# ARABINOXYLAN IN WESTERN CANADIAN BARLEY AND ITS EFFECTS ON BROILER CHICK PERFORMANCE

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Michelle Dawn Fleury

In Partial Fulfilment of the

Requirements for the Degree

of

Master of Science

Department of Animal Science

May, 1994

ISBN 0-315-92262-1

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# ABSTRACT

FLEURY, MICHELLE DAWN. M.Sc., The University of Manitoba, May, 1994. Arabinoxylan in Barley and Its Effects on Broiler Chicks. Major Professor; Lloyd D. Campbell.

A series of studies were performed to determine the effects of arabinoxylan (AX) in western Canadian barley on the performance and digesta viscosity of young broiler chickens. Breeder lines and cultivars from the 1991 Western Canadian Barley Cooperative Program were surveyed, using a modification of the orcinol technique, to determine the range in total, water-soluble and acid-soluble AX concentrations expressed by modern genetic materials. The six-rowed barley type contained greater total, acid-soluble and water-soluble AX than hulless or two-rowed cultivars ($P \leq .01$). The AX in hulless barley was less than the total, greater than the water-soluble ($P<0.01$), and not different from the acid-soluble ($P>0.05$) level of corresponding fractions in two-rowed barley. A genetic effect on AX concentration was seen for all cultivar and breeder line fractions ($P<0.05$). Environment also contributed to the variation in water- and acid-soluble AX content ($P<0.05$). Broiler chicken diets were formulated from cultivars with high (Stacey), moderate (Manley) and low (CDC Richard) water- and acid-soluble arabinoxylan, and supplemented with purified

xylanase (PX) or ß-glucanase-free xylanase (XBG) at 0, 475 or 950 IU/kg. Birds consuming the PX-supplemented Stacey-based diets had increased weight gain ($P<0.05$) and feed conversion ($P<0.05$ for 950 IU/kg), with a corresponding reduction in proximal (PSIV; $P<0.05$) and distal small intestine digesta viscosities (DSIV; $P<0.05$). Other cultivar-xylanase combinations reduced DSIV ($P<0.05$), but did not affect chick performance or PSIV. The results indicated that xylanase supplementation of barley-based diets, which contain elevated soluble AX, can improve the performance of young broiler chickens consuming these diets, and that the effect is associated with reduced PSIV.

# ACKNOWLEDGMENTS

Thanks to Dr. L.D. Campbell and Dr. M.J. Edney for their assistance, corrections and support throughout my program.

Thanks to Dr. A.W. MacGregor and the people on the 13th floor of the CGC for sharing their laboratory facilities, their knowledge and their friendship.

I am also thankful for the assistance of Dana Boros during the chick trial portion of my program.

I am grateful to the Natural Science and Engineering Research Council for providing the graduate scholarship which allowed me to perform this research.

Special thanks to Dr. M.R. Bedford of Finnfeeds International Ltd., and to G. Moser and Dr. R.L. Bernier of ICI Bioproducts, for providing specialized enzyme sources and advice regarding their use.

I am especially thankful for my husband, Michael, and for my family, without the support of whom I could not have succeeded.

# FOREWARD

The manuscript style was used in the preparation of this thesis. Two manuscripts are presented, the first of which will be submitted for publication to the the Canadian Journal of Plant Science, and the second to the Canadian Journal of Animal Science.

Manuscript I:

FLEURY, M.D., EDNEY, M.J., CAMPBELL, L.D and CROW, G.H. Total, water-soluble and acid-soluble arabinoxylan concentrations in western Canadian barley cultivars and breeder lines. Can. J. Plant Sci. (In preparation).

Manuscript II:

FLEURY, M.D., CAMPBELL, L.D., EDNEY, M.J. and CROW, G.H. Effect of endo-1,4-ß-xylanase on the performance of broiler chicks fed barley cultivars varying in arabinoxylan content. Can. J. Anim. Sci. (In preparation).

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# ABBREVIATIONS

AEV - Acid-extract viscosity
AME - Apparent metabolizable energy
A:X - Arabinose to xylose (ratio)
AX - Arabinoxylan
AXX - α-L-arabinofuranosyl-(1,3)-ß-D-xylopyranosyl-(1,4)-D-xylopyranose
BT - six-rowed barley
CMC - carboxymethylcellulose
CP - crude protein
DM - dry matter
DNSA - dinitrosalicylic acid
DSIV - distal small intestine viscosity
EC - enzyme category
FAXX - O-[feruloyl-(O-5)-α-L-arabinofuranosyl]-(1,3)-ß-D-xylopyranosyl-(1,4)-D-xylopyranose
FCE - feed conversion efficiency
GC - gas chromatography
HB - hulless barley
HMW - high molecular weight
HPLC - high performance liquid chromatography
IU - international units
IUBMB - International Union of Biochemistry and Molecular Biology
ME - metabolizable energy
MW - molecular weight
NSP - non-starch polysaccharide
NRC - National Research Council
PSIV - proximal small intestine viscosity
PX - purified xylanase
TME - true metabolizable energy
TR - two-rowed barley
XBG - beta-glucanase-free xylanase

# INTRODUCTION

The viscosity produced by soluble cell wall polymers in barley has been associated with the reduced performance of young chickens (White et al, 1983). The beer manufacturing industry has also encountered viscosity-related problems, including increased wort run-off time and lowered extract yield (Bourne et al, 1976). Viscosity production is usually attributed to the ß-glucan component of barley (White et al, 1981; Bourne and Pierce, 1972); however, the viscous polysaccharide, arabinoxylan, also constitutes a major portion of the endosperm cell wall (25%; Fincher, 1975). Arabinoxylan contributes to the digesta viscosity of other cereals, including rye and wheat (Fengler and Marquardt, 1988; Bedford and Classen, 1992), and speculation exists that it may also significantly enhance the gut viscosity of chicks consuming barley-based diets (de Silva et al, 1983; Classen and Bedford, 1991).

Elevated digesta viscosity reduces nutrient absorption from the chick gut (Choct and Annison, 1992). Studies using rats indicate that this may be due to a thickening of the unstirred water layer at the mucosal surface, resulting in a decrease monosaccharide and amino acid absorption (Elsenhans et al, 1980). Diffusion is reduced within the viscous media

(Fengler and Marquardt, 1988), and since diffusion rate is proportional to the square root of a substance's mass, absorption of lipid and fat-soluble vitamins is severely depressed (Campbell et al, 1983). Feed transit time is decreased in viscous diets (Salih et al, 1991), which may prevent chicks from consuming greater quantities of feed to compensate for the poor nutrient absorption.

Arabinoxylan contributes to the viscosity of acid extracts from barley, as indicated by the ability of purified xylanase to produce a modest viscosity reduction in this solution. The pentose content of hulless barley is also moderately correlated with acid extract viscosity ($r=0.61$; $P<0.05$; Bhatty et al, 1991). Only the concentration of high molecular weight (MW) arabinoxylan polymers (>500,000 MW) is correlated with gut viscosity (Bedford and Classen, 1992). Gel filtration of barley extract indicates that arabinoxylan molecules elute before ß-glucan during size-exclusion chromatography, and may therefore have a high MW (de Silva et al, 1983).

Enzyme supplementation of barley-based diets using crude bacterial or fungal preparations improves the performance of young chickens (Edney et al, 1989; Friesen et al, 1992; Hesselman et al, 1982). The fore gut viscosity reduction, associated with enzyme supplementation of barley diets, is also significantly correlated with increased live weight and feed conversion (Graham et al, 1993). Crude enzyme

preparations are likely to contain significant levels of endoxylanase activity (Graham and Pettersson, 1992), and may already eliminate arabinoxylan-associated viscosity problems in barley diets. However, breeding programs are currently aimed at reducing barley ß-glucan (B. Rossnagel, personal communication), which may indirectly select for increased levels of the other soluble cell wall component, arabinoxylan. In addition, substrate induction may be required for optimal xylanase expression by enzyme cultures (Royer and Nakas, 1990), if arabinoxylan were determined to have a significant adverse effect on the performance of young poultry consuming barley diets.

A brewing study (Canales et al, 1988) indicated that a crude ß-glucanase preparation, which showed activity against arabinoxylan, improved wort filtration rates more effectively than an enzyme source without xylanase. A multi-enzyme preparation was also thought to improve the performance of young broiler chickens relative to those consuming diets supplemented with ß-glucanase (Graham and Pettersson, 1992). The study was inconclusive, however, because the ß-glucanase source contained xylanase, and the treatments did not differ significantly. The objective of the current study was to determine the range in total, acid-soluble and water-soluble arabinoxylan present in two-rowed, six-rowed and hulless cultivars. The data from the first study will allow the selection of barley cultivars containing high, moderate and

low arabinoxylan levels for inclusion in a chick trial examining supplementation with a purified xylanase source or a crude, ß-glucanase-free xylanase source. Chick performance and gut viscosity will be measured to determine if xylanase supplementation and, conversely, arabinoxylan, affects the performance of broiler chicks.

# LITERATURE REVIEW

## Arabinoxylan in Small-seeded Cereals

Arabinoxylan (AX) is a ubiquitous component of primary and secondary plant cell walls (Bacic et al, 1988), and constitutes the major non-starch polysaccharide (NSP) found in monocots (Varner and Lin, 1989). Consequently, AX is a constituent of some of the most economically important cereal crops grown in western Canada: wheat, barley, oats and rye. Levels of AX typically found in these cereals are shown in Table 1.

TABLE 1. Arabinoxylan concentrations (% dry matter; DM) determined for wheat, barley, oats and rye grain samples.

| | Barley | Oats | Rye | Wheat |
|---|---|---|---|---|
| Henry (1987) | 6.60 | 5.74 | 8.96 | 6.59 |
| Hesselman (1989)[1] | 7.61 | 7.84 | 8.07 | 6.14 |

[1] Cited in Classen and Bedford (1991).

Arabinoxylan is composed mainly of the 5-carbon sugars, xylose and arabinose, and is frequently referred to as pentosan. The name 'heteroxylan' acknowledges that other

substituents may be present on the xylan backbone (Bacic et al, 1988), and is therefore more accurate. However, the name 'arabinoxylan' best describes the polysaccharide, as found in primary cell walls of the Gramineae family of small-seeded cereal crops, and will be used throughout the remainder of the text. The usage of the term in the present paper is concurrent with other reviewers (Fincher and Stone, 1986).

## Structure of Barley Arabinoxylan

Arabinoxylan consists of a ß-(1,4)-linked D-xylopyranosyl backbone that is periodically substituted at the O-2- and/or O-3-xylosyl positions. Evidence suggests AX exists in solution as a combination of randomly coiled (Dea et al, 1973) and linear ribbon-like regions (Andrewartha et al, 1979; Fig. 1), the ratio of which may be temperature-dependent (Dea et al, 1973). Equally important in determining the characteristics of the AX polymer are the type, degree and distribution of substituents (Andrewartha et al, 1979).

### Xylan Substituents

The plant kingdom contains a wide variety of heteroxylan substituents (Wilkie, 1979; Darvill et al, 1980), some of which are shown in Fig. 2. In barley grain, substituent composition can be characterized roughly on the basis of

parent tissue. The main substituent of all molecules is α-L-arabinofuranose, linked at the O-2- and/or O-3-xylopyranosyl positions. In the past, barley aleurone was the only tissue known to contain the lone O-2 linkage (McNeil et al, 1975); however, Vietor et al (1992a) detected equivalent levels of substitution in cell walls from dehusked barley flour, indicating that the lone O-2 linkage may be common throughout the kernel. Husk AX has a more complex structure, including 2-O-D-xylopyranosyl-L-arabinose and frequent glucuronic acid residues (Aspinall and Ferrier, 1957). Most endosperm substituents are monomeric; however, oligomeric arabinofuranosyl sidechains, with or without terminal xylopyranose, have been identified (Vietor et al, 1992a). Barley aleurone (Gubler et al, 1985) and endosperm (Ahluwahlia and Fry, 1986) contain O-5-O-feruloyl-arabinofuranose sidechains (Fig. 3). Arabinoxylan from immature vegetative barley tissue contains xylose, arabinose, galactose, glucose, glucuronic acid and galacturonic and in the molar ratio 59:28:5:1:8:trace (Kato et al, 1988).

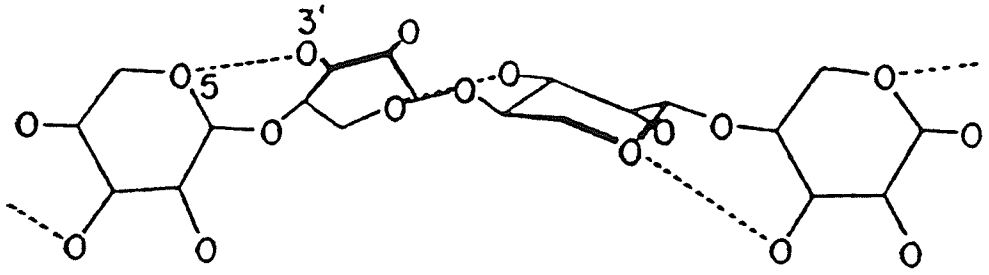

Figure 1. Twisted ribbon conformation of a ß-(1-4)-xylan, showing hydrogen bonds between O5 and O3′ atoms on contiguous residues (Winterburn, 1974).

α-L-Ara *f*-(1→3[α 2])-

[4-Me]-α-D-Glc*p*A-(1→2)-

α-D-Xyl*p*-(1→3)-L-Ara *f*-(1→

Figure 2. Some arabinoxylan substituents (Bacic et al, 1988).

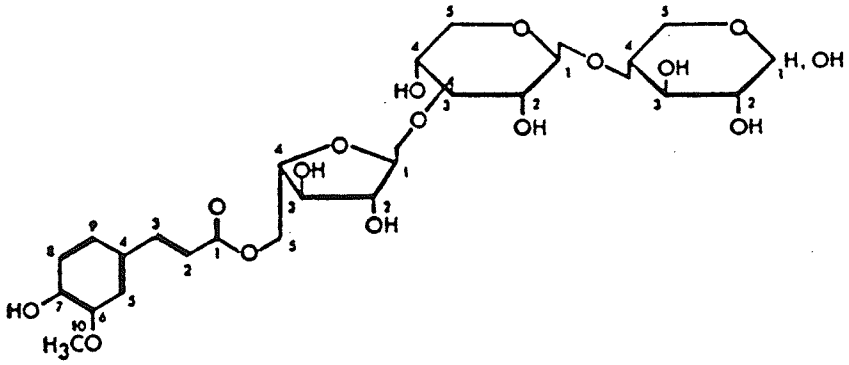

Figure 3. Proposed structure for feruloyl-O-5-arabinosyl sidechains of arabinoxylan (Gubler et al, 1985).

Degree of Substitution

The degree of substitution is also related to AX function and parent tissue. Studies of Australian barley varieties showed endosperm arabinose:xylose ratios of 0.67 (Henry, 1987) and aleurone ratios of 0.35 (Bacic and Stone, 1981b). Husk contains a ratio of 0.17 (Aspinall and Ferrier, 1957). Elevated arabinose:xylose ratios indicate increased xylan substitution. Highly substituted arabinoxylan binds cellulose less tightly (McNeil et al, 1975) and is more water-soluble, as illustrated by the reduced arabinose/xylose ratios of barley flour fractions solubilized by successive aqueous extraction procedures (Table 2). Arabinose subunits restrict noncovalent bonding between AX molecules, but arabino-furanosidase digestion allows fibres to aggregate and precipitate from solution (Andrewartha et al,1979). Previous reviewers (Fincher and Stone, 1986), citing barley cell wall fractionation and analysis data (Mares and Stone, 1973), indicated that water-soluble and -insoluble AX have similar arabinose:xylose (A:X) ratios. A recent study (Vietor et al, 1991; Table 2) indicated that the initial ethanol extraction performed by Mares and Stone (1973) removed AX which had an elevated A:X ratio. The fraction accounted for 25% of the total AX; therefore, its removal would result in an underestimation of the A:X ratios of the soluble fraction.

TABLE 2. Arabinose/xylose ratios of cell wall material extracted from defatted, dehusked barley flour using successively rigorous aqueous extraction procedures.

| Fraction analyzed | Arabinose/xylose |
|---|---|
| Total flour | 0.71 |
| 70% aqueous EtOH extract | 2 |
| Seived starch particles | 0.80 |
| α-amylase digest extract #1 | 0.78 |
| α-amylase digest extract #2 | 0.75 |
| 70 C $H_2O$ extract | 0.67 |
| Water-insoluble cell wall | 0.68 |

(Vietor et al, 1991)

Substituent Distribution

The distribution of substituents is also critical in determining AX solubility and interaction with other cell-wall components (McNeil et al, 1975). An AX molecule containing both highly substituted areas and long, unsubstituted areas will covalently bond differently from one in which the substituents are dispersed evenly because the unbranched areas of the xylan backbone tend to associate first (Dea et al, 1973). Recent work by Vietor et al (1992c) produced a model of barley AX structure (Fig. 4) similar to that which Goldschmid and Perlin (1963) constructed for wheat illustrating branching patterns on the xylan backbone. Methylation analysis of linkage units (Vietor et al, 1992a) and $^1$H-n.m.r. spectroscopy of xylanase-liberated oligosaccharides (Vietor et al, 1992b) indicated AX is

composed of two types of sequences. The most common sequence consists of lone xylosyl residues separated by one or two substituted residues (a in Fig. 3). The 'a' sequences are in turn separated by clusters of two or more unsubstituted xylosyl residues, denoted 'b'.

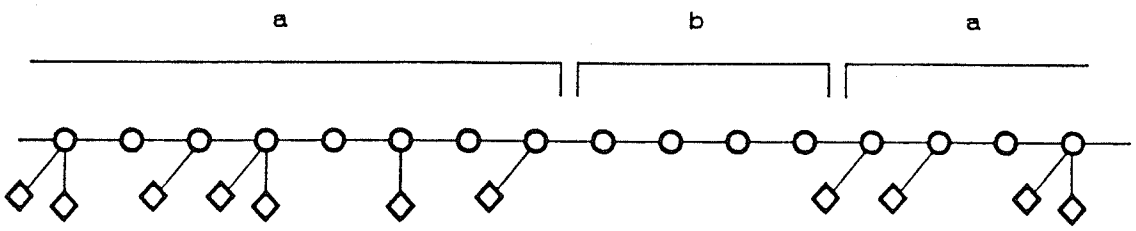


Figure 4. Schematic view of a model for the distribution of substituents over an arabinoxylan chain. (Vietor et al, 1992c).

