Influence of waxy and high-amylose starch genes on the composition of barley and the cholesterolemic and glycemic responses in chicks and rats
by Qi Xue

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University
© Copyright by Qi Xue (1992)

Abstract:
The ratio of amylose vs amylopectin (waxy) starch in barley grain affects its chemical composition and biological properties. To investigate the effects of waxy (ww) and high-amylose (amol) starch genes on chemical composition of barley and the cholesterolemic and glycemic responses in an animal model, a series of barley isotypes having non-waxy, waxy starch and another series of Glacier barley having high-amylose (HA) starch in covered, and hull-less lines were tested. Waxy gene increased ($P< .01$) total and soluble dietary fiber, total and soluble β-glucans, extract viscosity, free sugars, and reduced starch content of barley. Extract viscosity was correlated with total β-glucan and free sugars ($r=.55$ and .76, $P<.001$). High-amylose gene increased amylose content (40%-44%) and β-glucan content (7.4%) over normal barley (29%, 5.2%). Means of postprandial blood glucose levels were not different for chicks fed uncooked (raw) high-amylose barley flour or red dog (RD) milling fractions compared with normal barley but were higher ($P < .05$) compared with com controls. However, total and LDL plasma cholesterol were lower for chicks fed the HA flours and all barley RD diets compared to com controls. Starches were purified from barley flours of waxy, normal, and high-amylose cultivars. These starches were moisture-autoclaved 3 and 12 times with subsequent cooling. Only marginal effects occurred on hydrolysis rates with no effects on glucose levels in rats when boiled starches of different origins (amylose vs amylopectin) were tested. However, the effects of starch types on digestibility and blood glucose responses were significantly altered by autoclaving. Digestibility of waxy starches was not changed in vitro ($P>.05$) but glucose in rats was significantly ($P < .05$) increased after ingestion of autoclaved waxy barley starch. In contrast, the digestibilities of high-amylose starches were reduced by 14% and 20% after 3 and 12 autoclave-cool cycles, respectively. Autoclaved HA starch significantly lowered the glucose peaks in rats compared to waxy and normal starches at 30 min ($P< .01$). The in vivo results corresponded to that in vitro study which demonstrated that the digestibility of different cereal starches followed the pattern: waxy > normal > high-amylose starches after heat-moisture autoclaving, possibly due to the formation of enzyme resistant starch from the amylose component. Enzyme resistant starch formation was highly ($P < .001$) correlated with digestibility ($r= -.98$) and amylose contents ($r= .96$) of autoclaved starches. These data indicate that the expression of the waxy and high-amylose genes in barley is desirable to increase the levels of soluble dietary fiber, particularly β-glucans and also implied that high amylose barley has potential for specialty food product development.

INFLUENCE OF WAXY AND HIGH-AMYLOSE STARCH GENES ON THE COMPOSITION OF BARLEY AND THE CHOLESTEROLEMIC AND GLYCEMIC RESPONSES IN CHICKS AND RATS

by

Qi Xue

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Crop and Soil Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 1992

D378
X811

APPROVAL

of a thesis submitted by

Qi Xue

This thesis has been read by each member of the graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

11/23/92
Date

Rosemary K Sturman
Co-Chairperson, Graduate Committee

11/23/92
Date

Charles F McGuire
Co-Chairperson, Graduate Committee

Approved for the Major Department

11/23/92
Date

Head, Major Department

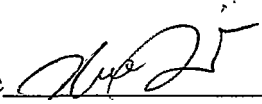
Approved for the College of Graduate Studies

11/24/92
Date

Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S Copyright law. Requests for extensive copying or reproduction of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted " the exclusive right to reproduce and distribute copies of the dissertation in and from microfilm and the right to reproduce and distribute by abstract in any format."

Signature 

Date 11/23/92

ACKNOWLEDGEMENTS

My sincere thanks to the following:

Drs. Rosemary K. Newman, C. Walt Newman and Charles F. McGuire for serving as my major professors and providing guidance, encouragement, friendship, patience and support throughout this project.

Drs. Samuel J. Rogers, R. Thomas Ramage and Joe Anderson for their suggestions and assistance while serving on my graduate committee.

Alan D. Danielson for his excellent assistance and for being a good friend throughout my program.

Petrea Hofer, Jill Abbott, Kibbie Horsley, Adam Fengler, Ted Mori, Virginia Martinez, Gayle Watts and Linji Wang for suggestions, collaboration, laboratory assistance, collecting data and friendship.

The Montana Wheat and Barley Committee for financial support during the course of my study.

Appreciation is also extended to the staff in our department, Animal Resource Center and Animal Nutrition Center for providing assistance in all aspects of my graduate study.

Finally, I thank my wife, Huijun, and my parents for their love and support.

TABLE OF CONTENTS

Page

APPROVAL ii

STATEMENT OF PERMISSION TO USE iii

VITA iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

LIST OF TABLES ix

LIST OF FIGURES xii

ABSTRACT xiii

CHAPTER:

1. INTRODUCTION 1

2. LITERATURE REVIEW 4

Starch 4
Starch Structure and Composition 4
Some Properties of Starch 7
Nutritional Implications of Starch 9
Dietary Fiber 12
Composition of Dietary Fiber in Barley 12
Nutritional Implications of Dietary Fiber 14

TABLE OF CONTENTS----Continued

CHAPTER: Page

3. INFLUENCE OF THE WAXY, HULL-LESS AND SHORT-AWNED GENES ON THE COMPOSITION OF BARLEYS: A 3-YEAR STUDY 16

Introduction 16
Materials and Methods 18
Results and Discussion 20
Hull-less Gene Effects 20
Waxy Gene Effects 25
Cultivar Background Effects 31
Year Effects 33
Short-awned Gene Effects 33
Correlation Coefficients of the Components 36
Conclusions 40

4. GLYCEMIC RESPONSES AND HYPOCHOLESTEROLEMIC EFFECTS OF GLACIER BARLEYS FED TO BROILER CHICKS 41

Introduction 41
Materials and Methods 44
Barley, Milling Fraction and Chick Diets 44
Feeding Trials 49
Chemical Analysis 50
Statistical Analysis 50
Results and Discussion 50
Chemical Analysis of Barley Grain and Milling Fractions 50
Glucose Responses of Chicks 52
Hypocholesterolemic Effect 56
Conclusions 61

TABLE OF CONTENTS----Continued

CHAPTER: Page

5. DIGESTIBILITIES *IN VITRO* AND GLUCOSE RESPONSES IN RATS WITH DIFFERENT TYPES OF BARLEY STARCHES SUBJECTED TO HEAT-TREATMENTS 62

Introduction 62
Materials and Methods 65
Barley Flour 65
Starch Isolation 66
Starch Treatment 66
Enzyme Resistant Starch Determination 67
Starch Hydrolysis *in vitro* with α-amylase 67
Glucose Tolerance Test in Rats 68
Chemical Analysis 69
Statistical Analysis 69
Results and Discussion 69
Starch Isolation 70
Formation of Enzyme Resistant Starch 73
In vitro Digestibilities and Hydrolysis Rates of Starches 75
Glucose Responses in Rats 81
Conclusions 85

6. SUMMARY 86

LITERATURE CITED 90

APPENDIX 102

LIST OF TABLES

Table — Page

1. Isotype Series of Waxy and Non-waxy, Covered and Hull-less, Short-awned and Long-awned Barleys in Compana and Betzes Barleys 18

2. Analysis of Variance Mean Squares of Chemical Composition for Different Years, Parents, Hull and Starch Types of Barleys 21

3. Effect of the Hull-less Gene on the Chemical Composition and Physical Characteristics of Barleys 24

4. Effect of the Waxy Gene on the Chemical Composition and Physical Characteristics of Barleys 27

5. Effects of the Waxy Gene on the β-glucan, Dietary Fiber Content and Extract Viscosity of Six Genotypes of Compana and Betzes Barleys 28

6. Interaction Effects of Hull-less and Waxy Genes on TDF, Cellulose, Ash and Extract Viscosity 30

7. Effect of the Cultivar Background on the Chemical Composition and Physical Characteristics of Barleys 32

8. Effect of the Years on the Chemical Composition and Physical Characteristics of Barleys 34

9. Effect of the Short-awned Gene on the Chemical Composition and Physical Characteristics of Barleys 35

10. Correlation Coefficients (r) of Chemical Composition of Barleys 37

LIST OF TABLES----Continued

Table Page

11. Chemical Analysis of Barley Grains 44

12. Chemical Analysis of Barley Flour Fraction and Corn Meal for *Trial I* Diets 45

13. Chemical Analysis of Barley Red Dog Fraction and Corn Meal for *Trial II* Diets 46

14. Composition and Chemical Analysis of Chick Diets for *Trial I* 47

15. Composition and Chemical Analysis of Chick Diets for *Trial II* 48

16. Chick Glucose Response (mg/dl) and Feed Intake (g) of *Trial I* (Barley Flour or Corn Control-1 Diets) 52

17. Chick Glucose Response (mg/dl) and Feed Intake (g) of *Trial II* (Barley Red Dog Fraction or Corn Control-2 Diets) 54

18. Plasma Lipid Concentrations (mg/dl) and Average Daily Gain of Chicks Fed *Trial I* Diets 57

19. Plasma Lipid Concentrations (mg/dl) and Average Daily Gain of Chicks Fed *Trial II* 59

20. Yield and Chemical Composition of Starchy Materials Isolated from Barleys 71

21. Chemical Composition of Starches from Sigma Chemical Company. 72

22. Yield of Enzyme Resistant Starch from Autoclaving-cooling Cycles 73

LIST OF TABLES----Continued

Table Page

23. Digestibilities of Starches *in vitro* with Different Heat-Treatments 76

24. Selected Correlation Coefficients (r) of Amylose Content, Enzyme Resistant Starch and Digestibility *in vitro* 80

25. Correlation Coefficients (r) of Glucose Responses in Rats and Amylose Content, Enzyme Resistant Starch, Digestibility *in vitro* with Autoclaved Treatments 85

LIST OF FIGURES

Figure — Page

1. Glucose Responses of Chicks Fed Barley Flour or Corn-1 Diets 53
2. Glucose Responses of Chicks Fed Barley Red Dog or Corn-2 Diets 55
3. Blood Lipid Values of Chicks Fed Barley Flour and Corn-1 Diets 58
4. Blood Lipid Values of Chicks Fed Barley Red Dog or Corn-2 Diets 60
5. Starch Hydrolysis Rates (%) with Boiled Treatment 77
6. Starch Hydrolysis Rate (%) with 3 Cycle Autoclaved Treatment 78
7. Starch Hydrolysis Rate (%) with 12 Cycle Autoclaved Treatment 79
8. Glucose Responses in Rats Fed Boiled Starches 82
9. Glucose Responses in Rats Fed 3 Cycle Autoclaved Starches 83
10. Glucose Responses in Rats Fed 12 Cycle Autoclaved Starches 84
11. Milling Flow Sheet for MIAG MULTOMAT 8-Roller Dry Mill 103

ABSTRACT

The ratio of amylose vs amylopectin (waxy) starch in barley grain affects its chemical composition and biological properties. To investigate the effects of waxy (*ww*) and high-amylose (*amo1*) starch genes on chemical composition of barley and the cholesterolemic and glycemic responses in an animal model, a series of barley isotypes having non-waxy, waxy starch and another series of Glacier barley having high-amylose (HA) starch in covered, and hull-less lines were tested. Waxy gene increased ($P<.01$) total and soluble dietary fiber, total and soluble β-glucans, extract viscosity, free sugars, and reduced starch content of barley. Extract viscosity was correlated with total β-glucan and free sugars ($r=.55$ and .76, $P<.001$). High-amylose gene increased amylose content (40%-44%) and β-glucan content (7.4%) over normal barley (29%, 5.2%). Means of postprandial blood glucose levels were not different for chicks fed uncooked (raw) high-amylose barley flour or red dog (RD) milling fractions compared with normal barley but were higher ($P<.05$) compared with corn controls. However, total and LDL plasma cholesterol were lower for chicks fed the HA flours and all barley RD diets compared to corn controls. Starches were purified from barley flours of waxy, normal, and high-amylose cultivars. These starches were moisture-autoclaved 3 and 12 times with subsequent cooling. Only marginal effects occurred on hydrolysis rates with no effects on glucose levels in rats when boiled starches of different origins (amylose vs amylopectin) were tested. However, the effects of starch types on digestibility and blood glucose responses were significantly altered by autoclaving. Digestibility of waxy starches was not changed *in vitro* ($P>.05$) but glucose in rats was significantly ($P < .05$) increased after ingestion of autoclaved waxy barley starch. In contrast, the digestibilities of high-amylose starches were reduced by 14% and 20% after 3 and 12 autoclave-cool cycles, respectively. Autoclaved HA starch significantly lowered the glucose peaks in rats compared to waxy and normal starches at 30 min ($P<.01$). The *in vivo* results corresponded to that *in vitro* study which demonstrated that the digestibility of different cereal starches followed the pattern: waxy > normal > high-amylose starches after heat-moisture autoclaving, possibly due to the formation of enzyme resistant starch from the amylose component. Enzyme resistant starch formation was highly ($P < .001$) correlated with digestibility ($r= -.98$) and amylose contents ($r= .96$) of autoclaved starches. These data indicate that the expression of the waxy and high-amylose genes in barley is desirable to increase the levels of soluble dietary fiber, particularly β-glucans and also implied that high amylose barley has potential for specialty food product development.

CHAPTER 1

INTRODUCTION

Coronary heart disease (CHD) and diabetes mellitus are not only the major causes of death in the industrialized counties, but they account for enormous costs for treatment and care (Anonymous, 1981; NDDG, 1985; Anderson et al., 1987a). Individuals with diabetes die from CHD two to three times more frequently than those who do not have diabetes (Anderson et al., 1987b). Hypercholesterolemia and hyperglycemia were recognized as significant risk factors for CHD (Connor and Connor, 1984) and for diabetes (Anderson et al., 1987b). Reductions in cholesterol and postprandial glucose and insulin in the blood are considered to be beneficial in preventing and treating hyperlipidemia and hyperglycemia as well as related disorders including obesity and hypertension, thereby reducing the risk of CHD and diabetes (Amelsvoort and Weststrate, 1992).

Diets high in fiber and starches have been reported to normalize blood glucose and lipid levels in carbohydrate-sensitive diabetic and hyperlipidemic individuals (Behall and Scholfield, 1989; Jenkins and Jenkins, 1985). Soluble fiber such as β-glucan in barley has been shown to have hypocholesterolemic effects in rats (Klopfenstein and Hoseney, 1987; Mori, 1990), chicks (Fadel et al., 1987), and humans (Newman et al.,

1989a; McIntosh et al., 1991). Bengtsson et al. (1990) concluded that the hypocholesterolemic response observed in a waxy barley, but not in a non-waxy barley was due to the higher level of β-glucan and viscosity in waxy barley. Higher levels of total dietary fiber (TDF), β-glucan and relative extract viscosity have been reported by Ullrich et al. (1986), Mori (1990) and Han and Froseth (1992). Soluble fiber, including β-glucan was demonstrated to flatten glucose curves in humans (Jenkins and Jenkins, 1985) and in rat (Vachon et al., 1988).

The bioavailability of starch, especially the effect of amylose/amylopectin ratio, has recently been an issue of nutritional concern. Research reports on the glucose-lowering response of high-amylose corn starch (Behall and Scholfield, 1989; Amelsvoort and Weststrate, 1992) and rice starch (Goddard et al, 1984; Panlasigui et al, 1992) have been reported. Behall and Scholfield (1989) also reported a lowering of blood triglycerides and cholesterol in subjects consuming high-amylose corn starch.

Few nutritional studies on barley starch have been reported although some barley cultivars are known to have widely varying ratios of amylose to amylopectin. An amylose level of 44% was discovered in a mutant of Glacier. In addition to the starch ratio, high-amylose Glacier barley also contains relatively high β-glucan, indicative of hypocholesterolemic responses.

The objectives of these studies were to (1) determine if increased levels of β-glucans, TDF and extract viscosity in waxy barley are consistent in more than one genotype grown in different environments; (2) evaluate the effects of hull-less and short-awned genes on other nutrient components in addition to the effects of the waxy gene;

(3) investigate glucose and cholesterol responses of broiler chicks fed two uncooked barley milling fractions from three different Glacier barleys; (4) estimate the formation of enzyme resistant starch during the autoclaving process with different ratios of amylose and amylopectin in barley and corn; (5) determine *in vitro* digestibility and hydrolysis rate of barley and corn starches with different amylose and amylopectin content and heat treatments (autoclaved vs non-autoclaved); and (6) evaluate the glucose responses of autoclaved barley starch compared to wheat starch in rats.

CHAPTER 2

LITERATURE REVIEW

Barley is an important agricultural crop in the world, and particularly in the Northern Plains States. The grain is used mainly as an animal feed and for malt but there is a growing interest in barley for food and industrial use (Åman and Newman, 1986). A better understanding of the relationship between grain chemical composition and physical characteristics as well as the nutrition value would be helpful in successful utilization of barley for new uses (Henry, 1988). A knowledge of the contribution of genetic and environmental factors for grain composition is also required. Carbohydrates, major components of the kernel, have a significant influence on grain quality. The carbohydrates are generally subdivided into starch, sugar and dietary fiber (DF). This review will focus on aspects of carbohydrate nutrition related to barley starch genotypes.

Starch

Starch Structure and Composition

Starch makes up about 50 to 68% of barley grain (Åman and Newman, 1986), occurring as water insoluble granules. The shape and size of the granules are markedly different in plants, dependent on the botanical source. For example, normal Glacier

barley starch exhibits bimodal distribution (Banks and Muir, 1980). In these starch granules, there is a population of large lenticular granules, ranging in diameter from 15 - 35 μm, which comprise some 90% by weight but less than 10% by number of the total starch, and a separate population of small spherical granules ranging from 1 to 10 μm. However, high-amylose Glacier barley is quite different from the normal Glacier, having smaller sized granules with less regular shaping and no clear evidence of the bimodal distribution.

Starch granules are semicrystalline and exhibit birefringence in polarized light. Starch granules can give a number of distinct types of X-ray patterns (designated A, B, and C). Information on the organization of the starch granule has been obtained from different microscopy techniques (Lineback, 1984; Zobel, 1992). X-ray diffraction of crystalline amylose helical inclusion compounds results in the V amylose pattern (Hoseney, 1986).

Starch granules consist of a mixture of amylose and amylopectin in proportions which vary from one starch to another. Amylose is essentially a linear polymer composed of α-(1→4)-linked D-glucose while amylopectin is highly branched with α-(1→4), α-(1→6)-linkages (Briggs, 1978). In normal barley, about 22-30% of the starch is amylose and 70-78% is amylopectin (Morrison et al., 1986). However, the genetic variation in the amylose/amylopectin ratio is varied, including both high amylose and high amylopectin (waxy) genotypes. A high amylose level of 41-44% has been found in a mutant of Glacier (CI 9676) barley, which is designated Glacier Ac38 (Merritt, 1967). This character was shown to be under a single recessive gene (*amo1*) which is

located on chromosome 3 (von Wettstein-Knowles, 1992). Walker and Merritt (1969) reported this gene with a dose effect of allele. Endosperm which are triploid, were accordingly produced representing 0, 1, 2, and 3 doses of mutant allele by reciprocal hybridization between the mutant (Ac38) and the its parent, Glacier (CI9676). The high-amylose gene from high-amylose Glacier differs fundamentally from the *amylose extender (ae), dull (du) and sugary (su)* genes that increase the proportion of amylose to amylopectin in maize. In contrast, waxy starch, with amylose content as low as 0-8% (Morrison et al., 1986), is controlled by the recessive waxy endosperm gene *ww* in maize (Nakao, 1950) and barley (Goering and Eslick, 1976). The waxy barley was native to Asia (Banks et al., 1970), and the waxy gene in it was shown to be located on chromosome 1 (Hockett and Nilan 1985). This gene was introduced into two parent barley cultivars, Compana and Betzes (Goering and Eslick, 1976). Various other traits such as nude and short awn were also introduced, resulting in isogenic series. Åman and Newman (1986) first suggested that waxy barleys contain less starch and a greater quantity of sucrose, but did not measure maltose. Therefore, the waxy gene may affect not only the starch type but also other components of the waxy barleys, such as free sugar, β-glucan and dietary fiber (Newman and Newman, 1992).

Amylopectin in the starch granule constitutes the crystalline region, whereas amylose is mainly amorphous, filling the spaces between the clusters of amylopectin branches (Eliasson et al., 1987). The waxy varieties of starch give the same X-ray diffraction patterns as the normal starch, whereas high amylose varieties exhibit very low crystallinity. Further, amylose can be leached from the intact starch granule without

affecting the crystal state of the granule (Zobel, 1992). The consequence of the differences in structure between the two major constituents of starch is that their properties are quite different, as described by Marshall (1972).

Some Properties of Starch

Starch granules are insoluble in cold water, but by heating above 50^0C in excess water, the structure of the granule is altered by swelling, hydration and solubilization. French (1984) has theorized that the swelling of the amorphous phase contributes to the disruption of the crystalline regions by tearing molecules from the crystallites. This process called gelatinization involves melting of the crystallites that are present in the native granule. It is known that the gelatinization behavior of the starch granule is affected by its amylose content. According to several reports, the gelatinization temperature of maize and pea starches is generally increased (135^0C to 150^0C) at a higher amylose content (50% to 80%) as compared that of 75^0C to 85^0C in normal starches (Colonna and Mercier, 1985; Manners, 1985; 1988). It was demonstrated that a close correlation exists between the degree of starch gelatinization and the rate of enzymic hydrolysis both *in vitro* and *in vivo* (Holm et al., 1988). When gelatinized amylose starch is cooled, retrogradation may occur, causing a return from a solvated dispersed, amorphous state to an insoluble, aggregated or crystalline state. Retrogradation is partly reversible by heating the retrograded starch, which dissolves the crystalline nuclei. However, starch may retrograde so firmly that it becomes irreversible and totally resistant to α-amylolysis and thus it can escape digestion both *in vitro* and *in vivo* (Englyst and Cummings, 1985; Björck et al., 1987; Ring et al., 1988). This enzyme

resistant starch (ERS) is generally defined as the component of native or processed starch that is able to survive exhaustive digestion with amylolytic enzymes, but which nevertheless can be measured as α-glucan after solubilization with alkaline dimethyl sulfoxide (Berry, 1986). Processed foods such as bread, breakfast cereals and biscuits (Englyst and Cummings, 1985; Björck et al., 1986; Crawford, 1987;) or cooked amylomaize starch (Berry, 1986) contain appreciable quantities of ERS. The formation of this ERS is influenced by the amylose content, processing temperature and water content. The association between the amylose content and yield of ERS suggests that the linear components of starch and the heating process are involved, hence debranched amylopectin yielded 32-46% ERS by autoclaving and drying (Berry, 1986). Twenty cycles of autoclaving at 121^0C-134^0C with excess water and subsequent cooling increased the ERS formation up to 25% in high-amylose barley starch and 40% in amylomaize (Sievert and Pomeranz, 1989; Szczodrak and Pomeranz, 1991). Under these conditions, waxy maize starch yielded less than 1% resistant starch (Berry, 1986; Sievert and Pomeranz, 1989). This ERS is believed to consist mainly of retrograded amylose, as the amount formed increases with increasing amylose content (Russell et al., 1989; Berry, 1986; Ring et al., 1988; Siljeström et al., 1989; Szczodrak and Pomeranz, 1991). Since ERS is reported to have reduced bioavailability in the human gastrointestinal tract and to exert physiological effects similar as those of dietary fiber (Björck et al., 1987; Jenkins and Jenkins, 1985; Schneeman, 1989), it has been proposed that it be considered part of the total dietary fiber (Englyst and Cummings, 1987).

Nutritional Implications of Starch

A wide variation in the digestibility of starch granules both *in vitro* and *in vivo*, depending on starch source, structure and food processing and storage conditions, have been reported (Dreher et al., 1984). The efficient digestion of starch has been shown to be critical for formula-fed infants 6 months of age or younger (Auricchio et al., 1967) as well as elderly people or those with medical disorders who have reduced digestive capacity (Sugimoto et al., 1980). In animal feedstuffs, maximum efficiency of feed utilization is needed to reduce production cost, and starch is the most important source of energy (Hale, 1973). On the other hand, a somewhat incomplete starch digestibility may be nutritionally advantageous in certain conditions. Malabsorbed starch reaching the colon could physiologically function like dietary fiber as mentioned above and have therapeutic applications such as blood glucose control in diabetics or as an aid in weight control (Jenkins et al., 1987).

