2003

The metabolizable energy value and physiologic effects of Hi-Maize resistant starch in male rats

Tanya A. Garcia

Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Human Ecology Commons

Recommended Citation


https://digitalcommons.lsu.edu/gradschool_theses/2972

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
THE METABOLIZABLE ENERGY VALUE
AND PHYSIOLOGIC EFFECTS OF
HI-MAIZE® RESISTANT STARCH IN MALE RATS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Human Ecology

by
Tanya A. Garcia
B.S., Louisiana State University, 2001
December 2003
ACKNOWLEDGMENTS

This thesis was created with the support and efforts of my major professor, Dr. Maren Hegsted, and my committee members, Dr. Michael Keenan and Dr. Carol O’Neil, for which I am very thankful for their time and assistance. In addition, I would like to thank Mrs. Anne Francis, Ms. Kathy McCutcheon, and the student workers for their help with the animals and statistical analysis. Special thanks should also be given to Ms. Millie Williams who assisted me with the bomb calorimeter. Finally, I would like to thank my fiancé, John, and my parents, without their support I would not have been able to complete this Master’s thesis.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................. ii

LIST OF TABLES .................................................................................. v

LIST OF DEFINITIONS ......................................................................... vi

LIST OF ABBREVIATIONS ..................................................................... viii

ABSTRACT ............................................................................................ ix

INTRODUCTION ....................................................................................... 1
Justification ............................................................................................ 1
Objectives ............................................................................................... 2
Hypotheses .............................................................................................. 3
Assumptions ............................................................................................ 4
Limitations ............................................................................................... 4
References ............................................................................................... 4

REVIEW OF LITERATURE ................................................................. 6
Starch ...................................................................................................... 6
Resistant Starch ...................................................................................... 7
Physiologic Effects of RS ....................................................................... 9
Adverse Effects of RS Metabolism ........................................................ 12
Fermentation Process of RS ................................................................... 13
Metabolizable Energy ............................................................................ 18
Metabolizable Energy of RS ................................................................. 23
Summary ................................................................................................. 25
References ............................................................................................... 25

THE METABOLIZABLE ENERGY VALUE AND PHYSIOLOGIC EFFECTS
OF HI-MAIZE® RESISTANT STARCH IN MALE RATS .................... 30
Introduction............................................................................................. 30
Materials and Methods ......................................................................... 32
Results .................................................................................................... 37
Discussion ............................................................................................... 43
References ............................................................................................... 49

CONCLUSION .......................................................................................... 52

APPENDIX ............................................................................................... 54
A: DIET MIXING PROCEDURE ........................................................... 54

B: TIMELINE OF PROCEDURES ....................................................... 56

C: DATA COLLECTION ........................................................................... 57
LIST OF TABLES

1. Timeline of Procedures ................................................................. 32

2. Modified American Institute of Nutrition (AIN)-93G Diet for Growing Rodents................................................................. 33

3. Body Weight, Diet Intake, and Fecal Excretion per Period (Mean ± SD).................................................................................. 37

4. Gastrointestinal Tract Organ and Content Weight, and Abdominal Area Adipose Tissue Weight (Mean ± SD)............................. 38

5. Fecal, Gross, and Digestible Energy Values for each Period (Mean ± SD).................................................................................. 39

6. Energy Values (kcal/g) for Diets at the End of the Third Period (Mean ± SD).......................................................................... 40

