of oat β-glucan in moderating the postprandial blood glucose and insulin response in humans.

β-GLUCAN CONCENTRATION

Table 1 summarizes data on β-glucan concentration in cereals. Oats and barley have the highest concentrations. Wide ranges in values have been reported reflecting, for the most part, cultivar diversity. However, some differences are related to the presence or absence of hull,

Table 1. β-Glucan concentration (dry weights basis, dwb) in some common cereals

Cereal	β-Glucan concentration % dwb[a]	Reference
Barley	3·0–6·9	Aman & Graham (1987)
	3·6–6·4	Anderson *et al.* (1978)
	5·0–10·7	Bengtsson *et al.* (1990)
	2·7–4·7	Gill *et al.* (1982)
	2·8–5·6	Lehtonen & Aikasalo (1987)
	3·8–4·8	McCleary & Glennie-Holmes (1985)
	2·7–3·8	Palmer & Mackenzie (1986)
Corn	0·1	McCleary & Glennie-Holmes (1985)
Oats (with hull)	2·7–3·6	Aman (1987)
	2·2–4·2	Aman & Graham (1987)
	2·5	Anderson *et al.* (1978)
	3·4	Henry (1985)
Oat groats	2·7–5·4	McCleary & Glennie-Holmes (1985)
	3·2–6·3	Welch & Lloyd (1989)
	3·9–6·8	Wood *et al.* (1991*b*)
Rice	0·1	Anderson *et al.* (1978)
	0·1	Henry (1985)
	0·04	McCleary & Glennie-Holmes (1985)
Rye	1·3	Aman & Hesselman (1985)
	1·9	Anderson *et al.* (1978)
	1·9	Henry (1985)
	1·4–2·1	McCleary & Glennie-Holmes (1985)
	1·6–2·0	Saastamoinen *et al.* (1989)
Wheat	0·5	Aman & Hesselman (1985)
	0·5–1·0	Beresford & Stone (1983)
	0·6	Henry (1985)
	0·5–0·7	McCleary & Glennie-Holmes (1985)

[a]Range shown if more than two samples analysed.

which for oats may be as much as 30% by weight of the seed. Oats for human food are always dehulled and data related to human nutrition should be reported on a hull-free, or groat, basis.

Good quality commercial oat bran normally contains about 7–10% β-glucan, but since there was no well-established commercial practice, quality was variable and some products did not have increased dietary fibre or β-glucan concentrations. In an effort to introduce some order into this situation, in 1989 the American Association of Cereal Chemists (AACC) adopted a definition of oat bran with 5·5% (dry weight basis) as the lower limit for β-glucan and 16·0% for dietary fibre (Anonymous, 1989). In addition to variability arising from different process methods, commercial milling may give variable yields of oat bran with variable β-glucan concentration (Table 2) because of differences in the quality of the raw material (Ganssmann, 1990).

We developed a simple, small-scale milling process to produce coarse (bran), and fine fractions from a selection of oat cultivars and analysed these for β-glucan (Wood *et al.* 1991*b*). The results (Table 3) show the variation in the β-glucan content of groats prior to milling. Cultivar differences were significant and values ranged from 3·9 to 6·8%. This just overlapped with the lowest β-glucan brans which also showed significant differences in the range 5·8–8·9%. These values illustrate the problem with assigning analytical limits in a definition of oat 'bran'. The mean β-glucan content of all the brans was 7·4% with an average enrichment factor of 1·5 from an average bran yield of 53·3% (close to the maximum, 50%, suggested in the AACC definition). Although there was a correlation between groat β-glucan and bran β-glucan ($r^2 = 0·82$), there was sufficient variation in the ratio of bran:groat β-glucan (1·28–1·60) to observe significant changes in the ranking of cultivars after milling.