Starch digestibility and absorption can be measured by different methods, each having its own advantages and limitations and each providing specific information. Both the rate and extent of starch hydrolysis *in vitro* are regarded as predictors of metabolic responses to complex carbohydrate *in vivo* (O'Dea and Holm, 1988). A commonly used method for the investigation of rates of starch hydrolysis in foods is simulated digestion *in vitro*. It has been well established that such differences correlate with postprandial blood glucose changes in humans under experimental conditions (Lund and Johnson, 1991; Brand et al., 1985; Bornet et al., 1989). The glucose tolerance test monitors the rise in plasma levels of glucose and insulin following oral ingestion of carbohydrate

meals. The rapidity, duration and the degree of rise of plasma glucose and insulin represent the end result of gastric emptying, digestion, absorption and also that of glucose utilization. Over the years, it has frequently been demonstrated that the ingestion of various types of starchy foods has various effects on the postprandial glucose and insulin responses in healthy and diabetic humans. These observations led to the development of the glycemic index which is defined as the ratio of the area under the incremental blood glucose response curve after 2 h for healthy subjects and 3 h for diabetics (Jenkins et al., 1981). The standard was originally pure glucose and later white bread was used (Wolever et al., 1986). The glycemic index obtained for the various starchy foods clearly shows that this index is affected by many factors including the source of the food, particle size and type of preparation dietary mixtures (Thorne et al. 1983). The chemical composition of the starch (amylose vs amylopectin ratio) appears to be an important influence (Björck et al., 1990).

Waxy starch from maize, barley and sorghum in the uncooked state has been shown to be more readily digested by α-amylase *in vitro* than normal starch or high amylose starch (Sandstedt and Hites, 1968; Goering et al., 1973; Sullins and Rooney, 1974; Björck et al., 1990). However, this has not been observed *in vivo* when waxy barley starch was fed to rats (Calvert et al., 1976) or when whole grain waxy barleys were compared with normal barleys for ruminants (Moss et al, 1980), rats and swine (Calvert et al, 1977) and broiler chickens (Moss et al, 1983). The data from these studies varied with experiments and therefore left an open question about waxy starch digestibility.

High-amylose barley (HAG) and amylomaize starch were less susceptible to α-amylase than normal starch (Pomeranz et al., 1972; Dreher et al., 1984). Calvert et al. (1976) reported that rats consumed less of a purified diet prepared with HAG starch than a similar diet containing Glacier starch and as a consequence gained at a slower rate. Amylomaize starch has lower blood glucose and insulin levels in humans (Behall et al., 1988; Behall and Scholfield, 1989; Amelsvoort and Weststrate, 1992) and high-amylose rice has similar effects (Goddard et al., 1984; Panlasigui, et al., 1992). Behall and Scholfield (1989) also reported a lowering of blood triglycerides and cholesterol in subjects consuming amylomaize starch and concluded that long-term intake of high-amylose starch may benefit hyperlipidemic individuals as well as those with adult-onset diabetes.

Available data on the nutritional properties of amylose starch are not consistent. Calvert et al. (1981) found that barley starch type did not significantly affect gain or feed consumption of swine. Rubin et al. (1974) demonstrated HAG to be nutritionally superior to five other barleys including Glacier as measured by growth of weanling rats. In a study with rice, a slightly higher amylose content (22 vs 15%) resulted in an increased rate of *in vitro* α-amylolysis and an increase in postprandial glucose in healthy subjects (Srinivasa Rao, 1971).

Björck et al., (1990) reported that the amylose/amylopectin ratio in different barley genotypes (waxy, normal and high-amylose starch) produced no differences in *in vitro* digestibility when the barley flour was boiled even though results were significantly different when the raw starches were tested. Autoclaving the barley flour, however,

produced a concomitant decrease in *in vitro* digestibility with increased amylose content. This was explained by an increased amount of ERS (3%) and the formation of amylose-lipid complexes, which did not occur in autoclaved waxy barley or in any of the flours when boiled (Björck et al., 1990). Little is known about the long-term effects of amylose starch and the contribution of resistant starch to the total level of non starch-polysaccharides to human nutrition.

Dietary Fiber

Composition of Dietary Fiber in Barley

Dietary fiber (DF) has been defined as non-starch polysaccharides (NSP) plus lignin (Theander et al., 1989) or plant materials resistant to hydrolysis by digestive enzymes of mammals (Hipsley, 1953). Plant cell wall materials are the major source of DF. Three main NSP were found in barley seed cell walls: arabinoxylans, cellulose and mixed linked (1→3), (1→4)-β-glucans (Henry, 1988). Arabinoxylans are polysaccharides containing (1→4)-β-xylan chains with arabinose residues attached through (1→2) or (1→3)-linkages. Cellulose is composed of linear (1→4)-β-glucans. The β-glucans are also linear polymers and consist of β-cellotriosyl and β-cellotetraosyl residues separated by (1→3) linkages arranged in an independent or random way along the chain (Staute et al., 1983).

The composition of cell walls of barley seed can be quite different depending on the botanical tissue. Husk of barley grain consists of approximately equal amounts of arabinoxylans (50%) and cellulose residues (46%) (Salamonsson et al., 1980).

Arabinoxylans and β-glucans are concentrated in the aleurone and endosperm cell walls but the ratio between the two are different (Henry, 1988). Aleurone cell wall contains about 60% arabinoxylans and 20% β-glucans (Bacic and Stone, 1981) whereas endosperm cell walls contain 25% arabinoxylans and 70% β-glucans (Ballance and Manners, 1978).

The total β-glucan content of barley grain has been extensively studied and is shown to vary, depending on genotype or cultivars and the growing environmental conditions (Bourne and Pierce, 1970; Aastrup, 1979; Andersson et al., 1978; Newman and McGuire, 1985; Ullrich et al., 1986; Henry, 1986). Åman et al (1989) demonstrated an interaction of genotype x environment effect on β-glucan content of barley. Åman and Graham (1987) showed the average for Scandinavian barleys was 4.4% with a range of 3.0 - 5.6% of total β-glucan, of which 50-70% are soluble in water. Hockett et al. (1987) found a range in β-glucan of 4.9 - 6.2% with a mean of 5.5% in 12 North American cultivars over a 3-year period.

Powell et al. (1985) reported that the β-glucan content of barley is controlled by a simple additive genetic system. Waxy isotypes of Titan (CI 7055), Betzes (CI 6398) and Compana (CI 5438) barley contained 7.1, 7.3 and 4.7% β-glucan compared with those of 4.7, 5.7 and 3.8% β-glucan in non-waxy types (Ullrich et al., 1986). Azhul (a waxy hull-less six-rowed barley) was found to contain 10 - 11% β-glucan while the non-waxy parent barley contains 6-7% (Mori, 1990). The mechanism of the waxy gene effect on barley β-glucan content is currently unknown.

Total dietary fiber and soluble dietary fiber (SDF) in barley can be concentrated

in a fraction by milling and pearling (Newman and Newman, 1991). Mori (1990) reported that a milling fraction, red dog from barley was concentrated in TDF and SDF and had a marked hypocholesterolemic effect in rats. Up to 36% of TDF and 20% of SDF were obtained in a milling and air-classification fraction (Danielson et al., 1990).

Nutritional Implications of Dietary Fiber

The human health benefits of DF have been well documented and reviewed (Vahouny and Kritchevsky, 1986). Epidemiology studies have shown a relationship between inadequate consumption of DF and cancer of colon, hypercholesterolemia, atherosclerosis, and complications of diabetes, diverticulosis, constipation, hypertension, gallstones and obesity. Recently increased emphasis has been placed on the importance of soluble dietary fiber and its role in nutrition. Soluble fiber, primarily soluble β-glucans in barley, has hypocholesterolemic effects in rats (Klopfenstein and Hoseney, 1987; Mori, 1990), chicks (Fadel et al., 1987), and humans (Newman et al., 1989a; McIntosh et al., 1991). Bengtsson et al. (1990) reported that the hypocholesterolemic response observed in a waxy barley, but not in a non-waxy barley was due to the higher level of β-glucans and viscosity. Newman et al. (1991) demonstrated that β-glucans play a major role in the hypocholesterolemic effect of barley by showing that β-glucanase partially or completely eliminates the effect. Other soluble fiber components in barley such as soluble arabinoxylans may function like β-glucans (Newman and Newman, 1992). In addition, α-tocotrienol of barley oil was reported to lower serum cholesterol levels by inhibiting HMG-CoA reductase activity in the chick liver (Qureshi et al., 1986). However, factors other than β-glucans need to be further verified.

Studies have shown a beneficial effect of DF on the postprandial glucose responses in diabetes patients (Anderson et al., 1987b; Jenkins and Jenkins, 1985; Hagander, et al, 1988;). Soluble fiber was demonstrated to be more effective in humans (Jenkins et al., 1976; 1978; Canon et al, 1980; Ebihara and Kiryiama, 1982) and in rats (Vachon et al., 1988). One hypothesis suggests that the beneficial effect of increased fiber consumption in diabetes might result from its ability to reduce (1) the rate of carbohydrate absorption; (2) the rate of gastric emptying; (3) the rate of digestion of foods and (4) to increase the effective thickness of the so-called unstirred water layer (Jenkins and Jenkins, 1985). Dietary fiber is therefore one factor which may influence glycemic responses.

The importance of barley as a food grain has declined during the last 100 years when compared with other cereal food grains, particularly wheat (Munck, 1981). However, because of the renewed interest in DF, high amylose starch and oil as important factors in health and disease, the future for barley as a food grain has bright prospects.

CHAPTER 3

INFLUENCE OF THE WAXY, HULL-LESS AND SHORT-AWNED GENES ON THE COMPOSITION OF BARLEYS: A 3-YEAR STUDY

Introduction

Waxy (*ww*), hull-less (*nn*) and short-awned (*lklk*) genes are recessive characters located on chromosome one in barley (Hockett and Nilan, 1985). The three genetic characters were introduced as a series into several of barley cultivars, through a back crossing breeding program at the Montana Agricultural Experiment Station (MAES). The waxy gene produces starch that is 96-100% amylopectin (branched chain as opposed to that in "normal" barley which has a 3:1 ratio of amylopectin and amylose (straight chain) starch (Banks et al., 1970). As indicated by the terms, the hull-less gene produces kernels without adhering hulls (hull-less barley) and the short-awned gene produces awns that are approximately 50% shorter than normal. Nutritional evaluations of waxy barley have shown no advantage or disadvantage over normal barley as a feed for swine (Calvert et al., 1977; Han and Froseth, 1992) or ruminants (Moss et al., 1980). Studies with broiler chicks, however, indicate that waxy barley is inferior to normal barley as a feed grain for poultry (Moss et al., 1983; Newman and Newman, 1987; Fadel et al., 1987; Wang et al., 1992). In these studies, supplemental β-glucanase in the waxy and

non-waxy barley diets improved the performance of the birds to near that of those fed maize based diets. The beneficial effect of supplemental β-glucanase was most pronounced in barleys having both waxy and hull-less characteristics.

Analyses of waxy and waxy hull-less barleys in our laboratory have generally indicated increased total dietary fiber (TDF), β-glucans and relative extract viscosity compared to covered and non-waxy barleys (Mori, 1990; Xue et al., 1991). Similar findings have been reported by Ullrich et al. (1986) and Han and Froseth (1992). In some instances in our studies, short-awned waxy barleys appear to have an even further increase in TDF and β-glucans. This was first inferred by Fox (1981) who reported increased extract viscosity of short-awned compared to long-awned isotypes. Studies with hypercholesteremic chicks and rats have shown that the β-glucan component of barley is as effective as oats in lowering serum cholesterol (Fadel et al., 1987; Mori, 1990; Newman et al., 1989b). Three studies have confirmed these findings with barley in human subjects (Newman et al., 1989a; McIntosh et al., 1991).

The main objective of this study was to determine if increased levels of β-glucans, TDF and extract viscosity in waxy barley are consistent in more than one genotype grown in different environments. In addition, the effects of the waxy, hull-less and short-awned genes on other nutrient components were evaluated.

Materials and Methods

A series of barleys developed from Compana (CI 5438) and Betzes (CI 6398) regarded as parents with varying combinations of the non-waxy (*WW*), waxy (*ww*), covered (*NN*), hull-less (*nn*) and long (*LKLK*) and short-awned (*lklk*) genes were obtained from Dr. E. A. Hockett, USDA-ARS barley breeder at the MAES. These barleys were developed in a backcross barley breeding program by Dr. Hockett and Professor emeritus R. F. Eslick and are illustrated in Table 1.

Table 1. Isotype Series of Waxy and Non-waxy, Covered and Hull-less, Short-awned and Long-awned Barleys in Compana and Betzes Barleys

Genotype	Phenotype		
	Starch	Hull	Awn
WWNNLKLK[a]	Non-waxy	Covered	Long
wwNNLKLK[b]	Waxy	Covered	Long
WWnnLKLK[c]	Non-waxy	Hull-less	Long
wwnnLKLK[d]	Waxy	Hull-less	Long
WWnnlklk[e]	Non-waxy	Hull-less	Short
wwnnlklk[f]	Waxy	Hull-less	Short

[a] Derived parent barleys, Compana (CI 5438) and Betzes (CI 6398).
[b] Wapana and Wabet.
[c] Nupana and Nubet.
[d] Wanupana and Wanubet.
[e] Shonupana and Shonubet.
[f] Washonupana and Washonubet.

The covered types were long-awned and the hull-less types were expressed in both long and short-awned lines.

The barleys were grown in small plots in 1987, 1988 and 1989 at the MAES agronomy farm west of Bozeman, MT. Irrigation was provided as needed to prevent drought stress. The barleys were harvested with a small plot combine and seed cleaned of foreign material prior to chemical analyses. Analyses were made for protein, ether extract, ash (AOAC, 1984), starch (Åman and Hesselman, 1984), non-starch polysaccharide residues including TDF and SDF and Klason lignin (Theander and Westerlund, 1986) and total and soluble mixed-linked β-glucans (Åman and Graham, 1987). Cellulose was calculated as the difference between non-starch glucose residues and arabinoxylans as the sum of arabinose, xylose and uronic acid residues. Soluble sugars including fructose, glucose, sucrose, and maltose, were quantified by HPLC (Wade and Morris, 1982). Physical characteristics (kernel weight and test weight) were also measured in the MAES Cereal Quality Laboratory. Alkaline extract viscosity of the kernels was measured by the falling ball technique of Bendelow (1975) using a model No. B/H 72102 Haake viscometer.

Data were analyzed by the General Linear Models procedure (SAS, 1988) for analysis of variance (ANOVA). Main effects and interactions for year, cultivar background, hull and starch type were tested. The effects of awn type were tested in separate analyses, comparing the long-awned barleys with the short-awned barleys within the hull-less type. Differences between means were analyzed by Least-Squares Means. Pearson's correlations were determined for each measurement of chemical

composition and physical characteristics of barleys using CORR Procedure (SAS, 1988).

Results and Discussion

Analysis of variance mean square and significance for the effects in the model for hull, starch type, cultivar background (parent), year and the two- and three-way interactions on composition and physical characteristics of Compana and Betzes derived barleys are shown in Table 2. The analysis showed no significant effects for protein and phosphorus contents or percentage of thin kernel in the model, therefore these measurements will not be discussed. Means and the standard error of means for each measurement and the probability values for the effects of the hull-less gene, waxy gene, cultivar background, year and awn type are reported in Tables 3 through 9, respectively. Pearson's correlation coefficients are shown in Table 10 for each measurement.

Hull-less Gene Effects

The hull-less gene had significant effects on most of the measurements reported (Tables 2 and 3). The most obvious and important effects of the hull-less gene were the reduction ($P < .001$) of TDF and increase ($P < .001$) in starch. All components of TDF, with the exception of β-glucans, were significantly decreased in the hull-less barleys. This was to be expected since Klason lignin, arabinoxylans and cellulose are concentrated in the hull and β-glucans are found in principally in endosperm cell walls (Henry, 1988). The high correlations ($P < .001$) between TDF and Klason lignin, arabinoxylans and cellulose, confirmed these results (Table 10). The increased ($P < .003$) level of β-glucans in the hull-less types was partially responsible for the increase

Table 2. Analysis of Variance Mean Squares of Chemical Composition for Different Years, Parents, Hull and Starch Types of Barleys[a]

Source	df	Mean Square						
		Protein	EE[b]	Starch	Total sugar	Sucrose	Maltose	Hexoses
Model	23	110.14	18.04**	1853.05***	493.01***	12.78**	352.66***	2.30***
Hull[c]	1	276.12	14.22*	28520.68***	210.12*	14.22*	100.34*	0.50
Waxy[d]	1	177.34	186.88***	11275.01***	5425.34***	84.50***	3916.12***	2.72*
Parent[e]	1	351.12	1.38	316.68	78.12	3.55	105.12*	0.05
Year[f]	2	398.62	4.66	42.12	1781.51***	56.26***	1197.68***	17.05***
Hull×waxy	1	7.34	8.00	316.68	3.12	0.50	2.34	1.38
Hull×parent	1	8.68	29.38**	0.12	36.12	8.0	7.34	0.05
Hull×Year	2	48.79	20.22*	114.34	24.12	3.43	14.18	2.16*
Waxy×parent	1	0.68	0.05	23.34	45.12	1.38	23.34	0.50
Waxy×year	2	14.93	0.38	21.18	241.76**	4.29	215.29***	0.72
Parent×Year	2	155.04	11.72	303.43	10.04	0.01	10.79	0.05
Hull×waxy×parent	1	23.34	0.05	6.12	1.12	4.5	0.68	0.05
Hull×waxy×year	2	69.09	0.16	215.84	9.04	0.12	8.34	0.72
Hull×parent×year	2	90.09	22.72**	57.54	4.54	2.62	2.51	0.05
waxy×parent×year	2	6.34	5.38	0.26	5.79	0.26	4.51	0.16
Hull×waxy×parent×year	2	10.51	2.38	156.37	10.29	0.87	3.01	0.38
Experimental error	12	52.29	3.00	130.29	22.95	3.00	11.54	0.33
Total	35							
C.V. %		4.43	7.53	1.97	12.2	17.22	12.72	23.09

[a] Mean Squares of each source of variation and the significant effect is shown, *, **, ***, at 5%, 1%, 0.1% levels.
[b] EE = ether extract.
[c] Hull-less gene effect.
[d] Waxy gene effect.
[e] Compana vs Betzes barley series as cultivar background effects.
[f] Year effects of 1987, 1988 and 1989.

Table 2. (Continued 1)[a]

Source	df	Mean Square						
		TDF	SDF	ß-glucan	Sol-ß-g	K Lignin	Arabinoxylans	Cellulose
Model	23	1007.21***	213.78*	102.29***	46.44***	48.75***	207.50***	173.83***
Hull[b]	1	18400.01***	19.36	150.22**	9.41	953.38***	3173.38***	3528.00***
Waxy[c]	1	2652.34***	970.11**	1250.00***	467.77***	0.00	93.38	32.00
Parent[d]	1	268.34*	398.45*	440.05***	29.21*	6.72	4.5	10.88
Year[e]	2	384.05*	1289.51***	55.93*	178.28***	42.05***	574.01***	45.79
Hull×waxy	1	284.01*	75.14	0.88	5.84	0.22	29.38	128.00*
Hull×parent	1	7.34	86.00	2.72	1.62	0.05	16.05	26.88
Hull×Year	2	46.05	203.30	3.10	10.37	13.72*	0.68	9.12
Waxy×parent	1	48.34	43.32	40.50	4.41	0.22	12.50	8.00
Waxy×Year	2	17.05	45.36	2.54	3.10	4.50	1.84	11.29
Parent×Year	2	38.22	136.81	11.68	17.97	11.72*	1.29	4.18
Hull×waxy×parent	1	147.34	14.56	0.50	0.40	3.55	2.70	72.00
Hull×waxy×year	2	9.05	11.42	3.93	2.49	7.38	2.18	7.62
Hull×parent×year	2	6.2	69.63	4.51	0.37	1.05	7.51	6.51
Waxy×parent×year	2	193.05	21.61	2.62	0.85	2.72	58.62	38.79
Hull×waxy×parent×year	2	40.38	7.60	0.12	0.92	2.38	15.18	6.125
Experimental error	12	56.20	77.22	11.08	5.16	2.58	20.00	21.50
Total	35							
C.V. %		4.87	16.71	6.10	7.42	12.71	8.70	17.53

[a] Mean Squares of each source of variation and the significant effect is shown, *, **, ***, at 5%, 1%, 0.1% levels.
[b] Hull-less gene effect.
[c] Waxy gene effect.
[d] Compana vs Betzes barley series as cultivar background effects.
[e] Year effects of 1987, 1988 and 1989.

Table 2. (Continued 2)[a]

Source	df	Mean Square							
		Ash	Ca	P	Kernel WT	Test WT	Thin Kernel	Plump	Viscosity
Model	23	21.54***	.014*	0.07	94.29***	48.26*	644.75	1117.55**	6.00***
Hull[b]	1	440.05***	.094**	0.50	190.45***	361.80***	2907.03**	7381.12***	15.17***
Waxy[c]	1	0.00	.008	0.07	14.13	14.40	0.28	90.22	16.59***
Parent[d]	1	1.38	.005	0.14	1265.88***	1.86	4391.40**	9189.16***	2.30
Year[e]	2	1.16	.001	0.13	213.78***	170.73***	1230.50*	826.36	20.98***
Hull×waxy	1	5.55**	.020	0.05	3.08	0.02	0.33	118.06	4.64*
Hull×parent	1	2.72	.005	0.04	8.6	0.32	1148.00	1452.60*	1.87
Hull×year	2	0.38	.037**	0.01	6.85	56.97*	138.19	108.97	3.29*
Waxy×parent	1	3.55*	.000	0.05	2.17	0.04	36.83	12.00	0.62
Waxy×year	2	2.16	.001	0.05	19.26	8.90	57.96	24.67	3.93*
Parent×year	2	0.72	.023*	0.11	29.62	0.50	110.57	95.60	0.05
Hull×waxy×parent	1	2.00	.002	0.01	7.93	5.78	81.70	7.09	0.11
Hull×waxy×year	2	9.38***	.016	0.03	7.77	3.67	30.44	14.15	1.50
Hull×parent×year	2	5.05**	.020*	0.01	1.09	16.41	50.44	124.43	0.67
Waxy×parent×year	2	1.38	.003	0.02	5.22	10.84	92.35	87.46	0.14
Hull×waxy×parent×year	2	2.16	.001	0.01	6.30	1.71	9.40	11.14	0.01
Experimental error	12	0.58	.01	0.07	10.39	12.76	294.23	225.51	0.72
Total	35								
C.V. %		3.30	37.99	6.15	8.06	4.94	63.06	31.04	21.17

[a] Mean Squares of each source of variation and the significant effect is shown, *, **, *** at 5%, 1%, 0.1% levels.
[b] Hull-less gene effect.
[c] Waxy gene effect.
[d] Compana vs Betzes barley series as cultivar background effects.
[e] Year effects of 1987, 1988 and 1989.

Table 3. Effect of the Hull-less Gene on the Chemical Composition and Physical Characteristics of Barleys[a]

Item	Covered	Hull-less	P<
Protein, g/kg	159.0 ± 2.7	164.9 ± 1.8	NS
Ether extract, g/kg	22.1 ± 1.0	23.4 ± 0.7	.05
Starch, g/kg	537.1 ± 7.0	596.8 ± 4.1	.001
Free sugars, g/kg			
Total	35.8 ± 5.2	41.0 ± 3.8	.01
Hexoses[b]	2.3 ± 0.4	2.6 ± 0.3	NS
Sucrose	9.2 ± 0.8	10.5 ± 0.7	.05
Maltose	24.3 ± 4.3	27.9 ± 3.2	.02
Dietary Fiber, g/kg			
Total	185.8 ± 4.4	137.8 ± 2.3	.001
Soluble	51.5 ± 4.6	53.1 ± 2.3	NS
β-Glucan, g/kg	51.9 ± 2.2	56.3 ± 1.7	.003
soluble	29.9 ± 1.6	31.0 ± 1.2	NS
Klason lignin, g/kg	19.9 ± 0.9	9.0 ± 0.4	.001
Arabinoxylans, g/kg	64.6 ± 2.2	44.8 ± 1.5	.001
Cellulose, g/kg	40.5 ± 1.6	19.5 ± 0.8	.001
Minerals, g/kg			
Ash	28.1 ± 0.5	20.6 ± 0.2	.001
Calcium	.3 ± 0.0	.2 ± 0.0	.001
Phosphorus	4.1 ± 0.1	4.4 ± 0.1	NS
Kernel wt, mg	43.3 ± 2.5	38.4 ± 1.5	.002
Test wt, kg/hl	67.8 ± 0.8	74.5 ± 1.2	.001
Thin kernels, %	14.5 ± 3.6	33.6 ± 5.0	.01
Plump kernels, %	68.6 ± 3.8	38.3 ± 5.9	.009
Viscosity, cP[c]	3.1 ± 0.3	4.5 ± 0.5	.001

[a] Each mean and SEM represent duplicate analyses of 12-24 samples.
[b] Glucose and fructose.
[c] Centipoise units.