Analysis of substitution patterns shows that the process is non-random and under regulatory control by the plant. In ethanol gradient precipitates of barium hydroxide-extracts from barley, O-3-arabinosylated xylose residues constitute a static 15 ± 1 % of all fractions (Vietor et al, 1992a). Differences in A:X (arabinose to xylose) ratios result from changes in the frequency of O-2- and O-2,3-linked xylosyl residues (Vietor et al, 1992a). Computer-generated models based on random substitution predict substituent levels would be inversely proportional to xylobiose and xylotriose release by endoxylanase. However, xylan oligosaccharide levels remain

relatively constant despite changes in the A:X ratio (Vietor et al, 1992c). The constant level of O-3-substituted and of polyunsubstituted xylose residues indicates plant cells may be able to control substitution patterns. Although templates (ie:mRNA) do not control the synthesis of carbohydrate structures, the pattern of arabinoxylan substitution may be manipulated by a system similar to that found in glycoprotein glycosylation (Schachter, 1986). In glycoprotein synthesis, the degree of protein glycosylation and the structure of the carbohydrate sidechains formed is a product of, and therefore directly proportional to, the glycosyltransferases present to catalyze the reaction. The cell controls the glycosylation event by regulating the number and proportion of glycosyl-transferases transcribed. Since non-random AX structures are present in barley and their formation requires biosynthetic regulation, it is reasonable to assume that these structures perform specific functions in the cell wall.

Molecular size

Relatively little is known about the molecular size of barley arabinoxylan because most polymer sizing techniques (ie: gel filtration) involve the use of standards, and due to the heterogeneity of carbohydrates, a truly representitive standard is not available. Molecular weights ranging from 10,500 in barley husk (Aspinall and Ferrier, 1957) to 58,800

for sedimentation velocity analysis of barley flour extract (Podrazky, 1964) have been reported. Molecular seive chromatography indicated values of $10^6$ daltons for water-soluble and 5 x $10^6$ daltons for alkali-extracted arabinoxylan (Forrest and Wainwright, 1977).

## Arabinoxylan Synthesis and Degradation

### Arabinoxylan Synthesis

Studies of barley AX synthesis have been limited. However, the molecular biology of other plant systems is better understood, and likely to be similar. A review of the literature (Northcote, 1985) indicated that all matrix polysaccharides are synthesized by the endomembrane system, from which they are transferred via vesicles to the site of deposition in the cell walls. Radioautographic study of wheat root caps (Northcote and Pickett-Heaps, 1966) showed that nucleotide diphosphate precursors formed from UDP-Glc (Fig. 5) were assembed in the Golgi apparatus to form a diverse group of polysaccharides, some of which were deposited in the cell wall as labelled arabinose and xylose. Polysaccharides are synthesized by enzyme complexes situated within the endoplasmic reticulum and Golgi apparatus. The enzymes involved in the process include transporters, glycosyl-transferases, epimerases and binding proteins to hold the

acceptor molecule (Northcote, 1985). Hardwood and softwood xylan has the reducing end-group structure, ß-D-Xylo*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xyl (Andersson et al, 1983), which may be an AX synthesis-initiation sequence that attaches to the binding protein. The scenario described is in agreement with that observed for glucuronoxylan formation in peas - a coordinated event of xylan elongation and glucuronidation, not glucuronosylation of a preformed xylan (Hobbs et al, 1991).

## Arabinoxylan Degradation

Efficient degradation of AX is important for seedling germination and ruminant nutrition. Cell wall degradation improves the access of hydrolytic enzymes to starch and protein, and releases xylose and arabinose for metabolism or structural synthesis.

Alpha-amylase- and hemicellulase-degrading enzymes are produced in the aleurone and scutellum during germination (Gibbons and Nielsen, 1983), but diffusion to surrounding tissues is limited by the cell wall (Varner and Mense, 1972). In animal nutrition, the cell wall may also restrict the access of gallian (55,000 ± 600 Mr) and porcine (53,000 Mr) α-amylase (Lehrner and Malacinski, 1975) to endospermal starch; however, diffusion assays using dextran and globular proteins

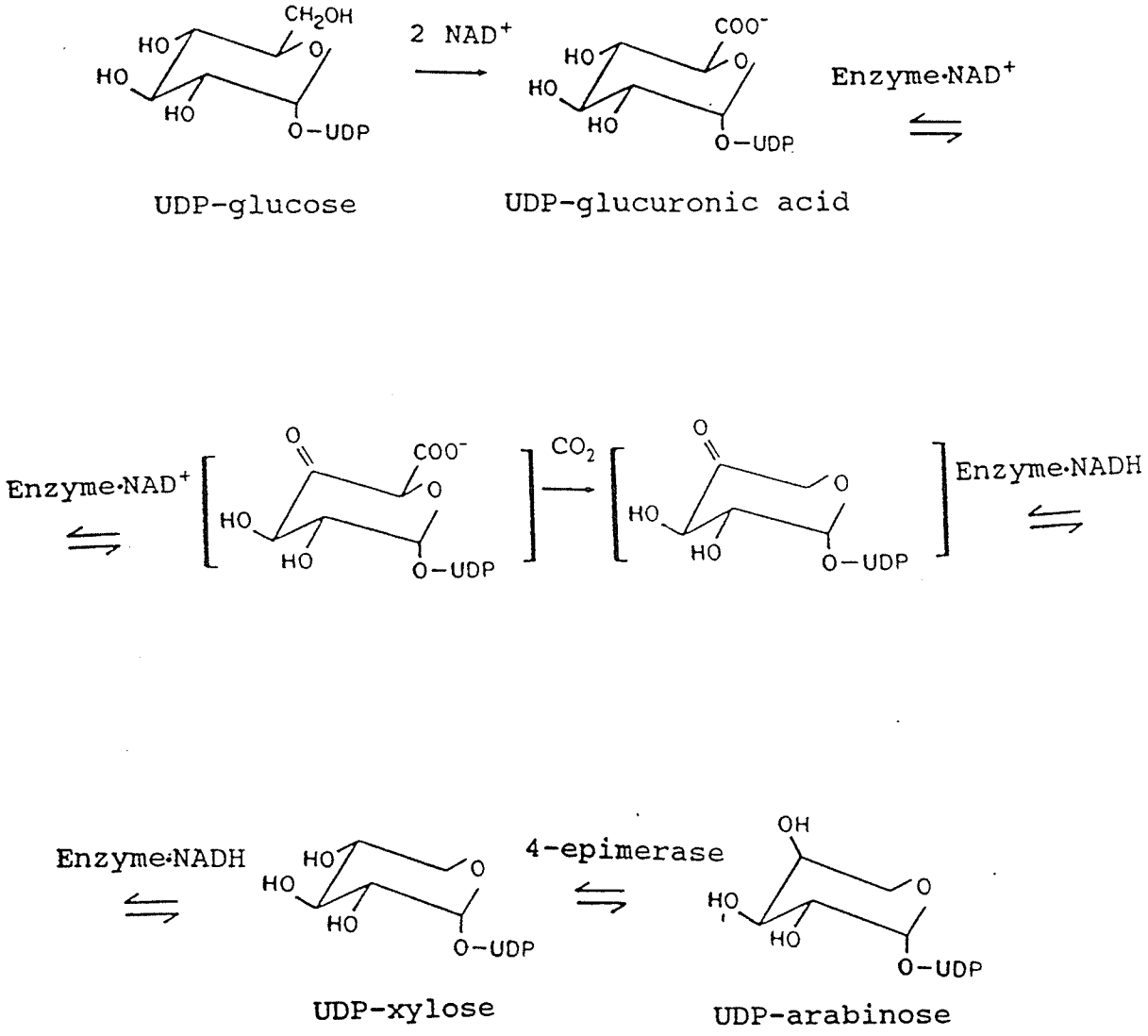


Figure 5. The reactions involved in the formation of UDP-xylose and UDP-arabinose from their UDP-glucose precursor (Hori and Elbein, 1985).

(Baron-Epel et al, 1988; Shedletzky et al, 1992) indicated that the cell wall is permeable to proteins of greater mass than α-amylase (bovine serum albumin; 67,000 Mr). A large proportion of barley ß-glucan (23-67%; Smith et al, 1980a) is acid-soluble, which may increase cell wall porosity and enhance α-amylase diffusion during digestion. Ileal starch digestibilities in barley-based broiler chick diets range from 85 to 88.5% (Hesselman and Åman, 1986), indicating that α-amylase has access to a majority of starch granules. However, starch solubilization by duodenal inoculum is reduced in course-ground barley (Lowgren et al, 1992), presumably because more starch granules and cell walls are intact. Therefore the effects of cell walls should not be underestimated.

Arabinoxylan hydrolysis is achieved by a variety of enzymes acting in concert. Endo-1,4-ß-xylanase (EC 3.2.1.8) cleaves non-terminal 1,4-ß-D-xylosidic linkages. Xylan 1,4-ß-xylosidase (EC 3.2.1.37) removes successive D-xylose residues from the non-reducing termini of 1,4-ß-D-xylans, and hydrolyzes xylobiose. Alpha-N-arabinofuranosidase (EC 3.2.1.55) removes terminal, non-reducing α-L-arabinofuranose residues from the xylan backbone (IUBMB, 1992). The nomenclature of these enzymes is variable, as shown by their common and systematic names in Table 3. These enzymes act in concert (Fig. 6) in a wide variety of plants, bacteria, yeast and fungi that require xylose and arabinose for various life processes.

TABLE 3. Nomenclature of primary xylanolytic enzymes, including the recommended, systematic and discouraged alternative names cited by the International Union of Biochemistry and Molecular Biology (1992).

| Recommended name | Systematic name | Other name(s) |
|---|---|---|
| Endo-1,4ß-xylanase (EC 3.2.1.8) | 1,4-ß-D-xylan xylanohydrolase | Xylanohydrolase Xylanase |
| Xylan 1,4-ß-xylosidase (EC 3.2.1.37) | 1,4-ß-D-xylohydrolase | (exo-1,4-) ß-xylohydrolase Xylobiase |
| α-N-arabinofuranosidase (EC 3.2.1.55) | α-L-arabinofuranoside arabinofuranohydrolase | Arabinosidase |

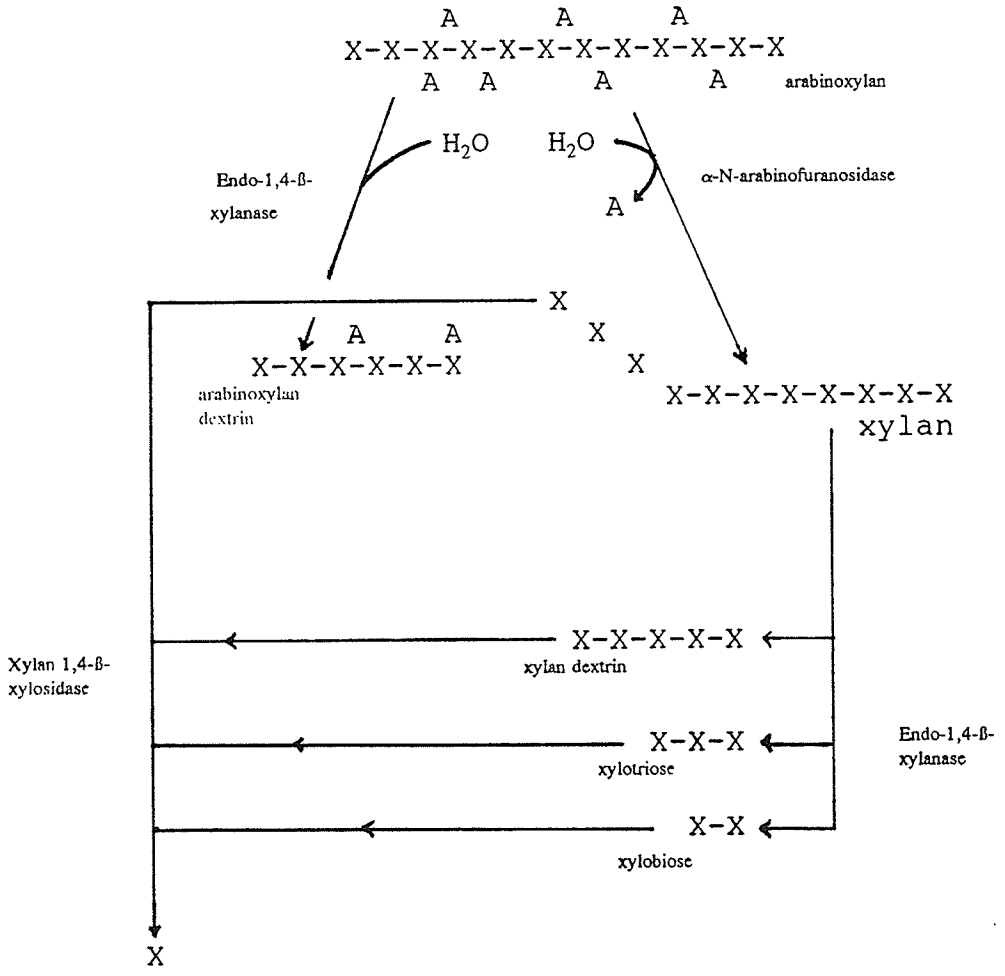


Figure 6. Possible routes by which enzymes degrade arabinoxylan.

The enzyme classification system of the International Union of Biochemistry and Molecular Biology (IUBMB) defines arabinoxylan-hydrolyzing enzymes on the basis of substrate specificity. Endo-1,4-ß-xylanase (pI 5.2), from germinated barley extract (Slade et al, 1989), complies with the EC 3.1.2.8. definition, cleaving xylan and AX substrate with at least three contiguous (1,4)-ß-xylosyl residues. The current system is convenient, but fails to recognize that some cellulase/hemicellulase families are polyfunctional. Another system which may replace it, classifies enzymes based on amino acid sequence similarity. Active site structure determines the substrate specificity of an enzyme, therefore amino acid sequence conservation, within a family of enzymes, may indicate similar substrate requirements. For example, sequence homology between a mannase and a cellulase may indicate that the cellulase would also be active against mannan (Henrissat, 1992). A combination of these two systems would be ideal, allowing the classification of enzymes based on their known and potential substrates, while avoiding the laborious process of testing each possible combination.

The field of enzymology is rapidly expanding, with new knowledge allowing clarification of that gained previously. Multisubstrate activity involving xylose and arabinose is frequently reported. An α-L-arabino/ß-D-xylosidase produced by Clostridium stercorarium (Schwarz et al, 1990), and an endo-1,4-ß-xylanase I purified from Fibrobacter succinogenes,

are able to cleave the xylan backbone as well as remove the arabinose substituents from rye flour arabinoxylan. Most recent studies do not support xylanase release of arabinose, and it has never been reported to hydrolyze other xylan substituents (Biely et al, 1992). Reports of xylanase activity against carboxymethylcellulose (CMC) (Matte and Forsberg, 1992) are frequently published; however, hydrolysis of CMC is only an indicator of activity against a man-made substrate, and should not be interpreted otherwise (Henrissat, 1992).

## Arabinoxylan in Barley Cell Walls

Arabinoxylan is a major component of graminaceous primary cell walls. In barley this includes the endosperm, aleurone and inner, unlignified walls of the husk and other vegetative tissues. Arabinoxylan provides structural support in combination with the flexibility required for normal cell growth and development, because its size and substituents can be manipulated to produce a wide variety of components. The primary cell wall also contains many other constituents that interact with AX, defining its architectural and functional roles in the plant.

Structural Role

The primary cell wall is a carefully orchestrated complex of many molecular subunits. Cellulose microfibrils consisting of linear beta-4-D-glucan aggregates are deposited onto the surfaces of newly formed cells, creating a framework which is later cemented together by various hemicellulosic components (Fincher, 1975; Varner and Lin, 1989). In dicotyledons, xyloglucan provides the bulk of the cementing matrix (20%), penetrating and H-bonding to crosslink the cellulose skeleton. Arabinoxylan usurps the role in monocotyledons (Varner and Lin, 1989), by noncovalently bonding with cellulose in regions that have at least 4 contiguous unsubstituted residues (McNeil et al, 1975). Evidence suggests AX further crosslinks the matrix by bonding with itself (McNeil et al, 1975) in a process to which the arabinosyl substituents may contribute by replacing intermolecular $H_2O$ (Dea et al, 1973). Beta-glucan is associated with these cell wall components, as indicated by their co-extraction under both aqueous and alkali conditions (Fincher, 1975). Immunogold labelling, using antibodies against AX (Barry et al, 1991), will allow visualization of their relative positions within the cell wall.

Both AX and ß-glucan are matrix components, and may share duplicity in their function. Arabinoxylan is more common than ß-glucan in barley aleurone (67 vs. 28%; Bacic and Stone, 1981b) but is less common in endosperm walls (25 vs 75%;

Fincher, 1975). These values may be related to critical structural functions which only these ratios of components can satisfy, or they may simply be the result of random selection between two equally satisfactory subunits. The structural role of AX may be modified by the presence of feruloyl substituents (Gubler et al, 1985; Ahluwalia and Fry, 1986). Peroxidase-catalyzed oxidative coupling of feruloylated residues on adjacent AX molecules (Fig. 7) is thought to strengthen the cell wall (Markwalder and Neukom, 1976; Fry, 1986). The theory is supported by the presence of trans-feruloyl esterase in wheat (McCallum et al, 1991), and of diferulic acid crosslinkages in bamboo (Ishii, 1991). Barley endosperm contains 60 ug ferulic acid/100mg of cell wall, or an estimated 80 esterifications per average AX molecule (Ahluwalia and Fry, 1986). Analysis of total, water-soluble and water-insoluble endosperm cell wall components indicates water-extractable components do not contain ferulic acid (Table 4), although in other studies feruloyl distribution is not as clearly defined (Vietor et al, 1991). Fluorescence microscopy of barley aleurone showed autofluorescent feruloyl groups were evenly distributed throughout the cell wall (Bacic and Stone, 1981a), which agrees with the incorporation process of AX as described by Varner and Lin (1989).

