# Nutritional implications of amylose-amylopectin ratio in barley cultivars for rats and swine

by Campbell Christopher Calvert

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University


Abstract:
Weanling rats were fed purified starch diets in each of two nitrogen and energy balance trials and one growth trial. The starches were extracted from normal Compana (NC), waxy Compana (WC), normal Glacier (NG) and high-amylose Glacier (HAG) barleys. Diets were balanced with vitamins, minerals and casein as the source of protein. Rats fed 14.5% protein diets showed no differences for retained nitrogen or digestible energy. However, digestible nitrogen was increased (P<.01) in rats fed the WC diet compared to those fed the NC and HAG starches. Rats fed the NG diets were not significantly different in these parameters from those fed the other three diets. Similar results were noted when rats were fed 10% protein diets except digestible nitrogen was lowered (P<.01) in rats fed the HAG starch diet compared to the other three diets. Rats in the growth trial fed 14.5% protein diets showed no differences in feed efficiency or feed consumption, however, gain was higher (P<.10) in rats fed the NG starch as compared to those fed the NC or HAG. Rats fed the WC were not different in rate of gain from those fed the other three diets. Protein efficiency ratios were higher (P<.05) for rats fed the NG and NC compared to those fed the HAG starch diet.

Isonitrogenous, isocaloric 10% protein diets were prepared from isogenic WC and NC barleys and fed to rats in growth and balance trials for nitrogen and energy. There were no differences in rat performance or nitrogen and energy digestibility due to barley. Thirty-two pigs were fed NC or WC barley diets with and without supplemental protein in each of two trials. Those fed the supplemented diets gained faster (P<.01). Pigs fed the unsupplemented WC diet gained faster as compared to pigs fed the normal isogene in the first trial but the results were reversed in the second trial. The WC contained more total protein in the first trial than the NC whereas the isogenes were nearly iso-nitrogenous in the second trial. Carcass characteristics were in general improved by supplemental protein although loin area was not affected by added protein in the second trial.

Isocaloric, isonitrogenous 10% protein diets were prepared from HAG and NG isogenic barleys and fed to rats in growth and balance trials for nitrogen and energy. Rats fed HAG barley had a greater rate of gain (P<.01), consumed more feed (P<.10), were more efficient. (P<.10) and had higher PER (P<.10) than rats consuming the NG barley diets. Thirty-two pigs were fed protein supplemented growing-finishing diets prepared from NG or HAG in each of two trials. There were no differences in growth data or carcass characteristics with the exception of an increase in percentage of ham (P<.05) in pigs fed the NG diet in the first trial. In both trials, pigs fed the NG barley had a slight increase in feed utilization (P<.01).

NUTRITIONAL IMPLICATIONS OF AMYLOSE-AMYLOPECTIN RATIO IN BARLEY CULTIVARS FOR RATS AND SWINE

by

CAMPBELL CHRISTOPHER CALVERT

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

of

MASTER OF SCIENCE

in

Animal Science

Approved:

C. W. Newman
Chairman, Examining Committee

R. L. Blackwell
Head, Major Department

H. Goering
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1975

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. C. W. Newman for his assistance and encouragement with this thesis, the course work of both undergraduate and graduate studies and his helpful suggestions in many other areas.

A special thanks to Dr. Ken Goering for his help in extracting the starches and for his valuable suggestions and comments concerning the thesis research.

Thanks is also due Professor Bob Eslick for the breeding and increasing of those barleys used in this research. His comments and suggestions have proved very helpful. I also wish to thank Dr. R. L. Blackwell for supporting this research and giving encouragement throughout the project. A special thanks is due to Dr. Larry Jackson and Dr. Ray Ditterline for their time and help with my graduate program. Appreciation is also expressed to the rest of my graduate committee, Dr. Pete Moss and Dr. Jane Lease.

The help of Dr. Ervin Smith, Dr. Don Kress and Mr. Bob Friedrich with statistical analysis is greatly appreciated. The staff at the Nutrition Center were very helpful with chemical analysis and care of rats. Thanks to Don McCarl for assisting with care of the pigs.

I wish to thank my wife and my family for their support throughout my graduate studies.

# TABLE OF CONTENTS

Page

VITA . . . . . . . . . . . . . . . . . . . . . . . . . . . . ii

ACKNOWLEDGEMENTS . . . . . . . . . . . . . . . . . . . . . . iii

INDEX TO TABLES . . . . . . . . . . . . . . . . . . . . . . vi

INDEX TO FIGURES . . . . . . . . . . . . . . . . . . . . . . viii

INDEX TO APPENDIX . . . . . . . . . . . . . . . . . . . . . ix

ABSTRACT . . . . . . . . . . . . . . . . . . . . . . . . . . xi

CHAPTER I . . . . . . . . . . . . . . . . . . . . . . . . . 1

INTRODUCTION . . . . . . . . . . . . . . . . . . . . . . . 1

CHAPTER II . . . . . . . . . . . . . . . . . . . . . . . . 3

LITERATURE REVIEW . . . . . . . . . . . . . . . . . . . . 3

Chemical Characteristics . . . . . . . . . . . . . . . . . 3
Starch Degradation . . . . . . . . . . . . . . . . . . . . 5
Amylose Action on Starch . . . . . . . . . . . . . . . . . 7
High-Amylose Starch . . . . . . . . . . . . . . . . . . . 8
Feeding Trials with High-Amylose Grains. . . . . . . . . . 11
Waxy Starch. . . . . . . . . . . . . . . . . . . . . . . . 12
Feeding Trials with Waxy Grains. . . . . . . . . . . . . . 15