Table 2. β-Glucan concentration (% dry weight) of rolled oats and bran from three different supplies of oats[a]

Source of oat	Yield of bran[b] (%)	β-Glucan concentration (%)		EF[c]
		Groat	Bran	
West Germany	35	4·7	9·4	2·0
East Germany	40	4·3	8·7	2·0
Australia	50	3·3	5·6	1·7

[a]From Ganssmann (1990).
[b]As obtained by a commercial milling and sieving procedure.
[c]Enrichment factor: β-glucan in bran/β-glucan in groat.

Table 3. Summary of analytical data from duplicate milling of 11 oat cultivars[a] (Adapted from Wood *et al.*, 1991*b*)

Cultivar	β-Glucan (% dry weight basis)[b]		Bran yield (%)	EF[c]
	Groat	Bran		
Marion	6·8a	8·8a	58·0	1·28
Capital	6·0b	8·9a	57·3	1·49
Woodstock	5·5c	7·8b,c	57·5	1·42
Sentinel	5·4c,d	8·1b	54·9	1·51
Ogle	5·1d,e	7·7c	50·2	1·52
Hinoat	5·0d,e	8·0b,c	48·4	1·60
Tibor	4·7e,f	6·7	50·8	1·41
NO-1	4·7e,f	7·2d	55·4	1·52
Donald	4·6f	6·3f	53·0	1·38
OA 516-2	4·3f,g	6·5e,f	52·7	1·51
Harmon	3·9g	5·8g	48·3	1·49
Overall mean[d]	5·1 ± 0·8	7·4 ± 1·03	53 ± 4	1·5 ± 0·1

[a]Numbers in column not followed by same superscript letter are significantly different ($P < 0·05$).
[b]Determined by method of McCleary & Glennie-Holmes (1985).
[c]EF (Enrichment factor) is the ratio of β-glucan concentration in bran to β-glucan concentration in groat.
[d]Mean ± standard deviation of the 11 cultivar means.

STRUCTURE

Purified β-glucan from oats is a linear, unbranched polysaccharide composed of 4-O-linked β-D-glucopyranosyl units (~70%) and 3-O-linked β-D-glucopyranosyl units (~30%) (Parrish *et al.*, 1960; Aspinall & Carpenter, 1984). To determine the sequence of these linkages we recently applied a method (Woodward *et al.*, 1983) of structural analysis based on characterization of oligosaccharide products isolated following the action of a (1 → 3)(1 → 4)-β-D-glucan-4-glucanohydrolase (EC 3.2.1.73; lichenase) which cleaves the (1 → 4)-linkage of the 3-O-substituted glucopyranosyl units in the β-glucan. These released oligosaccharides are the building blocks of the polysaccharide, β-(1 → 4)-linked in the original chain.

Methylation analysis was used for structural analysis of the oligosaccharides. The first methylation identifies the linkage positions in the oligosaccharide and the reducing end is identified by a second methylation of the pre-reduced oligosaccharide. The results (Wood *et al.*, 1991*a*), summarized in Table 4, confirmed the major structural feature of oat β-glucan, first established over 30 years ago (Parrish *et al.*, 1960) as (1 → 3)-β-linked cellotriosyl and cellotetraosyl units. The analysis showed a close similarity between oat and barley β-glucan (Woodward *et al.*, 1983) including small amounts of higher degree of polymerization (DP) β-(1 → 3)-linked cello-oligosaccharides. Analysis of the insoluble material (~5% by weight) produced by the enzyme indicated 7–8 as the average number of consecutive (1 → 4)-linkages, 1–2 units less than suggested for similar material from barley (Woodward *et al.*, 1983).

Despite their similar results from methylation analysis and almost identical ^{13}C-NMR spectra (Wood *et al.*, 1991*a*; Dais & Perlin, 1982), oat and barley β-glucan are structurally distinct. This was demonstrated by quantitative HPLC analysis of lichenase released oligosaccharides (Wood *et al.*, 1991*a*). Consistent differences were observed between oats, in which approximately one-third molar proportion of the structure was β-(1 → 3)-linked cellotetrasyl units, and barley and wheat where this proportion was closer to one-quarter (Table 5).