($P < .001$) in extract viscosity as indicated by the significant correlation of these two measurements ($r = .55$, $P < .001$, Table 10). Total free sugar was increased in hull-less barleys ($P < .01$) which was primarily due to an increase in maltose content($P < .02$) as the other components were principally unchanged. A high correlation ($r = .76$, $P < .001$) was observed between extract viscosity and total free sugar content. This was principally due to the high correlation between extract viscosity and maltose ($r = .78$, $P < .001$) as the other free sugar components were less related to extract viscosity (Table 10). It may be hypothesized that the cause of the increased viscosity may not be due the maltose but to some unidentified component that is associated with increased maltose, such as α-amylase or dextrins occurring from the hydrolysis of starch.

Most of the effects of the hull-less gene, such as higher starch, ether extract ($P < .05$), free sugar ($P < .01$) levels in the hull-less barleys, can be explained by the removal of the fibrous hull that has a dilution effect on nutrients as reported earlier by Bhatty et al. (1986a), McGuire and Hockett (1981). These data support the conclusions of Bhatty (1986b) that hull-less barleys have greater concentrations of digestible nutrients than covered barleys. With the exception of the higher levels of β-glucans ($P < .003$), also reported by Bhatty (1986b), hull-less barley has greater potential for feed and food than covered types.

Waxy Gene Effects

The most dramatic effect of the waxy gene observed in this study was the reduction of starch content and increase ($P < .001$) in free sugar, especially the maltose and sucrose levels (Table 4). Maltose content was increased by 240% in waxy barleys

compared to the non-waxy types. According to Macleod et al (1953), maltose is only produced in barley during germination and Henry (1988) stated that maltose in barley is produced by the action of amylases. Åman and Newman (1986) first suggested that waxy type barleys contain less starch and greater amounts of sucrose, but did not measure maltose. The significant increase in sucrose in waxy types may be a reflection of altered starch synthesis; however, the presence of relatively high levels of maltose is possibly due to a high level of α-amylase activity in the waxy types such as that reported by Goering and Eslick (1976) in a hull-less, short-awned, waxy Compana barley. Additionally, Harlan (1957) stated that waxy barleys deposit amylodextrin instead of starch during the final stage of carbohydrate deposition. This may be a partial explanation of the phenomenon as amylodextrin is possibly more susceptible to amylase activity than starch in the intact kernel.

Total and soluble dietary fiber were increased in waxy types ($P < .001$ and .004) compared to non-waxy barleys, respectively. The increased TDF and SDF were associated with increases ($P < .001$) in total and soluble β-glucans, as other fiber components were not different between the two starch types (Table 4). Means of TDF, SDF, total, soluble β-glucan and extract viscosity of each of six isogenic barley pairs in Compana and Betzes cultivar background with normal or waxy starch are shown in Table 5. TDF, total, soluble β-glucan and viscosity were consistently higher in waxy barleys than in normal barleys. These results confirm the earlier findings of Ullrich et al. (1986) with waxy and non-waxy isotypes of Compana, Betzes and Titan barleys and that of Han and Froseth (1992) with Steptoe barley and a waxy mutant of Steptoe. Han and Froseth

Table 4. Effect of the Waxy Gene on the Chemical Composition and Physical Characteristics of Barleys[a]

Item	Normal	Waxy	P<
Protein, g/kg	160.7 ± 2.3	165.1 ± 2.1	NS
Ether extract, g/kg	20.4 ± 0.5	25.6 ± 0.7	.001
Starch, g/kg	594.6 ± 6.5	559.2 ± 8.1	.001
Free sugars, g/kg			
Total	26.3 ± 2.0	52.2 ± 3.8	.001
Hexoses[b]	2.3 ± 0.3	2.7 ± 0.3	.02
Sucrose	8.4 ± 0.5	11.7 ± 0.7	.001
Maltose	15.7 ± 1.4	37.7 ± 3.2	.001
Dietary Fiber, g/kg			
Total	145.7 ± 5.2	161.9 ± 6.6	.001
Soluble	47.6 ± 2.8	57.6 ± 2.9	.004
β-Glucan, g/kg	48.5 ± 1.1	61.1 ± 1.5	.001
soluble	26.6 ± 0.9	34.6 ± 1.0	.001
Klason lignin, g/kg	12.7 ± 1.3	12.6 ± 1.4	NS
Arabinoxylans, g/kg	50.0 ± 2.7	52.8 ± 3.3	NS
Cellulose, g/kg	26.2 ± 2.1	26.8 ± 3.1	NS
Minerals, g/kg			
Ash	23.0 ± 1.0	23.3 ± 0.8	NS
Calcium	.2 ± 0.0	.2 ± 0.0	NS
Phosphorus	4.3 ± 0.1	4.3 ± 0.1	NS
Kernel wt, mg	40.6 ± 2.0	49.4 ± 1.9	NS
Test wt, kg/hl	73.0 ± 1.5	71.6 ± 1.3	NS
Thin kernels, %	27.3 ± 4.9	27.1 ± 6.0	NS
Plump kernels, %	46.1 ± 6.8	50.7 ± 6.7	NS
Viscosity, cP[c]	3.2 ± 0.2	4.9 ± 0.6	.001

[a] Each mean represents duplicate analyses of 18 samples.
[b] Glucose and fructose.
[c] Centipoise units.

Table 5. Effects of the Waxy Gene on the β-glucan, Dietary Fiber Content and Extract Viscosity of Six Isotypes of Compana and Betzes Barleys[a]

Genotype[b]	TDF		SDF		Total β-glucan		Soluble β-glucan		Viscosity (cP)[c]	
Normal/Waxy	NWx[d]	Wx[d]	NWx	Wx	NWx	Wx	NWx	Wx	NWx	Wx
Compana/Wapana	17.03	19.63	4.08	5.16	4.37	5.33	2.58	3.16	2.82	3.33
Nupana/Wanupana	13.23	13.47	4.83	4.82	4.70	5.63	2.60	3.21	3.15	4.46
Shonupana/Washonupana	13.10	13.97	4.69	6.09	4.66	6.90	2.65	3.64	2.97	5.28
Betzes/Wabet	17.70	19.93	4.78	6.56	4.80	6.27	2.72	3.49	2.70	3.55
Nubet/Wabet	12.83	15.47	5.03	6.69	5.13	6.90	2.64	3.61	3.21	5.85
Shonubet/Washonubet	13.50	14.67	5.07	5.19	5.43	6.63	2.80	3.63	4.17	6.71
Overall Mean	14.57	16.19**	4.76	5.76**	4.85	6.11**	2.67	3.46**	3.17	4.86**

[a] Percentage of dry matter. Each value is mean of 1987, 1988 and 1989.
[b] Wa- = waxy, Nu- = hull-less, and Sho- = short-awned.
[c] cP, centipoise.
[d] NWx = non-waxy, Wx = waxy.
** Significant ($P < .01$) difference between NWx and Wx isotypes of the overall means.

(1992) also found an increase in TDF in the waxy Steptoe mutant. Bhatty (1992) showed that five Canadian waxy barley cultivars had higher β-glucan than normal barleys but higher acid extract viscosity was not consistent with the waxy type. These data and the earlier reports (Xue et al., 1991) illustrate the consistent increase in β-glucans in waxy barley having different genetic backgrounds. Starch type was not associated with varying levels of Klason lignin, arabinoxylans or cellulose.

Extract viscosity was increased ($P < .001$) in waxy barleys compared to the non-waxy types. The increase in extract viscosity in waxy barleys was related to the increased levels of total and soluble β-glucans and maltose in waxy barleys, as discussed earlier in regard to the effects of the hull-less gene. Major differences were not apparent in the ash and calcium contents due to starch type. Physical characteristics (kernel weight, test weight, %plump) were not different between the two starch types of barleys.

The interactions between hull-less and waxy genes for TDF, cellulose, ash and viscosity were significant (Tables 2 and 6). The hulls of covered barley had an additive effect in the TDF of the waxy cultivars. This was mainly due to an increase in the insoluble fiber components, cellulose, arabinoxylans and lignin (Henry 1988). Therefore, significant increases ($P < .05$) in cellulose and a greater increase in TDF in covered, waxy barley than that in hull-less waxy type were observed. Extract viscosity was increased in the waxy barley but the effect was more pronounced in the hull-less ($P < .001$) than in the covered types ($P = .05$, Table 6). This is more evidence that the hull-less gene increases barley viscosity as stated earlier. The ash content of waxy barley was higher than non-waxy type within hull-less barley, but not in covered barleys (Table

Table 6. Interaction Effects of Hull-less and Waxy Genes on TDF, Cellulose, Ash and Extract Viscosity

	Covered			Hull-less		
Measurements	NWx[a]	Wx[a]	P<	NWx	Wx	P<
TDF[b], g/kg	170.7	197.8	.001	131.7	143.9	.001
Cellulose, g/kg	37.5	43.5	.045	20.5	18.5	NS
Ash, g/kg	28.5	27.7	NS	20.3	21.1	.02
Viscosity, cP[c]	2.8	3.4	.05	3.4	5.6	.001

[a] NWx = nonwaxy, Wx = waxy.
[b] TDF = total dietary fiber.
[c] Centipoise units.

6).

Cultivar Background (Parent) Effects

Betzes barleys contained more ether extract ($P < .05$) and less maltose ($P < .01$) than Compana barleys while the starch and total free sugars were not different (Table 7). Total dietary fiber and SDF were higher in Betzes ($P < .05$) compared to Compana barleys. The increased TDF and SDF in Betzes were associated with increases ($P < .001$) in total, and soluble β-glucans ($P < .04$) as other fiber components were not different between the two cultivars. This followed the pattern of the waxy gene effects. Both cultivars were similar in ash and calcium. Compana genotypes had heavier ($P < .001$) kernel weights with correspondingly greater ($P < .001$) percentages of plump kernels and fewer thin kernels. Test weights were similar between Compana and Betzes barleys irrespective of differences in kernel weights and percentage of plump kernels. Extract viscosity was not different between the two barleys even though the Betzes types contained a higher level of total and soluble β-glucans. This may be explained by the correspondingly lower level of maltose in Betzes barleys as well as smaller kernels. As β-glucans and maltose have major significant effects on extract viscosity as indicated by the correlation coefficients, the higher and lower levels of these two components in Compana and Betzes types apparently had offsetting effects on extract viscosity.

The hull-less gene had an additive (modifying) effect on Compana barleys in percentage of plump kernels and an additive effect on Betzes barleys in ether extract (data not shown), which were reflected in the interaction effects of hull $\times$ parent (Table 2).

Table 7. Effect of the Cultivar Background on the Chemical Composition and Physical Characteristics of Barleys[a]

Item	Compana	Betzes	P<
Protein, g/kg	166.1 ± 2.1	159.8 ± 2.2	NS
Ether extract, g/kg	22.4 ± 0.7	23.5 ± 0.9	.05
Starch, g/kg	580.1 ± 8.4	573.7 ± 8.5	NS
Free sugars, g/kg			
Total	41.2 ± 4.7	37.3 ± 3.9	NS
Hexoses[b]	2.6 ± 0.3	2.4 ± 0.3	NS
Sucrose	9.9 ± 0.8	10.2 ± 0.6	NS
Maltose	28.7 ± 3.9	24.7 ± 3.3	.01
Dietary Fiber, g/kg			
Total	150.7 ± 6.2	156.8 ± 6.2	.05
Soluble	49.6 ± 3.1	55.6 ± 2.9	.05
β-Glucan, g/kg	51.0 ± 1.6	58.6 ± 2.0	.001
soluble	29.7 ± 1.1	31.5 ± 1.5	.04
Klason lignin, g/kg	12.2 ± 1.3	13.1 ± 1.4	NS
Arabinoxylans, g/kg	52.0 ± 2.7	50.8 ± 3.0	NS
Cellulose, g/kg	26.8 ± 2.9	26.2 ± 2.4	NS
Minerals, g/kg			
Ash	23.4 ± 0.9	22.8 ± 0.9	NS
Calcium	.2 ± 0.0	.2 ± 0.0	NS
Phosphorus	4.4 ± 0.1	4.2 ± 0.1	NS
Kernel wt, mg	46.1 ± 1.2	33.9 ± 1.3	.001
Test wt, kg/hl	72.1 ± 1.4	72.5 ± 1.4	NS
Thin kernels, %	13.5 ± 2.5	40.9 ± 5.6	.003
Plump kernels, %	67.6 ± 4.0	29.2 ± 5.8	.001
Viscosity, cP[c]	3.6 ± 0.4	4.4 ± 0.5	NS

[a] Each mean represents duplicate analyses of 18 samples.
[b] Glucose and fructose.
[c] Centipoise units.

Year Effects

Uncontrolled environmental factors which vary from year to year such as rainfall, temperature, light and soil fertility, affect plant growth and development. Therefore, variation in chemical composition and physical characteristics are expected to be significant (Table 2 and 8). The most sensitive components affected by year were all of the free sugars (hexoses, sucrose, maltose; $P < .001$) and all dietary fiber components ($P < .02$) except cellulose. The total free sugar increase was mainly due to maltose content increase since total sugar was increased 195% while maltose increased 207% from samples taken in 1987 to those of 1989. Extract viscosity was increased in 1989 compared to 1987 barleys while the total β-glucan content was not significantly different and soluble B-glucans actually decreased. The variation of viscosity can be accounted for by the large increase in maltose content of the 1989 barleys. This observation confirms the results that were seen with the effects of the hull-less and waxy genes on maltose and viscosity. Kernel weight and test weight were different from year to year (Table 8).

Short-awned Gene Effects

Effects of awn type within the hull-less genotypes are shown in Table 9. The significant effects of the short-awned gene in these data were an increase ($P < .04$) in extract viscosity, confirming the earlier findings of Fox (1981), and reduced test weight ($P < .008$) and percentage of plump ($P < .03$) kernels and an increase in thin ($P < .03$) kernels. Fox (1981) concluded that the increased extract viscosity he observed in

Table 8. Effect of the Years on the Chemical Composition and Physical Characteristics of Barleys[a]

Item	1987	1988	1989	P<
Protein, g/kg	169.3±2.1	162.7±2.8	156.8±2.1	NS
Ether extract, g/kg	23.0±0.9	23.2±1.0	22.8±1.2	NS
Starch, g/kg	576.1±11.5	579.2±10.4	575.4±9.6	NS
Free sugars, g/kg				
Total	27.3±2.9 c	37.1±3.6 b	53.3±5.9 a	.001
Hexoses[b]	1.0±0.0 b	3.3±0.3 a	3.2±0.2 a	.001
Sucrose	7.3±0.5 b	11.8±0.8 a	11.1±0.7 a	.001
Maltose	18.9±2.5 b	22.0±2.7 b	39.2±5.2 a	.001
Dietary Fiber, g/kg				
Total	150.8±8.4 a	149.8±6.9 a	160.7±7.5 b	.02
Soluble	60.3±2.9 a	56.1±2.4 a	41.3±3.4 b	.001
β-Glucan, g/kg	52.3±2.4 b	57.1±2.3 a	55.0±2.5 ab	.009
soluble	34.0±1.3 a	26.3±1.3 c	31.5±1.6 b	.008
Klason lignin, g/kg	14.6±1.9 a	11.8±1.4 b	11.5±1.6 b	.001
Arabinoxylans, g/kg	45.8±3.0 b	48.7±3.1 b	59.7±3.1 a	.001
Cellulose, g/kg	27.1±3.5	24.3±3.0	28.1±3.2	NS
Minerals, g/kg				
Ash	23.1±1.0	23.5±1.2	22.8±1.1	NS
Calcium	.2±0.0	.2±0.0	.2±0.0	NS
Phosphorus	4.2±0.1	4.4±0.1	4.4±0.1	NS
Kernel wt, mg	35.3±2.0 b	41.2±2.6 a	43.6±1.8 a	.002
Test wt, kg/hl	67.4±0.9 b	76.4±2.0 a	73.1±1.0 a	.004
Thin kernels, %	39.7±6.4 a	20.5±5.6 b	21.4±6.7 b	.04
Plump kernels, %	40.6±7.5	58.9±9.0	45.7±7.8	NS
Viscosity, cP[c]	2.9±0.2 b	3.3±0.3 b	5.8±0.7 a	.001

[a] Each mean and SEM represent duplicate analyses of 12 samples. Means in a row followed by the different letters are significant different at the levels of indication (P).
[b] Glucose and fructose.
[c] Centipoise units.

Table 9. Effect of the Short-awned Gene on the Chemical Composition and Physical Characteristics of Barleys[a]

Item	Long	Short	P<
Protein, g/kg	167.7 ± 2.6	162.5 ± 2.5	NS
Ether extract, g/kg	22.3 ± 1.1	23.5 ± 1.0	NS
Starch, g/kg	597.7 ± 6.3	595.9 ± 5.5	NS
Free sugars, g/kg			
Total	40.8 ± 5.5	41.1 ± 5.5	NS
Hexoses[b]	2.7 ± 0.4	2.5 ± 0.3	NS
Sucrose	10.3 ± 1.0	10.8 ± 0.9	NS
Maltose	28.0 ± 4.7	27.8 ± 4.6	NS
Dietary Fiber, g/kg			
Total	137.5 ± 3.9	138.1 ± 2.6	NS
Soluble	53.4 ± 3.2	52.7 ± 3.5	NS
β-Glucan, g/kg	55.9 ± 2.7	56.6 ± 2.3	NS
soluble	30.1 ± 1.5	31.8 ± 1.8	NS
Klason lignin, g/kg	9.3 ± 0.7	8.7 ± 0.5	NS
Arabinoxylans, g/kg	43.3 ± 2.3	46.2 ± 1.9	NS
Cellulose, g/kg	20.8 ± 1.1	18.3 ± 1.2	NS
Minerals, g/kg			
Ash	20.9 ± 0.3	20.4 ± 0.3	NS
Calcium	.2 ± 0.0	.1 ± 0.0	NS
Phosphorus	4.5 ± 0.1	4.2 ± 0.1	.003
Kernel wt, mg	39.3 ± 2.1	37.4 ± 2.3	NS
Test wt, kg/hl	76.6 ± 1.4	72.5 ± 1.8	.008
Thin kernels, %	25.7 ± 4.9	41.4 ± 8.3	.009
Plump kernels, %	44.6 ± 7.0	31.9 ± 9.4	.03
Viscosity, cP[c]	4.2 ± 0.5	4.8 ± 0.8	.04

[a] Each mean represents duplicate analyses of 18 samples.
[b] Glucose and fructose.
[c] Centipoise units.

the kernels from short-awned barleys was associated with the smaller kernel size which was also observed in this study as noted above.

Correlation Coefficients of the Components

As expected, starch had a highly negative correlation with TDF ($r = -.91$, $P < .001$) and insoluble fiber ($r = -.70$, $P < .01$). Total free sugar was correlated to its components (hexoses, $r = .59$, $P < .001$; sucrose, $r = .76$ $P < .001$; maltose, $r = .98$ $P < .001$). Some unexpected r values were also observed (Table VIII). Ether extract was correlated with total free sugar ($r = .50$, $P < .01$), maltose ($r = .51$, $P < .01$), total β-glucan ($r = .67$, $P < .001$) , soluble β-glucan($r = .55$, $P < .001$) and viscosity ($r = .46$, $P < .01$). The r values between total and soluble β-glucan (.55 and .47, $P < .01$) and extract viscosity were lower than that reported by Aastrup (1979) and Xue et al (1991). Extract viscosity is due to several factors other than β-glucans. Free sugars, especially maltose was highly correlated with extract viscosity in this study. Plotting the means of β-glucan and extract viscosity from the first years data did show a higher r value ($r = .86$ for total β-glucan and $r = .82$ for soluble β-glucan, $P < .001$) (Xue et al., 1991) but with the addition of two more years data, the correlation coefficients were much lower, due to other mediating factors, such as maltose or unidentified compounds that are related to maltose.

Table 10. Correlation Coefficients (r) of Barley Chemical Composition[a]

	Protein	EE	Starch	TOTSUG	Hexoses	SUCR	MALT	TDF	SDF	T-β-G
EE	.186									
Starch	.046	-.238								
TOTSUG	-.025	.500**	-.281							
Hexoses	-.242	.047	-.033	.593***						
SUCR	-.150	.397*	-.141	.760***	.773***					
MALT	.018	.507**	-.302	.984***	.469**	.636***				
TDF	-.315	.063	-.909***	.204	.044	.032	.231			
SDF	.380*	.283	-.259	-.273	-.505**	-.138	-.260	.074		
T-β-G	.051	.673***	-.249	.618***	.381*	.696***	.561***	.091	.184	
S-β-G	.253	.552***	-.361*	.480**	-.248	.157	.556***	.209	.319	.561***

[a] Pearson's correlation coefficients. EE=ether extract, TOTSUG=total sugar, Hexoses=glucose+fructose, SUCR=sucrose, MALT=maltose, TDF=total dietary fiber, SDF=soluble dietary fiber, T-β-G=total β-glucan, S-β-G=soluble β-glucan.
*, **, ***, Significant at 5%, 1%, 0.1% levels.

Table 10. (Continued 1)[a]

	Protein	EE	Starch	TOTSUG	Hexoses	SUCR	MALT	TDF	SDF	T-β-G	S-β-G
KL	-.150	-.150	-.784***	-.248	-.256	-.320	-.209	.812***	.229	-.233	.028
ARAB	-.515**	-.104	-.700***	.280	.200	.088	.298	.885***	-.232	-.099	.019
CELL	-.248	-.179	-.772***	-.075	-.118	-.282	-.022	.911***	.027	-.276	-.024
ASH	-.194	-.138	-.755***	-.116	-.133	-.165	-.095	.794***	.153	-.243	-.113
CA	-.286	-.062	-.372*	.027	-.032	-.051	.045	.439**	-.018	-.178	-.043
P	.313	.096	.297	.397*	.490**	.446**	.341*	-.351*	-.279	.120	-.177
KWT	-.051	-.201	-.129	.203	.383*	.160	.176	.184	-.342*	-.354*	-.333*
TW	-.072	-.017	.515**	.195	.630***	.472	.087	-.519**	-.358*	.233	-.422*
THIN	.160	.209	.249	-.213	-.398*	-.285	-.161	-.290	.122	.269	.329*
PLUMP	.019	-.189	-.381*	.093	.226	.157	.057	.364*	.089	-.279	-.232
VI	-.116	.464**	-.002	.755***	.351*	.461*	.775***	-.021	-.396*	.547***	.474**

[a] Pearson's correlation coefficients. EE=ether extract, TOTSUG=total sugar, Hexoses=glucose+fructose, SUCR=sucrose, MALT=maltose, TDF=total dietary fiber, SDF=soluble dietary fiber, T-β-G=total β-glucan, S-β-G=soluble β-glucan; KL=Klason lignin, ARAB=arabinoxylans, CELL=cellulose, KWT=kernel weight, TW=test weight, VI=viscosity.
*, **, ***, Significant at 5%, 1%, 0.1% levels.

Table 10. (Continued 2)[a]

	KL	ARAB	CELL	ASH	CA	P	KWT	TW	THIN	PLUMP
ARAB	.612***									
CELL	.865***	.816***								
ASH	.841***	.710***	.822***							
CA	.439**	.445**	.477**	.387*						
P	-.458**	-.251	-.356*	-.350*	-.216					
KWT	.077	.433**	.251	.319	.262	.216				
TW	-.551***	-.407*	-.560***	-.465**	-.228	.535***	.105			
THIN	-.235	-.454**	-.318	-.442**	-.347*	-.241	-.844***	-.161		
PLUMP	.374	.434**	.402*	.561***	.310	.109	.804***	-.059	-.909***	
VI	-.398	.098	-.268	-.345*	.040	.110	-.091	.150	.237	-.344*

[a] Pearson's correlation coefficients. KL=Klason lignin, ARAB=arabinoxylans, CELL=cellulose, KWT=kernel weight, TW=test weight, VI=viscosity.