CHAPTER III . . . . . . . . . . . . . . . . . . . . . . . 19

COMPARISON OF WAXY, HIGH-AMYLOSE AND NORMAL BARLEY
STARCHES IN PURIFIED RAT DIETS . . . . . . . . . . . . . 19

SUMMARY . . . . . . . . . . . . . . . . . . . . . . . . . 19

Introduction . . . . . . . . . . . . . . . . . . . . . . . 20
Materials and Methods . . . . . . . . . . . . . . . . . . 21
Results and Discussion . . . . . . . . . . . . . . . . . . 22

Page

CHAPTER IV . . . . . . . . . . . . . . . . . . . . . . . . . . 28

WAXY COMPANA VS NORMAL COMPANA BARLEY IN RAT AND PIG DIETS . . 28

SUMMARY . . . . . . . . . . . . . . . . . . . . . . . . . . 28

Introduction . . . . . . . . . . . . . . . . . . . . . . . . 29
Experimental Procedure . . . . . . . . . . . . . . . . . . . 30
Results. . . . . . . . . . . . . . . . . . . . . . . . . . . 33
Discussion . . . . . . . . . . . . . . . . . . . . . . . . . 40

CHAPTER V. . . . . . . . . . . . . . . . . . . . . . . . . . 43

HIGH-AMYLOSE GLACIER VS NORMAL GLACIER BARLEY IN RAT AND PIG DIETS . . . . . . . . . . . . . . . . . . . . . 43

SUMMARY. . . . . . . . . . . . . . . . . . . . . . . . . . . 43

Introduction . . . . . . . . . . . . . . . . . . . . . . . . 44
Experimental Procedures. . . . . . . . . . . . . . . . . . . 45
Results. . . . . . . . . . . . . . . . . . . . . . . . . . . 48
Discussion . . . . . . . . . . . . . . . . . . . . . . . . . 49

CHAPTER VI . . . . . . . . . . . . . . . . . . . . . . . . . 58

SUMMARY AND CONCLUSIONS. . . . . . . . . . . . . . . . . . . 58

APPENDIX . . . . . . . . . . . . . . . . . . . . . . . . . . 61

LITERATURE CITED . . . . . . . . . . . . . . . . . . . . . . 69

# INDEX TO TABLES

TABLE - CHAPTER III — Page

1 COMPOSITION OF BARLEY STARCH DIETS . . . . . . . . . . . . . . 23

2 PERCENT COMPOSITION OF EXTRACTED STARCHES USED IN FORMULATING PURIFIED STARCH DIETS, TRIAL 1. . . . . . . . 23

3 PERFORMANCE OF RATS FED COMPANA AND GLACIER BARLEY STARCHES. . . . . . . . . . . . . . . . . . . . . . . . 27

CHAPTER IV

1 PERCENTAGE COMPOSITION OF BARLEY AND CORN STARCH CASEIN DIETS FED TO RATS . . . . . . . . . . . . . . . . . . . 31

2 PERCENTAGE COMPOSITION OF SWINE DIETS. . . . . . . . . . . . . 34

3 PROXIMATE ANALYSIS, CALCIUM, PHOSPHORUS, AMYLOSE AND AMYLOPECTIN CONTENT OF THE COMPANA BARLEY ISOGENIC PAIRS . . 35

4 AMINO ACID COMPOSITION OF THE COMPANA BARLEY ISOGENIC PAIRS. . . . . . . . . . . . . . . . . . . . . . . . . . . . 36

5 PERFORMANCE OF RATS FED DIETS PREPARED FROM NORMAL (NC) AND WAXY COMPANA (WC) ISOGENIC BARLEY PAIRS, TRIAL 1 . . . 38

6 FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF PIGS FED NORMAL AND WAXY COMPANA ISOGENIC BARLEYS (PAIR 2), WITH AND WITHOUT SUPPLEMENTAL PROTEIN, TRIAL 2 . . . . . . 39

7 FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF PIGS FED NORMAL AND WAXY COMPANA ISOGENIC BARLEYS (PAIR 3,) WITH AND WITHOUT SUPPLEMENTAL PROTEIN, TRIAL 3 . . . . . . 41

CHAPTER V

1 PERCENTAGE COMPOSITION OF BARLEY AND CORN STARCH CASEIN DIETS FED TO RATS. . . . . . . . . . . . . . . . . . . . . . . 51