Reported differences in solubility and viscosity of cereal β-glucans may, in part, be due to subtle differences in linkage sequences. Conformational analysis and calculations of theoretical molecular extension in solution showed that experimentally determined values for molecular dimensions could not be explained on the basis of β-(1 → 3)-linked cellotriosyl and cellotetrasyl units alone (Buliga *et al.*, 1986). Relatively minor amounts (2 M%) of sequences of more than three consecutive (1 → 4)-linkages significantly increased, whereas sequences of more than one consecutive (1 → 3)-linkage significantly decrease, theoretical molecular dimensions of β-glucans of otherwise similar DP and structure. These structural features would, therefore, be expected to exert a major influence on viscosity.

Periodate oxidation of oat β-glucan, followed by reduction and controlled acid hydrolysis (Smith degradation) reportedly showed the presence of consecutive (1 → 3)-linked units (Goldstein *et al.*, 1965); a more recent study did not detect this feature (Vårum & Smidsrød, 1988). The methodology we have used to date has not detected consecutive (1 → 3)-linkages (Wood *et al.*, 1991*a*). Although problems inherent in the Smith degradation (Woodward *et al.*, 1983) might lead to misidentification of consecutive (1 → 3)-linkages, other techniques have revealed this feature in β-glucan from *Zea mays* (Kato & Nevins, 1986). Since this structure might profoundly affect physical properties (Buliga *et al.*, 1986; Kato & Nevins, 1986), further study would be worthwhile.

Our original HPLC method (Wood *et al.*, 1991*a*), for analysis of oligosaccharides released by lichenase from β-glucans, was unable to accurately analyse products of DP > 5. We have recently developed a HPLC technique using high-performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (Dionex system), which allows rapid analysis of the

Table 4. Results of methylation analysis of oligosaccharides produced by the action of lichenase on the (1 → 3)(1 → 4)-β-D-glucan of oats (Adapted from Wood *et al.*, 1991*a*)

Oligosaccharide	Glycosyl residue (molar proportions)[a]		
	T-Glc	3-Glc	4-Glc
Trisaccharide	1	1·03[b]	1·02
Tetrasaccharide	1	0·98[b]	1·97
Pentasaccharide	1	0·81[b]	2·66
Hexasaccharide	1	1·36[c]	3·42
Water insoluble	1	0·80	6·19

[a]T-Glc-, terminal glucose, non-reducing end; 3-Glc, 3-linked glucose; 4-Glc, 4-linked glucose.
[b]Identified as residue on reducing end; corrected for losses during methylation.
[c]Evidence for ~0·6 mole proportion non-reducing 3-Glc.

Table 5. Molar ratio of tri-/tetrasaccharide released by lichenase from oats, barley and wheat, determined by anion exchange chromatography with pulsed amperometric detection (Wood *et al.*, 1991*a* and unpublished data)

Cereal	Number of samples	Molar ratio	
		Mean ± SD	Range
Oats *Avena sativa*	11[a]	2·1 ± 0·1	1·9 – 2·2
Barley *Hordeum vulgare*	6[a]	2·8 ± 0·2	2·5 – 3·2
Wheat *Triticum aestivum*	3[b]	3·1 ± 0·4	2·6 – 3·3

[a]Number of different cultivar.
[b]Soft white winter wheat, hard red winter wheat and hard red spring wheat.