7. Metabolizable Energy (kcal) of the Diets (Mean ± SD).................... 41

8. Gross Energy Values within the Carcass (Mean ± SD)..................... 42

9. Previously Reported Energy Values (kcal/g)...................................... 44
### LIST OF DEFINITIONS

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>Digestive enzyme in the small intestine that breaks down glycosidic bonds within polysaccharide molecules to release glucose molecules for absorption into the blood.</td>
</tr>
<tr>
<td>Amylopectin</td>
<td>Starch component with glucose monomers linked in α-(1-4) glycosidic bonds in straight chain configuration with branches of glucose monomers in α-(1-6) glycosidic bonds. Easily digested by amylase in the small intestine by quick release of glucose molecules for absorption.</td>
</tr>
<tr>
<td>Amylose</td>
<td>Starch component with glucose monomers linked in α-(1-4) glycosidic bonds in straight chain configuration. Resistant to digestion by amylase in the small intestine with little or slow release of glucose for absorption.</td>
</tr>
<tr>
<td>Cecum</td>
<td>Organ of the gastrointestinal tract containing bacteria that produces short chain fatty acids (SCFA) when undigested carbohydrate passes into the large intestine. The first section of the large intestine within the human body; the organ in between the small intestine and the large intestine of the rat.</td>
</tr>
<tr>
<td>Fermentation</td>
<td>Process in the cecum or large intestine that produces short chain fatty acids (SCFA) by colonic bacteria from glucose molecules not digested in the small intestine.</td>
</tr>
<tr>
<td>Gastrointestinal Tract</td>
<td>Organ system in the body divided into the esophagus, stomach, small intestine, cecum, and large intestine, which digests and absorbs dietary nutrients. For the purpose of this study, the gastrointestinal tract includes all but the esophagus.</td>
</tr>
<tr>
<td>Metabolizable energy</td>
<td>Chemical energy that is available for the production of heat, basal metabolism, or work that is used for catabolism and anabolism.</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Three-carbon molecule metabolized from a six-carbon glucose molecule; an intermediate to normal carbohydrate metabolism.</td>
</tr>
<tr>
<td>Resistant Starch (RS)</td>
<td>Carbohydrate that avoids digestion in the small intestine and is subject to fermentation in the cecum or large intestine. High amylose starch is one of the natural resistant starches.</td>
</tr>
</tbody>
</table>
Short Chain Fatty Acids (SCFA)  
Products of colonic bacteria that metabolize glucose in the colon, which produce energy for the body. The primary SCFA are acetate, butyrate, and propionate.
LIST OF ABBREVIATIONS

BTU……………………………………………………….British Thermal Unit
CH₃………………………………………………………..Methane
cm…………………………………………………………Centimeter
CO₂……………………………………………………..Carbon Dioxide
et al………………………………………………………..Et alia; and others
g…………………………………………………………...Gram
IBD……………………………………………………….Inflammatory Bowel Disease
i.e………………………………………………………….Id est; that is
kcal……………………………………………………...…Kilocalorie
kg…………………………………………………………..Kilogram
kJ…………………………………………………………..Kilojoule
L…………………………………………………………...Liter
ml………………………………………………………….Milliliter
p…………………………………………………………...Probability
pH…………………………………………………………Measure of acidity or alkalinity
psi…………………………………………………………Pounds per square inch
SCFA……………………………………………………...Short Chain Fatty Acids
RS…………………………………………………………Resistant starch
µmol……………………………………………………Micromoles
ABSTRACT

The purpose of this study was to calculate the metabolizable energy value of Hi-Maize® RS (60% amylose), to observe if consumption of RS alters adiposity, and to examine the effects of RS on fermentation and fecal excretion. Eighteen four-week old male Sprague-Dawley rats consumed either a 20% amylose Hi-Maize® RS diet (n=6) or a control diet (baseline group, n=6; control group, n=6). The baseline group was sacrificed at the beginning of the study; the RS and control groups were transferred to metabolic cages and fed the respective diets for the next six weeks. Feces and urine from each individual rat was collected daily and stored separately for each of the periods. At sacrifice, fat pads were weighed; gastrointestinal tract organs were cleaned and weighed. Baseline rat data were used to determine the total energy gained in RS and control rats. Metabolizable energy value of Hi-Maize® RS was determined by a calculation by Livesey (1995) using energy data from bomb calorimetry of the diet, urine and feces. At the end of the third period, the metabolizable energy value for Hi-Maize® RS was 1.55 kcal/g. The value for the RS diet was 3.66 kcal/g, which was significantly lower than the control diet of 4.29 kcal/g (p < 0.001). Adaptation to the RS diet occurred over time as seen by changes in the digestible energy values of RS per each period. For the RS group, abdominal fat was lower (p < 0.05) possibly due to lower metabolizable energy of the RS diet, cecum and large intestine weights were greater (p < 0.001), and pH of the cecal contents was lower than the control rats (p < 0.001) due to greater fermentation of the RS diet. Fecal weight for each of the periods was greater in the RS group (p < 0.001) compared to the control group. Compared to the consumption of a highly digestible starch diet, Hi-Maize ® RS provides less energy to the body and increases fermentation and fecal excretion, which may provide a healthier colonic environment.
INTRODUCTION

Justification

Generally, carbohydrates can be separated into two groups: (1) those that are digestible and (2) those that are non-digestible in the body. Digestible carbohydrate can be divided into two types, rapidly and slowly digestible (Englyst, Kingman, & Cummings, 1992). Intestinal enzymes release glucose and other monosaccharides from digestible carbohydrates for absorption into small intestine cells. These same enzymes cannot release glucose from non-digestible carbohydrates, causing glucose not to be available for absorption into small intestine cells. The non-digested carbohydrate is then passed into the large intestine where fermentation by bacteria takes place (Englyst et al., 1992; Vitapole, 2001).

All starch is composed of two types of polysaccharide chains: amylose and amylopectin. Starch that contains a high amylose content is associated with poor digestion and lower absorption of glucose into the blood. Starch with a high amylose content is referred to as resistant starch (RS). Starch that contains a high amylopectin content is associated with great digestion and absorption of glucose into the body (Haralampu, 2000). Hi-Maize® RS is composed of approximately 60% amylose/40% amylopectin, as compared to the “standard starch” consumed, which is generally composed of approximately 30% amylose/70% amylopectin (Haralampu, 1998).