TABLE 4. Ferulic acid content of barley cell walls and each of the cell-wall fractions.

| Cell-wall fraction | Total material | Arabinoxylan component |
|---|---|---|
| Total cell wall | 0.06 | 0.24 |
| Water-extractable | Trace | -- |
| Total water-insoluble | 0.18 | 0.40 |
| Water-insoluble ß-glucan | Trace | -- |
| Water-insoluble arabinoxylan | 0.34 | 0.37 |

(Ahluwalia and Fry, 1986)

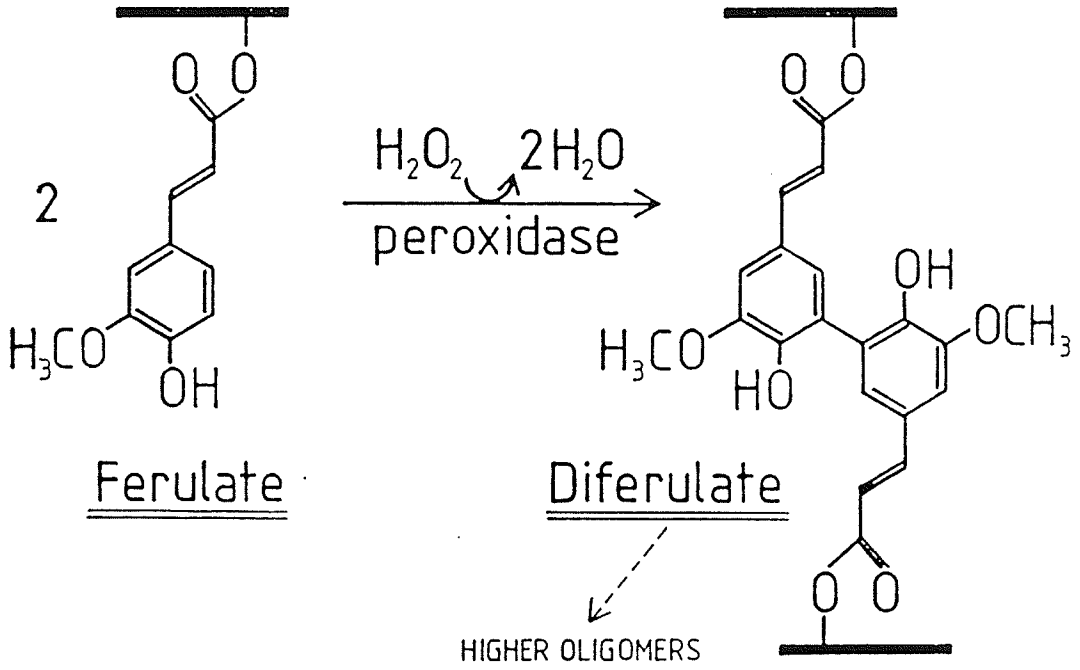


Figure 7. Oxidative coupling of ferulate residues to produce diferulic cross-links (Fry, 1986).

Functional Role

Arabinoxylan plays an important physiological role in cell growth regulation since plant cell expansion must occur within the boundaries of cell walls. A peroxidase-coupled reaction between ferulic acid residues on adjacent AX residues (Markwalder and Neukon, 1976) may control growth by covalently bonding the matrix into rigid, inelastic dimensions. A correlation exists between diferulic acid content and decreased colewtile extensibility in Avena spp. (Kamisaka et al, 1990) and Oryza spp.(Tan et al, 1991). Studies indicate that irradiation with white light inhibits growth in Oryza coleoptiles (Tan et al 1992). Coleoptiles grown in light have an increased ferulic and diferulic acid content, which correlates closely with reduced cell wall extensibility and increased minimum stress-relaxation times (Tan et al, 1992). Irradiation is thought to enhance crosslinking by stimulating the production of phenylalanine ammonia-lyase (Schopfer and Mohr, 1972), which is involved in ferulic acid synthesis.

Arabinoxylan oligosaccharides may also serve a hormone-like function by limiting the auxin-stimulated elongation of cells. Auxin-stimulated Oryza coleoptiles show reduced extensibility if either O-[O-5-feruloyl-α-L-arabinofuranosyl]-(1,3)-ß-D-xylopyranosyl-(1,4)-D-xylopyranose (FAXX) or ferulic acid are present in the media (Ishii and Saka, 1992).

TABLE 5. Inhibitory effects of FAXX[a], AXX[b] and ferulic acid on auxin-stimulated elongation of cells in Oryza lamina joints.

| Test solution | Concentration ppm | uM | % Inhibition with IAA[c] (50 uM) |
|---|---|---|---|
| FAXX | 20 | 32 | 16 |
| | 200 | 320 | 46 |
| AXX | 20 | 48 | 3 |
| | 200 | 483 | 0 |
| Ferulic acid | 20 | 103 | 26 |
| | 200 | 1013 | 59 |

[a] O-[feruloyl-(O-5)-α-L-arabinofuranosyl]-(1,3)-ß-D-xylopyranosyl-(1,4)-D-xylopyranose
[b] α-L-arabinofuranosyl-(1,3)-ß-D-xylopyranosyl-(1,4)-D-xylopyranose
[c] indole acetic acid

(Ishii and Saka, 1992)

Arabinoxylans that affect ice-crystal formation have been isolated from rye, wheat and barley (Kindel et al, 1989). The antifreeze protein produced endogenously in rye leaves increases thermal hysteresis, the difference between melting and freezing points (Griffith et al, 1992). Arabinoxylan, however, acts as an inhibitor of ice formation by competing with free water for positions in the forming ice lattice, disrupting its harmful structure (Olien, 1967).

## Barley arabinoxylan content

Various terms are used to describe barley AX content, including whole kernel, extracted or tissue-specific values. These are also assayed using a variety of methods for which accuracy can differ. The following examples characterize AX occurrence within the barley kernel and its tissues.

### Husk, Aleurone and Endosperm Contents

The barley endosperm cell wall contains about 20% AX (Ballance and Manners, 1978), of which approximately 14% is water-soluble, 78% is NaOH-soluble (1M) and 7% is unextractable (Fincher, 1975). In total, AX constitutes 1.42% of endospermal tissue (Henry, 1987). Aleurone walls are about 67-85% AX; 15% is water-soluble and 85% is alkali-soluble (Bacic and Stone, 1981a; McNeil et al, 1975). Husk also contains AX which is soluble in aqueous NaOH (Aspinall and Ferrier, 1957). Of the total barley AX, approximately 46% is located in the husk, 6% in the pericarp, 24% in the aleurone and subaleurone layers, and 24% in the endosperm (Angelino, 1989; cited in Vietor et al, 1992a).

Tissue and Extract Values

Whole grain, or total, barley AX values representing different locations, varieties and analytical techniques are illustrated in Table 6. Total values indicate the amount of AX present in the entire barley kernel and, because most AX is located in the husk (46%; Angelino, 1989; cited in Vietor et al, 1992a), vary widely between two-rowed, six-rowed and hull-less varieties. The soluble AX fractions (Table 6) are an important measure of quality in cereal grains such as wheat and rye because they significantly affect the baking quality (Shogren et al, 1987; Delcour, 1991) and the nutritional properties of poultry diets (Choct and Annison, 1990; Fengler and Marquardt, 1988). Acid-soluble values best assess barley used in chick diets because the analysis is designed to simulate fibre solubilization in the chick gut (Rotter et al, 1989a). Soluble arabinoxylan concentration in barley (0.72%) is lower than in wheat (1.04%) or rye (2.45%) (Henry, 1985).

TABLE 6. Arabinoxylan content (% DM) of barley from different locations.

| Location | Arabinoxylan | Method | Reference |
|---|---|---|---|
| Total | | | |
| Australia | 4.38-7.79 | GC | Henry (1986) |
| Finland | 6.7-9.8[a]<br>7.3-11.0[b] | Duffau | Lehtonen and Aikasalo (1987a) |
| Canada[d] | 2.7<br>5.5 | GC<br>Orcinol | Bhatty et al (1991) |
| Water-soluble | | | |
| Australia | 0.72 | PG[c] | Henry (1985) |
| Acid-extracted | | | |
| United Kingdom | 0.6-0.84 | Orcinol | Smith et al (1980a) |

[a,b] two-rowed and six-rowed barley, respectively
[c] Phloroglucinol method
[d] Hulless barley

TABLE 7. Beta-glucan content (% DM) of barley from different locations.

| | Hull | ß-glucan | Reference |
|---|---|---|---|
| Total | | | |
| Denmark | Hulled | 2.0-6.4 | Aastrup (1979) |
| Canada | Hulless | 3.9-5.4 | Bhatty et al (1991) |
| Australia | Hulled | 3.9-4.8 | Henry (1985) |
| Finland | Hulled[a] | 3.5-5.3 | Lehtonen and Aikasalo (1987b) |
| | Hulled[b] | 3.0-5.6 | |
| Acid-soluble | | | |
| Denmark | Hulled | 0.14-2.34 | Aastrup (1979) |
| Canada | Hulless | 1.0-2.7 | Bhatty et al (1991) |
| Water-soluble | | | |
| Australia | Hulled | 2.89 | Henry (1985) |

[a,b] two-rowed and six-rowed barley, respectively

## Varietal and Environmental Effects

A survey of Australian barley cultivars (Henry, 1986) showed that total AX content was significantly affected by both genetics and environment. The genetic effect indicates that plant breeding may successfully be used to manipulate these levels. The total AX concentration of Finnish barley cultivars differed statistically among three locations at which they were grown (Lehtonen and Aiksalo, 1987a). Although the authors stated that the AX levels differed due to varietal effects, the experiment was not well designed to measure the environmental impact.

## Correlation with ß-glucan Content

Beta-glucan (Table 7) and AX are the most common matrix polysaccharides in barley (Fincher, 1975; Henry, 1986; Vietor et al, 1991), and presumably act to strengthen the cell wall. Therefore, current breeding programs to reduce the ß-glucan content of malting and feed barley may indirectly select for reduced cell wall strength, or increased AX content. When cellulose production is inhibited in barley endosperm, crosslinking increases between the elevated ß-glucan (4X) and AX components, producing cell walls with greater tensile strength and turgor pressure resistance (Shedletzky et al, 1992). The observations by Schedletzky et al (1992) suggest

that monocot cell walls are not completely dependent on hemicellulose-cellulose interactions for strength, and that the ß-glucan-AX interaction is very important. A correlation between total ß-glucan and AX content has not been determined in barley (Henry, 1986; Lehtonen and Aikasalo, 1987a,b), possibly because the high level of AX present in the husk is required for lignification. Therefore, a correlation among acid- or water-soluble ß-glucan and AX values would better determine the role of these carbohydrates in the endosperm cell wall, and whether they are structurally equivalent or interchangeable.

## Analytical Methods

Arabinoxylan has been quantified in cereal grains using colorimetric, chromatographic and enzymatic techniques. The variety of methods available provides flexibility depending on the accuracy, speed and economy of analysis required.

### Colorimetric Analyses

Colorimetric techniques were developed as rapid and inexpensive analyses that could be performed in most laboratories (Douglas, 1981; Bell, 1985). Polysaccharides containing pentose sugars are hydrolyzed and the constituents are quantified using several alternative methods. Pentoses

may be converted to furfural (2-furancarboxyaldehyde, $C_5H_4O_2$), then distilled and reacted with aniline acetate to yield a product which is measured by absorbance (Cerning and Guilbot, 1973). Phloroglucinol may be reacted with the pentose sugars to produce a chromagen for which the absorbance is read directly, without distillation (Dische and Borenfreund, 1957). However, the reproducibility of the phloroglucinol method is not reported to be satisfactory (Edney, personal communication). The orcinol method (Albaum and Umbreit, 1947) measures aldopentoses after their conversion to furfural (Dische, 1962) and reaction with 5-methyl-1,3-benzenediol. Glucose is also detected at the absorbance optimum for orcinol determination; therefore, the hexose must be removed by fermenting samples with fresh yeast (Hashimoto et al, 1987). An easier method involves determining the absorbance of the solution at two wavelengths which have differential absorption by the pentose chromagens. However, the wavelengths give equivalent readings for glucose, allowing pentose determination by difference (Delcour et al, 1989). Dichromatic readings may not compensate for uronic acids and the ribose component of nucleosides and nucleotides, which are also detected by orcinol reagents (Brown and Hayes, 1952). Frequent discrepencies from GC values have been illustrated for colorimetric methods (Henry, 1985; Bhatty et al, 1991). Therefore, despite the low labor and capital inputs required, analysis by colorimetry is restricted for purposes where

absolute values are required (ie: comparison between laboratories).

## Chromatographic Methods

Although paper chromatography can be used to identify NSP components (Grant and D'Appalonia, 1991), gas chromatography (GC) and high performance liquid chromatography (HPLC) are the most common chromatographic methods used for determining xylose and arabinose content. Modification of the hydrolysis procedure (Henry, 1986) has reduced the largest source of error for each, and resulted in better agreement between methods (Henry, 1987; Table 8). The HPLC analysis does not require acetylation (Izydorczyk et al, 1991), and the elimination of the inherent errors associated with this step, makes this method the most reproducible. Carbohydrates are

TABLE 8. Total arabinoxylan concentrations (% dry matter; DM) determined for the same Australian wheat, barley, oats and rye grain samples using phloroglucinol and gas chromatographic assays.

| | Barley | Oats | Rye | Wheat |
|---|---|---|---|---|
| Henry (1985)[a] | 4.07 | 5.05 | 5.36 | 3.32 |
| Henry (1985)[b] | 5.69 | 7.65 | 8.49 | 6.63 |
| Henry (1987)[c] | 6.60 | 5.74 | 8.96 | 6.59 |

[a] Gas chromatography using unmodified hydrolysis technique
[b] Phloroglucinol analysis
[c] Gas chromatography using modified hydrolysis technique

usually separated on an anion exchange column, and quantified using pulsed amperometric detection, because other HPLC equipment is found to be ineffective (Rocklin and Pohl, 1983). Despite the accuracy and acceptance of these methods, high capital (HPLC) and labor (GC) requirements may be prohibitive for some labs.

Enzymatic Analyses

Specific enzymic methods have been used to quantify xylose and arabinose produced from AX hydrolysis by rumen fungi (Williams and Orpin, 1987). An NAD-dependent ß-D-galactose dehydrogenase oxidizes arabinose to arabinonic acid, producing NADH + $H^+$ which is measured spectro-photometrically (Melrose and Sturgeon, 1983). Although this method appears promising, improvements must be made before it is widely accepted. The α-N-arabinofuranosidase used cleaved 1,3 linkages between arabinose and xylose, but a substantial portion of arabinose is α-1,2- bonded to the xylan backbone (Vietor et al, 1992a), and may escape detection. Free galactose in samples contribute to error by producing NADH + $H^+$; however, this can be reduced by using a substrate blank and purified α-N-arabinfuranosidase.

Xylose may also be enzymatically quantified using commercially available reagents (Williams and Withers, 1986). The monosaccharide is reduced to xylitol using sodium

borohydride, and then converted to xylulose by sorbitol dehydrogenase in the presence of NAD. The amount of reduced NAD in solution is proportional to the initial xylose concentration, and is measured either directly by its absorbance at 340nm, or by its production of formazan following a second enzymic reaction. Sorbitol dehydrogenase also reacts with glucose; therefore, hydrolysis methods currently used to quantitatively extract xylose and other components from the cell wall, will overestimate the xylose content. As with the enzymatic ß-glucan assay (McCleary and Glennie-Holmes, 1985), an enzyme system which hydrolyzes AX specifically and completely will have to be developed before enzymatic quantification can be fully utilized.

Arabinoxylan quantification is based on the amount of arabinose and xylose released by the hydrolysis of a sample, regardless of whether the pentose sugars originate from AX, xyloglucan or arabinogalactan. Enzymic analyses have the potential to increase the accuracy of these analyses, because the substrate specificity of enzymes would allow selective hydrolysis of AX.

## Arabinoxylan Viscosity

Viscosity is a description of the flow behavior of a solution to which a shear force is applied. It measures the internal friction of a solution resulting from the mutual, noncovalent interactions between the solvent and solute molecules. Solubilized polysaccharides increase viscosity in solution because and hydrogen bonds are common, and hydrogen bond cooperativity dictates that the binding energy in a structure is greater than the sum of its bonds (Jeffrey and Saenger, 1991). Viscosity is mainly affected by the size and shape of molecules (Dunstan and Thole, 1914).

### Effect of Molecular Size and Substitution

Many polysaccharides adopt a random coil structure in solution (Morris et al, 1981); however, highly substituted molecules such as hydroxyethylcellulose (Castelain et al, 1987) and AX (Andrewartha et al, 1979; Izydorczyk and Biliaderis, 1992b) assume a more linear form due to the steric hindrance of their substituents. Linearity affords AX molecules an increased opportunity to come into contact and become entangled in solution. Unusually high viscosities at relatively low concentrations result (Morris et al, 1981), in comparison to globular molecules like proteins. Substituent

groups may also contribute indirectly to viscosity through increased solute-solvent (Castelain et al, 1987) and solute-solute interactions. When arabinosyl groups are removed from wheat AX by α-N-arabinofuranosidase, intrinsic viscosity decreases with time, indicating that the substituents contribute to extract viscosity (Andrewartha et al, 1979). Interpretation of this type of data is very difficult because there is more than one explanation for what is observed. Cross-contamination of the α-N-arabino-furanosidase with endo-1,4-ß-xylanase would also explain the drop in viscosity. Additionally, an initial increase in viscosity would normally be expected following this type of digest procedure due to localized aggregation of fibres, unless substituent removal had resulted in a significant reduction in molecular linearity (E.A. Macgregor, personal communication).

The effects which AX structural differences have on the viscosity of a complex barley extract can also be determined by separating the molecules into discrete fractions based on solubility (Vietor et al, 1991; Izydorczyk et al, 1992b). Fraction characteristics including molecular weight, substitution patterns and viscosity indicate how these properties are correlated and how they contribute to extract viscosity as a whole. Izydorczyk and Biliaderis w992a) used molecular seive chromatography to fractionate water-soluble wheat AX and to determine the relationship between chain length and viscosity. Although the A:X ratio decreased with

increasing molecular weight, viscosity increased substantially, indicating molecular length was its most important determinant. The relationship of molecular size and viscosity was confirmed by stepwise addition of ammonium sulphate to wheat AX extract: the large molecules which precipitated first had fewer substituents and higher viscosity than those which remained in solution (Izydorczyk and Biliaderis, 1992b). Smaller polymers are more substituted and linear in solution, ensuring a uniform contribution to viscosity by all molecules in the extract. Molecular size and viscosity determinations have not been performed for barley AX, but stepwise ethanol precipitation indicates that A:X ratios increase with solubility (Vietor et al, 1992a).