*, **, ***, Significant at 5%, 1%, 0.1% levels.

Conclusions

The waxy gene in barley has a major effect on carbohydrate metabolism in the developing kernel. It appears that a portion of the starch is shifted to free sugars, especially maltose and sucrose, and also to an increase in β-glucans. The hull-less gene reduces TDF and increases other nutrients due primarily to the removal of the dilution effect of the fibrous hull. The higher concentration of β-glucans and maltose in hull-less, waxy barleys increases extract viscosity. Major effect of the short-awned gene was to increase extract viscosity possibly due to the decrease in kernel size. The highly significant positive correlation between extract viscosity and maltose content of the barley kernel has not been reported previously. This phenomena is probably an artifact in that the cause of the increased viscosity may not be due to the maltose but to some unidentified component that is associated with increased maltose, such as α-amylase or larger molecular weight oligosaccharides occurring from starch hydrolysis. The increased level of β-glucans in waxy barley, makes this cereal grain type undesirable for poultry feed but extremely desirable and useful as a source of soluble dietary fiber in human diets.

CHAPTER 4

GLYCEMIC RESPONSES AND HYPOCHOLESTEROLEMIC EFFECTS OF GLACIER BARLEYS FED TO BROILER CHICKS

Introduction

Starch and dietary fiber are the major constituents of barley. Starch is a ready source of energy to animals and man and can also be used as a substrate for many technological processes. Structurally, starch is composed of a mixture of amylose and amylopectin, organized into semicrystalline granules (Dreher et al., 1984). Differences in structure between the two major constituents of starch affect their biological properties. Some of the major differences were described by Marshall (1972). Epidemiological studies have shown a relationship between inadequate consumption of dietary fiber and cancer of colon, hypercholesterolemia, atherosclerosis and complications of diabetes, diverticulosis, constipation, hypertension, gallstones and obesity (Southgate, 1982). Recently, increased emphasis has been placed on the importance of SDF and its role in nutrition.

Wide variations in the digestibility of starch granule both *in vitro* and *in vivo*, depending on starch source, structure and food processing and storage conditions, have been reported (Dreher et al., 1984). The source and form of carbohydrate given as a

meal in tolerance tests were reported to affect the resultant blood glucose response curves differently. Increase in blood glucose levels after consumption of glucose or potatoes was similar whereas consumption of fructose, rice, corn, wheat bread or legumes produced a much flatter response curve (Behall et al., 1988).

Amylomaize starch digestibility has been extensively studied *in vitro* and *in vivo* with chicks, rats, mice and humans. Amylomaize starch is less susceptible to amylolysis than normal maize (Dreher et al., 1984). High-amylose maize starch has a positive lowering effect on blood glucose and insulin levels in humans (Behall et al., 1988; Behall and Scholfield, 1989; Amelsvoort and Weststrate, 1992) as well as high-amylose rice (Goddard et al., 1984; Panlasigui et al., 1992). Behall and Scholfield (1989) concluded that long-term intake of high-amylose starch may benefit individuals who show elevated glucose and insulin levels and apparent insulin resistance, as in early adult-onset diabetes. Behall and Scholfield (1989) also reported a lowering of blood triglycerides and cholesterol in subjects consuming high-amylose maize starch. The flattened insulin response observed with amylose may also be beneficial for hyperlipidemic subjects because high insulin levels have been implicated in the development of thickened arterial walls and lesions.

High-amylose barley starch was also found to be less susceptible to α-amylase than normal barley starch (Pomeranz et al., 1972). Calvert et al. (1976) reported that rats consumed less of a purified diet prepared with high-amylose Glacier (HAG) starch than a similar diet containing Glacier starch and as a consequence gained at a slower rate. However, available data on the nutritional properties of amylose starch are by no

means clear cut. In another study, Calvert et al. (1981) found that barley starch type did not significantly affect gain or feed consumption of swine. Rubin et al. (1974) demonstrated HAG to be nutritionally superior to five other barleys including Glacier as measured by growth of weanling rats. In a study with rice, a slightly higher amylose content (22 vs 15%) resulted in an increased rate of *in vitro* α-amylolysis and an increase in postprandial glucose in healthy subjects (Srinivasa Rao, 1971). Björck et al. (1990) reported that uncooked barley flour (waxy, normal and high-amylose) showed much higher availability to α-amylase than a wheat flour reference on an equivalent starch basis. Data from our laboratory (Chapter 5) showed the raw *in vitro* digestibilities of normal Glacier and HAG barley starch were similar to normal corn starch but that of hull-less high-amylose Glacier (HHAG) was lower than corn starch.

Dietary fiber is known to flatten glucose curves in humans (Jenkins and Jenkins, 1985). β-glucan (a fraction of the SDF) was reported to be a factor in lowering blood glucose and insulin in rats (Vachon et al., 1988) and lowering blood cholesterol in hypercholesterolemic individuals (Newman et al., 1989b). Certain barley cultivars have been shown to lower serum cholesterol in chicks, rats, and humans. β-glucans and oil in barley grain are believed to be two components responsible for the effect. Dietary fiber or β-glucans in barley can be concentrated by milling processing (Newman and Newman, 1991).

Glacier barleys are known to have widely varying ratios of amylose to amylopectin and should be tested for their glycemic response and the lipid metabolic effect in order to enhance the nutritional value of barley. The objective of this study was

to investigate glucose and cholesterol responses of broiler chicks fed two uncooked barley milling fractions from three different Glacier barleys.

Materials and Methods

Barley, Milling Fraction and Chick Diets

Three barley cultivars, normal Glacier (Glacier), covered high-amylose Glacier (HAG) and hull-less high-amylose Glacier (HHAG) barleys were grown and harvested in Arizona in 1990. Chemical composition of each barley cultivar is shown in Table 11.

Table 11. Chemical Analysis of Barley Grains[a]

Item	Glacier[b]	HAG[c]	HHAG[d]
Ether extract	2.28	3.01	2.92
Crude protein	11.24	11.09	12.89
Ash	2.71	2.69	2.27
Starch	64.2	56.99	63.78
Amylose[e]	29.4	40.3	41.5
Total Dietary Fiber	17.70	20.15	15.16
Soluble	3.08	3.95	4.74
Insoluble	14.62	16.20	10.42
Total β-glucans	5.19	7.43	7.45
Soluble	1.07	0.93	1.24
Insoluble	4.12	6.50	6.21

[a] % of dry matter.
[b] Glacier barley flour.
[c] HAG = high-amylose Glacier barley flour.
[d] HHAG = hull-less high-amylose Glacier barley flour.
[e] % of starch.

The barleys were milled through an 8-roller MIAG multomat dry mill (Figure 11) at the Western Wheat Quality Laboratory (Washington State University, Pullman WA). Two fractions, uncooked flour (2B-4M, 2nd break flour to 4th middling) and red dog (RD) from each cultivar were used in this study. Corn was ground through a 1.59 mm hammer mill screen. The chemical analysis of barley flour and RD fractions for each of the cultivars and corn meal are reported in Table 12 and 13. Three barley flour fractions plus a corn control diet (corn-1) were fed in *Trial I* and three barley RD

Table 12. Chemical Analysis of Barley Flour Fraction and Corn Meal for *Trial I* Diets[a]

Item	Glacier[b]	HAG[c]	HHAG[d]	Corn Meal
Ether extract	1.9	2.8	2.5	4.2
Crude protein	10.08	11.56	12.82	9.9
Ash	1.3	1.6	1.5	1.4
Starch	76.8	73.7	72.7	68.0
Amylose[e]	29.4	40.2	44.5	27.3
Total Dietary Fiber	4.37	5.91	5.22	11.53
Soluble	1.45	2.10	1.62	0.50
Insoluble	2.92	3.80	3.60	11.03
Total β-glucans	2.73	2.41	2.39	0.13
Soluble	0.66	0.51	0.39	ND[f]
Insoluble	2.07	1.90	1.80	ND

[a] % of dry matter.
[b] Glacier barley flour.
[c] HAG = high-amylose Glacier barley flour.
[d] HHAG = hull-less high-amylose Glacier barley flour.
[e] % of starch.
[f] ND = not determined.

Table 13. Chemical Analysis of Barley Red Dog Fraction and Corn Meal for *Trial II* Diets[a]

Item	Glacier RD[b]	HAG RD[c]	HHAG[d]	Corn Meal
Ether extract	2.8	2.8	3.1	4.2
Crude protein	12.55	11.36	12.76	9.9
Ash	2.6	2.2	2.3	1.4
Starch	58.7	60.0	56.3	68.0
Amylose[e]	29.4	40.2	44.5	27.3
Total Dietary Fiber	19.97	22.20	19.71	11.53
Soluble	6.37	9.85	9.06	0.50
Insoluble	13.60	12.35	10.65	11.03
Total β-glucans	8.93	10.61	10.73	0.13
Soluble	1.37	1.27	1.88	ND[f]
Insoluble	7.56	9.34	8.85	ND

[a] % of dry matter.
[b] Glacier barley red dog fraction.
[c] HAG RD = high-amylose Glacier barley red dog fraction.
[d] HHAG RD = hull-less high-amylose Glacier barley red dog fraction.
[e] % of starch.
[f] ND = not determined.

fractions plus another corn control diet (corn-2) were fed in *Trial II*. Therefore, the study was divided into two feeding trials according to treatment diets that were made of either barley flour or RD as the major components. All diets were formulated to be isonitrogenous using high-protein soybean isolate, containing 23% crude protein and 5% fat in accordance to Nutrient Requirements of Poultry (NRC, 1984). The formulation and chemical analyses of the diets for *Trial I* and *Trial II* are shown in Table 14 and 15. The *Trial I* flour diets were considered as low fiber diets containing 5 - 6% of TDF and

Table 14. Composition and Chemical Analysis of Chick Diets for *Trial I*[a]

Ingredient	Glacier[b]	HAG[c]	HHAG[d]	Corn-1
Corn meal	0	0	0	50.00
Barley Flour	71.00	74.00	75.00	0
Soybean isolate[e]	18.33	16.71	15.48	20.88
Supplement[f]	6.38	6.38	6.38	6.38
Soybean oil	2.85	2.13	2.33	2.10
DL-Methionine	0.27	0.30	0.32	0.30
Corn starch	0.70	0	0	19.84
Cholesterol	0.50	0.50	0.50	0.50
Chemical Analysis				
Ether extract	5.01	6.28	5.12	4.09
Crude protein	19.71	24.57	24.52	24.63
Ash	5.7	6.2	6.0	5.9
Starch	50.3	51.0	49.4	44.8
Total Dietary Fiber	5.33	5.69	5.19	6.22
Soluble	1.32	1.14	1.24	0.48
Insoluble	4.02	4.53	3.93	5.75
Total β-glucans	2.64	2.60	2.33	0.33
Soluble	1.14	0.90	0.83	ND
Insoluble	1.5	1.7	1.5	ND

[a] % of dry matter. ND = not determined.
[b] Glacier barley flour diet.
[c] HAG = high-amylose Glacier barley flour diet.
[d] HHAG = hull-less high amylose Glacier barley flour diet.
[e] Composition of soybean protein isolate (%) produced by ICN Biochemicals: protein, 92; moisture, 6.0; ash, 4.0; fat, 0.8; fiber, 0.25; Ca, 0.15; P, 0.8; Na, 1.3; K, 0.05.
[f] Supplement contents (%): $Ca_3(PO_4)_2$, 2.80: $CaCo_3$, 1.50; vitamin diet fortification (ICN Biochemicals, Cleveland OH), 1.00; salt, 0.50; trace mineral mixture (ConAgra-Westfeeds, Billings, MT), 0.15; choline chloride, 0.20; DL-methionine, 0.125; antibiotic, 0.10; biotin, 0.01.

Table 15. Composition and Chemical Analysis of Chick Diets for *Trial II*[a]

Ingredient	Glacier-RD[b]	HAG-RD[c]	HHAG-RD[d]	Corn-2
Corn meal	0	0	0	64.00
Barley RD	74.00	73.00	75.00	0
Soybean isolate[e]	15.33	17.01	15.53	19.29
Supplement[f]	6.38	6.38	6.38	6.38
Soybean oil	2.13	2.15	1.89	1.51
DL-Methionine	0.31	0.29	0.30	0.28
Corn starch	0.82	0.67	0.42	0
Cellulose	0	0	0	8.04
Cholesterol	0.50	0.50	0.50	0.50
Chemical Analysis				
Ether extract	4.84	5.31	4.87	4.57
Crude protein	23.82	24.03	23.86	25.05
Ash	6.7	6.7	6.3	6.1
Starch	37.1	36.1	33.4	37.2
Total Dietary Fiber	14.48	16.02	14.83	15.17
Soluble	5.11	6.40	6.24	0.42
Insoluble	9.57	9.62	8.58	14.74
Total β-glucans	6.04	6.43	5.36	0.50
Soluble	1.64	0.43	0.86	ND
Insoluble	4.4	6.0	4.5	ND

[a] % of dry matter. ND = not determined.
[b] Glacier-RD = Glacier barley red dog fraction diet.
[c] HAG-RD = high-amylose Glacier barley red dog fraction diet.
[d] HHAG-RD = hull-less high-amylose Glacier barley red dog fraction diet.
[e] Composition of soybean protein isolate (%) produced by ICN Biochemicals: protein, 92; moisture, 6.0; ash, 4.0; fat, 0.8; fiber, 0.25; Ca, 0.15; P, 0.8; Na, 1.3; K, 0.05.
[f] Supplement contents (%): $Ca_3(PO_4)_2$, 2.80: $CaCo_3$, 1.50; vitamin diet fortification (ICN Biochemicals, Cleveland OH), 1.00; salt, 0.50; trace mineral mixture (ConAgra-Westfeeds, Billings, MT), 0.15; choline chloride, 0.20; DL-methionine, 0.125; antibiotic, 0.10; biotin, 0.01.

45 -50% of starch while the *Trial II* RD diets were regarded as high fiber diets containing 14 -16% of TDF and 33 - 37% of starch on dry matter basis.

Feeding Trials

One hundred ninety-two one-day old male cockerel broiler chicks were obtained from Fors Farms Inc., Puyallup, WA and fed a standard corn-soybean based diet for 3 d prior to initiating the experiment. This diet was then supplemented with 0.5 % cholesterol for 7 d to elevate the plasma cholesterol level. The chicks were randomly divided into 8 diet treatments at that time. Birds for each diet were further randomly assigned into 3 battery cages with 8 birds per cage. Chicks had light 24 h a day with room temperature at 26.7^{0}C and brooder (Petersime Incubator Co. Gettysburg, Ohio) temperature at 33.3^0 C, respectively. The protocol of the experiment was approved by Montana State University's Animal Care Committee.

The chicks had free access to water throughout the entire experiment and were fed experimental diets ad libitum except for d 16 and d 17. Glucose tolerance testing was performed by allowing the chicks to eat diets for 30 min and taking blood (1 ml) at 30, 60, 120, 150 min after feeding at d 16 for *Trial I* and d 17 for *Trial II*. The 0 min blood samples were also removed from fasted chicks before feeding at d 16 and d 17. Four chicks were used as duplications for each time interval within each diet treatment. Blood for lipid measurements was drawn at d 24 with 22 to 24 chicks for each diet treatment. All blood samples for both glucose and lipid studies were taken from the branchial veins after a 12 h overnight fast. Feed intake in each cage and individual chick weights were measured and average daily gain (ADG) was calculated on a cage basis.

Chemical Analysis

Dry matter, ash, crude protein and crude fat (AOAC, 1984), starch (Åman and Hesselman, 1984), SDF, IDF (Prosky et al., 1988) and soluble and insoluble β-glucans for barley fractions (McLeary and Glennie-Holms, 1985) and for diet (Åman and Graham, 1987) were analyzed. Amylose content of starch was measured by gel filtration chromatography (Torneport et al., 1990). The chemical analyses were carried out in duplicates and are reported on a dry matter basis. Plasma was separated from the whole blood with EDTA as an anticoagulating factor in vacuum tubes by centrifugation at 2400 rpm for 20 min. Blood plasma glucose, total, high density lipoprotein (HDL) cholesterol and triglyceride concentration were determined on a Kodak DT 60 Blood Analyzer. Plasma low density lipoprotein (LDL) cholesterol concentration was calculated (Friedwald et al., 1972).

Statistical Analysis

Data were analyzed using SAS General Linear Models Procedure and differences between means were analyzed by Least-Squares Means. Correlation coefficients (r) were determined by Pearson's CORR Procedure (SAS, 1988).

Results and Discussion

Chemical Analysis of Barley Grain and Milling Fractions

The most important characteristic of high-amylose barley is the high-amylose starch content. High-amylose Glacier and HHAG had 40% amylose starch while normal

Glacier contained 29% (Table 11), which is similar to the results of Morrison et al. (1986). The β-glucan content in HAG and HHAG was also higher (7.4%) compared to that of normal Glacier barley (5.2%). From this point of view, the high-amylose gene may have a similar effect to the waxy gene in increasing β-glucan content (Chapter 3). The hull-less gene in HHAG barley reduced the total dietary fiber content (15.2%) and increased the starch content (63.8%) compared with covered HAG barley (20.2%, 57.0%), respectively. Similar results were reported in hull-less Compana and Betzes barleys in Chapter 3.

The milling process separated the grain into different milling fractions according to particle size which depend on the botanical source and chemical composition of kernel. Therefore, uneven distribution of chemical components was found in different fractions. TDF, SDF and total β-glucans were higher in RD and starch content was higher in flour (Tables 12 and 13). Mori (1990) found that the RD fractions of Azhul (waxy, hull-less) and Azhul sister line (non-waxy) barley cultivars were the highest in TDF, SDF and β-glucan content among the milling fractions. The RD containing higher levels of SDF and β-glucan indicated that it consists of more endosperm cell walls than other milling fractions as barley β-glucan concentration is highest in endosperm cell walls (Fincher, 1975; Ballance and Manners, 1978; Åman and Graham, 1987). The β-glucan content of high-amylose RD fractions was higher (10.6%) than that of Glacier RD (8.9%). However, no difference in β-glucan content was observed among the three Glacier barley flours. These data indicate that high-amylose barley contains not only a higher level of amylose starch, but also a higher level of β-glucan.

Glucose Responses of Chicks

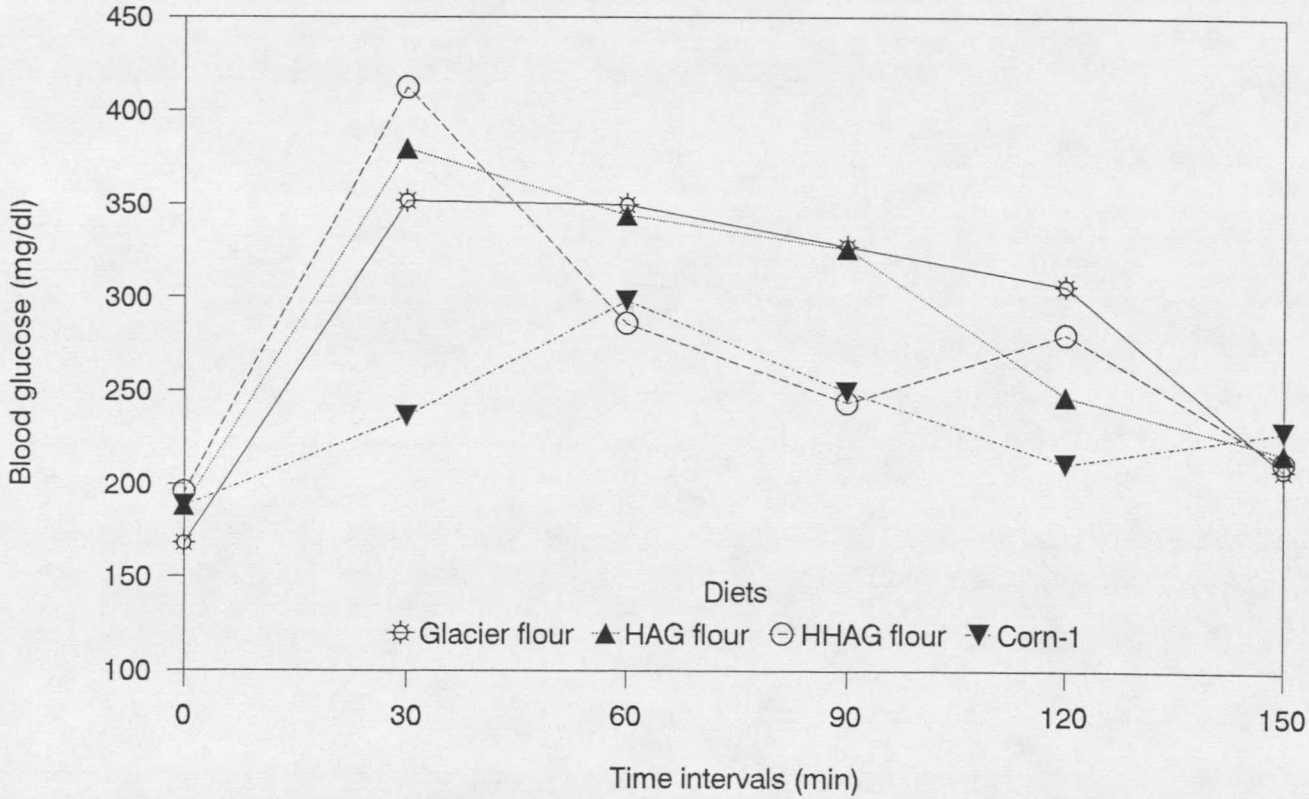
Postprandial blood plasma glucose responses in chicks after consumption of treatment diets over a period of 150 min are shown in Tables 16, 17 and Figs 1 and 2. No significant differences were found in fasting (0 min) blood glucose levels or following feed intake or blood glucose at 150 min among the 4 treatment diets within each flour group (*Trial I*) and RD fraction group (*Trial II*). Means of blood glucose levels over all time intervals were significantly higher ($P < .05$) for the chicks fed the uncooked barley

Table 16. Chick Glucose Response (mg/dl) and Feed Intake (g) of *Trial I* (Barley Flour or Corn Control-1 Diets)[a]

Time	Glacier[b]	HAG[c]	HHAG[d]	Corn-1
0 Min.	168.0±6.0	187.8±6.4	195.8±9.0	188.3±4.3
30 Min.	351.8±37.4 b	379.5±13.6 b	412.3±22.8 a	236.3±16.0 c
60 Min	349.8±33.7	344.0±44.0	285.8±21.1	298.0±36.9
90 Min	327.3±43.1 a	326.0±35.8 a	243.5±11.0 b	249.5±23.3 ab
120 Min	306.0±58.9 a	247.0±28.2 b	280.0±40.8 ab	211.0±13.9 b
150 Min	208.3±20.4	217.3±14.5	212.5±16.0	228.8±13.7
Mean[e]	285.2±20.0 a	283.6±17.6 a	271.6±10.3 a	235.3±4.0 b
Feed Intake[f]	4.1±0.4	4.3±0.5	3.8±0.7	4.1±1.2

[a] Numbers in rows are Mean±SEM of 4 replications, following unlike letters significantly differing at $P < .05$.
[b] Glacier barley flour diet.
[c] HAG = high-amylose Glacier barley flour diet.
[d] HHAG = hull-less high-amylose Glacier barley flour diet.
[e] Mean of glucose levels of all time internals.
[f] Feed intake of per chick allowing to eat for 30 min.