Carbohydrate that is absorbed into the body is used as energy for basal metabolism, growth, and activity. The total energy available from carbohydrate, which is analyzed by bomb calorimetry, is termed gross energy (Miller & Judd, 1984). The value of gross energy retained in the body not excreted in feces or urine is called metabolizable energy of a food or diet (Haralampu, 1998). Digestible energy is gross energy retained
minus the energy excreted from the feces only. The consumption of easily digestible carbohydrate provides the body with greater digestible and metabolizable energy available compared to non-digestible carbohydrate. The body cannot gain the total gross energy from the carbohydrate consumed if it is neither digested nor absorbed fully within the small intestine (Vitapole, 2001).

In 1902, General Atwater Factors stated that all carbohydrate provides four kilocalories (kcal) of metabolizable energy, before RS was discovered as a component of food (Livesey, 1991; Moe, 1994). If resistant starch resists digestion in the small intestine, the value of metabolizable energy provided from RS would be close to zero. However, RS is fermented in the colon to produce short chain fatty acids (SCFA) that can be absorbed as energy. The amount of metabolizable energy contributed by fermentation of RS varies depending on type and amount of RS consumed, including other complicating factors (Mathers, 1992). Therefore the metabolizable energy value of Hi-Maize® RS has not been reported in previous RS studies.

Dietary RS has been observed to benefit health in numerous ways. The potential decrease in metabolizable energy may reduce body weight and the risk of obesity. The addition of RS to the diet not only reduces energy intake it may also improve bowel health (Ferguson, Tasman-Jones, Englyst, & Harris, 2000; Haralampu, 2000). However, not all types of RS have been observed to produce the same health characteristics (Cummings, Beatty, Kingman, Bignham, & Englyst, 1996; Ferguson et al., 2000).

**Objectives**

1. To determine the digestible and metabolizable energy value of Hi-Maize® RS.
2. To measure the digestible and metabolizable energy value of a Hi-Maize® RS diet and a highly digestible carbohydrate (control) diet.
3. To measure food intake and calculate energy intake for each metabolic period.

4. To measure the effects of the two diets on body fat accumulation and gastrointestinal characteristics.

5. To determine if consumption of the RS diet induces fermentation in the cecum.

6. To see if digestible energy increases over time as the animals adapt to the RS diet and increase the rate of fermentation.

7. To measure the gross energy retained in rats consuming either the Hi-Maize® RS diet or control diet.

**Hypotheses**

1. The digestible and metabolizable energy of Hi-Maize® RS will be less than its gross energy value.

2. The digestible and metabolizable energy value of the Hi-Maize® RS diet will be less than the control diet.

3. RS fed rats will consume the same amount of diet (g) but have a lower energy intake.

4. The RS fed rats will accumulate less body fat but have greater cecum and large intestine weights.

5. Fermentation will be greater in rats consuming the Hi-Maize® RS diet compared to the control diet.

6. Adaptation over time to the Hi-Maize® RS diet will increase the digestible energy value of the diet.

7. Less energy will be retained in the rats consuming the Hi-Maize® RS diet compared to the rats consuming the control diet.
Assumptions

1. Male Sprague-Dawley rats were appropriate models for human fermentation of RS and energy absorption.

2. The feces, urine, diet, and body samples were not contaminated with extraneous matter that would bias the results.

3. The equation for the calculation of digestible and metabolizable energy was precise and unbiased.

Limitations

The high dose of RS consumed by the rat model in the study is not comparable to the amount of RS likely to be consumed by humans.

References


REVIEW OF LITERATURE

Starch

Starch is a complex carbohydrate composed of polyglucans, which are repeating glucose monomers. The two types of starch, amylose and amylopectin, are joined together by $\alpha$-D- (1-4) linkages. Amylose, containing approximately 1000 glucose units, is a straight chain polyglucan, which curls inward. Amylopectin, containing 4000 glucose units, has branches of polyglucans coming off the straight chain in $\alpha$-D- (1-6) linkages (Behall & Howe, 1996; Haralampu, 2000).

Amylose and amylopectin have different structural and physiologic characteristics. Because of their unique structural configurations, amylose and amylopectin exhibit different reactions within the body during digestion and absorption. The straight chain of amylose limits the access of $\alpha$-amylase enzyme to the two terminal glucose units on the amylose chain in the small intestine. In addition, amylose is also resistant to digestion because the two terminal ends may not be accessible due to folding of the polymer (Haralampu, 1998). The highly branched amylopectin molecule allows for quick hydrolysis of glucose units because the branched structure provides multiple terminal end glucose units that $\alpha$-amylase enzymes can contact readily. Amylopectin is rapidly hydrolyzed to glucose units that are quickly absorbed into the body whereas amylose hydrolysis takes longer (Vitapole, 2001).