Contribution to Barley Extract Viscosity

Beta-glucan, the major non-starch polysaccharide in barley endosperm walls (Fincher, 1975), is the barley extract component to which increased viscosity has historically been attributed (Aastrup, 1979; Scott, 1972). Initial studies of water-soluble and -insoluble barley ß-glucan concentrations suggested that discrepencies between the viscometric and enzymatic values were due to the presence of AX (Anderson et al, 1978). However, the methodology used in this experiment has been questioned and the assay results are not widely accepted (Bhatty, 1987). In later studies, barley extract was

separated into high and low molecular weight components and, based on the elimination of viscosity using only ß-glucanase and α-amylase, it was concluded that AX did not significantly contribute to viscosity (Smith et al, 1980a; 1980b). Critical review of this literature revealed that both the ß-glucanase and the α-amylase preparations were impure or non-specific, and exhibited acitivity against arabinoxylan standards (Smith et al, 1980b). The viscosity reduction (Smith et al, 1980a) therefore overestimated both ß-glucan and starch contributions to viscosity, and deleted that which should have been attributed to AX.

Current component analysis and viscosity reduction studies indicate that AX contributes to acid extract viscosity (AEV), especially in high viscosity barley (Bhatty, 1987). The AX (r=0.61), arabinose (r=0.60) and xylose (r=0.63) content of barley acid extract are significantly correlated with AEV (Bhatty et al, 1991), indicating either a direct contribution to viscosity or a strong correlation with another viscosity-producing component, such as ß-glucan. The AX- and arabinose-AEV correlations may be weakened because they include arabinose from nonviscous arabinogalactan (Fincher and Stone, 1974). Conclusive evidence of AX's contribution to AEV is provided by the ablility of purified endo-1,4-ß-xylanase to reduce AEV through selective AX hydrolysis, as indicated by the rapid reduction in barley extract viscosity, and the subsequent stabilization of the viscosity baseline (Bhatty et

al, 1991). The relative contributions to viscosity by beta-glucan and AX are illustrated in Figure 8.

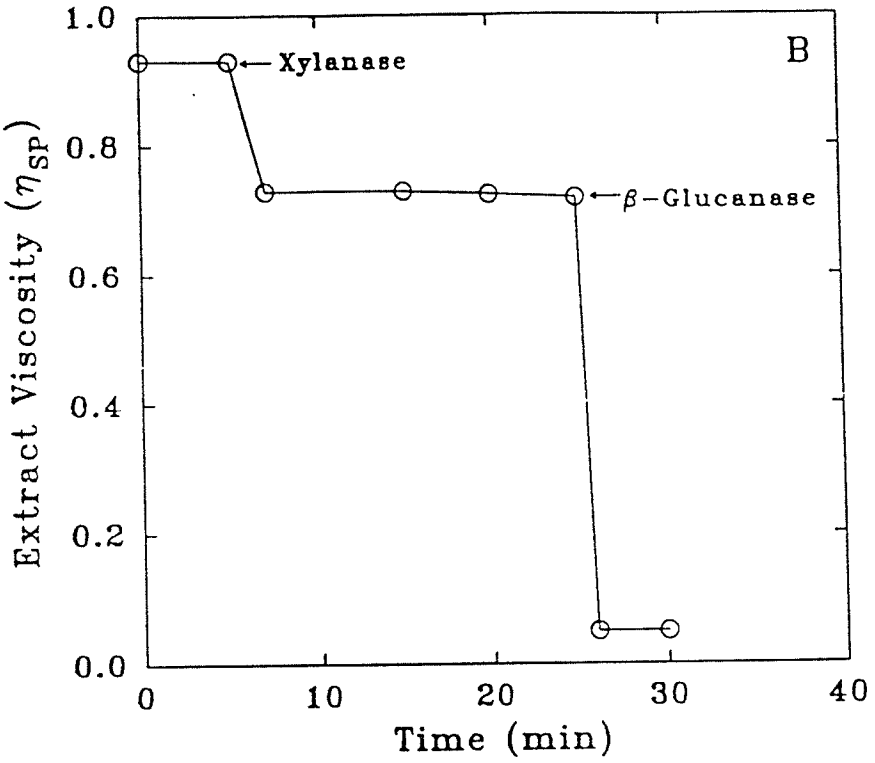


Figure 8. Effect of sequential addition of xylanase and ß-glucanase on the acid extract viscosity of hulless barley solution containing 2.5% ß-glucan and 0.5% arabinoxylan, by weight (Bhatty et al, 1991).
[a] $\eta_{sp}=(t_-t_o)/t_o$ where t and $t_o$ are running times of the sample and extraction buffer, respectively.

Antinutritional Effects of the Primary Viscous Polysaccharides

Arabinoxylan in wheat and rye, and ß-glucan in oats and barley, exert antinutritional effects when these cereals are used in the diets of young poultry. This is confirmed by the ability of isolated viscous polysaccharide fractions to reproduce the deleterious effects when added to control diets. Water- and alkali-extracted AX preparations from wheat (Choct and Annison, 1990; 1992) and rye (Ward and Marquardt, 1987; Fengler and Marquardt, 1988) reduce performance parameters including growth rate, AME value, and nitrogen and lipid retention. Beta-glucan supplementation of broiler diets has been too brief for accurate appraisal of its affects on growth performance (White et al, 1981); however, supplemental hydroxyethylcellulose, which has similar physical properties, depresses feed efficiency and growth in broiler chicks (White et al, 1983). The antinutritional effects of viscous polysaccharides are seen mainly in young birds (0-4 wks) and disappear with ageing (Salih et al, 1991).

Reduced Nutrient Absorption

The viscosity of AX and ß-glucan solutions may be responsible for their antinutritional effects in young poultry fed diets containing barley. Beta-glucan supplementation of broiler chick diets increases the viscosity of the aqueous digesta phase (White et al, 1981). From studies in rats, elevated viscosity is proposed to increase the thickness of the unstirred layer at the gut lumenal surface, resulting in the observed decrease in monosaccharide and amino acid absorption (Elsenhans et al, 1980). The size of digestive organs and the volume of their secretions increases as a result (Ikegami et al, 1990, Campbell et al, 1983). Alkali-extracted wheat arabinoxylan (10 to 40 g/kg diet) reduces AME and ileal digestibilities of starch, protein and lipid by up to 17.1%, 14.6%, 18.7% and 25.8%, respectively, in a manner significantly correlated to the dietary AX concentration (Choct and Annison, 1992). Rye-based diets, in comparison to wheat, elevate fore and hind gut viscosities (Bedford et al, 1991). Calcium retention in rye-based diets is reduced, and vitamin $D_3$ provided in the diet at 1000 times NRC-recommended levels is nontoxic, presumably due to the impairment of gut absorption (Antoniou et al, 1980).

The mechanism by which AX reduces nutrient absorption is not fully understood, it is presumably related to its ability to limit dialysis of glucose and salt in a concentration-

dependant manner (Fengler and Marquardt, 1988). Arabinoxylan may restrict diffusion by seiving molecules with its network of fibres. The "seive" model indicates that molecules pass through holes in the AX lattice, a process termed "effusion". The rate of effusion is inversely proportional to the square root of the substance's molecular mass (Gillespie et al, 1986), and explains why NaCl dialyzes more rapidly than glucose from an AX solution (Fengler and Marquardt, 1988) and why lipid absorption from rye diets is severely depressed (Campbell et al, 1983).

A recent methodological development enables an accurate assessment of the in vivo problems produced by wheat and rye AX (Bedford and Classen, 1992: Bedford et al, 1991). The amount of soluble fibre dissolved in the liquid portion of the chick gut content correlates well with its observed log viscosity (Bedford and Classen, 1992). Therefore, measurements to determine the relationship of digesta viscosity with chick performance, or with dietary level of enzyme supplementation, are made on the fluid portion of the fresh digesta, as isolated by centrifugation. Results from gel-filtration chromatography indicated that only the high molecular weight fibre fraction (HMW), which co-eluted with dextrans of ≥500,000 MW, was correlated ($P<0.01$) with digesta viscosity in the fore ($r=0.58$) and hind ($r=0.66$) gut, and that log viscosity was negatively correlated ($P<0.01$) with both feed conversion efficiency (FCE; 0.63) and weight gain (0.71).

Enzyme supplementation reduced the HMW fraction and viscosity in the foregut ($P<0.01$), which correlated with increased FCE and weight gain (Bedford and Classen, 1992; Bedford et al, 1991). Young broiler chickens fed barley-based diets supplemented with a multi-enzyme preparation showed a similar improvement in performance, correlated with gut viscosity ($P<0.05$; Graham et al, 1993).

Increased Digesta Transit Time and Bacterial Proliferation

Elevated gut viscosity increases feed transit time in young poultry, and is usually associated with a proliferation of bacteria in the upper small intestine (Salih et al, 1991). Poorly absorbed nutrients would be more readily available to microorganisms, and this would assist their colonization and growth. Antibiotic supplementation of viscous diets improves chick performance and tibia ash content (Classen et al, 1985), implicating a microbial contribution to the antinutritional effects of viscous polysaccharides. Bile salt supplementation restores tibia ash levels, therefore bile salt deconjugation may be one mechanism through which this occurs (Campbell et al, 1983).

Beta-glucan (Graham and Pettersson, 1992) and AX produce sticky feces and poor litter quality in intensive, litter-housed poultry operations (Classen and Bedford, 1991). This has presented a serious obstacle to the use of cereals

containing high concentrations of viscous polysaccharides in poultry diets. Enzyme-supplementation reduces fecal ß-glucan viscosity (Rotter et al, 1989b; Burnett, 1966) and barley-based diets are now common in western Canada. Litter quality is adversely affected by AX in rye and, despite dramatic increases in chick performance associated with enzyme supplementation (Friesen et al, 1991, 1992; Bedford et al, 1991), its use in broiler diets is still not practical.

Enzyme supplementation will not substantially enhance the utilization of xylose and arabinose residues by poultry, because only partial AX hydrolysis is expected during the brief digesta transit time of young poultry (Classen and Bedford, 1991). In addition, only a fraction of ingested arabinose is utilized (62%) by adult poultry, and the unabsorbed portion increases fecal moisture (Schutte et al, 1991), which may contribute to poor litter quality.

## Antinutritional Effects of Arabinoxylan in Barley

Beta-glucan is the most common NSP in barley endosperm (Fincher, 1975) and extracts (see Table 6 and 7); however, the concentration of AX in barley is sufficient to produce speculation that it may contribute significantly to gut viscosity and the associated antinutritional effects (Classen and Bedford, 1991; de Silva et al, 1983). Barley diets supplemented with either a preparation which primarily

contained ß-glucanase (50 IU endoxylanase/kg feed; H. Graham, personal communication), or a multi-enzyme product containing both ß-glucanase and xylanase (300 IU endoxylanase/kg feed), supported equivalent levels of production (Graham and Pettersson, 1992). The workers noted that the multienzyme complex produced numerically superior values for weight gain, intake and FCE, and reduced body weight variability (CV=7%) relative to ß-glucanase. The difference was attributed to the secondary activities, including xylanase, of the multienzyme complex; however, the conclusion is questionable because endoxylanase was present in both enzyme supplements, the improvements were non-significant, and the coefficient of variation (CV) for the untreated barley control (9%) was also less that that of the ß-glucanase-supplemented diet (15%).

## Viscosity-related Problems in Malting Systems

Malting of barley is a controlled germinative process which produces hydrolytic enzymes required for the modification of barley endosperm contents. Various ß-glucan- and arabinoxylan-hydrolyzing enzymes digest cell walls, exposing the endosperm to the various amylases and proteases required for the extraction of starch and protein reserves. If enzyme activity is insufficient for the hydrolysis of ß-glucan and AX, viscosity-related problems may occur, including reduced extract yield and increased filtration time (Table 9).

TABLE 9. Results of mashes on high and low glucanase grists.

| | Malt A | Malt B |
|---|---|---|
| Glucanase units | 170 | 2770 |
| Predicted extract | 131.0° | 131.6° |
| Actual extract | 123.8° | 130.6° |
| Wort run-off time | 604 min | 504 min |
| Viscosity 1st worts | 6.2 cp | 4.1 cp |

° litre degrees/kg
cp centipoise

(Bourne et al, 1976)

Viscosity-related problems have traditionally been attributed to ß-glucan because of its predominance in endosperm cell walls (Fincher, 1975) and preferential solubilization during modification (Palmer, 1989). However, in recent studies of wort NSP composition, AX content (1.7 g/L) far exceeded that of ß-glucan (0.26 g/L) (Vietor et al, 1992d), indicating that AX could have a significant effect on endproduct quality.

Arabinoxylan is a high molecular weight component of barley extracts (Smith et al, 1980b), that degrades at a slower rate than ß-glucan during malting (Vietor et al, 1991). Solubilized AX has been implicated in impaired wort filtration (Vietor et al, 1992d) and haze production in beer (Coote and Kirsop, 1976). Arabinoxylan contributes to the viscosity of barley acid extracts (Bhatty et al, 1991), and reports indicate that crude ß-glucanase preparations which liberate arabinose and xylose, improve wort filtration rates more effectively than those without similar activity (Canales et

al, 1988). Complete hydrolysis of AX during malting and brewing may also be undesirable, because AX is thought to contribute to beer attributes like foam stabilization (Dale, 1986).

## Future Research Involving Barley Arabinoxylan

The importance of AX in barley-based diets for young poultry, and of xylanase in the multi-enzyme preparations commonly used to remove polysaccharide ANFs, cannot be adequately assessed from the information currently available. The knowledge that AX significantly affects diet utilization would indicate that xylanase activity should not be lost from enzyme cultures through random mutation. Enzyme levels can also be tailored by manipulation of the culture substrate (Royer and Nakas, 1990) once the dietary requirements are known. Conversely, if AX does not affect performance, resources which would be directed toward xylanase production could be used elsewhere. Current barley breeding programs, designed to reduce the soluble ß-glucan content of barley (B. Rossnagel, personal communication), may alter endospermal cell wall characteristics, including the solubility of AX. Analysis of AX concentrations in the soluble and total fractions of barley cultivars would allow plant breeders to assess its status and the potential for genetic manipulation of this trait.

Beta-glucan is recognized as the most important ANF in barley for poultry; therefore, the ß-glucanase level is the most critical component in enzyme products used in the supplementation of young poultry diets (Graham and Pettersson, 1992). In comparison to ß-glucan concentrations, the AX content of barley endosperm is much lower (Fincher, 1975). Arabinoxylan also contributes less to barley AEV (Bhatty et al, 1991). Therefore the effects of AX, and the improvements resulting from its enzymatic hydrolysis, are anticipated to be smaller than those relating to ß-glucan. However, considering the size of the poultry industry, and the potential economic benefit from using barley as a cereal source in diets for young poultry in western Canada, any significant improvement in performance has important financial implications. Information regarding the nutritional effects of AX and xylanases may also add foresight to future decisions affecting barley and enzyme composition.

# MANUSCRIPT I: TOTAL, WATER-SOLUBLE AND ACID-SOLUBLE ARABINOXYLAN CONCENTRATIONS IN WESTERN CANADIAN BARLEY CULTIVARS AND BREEDER LINES

## ABSTRACT

Total, water-soluble and acid-soluble arabinoxylan (AX) levels were determined for six-rowed, two-rowed and hulless barley (Hordeum vulgare) breeder lines and cultivars grown in the 1991 Western Canadian Barley Cooperative program. Analyses were performed using a dichromatic modification of the orcinol method. The six-rowed barley type contained greater total, water-soluble and acid-soluble AX concentrations than hulless or two-rowed types ($P \leq 0.05$). The AX in hulless barley was less than the total ($P < 0.01$), greater than the water-soluble ($P < 0.01$), and equal to the acid-soluble ($P > 0.05$) level of corresponding fractions from two-rowed barley. Environment had a significant effect on water- ($P < 0.01$) and acid-soluble ($P < 0.05$) AX. Genetic differences affected the AX content of breeder lines and cultivars within each the barley types ($P < 0.05$). Acid-soluble AX levels were positively correlated with log acid-extract viscosity ($r = 0.55$; $P < 0.01$; $n = 51$).


KEY WORDS: arabinoxylan, barley, viscosity, acid-extract

# INTRODUCTION

Barley (Hordeum vulgare) ß-glucan and arabinoxylan are major structural components of the endospermal cell wall (Fincher, 1975). The viscosity of barley acid-extract is significantly correlated to the levels of solubilized ß-glucan and arabinoxylan (r=0.71 and r=0.63, respectively; Bhatty et al. 1991). Viscosity is an important indicator of malting (Morgan, 1977) and nutritional quality (Rotter et al. 1989), because it is negatively correlated with performance. Viscosity-related problems have traditionally been associated with the solubilization of endospermal ß-glucan, and eliminated by beta-glucanase supplementation of the diet (Classen and Bedford, 1991) or wort (Canales et al. 1979). However, evidence suggests that the other major component of barley endosperm cell walls, arabinoxylan, may also contribute to extract viscosity problems. Purified xylanase reduces the viscosity of acid-extracts from hulless barley (Bhatty et al. 1991), and ß-glucanase preparations which contain xylanase are thought to be more effective at reducing viscosity-related problems in wort (Canales, 1988) and poultry diets (Graham and Pettersson, 1992).

Arabinoxylan content, and its relationship to acid-extract viscosity, has been determined for a number of Canadian hulless cultivars (Bhatty et al. 1991); however, less is known about two- and six-rowed barley. In the current

study, total, water- and acid-soluble arabinoxylan levels were determined in several cultivars and breeder lines of barley grown throughout western Canada to establish the range in values and to evaluate environmental and genetic effects. The correlation between viscosity and arabinoxylan levels of acid-extract solutions was determined.

# MATERIALS AND METHODS

## Plant Material

Barley (1990, cv. Harrington) and wheat (Western Canada Composite, No. 1 CWRS Wheat, 1990) were used to develop laboratory methods. Cultivars of hulless, six-rowed and two-rowed barley were selected from those grown in the 1991 Western Canadian Barley Cooperative Program (Appendix 3). Samples were ground (Udy cyclone mill; 0.5 mm screen) and analyzed for dry matter (DM) content under vacuum (29 atm at 50°C) overnight. Cultivars and breeder lines which contained the highest water-soluble arabinoxylan levels were analyzed to determine acid-soluble and total concentrations.

## Extraction Procedure

Samples (0.5 g) for water-extraction of arabinoxylan were weighed into screw-capped centrifuge tubes[1] (10 mL) and suspended in 5 mL Nanopure[2] water. The tubes were secured horizontally in a shaking bath (39°C) and agitated (120 oscillations/min) for 60 min. The samples were centrifuged (7500 x g; 20 min), the supernatants were filtered through glass wool, and the pellets were re-extracted for 10 min under

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1 Nalgene Co., Rochester, NY 14602-0365.