2 PERCENTAGE COMPOSITION OF SWINE DIETS. . . . . . . . . . . . . 52

3 PROXIMATE ANALYSIS, CALCIUM, PHOSPHORUS, AMYLOSE AND AMYLOPECTIN CONTENT OF GLACIER BARLEY ISOGENIC PAIRS . . . 53

| Table | | Page |
|---|---|---|
| 4 | AMINO ACID COMPOSITION OF THE GLACIER BARLEY ISOGENIC PAIRS. . . . . . . . . . . . . . . . . . . . . . . . . . | 54 |
| 5 | PERFORMANCE OF RATS FED DIETS PREPARED FROM NORMAL AND HIGH-AMYLOSE GLACIER ISOGENIC BARLEYS, TRIAL 1 . . . | 55 |
| 6 | FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF PIGS FED NORMAL AND HIGH-AMYLOSE GLACIER ISOGENIC BARLEYS (PAIR 3), TRIAL 2. . . . . . . . . . . . . . . . . . | 56 |
| 7 | FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF PIGS FED NORMAL AND HIGH-AMYLOSE GLACIER ISOGENIC BARLEYS (PAIR 2), TRIAL 3. . . . . . . . . . . . . . . . . . | 57 |

## INDEX TO FIGURES


| Figure | | Page |
|---|---|---|
| 1 | Brabender amylograms of starches from Glacier and Compana isogenes . . . . . . . . . . . . . . . . . . . . . . | 24 |

INDEX TO APPENDIX


| TABLE | | Page |
|---|---|---|
| 1 | MEAN SQUARES OF DATA FROM PURIFIED BARLEY STARCH DIETS FED TO RATS, CHAPTER 3, TRIAL 1. . . . . . . . . . . | 62 |
| 2 | MEAN SQUARES CALCULATED FROM NITROGEN AND ENERGY BALANCE OF BARLEY STARCH DIETS, CHAPTER 3, TRIAL 1 AND 2. . . . . . . . . . . . . . . . . . . . . . . . | 62 |
| 3 | MEAN SQUARES OF DATA FROM COMPANA BARLEY DIETS FED TO RATS, CHAPTER 4, TRIAL 1. . . . . . . . . . . . . . . . . | 63 |
| 4 | MEAN SQUARES COMPUTED FROM NITROGEN AND ENERGY BALANCE OF COMPANA BARLEY DIETS, CHAPTER 4, TRIAL 1. . . . | 63 |
| 5 | MEAN SQUARES OF GROWTH DATA FROM COMPANA BARLEY FED TO PIGS, CHAPTER 4, TRIAL 2. . . . . . . . . . . . . . . . | 64 |
| 6 | MEAN SQUARES OF CARCASS DATA FROM COMPANA BARLEY FED TO PIGS, CHAPTER 4, TRIAL 2. . . . . . . . . . . . . . . . | 64 |
| 7. | MEAN SQUARES OF GROWTH DATA FROM COMPANA BARLEY FED TO PIGS, CHAPTER 4, TRIAL 3. . . . . . . . . . . . . . . . | 65 |
| 8. | MEAN SQUARES OF CARCASS DATA FROM COMPANA BARLEY FED TO PIGS, CHAPTER 4, TRIAL 3. . . . . . . . . . . . . . | 65 |
| 9. | MEAN SQUARES OF DATA FROM GLACIER BARLEY FED TO RATS, CHAPTER 5, TRIAL 1 . . . . . . . . . . . . . . . . . . | 66 |
| 10. | MEAN SQUARES COMPUTED FROM NITROGEN AND ENERGY BALANCE OF GLACIER BARLEY FED TO RATS, CHAPTER 5, TRIAL 1. . . . . . . . . . . . . . . . . . . . . . . . | 66 |
| 11. | MEAN SQUARES OF GROWTH DATA FROM GLACIER BARLEY FED TO PIGS, CHAPTER 5, TRIAL 2. . . . . . . . . . . . . . . . | 67 |
| 12. | MEAN SQUARES OF CARCASS DATA FROM GLACIER BARLEY FED TO PIGS, CHAPTER 5, TRIAL 2. . . . . . . . . . . . . . . . | 67 |

TABLE Page

13. MEAN SQUARES OF GROWTH DATA FROM GLACIER BARLEY FED TO PIGS, CHAPTER 5, TRIAL 3 . . . . . . . . . . . . . . 68

14. MEAN SQUARES OF CARCASS DATA FROM GLACIER BARLEY FED TO PIGS, CHAPTER 5, TRIAL 3 . . . . . . . . . . . . . . 68

## ABSTRACT

Weanling rats were fed purified starch diets in each of two nitrogen and energy balance trials and one growth trial. The starches were extracted from normal Compana (NC), waxy Compana (WC), normal Glacier (NG) and high-amylose Glacier (HAG) barleys. Diets were balanced with vitamins, minerals and casein as the source of protein. Rats fed 14.5% protein diets showed no differences for retained nitrogen or digestible energy. However, digestible nitrogen was increased ($P<.01$) in rats fed the WC diet compared to those fed the NC and HAG starches. Rats fed the NG diets were not significantly different in these parameters from those fed the other three diets. Similar results were noted when rats were fed 10% protein diets except digestible nitrogen was lowered ($P<.01$) in rats fed the HAG starch diet compared to the other three diets. Rats in the growth trial fed 14.5% protein diets showed no differences in feed efficiency or feed consumption, however, gain was higher ($P<.10$) in rats fed the NG starch as compared to those fed the NC or HAG. Rats fed the WC were not different in rate of gain from those fed the other three diets. Protein efficiency ratios were higher ($P<.05$) for rats fed the NG and NC compared to those fed the HAG starch diet.