Figure 1. Glucose Responses of Chicks Fed Barley Flour or Corn-1 Diets



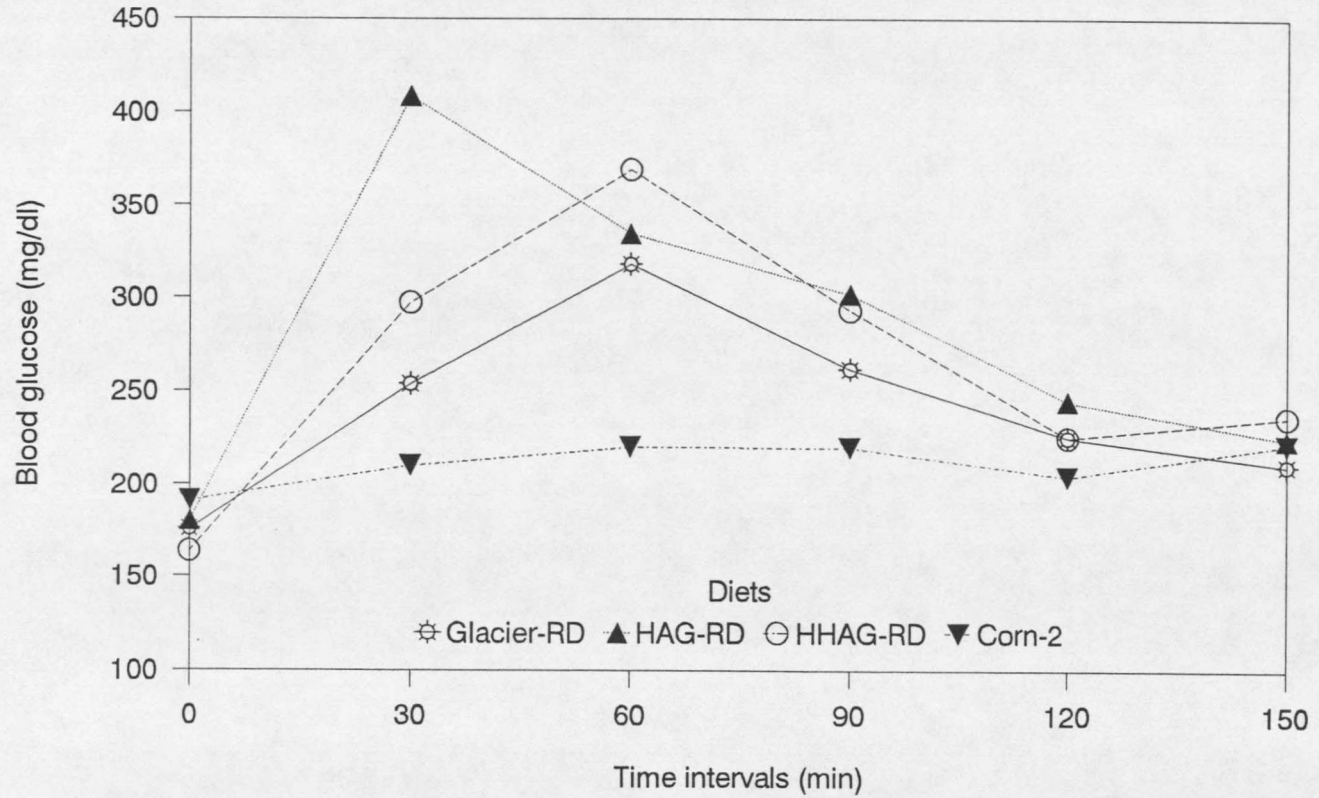
flour and RD fraction diets compared with corn diets but there were no differences among barley flour treatments. All barley flour diets produced significantly higher ($P < .01$) blood glucose peaks than the corn control while HHAG flour diet showed the highest glucose peak compared to Glacier and HAG flour diets at 30 min (Table 16). Differences were not significant in glucose levels among 4 flour treatments at 60 min. However, the glucose levels were higher ($P < .05$) for chicks fed Glacier flour compared with that of HHAG flour at 90 min and HAG flour at 120 min.

Table 17. Chick Glucose Response (mg/dl) and Feed Intake (g) of *Trial II* (Barley Red Dog Fraction or Corn Control-2 Diets)[a]

Time	Glacier-RD[b]	HAG-RD[c]	HHAG-RD[d]	Corn-2
0 Min.	175.5±4.4	180.0±7.5	163.5±9.1	191.0±4.3
30 Min.	253.8±11.0 bc	408.3±22.0 a	297.3±28.8 b	209.8±4.8 c
60 Min	318.0±30.5 a	334.5±34.0 a	369.0±19.0 a	219.8±10.3 b
90 Min	261.8±28.9 ab	301.8±34.8 a	293.3±33.5 a	219.5±8.3 b
120 Min	225.0±4.7	244.5±14.1	225.0±17.0	204.0±11.9
150 Min	210.0±8.2	224.0±25.1	236.0±12.9	221.5±9.2
Mean	240.7±11.4 b	282.2±18.2 a	264.0±16.2 a	210.9±3.84 c
Feed Intake[e]	3.1±0.3	3.1±0.1	2.5±0.2	3.3±0.2

[a] Numbers in rows are Mean±SEM of 4 replications, following unlike letters significantly differing at $P < .05$.
[b] Glacier barley red dog fraction diet.
[c] HAG = high-amylose Glacier barley red dog fraction diet.
[d] HHAG = hull-less high-amylose Glacier barley red dog fraction diet.
[e] Mean of glucose levels of all time internals.
[f] Feed intake of per chick allowed to eat for 30 min.

Figure 2. Glucose Responses of Chicks Fed Barley Red Dog Fraction or Corn-2 Diets



All barley RD fraction diets produced significantly higher ($P < .01$) blood glucose peaks than the corn control at 30, 60 and 90 min while the HAG RD diet showed the highest glucose peak compared with Glacier and HHAG flour diets at 30 min (Table 17). There were no significant differences in blood glucose levels within 3 RD treatment diets after 60 min. The HAG and HHAG produced significantly higher ($P < .05$) overall mean blood glucose levels than that of Glacier RD diet (Table 17).

These data demonstrate that chicks fed uncooked high amylose barley flour and RD fractions do not have a flattened postprandial glucose curve compared with normal barley and corn. These uncooked high amylose barley flour and RD fractions appeared to be equally or as easily digested and absorbed by chicks as that of normal Glacier barley. The larger particle size of the corn meal, produced through the grinding process, could have been the cause of the reduced or slower digestibility of the corn diet which resulted in the lowest glucose peaks as reported by Heaton et al.(1988).

Hypocholesterolemic Effects

Table 18 and Fig 3 show the chick blood lipid profiles for barley flour and corn-1 treatments. Total and LDL plasma cholesterol concentrations were significantly ($P < .01$) lower for chicks fed the HAG and HHAG flour diets compared with Glacier flour and corn control diets. The HDL cholesterol values for chicks fed Glacier and HHAG flour diets were higher ($P < .05$) than the corn control but similar to the HAG diet. The LDL to HDL cholesterol ratios for chicks fed HAG and HHAG diet were significantly lower ($P < .01$) than the control but similar to the Glacier flour diet. Plasma triacylglycerol was significant lower for chicks fed HAG and HHAG flour diets

compared with the Glacier diet. Average daily gain (ADG) of chicks fed barleys was significantly lower ($P < .01$) than the corn control while no differences were found among the barley diets. The HAG and HHAG flour diets contained the same amount of TDF, SDF and β-glucans (Table 14) as the Glacier flour diet except for the higher amylose content. These results suggest that the hypocholesterolemic effect of HAG and HHAG flour may partially be due to the starch structure and its interaction with barley SDF. Similar results were reported by Behall and Scholfield (1989), in which blood triacylglycerol and cholesterol were lowered in subjects consuming high amylose maize starch.

Table 18. Plasma Lipid Concentrations (mg/dl) and Average Daily Gain (ADG) of Chicks Fed Barley Flour or Corn Control-1 Diets (*Trial I*)[a]

Measurements	Glacier[b]	HAG[c]	HHAG[d]	Corn-1
Cholesterol				
Total	301.5±14.7 a	244.1±13.9 b	221.2±11.8 b	305.9±12.0 a
LDL	113.6±15.2 a	81.0±11.5 b	56.6±12.0 b	131.7±13.3 a
HDL	190.9±10.0 a	166.8±11.3 ab	192.6±8.5 a	158.5±7.7 b
LDL/HDL	0.7±0.1 ab	0.6±0.2 b	0.4±0.1 b	0.9±0.1 a
Triacylglycerol	120.9±5.7 a	89.3±3.7 c	106.3±5.2 b	79.3±3.9 c
ADG (g)	15.7±0.7 b	17.0±0.5 b	16.1±0.7 b	24.7±0.9 a

[a] Numbers in rows are Mean±SEM of 22 to 24 observations, following with unlike letters significantly differing at $P < .05$.
[b] Glacier barley flour diet.
[c] HAG = high-amylose Glacier barley flour diet.
[d] HHAG = hull-less high-amylose Glacier barley flour diet.

Figure 3. Blood Lipid Values of Chicks Fed Barley Flour or Corn-1 Diets

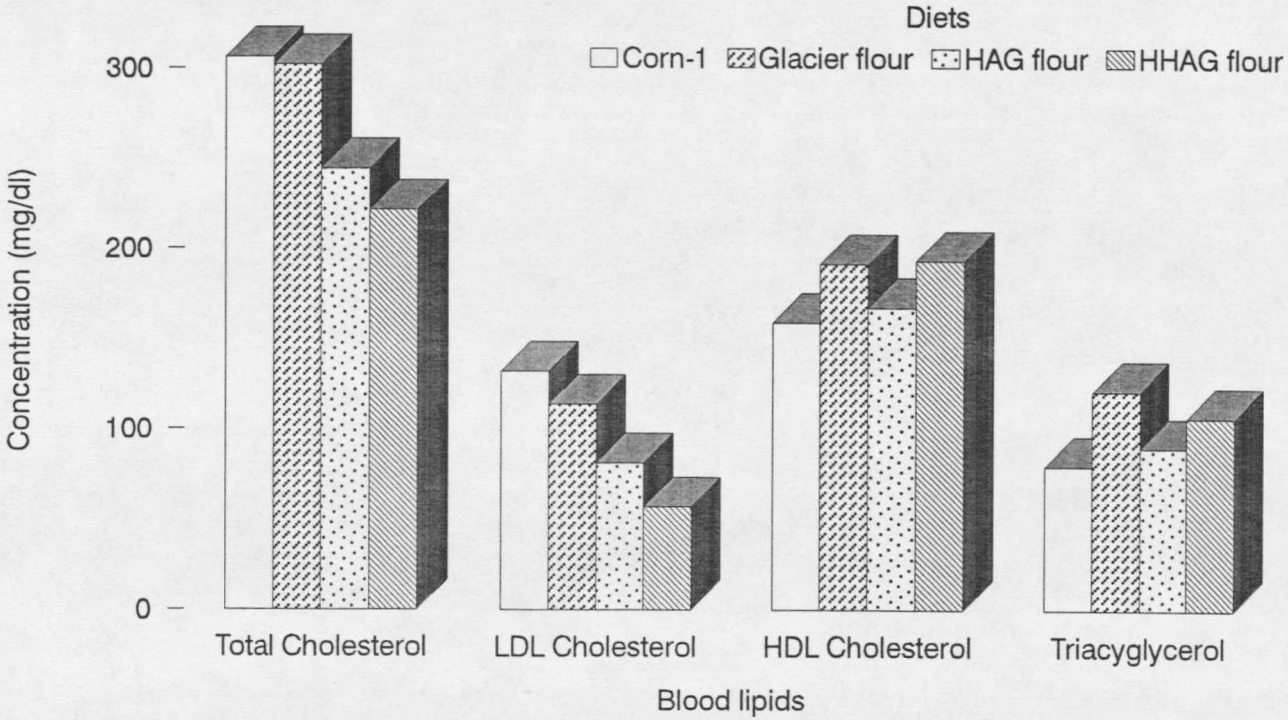


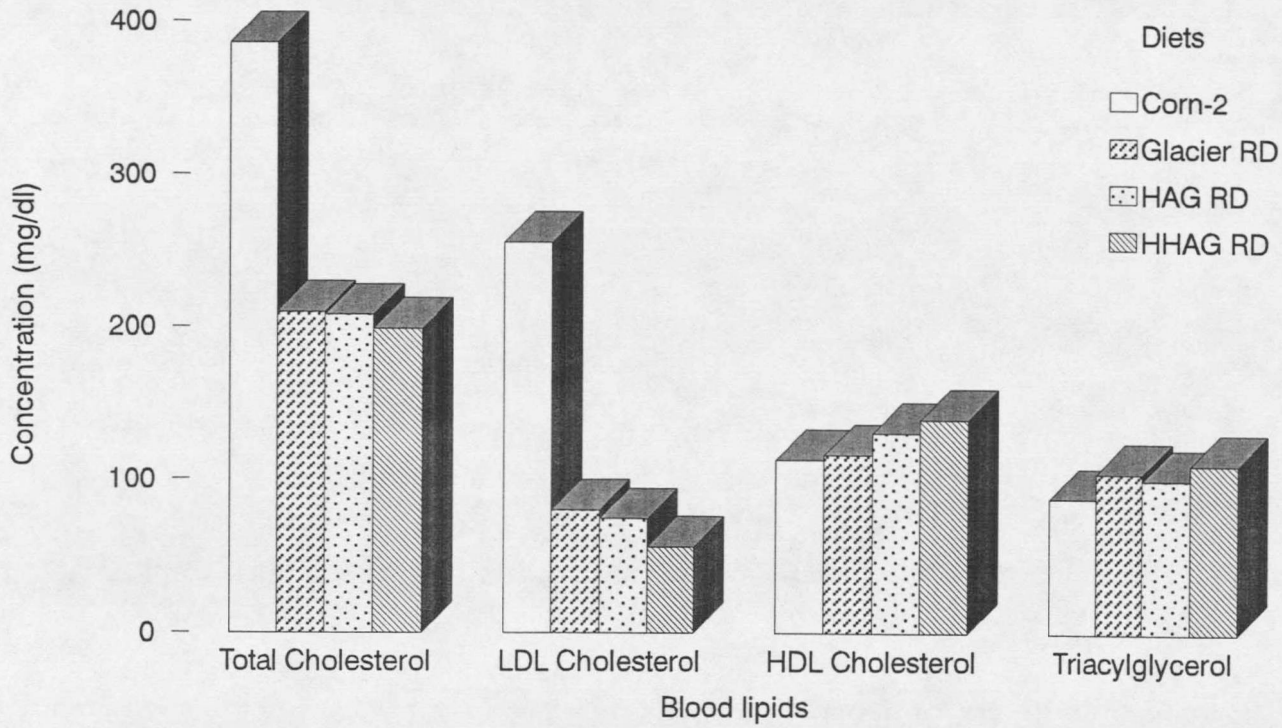
Table 19 and Fig 3 show the chick plasma lipid concentrations for the barley RD treatments and corn-2 diets. Total and LDL plasma cholesterol were significantly lower ($P < .001$) for chicks fed all barley RD diets compared to the corn control. The HDL cholesterol values for chicks fed HHAG barley RD were significantly higher ($P < .05$) while the other two barley diets were the same as the corn control. The LDL to HDL cholesterol ratios for chicks fed all barley RD diets were significantly lower ($P < .05$) than the corn control. The blood triacylglycerol was not different among barley diets but higher ($P < .05$) in Glacier and HHAG RD diets compared with the control. Chicks gained less weight when fed all barley RD diets. Average daily gain of chicks was

Table 19. Plasma Lipid Concentrations (mg/dl) and Average Daily Gain (ADG) of Chicks Fed Barley Red Dog Fraction or Corn Control-2 Diets (*Trial II*)[a]

Measurements	Glacier-RD[b]	HAG-RD[c]	HHAG-RD[d]	Corn-2
Cholesterol				
Total	209.1±6.7 b	207.2±13.1 b	198.2±9.1 b	385.0±15.9 a
LDL	79.7±8.7 b	74.0±11.5 b	55.8±7.1 b	254.6±16.4 a
HDL	116.3±9.6 ab	130.6±10.7 ab	139.3±7.6 a	112.9±7.5 b
LDL/HDL	0.9±0.1 b	0.7±0.1 b	0.5±0.1 b	2.56±0.3 a
Triacylglycerol	104.1±3.6 a	99.8±5.5 ab	109.9±5.7 a	87.5±5.0 b
ADG	10.5±0.8 b	10.7±0.7 b	9.6±0.4 b	23.6±1.1 a

[a] Numbers in rows are Mean±SEM of 22 to 24 observations, following with unlike letters significantly differing at $P < .05$.
[b] Glacier barley red dog fraction diet.
[c] HAG = high-amylose Glacier barley red dog fraction diet.
[d] HHAG = hull-less high-amylose Glacier barley red dog fraction diet.

Figure 4. Blood Lipid Values of Chicks Fed Barley Red Dog Fraction or Corn-2 Diets



highly correlated with total and LDL cholesterol (r = 0.69 and 0.72). These results confirm that soluble fiber, mainly β-glucans, in barley may be responsible for the hypocholesterolemic effect (Newman et al., 1989b) because barley RD fraction contained much higher SDF and β-glucans than corn meal (Table 15).

Conclusions

Both HAG and HHAG barleys contained higher levels of amylose starch and β-glucans compared with normal Glacier barley. Total dietary fiber, SDF and β-glucans were concentrated in the barley milling fraction, RD. Significantly higher blood glucose levels were found in chicks fed two uncooked (raw) barley fractions (flour and RD) from all barleys compared with corn. Uncooked high amylose milling fractions did not produce lower postprandial glucose curves, but rather, increased blood glucose for chicks fed high amylose RD fractions compared with normal Glacier. However, plasma total and LDL cholesterol were significantly reduced in chicks fed two high-amylose flours and all barley RD fractions while the HDL cholesterol remained the same or higher compared with normal Glacier flour or the corn control, respectively. Loss of chick body weights were observed from all barley diets. The results suggested that the soluble fiber, mainly β-glucans and starch structure in barley are probably responsible for the hypocholesterolemic effect but the mechanism of high amylose barley lowering cholesterol needs to be further clarified. The data also demonstrates that the digestion and absorption for chicks fed the uncooked high amylose barleys were similar to Glacier normal barley in a short term study.

CHAPTER 5

DIGESTIBILITIES *IN VITRO* AND GLUCOSE RESPONSES IN RATS WITH DIFFERENT TYPES OF BARLEY STARCHES SUBJECTED TO HEAT-TREATMENTS

Introduction

Both rate and extent of starch hydrolysis *in vitro* are regarded as predictors of metabolic responses to complex carbohydrate *in vivo* (O'Dea and Holm, 1988). A commonly used method for the investigation of rates of starch hydrolysis in foods is simulated digestion *in vitro*. Marked differences in both the rate and extent of digestion of complex carbohydrate foods have been observed. It has been well established that such differences correlate with postprandial blood glucose changes in humans under experimental conditions (Lund and Johnson, 1991; Brand et al., 1985; Bornet et al., 1989).

Amylomaize starch digestibility has been extensively studied *in vitro* and *in vivo* with chicks, rats, mice and humans. Amylomaize starch is less susceptible to amylolysis than normal maize (Dreher et al., 1984). High-amylose maize starch has been shown to have a positive lowering effect on blood glucose and insulin levels in humans (Behall et al., 1988; Behall and Scholfield, 1989; Amelsvoort and Weststrate, 1992) as well as

high-amylose rice (Goddard et al., 1984). Behall and Scholfield (1989) concluded that long-term intake of a high-amylose cereal grain may benefit individuals who show elevated glucose and insulin levels and apparent insulin resistance, as in early adult-onset diabetes. Behall and Scholfield (1989) also reported a lowering of blood triglycerides and cholesterol in subjects consuming high-amylose maize starch. The flattened insulin response observed with amylose may also be beneficial for hyperlipidemic subjects because high insulin levels have been implicated in the development of thickened arterial walls and lesions.

High-amylose barley starch was also found to be less susceptible to α-amylase than normal barley starch (Pomeranz et al., 1972). Calvert et al. (1976) reported that rats consumed less of a purified diet prepared with HAG starch than a similar diet containing Glacier starch and as a consequence gained at a slower rate. However, available data on the nutritional properties of amylose starch are by no means clear cut. In another study, Calvert et al. (1981) found that barley starch type did not significantly affect gain or feed consumption of swine. Rubin et al. (1974) demonstrated HAG to be nutritionally superior to five other barleys including Glacier as measured by growth of weanling rats. In a study with rice, a slightly higher amylose content (22 vs 15%) resulted in an increased rate of *in vitro* α-amylolysis and an increase in postprandial glucose in healthy subjects (Srinivasa Rao, 1971).

Data from our laboratory (Xue et al., 1991) showed that blood glucose levels in broiler chicks fed two uncooked (raw) milling fractions (flour and red dog) from HAG and HHAG barleys were not significantly flattened compared with normal Glacier barley

milling fractions and were higher than that of corn control. Plasma cholesterol, however, was significantly reduced in chicks fed the high-amylose flour and red dog fractions, respectively.

Enzyme resistant starch (ERS) formation in wheat, maize, and barley during moisture-heat processing may explain the lowered glucose response to high-amylose starch, because ERS is believed to consist of retrograded amylose (Berry, 1986; Ring et al., 1988; Siljeström et al., 1989; Szczodrak and Pomeranz, 1991). Moreover, ERS is less bioavailable in the human gastrointestinal tract and exerts physiological effects similar to those of dietary fiber (Björck et al., 1987; Englyst and Cummings, 1987; Jenkins and Jenkins, 1985; Schneeman, 1989).

Björck et al., (1990) reported that the amylose/amylopectin ratio in different barley genotypes (waxy, normal and high-amylose starch) produced no differences in enzymatic hydrolysis when the barley flour was boiled. Autoclaving the flour, however, produced a concomitant decrease in *in vitro* digestibility with increased amylose content. This was explained by an increased amount of ERS (3%) and the formation of amylose-lipid complexes, which did not occur in autoclaved waxy barley flour or in any of the flours when boiled. However, the flour or starch-containing meal used in past experiments contained not only starch, but also dietary fiber, protein, lipid and lesser constituents. Dietary fiber is known to flatten glucose curves in humans (Jenkins and Jenkins, 1985). β-glucan (a fraction of the soluble fibers) was reported to be a factor in lowering blood glucose and insulin in rats (Vachon et al., 1988) and lowering blood cholesterol in hypercholesterolemic individuals (Newman et al., 1989b). In addition to

the high-amylose content, high-amylose Glacier barley also contains relatively high β-glucan (7%). Therefore, effect of DF and other components in the barley may confound starch effects on glucose responses. In order to identify the effect of starch structure (ratio of amylopectin to amylose) on the glucose responses, the glucose response test must be verified with purified starches *in vitro* and *in vivo*.

The objectives of these studies were to (1) estimate the formation of enzyme resistant starch during the autoclaving process with different ratios of amylose and amylopectin in barley and corn; (2) determine *in vitro* digestibility and hydrolysis rate of barley and corn starches with different amylose and amylopectin content and heat treatments (autoclaved vs non-autoclaved); and (3) evaluate the glucose responses of autoclaved barley starch compared to wheat starch in rats.

Materials and Methods

Barley Flour

Waxbar, Glacier, covered high-amylose Glacier (HAG) and hull-less high-amylose Glacier (HHAG) barleys were grown in Arizona in 1990. The barleys were milled through a MIAG MULTOMAT 8-roll dry mill (Figure 11) at the Western Wheat Quality Laboratory (Washington State University, Pullman WA). Flour was made up of 2nd break (B), 3rd B, 1st middling (M), 2nd M, 3rd M and 4th M streams (2B-4M, 2nd break flour to 4th middling) from each cultivar.

Starch Isolation

Starches were isolated from barley flours (2B-4M) by a modification of the method of Morrison and Laignelet (1983) and Szczodrak and Pomeranz (1991). After steeping briefly in 0.02 M HCl and neutralizing with 0.02 M NaOH at pH 7.6, the starch suspensions were incubated for 24 hr at 37^{0}C with protease (Sigma Chemical Co., Type XIV, 5 mg/g) and 0.01% thiomersal. The suspension was filtered through 88 and 61 μm mesh sieves. The residue was homogenized with a Brinkmann homogenizer (Brinkmann Instruments, Co. Westbury NY 11590) with water and screened again. The filtered suspension was centrifuged at 2000 rpm for 15 min and the supernatant was discarded. The brown layer and white layers were combined and purified with 8:1 water:toluene by a shaking procedure. As a result of this treatment the protein was denatured and concentrated in the supernatant toluene layer while the purified starch granules precipitated in the aqueous layer. The toluene layer was separated from the aqueous layer and the procedure was repeated until a completely clear toluene layer was obtained. All wet starches were air-dried.

Wheat starch (unmodified), corn amylopectin, corn starch and corn amylose were purchased from Sigma Chemical Co. St. Louis MO.

Starch Treatment (Formation of ERS)

The formation and determination of ERS were performed according to the methods described by Szczodrak and Pomeranz (1991). The starch-water ratio used for ERS formation was 1:5. The suspension was autoclaved for 1 hr at 121^{0}C under 1.46 b pressure using a thermostatically controlled autoclave and cooled overnight in a

refrigerator (4°C). Numbers of autoclaving-cooling cycles were 3 and 12, respectively. The treated samples were vacuum-dried from -40°C to room temperature (Freeze dryer, FTS, Systems Co., Stone Ridge NY). All the material was ground on a Wiley mill (A. H. Thomas Co., Philadelphia PA) to pass a screen with .5 mm diameter openings.