Heating disrupts the physical structure of the starch granule when submerged in water. This process called gelatinization produces the thickening characteristic of cooked starch, which is commonly used to make puddings and sauces. Gelatinization causes starch to be fully accessible to $\alpha$-amylase to easily digest the starch granule in the small intestine. When starch cools, it undergoes a process of retrogradation, which is the slow
re-association of the starch granule to the pre-gelatinized state. Retrogradation causes starch to stabilize through increasing hydrogen bonding preventing quick degradation from α-amylase (Haralampu, 2000).

The percentage of amylose to amylopectin in typical dietary starch varies. The most common commercial cornstarch composition of the Western diet is 70% amylopectin/30% amylose cornstarch (Behall & Howe, 1996). Amylopectin portion of standard digestible starch ranges from 70 to 80%, though any percentage can be produced (Bird, Brown, & Topping, 2000).

**Resistant Starch**

Resistant starch is a carbohydrate that avoids digestion is the small intestine (Haralampu, 1998). Resistant starch is defined as the undigested and unabsorbed starch product that reaches the large intestine in healthy adults (Ferguson, et al., 2000). In the large intestine, RS is subject to fermentation by colonic bacteria to produce short chain fatty acids (SCFA) (Bjork, Nyman, Pedersen, Siljestrom, Asp, & Englyst, 1986).

The amylose component of cornstarch is referred to as resistant starch (RS). The general composition of resistant cornstarch is approximately 70% amylose/30% amylopectin (Bird et al., 2000). However, researchers have experimented with a range of 10 to 65% amylose cornstarch composition in experimental diets (Behall & Howe, 1996). In RS studies, animal diets are generally comprised of 10 to 30% amylose content as percentage of the total weight of the diet. Researchers have not attempted to go beyond this range of amylose content to keep potential symptoms and side effects of RS low because not all physiologic aspects of RS have been well studied.

Researchers debate whether RS, a starch polysaccharide, should be defined as a dietary fiber. Fiber is classified as the non-starch polysaccharide component of plant
cells that is resistant to digestion by human enzymes (Behall & Howe, 2002; Englyst, Trowell, Southgate, & Cummings, 1987; Food and Nutrition Board, 2000; Haralampu, 2000). Resistant starch was previously identified as a complicating factor in the determination of fiber content in foods because it assays as an insoluble fiber. If RS were not distinguished from fiber, the fiber content of foods would be falsely elevated (Englyst et al., 1987). The physiologic fate of RS is similar to that of soluble fiber, i.e., escaping small intestine digestion, fermenting in the large intestine, increasing bulk, and lowering the pH of the contents of the colon (Haralampu, 1998). In rats, consumption of RS decreased transit time (Ferguson et al, 2000). Insoluble fiber decreases transit time more than RS. On the contrary, RS has no significant water holding properties similar to insoluble fiber (Cummings et al., 1996). The problem of including RS as a fiber is because RS does not have all the properties of soluble and insoluble fiber together (Haralampu, 2000).

Resistant starch is categorized into four groups. Each of the four groups has a different structure. The RS$_1$ is a tightly bound molecule wrapped in a fiber shell that does not allow the digestive enzymes access to the starch molecule (Bird et al., 2000; Haralampu, 1998). The RS$_2$ molecule is termed raw ungelatinized starch because it is not cooked or gelatinized similar to most starch sources (Food and Nutrition Board, 2000). The RS$_2$ has terminal glucose ends of the starch structure wrapped tightly within its structure, resisting breakdown by amylase. Retrograded starch, RS$_3$, is a starch molecule formed during heating and then cooling of the starch. This process called retrogradation produces crystalline amylopectin molecules making the starch highly heat stable. The chemically modified starch, RS$_4$, is a starch molecule that cannot be broken down since
the modification process rendered the structure inaccessible for digestion by $\alpha$-amylase (Cummings et al., 1996; Haralampu, 1998; Haralampu, 2000).

There are varieties of sources of RS in foods available. Generally, RS comprises at the most five percent of all starch consumed in Western diets (Behall & Howe, 1996; Liljeberg & Bjork, 1994; Roberfroid, 1999). Legumes are the most common natural form of RS in the Western diet. Other sources are high amylose corn, peas, and whole grain cereals (Cummings et al., 1996; Faulks, Southon, & Livesey, 1989). Green bananas and raw potatoes are also sources of RS, but are not generally consumed (Behall & Howe, 1996; Cummings et al., 1996). Specifically, legumes and whole or partly milled grains and seeds are sources of RS$_1$. Green bananas, raw potatoes, and legumes are sources of RS$_2$. Cooked and cooled potato, bread and flaked corn cereal are sources of RS$_3$. Esterified, or cross-bonded, starches that are used in chemically synthesized processed foods are sources of RS$_4$ (Bird et al., 2000; Food and Nutrition Board, 2000).