Isonitrogenous, isocaloric 10% protein diets were prepared from isogenic WC and NC barleys and fed to rats in growth and balance trials for nitrogen and energy. There were no differences in rat performance or nitrogen and energy digestibility due to barley. Thirty-two pigs were fed NC or WC barley diets with and without supplemental protein in each of two trials. Those fed the supplemented diets gained faster ($P<.01$). Pigs fed the unsupplemented WC diet gained faster as compared to pigs fed the normal isogene in the first trial but the results were reversed in the second trial. The WC contained more total protein in the first trial than the NC whereas the isogenes were nearly isonitrogenous in the second trial. Carcass characteristics were in general improved by supplemental protein although loin area was not affected by added protein in the second trial.

Isocaloric, isonitrogenous 10% protein diets were prepared from HAG and NG isogenic barleys and fed to rats in growth and balance trials for nitrogen and energy. Rats fed HAG barley had a greater rate of gain ($P<.01$), consumed more feed ($P<.10$), were more efficient ($P<.10$) and had higher PER ($P<.10$) than rats consuming the NG barley diets. Thirty-two pigs were fed protein supplemented growing-finishing diets prepared from NG or HAG in each of two trials. There were no differences in growth data or carcass characteristics with the exception of an increase in percentage of ham ($P<.05$) in pigs fed the NG diet in the first trial. In both trials, pigs fed the NG barley had a slight increase in feed utilization ($P<.01$).

CHAPTER I

# INTRODUCTION

The world's growing population is demanding more of agricultural production than ever before in the history of man. Science is continually trying to learn how to produce greater quantities of higher quality food on a smaller area of tillable land.

It is first necessary to define those characteristics desirable for increasing food value in order that plant breeders may incorporate those qualities in new varieites. There has been considerable research interest in recent years with new varieties of feed grains that contain a more optimum amino acid balance. Opaque-2 corn and Hiproly barley which contain more total protein, lysine, methionine and other essential amino acids than standard varieties are receiving increased attention by nutritionists and plant breeders.

The primary nutritional energy source of man and domestic farm animals is carbohydrate. Starch represents the greatest percentage of the carbohydrate in energy feeds such as cereal grains. The importance of increasing starch digestion resulting in increased energy utilization becomes very apparent under the present economic situation. The starch of cereal grains is known to vary chemically in a number of ways. Mutant strains of corn, sorghum, maize and barley are known to have different amylose amylopectin ratios. It is possible that one ratio may prove to be better utilized and have interactions with other nutrients. Molecular structure and granule size are also known to vary with cereal species which may affect nutritional value.

In Montana, the major feed grain is barley, a large portion of which is marketed through cattle and hogs. Barley is well suited for semi-arid regions of the world such as Montana as it can be grown further north, at higher elevations and with less water than any other cereal. Barley starch has not been investigated as closely as corn starch and much of what is known about corn starch may not be applicable to barley starch. This thesis is concerned with evaluating different starch types and associated nutritional characteristics in two barley cultivars.

# CHAPTER II

## LITERATURE REVIEW

The average barley (IRN 4-00-534) contains 64.5% starch (N.R.C. 1971). In many feedlots, the diet for finishing cattle may contain up to 75% barley, or about 50% barley starch. Likewise, a finishing swine diet may have as much as 85% barley or about 60% barley starch. It is easy to see the importance of improving the efficiency of starch utilization. The concern of this thesis is the correlation of the nutritional responses with starch properties expressed by two isogenic lines of two barley cultivars.

### Chemical Characteristics

Starch consists of two distinct molecular forms of polyemerized glucose molecules; a linear homopolysaccharide, and a branched homopolysaccharide. The linear fraction is called amylose and the branched fraction called amylopectin (Pasur, 1965; Greenwood, 1970). The two fractions of starch can be separated by selective precipitation of starch solutions (Guthrie and Honeyman, 1968). The addition of n-butyl alcohol to an aqueous solution of starch will precipitate out the amylose. This fraction is obtained in the form of needles and makes up about 28% of the corn starch. The second fraction, amylopectin, can then be precipitated by the addition of methanol. Industrially, the separation of starch is done by salting out the amylose with magnesium sulphate. Amylose (90% pure) separates out by keeping an aqueous solution of maize starch below its boiling point for 8 hours. Amylopectin

(87% pure) is recovered by evaporation of the residual solution.

The two fractions of starch are associated in a starch granule by hydrogen bonding. These bonds may be direct hydrogen bonds or water hydrate bridges that form radially oriented micelles or crystalline areas (Leach, 1965). In cereal grains, these starch granules are found in the endosperm and are surrounded by a thin layer of protein (Greenwood, 1970). As the starch granule grows, it takes on a layered effect showing a shell type structure, (Badenhuizen, 1965; Greenwood, 1970). The actual method of starch synthesis and deposition of the starch in the granule is not known. The layers of the starch molecule contain the two fractions. They are held together by hydrogen bonds (Badenhuizen, 1965; Pazur, 1965). The linear fraction, amylose, is composed of α-D-glucose units linked by α-(1-4) bonds (Sandstedt, 1965). Molecular weights of amylose indicate it contains 200 to 350 α-D-glucose residues.