ERS Determination

ERS was estimated by an enzymatic-gravimetric assay as described by Sievert and Pomeranz (1989). It was considered as the residue remaining after incubation of the sample with a heat-stable α-amylase (A-3306, Sigma Chemical Co. St Louis MO) and amyloglucosidase (from *Aspergillus niger*, A-9913). This assay was a modification of the AOAC method for the determination of insoluble dietary fiber (AOAC 1985).

Starch Hydrolysis *in vitro* with α-amylase

The amounts of maltose released by treatment with pancreatin were determined spectrophotometrically with 3,5 - dinitrosalicylic acid (1% in 0.4 N NaOH containing 30% sodium potassium tartrate) according to the method as described by Björck et al (1987) and Wootton and Chandhry (1979) with modifications. About 500 mg (dwb) of starch was added to 50 ml 0.05 M K-Na phosphate buffer (pH 6.9), containing 0.04%(w/v) NaCl. The solution was boiled in a water bath for 10 min and cooled to 37°C. Porcine pancreatic α-amylase (Product #A-6255, containing 27 mg protein/ml and 1260 U/mg, (1 U liberates 1 mg maltose from starch in 3 min at pH 6.9 and 20°C), Sigma Chemical Co., St Louis MO), was added after dilution to contain 108 U in 0.2 ml. The starch suspension was incubated at 37°C for up to 1 hr. Samples (.2 ml) were

withdrawn at time intervals and analyzed for reducing sugar with a dinitrosalicylic acid reagent. Maltose was used as a standard. Percent hydrolysis was calculated (mg of maltose from standard curve/ mg of starch × 100). The digestibility of the starches was calculated on the basis of % hydrolysis measured after 1 hr of incubation, at which point the rate of increase in reducing power was constant.

Glucose Tolerance Test in Rats

Treatment diets: four starches (Waxbar, Glacier, HAG barley and wheat starches) with different heat-moisture treatments (non-autoclaved, autoclaving-cooling 3 and 12 cycles) were boiled for 15 min with stirring (7.5% starch solution) and cooled overnight at room temperature. Wheat starch was used as control and glucose was used as a reference.

Sprague-Dawley male rats (8 wk old) from Harlan Sprague-Dawley, Inc. Indianapolis, IN (average weight 240 g, n=56) were individually housed, adapted for 5 days to a 12 h light/ 12 hr dark cycle and fed Purina laboratory chow. Feed was withdrawn 24 h prior to the gavaging of starch solution. During the starvation period free access to water was provided. Glucose tolerance tests were performed by giving glucose or other diets (10 ml of 7.5 starch percent solution, approximately 300 mg dry matter/100 g body weight) to rats by stomach tube and taking 1 ml blood from the tail artery of each rat at 0, 30, 60, 120 and 240 min. Blood glucose and insulin levels were determined. The rats were then fed Purina rat chow for two weeks and by that time they were randomized and the test repeated as described. Eight rats were in each treatment group and the glucose reference.

Chemical Analysis

Analyses were made for protein, ether extract, ash (AOAC, 1984) and starch (Åman and Hesselman, 1984). Amylose starch was measured by gel filtration chromatography (Torneport et al., 1990). Serum glucose was determined on a Kodak DT 60 Blood Analyzer (Eastman Kodak Co., Rochester NY).

Statistical Analysis

Main effects were analyzed using SAS General Linear Models Procedure for repeated measurement and differences between means were analyzed by Least-Squares Means. Correlation coefficients (r) were determined by Pearson's CORR Procedure (SAS, 1988).

Results and Discussion

The experiment was designed to study the effects of starch type on the rates and extent of reducing sugars released from starches and glucose absorption in the animal model. Several aspects will be discussed. Firstly, purified starch was used for this study in order to avoid interference from other components, such as DF, lipid, and protein in the flour. Using a purified starch, an *in vitro* study was conducted, and the ERS was measured as the residue after starch was hydrolyzed by α-amylase. In contrast, the reducing sugar released from starches by α-amylolysis was determined and expressed by *in vitro* hydrolysis rates and digestibility. Finally, *in vivo*, a rat glucose tolerance study was completed to confirm the *in vitro* study.

Starch Isolation

The yield and chemical composition of purified barley starch are shown in Table 20. The yields of starches isolated from barley flour were about 50% and were similar to those of Szczodrak and Pomeranz (1991). Barley starches isolated in our experiment were considered to be pure (>92.3%) compared to that of starch from Sigma Chemical Co. (Table 21). By this method it assayed 92.5% starch dry matter basis when soluble starch from Sigma Chemical Co. (S-9765) was used as control in starch determination. In addition, the crude protein, ether extract and ash contents of each starch were less than 0.3%, which was similar to the starches from Sigma Chemical Co. The lower starch value (74.3%) of corn amylose starch could be due to the high level of ERS (23%) resulting from the extraction process (Table 22). The amylose content of barley starches varied from 7.3% in Waxbar to 40% in HAG and HHAG starches. In contrast, corn starches varied from 0% amylose in corn amylopectin to 70% amylose in corn amylose starch reported by Sigma Chemical Co.

Starch granule size and amylose content were considered during starch isolation. Szczodrak and Pomeranz (1991) reported that the purified brown starch layer contained a higher percentage of small (2-5 μm in diameter) round granules high in amylose content (47.9%) while the white layer contained mainly large lenticular granules with an amylose content of 44.5%. However, Stark and Yin (1986) reported that small granules (brown layer) of regular (about 25% amylose) barley contained 4% less amylose than large granules (white layer). Based on this information, a combination of brown layer and white layer would not change the granule size distribution and amylose content in

Table 20. Yield and Chemical Composition of Starchy Materials Isolated from Barleys[a]

Source of Starch	Yield	Starch	Amylose[b]	Protein	Lipid	Ash
Waxbar	51.3	94.1	7.3	.05	.13	.09
Glacier	56.0	92.6	29.4	.16	.07	.21
HAG[c]	50.8	92.3	40.3	.25	.07	.25
HHAG[d]	43.3	92.6	40.5	.19	.04	.22

[a] % of dry matter.
[b] % of starch.
[c] Covered high amylose Glacier.
[d] Hull-less high amylose Glacier.

Table 21. Chemical Composition of Starches from Sigma Chemical Co.[a]

Starch Type	Product #	Starch	Amylose[b]	Protein	Lipid	Ash
Wheat starch	S 5127	90.4	25	.03	.00	.18
Corn amylopectin	A 7780	93.6	0	.05	.03	.10
Corn starch	S 4126	94.2	25	.09	.05	.06
Corn amylose	A 7043	74.3	70	.69	.01	.09

[a] % of dry matter.
[b] % of starch and information from Sigma Chemical Co.

our study. The incubation temperature of 37°C for protein degradation during starch purification would avoid gelatinization which occurred above 50°C (Szczodrak and Pomeranz, 1991).

Formation of ERS

Yields of ERS from the different cereal starches with 0, 3 and 12 autoclaving-cooling cycles are shown in Table 22. Enzyme resistant starch formation varied depending on the amylose content and the heat treatments. Greater than 1% of ERS formation was measured in HAG and HHAG starches that did not undergo the

Table 22. Yield of Enzyme Resistant Starch from Autoclaving-cooling Cycles[a]

Starch	Non-autoclaved	3 Cycles	12 Cycles
Corn amylopectin[b]	0.24	0.34	0.52
Waxbar barley	0.24	0.19	0.31
Corn starch[b]	0.24	8.14	14.07
Wheat[b]	0.52	8.83	13.98
Glacier barley	0.66	7.40	12.14
HAG barley[c]	1.16	12.40	18.59
HHAG barley[d]	1.82	11.34	16.71
Corn amylose[b]	23.05	33.05	44.17

[a] % of dry matter.
[b] Starch from Sigma Chemical Co.
[c] Covered high-amylose Glacier.
[d] Hull-less high amylose Glacier.

autoclaving-cooling process as observed by Szczodrak and Pomeranz (1991). However, the extremely high ERS of 23% in native corn amylose from Sigma Chemical Co. was found but there is no readily apparent explanation for this ERS. Yield of ERS from waxy starches (corn amylopectin and Waxbar barley starch) which was less than 0.6% even after 12 cycles of autoclaving, was not affected by autoclaving-cooling treatments. In contrast, non-waxy starches were strongly affected by the autoclaving heat treatment, with the high-amylose starches affected most. The ERS was less than 0.7% in native normal starches (corn starch, wheat starch, Glacier barley starch) and increased to approximately 8% and 13% after 3 and 12 cycles of autoclaving, respectively. The ERS levels from HAG and HHAG barley starches were increased from 1.8% in native starch to 11 and 17% after 3 and 12 cycles of autoclaving. This result was close to the 21% of ERS formation from HAG barley with 12 autoclaving-cooling cycles, reported by Szczodrak and Pomeranz (1991). Although ERS in native corn amylose was exceptionally high, it was still increased from 23% to 44% after 12 cycles of autoclaving.

A significant positive correlation between amylose content and ERS formation was found ($r = .79$, $P < .05$ for native starch, $r = .96$, $P < .001$ for autoclaved starch), as earlier reported by Björck et al. (1990), Berry (1986) and Szczodrak and Pomeranz (1991). With the number of autoclaving cycles increased, ERS formation increased in non-waxy starch, which confirmed the earlier hypothesis that retrograded amylose is mainly responsible for the generation of ERS as indicated by several authors who used different methods (Berry, 1986; Ring et al., 1988; Berry et al., 1988; Siljeström et al., 1989;, Sievert and Pomeranz, 1989; Sievert et al., 1991; Szczodrak and Pomeranz

1991). An amylose-lipid complex should be ignored during the ERS formation since the lipid content was less than 0.07% in HAG and 0.01% in corn amylose in our study.

In vitro Digestibilities and Hydrolysis Rates of Starches

The *in vitro* digestibilities of starches with different pre-heat treatment following 60 min of incubation with α-amylase are shown in Table 23. Raw starches had a much lower digestibility value than boiled starches because they were under gelatinization temperature. Waxy barley starch showed the highest susceptibility to α-amylolysis when tested raw. The degree of hydrolysis in raw wheat was lower than waxy, similar to corn amylopectin, but higher than non-waxy barley starches. These results differ from those of Björck et al. (1990). The lowest digestibility of raw corn amylose was expected because of the higher content of ERS (Table 22).

Hydrolysis rates of all starches subjected to 0, 3, and 12 autoclaving-cooling cycles are illustrated in Figs 5, 6 and 7. There were no significant differences in hydrolysis rates of boiled starches (except corn amylose) until 60 min incubation (Table 23). The digestibilities of boiled high-amylose starch suspensions were lower ($P < .05$) than that of waxy and normal starches. Autoclaving significantly changed hydrolysis rate and digestibility of non-waxy starches (Figs 6, 7 and Table 23). Hydrolysis rates and digestibilities of waxy starches were not affected ($P > .05$) by the autoclaving-cooling treatment (Figs 5, 6, 7 and Table 23). In contrast, digestibilities of non-waxy starches were significantly lowered by autoclaving ($P <. 001$). Similar results from barley flour have been reported by Björck et al. (1990). The digestibilities of HAG and HHAG starches were reduced by 14% after 3 autoclaving-cooling cycles and by 20% after 12

Table 23. Digestibility of Starches *in vitro* with Different Heat-Treatments[a,b]

Starch	Raw[c]	Boiled[d]	3-Cycles[e]	12-Cycles[f]	P-value[g]
Corn amylopectin	19.7±0.36 b[h]	76.6±0.31 a	77.5±0.92 a	76.9±0.58 a	>.05
Waxbar barley	24.3±0.28 a	74.9±0.76 ab	75.8±0.76 a	73.3±0.56 b	>.05
Corn	14.1±0.20 d	75.5±0.65 a	70.8±0.65 b	64.2±1.02 c	<.001
Wheat	18.8±0.44 b	76.8±0.55 a	69.0±0.49 b	64.4±0.20 c	<.001
Glacier barley	15.7±0.29 c	73.4±0.78 bc	70.4±1.30 b	64.3±1.48 c	<.01
HAG barley[i]	13.4±0.21 d	72.1±0.73 c	61.4±0.53 c	57.2±1.11 d	<.001
HHAG barley[j]	11.5±0.20 e	72.0±0.56 c	62.4±0.30 c	57.8±0.38 d	<.001
Corn amylose	5.3±0.41 f	44.8±0.68 d	49.1±0.80 d	38.6±0.87 e	<.001

[a] Measured as starch hydrolysis rate (%) with α-amylase after 60 min incubation at 37°C.
[b] Data are means (± SEM) of 4 to 14 observations.
[c] Native starch without heat-treatment.
[d] Boiled before incubation.
[e] Autoclaved 3 times, dried and reboiled before incubation.
[f] Autoclaved 12 times, dried and reboiled before incubation.
[g] Significance levels of autoclaving effects.
[h] Numbers in column with superscripts differ at P < .05.
[i] Covered high-amylose Glacier.
[j] Hull-less high-amylose Glacier.

Figure 5. Starch Hydrolysis Rates (%) with Boiled Treatment

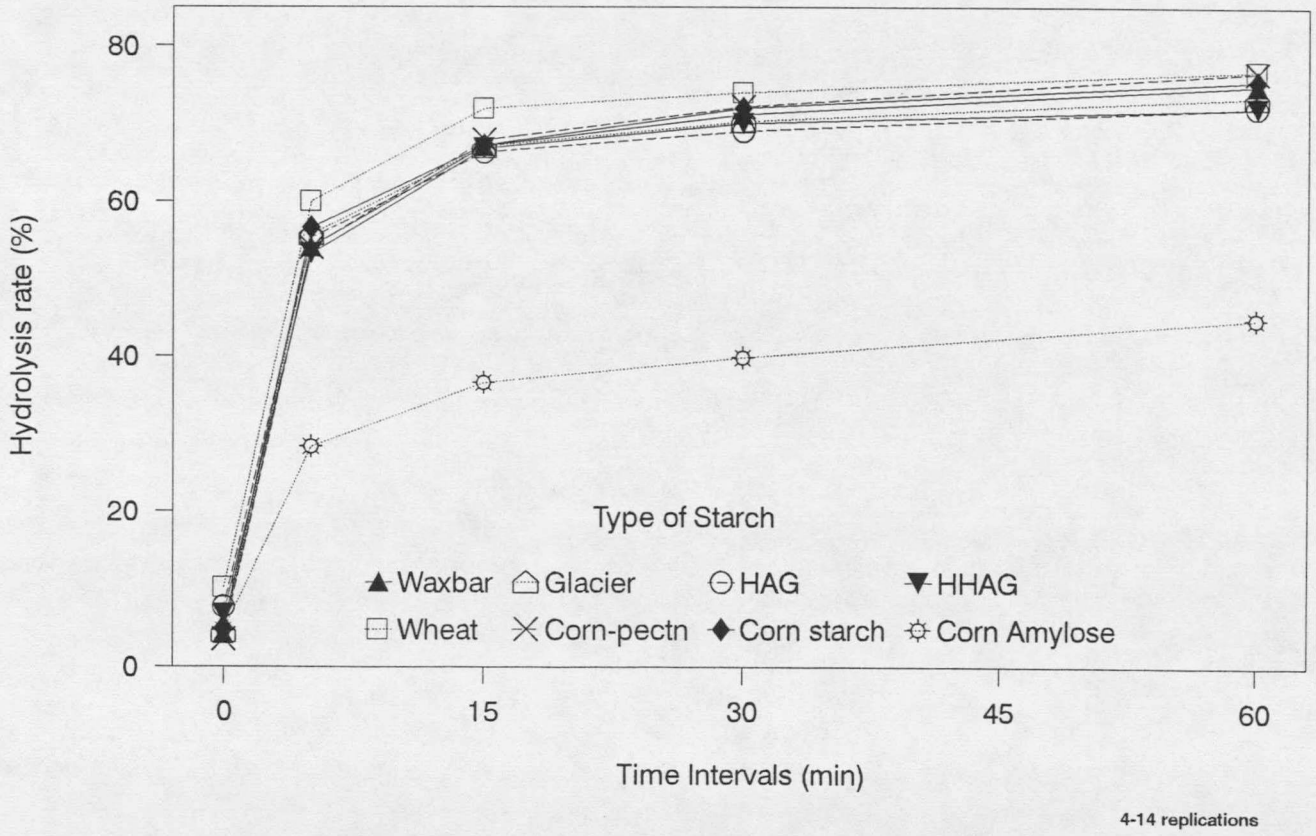


Figure 6. Starch Hydrolysis Rate (%) with 3 Cycle Autoclaved Treatment

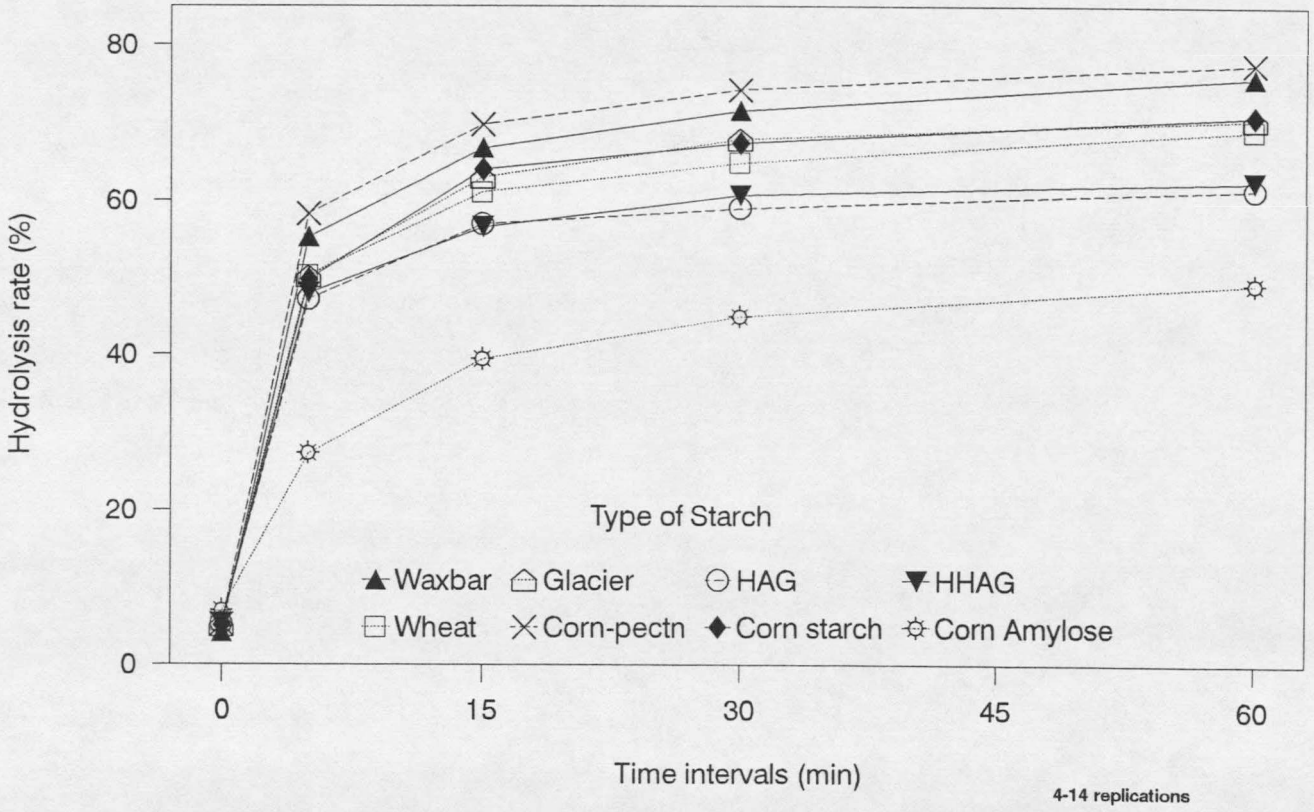
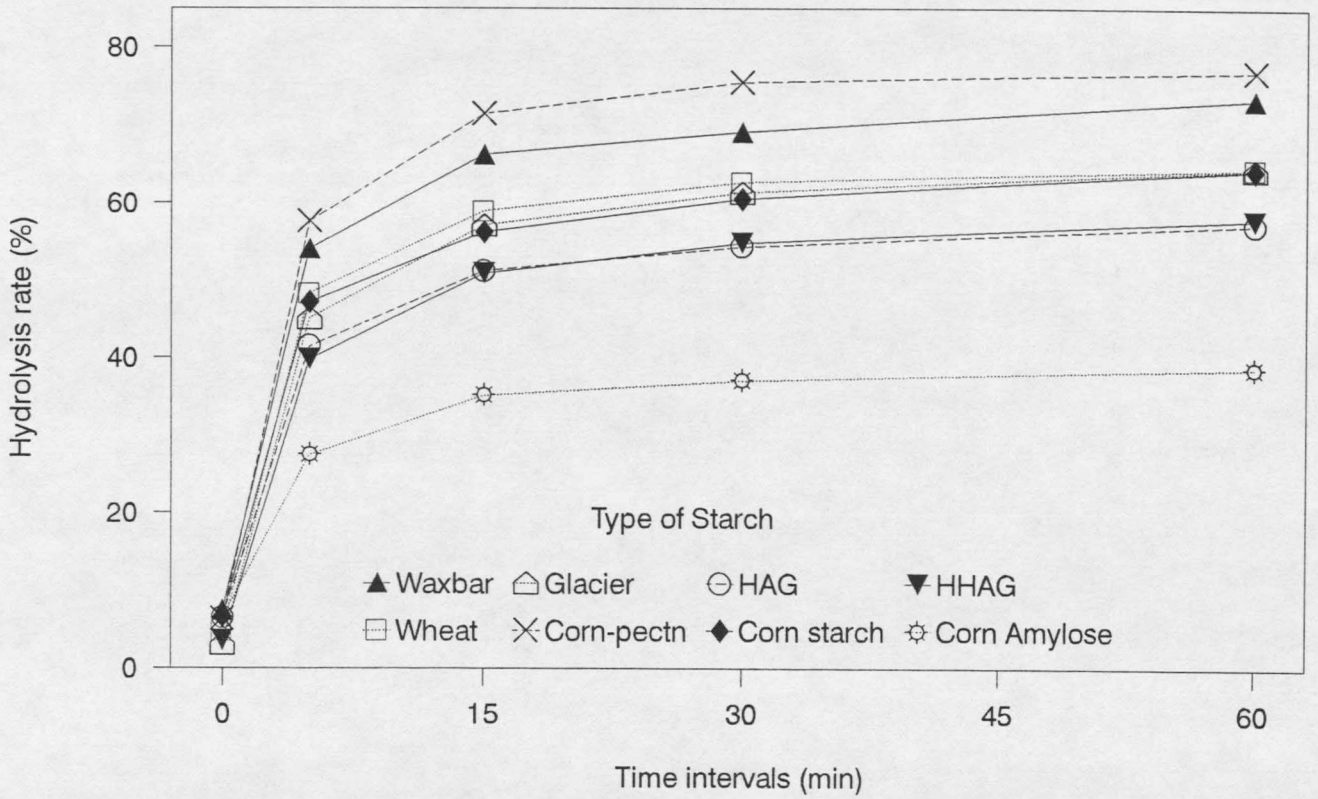


Figure 7. Starch Hydrolysis Rate (%) with 12 Cycle Autoclaved Treatment



autoclaving-cooling cycles compared to the starches without autoclaving (Table 23). The autoclaving effect on bio-availability of starch depended on the amylose level of starches. Therefore, with increasing amylose content, a concomitant decrease occurred in *in vitro* digestibility of autoclaved starch. Digestibility of starch suspension was significantly ($P < .001$) negatively correlated with amylose content and ERS formation (Table 24).

These data demonstrate that the hydrolysis rates of different cereal starches followed the pattern: waxy > normal > high-amylose starches after heat-moisture autoclaving, possibly due to the formation of ERS from the amylose component.