**Physiologic Effects of Resistant Starch**

When food is consumed, it travels down the esophagus into the stomach for degradation by the acidic environment provided by the secretion of hydrochloric acid and enzymes. The degraded nutrients are passed into the small intestine. The small intestine is divided into three sections: duodenum, jejunum, and ileum. The small intestine contains pancreatic enzymes that digest dietary polysaccharides for absorption as monosaccharides into the blood. The intestinal wall contains brush border enzymes, which degrade di- and oligo- saccharides, polypeptides, and di- and tri- glycerides. Other enzymes within the small intestine assist in the absorption of vitamins and minerals (Nordgaard, 1998). The undigested and unabsorbed nutrients are passed into the large intestine. The human large intestine is divided into the cecum, ascending colon,
transverse colon, descending colon, and sigmoid colon. The large intestine absorbs only a small amount of nutrients, relative to the small intestine, and moves the undigested material to the rectum for excretion (Klein, Cohn, & Alpers, 1999). In many animals, specifically rats, the cecum is a separate organ of the gastrointestinal tract. The cecum is located before the large intestine and is more active in absorption of undigested nutrients.

There are physiologic differences between the four categories of RS (RS₁-RS₄) (Cummings et al., 1996; Faulks et al., 1989; Haralampu, 2000). There are also physiological differences found between the types of food within each RS category, such as the digestibility rate of RS₂ sources from corn, pea, and potato. There is no clear evidence explaining why the many sources of RS obtain physiologic differences.

**Human Studies**

Thirty to 70% of RS is metabolized overall in the small and large intestine combined. Eighty to 90% of standard starch is metabolized (Haralampu, 1998; Vonk, Hagoedorm, de Graaff, Elzinga, Tabak, Yang et al., 2000). The overall digestibility of RS depends on the category and source of the RS consumed: 84% RS₃ (corn), 89% RS₂ (potato), 96% RS₂ (banana) and 65% RS₃ (wheat). Digestibility of RS was also found to vary per individual (Cummings et al., 1996).

The digestion of RS in humans takes five to seven hours, compared to the almost immediate digestion of a standard starch (Haralampu, 1998). The RS remains undigested in the small intestine and passes into the large intestine for the excretion process. The duration of time for the fecal bulk to pass through the large intestine, i.e. transit time, is prolonged in humans consuming RS (Cummings et al., 1996; Hylla, Gostner, Dusel, Anger, Bartram, Christi et al., 1998).
Fecal bulk increases in humans who consume RS (Heijnen, Amelsvoort, Deurenberg, & Beynen, 1996; Hylla et al., 1998). Given that less dietary RS is metabolized within the body compared to standard digestible starch, the amount of RS excretion should be greater when compared to the excretion of standard dietary starch (Food and Nutrition Board, 2000; Jenkins, Vuksan, Kendall, Wursh, Jeffcoat, Waring, et al., 1998; Nordgaard, 1998). Humans consuming a high-RS diet have demonstrated that undigested RS can make up as much as ten percent of the feces, whereas excretion of digestible starch in the feces is generally insignificant (Cummings et al., 1996).

The increased amount of the fecal bulk contains undigested carbohydrate. Additionally, a large percent of nitrogen is included from the colonic bacteria being excreted (Cummings et al., 1996). These colonic bacteria proliferate in the large intestine though large amounts are excreted due to the increase in undigested starch reaching the large intestine (Hylla et al., 1998).

The level of RS consumed by human participants in multiple studies ranged from 17 to 30 grams per day (Cummings et al., 1996), 26 to 50 grams per day (Phillips et al., 1995), and an average amount of 29.7 grams per day (Behall & Howe, 1996). In all studies, the diet was composed of approximately 50% of the energy intake as carbohydrate, comparable to a general diet. These high levels of RS gave the participants healthy, normal bowel movements with minimal side effects such as bloating, gas, and abdominal pain.

**Rat Studies**

Rats show similar findings to humans for digestion of RS (DeSchrijver, Vanhoof, & Vande-Ginste, 1999; Faulks et al., 1989). The digestibility of RS in rats was 55 to 77% (Faulks, Roe, & Livesey, 1992). Resistant pea starch, RS3, was observed to have a
lower degree of digestibility compared to resistant cornstarch, RS₂ (Faulks et al., 1989; Livesey, Davies, Brown, Faulks, & Southon, 1990).