2 Barnstead Thermolyne Corp., Debuque, IA 52004-0797.

the same conditions. The supernatants from the re-extraction were filtered through the original glass wool and pooled with the first supernatants. The acid extraction procedure for arabinoxylan was similar to that described above except for the following: the extraction solution contained 82.8 mL 1M HCl + 7.46 g KCl/L, and samples were centrifuged at 1000 x g (Bhatty et al. 1991). The procedural change facilitated the comparison of arabinoxylan and viscosity values.

Procedure Development

The arabinoxylan assay was based on the orcinol method (Hashimoto et al. 1987) using differential spectrophotometry (Delcour et al. 1989) which reduces the absorbance interference. Standard curves, using either xylose or arabinose (0 to 60 μg/ml), were compared. Glucose interference was measured at a range of concentrations (0-500 μg/ml) in a xylose (30 μg/ml) solution. The effects of incubation time (30 and 60 min) on arabinoxylan detection in wheat and barley water-extracts were determined. Optimization of the prehydrolysis time was determined by incubating 1.5 ml extract with 1.5 ml 4N HCl at 100°C for 0 to 180 min., and then performing the orcinol analysis on the hydrolysate. All materials used were analytical grade.

Arabinoxylan Determination

Whole barley or its water- or acid extracts were analyzed to determine the levels of total, water-soluble and acid-soluble arabinoxylan, respectively. Ground grain (5 mg), water-extract (0.4 mL) or acid-extracts (0.4 mL) were diluted with water to 3 mL total volume in screw-capped test tubes. Chromagen reagents were added as described by Hashimoto et al. (1987) and samples were incubated in boiling water for 30 min. Absorbance was measured using differential spectrophotometry ($A_{670}$ minus $A_{580}$) (Delcour et al. 1989), and pentose sugar content was determined by comparison with xylose standards. A standard barley sample (1991, cv. Harrington) was analyzed concurrently to monitor day-to-day variation. Arabinoxylan was calculated as the pentose sugar content X 0.88.

The variance within each day of analysis was monitored using sample replicates. The analysis was repeated if the four total arabinoxylan replicate values did not lie within 5% of their mean. Acid- and water-soluble values were based on duplicate analysis of each of 2 separate extractions per sample. The duplicate analyses had to lie within 3% of their mean, as did the duplicate extraction means, or the sample was reanalyzed.

## Hydrolysate Monosaccharide Analysis

The xylose and arabinose contents of barley and its extracts were analyzed using the method adapted by Slominski and Campbell (1990) from Englyst and Cummings (1984), with the following modifications. Ground grain (0.1 g), water-extract (6 ml) and acid-extract (6 ml) samples were prepared as described above, and placed in screw-capped test tubes. The ground grain samples were preincubated with 1 ml $H_2SO_4$ (12 M) at 35°C for 1 hr, and then diluted with water (6 ml). Extracted samples were combined with 12 M $H_2SO_4$ (1 ml) and an internal standard (5 ml 0.1% w/vol myoinositol in saturated benzoic acid solution), vortexed gently, and incubated (100°C) for 2 hr.

## Determination of Total ß-glucan and Acid Extract Viscosity

The total ß-glucan content of barley samples was determined using the method of Jørgensen and Aastrup (1988). The viscosity of the acid-extracted samples was measured using a flow-through viscometer (Glennie-Holmes, 1989).

Statistics

The cultivars of barley and the locations where they were grown are shown in Table 10. This was a randomized incomplete block design, where all cultivars were grown in two or more locations (ie:blocks), but not at all locations. An additional blocking factor was introduced to account for the day-to-day variation of chemical analysis, because Brown and Hayes (1952) indicated that the orcinol values were extremely sensitive to variations in the boiling procedure.

The barley cultivars were grown as part of the 1991 Western Canadian Barley Cooperative Program, and consequently provided a broad and current range of material from which to determine the environmental and genetic effects on barley AX levels. The effect of location, type, cultivar and day of chemical analysis on arabinoxylan concentrations was analyzed using the general linear models for ANOVA (SAS Institute Inc., 1982). The values were reported as least square means (LSMs), as necessitated by the incomplete block design. In analyzing total arabinoxylan data, the values were preadjusted for day of analysis effect: the average of the day LSMs was subtracted from the average for each day of analysis, and the difference was added to the sample values corresponding to each day of analysis. The data was preadjusted because the factors of interest (ie: location, type, cultivar(type)) could not be analyzed separately from day in the same analysis (SAS

TABLE 10. The number of hulless, two-rowed and six-rowed barley cultivars analyzed for total (T), water-soluble (W-S) and acid-soluble (A-S) arabinoxylan levels at each growing location.

| | Hulless barley | Two-rowed barley | Six-rowed barley |
|---|---|---|---|
| Beaverlodge, Alberta. | W-S = 15<br>A-S = 3<br>T = 3 | | W-S = 20<br>A-S = 8<br>T = 8 |
| Lacombe, Alberta. | | W-S = 18<br>A-S = 7<br>T = 7 | |
| Trochu, Alberta. | W-S = 15<br>A-S = 3<br>T = 3 | | |
| Watrous, Saskatchewan | | | W-S = 20<br>A-S = 8<br>T = 8 |
| Elrose, Saskatchewan | | W-S = 18<br>A-S = 7<br>T = 7 | |
| Melfort, Saskatchewan | | W-S = 18<br>A-S = 7<br>T = 7 | W-S = 20<br>A-S = 8<br>T = 8 |

Institute, 1982). Differences between means were analyzed using contrasts (Steel and Torrie, 1980).

The day LSMs were correlated with the Harrington control values from each day of analysis. The correlation indicated the accuracy of the statistical analysis in estimating the observed day effect.

Cultivar x location interaction was estimated using Spearman correlations (Steel and Torrie, 1980). A high correlation indicated that the varieties behaved similarly between locations, and that mean differences were not due to random reranking of varieties. A Pearson correlation between total ß-glucan, viscosity and arabinoxylan values was used to determine their relationships.

## RESULTS AND DISCUSSION

### Method Modifications

The orcinol assay was modified to improve its efficiency. The water-extracted arabinoxylan levels (% DM) detected in barley did not differ ($P<0.01$) when boiled with the orcinol reagents for 30 or 60 min (0.29%, SD=0.01 vs. 0.32%, SD=0.02; n=8). Wheat arabinoxylan values were also the same ($P<0.05$) at 30 and 60 min (0.58%, SD=0.03 vs. 0.62%, SD=0.03; n=8); therefore, the shorter incubation period was chosen. The two-hour prehydrolysis (Hashimoto et al. 1987) was omitted because

maximal arabinoxylan content had been determined following color development in boiling concentrated HCl (30 min). Xylose was used to construct the standard curve in all assays because its equation (Pentose = 148.43(Absorbance) + 0.6736; $r^2$=0.999) was nearly identical to that of arabinose (Pentose = 148.39(Absorbance) - 0.5199; $r^2$=0.997). The concentration of glucose (0 to 500 μg/ml) in a xylose solution (30 μg/ml) did not affect its absorbance curve, as indicated by its zero slope (X coeff= -0.003). This verified that differential spectrophotometry could adequately correct for glucose interference. Using the modified method, percent recovery of xylose averaged 89.2% over 5 days of analysis, with values ranging from 86.8 to 92.1%.

Comparison of Orcinol and Gas Chromatography Results

Gas chromatography analysis for total, water-soluble and acid-soluble arabinose and xylose levels in BT 544, TR 630 and TR 561 (Table 11) yielded similar, but not identical values to those obtained with the orcinol method. Missing data made orcinol-GC comparisons of the total and acid-extracted arabinoxylan values impossible, but the GC values were ranked as predicted by location and type pentose LSMs (Appendix 1 and 2).

TABLE 11. Arabinoxylan (% DM) present in BT 544, TR 630, and TR 561 cultivars as determined by orcinol (Orc) and gas chromatographic (GC) analysis of whole barley and its water or acid extracts.

| | Whole barley | | Water extract | | Acid extract | |
|---|---|---|---|---|---|---|
| | Orc | GC | Orc | GC | Orc | GC |
| BT 544 | 6.36 | 8.58 | 0.808 | 0.788 | 0.597 | 0.701 |
| TR 630 | NA[1] | 6.93 | 0.575 | 0.560 | NA | 0.477 |
| TR 561 | NA | 6.96 | 0.413 | 0.379 | NA | 0.430 |

[1] Not available

## Barley Arabinoxylan Values

Parameters of the Canadian six-rowed, two-rowed and hulless barley arabinoxylan LSMs are shown in Table 12. Within each analysis of barley type, the coefficient of variability did not exceed 15%. The range in cultivar values was greater within the acid-extracted and water-extracted fractions, possibly due to cultivar differences in arabinoxylan solubilization from the cell walls.

The concentrations of total and acid-extracted pentose in hulless barley (Table 12; Appendices 1 and 2) corresponded to results from previous GC work (1.2-4.7% and 0.2-0.6%, respectively; Bhatty et al. 1991). Bhatty et al. (1991) also performed orcinol analysis of the same material (4.4-7.3% and 1.1-2.3%, respectively), but reported that the method was unsatisfactory because it failed to prevent interference from

starch hydrolysis byproducts like oligo-saccharides (R.S. Bhatty, personal communication). The water-soluble arabinoxylan values were in agreement with those previously reported (0.65-0.79%; Henry, 1985) for hulled barley.

Water-soluble arabinoxylan levels were consistantly higher than those resulting from acid-extraction (Table 12). In contrast, ß-glucan has been reported to be preferentially solubilized by acid buffer (Bhatty et al. 1991). The difference between water- and acid-extracted levels in the current study may reflect the solubilization characteristics of arabinoxylan. Alternatively, the orcinol reagents react with other compounds, including nucleosides, nucleotides (Albaum and Umbreit, 1947), and glucuronic acid (Brown and Hayes, 1952), for which differential spectrophotometry may not compensate. If they were extracted at greater levels by the acid buffer, the interference may have reduced the differential absorbance.

TABLE 12. Arabinoxylan least square means from the analysis of acid-extracted, water-extracted or total grain fractions of six-rowed, two-rowed or hulless barley cultivars (% DM).

| | Mean | Range | SD[a] | CV[b] |
|---|---|---|---|---|
| Water-extracted arabinoxylan | | | | |
| Six-rowed (n=20) | 0.634 | 0.484 - 0.816 | 0.075 | 11.9 |
| Hulless (n=15) | 0.580 | 0.502 - 0.696 | 0.061 | 10.5 |
| Two-rowed (n=18) | 0.515 | 0.433 - 0.624 | 0.059 | 11.5 |
| Acid-extracted arabinoxylan | | | | |
| Six-rowed (n=8) | 0.525 | 0.419 - 0.589 | 0.057 | 10.9 |
| Hulless (n=3) | 0.380 | 0.361 - 0.391 | 0.017 | 4.4 |
| Two-rowed (n=7) | 0.353 | 0.267 - 0.415 | 0.051 | 14.5 |
| Total arabinoxylan | | | | |
| Six-rowed (n=8) | 6.12 | 5.66 - 6.42 | 0.30 | 4.9 |
| Hulless (n=3) | 4.07 | 3.72 - 4.30 | 0.31 | 7.6 |
| Two-rowed (n=7) | 5.61 | 5.41 - 5.81 | 0.17 | 3.0 |

[a] standard deviation
[b] coefficient of variation

## Genetic and Environmental Effects on Barley Arabinoxylan Content

The LSM averages for total, acid-soluble and water-soluble arabinoxylan concentrations of the two-rowed, six-rowed and hulless barley are shown in Table 12. The environmental and genetic effects for these values are described in Table 13. The total arabinoxylan LSM average for hulless barley was significantly less ($P<0.01$) than that of the six-rowed or two-rowed types. The two-rowed total barley

arabinoxylan LSM was also less ($P<0.05$) than that of six-rowed cultivars. Hull contains up to 55% of the whole barley arabinoxylan (Palmer, 1989), therefore even small differences in hull content could affect total levels. Six-rowed barley contains more hull than the two-rowed cultivars, and hulless barley contains none, which may explain why type accounted for the majority of variance within water-extract, acid-extract and whole barley analyses (Table 13). Lehtonen and Aikasalo (1987) also indicated that six-rowed cultivars contain significantly higher arabinoxylan levels than two-rowed cultivars (9.3 vs. 8.1% DM).

The six-rowed, acid-extracted arabinoxylan levels were higher than those of the hulless ($P<0.01$) or two-rowed ($P<0.05$) barleys (Table 12). Acid-extract arabinoxylan from hulless and two-rowed types did not differ ($P>0.05$). Water-soluble arabinoxylan was greater in the six-rowed type than in the two-rowed or hulless types ($P<0.01$). The hulless water-soluble arabinoxylan level was higher than that of the two-rowed type ($P<0.05$).

TABLE 13. Influence of environment, type, cultivar, and date of analysis on the arabinoxylan content of whole, water-extracted and acid-extracted barley fractions analyzed using the orcinol method.

| | df | F-value |
|---|---|---|
| Water-extracted arabinoxylan | | |
| Type | 2 | 25.09** |
| Cultivar(type) | 50 | 5.56** |
| Location | 5 | 16.42** |
| Day | 17 | 3.99** |
| Error (cultivar x location) | 69 | (EMS=0.0023) |
| Acid-extracted arabinoxylan | | |
| Type | 2 | 13.19** |
| Cultivar(type) | 15 | 2.64* |
| Location | 5 | 3.38* |
| Day | 7 | 2.95* |
| Error (cultivar x location) | 21 | (EMS=0.0028) |
| Whole-grain arabinoxylan | | |
| Type[a] | 2 | 109.44** |
| Cultivar(type)[a] | 15 | 2.20NS |
| Day[a] | 5 | 9.40** |
| Error(cultivar x location)[a] | 28 | (EMS=0.0926) |
| Type[b] | 2 | 79.32** |
| Cultivar(type)[b] | 15 | 2.92** |
| Location[b] | 5 | 0.77NS |
| Error(cultivar x location)[b] | 28 | (EMS=0.0814) |

*, ** ($P < 0.05$, 0.01) using ANOVA
NS ($P > 0.05$) using ANOVA
[a] Analysis of variance used to estimate the day effect
[b] Analysis of variance using day-corrected data

Cultivar had a highly significant effect on the arabinoxylan content of whole barley (Table 13), as determined using data corrected for the day of analysis effect. Henry (1986) observed a similar effect for Australian barley. Genetic differences were also significant for water-soluble ($P<0.01$) and acid-soluble ($P<0.05$) arabinoxylan.

The locations at which barley cultivars were grown affected water-extracted ($P<0.01$) and acid-extracted ($P<0.05$) arabinoxylan. However, in contrast to a previous report (Henry, 1986), whole barley levels did not differ ($P>0.05$) between environments.

The environmental effect on arabinoxylan solubility may have been related to diferulic function in the cell wall. Evidence suggests that diferulic-acid crosslinks may cohese the cell wall structure and limit component solubility (Shedletzky et al. 1992). Irradiation with white light increases the ferulic and diferulic acid content of rice (Oryza sativa) coleoptiles (Tan et al. 1992). Therefore, the amount of light to which a plant is exposed may alter its ferulic and diferulic acid content, affecting cell wall solubility. Locations received varying amounts of sunlight during grain maturation, and this may partially explain the environmental effect on arabinoxylan solubility.

Relationship between Acid-extracted Arabinoxylan, Log AEV and Total ß-glucan

A significant relationship between log AEV and acid-soluble arabinoxylan was present in the current study ($r=0.55$, $P<0.0001$; $n=51$). Previous studies by Bhatty et al. (1991) produced comparable GC ($r=0.61$) and orcinol-determined ($r=0.39$) correlations. The total ß-glucan and log AEV correlation was much lower in this study ($r=0.42$, $P<0.01$, $n=51$) than previously reported ($r=0.94$, $P<0.01$, Bhatty et al. 1991). Analytical and sample differences may have accounted for differences between the current study and those previously reported.

Correlation Between Harrington Standard Values and Day of Chemical Analysis LSM

Statistical estimates of day-to-day variance were confirmed by correlating day LSMs with Harrington standard values (Table 14). The acid-extract correlation was highly significant, and the water-extract correlation was near significance. This indicated that day-to-day variation was responsible for some of the differences between values, and was reasonably included as a correction factor. The significance of the whole barley correlation may have increased if the calculations had included a greater number of

observations. The large day-to-day variation indicated that the orcinol method is only suitable for analyzing samples if the statistical estimation and correction of day-to-day variance can be performed, as in the current study.

TABLE 14. Pearson correlations between day-to-day analysis variance and Harrington standard values for each of the barley fractions assayed.

| Water-extract | Acid-extract | Whole barley |
|---|---|---|
| n=12 | n=8 | n=6 |
| r=0.563 | r=0.958 | r=0.615 |
| P=0.0567 | P=0.0002 | P=0.1934 |

## Spearman Correlation to Estimate Location x Cultivar Interactions

The barley cultivars analyzed were grown only once at any location and therefore, the cultivar x location interaction could not be determined directly. Although both cultivar and location significantly affected the level of arabinoxylan detected in the samples, it was important to estimate the interaction effect to prove that mean differences did not result from random reranking of cultivars. A Spearman correlation was used to compare the cultivar ranking of LSM and location values (Steel and Torrie, 1980). The cultivar ranking at most locations was significantly correlated to that of the LSM values; however, total arabinoxylan comparisons of

two-rowed cultivars at Melfort and Lacombe (Table 15) indicated that a cultivar x location interaction may have been present. Hulless barley results should be interpreted with caution because of the small number of samples analyzed (n=3).