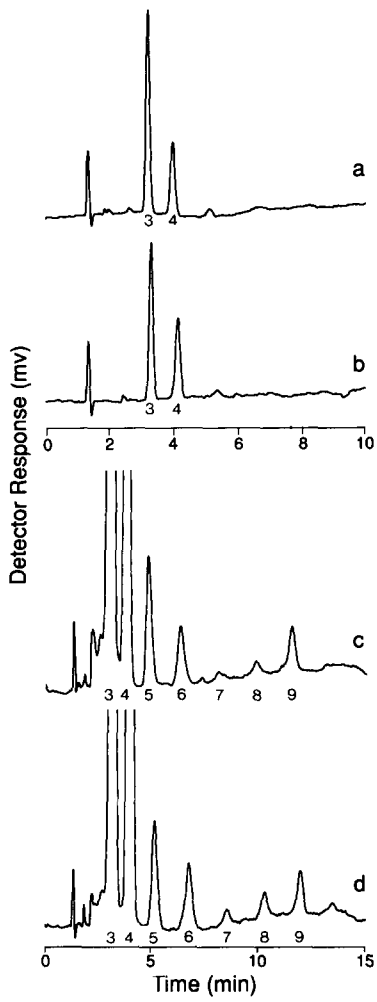

Fig. 1. High-performance anion exchange chromatography, with pulsed amperometric detection, of oligosaccharides released by lichenase from barley (a and c) and oat (b and d) β-glucan; c and d at increased concentration to show oligosaccharides DP 5–9.

higher DP oligosaccharides (Wood *et al.*, 1994*a*). We are, therefore, able to analyse those minor structural features with more than three consecutive (1 → 4)-linkages which may have a disproportionate influence on physical properties. Figure 1 shows the fractionation of oligosaccharides obtained from oat and barley β-glucan at two sensitivities. Having established the tri/tetrasaccharide ratio we used the higher sensitivities to look at the higher DP oligosaccharides. There were no major differences between oat and barley β-glucan apart from the tri/tetrasaccharide ratio. The oligosaccharide reaction products of DP > 4 accounted for 9% by weight and were present in similar amounts. There appeared to be a slight increase in amount of the DP9 oligosaccharide.

The fraction (~5% by weight) made insoluble by lichenase showed 6–7 consecutive (1 → 4)-linked units by methylation analysis. This material was dissolved in dimethylsulfoxide and analysed by HPAEC. Approximately 40% by weight (as estimated from peak areas) of this oligosaccharide mixture was of DP9, accounting for the increase at this DP in the soluble fraction. Methylation evidently underestimated the average DP of this product. In total, about 15% by weight of oat β-glucan contained sequences of more than 3 consecutive (1 → 3)-linked units.

Some care is required in generalizing from studies of purified material since differences, or similarities, observed may be a function of method of extraction and purification or cultivar and environment, as much as species. The HPLC methods of analysis of lichenase-released oligosaccharides described allows rapid evaluation of the overall average structure without prior extraction and purification. Analysis of whole groat flour, bran, crude oat gum and derived purified β-glucan all showed similar tri/tetrasaccharide ratios (Wood *et al.*, 1991*a*), indicating no variation in average structure between oat groat and bran, or preferential isolation of a specific structure during purification.

MOLECULAR WEIGHT

Size exclusion chromatography (SEC) using a high performance gel column and calcofluor post-column detection was recently used to estimate molecular weight (MW) of oat β-glucans (Wood *et al.*, 1991*b*). It was shown that use of the commercially available pullulans as MW standards would lead to overestimation of the MW of the oat β-glucans, and therefore β-glucans of different MW were used as chromatographic standards. The MWs of the chromatographic peaks of these standards were determined by SEC with low angle laser light scattering detection. The results (Table 6) showed a MW for the chromatographic peak of crude extracts from either whole groat or bran of $\sim 3 \times 10^6$ Da. The process of isolation and purification seems to result in a decrease in MW.

Table 6. Molecular weights[a] of partially purified oat β-glucan and oat β-glucan extracted in pH 10 carbonate buffer at 60°C (from Wood *et al.*, 1991*b* and unpublished data)

Sample	MW × 10^{-6}
Pilot plant oat gum	1·2
Bench oat gum	2·2
Purified oat β-glucan	0·9
Commercial barley β-glucan (Biocon)	0·2
Extract from oat groats[b]	2·9
Extract from oat bran[b]	3·0
Extract from rat small intestinal contents[c]	0·10
Extract from chick small intestinal contents[d]	0·14

[a]MW determined from retention time of chromatographic peak using high performance size exclusion chromatography.
[b]Average of four cultivars.
[c]Average from rats fed Pilot plant oat gum ($n = 4$).
[d]Average from chicks fed oat bran ($n = 4$).