In general, 10 to 35 grams of any RS source for 100 grams of total diet intake is provided to rats in research settings. In human studies, less than 10% of the diet consumed is RS (Faulks et al., 1989; Ferguson et al., 2000; Le Blay, Michel, Blottiere, & Cherbut, 1999; Leu, Hu, & Young, 2002; Younes, Coudray, Bellager, Demigne’, Rayssiguier, & Remesey, 2001). The percent of dietary RS is not generally comparable for rat versus human studies.

Fecal excretion of rats is similar to humans. An increase in fecal bulk was seen with the consumption of RS (De Schrijver, 1999; Ferguson et al., 2000; Haralampu, 1998). A positive correlation was observed between rats consuming RS and fecal bulk; the greater the RS amount consumed the greater the excretion of the starch was in the feces (Silvester, Englyst, & Cummings, 1995). Conversely, one study reported that there was no significant fecal excretion of RS, even at high concentrations (Mathers, 1992). This result has not been observed in human studies. When rats consumed a digestible starch diet less than one percent starch was excreted in the feces (Ferguson et al., 2000).

A slightly decreased transit time was observed for rats fed Hi-Maize® RS diet compared to the transit time resulting from rats fed a control, standard starch diet. Transit time reduction may be associated with a reduction of colon cancer in rats (Ferguson et al., 2000). However, transit time was prolonged in humans consuming RS (Cummings et al., 1996).

**Adverse Effects of RS Metabolism**

Flatulence and bloating are the main gastrointestinal problems from consuming RS. Consumption greater than 32 grams of RS₂ per day has been observed to produce
these effects in humans (Behall & Howe, 1996; Heijnen et al., 1996; Phillips et al., 1995). An enlarged cecum and large intestine have been observed in animals consuming RS diets, yet little research has stated the negative effects of this enlargement and the level of comfort this entails. Gastrointestinal distress was seen to a smaller extent with the ingestion of less than 30 grams of RS₂ per day (Heijnen et al., 1996). Bakery products prepared with RS₃, such as cookies, did not create as much flatulence and bloating as RS₂ (Hylla et al., 1998).

Similar to many food and disease state interactions, consumption of RS by animals and humans can induce negative physiologic effects on morbidity and mortality in certain situations. Weanling piglets with dysentery demonstrated an increase in symptoms when fed a RS diet (Pluske, Durmic, Pethick, Mullan, & Hampson, 1998). Similarly, high-RS diets have been found to cause greater pain and other associated symptoms in people suffering from Inflammatory Bowel Disease (IBD). The symptoms of IBD in humans decreased when RS was decreased or removed from the diet (Bird et al., 2000).

**Fermentation Process of RS**

A healthy large intestine contains hundreds of bacterial species that degrade undigested carbohydrate within an anaerobic environment to produce SCFA (Silvi, Rumney, Cresci, & Rowland, 1999). Colonic bacteria metabolize the undigested carbohydrate passed from the small intestine into the large intestine (Klein et al., 1999). The anaerobic bacteria, i.e., bacteria functioning without oxygen, hydrolyze RS producing monosaccharides. Monosaccharides are single, six-carbon sugar molecules that are metabolized further to produce pyruvate, a three-carbon intermediate product of normal carbohydrate metabolism that is generally produced within the body’s cells.
In the large intestine or cecum, pyruvate is further metabolized into SCFA, specifically propionate, acetate and butyrate. Absorption of SCFA in the large intestine results in the eventual digestion and absorption of RS energy in the colon. On a high-RS diet, not more than ten percent of dietary RS is excreted in the feces since most is fermented and absorbed (Cummings et al., 1996; Phillips et al., 1995).

The fermentation of RS produces an acidic environment (Younes et al., 2001). In turn, an acidic environment in the cecum and large intestine promotes healthy bacterial proliferation and inhibits pathogenic bacteria compared to a neutral pH value \(7.2 \pm 0.2\) in humans consuming a low-RS diet (Leu, Hu, & Young, 2002). Alkaline-based toxic compounds are dissociated in a low pH environment inhibiting the absorption of these toxins into the body (Bird et al., 2000).

The cecum and ascending colon promote the greatest bacterial fermentation rate of non-digestible carbohydrate compared to the other sections of the large intestine. During fermentation, the pH of the cecum and the ascending colon ranges from 5.4 to 5.9. In the transverse colon, the pH increases to 6.2, due to the reduction of carbohydrate available for fermentation. The bacteria in the descending colon produce less fermentation from carbohydrate. The pH ranges from 6.6 to 6.9 in the descending colon. The sigmoid colon contributes to relatively little or no fermentation (Nordgaard, 1998).