TABLE 15. Spearman correlations between cultivar least square means and day-adjusted values at each location from which total, water-soluble, and acid-soluble barley arabinoxylan was analyzed.

| | Water extract | | Acid extract | | Whole barley | |
|---|---|---|---|---|---|---|
| | r | n[1] | r | n | r | n |
| BT[2] - WATR[3] | 0.708** | 20 | 0.762* | 8 | 0.833* | 8 |
| BT - MELF[3] | 0.803** | 20 | 0.714* | 8 | 0.786* | 8 |
| BT - BEAV[3] | 0.752** | 20 | 0.643 | 8 | 0.929** | 8 |
| HB[2] - TROC[3] | 0.839** | 15 | 0.500 | 3 | 0.500 | 3 |
| HB - BEAV | 0.696** | 15 | 1.000* | 3 | 1.000** | 3 |
| TR[2] - MELF | 0.884** | 18 | 0.964** | 7 | 0.393 | 7 |
| TR - LACO[3] | 0.637** | 18 | 0.893** | 7 | 0.357 | 7 |
| TR - ELRO[3] | 0.860** | 18 | 0.901** | 7 | 0.893** | 7 |

[1] number of observations
[2] HB = Hulless barley, TR = Two-rowed barley, BT = Six-rowed barley
[3] MELF = Melfort, WATR = Watrous, ELRO = Elrose, LACO= Lacombe, TROC = Trochu, BEAV = Beaver Lodge
* $P < 0.05$
** $P < 0.01$

# CONCLUSION

Arabinoxylan is a major component of barley hull (Henry, 1987) and endosperm cell walls (Fincher, 1975), but little information has been available regarding how these levels vary among cultivars or growing location. The current study revealed that the environment had significant effects on acid and water-soluble arabinoxylan concentrations. Significant genetic effects were present for all fractions analyzed, illustrating the potential to manipulate arabinoxylan levels through plant breeding. This survey assessed the arabinoxylan content of existing breeder lines and cultivars of barley, providing a basis for future analysis and selection.

Acid-extracted arabinoxylan was positively correlated with log AEV in this study ($r=0.55$, $P<0.01$) as in others ($r=0.61$, $P<0.05$; Bhatty et al. 1991), which supports the theory that soluble arabinoxylan in barley may contribute to viscosity-related problems in poultry (Classen and Bedford, 1991; de Silva et al. 1983) and beer (Bamforth, 1986) production. However, the moderate correlation indicates that genetic selection against soluble arabinoxylan would not be likely to effect large changes in viscosity.

# MANUSCRIPT II: EFFECT OF ENDO-1,4-ß-XYLANASE ON THE PERFORMANCE OF BROILER CHICKENS FED BARLEY CULTIVARS VARYING IN ARABINOXYLAN CONTENT

# ABSTRACT

The effect of barley arabinoxylan on the performance and digesta viscosity of broiler chickens was examined by supplementing barley-based diets with a purified fungal xylanase (PX), or a bacterial, ß-glucanase-free xylanase (XBG) source. Barley cultivars used in the diets contained high (Stacey), moderate (Manley) or low (CDC Richard) levels of water- and acid-soluble arabinoxylan. The PX enzyme was added at 0, 475 or 950 IU/kg to treatments from each of the cultivar-based rations, and XBG was also added at 475 or 950 IU/kg to two Stacey diets. The birds consuming the PX-supplemented Stacey-based diets had increased weight gain ($P<0.05$) and feed conversion ($P<0.05$ at 950 IU/kg), and a corresponding reduction in proximal (PSI; $P<0.05$ at 950 IU/kg) and distal small intestine ($P<0.05$) viscosity, compared to the control Stacey diet. Distal small intestine viscosity was the only parameter affected by enzyme supplementation in the Manley, Richard and XBG-supplemented Stacey diets ($P<0.05$). The data indicated that the gain and feed efficiency of young chickens consuming a barley cultivar with elevated arabinoxylan solubility can be improved by endo-1,4-ß-xylanase supplementation, and that the effect is associated with reduced PSI viscosity.

KEY WORDS: arabinoxylan, endo-1,4-ß-xylanase, viscosity, broiler

# INTRODUCTION

Beta-glucan and arabinoxylan constitute approximately 71% and 23% of the barley endosperm cell wall, respectively (Briggs et al. 1981). In Canadian barley, nearly one third of this ß-glucan is acid-soluble (Bhatty, 1987), and can produce a dramatic increase in chick digesta viscosity when ingested (White et al. 1981). Dietary ß-glucanase supplementation hydrolyzes ß-glucan (Hesselman and Åman, 1986), reducing gut viscosity (Salih et al. 1991) and improving nutrient digestibility and chick performance (Edney et al, 1989). Crude bacterial and fungal ß-glucanase preparations are therefore routinely added to barley-based diets for young chicks (Ab Freig, personal communication).

Research has focused on the antinutritional effects of ß-glucan, which is present in acid extract at concentrations up to five times that of arabinoxylan (Bhatty et al. 1991). However, the effects which the other soluble and viscous endospermal component, arabinoxylan, may have on poultry performance are not known. Barley acid-extract viscosity is significantly correlated with arabinoxylan content, and is reduced by the addition of purified xylanase (Bhatty et al. 1991). These results have led to speculation that barley arabinoxylan may have an impact on broiler chick performance (Classen and Bedford, 1991; de Silva et al. 1993). A recent study involving barley-based diets found that a multi-enzyme

product containing ß-glucanase and xylanase did not improve chick performance relative to supplementation with equal ß-glucanase activity in a purified form (Graham and Pettersson, 1992). However, arabinoxylan is responsible for a relatively small proportion of total acid-extract viscosity in barley (Bhatty et al. 1991), and hydrolysis of the ß-glucan component may have reduced viscosity below the threshold for antinutritive expression, a phenomenon observed in wheat diets (Bedford and Classen, 1992).

The objective of the present study was to determine the antinutritive effect of arabinoxylan in western Canadian barley cultivars used in broiler chick diets. Barley-based diets were supplemented with endo-1,4-ß-xylanase, to hydrolyze solubilized arabinoxylan in the chick gut. Chick performance was measured to determine the response to xylanase supplementation, and gut viscosity was analyzed to link the growth and efficiency effects with changes in the intestinal environment.

# METHODS AND MATERIALS

## Plant Material

Hulless CDC Richard (cv.) was foundation barley grown at Sturgess, Saskatchewan during 1991. Stacey was a six-rowed, registered seed grown in 1992 at Nipawin, Saskatchewan; the region reported an early frost and poor harvest conditions. Manley barley was procured from Dominion Malt, and represented malting quality two-rowed barley which had been grown throughout western Canada in 1992. Samples used for chemical analysis were ground (Udy cyclose mill; 0.5 mm screen) and analyzed for dry matter content under vacuum (29 atm at 50°C) overnight.

## Xylanase Sources

A purified endo-1,4-ß-xylanase (PX; Finnfeeds International Ltd) and a crude ß-glucanase-free endo-1,4-ß-xylanase (XBG; ICI Bio Products) were used in the feeding trial. The PX preparation was purified from Trichoderma reesei, and had an isoelectric point of 9.0 (Tenkanen et al. 1992). It was supplied in liquid form and, when stored at 4°C, was stable for an extended period of time (M.R. Bedford, personal communication), as reported for other purified fungal xylanases (Gomes et al. 1993). The XBG product was a coarse, freeze-dried supernatant from a ß-glucanase-negative mutant of

*Streptomyces lividans*, into which ß-1,4-endo-xylanase had been cloned using a multicopy plasmid (Mondou et al. 1986). The XBG preparations did not display carboxymethyl- or endo-cellulase activity (R.L. Bernier, personal communication).

ß-glucanase Detection in the PX and XBG Sources

A modification of the McCleary and Glennie-Holmes (1985) ß-glucan assay was used to confirm the zero ß-glucanase activity levels reported by the suppliers of the PX and XBG sources. The substrate used in the method was ß-glucan (Sigma), dissolved (1% w/w) in sodium phosphate buffer (20 mM, pH 6.5) according to the procedure of Bathgate (1979). Each xylanase source was prepared in sodium citrate buffer (pH 5.3, 50 mM). The ß-glucan solution (2.0 ml) and ethanol (50% v/v; 0.4 ml) were pipetted into 50 ml polypropylene centrifuge tubes and vortexed. The tubes were incubated in a boiling water bath (2 min), vortexed, placed back into the boiling water (3 min), and removed to cool at room temperature. Purified xylanase (0.2 ml; 5 U), XBG (0.2 ml; 1U) and citrate buffer were placed into separate tubes of ß-glucan solution, and allowed to digest (40°C, 60 min). Three aliquots (0.1 ml) from each digest were pipetted into the bottom of test tubes.

Beta-glucosidase[1] (0.1ml) was added to two test tubes from each treatment, and acetate buffer (50 mM, pH 4.0, 0.1 ml) was placed in the third. The tubes were incubated (40°C, 20 min), allowed to cool, and water was added (0.8 ml). Hexokinase solution[2] (4 ml) was pipetted into these tubes, and those containing the standard solutions. The tubes were vortexed and allowed to sit at room temperature (15 min) before the absorbance was read (340 nm). The ß-glucan content of the solution was measured using the same procedure, but with the following changes: ethanol (50% v/v; 1 ml), water (2.5 ml) and ß-glucan solution (2.5 ml) were placed in the 50 ml centrifuge tube; the ß-glucan preparation was digested with lichenase[1] (0.2 ml); and following lichenase digestion, water (24 ml) was added to the sample, and vortexed. Three 0.1ml samples were then removed for glucose analysis.

Endo-1,4-ß-xylanase Activity

The arabinoxylan-hydrolyzing activity of PX and XBG was expressed in terms of reducing sugar production from oat spelt xylan. One unit of activity was defined as the amount of

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[1] Megazyme (Aust) Pty. Ltd. North Rocks, N.S.W. Australia. 2151.

[2] Gluco-Quant kit. Boehringer Mannheim Biochemicals. Indeanapolis, IN. 46250

enzyme required to produce one μmol of reducing sugar in one min, per ml of substrate solution (pH 5.3) at 50°C.

Reagents

Oat spelt xylan (Sigma) was solubilized (1% w/vol; Bathgate, 1979) in sodium citrate buffer (pH 5.3; 50 mM), and centrifuged after cooling (1460g x 15 min). The supernatant was used for the xylanase activity assay. The enzyme sources were diluted (PX = 10 μl/1000 ml; XBG = 1 g/10,000 ml) volumetrically in sodium citrate buffer (pH 5.3; 50 mM).

Method

Reducing sugars were detected using the neocuproin method, as described by Dygert et al. (1965). Oat spelt xylan solution (4 ml) was placed in a test tube, covered, and equilibrated in a water bath (50°C). Diluted enzyme (1 ml, 50°C) was added to the xylan solution, and mixed gently. Before the enzyme-xylan solution was placed back into the water bath (50°C), an aliquot (0.1 ml) was transferred into a screw-capped test tube containing water (0.4 ml). Reagent A (4 ml) and reagent B (4 ml) were added to the sample, vortexed, and placed in a boiling water bath (15 min). The reducing sugar concentration was measured in a sample from the enzyme-xylan digest (0.1 ml) at exactly 20 minutes after

xylanase addition, using the same analytical procedure. The analysis was replicated in six separate enzyme-xylan digests. After boiling, samples were cooled in an ambient-temperature water bath, vortexed, and the absorbance (450 nm) was read against an enzyme-sodium citrate-reagent blank. Pentose sugar content was determined by comparison with xylose standards. A xylose standard curve was also prepared using the DNSA reducing sugar method of Miller (1959), to allow comparison of the DNSA and Neocuproine variability.

Barley Cultivar Analyses

Arabinoxylan levels for the total, acid-soluble and water-soluble fractions from Richard, Manley and Stacey were determined using a modification of the orcinol method (Manuscript I). Total barley ß-glucan was assayed using the enzymatic method described previously, with the following modifications: ethanol (1 ml) and ground barley (0.5 g) were placed in the 50 ml centrifuge tube; the barley preparation was digested with lichenase (0.2 ml); and following lichenase digestion, water (24 ml) was added to the sample and vortexed.

Crude protein levels in the barley cultivars were measured using the Kjeldahl method (Williams, 1973). Hundred-kernel weight was calculated for a cultivar from the mean weight of two 100-kernel samples. The true metabolizable energy (TME) of Stacey, Manley and Richard cultivars was

predicted using near-infrared reflectance spectroscopy (Campbell et al. 1992). Arabinoxylan and ß-glucans content were calculated using the conversion factors: % arabinoxylan = % pentoses x 0.88, and % ß-glucan = % glucose x 0.90, respectively.

Diet Preparation

The barley cultivars were autoclaved (121°C, 20 min), dried overnight at room temperature, and ground to pass through a 4 mm screen. The diets were formulated to be isocaloric and isonitrogenous, based on the determined crude protein (CP) and estimated apparent metabolizable energy (AME) content of Stacey (13.34%, 12.14 MJ/kg), Manley (10.94%, 12.14 MJ/kg) and Richard (12.66%, 12.66 MJ/kg) barley, respectively. The Stacey, Richard and Manley basal diets (Table 16) were formulated to meet minimum National Research Council nutrient requirements (NRC, 1984), and supplemented with 0, 475 or 950 IU PX/kg (see Table 17). The XBG xylanase was also added to two Stacey-based diets at 475 and 950 IU/kg. A corn-soy diet was included as a control.

Chick Trial Design

A chick trial was conducted to determine the effects of barley arabinoxylan, at levels present in western Canadian cultivars, on the performance of chicks consuming barley-based diets with or without supplemental endo-1,4-ß-xylanase. Day-old broiler males (Arbor Acre x Peterson) were placed on commercial Leghorn starter (18% CP) for a pre-trial equilibration period. Food and water were supplied *ad libitum* throughout the trial. At 5 d of age, starved birds (4 h) were weighed into equal pen replicates of 6 chicks, and housed in Petersime[3] battery brooders. Six replicates were assigned to each trial diet. The pen weight and feed intake were measured when chicks were 12 and 19 d old. At 20 d of age, the bird of median weight from each of 5 replicates per treatment was killed by cervical dislocation. Digesta from the proximal (PSI) and the distal (DSI) small intestine segments was immediately collected and analyzed as described by Bedford et al. (1991), using a Brookfield digital viscometer[4] maintained at 26°C. Digesta samples in the present study were centrifuged at 13,000 x g for 10 minutes.

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[3] Petersime Incubator Co., Gettysburg, OH 45328.

[4] Model LVTDCP; Brookfield Engineering Laboratories, Inc., Stoughton, MA 02072

TABLE 16. Composition of experimental diets.

| Ingredient | Corn | SB | RB | MB |
|---|---|---|---|---|
| Corn | 55.25 | | | |
| Stacey barley | | 59.12 | | |
| Richard barley | | | 56.00 | |
| Manley barley | | | | 54.38 |
| Soybean meal | 34.13 | 26.75 | 30.50 | 31.00 |
| Limestone | 1.00 | 1.30 | 1.30 | 1.30 |
| Biophos (21/18) | 0.90 | 0.80 | 0.80 | 0.80 |
| DL-Methionine | 0.09 | 0.09 | 0.09 | 0.09 |
| Vitamin premix[1] | 1.00 | 1.00 | 1.00 | 1.00 |
| Mineral premix[1] | 0.35 | 0.35 | 0.35 | 0.35 |
| Fishmeal | 5.00 | 5.00 | 5.00 | 5.00 |
| Sunflower oil | 2.00 | 5.50 | 4.88 | 6.00 |
| Salt | 0.08 | 0.09 | 0.08 | 0.08 |
| Calculated analysis | | | | |
| Metabolizable energy (MJ/kg) | 12.72 | 12.65 | 12.73 | 12.71 |
| Crude protein (%) | 23.0 | 22.9 | 23.0 | 23.0 |
| Crude fat (%) | 4.6 | 7.0 | 6.4 | 7.5 |
| Methionine (%) | 0.58 | 0.52 | 0.54 | 0.54 |
| Lysine (%) | 1.47 | 1.34 | 1.45 | 1.45 |
| Determined | | | | |
| Crude protein (%) | 24.1 | 23.8 | 23.5 | 23.5 |

[1] Vitamin and mineral premix supplied per kg diet: Vit A, 8250 IU; Vit D3, 1000 IU; Vit E, 10.9 IU; Vit $B_{12}$, 0.0115 mg; Vit K, 1.1 mg; Vit $B_2$, 5.5 mg; Ca-pantothenate, 11.0 mg; niacin, 53.3 mg; choline chloride, 1019.9 mg; folic acid, 0.75 mg; biotin, 0.25 mg; DL-methionine, 500 mg; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.46 mg; NaCl, 2.5 g; Strpetomycin, 27.5 mg; Penicillin, 82.6 mg.

Statistics

The experimental design for the chick performance variables was a repeated measures design with the subplot consisting of time. An experimental unit consisted of a pen of six chicks. The twelve treatments (Table 17) consisted of the following: two Stacey-based diets supplemented with either 475 of 950 IU XBG/kg; one corn-soy control diet; nine diets arranged in a 3 X 3 factorial of barley cultivar (Stacey, Manley and Richard) and PX enzyme level (0, 475 and 950 IU/kg). Contrasts (Table 18) were used to determine the effects of cereal type (corn vs. barley), xylanase source (PX vs. XBG), and factorial effects (i.e. main effects and interactions) of barley cultivar and enzyme level on chick performance.

The viscosity of digesta supernatant from the PSI and DSI was logarithmically transformed ($e^x$) prior to analysis (Steele and Torrie, 1980), because the data was significantly skewed. The treatments were analyzed in a completely randomized design, and the experimental unit consisted of individual chick values.

TABLE 17. Experimental design to determine the antinutritional effects of arabinoxylan in barley-based broiler diets.

| Xylanase (level) | Stacey barley | Manley barley | Richard barley | Corn-soy |
|---|---|---|---|---|
| PX (950) | X | X | X | |
| PX (475) | X | X | X | |
| None (0) | X | X | X | X |
| XBG (475) | X | | | |
| XBG (950) | X | | | |

TABLE 18. Contrasts used in analysis of treatment differences.

| Contrasts applied to treatments | |
|---|---|
| 1. Contrasts to determine factorial effects of barley cultivar and PX enzyme: | |
| a) Do barley cultivars differ? | 2 |
| b) Do PX levels differ? | 2 |
| c) Do the effects of PX levels differ between cultivars? | 4 |
| 2. Comparisons with corn: | |
| a) Does corn differ from barley? | 1 |
| 3. Xylanase source comparisons: | |
| a) Does XBG differ from PX? | 1 |
| 4. Comparison of xylanase levels within Stacey: | |
| a) Do diets with xylanase differ from unsupplemented diets? | 1 |

# RESULTS AND DISCUSSION

## Assay of ß-glucanase Activity in Xylanase Sources

The McCleary - Glennie-Holmes method (1985) was modified to detect ß-glucanase in xylanase preparations. The assay provided an enzyme system which was highly sensitive to glucose release from ß-glucan, but which was not affected by xylose or arabinose. The McCleary - Glennie-Holmes test was modified because commercially available ß-glucan sources were suspected to contain low levels of arabinoxylan which, when hydrolyzed by xylanase, would have given a false ß-glucanase positive using either the reducing sugar method or the viscometric method. In the McCleary - Glennie-Holmes method, the lichenase digestion hydrolyzes ß-glucan into smaller segments, producing polymer reducing ends which act as substrate for glucose production by ß-glucosidase. In the modified assay, the sodium acetate blank measured the level of free glucose in the initial sample. The free glucose value was then subtracted from the glucose levels detected in the ß-glucosidase treatments, and the difference was attributed to glucose arising from ß-glucan hydrolysis, in the PX, XBG or sodium citrate assays. No significant differences ($P>0.05$) were found between the PX, XBG and sodium citrate pretreatments, indicating that ß-glucanase activity was not detected in these enzyme preparations (Table 19). The

standard ß-glucan assay using lichenase detected 0.77 g ß-glucan/100 ml initial ß-glucan solution.