Table 24. Selected Correlation Coefficients (r) of Amylose Content, Enzyme Resistant Starch and Digestibility *in vitro*

	Amylose	Dig-Raw[a]	Dig-Boiled[b]	Dig-A3[c]	Dig-A12[d]
Amylose		- 0.92**	- 0.83*	- 0.98***	- 0.99***
ERS-NA[e]	0.79*		- 0.99***		
ERS-A3[f]	0.96***			- 0.97***	
ERS-A12[g]	0.97***				- 0.99***

[a] Dig-Raw = Digestibility of starch without heat treatment.
[b] Dig-Boiled = Digestibility of starch after boiling.
[c] Dig-A3 = Digestibility of starch after autoclaved 3 times, dried and reboiled.
[d] Dig-A12 = Digestibility of starch after autoclaved 12 times, dried and reboiled.
[e] ERS-NA = Enzyme resistant starch formed from native starch without autoclaving.
[f] ERS-A3 = Enzyme resistant starch formed from starch after autoclaved 3 times.
[g] ERS-A12 = Enzyme resistant starch formed from starch after autoclaved 12 times.
*, ** and *** denote statistical significance at $P < .05$, $P < .01$ and $P < .001$, respectively.

Glucose Responses in Rats

Postprandial glucose responses in rats after ingestion of 13 different diet solutions are shown in Figs 8, 9 and 10. No significant differences were measured in postprandial glucose levels in rats among these boiled starch solutions at all time intervals, while the glucose solution showed the highest peaks ($P < .05$) at 30 and 60 min (Fig 8). After autoclaving, Waxbar barley starch resulted in a significantly ($P < .05$) increased glucose peak at 30 min (3 cycles) and 60 min (12 cycles) compared to non-autoclaved Waxbar starch in rats (Figs 9 and 10). However, autoclaved HAG barley starch significantly lowered the glucose levels in rats compared to Waxbar (at 30 min $P < .01$, at 60 min $P < .05$) and Glacier starches (at 30 min $P < .01$) after 3 cycles autoclaving (Fig 9). After autoclaving, HAG starch reduced the glucose levels by 20 units at 30 min compared to non-autoclaved HAG starch and numerically lowered glucose compared to wheat starch at 30 and 60 min. Twelve cycles of autoclaved starches showed the same pattern as that of 3 cycles (Fig 10) except that the normal Glacier barley starch statistically produced the same glucose curve as HAG starch. Differences were not significant ($P > .09$) among any treatments at times 0, 120 and 240 min. These *in vivo* results corresponded to the *in vitro* study which demonstrated that glucose absorption in rats from non-waxy barley starches was reduced after heat-moisture autoclaving, possibly due to the formation of resistant starch from the amylose component. The glucose peaks in rats at 30 and 60 min after ingestion of autoclaved starches were closely ($P < .05$) correlated to digestibility *in vitro* ($r > 0.79$), amylose content ($r > - 0.76$) and ERS ($r > - 0.72$) (Table 25).

Figure 8. Glucose Responses in Rats Fed Boiled Starches

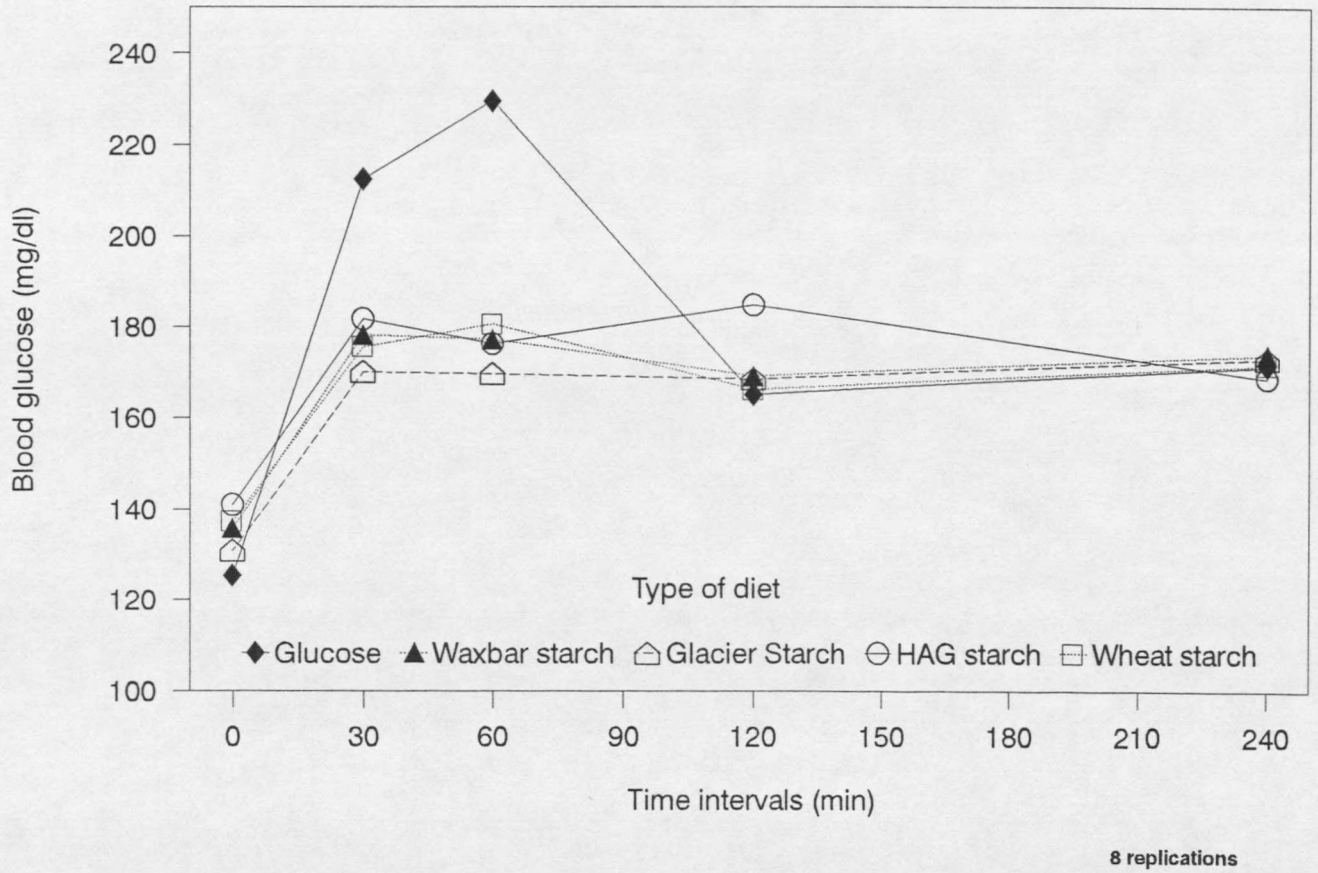


Figure 9. Glucose Responses in Rats Fed 3 Cycle Autoclaved Starches

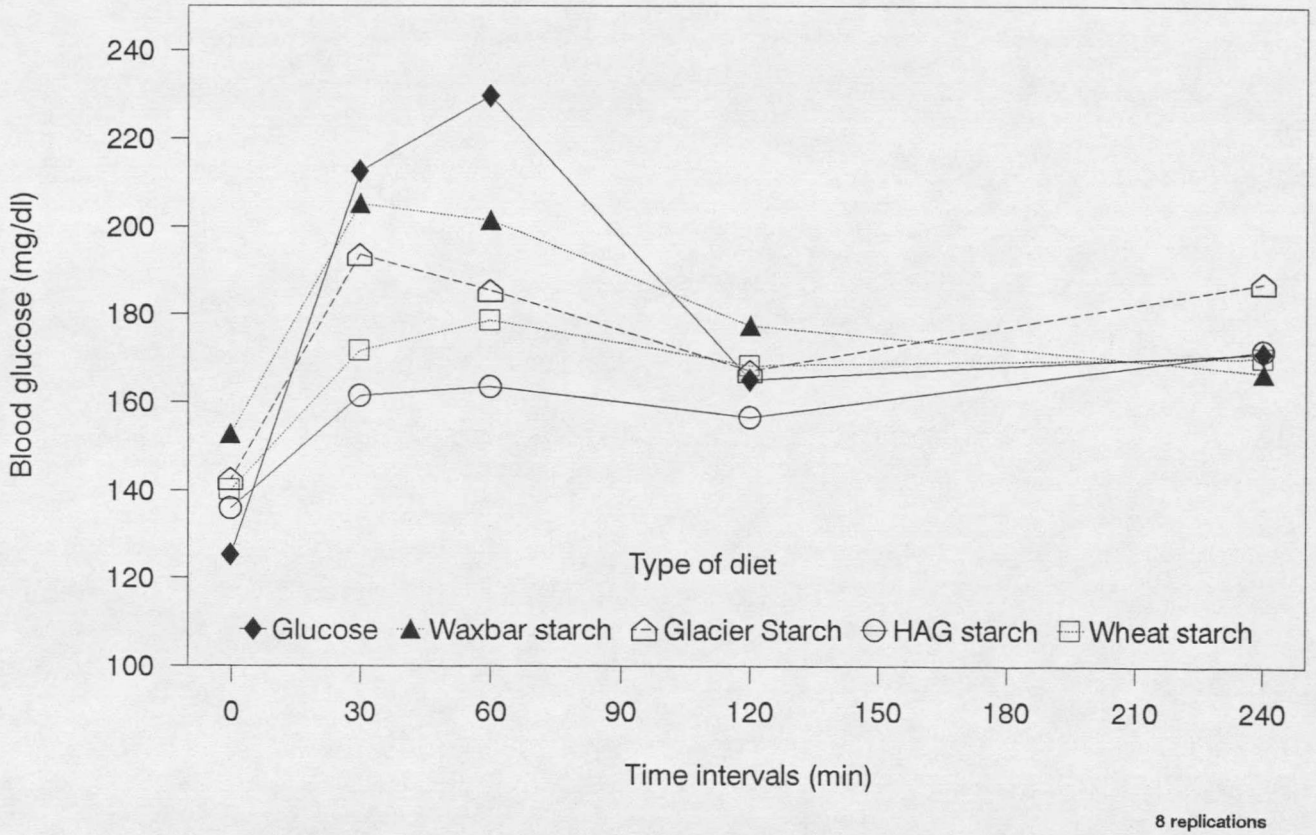


Figure 10. Glucose Responses in Rats Fed 12 Cycle Autoclaved Starches

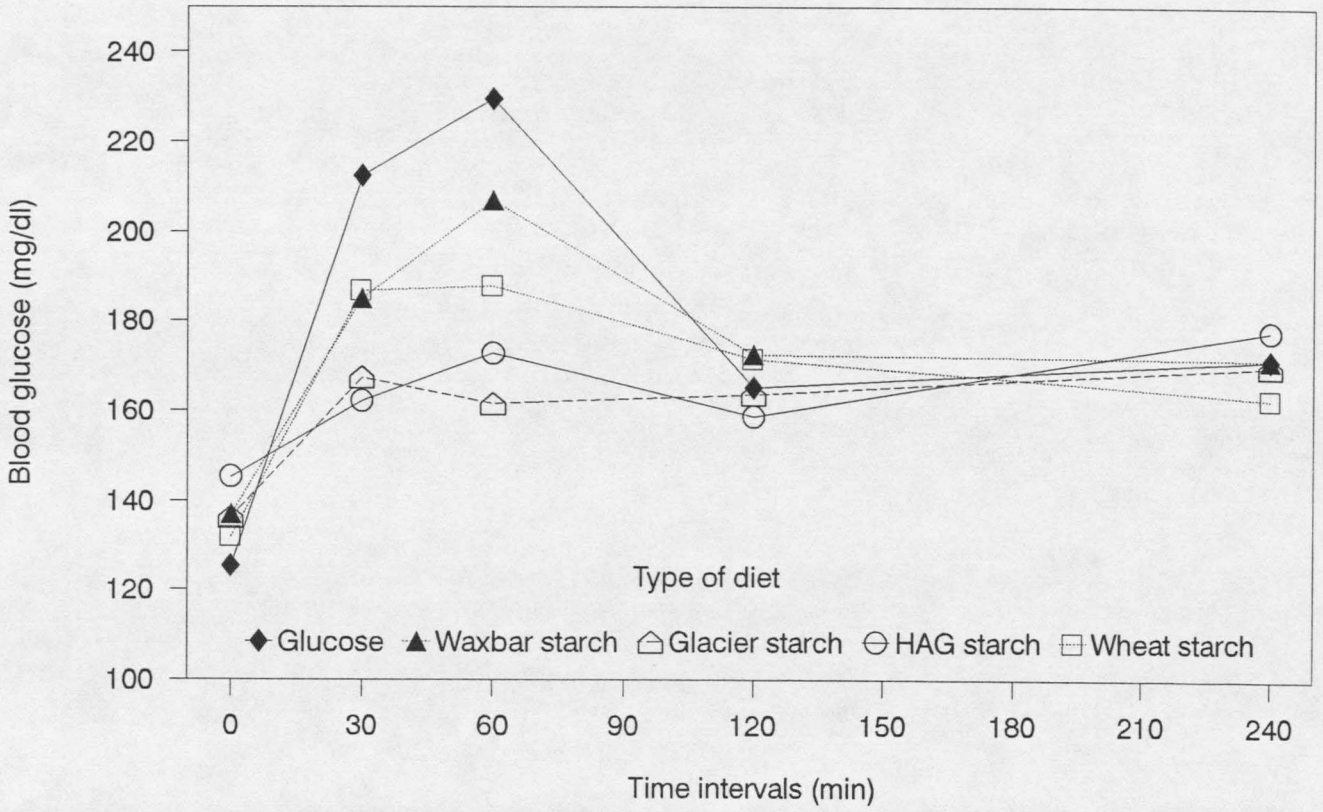


Table 25. Correlation Coefficients (r) of Glucose Responses in Rats and Amylose Content, Enzyme Resistant Starch, Digestibility *in vitro* with Autoclaved Treatments[a]

	Glucose at 30 min	Glucose at 60 min
Amylose content	- 0.76*	- 0.88**
Enzyme resistant starch	- 0.72*	- 0.78*
Digestibility *in vitro*	0.82*	0.79*

[a] Combined data from autoclaved 3 and 12 cycles, N=8.
* and ** denote statistical significance at $P < .05$ and $P < .01$, respectively.

Conclusions

From the purified cereal starches, effects were only marginal on digestibility and no effects on blood glucose levels in rats when boiled starches of different origins (amylose vs amylopectin) were tested. However, the effects of starch types on digestibility and blood glucose responses were significantly increased by autoclaving. Digestibility of waxy starches was not changed *in vitro* and blood glucose in rats was significantly increased after ingestion of autoclaved Waxbar barley starch. In contrast, when autoclaved non-waxy starches were gavaged, digestion rates were reduced, producing lower blood glucose levels. The high-amylose starches produced the lowest values in this respect. The effects of autoclaving on non-waxy starches were mainly due to the ERS formation from amylose components rather than amylose-lipid complexes forming since only trace amounts of lipids occurred in these starches. These results also implied that high-amylose barley has a great potential for food development for specialty markets, such as products for individuals with diabetes.

CHAPTER 6

SUMMARY

A series of six barley isotypes having non-waxy (*WW*) and waxy (*ww*) starch in covered (*NN*), hull-less (*nn*) and short-awned (*lklk*) lines were produced in a backcross breeding program. Isotypes were expressed in Compana (CI 5438) and Betzes (CI 6398). These barleys were grown and harvested near Bozeman, MT in 1987, 1988 and 1989. Analyses were done for protein, fat, starch, free sugars, total dietary fiber (TDF), components of TDF, β-glucans and extract viscosity. Data were analyzed to test the main effects of year, cultivar and isotype using the GLM procedure for ANOVA. The *nn* gene prevents high-fiber hulls from adhering to the kernel, thus reducing TDF. The *lk* gene had no effect on analyzed traits other than to increase ($P < .04$) viscosity. TDF and soluble dietary fiber (SDF) were increased ($P < .02$) in *ww* barleys, which was due to an increase ($P < .001$) in β-glucans. Extract viscosity increased with increased concentrations of β-glucans and free sugar. Total β-glucan, free sugar content and extract viscosity were correlated ($r = .55$ and $.76$, $P < .001$) over all genotypes. Total starch was lower ($P < .001$) where the *ww* gene was expressed; however, a concurrent increase ($P < .001$) in total free sugars (maltose, sucrose, glucose and fructose) resulted in near equal amounts of total starch + free sugars in *ww* and *WW* barleys with maltose

levels increased ($P < .001$) the most.

Normal Glacier (Glacier), covered high-amylose Glacier (HAG), hull-less high-amylose Glacier (HHAG) and Waxbar barleys were grown and harvested in Arizona in 1990. The HAG barley was discovered as a mutant of Glacier (CI 9676) and designated Glacier Ac38, which was suggested to be under control of a single recessive gene (*amo1*). The HHAG barley was an isotype of HAG with the hull-less (*nn*) gene. The barleys were milled through an 8-roller MIAG MULTOMAT dry mill. Two fractions, uncooked flour and red dog (RD) from each cultivar were used. The chemical analyses of barley grain, flour and RD fractions for each of the cultivars and corn meal are reported.

High-amylose Glacier and HHAG had 40% amylose starch while normal Glacier contained 29%. The β-glucan content in HAG and HHAG was also higher (7.4%) compared with that of normal Glacier barley (5.2%). From this point of view, the high-amylose gene could have an effect similar to the waxy gene in increasing β-glucan content. Total dietary fiber, insoluble dietary fiber (IDF) and total β-glucan were higher in RD, and starch content was higher in flour. The β-glucan content of high-amylose RD fractions was higher (10.6%) than that of Glacier RD (8.9%).

These data indicate that the expression of the waxy (*ww*) and high-amylose (*amo1*) genes in barley will increase levels of soluble dietary fiber, a desirable property when the end use will be human food products.

The glucose and lipid responses of barleys were investigated with chicks fed diets based on either flour or RD from each of three barley cultivars, Glacier, HAG and

HHAG, with two control corn diets. Mean time interval blood glucose levels were higher ($P < .05$) for chicks fed uncooked barley flour or RD, compared with controls. Differences among the barleys were not significant. Blood glucose levels were significantly higher at 30, 60, and 90 min after consumption of HAG and HHAG RD diets compared to controls. Total and LDL plasma cholesterol were lower ($P < .05$) for chicks fed the HAG and HHAG flour and all barley RD diets. HDL cholesterol values for chicks fed Glacier and HHAG flour and HHAG RD diets were higher than controls. The LDL/HDL cholesterol ratios for chicks fed HAG and HHAG flour and all barley RD diets were lower ($P < .05$) than controls. Lower body weights were observed in all chickens fed barley diets. The results suggest that the soluble fiber, mainly β-glucan and starch structure in barley may be responsible for the hypocholesterolemic effect. These data also demonstrate that the uncooked high amylose barleys were easily digested and absorbed by chicks in a short term study.

Starches were purified from barley flours of Waxbar, Glacier, HAG and HHAG cultivars. Wheat starch, corn amylopectin, corn amylose and normal corn starch were used for comparative purposes. These starches were moisture-autoclaved 3 and 12 times with subsequent cooling. An *in vitro* study was conducted to measure ERS, hydrolysis rates and digestibilities of these samples. Postprandial glucose responses in rats were investigated with Waxbar, Glacier, HAG , wheat starches and glucose. Only marginal effects were observed in hydrolysis rates, and there were no effects on glucose levels in rats when boiled starches of different origins (amylose vs amylopectin) were tested. However, the effects of starch types on digestibility and blood glucose responses were

significantly changed by autoclaving. Digestibility of waxy starches were not changed *in vitro* ($P > .05$) but glucose in rats was significantly ($P < .05$) increased after ingestion of autoclaved Waxbar barley starch. In contrast, the digestibility of HAG and HHAG starches was reduced by 14% and 20% after 3 and 12 autoclaving-cooling cycles, respectively. Autoclaved HAG starch significantly lowered the glucose peaks in rats compared with Waxbar and Glacier starches at 30 min ($P < .01$) and numerically lowered glucose compared to wheat starch at 30 and 60 min. The *in vivo* results corresponded to that *in vitro* study which demonstrated that the digestibilities of different cereal starches followed the pattern: waxy > normal > high-amylose starches after heat-moisture autoclaving, possibly due to the formation of ERS from the amylose component. ERS formation was highly ($P < .001$) correlated with digestibility ($R = -.98$) and amylose content ($R = .96$) of autoclaved starches. These results also implied that high-amylose barley is a potentially useful cereal grain for food product development which will be beneficial for health.

LITERATURE CITED

LITERATURE CITED

Aastrup, S. 1979. The relationship between the viscosity of an acid flour extract of barley and its β-glucan content. Carlsberg Res. Commun. 44:289-304.

Åman, P. and Hesselman, K. 1984. Analysis of starch and other main constituents of cereal grains. Swed. J. Agric. Res. 14:135-139.

Åman, P. and Graham, H. 1987. Analysis of total and insoluble mixed-linked (1→3), (1→4)-β-D-glucans in barley and oats. J. Agric. Food Chem. 35:704-709.

Åman, P., Graham, H. and Tilly, A.-C. 1989. Content and solubility of mixed-linked (1→3), (1→4)-β-D-glucan in barley and oats during kernel development and storage. J. of Cereal Sci. 10:45-50.

Åman, P. and Newman, C.W. 1986. Chemical composition of some different types of barley grown in Montana, USA. J. Cereal Sci. 4:133-141.

Amelsvoort, J.M. and Weststrate, J.A. 1992. Amylose-amylopectin ratio in a meal affects postprandial variables in male volunteers. Am. J. Clin. Nutr. 55:712-8.

Anderson, K., Castelli, W. and Levy, D. 1987a. Cholesterol and mortality: 30 years of follow-up from the Framingham study. J. Amer. Med. Assoc. 257:2176-2180.

Anderson, J.W., Gustafson, N.J., Bryant, C.A. and Tietyen, J. 1987b. Dietary fiber and diabetes: A comprehensive review and practical application. J. of the Amer. Dietetic Assoc. 87:1189-1198.

Andersson, M.A., Cook, J.A. and Stone, B.A. 1978. Enzymatic determination of (1→3), (1→4)-β-glucans in barley grain and other cereals. J. Inst. Brew. 84:233-239.

Anonymous. 1981. Arteriosclerosis. U.S. Department of HHS, PHS, NIH Publication, No. 81-2034.

AOAC, Association of Official Analytical Chemists. 1984. 'Official Methods of Analysis', 14th edn, Washington, D.C.

Auricchio, S., Pietra, D.D. and Vegnente, A. 1967. Studies on intestine digestion of starch in man, II: intestinal hydrolysis of amylopectin in infants and children. Pediatrics 39:853.

Bach Knudsen, K.E., Åman, P. and Eggum, B.O. 1987. Nutritive value of Danish grown barley varieties. I. Carbohydrates and other major constituents. J. Cereal Sci. 6:173-186.

Bacic, A. and Stone, B.A. 1981. Isolation and ultrastructure of aleurone cell walls from wheat and barley. Australian J. of Plant Physiology 8:453-474.

Ballance, G.M. and Manners, D.J. 1978. Structural analysis and enzymic solubilization of barley endosperm cell walls. Carbohydrate Research 61:107-118.

Banks, W. Greenwood, C.T. and Walker, J.T. 1970. Studies on the starches of barley genotypes: the waxy starch. Starch 22:149-152.

Banks, W. and Muir, D.D. 1980. Structure and chemistry of the starch granule. In: The Biochemistry of Plants. P.K. Stumpt and E.E. Conn, (eds). Vol 3:321-369. Academic Press, N.Y.

Behall, K. M. and Scholfield, D.J. 1989. Diets containing high amylose vs amylopectin starch: effects on metabolic variable in human subjects. Am. J. Clin. Nutr. 49:337-344.

Behall, K.M., Scholfield, D.J. and Canary, J. 1988. Effect of starch structure on glucose and insulin responses in adults. Am. J. Clin. Nutr. 47:428-432.

Bendelow, V.M. 1975. Determination of non-starch polysaccharides in barley breeding programmes. J. Inst. Brew. 81:127-130.

Bengtsson, S., Åman, P., Graham, H., Newman, C.W. and Newman, R.K. 1990. Chemical studies on mixed-linked β-glucans in hull-less barley cultivars giving different hypocholesterolemic responses in chickens. J. of Science of Food and Agriculture 52:435-445.

Berry, C.S. 1986. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. J. Cereal Sci. 4:301-314.

Berry, C.S., I'Anson, K., Miles, M.J., Morris, V.J. and Russell, P.L. 1988. Physical-chemical characterization of resistant starch from wheat. J. Cereal Sci. 8:203-206.

Bhatty, R.S. 1986a. Physiochemical and functional (breadmaking) properties of hull-less barley fractions. Cereal Chem. 63:31-35.

Bhatty, R.S. 1986b. The potential of hull-less barley - a review. Cereal Chem. 63:97-103.