Butyrate, propionate, and acetate are the most abundant SCFA produced in the large intestine, accounting for 90% of all production (Nordgaard, 1998). The SCFA concentrations differ among the sections of the large intestine. The greatest concentration of total SCFA produced is within the cecum. The concentration of SCFA decreases from the cecum to the sigmoid colon following the pattern of pH during fermentation, as described previously, (Cummings et al., 1996; Nordgaard, 1998).
conventional anaerobic stoichiometry, one mole of a six-carbon sugar molecule, i.e. glucose, produces two moles of acetate, two moles of propionate, or one mole of butyrate (Mathers, 1992).

Production and absorption of SCFA from RS is essential to utilize the available energy from the starch for the body. Monosaccharides initially produced during the process of fermentation by the colonic bacteria cannot be absorbed by the large intestine. The production of SCFA from monosaccharides is essential for regaining the lost energy from undigested carbohydrate. Without fermentation, energy from non-digestible starch would be lost in the feces (Roberfroid, 1999). Ninety-five percent of SCFA produced from the fermentation of undigested carbohydrate is absorbed in the large intestine (Cummings et al., 1996). The production and absorption of SCFA from undigested carbohydrate may contribute up to 12% of the body’s energy needs on a high-RS diet (Behall & Howe, 1996; Cummings et al., 1996). Livesey has estimated that SCFA have been reported to produce 1.7 kcal per gram of non-digestible carbohydrate that is fermented (1995).

The production of SCFA provides a benefit for not only the human or animal consuming the RS, but also the bacteria within the colon. Colonic bacteria use a small amount of the energy produced from undigested carbohydrate as a fuel source for their own multiplication, growth, and survival within the colon (Nordgaard, 1998). However this small amount of energy taken up by the bacteria is negligible compared to the amount absorbed in the body.

**Human Studies**

Resistant starch is thought to be the greatest contributor to large intestinal SCFA production (Bird et al., 2000). Compared to a standard starch diet, the production of
acetate, propionate, and butyrate has been found in greater concentration in the feces with a RS diet (Ahmed, Segal, & Hassan, 2000). Acetate is greatly increased by a RS diet and has been found frequently to be the predominant SCFA produced in the colon (Ahmed, et al., 2000; Phillips et al., 1995). Butyrate significantly increases with RS consumption as well as the ratio of butyrate to total SCFA. The increase in butyrate has been reported to have protective effects from colon cancer and has been determined to be a preferred energy source for colon cells (Jenkins et al., 1998). Propionate has been found in large amounts within the colon. Evidence that propionate can reduce cholesterol levels in humans has not been found conclusive in all studies (Ahmed et al., 2000; Bird et al., 2000; Phillips et al., 1995).

The production of SCFA from undigested carbohydrate, especially RS, is viewed as being beneficial to humans for maintaining normal bowel health and integrity (Bird et al., 2000; Haralampu, 2000). It is widely accepted that RS fermentation greatly increases fecal contents due to the increase in proliferation of bacteria (Ahmed et al., 2000; Cummings et al., 1996; Nordgaard, 1998; Phillips et al., 1995). An increase in stool weight and fecal bulk provides a potential anti-carcinogenic environment because the carcinogens are diluted by the increased fecal bulk (Hylla et al., 1998; Phillips et al., 1995).

On a high-RS diet, the cecum and large intestine grows allowing more room for the increased amount of fermentation (Younes et al., 2001). Lean mass increases due to hypertrophy of healthy cells, a decrease in cell atrophy, and an increase in butyrate uptake (Ahmad et al., 2000; De Schijver et al., 1999; Faulks et al., 1989; Livesey et al., 1990). A greater surface area of the large intestine increases absorption of SCFA and
other unabsorbed nutrients that may have been passed from the small intestine (Younes et al., 2001).

**Rat Studies**

Rats produce SCFA similar to humans when consuming RS diets as seen by the increased concentration of SCFA and decreased pH of feces (Ferguson et al., 2000, Hylla et al, 1998). Rat consumption of RS as a percentage of the diet may be greater than human consumption of RS. The total SCFA concentration is greatest within the cecum, with declining concentration through the mid-colon to the rectum. Elevated levels of acetate, butyrate, and propionate are found primarily in the cecum. In the cecum, concentrations of 75 micromoles (umol) SCFA per gram of cecal content (50 umol per gram fecal content) are produced from consuming a RS diet. Of the total cecal SCFA concentrations, acetate, propionate, and butyrate consisted of 90%, 9.3%, and 1.2%, respectively; total SCFA within the feces consisted of 93%, 6.1%, and 0.9%, respectively (Ferguson et al, 2000).

The ratio of acetate to total SCFA, produced within the colon and excreted, is greatest compared to butyrate or propionate (Leu, et al., 2002; Phillips et al., 1995; Younes et al., 2001). Butyrate production increases gradually over time. The acetate to butyrate ratio becomes smaller after a period under study (Le Blay et al., 1999). Butyrate production is suggested to increase over time; acetate production remains constant (Ferguson et al., 2000). In rats, increases in propionate have been found reduce serum and hepatic cholesterol levels (Cheng & Lai, 2000). This cholesterol lowering effect has not been observed in humans (Heijnen et al., 1996).