TABLE 19. Free glucose ($\mu g\ ml^{-1}$) in ß-glucan solutions treated with lichenase, purified xylanase, ß-glucanase-free xylanase or sodium citrate, and glucose levels ($\mu g\ ml^{-1}$) in the same solutions following digestion with ß-glucosidase.

| Preliminary treatment | Free glucose | ß-glucan glucose |
|---|---|---|
| Lichenase | 5 | 711 |
| Purified xylanase | 22 | 177[a] |
| ß-glucanase-free xylanase | 24 | 175[a] |
| Sodium citrate | 23 | 173[a] |

[a] Means within column not sharing a common letter are significantly different ($P<0.05$).

Xylanase Activity of PX and XBG Sources

The endo-1,4-ß-xylanase activity of the PX product was 22,700 IU/ml. The values ranged from 18,200 to 27,300 IU/ml, and had a standard deviation (SD) of 3800. The XBG sample contained 4800 IU of activity/g, and values ranged between 4500 and 5000 IU/g, with a SD of 200. The neocuproin determination of PX activity agreed reasonably well with an estimate supplied by Finnfeeds International Ltd. However, the XBG activity was 10-fold lower than that quoted by ICI Products, from analyses performed at pH 6.0 in McIlvaine buffer (G. Moser, ICI Bioproducts, personal communication).

The neocuproine method was used to assay reducing sugars in the present study; however, xylanase activities reported by

the source companies for PX and XBG were determined using the DNSA reducing sugar method (M.R. Bedford and G. Moser, personal communication). The DNSA method is known to give higher results than other reducing sugar methods (Bailey et al. 1992) by effecting the apparent hydrolysis of oligosaccharides which are present in solution (Bruell and Saddler, 1985). The neocuproin method has not been reported to contribute to an overestimation of reducing sugars, which may have partially accounted for the lower xylanase activities observed in the present study. The PX and XBG dilutions produced a linear increase in absorbance over time (Fig. 9); therefore, the enzyme/substrate ratio did not limit the activity of enzyme sources (Bruell and Saddler, 1985).

Despite its widespread acceptance (Bailey et al. 1992), the DNSA method was not used in the current study because of the reported variability of results (A.W. MacGregor, personal communication). Analysis of a set of xylose standards confirmed that the neocuproin assay (0 - 100 $\mu$g xylose/ml; $r^2$=0.998) was more precise than that using DNSA (0 - 200 $\mu$g/ml; $r^2$=0.934) (Fig. 10).

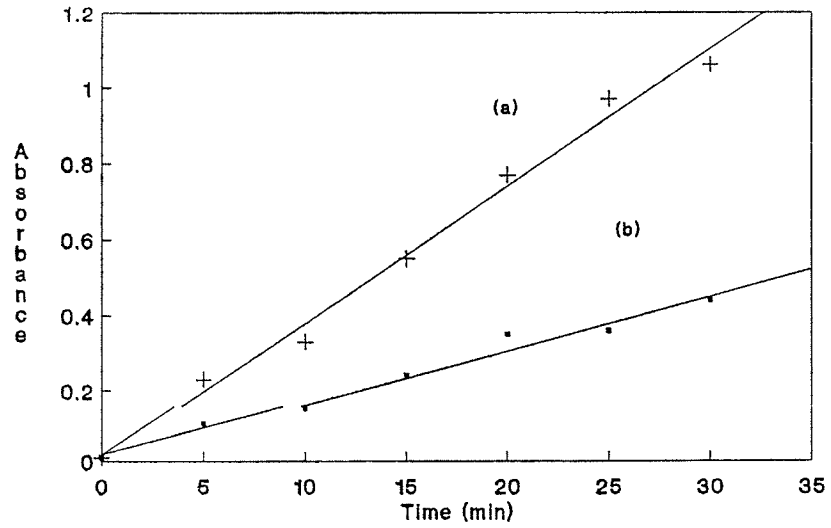


Figure 9. Absorbance (450 nm) versus time for (a) purifiew xylanase (1 $\mu$l / 100,000 ml), or (b) ß-glucanase-free xylanase (1 g/10,000 ml) digestion of arabinoxylan, as measured using the Neocuproine assay technique.

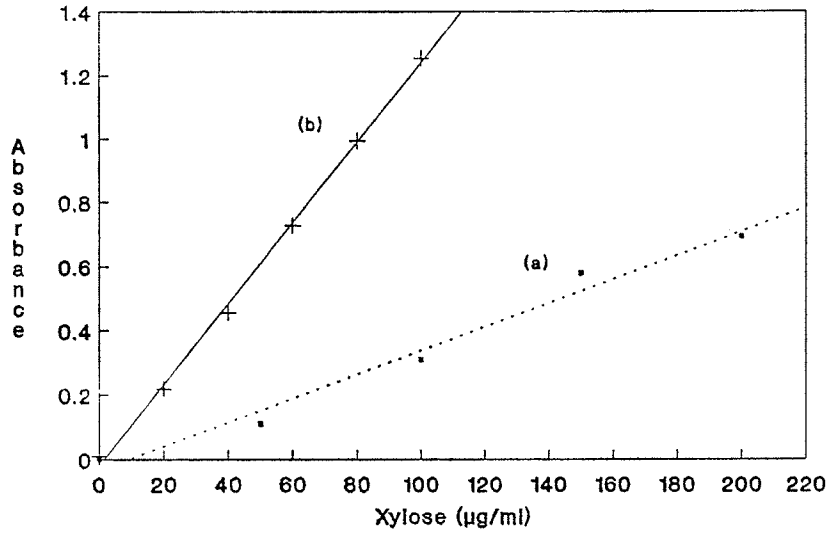


Figure 10. Absorbance versus xylose concentration ($\mu$g ml$^{-1}$) using (a) the DNSA (540 nm) or (b) the Neocuproine (450 nm) reducing sugar assays.

Arabinoxylan and ß-glucan Levels in Barley Cultivars

The Stacey, Manley and Richard cultivars were chosen for dietary xylanase-supplementation on the basis of a preliminary study of arabinoxylan levels in western Canadian barley cultivars (Manuscript I). A breeder line of six-rowed barley, BT 671, had the highest water- and acid-soluble pentose levels examined, but the nonregistered seed could not be acquired. The parent cultivar of BT 671, Stacey, was obtained instead, as a genetic effect on the variation of barley arabinoxylan levels has been shown to exist (Manuscript I). Manley and Richard were chosen as two-rowed malting and hulless cultivars, respectively, representative of those currently grown by industry. The water-extracted, acid-extracted and total barley arabinoxylan concentrations are shown in Table 20.

TABLE 20. Arabinoxylan (AX) and ß-glucan content (%DM) of total, water-soluble and acid-soluble fractions from Stacey, Manley, Richard and Harrington barley cultivars.

| | Stacey | Manley | Richard |
|---|---|---|---|
| Total AX | 6.37 | 5.78 | 4.09 |
| Water-soluble AX | 0.79 | 0.60 | 0.47 |
| Acid-soluble AX | 0.57 | 0.52 | 0.28 |
| Total ß-glucan | 4.91 | 4.86 | 4.64 |

Effect of Xylanase Supplementation on Chick Performance and Gut Viscosity

Exogenous xylanase supplementation did not improve measured growth traits for chicks across all barley diets (Table 21). However, weight gain and feed conversion of chicks consuming Stacey-based diets was significantly improved (Table 22), which indicated that the level of arabinoxylan in this barley cultivar was sufficient to produce antinutritional effects. The performance observations for Stacey were supported by viscosity measurements of digesta supernatant.

The Stacey cultivar was selected for xylanase supplementation in chick diets because BT 670, its offspring breeder line, contained the highest soluble arabinoxylan levels detected in a survey of western Canadian barley breeder lines and cultivars (Manuscript I). Orcinol assay of the three barley cultivars used in the current study indicated that Stacey also contained elevated levels of arabinoxylan (Table 20). Chick performance was not affected by xylanase supplementation of Richard and Manley-based diets, which contained lower arabinoxylan concentrations.

TABLE 21. Statistical summary[a] of results from contrasts used to determine treatment differences.

| | Split-plot analysis | | | | CRD[c] analysis | |
|---|---|---|---|---|---|---|
| Main plot analysis | Intake | F/G | Gain | df | PSI[e] | DSI[f] |
| Factorial effects | | | | | | |
| Barley cultivar | $0.95^{ns}$ | $5.95^{**}$ | $1.81^{ns}$ | 2 | $40.06^{**}$ | $56.52^{**}$ |
| PX levels | $0.87^{ns}$ | $1.10^{ns}$ | $2.59^{ns}$ | 2 | $1.01^{ns}$ | $7.15^{**}$ |
| Barley x PX level | $0.27^{ns}$ | $1.39^{ns}$ | $0.91^{ns}$ | 4 | $1.80^{ns}$ | $1.37^{ns}$ |
| Corn contrasts | | | | | | |
| Corn vs. barley | $1.77^{ns}$ | $13.86^{**}$ | $1.47^{ns}$ | 1 | $69.25^{**}$ | $80.93^{**}$ |
| Enzyme contrasts | | | | | | |
| PX vs XBG | $0.02^{ns}$ | $2.35^{ns}$ | $0.88^{ns}$ | 1 | $4.73^{*}$ | $2.07^{ns}$ |
| Xylanase in Stacey | | | | | | |
| 0 vs 475 & 950 IU/kg | $1.75^{ns}$ | $4.26^{*}$ | $6.76^{*}$ | 1 | $3.73^{ns}$ | $11.94^{**}$ |
| Sub plot analysis[d] | | | | | | |
| Factorial effects | | | | | | |
| Cultivar | $4.03^{*}$ | $3.82^{*}$ | $0.29^{ns}$ | 2 | | |
| PX level | $0.74^{ns}$ | $1.29^{ns}$ | $0.69^{ns}$ | 2 | | |
| Barley x PX level | $0.60^{ns}$ | $1.66^{ns}$ | $1.54^{ns}$ | 4 | | |
| Corn contrasts | | | | | | |
| Corn vs. barley | $6.46^{*}$ | $0.00^{ns}$ | $1.08^{ns}$ | 1 | | |
| Enzyme contrasts | | | | | | |
| PX vs. XBG | $2.03^{ns}$ | $1.00^{ns}$ | $1.38^{ns}$ | 1 | | |
| Xylanase in Stacey | | | | | | |
| 0 vs 475 & 950 IU/kg | $2.11^{ns}$ | $1.11^{ns}$ | $1.58^{ns}$ | 1 | | |

[a] F-values resulting from contrasts [b] Feed/gain ratio [c] Completely randomized design
[d] Contrasts are between the d 5-12 and d 13-19 periods [e] Proximal small intestine [f] Distal small intestine
$^{*},^{**},^{ns}$ $P<0.05$, $P<0.01$, $P>0.05$

TABLE 22. Intake, feed-to-gain and weight gain data of broiler chicks from 5 to 12 and from 13 to 19 d of age.

| Source of variation | Intake (g) | | | Weight gain (g) | | | Feed/gain (g/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5-12 d | 13-19 d | SEM | 5-12 d | 13-19 d | SEM | 5-12 d | 13-19 d | SEM |
| Factorial effects of barley cultivar and PX enzyme level | | | | | | | | | |
| Richard barley | 222 | 465 | 3 | 171 | 326 | 2 | 1.31 | 1.42 | 0.01 |
| Manley barley | 228 | 450 | 3 | 168 | 323 | 2 | 1.33 | 1.39 | 0.01 |
| Stacey barley | 226 | 458 | 3 | 166 | 318 | 2 | 1.37 | 1.44 | 0.01 |
| 0 PX[a] | 223 | 452 | 3 | 165 | 317 | 2 | 1.35 | 1.43 | 0.01 |
| 475 PX | 223 | 461 | 3 | 170 | 326 | 2 | 1.34 | 1.40 | 0.01 |
| 950 PX | 226 | 461 | 3 | 170 | 324 | 2 | 1.33 | 1.42 | 0.01 |
| Other effects | | | | | | | | | |
| Stacey 0 | 227 | 447 | 4 | 160 | 306 | 3 | 1.42 | 1.46 | 0.01 |
| Stacey (XBG[a]) | 233 | 458 | 3 | 169 | 316 | 2 | 1.43 | 1.45 | 0.01 |
| Stacey (PX[a]) | 226 | 464 | 3 | 168 | 325 | 2 | 1.35 | 1.38 | 0.01 |
| Corn-soy | 226 | 442 | 4 | 165 | 324 | 2 | 1.29 | 1.45 | 0.01 |

[a] Average of 475 and 950 IU/kg diet.

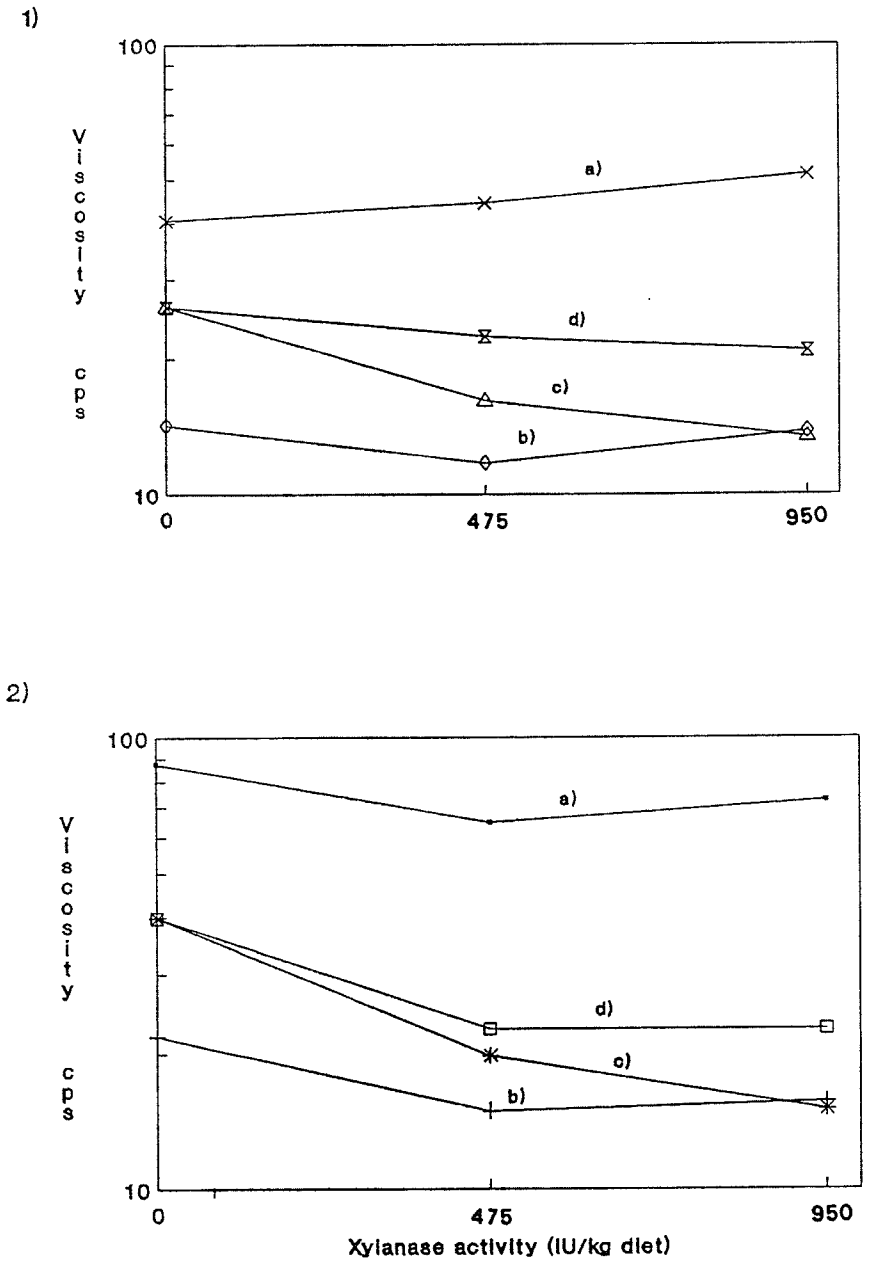


Figure 11. Viscosity of chick digesta supernatant from the 1) proximal and 2) distal small intestine versus xylanase concentration in PX-supplemented a) Richard b) Manley and c) Stacey and d) XBG-supplemented Stacey diets.

Main plot analysis indicated that weight gain and intake was similar for chicks consuming corn and barley; however, feed-to-gain ratios (F/G) differed among cultivars and between cereals (Table 22). Stacey was assimilated less efficiently ($P<0.01$) than other barley-based diets, and the 5 to 19 d feed conversion of the corn diet was significantly better than that of Manley, Richard ($P<0.05$) or Stacey ($P<0.01$). Sub plot comparisons between 5-12 and 13-19 d of chick age indicated that feed efficiency of Richard-based diets decreased relative to the change in Stacey and Manley treatments ($P<0.05$). The consumption of Richard-based diets increased over the same time period, in comparison to Manley.

The Stacey cultivar was examined to determine reasons for the reduced efficiency of chicks which consumed that barley. Near-infrared analysis indicated that the TME of Stacey was lower than that of Manley or Richard cultivars (13.8, 14.4 and 15.3 MJ/kg, respectively). Stacey and Manley had been assigned equivalent AME values for diet formulation (12.14 MJ/kg); therefore, the Stacey diet would have contained less metabolizable energy, forcing chicks to consume more diet to maintain performance levels.