Amylose preparations have irregularities related to the line structure of the starch molecule that limit the action of α-amylase (Weil and Bratt, 1967; Banks and Greenwood, 1967). The action of pullulanase, an enzyme specific for the α-(1-6) branched linkage, indicates that not all of the amylose is strictly straight chained but also has a limited degree of branching (Banks and Greenwood, 1967). The branched fraction, amylopectin, is composed of α-D-glucose units linked in straight chains by α-(1-4) linkages and the branch points being α-(1-6) linkages. Amylopectin's structure ranges from several thousand to millions of glucose units (Pazur, 1965). The branches of the amylopectin molecule contain twenty to thirty $\alpha$-glucose units (Wolfrom and Khadem, 1965; Greenwood, 1970). Full details of the fine structure of amylopectin are not yet complete (Greenwood, 1970).

By use of X-ray diffraction, starches have been sorted into two major groups with regard to their X-ray patterns. The A-type starch is found in most cereal starches, such as corn, wheat and barley. A-type starch crystallization is favored with conditions of high temperature and low water content, while B-type starch crystallization is more the result of low temperatures and high water content (Hizukure, 1961). The B-type starch is a tuber starch found in roots. As of yet, the significance of the differences between A and B type crystallization is not clear. However, a chain length dependence has been shown with long chained amylopectin favoring the B-type crystallization. Ordinary corn starch gives an A-type pattern, yet high amylose corn starch with long chain amylopectin gives a B pattern (Gallant _et al_. 1972 and French, 1973). Short chain amyloses, $G_9$-$G_{13}$, have been crystalized only in the A form (French and Youngquist, 1960).

Starch Degradation

The ability of the starch to be degraded determines the amount of energy received from the starch. The starch consists of a gel-like or amorphous region and an ordered crystalline region in approximately a

1:1 ratio (Hellman, et al. 1952). In an aqueous solution, the starch granule is limited in its ability to absorb cold water. By slightly heating the solution, the granule will imbibe more water with no harmful effect to the starch.

As the aqueous solution of starch is heated, more water is absorbed into the amorphous region and swelling continues to occur until the gelatinization temperature is reached (60 to 80$^{o}$ C for most starches). The gelantinization temperature varies with species of starch and the degree of molecular association of the starch (Leach, 1965; Rooney and Clark, 1968; Greenwood, 1970). As the increased swelling and subsequent gelatinization of the starch granule takes place, hydrogen bonds are broken with a resultant loss of crystalline structure and the development of fractures in the starch granule. Gelatinization of starch granules also occurs when the granule has been damaged by pressure, heat, sheer or strain and moisture (Sandstedt and Mattern, 1960; Sullivan and Johnson, 1964; Anstaett et al., 1969). When gelatinization has taken place, the starch has undergone irreversible swelling. If the starch is cooled after irreversible swelling the solution sets up in a "rigid" gel. However, if the aqueous starch solution is allowed to set in cold water or slightly heated water, reversible swelling takes place. The water is absorbed into the gel phase and does not effect the crystalline phase of the starch granule. After cooling and drying, the starch appears unaltered except that the gel phase has been leached out. In the same way, acid hydrolysis under cool conditions can leach out the gel phase and leave an insoluble crystalline residue behind (Kainuma and French, 1972). It has originally been thought that enzymatic digestion of starch proceeded in much the same way. Lathe and Ruthven (1956) demonstrated that the fine structure of the gel phase in the granule would not allow the large amylase molecule inside. Amylase is currently thought to work on the surface of the granule. Leach and Schock (1961) suggested that cereal grain starches have a coarse sponge-like structure or possess pores large enough to admit the enzyme molecule. Enzymatic digestion in this case would seem to cause the formation of pits in the starch granule. The pitted structure of the starch granule acted upon by amylase has been demonstrated with the scanning electron microscope (Dronzek et al. 1972). Gelatinization of starches causes the fragmentation of the starch granule. This fragmentation allows amylase to enter the starch molecule. This results in rapid starch digestion due to internal and external digestion. Cooked starch digests faster than raw starch (Schwemmer, 1945; Walker and Hope, 1963). Sandstedt and Mattern (1960) showed that rate of digestion was dependent upon the accessibility of the starch molecule to the amylase enzymes.

Amylase Action on Starch

Alpha-amylases are endo-enzymes attacking the linear starch molecule at any random interior point but only at the $\alpha$-(1-4) linkages.

The action of alpha-amylase on the linear amylose molecule causes the polysaccharide to be degraded to glucose, maltose and maltotriose (Robyt and French, 1967). Beta-amylase also hydrolyzes α-(1-4) glucosidic linkages, but only splits off maltose units from the non-reducing end of the starch molecule. This enzyme is found in plants, but not animals. The enzyme is important for its use in determining structure of starches; however, it is of little use in starch digestion in the animal. If the alpha-amylase is allowed to act on amylopectin *in vitro*, the α-(1-6) linkages will resist amylitic digestion and give rise to a well defined set of oligosaccharides containing the resistant branch linkage (French, 1973). These oligosaccharides are resistant to further digestion by alpha or beta amylases. Certain enzymes in microorganisms, plants and animals are capable of breaking down the α-(1-6) glucosidic linkages. These enzymes have been shown to hydrolyze a starch consisting of both amylose and amylopectin directly to α-D-glucose (Pazur, 1965). The extent and amount of action of α-(1-6) hydrolases in the animal is unclear at this time and is of limited importance when considering digestion of high amylopectin starch by the monogastric animal. Microorganisms in the rumen of ruminant animals can easily digest the α-(1-6) linkage.