At high MW, the system is very sensitive to small changes in retention volume; a difference of ~0·2 ml corresponds to a change in MW from 3 to $2{\cdot}5 \times 10^6$. The method is therefore most usefully applied to MW $< 2 \times 10^6$ and detecting changes in MW. The specific detection system allowed rapid assessment, for example, of crude extracts of cereals and of gastrointestinal contents of animals fed oat products. It was demonstrated that significant depolymerization of oat β-glucan takes place in the small intestine of the rat and chick (Wood *et al.*, 1991*c*), and unpublished data (Table 6). The basis for this loss is not certain, but may reflect microbiological activity. Clearly this complicates the assessment, in animal models, of the nutritional effects of viscosity of oat β-glucan.

EFFECT OF OAT β-GLUCAN ON GLYCAEMIC RESPONSE

Jenkins and colleagues (1978) established that postprandial blood glucose and insulin rise was reduced following meals containing viscous soluble fibres, such as agar gum and pectin, relative to the same meal without fibre. They further established that viscosity reduction by acid treatment of guar removed the ability of the gum to reduce the blood glucose peak. An insoluble fibre source (wheat bran) was without effect on postprandial glucose but the effect with four soluble fibres was correlated with viscosity.

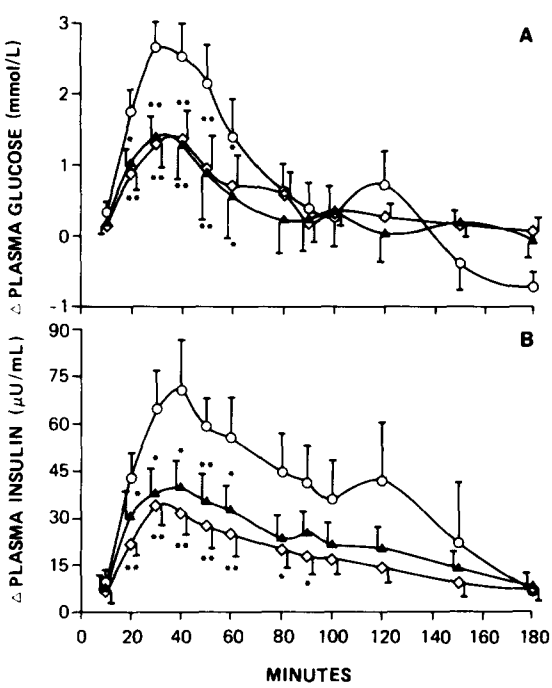

Fig. 2. Change from baseline in postprandial mean plasma glucose (A) and insulin (B), following a 50 g oral glucose load in the presence of oat and guar gum (14·5 g dose) in healthy human subjects: (n : 9): ○, glucose alone; ▲, oat gum + glu- ◇, guar gum + glucose (from Braaten *et al.*, 1991; reprinted with permission. *Am. J. Clin. Nutr.*). $\bar{x} \pm$ SEM; $^*P < 0{\cdot}05$. $^{**}P < 0{\cdot}01$ compared with glucose alone.

In other studies, there have been less clear relationships between polysaccharide viscosity and glycaemic response. O'Connor *et al.* (1981) found no significant difference in blood glucose response to an oral glucose load in the presence or absence of guar gums of different viscosities. However, there were significant reductions in insulin response and the least viscous of three preparations of gum was least effective. The gum dose (5 g in 500 ml) was less than that used by Jenkins and colleagues (14·5 g in ~440 ml). Edwards *et al.* (1987) measured viscosity of gums (1%) in water containing 20% glucose and did not find good correlation between viscosity and peak postprandial blood glucose, but there was correlation with viscosity measured in physiological saline or neutralized acid (i.e. digestive tract conditions). Some of the gums and gum mixtures used in this study, xanthan and mixtures of xanthan with locust bean gum, are capable of developing high viscosity, or gelling, at low concentration but the conformational properties which enable this are salt and pH-sensitive.