Bhatty, R.S. 1992. β-glucan content and viscosities of barleys and their roller-milled flour and bran products. Cereal Chem. 69:469-471.

Björck, I., Eliasson, A.-C., Drews, A., Gudmundsson, M. and Karlsson, R. 1990. Some nutritional properties of starch and dietary fiber in barley genotypes containing different levels of amylose. Cereal Chem. 67:327-333.

Björck, I., Nyman, M., Pedersen, B., Siljeström, M., Asp, N.-G. and Eggum, B.O. 1986. On the digestibility of starch in wheat bread - studies *in vitro* and *in vivo*. J. of Cereal Sci. 4:1-11.

Björck, I., Nyman, M., Pedersen, B., Siljeström, M., Asp, N.-G. and Eggum, B.O. 1987. Formation of enzyme resistant starch during autoclaving of wheat starch: Studies *in vitro* and *in vivo*. J. of Cereal Sci. 6:159-172.

Bornet, F.R.J., Fontvielle, A.-M., Rizkalla, S., Colonna, P., Blayo, A., Mercier, C. and Slama, G. 1989. Insulin and glycemic responses in healthy humans to native starches processed in different ways: correlation with *in vitro* α-amylase hydrolysis. Am. J. Clin. Nutr. 50:315-323.

Bourne, D.T. and Pierce, J.S. 1970. β-glucan and β-glucanase in brewing. J. Inst. Brew. 76:328-325.

Brand, J.C., Nicholson, P.L., Thorburn, A.W. and Truswell, A.S. 1985. Food processing and the glycemic index. Am. J. Clin. Nutr. 61:174-179.

Briggs, D.E. 1978. Barley. Chapman and Hall Ltd. New York.

Calvert, C.C., Newman, C.W., El-Negoumy, A.M. and Eslick, R.F. 1981. Amylose Glacier barley in swine diets. Nutr. Rep. Int. 23:29-36.

Calvert, C.C., Newman, C.W., Eslick, R.F. and Goering, K.G. 1976. Comparison of waxy, high amylose and normal barley starches in purified rat diets. Nutr. Rep. Int. 14:55-61.

Calvert, C.C., Newman, C.W., Eslick, R.F. and Goering, K.J. 1977. Waxy vs normal barley in rat and pig diets. Nutr. Rep. Int. 15:157-164.

Colonna, P. and Mercier, C. 1985. Gelatinization and melting of the maize and pea starch with normal and high-amylose genotypes. Phytochemistry 24:1667

Canon, M., Flenniken, A. and Track, N. 1980. Demonstration of acute and chronic effects of dietary fiber upon carbohydrate metabolism. Life Sci. 27:1397-1401.

Connor, W.E. and Connor, S.L. 1984. The dietary prevention and treatment of coronary heart disease. In: Coronary Heart Disease. W.E. Connor and J.D. Bristow, (eds). J.B. Lippincott, Philadelphia.

Crawford, C. 1987. Survey of resistant starch in processed foods. FMBRA Bull. 2:59-63.

Danielson, A.D., McGuire, C.F., Newman, R.K., Newman, C.W. and Schwarz, P.B. 1990. Dietary fiber content of air-classified fractions of hull-less barley (abstract). Cereal Foods World. 35:819.

Dreher, M.L., Dreher, C.J. and Berry, J.W. 1984. Starch digestibility of food: a nutritional perspective. CRC Critical Reviews in Food Sciences and Nutrition. 20:47-71.

Ebihara, K. and Kiryiama, S. 1982. Comparative effects of water-soluble and water insoluble dietary fibers on various parameters relating to glucose tolerance in rats. Nutr. Rep. Int. 26:193-201.

Eliasson, A.C., Larsson, K., Andersson, S. and Hyde, S.T. 1987. On the structure of native starch - an analogue to the quartz structure. Starch 39:147-152.

Englyst, H.N. and Cummings, J.H. 1987. Digestion of polysaccharides of potato in the small intestine of man, Am. J. Clin. Nutr. 45:423-431.

Englyst, H.N. and Cummings, J.H. 1985. Digestion of the polysaccharides of some cereal foods in the human small intestine. Am. J. Clin. Nutr. 42:778-787.

Fadel, J.G., Newman, R.K., Newman, C.W. and Barnes, A.E. 1987. Hypocholesterolemic effects of beta-glucans in different barley diets fed to broiler chicks. Nutr. Rep. Int. 35:1049-1058.

Fincher, G.B. 1975. Morphology and chemical composition of barley endosperm cell walls. J. Inst. Brew. 81:116-122.

Fox, G.J. 1981. The effect of the waxy endosperm, short awn, and hull-less seed genes upon biochemical and physiological seed characteristics important in barley (*Hordeum vulgare L.*) utilization. Ph.D dissertation. Montana State University, Bozeman, MT.

French, D. 1984. Organization of starch granules. pp184-247. In: Starch Chemistry and Technology, 2nd edn. R.L. Whistler, J.N. BeMiller, and E.F. Paschall, (eds). Academic Press, Orlando.

Friedwald, W.T., Levy, R.I. and Fredrickson, D.S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of

preoperative ultracentrifuge. Clin. Chem. 18:499-502.

Goddard, M.S.G., Young, G. and Marcus, R. 1984. The effect of amylose content on insulin and glucose responses to ingested rice. Am. J. Clin. Nutr. 39:388-392.

Goering, K.J. and Eslick, R.G. 1976. Barley starch VI. A self-liquefying waxy barley starch. Cereal Chem. 53:174-180.

Goering, K.J., Eslick, R.G. and DeHaas, B.W. 1973. Barley starch V. A comparison of the properties of waxy Compana barley starch with starches of its parents. Cereal Chem. 50:332-328.

Hagander, B., Asp, N-G., Efendić, S., Nilsson-Ehle, P. and Scherstén, B. 1988. Dietary fiber decreases fasting blood glucose levels and plasma LDL concentration in noninsulin-dependent diabetes mellitus patients. Am. J. Clin. Nutr. 47:852-858.

Hale, W.H. 1973. Influence of processing on the utilization grain by ruminants. J. Anim. Sci. 37:1075.

Han, M.S. and Froseth, J.A. 1992. Composition of pearling fractions of barleys with normal and waxy starch. Proc. West. Sec. Amer. Soc. Anim. Sci. 43:155-158.

Harlan, H.V. 1957. pp168-169. In: One Man's Life with Barley - The Memories and Observations of Harry V. Harlan. J.R. Harlan, (ed). Exposition Press, New York.

Heaton, K.W., Marcus, S.N., Emmett, P.M. and Bolton, C.H. 1988. Particle size of wheat, maize, and oat test meals: effects on plasma glucose and insulin responses and on the rate of starch digestion in vitro. Am. J. Clin. Nutr. 47:675-682.

Henry, R.J. 1986. Genetic and environmental variation in the pentosan and β-glucan contents of barley, and their relation to malting quality. J. of Cereal Sci. 4:269-277.

Henry, R.J. 1988. The carbohydrates of barley grains - a review. J. Inst. Brew. 94:71-78.

Hipsley, E.H. 1953. Dietary "fiber" and pregnancy toxaemia. Brit. Med. J. 42:420.

Hockett, E.A., McGuire, C.F., Newman, C.W. and Prentice, N. 1987. The relationship of barley β-glucan content to agronomic and quality characteristics. pp851-865. In: Barley Genetics V. S. Yasuda, & T. Konishi, (eds). Sanyo Press, Okayama, Japan.

Hockett, E.A. and Nilan, R.A. 1985. Genetics. pp187-230. In: Barley. D.C. Rasmusson, (ed). Agronomy Monograph No 26. ASA, SSSA, Madison, WI.

Holm J., Lundquist, I. Björck, I., Eliasson, A.-C., and Asp, N.-G. 1988. Relationship between degree of gelatinization, digestion rate *in vitro* and metabolic response in rats. Am. J. Clin. Nutr. 47:1010-1016.

Hoseney, R.C. 1986. Principles of Cereal Scicence and Technology. Amer. Assoc. of Cereal Chem. Inc. St. Paul, MN

Jenkins, D.J.A., Leeds, A.R., Gassull, M.A., Wolever, T.M.S., Goff, D.V., Alberti, K.G.M.M. and Hocaday, T.D.R. 1976. Unabsorbable carbohydrates and diabetes:decreased postprandial hyperglycemia. Lanet. 2:172-174.

Jenkins, D.J.A. and Jenkins, A.L. 1985. Dietary fiber and the glycemic responses. Proceeding of the Society for Experimental Biology and Medicine 180:422-431.

Jenkins, D.J.A., Jenkins, A.L., Wolever, D.M., Collier, G., Venkent Rao, A. and Thompson, L. 1987. Starchy foods and fiber reduced rate of digestion and improved carbohydrate metabolism. Scand. J. Gastroenterology 22:132.

Jenkins, D.J.A., Wolever, T.M.S., Taylor, R.H., Barker, H. M., Fielden , H., Baldwin, J.M., Bowling A.C., Newman, H.C., Jenkins A.L. and Goff, D.V. 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am. J. Clin. Nutr. 34:362-366.

Jenkins, D.J.A., Wolever, T.M.S., Leeds, A.R., Gassull, M.A., Delawari, J.B., Goff, D.C., Metz, G.L., and Alberti, K.G.M.M. 1978. Dietary fibers, fiber analogues and glucose tolerance: importance of viscosity. Br. Med. J. 1:1392-1394.

Klopfenstein, C.F. and Hoseney, R.C. 1987. Cholesterol-lowering effect of β-glucan enriched bread. Nutr. Rep. Int. 36:1091-1098.

Lineback, D.R. 1984. The starch granule organization and properties. Bakers Digest. March 13, 1984:16-21.

Lund, E.K. and Johnson, I.J. 1991. Fermentable carbohydrate reaching the colon after ingestion of oats in humans. J. Nutr. 121:311-317.

MacLeod, A.M., Travis, D.C. and Wreag, D.G. 1953. Studies on the free sugars of the barley grain III. Changes in sugar content during malting. J. Inst. Brew. 54:154-165.

Manners, D.J. 1985. Some aspects of the structure of starch. Cereal Foods World 30:461-467.

Manners, D.J. 1988. Recent developments in our understanding of amylopectin

structure. Carhy. Polym. 11:87-112.

Marshall, J.J. 1972. The structure, function and metabolism of the cereal carbohydrates. Wallerstein Communications 34:116.

McCleary, B.V. and Glennie-Holmes, M. 1985. Enzymatic Quantitation of (1→3)(1→4)-β-glucan in barley and malt. J. Inst. Brew. 91:285-295.

McGuire, C.F. and Hockett, E.A. 1981. Effect of awn length and naked caryopsis on malting quality of "Betzes" barley. Crop Sci. 21:18-21.

McIntosh, G.H., Whyte, J., McArthur, R. and Nestel, P.J. 1991. Barley and wheat foods: influence on plasma cholesterol concentrations in hypercholesterolemic men. Am. J. Clin. Nutr. 53:1205-1209.

Merritt, N.R. 1967. A new strain of barley with starch of high amylose content. J. Inst. Brew. 73:583-585.

Mori, T. 1990. Chemical characterization and metabolic function of soluble dietary fiber from select milling fractions of a hull-less barley and its waxy starch mutant. Masters Thesis. Montana State University, Bozeman, MT.

Morrison, W.R., and Laignelet, B. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. J. of Cereal Sci. 1:9-20.

Morrison, W.R., Scott, D.C. and Karkalas, J. 1986. Variation in the composition and physical properties of barley starches. Starch 38:374-379.

Moss, B.R., El-Negoumy, A.M. and Newman, C.W. 1983. The nutritional value of waxy barleys for broilers. Anim. Feed Sci. Tech. 8:25-34.

Moss, B.R., Laudert, S.B., Stockton, G., Miller, C. and Baldridge, D. 1980. Nutritional value of waxy vs normal barley for ruminants. Nutr. Rep. Int. 21:487-496.

Munck, L. 1981. Barley for food, feed and industry. pp427-459. In: Cereals: a renewable resource, theory and practice. AACC. St. Paul. MN.

Nakao, S. 1950. On the waxy barley in Japan. Seikin Ziko 4:111-113.

National Research Council. 1984. Nutrition requirements of poultry, 8th revised edition. National Academy of Science. National Academy Press. Washington D.C.

NDDG. 1985. National Diabetes Data Group: Diabetes in America. NIH Publication.

Newman, R.K., Lewis, S.E., Newman, C.W., Boik, R.J. and Ramage, R.T. 1989a. Hypocholesterolemic effect of barley foods on healthy men. Nutr. Rep. Int. 39: 749-759.

Newman, C.W. and McGuire, C.F. 1985. Nutritional quality of barley. pp403-456. In: Barley. D.C. Rasmusson (ed). Agronomy Monograph No. 26, ASA, CSA, SSSA. Madison, WI. USA.

Newman, C.W. and Newman, R.K. 1992. Nutritional aspects of barley seed structure and composition. pp351-368. In: Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. P.R. Shewry, (ed). C.A.B International, Oxon, UK.

Newman, R.K. and Newman, C.W. 1987. β-glucanase effect on the performance of broiler chicks fed covered and hull-less barley isotypes having normal and waxy starch. Nutr. Rep. Int. 36:693-699.

Newman, R.K. and Newman, C.W. 1991. Barley as a food grain. Cereal Foods World 36:800-805.

Newman, R.K., Newman, C.W. and Graham, H. 1989b. The hypocholesterolemic function of barley ß-glucans. Cereal Foods World 34:883-886.

Newman, R.K., Newman, C.W., Hofer, P.J. and Barnes, A.E. 1991. Growth performance and lipid metabolism of broiler chicks fed hull-less barleys with and without supplemental ß-glucans. Plant Foods for Human Nutrition 41:371-380.

O'Dea, K., and Holm, J. 1985. Rate of starch hydrolysis *in vitro* as a predictor for metabolic responses to complex carbohydrate *in vivo*. Am. J. Clin Nutr. 34:1991-1993.

Panlasigui, L.N., Thompson, L.U., Juliano, B.O., Perez, C.M., Jenkins, D.J.A. and Yiu, S.H. 1992. Extruded rice noodles: starch digestibility and glycemic response of healthy and diabetic subjects with different habitual diets. Nutrition Research 12:1195-1204.

Pomeranz, Y., Eslick, R.F. and Robbins, G.S. 1972. Amino acid composition and malting and brewing performance of high amylose and hiproly barleys. Cereal Chem. 49:629-635.

Powell, W., Galigari, P.D.S., Swanston, J.S. and Jinks, K.L. 1985. Genetical investigations into β-glucan content in barley. Theoretical and Applied Genetics. 71:461-466.

Prosky, L., Asp, N.-G., Schweizer, T.F., DeVries, J.W. and Furda, I. 1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products:

Interlaboratory Study. J. Assoc. Off. Anal. Chem. 71:1017-1023.

Qureshi, A.A., Burger, W.C., Peterson, D.M. and Elson, C.E. 1986. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J. Biol. Chem. 261:10544-10552.

Ring, S.G., Gee, J.M., Whittam, M, Orford, P., and Johnson, I.T. 1988. Resistant starch: Its chemical form in foodstuffs and effect on digestibility *in vitro*. Food Chem. 28:97.

Rubin, M., Johnson, J.W., Schoonover, D.R. and Smith, R.T. 1974. Relative nutritive value of barley, corn and oat cultivars. Barley Newsletter. 18:35.

Russell, P.L., Berry, C.S. and Greenwell, P. 1989. Characterization of resistant starch from wheat and maize. J. Cereal Sci. 9:1-15.

Salamonsson, A-C., Theander, O. and Åman, P. 1980. Composition of normal and high-lysine barleys. Swed. J. Agric. Rec. 10:11-16.

Sandstedt, R.M. and Hites, B.O. 1968. Genetic variations in maize: effects on the properties of starches. Cereal Science Today. 13:82-92.

SAS. 1988. Statistical Analysis Systems User's Guide: Basics. Version 6.03 edition. SAS Inst. Inc.: Cary, NC.

Schneeman, B.O. 1989. Dietary fiber. A scientific status summary by the institute of food technologists expert panel on food safety and nutrition. Food Technol. 43:133.

Sievert, D., Czuchajowska, Z. and Pomeranz, Y. 1991. Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII starch and enzyme-resistant starch residues. Cereal Chem. 68:86-91.

Sievert, D. and Pomeranz, Y. 1989. Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical and microscopic methods. Cereal Chem. 66:342-347.

Siljeström, M., Eliasson, A-C. and Björck, I. 1989. Characterization of resistant starch from autoclaved wheat starch. Starch 41:147.

Southgate, D.A.T. 1982. Definition and terminology of dietary fiber. In: Dietary Fiber in Health and Disease, G.V. Vahouny and D. Kritchevsky, (eds). Plenum Press, New York.

Srinivasa Rao, P. 1971. Studies on the nature of carbohydrate moiety in high yielding

varieties of rice. J. Nutr. 101:879-884.

Stark, J. R. and Yin, X.S. 1986. The effect of physical damage on large and small barley starch granules. Starch 38:369-374.

Staute, R.G., Woodward, J.R., Fincher, G.B. and Stone, B.A. 1983. Water soluble (1→3), (1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. III. Distribution of cellotriosyl and cellotetraosyl residue. Carbohydrate Polymers 3:299-312.

Sugimoto, Y., Fujita, S., Takaya, T. and Fuwa, H. 1980. *In vivo* digestion of banana starch granules. Starch 32:290-294.

Sullins, R.D. and Roony, L.W. 1974. Microscopic evaluation of the digestibility of sorghum lines that differ in endosperm characteristics. Cereal Chem. 51:134-142.

Szczodrak, J. and Pomeranz, Y. 1991. Starch and enzyme-resistant starch from high-amylose barley. Cereal Chem. 68:589-596.

Theander, O and Westerlund, E.A. 1986. Dietary fiber 3: Improved procedures for analysis of dietary fiber. J. Agric. Food Chem. 34:330-336.

Theander, O., Westerlund, E.A., Åman, P. and Graham, H. 1989. Plant cell walls and monogastric diets. Anim. Feed. Sci. Tech. 23:205-225.

Thorne, M.J., Thompson, L.U. and Jenkins, D.J.A. 1983. Factors affecting starch digestibility and the glycemic response with special reference to legumes. Am. J. Clin. Nutr. 38:481-488.

Torneport, L.J., Salomonsson, A.-C. and Theander, O. 1990. Chemical characterization of bromine oxidized potato starch. Starch 42:413-417.

Ullrich, S.E., Clancy, J.A., Eslick, R.F. and Lance, R.C.M. 1986. β-glucan content and viscosity of extracts from barley. J. Cereal Sci. 4:279-285.

Vachon, C., Jones, J.D., Wood, P.J. and Savoie, L. 1988. Concentration effect of soluble dietary fibers on postprandial glucose and insulin in the rat. Can. J. Physiol. Pharmacol. 66:801-806.

Vahouny, G.V. and Kritchevsky, D., (eds). 1986. Dietary Fiber Basics and Clinical Aspects. Plenum Press, New York.

von Wettstein-Knowles, P. 1992. Cloned and mapped genes: current status. pp73-98. In: Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. P.R. Shewry, (ed). C.A.B International, Oxon, UK.

Wade, N.L. and Morris, S.C. 1982. Rapid determination of sugars in cantaloupe melon juice by high-performance liquid chromatography. J. Chromatogr. 240:257-262.

Walker, J.L. and Merritt, N.R. 1969. Genetic control of abnormal starch granules and high amylose content in a mutant of Glacier barley. Nature 221:482-483.

Wang, L., Newman, R.K., Newman, C.W., and Hofer, P.J. 1992. Relationship of viscosity of the small intestine content to growth performance and plasma cholesterol concentration in broiler chicks fed barley diets with or without β-glucanase. J. Nutr. 122:2292-2297.

Wolever, T.M.S. and Jenkins, D.J.A. 1986. Effect of dietary fiber and foods on carbohydrate metabolism. pp87-119. In: Spiller, CRC Handbook of Dietary Fiber in Human Nutrition. CRC Press, Boca Raton.

Wootton, M. and Chandhry, M.A. 1979. Enzyme digestibility of modified starches. Starch 31:224-228.

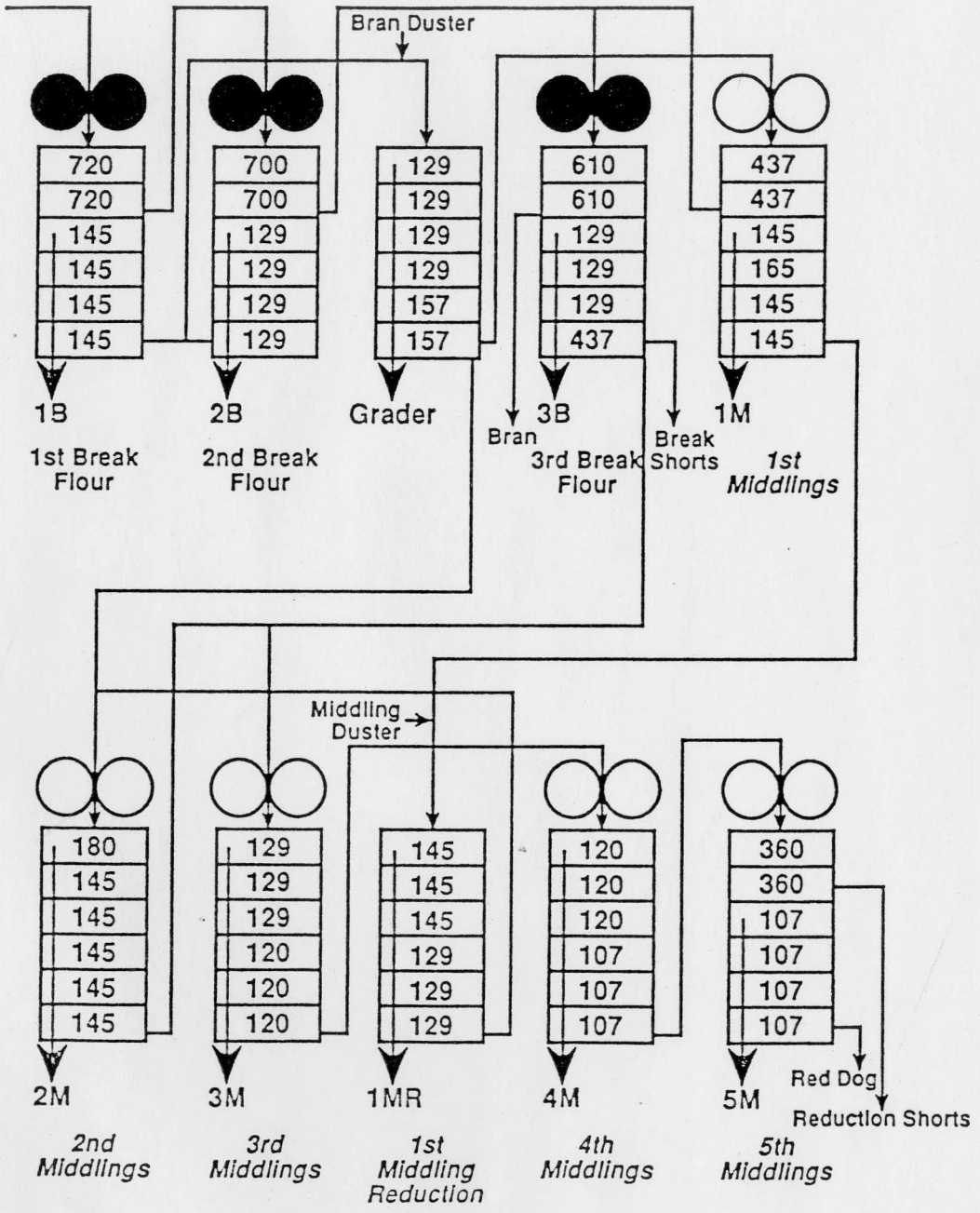
Xue, Q., Newman, R.K., Newman, C.W. and McGuire, C.F. 1991. Waxy gene effects on β-glucan, dietary fiber content and viscosity of barleys. Cereal Res. Comm. 19:399-404.

Zobel, H.F. 1992. Starch granule structure. pp 1-36. In: Development in Carbohydrate Chemistry. R.J. Alexander and H.F. Zobel, (eds). Am. Assoc. Cereal Chem. Inc., St Paul, MN.

APPENDIX

Figure 11

Milling Flow Sheet for MIAG MULTOMAT 8-Roller Dry Mill (Sieve Cloth Openings in Microns)



MONTANA STATE UNIVERSITY LIBRARIES
3 1762 10198975 2