The barley used in the chick diets was autoclaved to inactivate endogenous ß-glucanase and xylanase activities, which can reduce digesta viscosity in the chick gut (Burnett, 1966). A threshold level of gut viscosity must be present before chicks exhibit antinutritional effects (Bedford and

Classen, 1992). Arabinoxylan contributes a relatively small proportion to the extract viscosity of barley (Bhatty et al, 1991), possibly less than that of the antinutritional threshold. Therefore, had the barley not been autoclaved, endogenous ß-glucanase activity barley may have reduced total gut viscosity below the threshold level, and arabinoxylan effects on chick performance would not have been detected. Growth depression in the diets containing the autoclaved hulless barley was not observed, unlike in a previous study (Classen et al. 1985). However, the chick performance effects of autoclaving cereal grains are inconsistant, even within the same experiment (Teitge et al. 1991).

The log viscosity of barley digesta supernatant from the PSI and DSI was affected by barley cultivar and cereal type (Table 22). The log-transformed PSI values ranged from 2.58 to 3.81, and the DSI values from 2.84 to 4.31 centipoise (Table 21), indicating the cell wall had further solubilized in the hind gut, or that polymers were concentrated as the dry matter content of the digesta increased (Bedford et al. 1991). The PSI and DSI viscosities were comparable to values previously determined for barley-based diets, using the same method (H.L. Classen, personal communication). Factorial analysis indicated that Richard-based diets produced greater digesta viscosity than diets containing either Manley or Stacey cultivars ($P<0.01$). Hulless barleys are frequently associated with elevated viscosity and soluble ß-glucan levels

(Rotter et al. 1990); however, the Richard cultivar was designed to have low extract viscosity levels (B. Rossnagel, personal communication). The purified xylanase reduced hindgut viscosity at both 475 and 950 IU/kg supplementation levels ($P<0.01$). The supernatant from corn-based diets was less viscous than that of barley diets ($P<0.001$), in both the proximal and distal sections of the chick gut.

Proximal small intestine viscosity was reduced by PX-supplementation of Stacey-based diets ($P<0.05$), but was not affected when Stacey diets contained XBG (Fig. 11). This was confirmed by the significant PSI contrast between PX and XBG (Table 22) within Stacey. The pH of chick gut digesta increases during transit through the proventriculus (2.68), duodenum (5.63), ileum (6.34) and jejunum (6.85; Ward et al. 1987). The fungal Finnfeeds xylanase (PX), has been reported to have a pH optimum of about 5.0, and to retain over 60% activity at pH 4.0 (Tenkanen et al. 1992). The bacterium, *Streptomyces lividans*, has been shown to have a pH optimum near 7.0, with a greatly reduced activity at pH 4.5 (Kluepfel et al. 1986). Therefore, the PX may have initiated arabinoxylan hydrolysis at a lower pH than XBG, and produced a greater viscosity reduction in the PSI.

TABLE 23. The natural logarithm viscosity[a] of digesta from the proximal and distal small intestine.

| | Proximal | SEM | Distal | SEM |
|---|---|---|---|---|
| Factorial effects of barley cultivar and PX enzyme level | | | | |
| Richard barley (PX) | 3.81 | 0.10 | 4.31 | 0.10 |
| Manley barley (PX) | 2.58 | 0.10 | 2.84 | 0.10 |
| Stacey barley (PX) | 2.88 | 0.10 | 3.13 | 0.10 |
| 0 PX | 3.21 | 0.10 | 3.74 | 0.10 |
| 475 PX | 3.01 | 0.10 | 3.29 | 0.10 |
| 950 PX | 3.05 | 0.10 | 3.25 | 0.10 |
| Other effects | | | | |
| Stacey 0 | 3.26 | 0.18 | 3.69 | 0.18 |
| Stacey (XBG[a]) | 3.08 | 0.12 | 3.12 | 0.14 |
| Stacey (PX[a]) | 2.70 | 0.12 | 2.85 | 0.14 |
| Corn-soy | 1.56 | 0.18 | 1.68 | 0.18 |

[a] Average of 475 and 950 IU/kg diet.

Richard barley produced significantly greater gut viscosities than the other cultivars included in the chick diets. Although elevated digesta viscosity in both the PSI ($r=0.71$) and DSI ($r=0.79$) has been shown to be significantly correlated with reduced weight gain (Bedford and Classen, 1992) of chicks consuming rye diets, no differences were observed for the barley diets in the current experiment. The consumption of Richard-based diets increased from d 5-12 to d 13-19, relative to Stacey ($P<0.05$) and Manley ($P<0.01$) diets, indicating that chicks had to consume increasing amounts to maintain production levels.

## Conclusion

The viscosity of digesta supernatant from chicks fed barley diets were reduced when supplemented with endo-1,4-ß-xylanase. This suggested that arabinoxylan from the cell walls of barley contributes to the hind gut viscosity in broiler chicks. The fore gut viscosity of chicks consuming the cultivar Stacey was significantly lower in PX-supplemented diets; this was the only enzyme-cultivar combination which resulted in a significant increase in weight gain and feed efficiency. Therefore, improved chick performance may result from including xylanase in diets containing barley cultivars with elevated arabinoxylan levels. This scenario may be difficult to identify in the industry setting, but the level of endo-1,4-ß-xylanase in some crude feed enzyme preparations may ensure that chick performance and litter quality are being optimized.

# GENERAL DISCUSSION

The current study measured the range in total, water-soluble and acid-soluble arabinoxylan in western Canadian barley cultivars and breeder lines. Three cultivars were analyzed to determine the effects of barley arabinoxylan on the performance and digesta supernatant viscosity of young broiler chickens.

The arabinoxylan content of acid-extract from the six-rowed barley was 50% and 38% greater than that of two-rowed and hulless barley, respectively. Water-soluble values were higher, but showed less contrast between six-rowed versus two-rowed or hulless arabinoxylan contents (23% and 13%, respectively). Within each fraction analyzed, the highest value was nearly twice that of the lowest (Manuscript I, Table 12). Genetics, as indicated by differences between cultivars and/or breeder lines, contributed to extract arabinoxylan content ($P<0.05$). The location of growth had a significant effect on extract arabinoxylan content of all fractions analyzed ($P<0.05$). Maltsters, and producers who feed barley-based poultry diets, will probably encounter a range in barley arabinoxylan solubility.

The feeding trial indicated that the arabinoxylan level in the Stacey cultivar was sufficient to produce antinutritional effects in young broiler chickens. Xylanase supplementation improved the weight gain and feed conversion

($P<0.05$), and was associated with a reduction in the DSIV ($P<0.01$; Table 22) and PSIV ($P<0.05$; PX only). Therefore, improved chick performance may result from including xylanase in diets containing barley cultivars with elevated arabinoxylan levels.

To keep the implications of the present study in perspective, it must be noted that arabinoxylan contributes to a relatively small proportion of barley acid-extract viscosity (Bhatty et al, 1991). In addition, the range in soluble ß-glucan levels of hulless (Bhatty et al, 1991) and hulled barley (Smith et al, 1980) is greater than the concentration of soluble arabinoxylan.

# SUMMARY AND CONCLUSION

The current study illustrated the range in total, water-soluble and acid-soluble arabinoxylan found in western Canadian barley cultivars and breeder lines. An orcinol procedure was modified to reduce sample hydrolysis time, and to utilize a dichromatic absorbance measurement which eliminated interference from the large quantities of glucose present in samples. Genetic differences influenced arabinoxylan levels in total, water-soluble and acid-soluble barley fractions, and the location of growth affected the solubility of cell wall polymers. The arabinoxylan level of barley acid extract was moderately correlated with viscosity (0.55; $P<0.05$; $n=51$), which indicated that this polymer may be partially responsible for the reduced performance which can occur when young poultry are fed barley-based diets.

Barley cultivars were selected to represent a range of soluble arabinoxylan levels: high (Stacey), moderate (Manley) and low (Richard). Chick diets were formulated to meet NRC requirements, using barley as the sole cereal source. Purified endoxylanase (PX) and a ß-glucanase-free endoxylanase preparation did not hydrolyze barley ß-glucan, as determined by a modification of the McCleary-Glennie-Holmes ß-glucan assay (1985). Purified endo-1,4-ß-xylanase (0, 475 and 950 IU/kg) was supplemented to each of the cultivar-based diets, and XBG (475 and 950 IU/kg) was added to two Stacey

treatments. Enzyme activity was determined by Neocuproine assay of reducing sugar production in a xylan solution (1% w/vol; pH 5.3; 50°C) over a 20 min period. The trial data indicated that enzyme supplementation of the Stacey diet supported improved chick weight gain and feed efficiency ($P<0.05$).

The proximal small intestine digesta viscosity of Stacey-based diets was reduced by PX supplementation ($P<0.05$), but was not affected when XBG was included in the diet. Improvements in weight gain and feed conversion were also restricted to the PX-supplemented diets. This effect is possibly related to the lower pH optimum of the fungal PX product, which may permit polymer hydrolysis in more anterior segments of the gut. The distal small intestine viscosity was reduced in all treatments supplemented with endoxylanase ($P<0.01$).

Further research is suggested to:

1. determine if the same increment in performance occurs when PX is added to a Stacey-based diets supplemented with purified ß-glucanase
2. establish the contribution of arabinoxylan and ß-glucan to decreased wort filtration times, using purified xylanase and ß-glucanase
3. evaluate the relationship between the pH optima of feed enzymes and substrate hydrolysis in the chick gut

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# APPENDICES

Appendix 1. Least square means (LSM) of location, type, cultivar and analytical effects on the total pentose level of barley breeder lined and cultivars (named).

| Location | Pentose LSM | Std. Err. LSM |
|---|---|---|
| Beaver | 5.8525 | 0.0998 |
| Elrose | 5.9344 | 0.1596 |
| Lacombe | 6.1334 | 0.1596 |
| Melfort | 5.9438 | 0.0998 |
| Trochu | 6.0625 | 0.2148 |
| Watrous | 5.9832 | 0.1294 |
| **Type** | | |
| Six-rowed barley (BT) | 6.9455 | 0.0860 |
| Hulless barley (HB) | 4.6267 | 0.1700 |
| Two-rowed barley (TR) | 6.3828 | 0.1062 |
| **Cultivar(type)** | | |
| BT 120 (cv. Argyle) | 6.5322 | 0.1765 |
| BT 409 | 6.4317 | 0.1765 |
| BT 410 | 6.9967 | 0.1765 |
| BT 542 | 6.7990 | 0.1765 |
| BT 543 | 7.3003 | 0.1765 |
| BT 544 | 7.2830 | 0.1765 |
| BT 670 | 6.9514 | 0.1765 |
| BT 671 | 7.2695 | 0.1765 |
| HB 004 | 4.7676 | 0.2367 |
| HB 602 | 4.2262 | 0.2367 |
| HB 603 | 4.8862 | 0.2367 |
| TR 319 | 6.2131 | 0.1858 |
| TR 320 | 6.2924 | 0.1858 |
| TR 321 | 6.1512 | 0.1858 |
| TR 490 (cv. Manley) | 6.5694 | 0.1858 |
| TR 554 | 6.5945 | 0.1858 |
| TR 562 | 6.5771 | 0.1858 |
| TR 563 | 6.2817 | 0.1858 |
| **Analysis** | | |
| Day 1 | 5.9797 | 0.1267 |
| Day 2 | 5.6808 | 0.1315 |
| Day 3 | 6.0733 | 0.1173 |
| Day 4 | 6.3976 | 0.1281 |
| Day 5 | 6.2086 | 0.1456 |
| Day 6 | 5.4358 | 0.1278 |

Appendix 2. Least square means (LSM) of location, type, cultivar and analytical effects on the level of acid-soluble pentose in barley breeder lines and cultivars (named).

| Location | Pentose LSM | Std Err LSM |
|---|---|---|
| Beaver | 0.2934 | 0.0674 |
| Elrose | 0.5181 | 0.0492 |
| Lacombe | 0.5230 | 0.0631 |
| Melfort | 0.5973 | 0.0768 |
| Trochu | 0.4104 | 0.0829 |
| Watrous | 0.5168 | 0.0702 |
| **Type** | | |
| Six-rowed barley (BT) | 0.5969 | 0.0318 |
| Hulless barley (HB) | 0.4318 | 0.0432 |
| Two-rowed barley (TR) | 0.4008 | 0.0433 |
| **Cultivar(type)** | | |
| BT 120 (cv. Argyle) | 0.4761 | 0.0477 |
| BT 409 | 0.5729 | 0.0477 |
| BT 410 | 0.6313 | 0.0438 |
| BT 542 | 0.5687 | 0.0438 |
| BT 543 | 0.6619 | 0.0433 |
| BT 544 | 0.6379 | 0.0433 |
| BT 670 | 0.5577 | 0.0433 |
| BT 671 | 0.6687 | 0.0433 |
| HB 004 | 0.4096 | 0.0530 |
| HB 602 | 0.4444 | 0.0530 |
| HB 603 | 0.4414 | 0.0530 |
| TR 319 | 0.4165 | 0.0414 |
| TR 320 | 0.3597 | 0.0576 |
| TR 321 | 0.3850 | 0.0562 |
| TR 490 (cv. Manley) | 0.4589 | 0.0576 |
| TR 554 | 0.4102 | 0.0577 |
| TR 562 | 0.4724 | 0.0588 |
| TR 563 | 0.3031 | 0.0586 |
| **Analysis** | | |
| Day 1 | 0.3978 | 0.0606 |
| Day 2 | 0.3595 | 0.0835 |
| Day 3 | 0.5000 | 0.0618 |
| Day 4 | 0.6944 | 0.0672 |
| Day 5 | 0.5112 | 0.0365 |
| Day 6 | 0.3948 | 0.0731 |
| Day 7 | 0.4338 | 0.0666 |
| Day 8 | 0.5208 | 0.0510 |

Appendix 3. Least square means (LSM) of location, type, cultivar and analytical effects on the level of water-soluble pentose in barley breeder lines and cultivars (named).

| Location | Pentose LSM | Std Err LSM |
|---|---|---|
| Beaver | 0.4570 | 0.0322 |
| Elrose | 0.6238 | 0.0325 |
| Lacombe | 0.7530 | 0.0326 |
| Melfort | 0.7343 | 0.0265 |
| Trochu | 0.5237 | 0.0328 |
| Watrous | 0.8381 | 0.0409 |
| Type | | |
| Six-rowed barley (BT) | 0.7202 | 0.0121 |
| Hulless barley (HB) | 0.6599 | 0.0181 |
| Two-rowed barley (TR) | 0.5847 | 0.0157 |
| Cultivar(type) | | |
| BT 120 (cv. Argyle) | 0.5496 | 0.0310 |
| BT 371 | 0.5697 | 0.0333 |
| BT 373 | 0.7085 | 0.0333 |
| BT 409 | 0.7142 | 0.0319 |
| BT 410 | 0.7531 | 0.0355 |
| BT 417 | 0.7276 | 0.0314 |
| BT 419 | 0.7199 | 0.0313 |
| BT 420 | 0.7683 | 0.0310 |
| BT 483 (cv. Brier) | 0.7525 | 0.0313 |
| BT 542 | 0.7559 | 0.0324 |
| BT 543 | 0.8416 | 0.0316 |
| BT 544 | 0.7788 | 0.0310 |
| BT 545 | 0.6871 | 0.0330 |
| BT 546 | 0.7610 | 0.0301 |
| BT 636 | 0.6861 | 0.0298 |
| BT 670 | 0.7678 | 0.0323 |
| BT 671 | 0.9265 | 0.0461 |
| BT 672 | 0.6359 | 0.0290 |
| BT 930 | 0.6450 | 0.0323 |
| BT 934 | 0.6556 | 0.0326 |
| HB 003 | 0.6441 | 0.0416 |
| HB 004 | 0.5799 | 0.0393 |
| HB 005 | 0.7908 | 0.0418 |
| HB 302 | 0.6555 | 0.0396 |
| HB 303 | 0.6895 | 0.0449 |
| HB 309 | 0.6215 | 0.0423 |
| HB 310 | 0.7536 | 0.0396 |
| HB 311 | 0.5702 | 0.0415 |
| HB 312 | 0.6520 | 0.0423 |
| HB 313 | 0.6898 | 0.0393 |
| HB 314 | 0.7684 | 0.0418 |

Appendix 3 (cont). Least square means (LSM) of location, type, cultivar and analytical effects on the level of water-soluble pentose in barley breeder lines and cultivars (named).

| Location | Pentose LSM | Std Err LSM |
|---|---|---|
| HB 501 (cv. Falcon) | 0.6711 | 0.0406 |
| HB 601 | 0.6246 | 0.0396 |
| HB 602 | 0.6075 | 0.0396 |
| HB 603 | 0.5805 | 0.0385 |
| TR 122 | 0.6453 | 0.0321 |
| TR 318 | 0.5742 | 0.0333 |
| TR 319 | 0.5465 | 0.0346 |
| TR 320 | 0.4918 | 0.0347 |
| TR 321 | 0.5574 | 0.0361 |
| TR 441 (cv. Harrington) | 0.6081 | 0.0320 |
| TR 490 (cv. Manley) | 0.7091 | 0.0318 |
| TR 544 (cv. Bridge) | 0.5458 | 0.0323 |
| TR 554 | 0.5054 | 0.0334 |
| TR 561 | 0.5655 | 0.0352 |
| TR 562 | 0.7070 | 0.0337 |
| TR 563 | 0.4977 | 0.0314 |
| TR 621 | 0.5627 | 0.0316 |
| TR 628 | 0.5128 | 0.0334 |
| TR 630 | 0.6583 | 0.0321 |
| TR 631 | 0.6026 | 0.0333 |
| TR 943 | 0.6319 | 0.0323 |
| TR 955 | 0.6034 | 0.0361 |
| **Analysis** | | |
| Day 1 | 0.4597 | 0.0427 |
| Day 2 | 0.5639 | 0.0415 |
| Day 3 | 0.6034 | 0.0323 |
| Day 4 | 0.5954 | 0.0353 |
| Day 5 | 0.6027 | 0.0294 |
| Day 6 | 0.6160 | 0.0342 |
| Day 7 | 0.6626 | 0.0326 |
| Day 8 | 0.6016 | 0.0347 |
| Day 9 | 0.6462 | 0.0293 |
| Day 10 | 0.6545 | 0.0378 |
| Day 11 | 0.6648 | 0.0372 |
| Day 12 | 0.7507 | 0.0332 |
| Day 13 | 0.7551 | 0.0325 |
| Day 14 | 0.7193 | 0.0416 |
| Day 15 | 0.7436 | 0.0405 |
| Day 16 | 0.7930 | 0.0461 |
| Day 17 | 0.6328 | 0.0215 |
| Day 18 | 0.7239 | 0.0698 |