High-Amylose Starch

Grains typically have 25 to 30% of the starch present as amylose with barley starch having about 25% amylose (Merritt, 1967). Genotypes of pea (Nielsen and Gleason, 1945), maize (Deatherage et al., 1954), and barley (Walker and Merritt, 1969) have been reported to contain starches of high-amylose content. Most research with high-amylose starch seems to have been concerned with amylomaize starches. These starches were the result of breeding programs at the Northern Regional Laboratory of the U. S. Department of Agriculture designed to produce a starch with an amylose content of at least 85% (Langlois and Wagoner, 1967). The amylomaize starches possess characteristics very different from normal maize starch.

The higher the amylose content of a starch, the greater the irregularity of the granule shape (Mercier et al., 1970). The granules also seem to have decreased swelling properties with an increase in amylose. Banks et al. (1974) suggested that amylomaize starches and high-amylose pea starch also have a starch fraction that is neither amylose nor amylopectin in the conventional sense. Amylomaize starches have been shown to be rather unstable in aqueous solution (Greenwood and Hourston, 1971) with a tendency to aggregate while amylopectin showed no tendency to crystallize (Adkins and Greenwood, 1966).

Banks et al. (1974) has drawn five generalizations from the amylomaize research: (1) The amylose of amylomaize is the same as that of normal maize. (2) Normal amylopectin fractions have been demonstrated in all amylomaize starches, however, it is a very small part of the total starch in amylomaize. (3) Amylomaize starches contain a high degree of "intermediate material" that cannot be classified as amylose or amylopectin. (4) In many fractionation processes this intermediate material has been grouped with the normal branched material. It can now be separated from the normal branched material but linear and branched components of the intermediate material have not been separated as yet. (5) This intermediate material seems to be responsible for the granular properties and solution instability of amylomaize starches.

A mutant barley variety of Glacier, AC-38, was reported by Merritt (1967) to have an amylose content of 44% as compared to normal barley with 26% amylose. The high-amylose barley has very different granular properties from normal starch, specifically a smaller granule and higher gelatinization temperatures (Banks, et al., 1973). Banks et al. (1974) suggested that there is little or no "intermediate material" in the high-amylose barley starch.

The high amylose grains are of commercial interest. Amylose quickly forms edible films which are only slightly permeable to gases and could be used as containers for foodstuffs and present no waste disposal problems. Merritt (1967) has proposed that the high-amylose barley would improve the fermentability of barley by partial elimination of the $\alpha$-(1-6) glucoside linkage which is a source of residual carbohydrates. Pomeranz et al. (1972) reported that the tightly packed and bonded amylose reduced the availability of the amylose fraction to amylolytic action. The resistance to amylolytic action was so severe that the high-amylose barley could not be used in experimental procedures for brewing tests. This suggests that the high-amylose starch may not be as digestible as normal starch in the mammalian digestive system.

Feeding Trials with High-Amylose Grains

Sandstedt *et al*. (1962) digested cereal starches with pancreatin. The maize starches with greater than 47% amylose were very poorly digested, with digestion being only 22 to 50% as compared to the starches with lower amylose percentages.

Corn of high-amylose strains as compared to a normal variety were reported to have a decreased digestibility in rats (Borchers, 1962). Starch from the normal corn was 95.1% digestible while starch from the high-amylose strains was only 66.0 to 73.0% digestible. The digestibility of the starch decreased as the percent amylose increased. In chickens fed normal and high-amylose corn, the normal corn starch appeared to be totally digested while the chickens fed high-amylose starch had 58% starch in their feces (Ackerson, 1961). Preston *et al*. (1964) fed high-amylose and normal corn to lambs on a growth trial. There were only minor differences within each of the two treatments, none of which were significant. A digestibility trial with the lambs showed high-amylose corn to have a slightly greater total digestible nutrients (TDN) (71.2%) than the normal corn ration (64.0%). Krall (1972) reported that calves wintered for 140 days had slightly better gains and feed conversions when fed a high-amylose barley diet as compared to a normal barley diet. After wintering, the calves were put on a fattening diet for 156 days. The steers fed the normal barley gained slightly more efficiently. An average over the total trial, both wintering and fattening, showed that the high-amylose fed steers gained 0.93 kg/day and the steers fed the normal barley ration gained 0.91 kg/day.

