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

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

BY

MICHELLE DAWN FLEURY

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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).

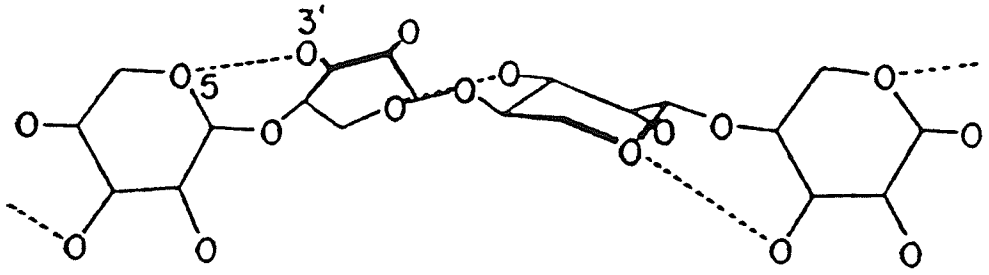

Figure 1. Twisted ribbon conformation of a ß-(1-4)-xylan, showing hydrogen bonds between O5 and O3′ atoms on contiguous residues (Winterburn, 1974).

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).

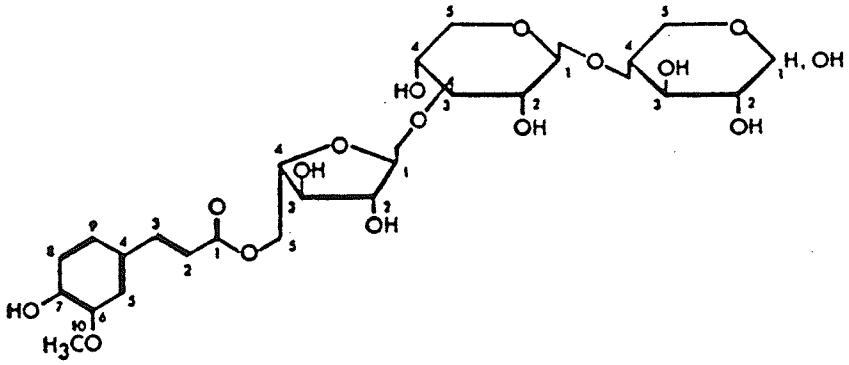

Figure 3. Proposed structure for feruloyl-O-5-arabinosyl sidechains of arabinoxylan (Gubler et al, 1985).

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'.

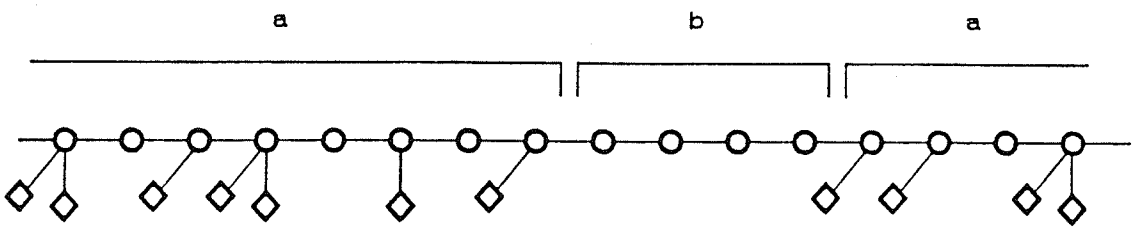

Figure 4. Schematic view of a model for the distribution of substituents over an arabinoxylan chain. (Vietor et al, 1992c).

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