Gatenby *et al.* (1991) have reported significant reductions in postprandial blood glucose and insulin responses to three different viscosity guar gums in non-insulin-dependent diabetic subjects. Lower viscosity did not reduce effectiveness. Other studies from the same group (Ellis *et al.*, 1988) did, however, establish an inverse relationship between postprandial insulin rise and dose of guar gum in crispbread biscuits fed to healthy volunteers.

The availability (Wood *et al.*, 1989) of large quantities of oat gum (80% β-glucan) allowed a comparison of the effect of oat and guar gum fed to healthy volunteers essentially as described by Jenkins *et al.* (1978). Blood glucose and insulin rise were both significantly reduced, relative to the gum-free control, to a similar extent by both gums (Braaten *et al.*, 1991) (Fig. 2).

In more recent dose response studies we have found (Wood *et al.*, 1992*b*) a progressive decrease in peak blood glucose increment in the presence of gum between 1·8 and 14·5 g per 500 ml. There was a highly significant ($P < 0{\cdot}0001$) inverse linear relationship ($r^2 = 0{\cdot}98$) between mean peak blood glucose increment and log [viscosity] (Fig. 3).

CONCLUSIONS

The (1 → 3)(1 → 4)-β-D-glucan of oats and barley may develop significant viscosity in aqueous systems, of potential benefit for human nutrition but deleterious to feed value and the brewing process. The magnitude of viscosity development is controlled, *inter alia*, by amount, or concentration of β-glucan, and at the molecular level by structure and molecular weight. Similar ranges of β-glucan concentration (3·5–6·0%) are gener-

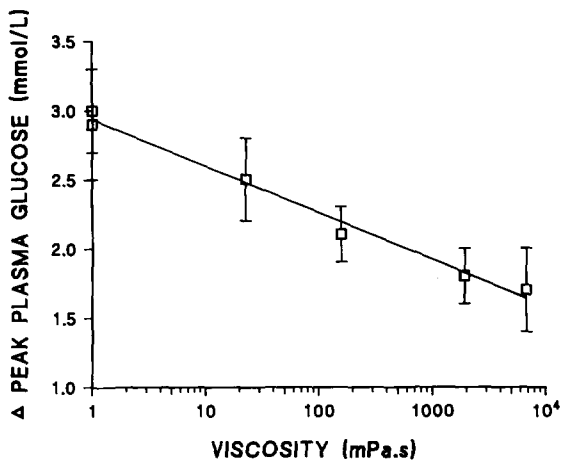

Fig. 3. Relationship between mean peak plasma glucose increment (peak plasma glucose ± SEM) and log [viscosity] in healthy subjects consuming a 50 g oral glucose load in 500 ml water in the presence of oat gum at doses of 0 (control) and 1·8, 3·6, 7·2 and 14·5 g.

ally reported for typical cultivars of oats and barley. Although there is some cultivar variability, simple dry milling can give, in approximately 50% yield, oat bran enriched in β-glucan 1·5-fold. Methods to rapidly assess molecular characteristics of cereal β-glucans have been developed. Some differences in structure of oat and barley β-glucan were shown. Oat β-glucan undergoes molecular weight loss during extraction and purification and significant depolymerization in the small intestine of the rat and chick.

Oat β-glucan moderated the blood glucose response to an oral glucose load in healthy humans. Within the range studied, the magnitude of the effect was proportional to the logarithm of the viscosity.

ACKNOWLEDGEMENTS

Much of the work reviewed here has depended on the collaboration, help and advice of B. Blackwell, J. Braaten, , V. Burrows, P. Fedec, W. Mahn, D. Riedel and F. Scott. Special thanks for technical and analytical assistance are due to Nicole Fillion and John Weisz. Clinical trials were funded by the Quaker Oats Co. Ltd. Contribution No. 2213 of the Centre for Food and Animal Research.

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