Waxy Starch

Waxy starches contain only the branched or amylopectin fraction of normal starch. Unlike the linear fraction, amylose, waxy starches will not cause retrogradation of a starch paste upon cooling. Retrogradation or "setback" is seen as an irreversible precipitate or gel network primarily caused by amylose (Katzbeck, 1972). For this reason, waxy starches make better gels and pastes and are of great interest to the prepared food manufacturers. Leach and Thomas (1961) found that the waxy starches were more susceptible to enzyme attack than the corresponding normal starch. Similarly, normal pea starch is attacked more rapidly than the high-linear starch from wrinkled peas (Schwimmer, 1945). Electron micrographs revealed that waxy sorghum starch digested *in vitro* by procine $\alpha$-amylase show considerable point hydrolysis while the yellow endosperm sorghum starch exhibited radial erosion (Davis and Harbers, 1974). The waxy sorghum starch was also shown to be digested at a faster rate when the substrate to enzyme ratio was less than one mg starch per I.U. procine $\alpha$-amylase. At ratios higher than this, the waxy and yellow endosperm sorghum starches were digested at the same rate.

These researchers also found that split grain from the waxy and yellow endosperm sorghum was digested differentially when suspended in rumen fluid for 75 minutes or longer. Surface attack on the waxy starch of the split grain was by point hydrolysis with alternate layers digested inside the starch granule. The yellow endosperm starch was attacked without relation to the starch structure, but had large irregularly shaped holes where digestion had taken place. Harbers and Davis (1974) found that split sorghum grain recovered from the jejunum of rats and pigs showed erosion patterns similar to those seen from hydrolysis in the rumen and by purified procine $\alpha$-amylase. The waxy variety showed the characteristic point hydrolsis while the yellow endosperm type showed a radial erosion pattern. These researchers postulated that amylase enzymes seemed to be capable of diffusing through at least one cell wall layer and attacking the underlying starch granule causing starch digestion, although this is a relatively slow process. Endosperm cell walls were not removed as digestion of the sorghum took place, but rather appeared to shrink and flatten.

In a feeding trial by Newman et al. (1968), hulless Glacier and covered Glacier were found to be equal as a diet for pigs, however, the hulless Compana was superior as compared to the covered Compana.

Goering et al. (1970) found that the naked Compana was more waxy than either covered Compana or the two Glacier varieties fed by Newman et al. (1968), thus the difference in the feeding trial reported by these authors might be explained on the basis that amylopectin is more readily available to animals. Royals and Meits (1968) reported that starches with a high amylopectin content appeared to be digested at higher rates by human digestive fluids than those starches with lower amylopectin contents. Sandstedt et al. (1968) found that maize exhibiting the waxy trait was more readily digested than those starches of normal amylopectin quantities.

Goering et al. (1973) characterized the waxy Compana barley starch. It was found that under high magnification the waxy barley starch granule appeared to show fractures which might have accounted for their susceptibility to enzyme action. The iodine affinities of the waxy starch indicated some contaminating amylose or that the amylopectin branches of the waxy Compana were very long. The waxy Compana starch had a typical waxy starch pasting peak when run on a Brabender for a cooking curve, and a high pasting peak, granule instability and very little setback on cooling.

The waxy starches are easily broken down by bacterial $\alpha$-amylase. It would seem that the non-waxy barley starches contain an enzyme resistant fraction. Goering et al. (1973) stated his results suggested that waxy Compana starch was unique. He believed that some natural cross bonding or extremely long amylopectin chains may be partially responsible for the waxy Compana starch differences. The ease by which this starch is attacked by enzymes would also suggest that it would make better feed and an ideal substrate for fermentation.

Feeding Trials with Waxy Grains

McGinty and Riggs (1968) found several differences in the digestibility of sorghum grain varieties. They noted that the waxy sorghum grain had higher coefficients of digestibility for dry matter than did some other varieties. Nishimuta et al. (1969) determined the digestibility of regular, waxy and white sorghum grain diets fed to sheep. The waxy and white had significantly higher ($P<.05$) digestibility of organic matter and non-protein organic matter than regular sorghum grain. The waxy and white also had significantly great ($P<.10$) digestibility of nitrogen-free extract and gross energy as compared to the regular sorghum grain. Crude fiber digestibility of the white grain was significantly higher ($P<.01$) than the other two diets. The crude and true protein digestibility and nitrogen retention were improved with the waxy and white grains as compared to the regular grain diet. These results indicated that the starch in both the waxy and white was more readily available than that of the regular grain. Sherrod et al. (1969) determined the net energy values of regular and waxy sorghum grains as fed to finishing steers. Net energy for maintenance of the grains was 1.43 and 1.50 megcal per kg, and the diets 1.35 and

1.41 megcal per kg for the regular and waxy sorghum grains, respectively. Net energy of gain values for the grains were 0.95 and 1.24 megcal per kg and 0.86 and 1.10 megcal per kg for the rations. Waxy sorghum fed steers had a more efficient feed utilization both on a live weight ($P<.10$) and a carcass ($P<.05$) basis as compared to the steers fed the regular sorghum. These results suggested that the glutenous starch in the waxy grain provided a more readily available energy source than the non-glutenous starch in the regular sorghum grain.

McCormick and Farlin (1974) found no significant difference ($P<.05$) of average daily gain, dry matter consumption, or feed efficiency of corn diets fed to sheep. The diets included Opaque-2 high moisture and dry, waxy high moisture and dry, genetic equivalent to waxy dry, average maturity corn high moisture and dry and silage corn high moisture. Waxy corn and its genetic counterpart as high moisture and dry were also fed to steers. Again there was no real difference in the performance of the animals. Braman et al. (1973) included waxy corn in the diets of finishing lambs and steers fed all-concentrate diets. In the first trial, no significant difference was noted in nitrogen retention of lambs fed the waxy or normal corn diets. Apparent nitrogen digestibility was higher ($P<.01$) with the waxy corn. In trial two, the apparent nitrogen digestibility was higher ($P<.01$) for lambs fed the waxy corn diet. The higher nitrogen digestibility is due to decreased ($P<.10$) fecal nitrogen loss on the waxy corn diet.

It was noted that there was an interaction ($P<.01$) of corn type by supplemental nitrogen for urinary nitrogen excretion. Excretion increased with added dietary urea when regular corn was fed but not when waxy corn was fed to lambs. It was felt that the higher digestibility of the waxy corn would increase the nitrogen requirement of the rumen microflora, thus the added non-protein nitrogen (urea) was more efficiently used by the microbes of the sheep fed the waxy corn diet. In a third trial, steers fed a waxy corn-soybean diet had greater and more efficient gains ($P<.05$) than steers fed a regular corn-soybean diet or a regular or waxy corn diet supplemented with soybean meal plus urea. In a fourth trial, steers fed a waxy corn diet produced more efficient daily gains ($P<.05$) than those fed a normal corn diet. This agrees with Hinders and Eng (1970) who reported a 9% increase in daily gains of steers fed waxy sorghum grain as compared to those receiving normal sorghum grain. Kent Feeds, Inc, (personal communication) reported a 10% improvement ($P<.025$) in average daily gains of steers fed waxy corn as compared to those fed normal corn. The waxy corn fed to steers also had a 9.5% increase ($P<.025$) in feed efficiency.

Hanson (1946) fed swine waxy and normal corn diets. In one trial, pigs fed waxy corn made more rapid and efficient gains than pigs fed the normal corn and a normal-waxy corn mixed diet. In the second trial, the normal waxy corn mixed diet produced better gains as compared to either the normal or waxy corn diets. Allowed a choice of either the waxy or normal corn, the pigs preferred the normal corn diet. Jensen *et al*. (1973) fed young pigs normal corn and waxy corn diets. In the first trial, rate of gain in pigs from 5 to 12 weeks of age were the same for the waxy corn and normal corn diets, however, gain per unit of feed consumed was significantly higher ($P < .05$) for the waxy corn diet. In the second trial, there were no statistical differences.

Cohen and Tanksley (1973) fed sorghum grain diets to growing swine. Pigs fed waxy and normal sorghum grain showed no significant difference in digestibility coefficients for dry matter, organic matter and gross energy and the metabolizable and digestible energy was the same for the two grain types.

## CHAPTER III

## COMPARISON OF WAXY, HIGH-AMYLOSE AND NORMAL BARLEY STARCHES IN PURIFIED RAT DIETS


### SUMMARY

Starches from high-amylose Glacier, waxy Compana and their normal sisterline barleys were extracted and purified. Isonitrogenous 14.5% protein diets were prepared with the purified starches, casein, minerals, and vitamins. The purified diets were fed to female weanling rats in a growth trial and a balance trial for nitrogen and energy. Ten percent protein diets were also fed to female weanling rats in a second balance trial.

Rats in the growth trial evidenced no difference in feed consumption or feed efficiency due to starch, whereas average gain was higher ($P<.10$) for rats fed the normal Glacier starch diet as compared to rats fed the normal Compana or high-amylose Glacier starch diets. Rats fed the waxy Compana starch diet were not different from the rats fed the other three diets for average gain or protein efficiency ration (PER). The normal Glacier and normal Compana starch fed rats had a higher ($P<.05$) PER as compared to the rats fed the high-amylose Glacier starch. No significant differences were observed for retained nitrogen or apparent digestible energy due to starch in the first metabolism trial. Waxy Compana starch fed rats digested more nitrogen ($P<.01$) than rats fed the normal Compana or high-amylose Glacier starches, while the normal Glacier starch fed rats were not different from the other three

groups in this regard. There were no differences in retained nitrogen or digestible energy between diets in the second balance trial. High-amylose Glacier starch diets were lower ($P<.01$) in digestible nitrogen as compared to the other three diets.

## Introduction

Preston et al. (1964) reported high-amylose corn to be similar in value to normal corn for ruminants, while Borchers (1962) has reported lower digestibility of high-amylose corn fed to rats. Pomeranz et al. (1972) found that high-amylose barley had a much lower susceptibility to amylolytic action, probably due to starch structure. Sandstedt et al. (1962) reported lowered susceptibility of high-amylose corn starch to amylase action. Waxy barley, as characterized by Goering et al. (1973) appeared very easy to liquify as compared to normal starch. The ease by which the enzymes attacked the waxy starch suggested that it would make a better feed and an ideal substrate for fermentation. It appears that high-amylose starch is less susceptible to enzyme degradation than normal or waxy starch. Waxy grains have been reported to provide more available energy, higher average daily gains and improved feed efficiency in ruminants (Nishimuta et al., 1969; Sherrod et al., 1969; Hinders and Eng, 1970; Braman et al., 1973) and monogastrics (Jensen et al., 1973). Both high-amylose and waxy barleys are available and interest has been shown in both. It is desirable to study the ability of the monogastric to degrade and