Rat studies have shown positive influences for RS on maintaining a healthy colon compared to the neutral effect of a diet containing no RS (Ferguson et al., 2000; Le Blay
et al., 1999). A ten percent increase of RS in the diet may provide a higher cecal content weight and higher cecal tissue weight in rats (De Schrijver et al., 1999; Faulks et al., 1989). An assumption is that fermentation of RS increases healthy epithelial cell proliferation in the colon. Second, fermentation may decrease epithelial cell atrophy (Haralampu, 2000). Another possibility is that the colon cells absorb butyrate to prevent energy deficiency diseases, such as ulcerative colitis (Jenkins et al., 1998). There has been evidence that butyrate is the preferred energy source for colon cells (Le Blay et al., 1999).

**Metabolizable Energy**

There are many different types of energy. Gross, digestible, and metabolizable, are all specific terms associated with the energy value of food. Fecal and urinary energy are terms used for the energy value from a food that is excreted (Livesey, 1991b; Miller & Judd, 1984). All food energy terms can be measured in kilocalories (kcal), kilojoules (kJ), or British Thermal Units (BTU) (Moe, 1994).

Gross energy is the energy acquired by the burning of a food. The heat produced is measured directly in a bomb calorimeter and is converted to the energy unit kcal, kJ, or BTU (Moe, 1994). Digestible energy is obtained from the gross energy value of a food minus the energy excreted in the feces. Metabolizable energy is the digestible energy minus the losses of energy through the urinary nitrogen (Livesey, 1991b; Miller & Judd, 1984).

The fecal and urinary energy excretion in healthy persons generally accounts for at least five to ten percent of the total energy from the diet. The energy lost from excretion not only pertains to the food ingested, but also endogenous or metabolic fecal and urinary nitrogen from the breakdown of bodily components (Kleiber, 1975). A small
proportion of the energy ingested is lost to growth and maintenance of beneficial colonic bacteria (Food and Nutrition Board, 2000). Fecal content in humans consuming a diet high in RS or non-starch polysaccharides, i.e. fiber, is expected to contain greater amounts of fecal energy than a diet with low amounts of RS or fiber. A significant amount of fecal energy coming from the consumption of high-RS diets is assumed to come from excreted nitrogen-containing bacteria (Cummings et al., 1996). The urinary energy losses are accountable by the loss of urea, which contains a small amount of energy as nitrogen. This small, though possibly significant value, can incorrectly affect the metabolizable energy value of a carbohydrate (Miller & Judd, 1984).

Metabolizable energy is the energy the body utilizes from a food for growth and maintenance in the tissues, basal metabolism, and physical activity. All the energy used for the total reactions can be quantified. This can be measured from the gross energy of the consumed diet by subtracting excreted energy values from the feces and urine, verifying the metabolizable energy value. Measuring the basal metabolism and physical activity is more difficult because it involves measuring the energy excreted from the breath and flatus excreted, which is hydrogen and methane, respectively (Heijnen et al., 1996). In addition, heat produced and lost is also a component of metabolizable energy. To determine the individual components of metabolizable energy would require metabolism chambers and other sophisticated equipment (Kleiber, 1975).

**Measurement of Metabolizable Energy**

Determining the metabolizable energy value for all foods and food combinations consumed would be very tedious. Models, i.e. equations, are commonly used to predict the energy value of all foods through calculations. The two most frequently used models
to estimate the metabolizable energy values of a food are the factorial and empirical models (Livesey, 1995).

The theories and procedures are different for the two types of models. Factorial models are based on the analysis of energy values from protein, fat, and carbohydrate that have been determined through experimental measures on apparent digestibility. Apparent digestibility is the balance between the intake of a food including the fecal loss as expressed as a fraction of the total intake. Recent factorial models include measures for all non-digestible carbohydrate, i.e. RS and fiber, which have been determined through experimental measures on apparent digestibility. The factorial method may be inadequate for some foods due to its generality. The empirical approaches are based on gross energy and factors that predict energy excretion. Presently, the empirical model is most commonly used to analyze the metabolizable or digestible energy values of mixed diets (Baer, Rumpler, Miles, & Fahey, 1997; Livesey, 1995).

In 1910, the Atwater factors were derived to determine the energy value of carbohydrate, protein, and fat. The values for carbohydrate, protein, and fat were calculated to be four, four, and nine kcal/g, respectively. These values have been considered constants for determining metabolizable energy values for protein, carbohydrate, and fat seen on food labels (Livesey, 1991b; Livesey, 1995). The Atwater factors for carbohydrate, protein, and fat are used in the factorial method:

\[ ME = 4P + 9F + 4C, \]

where the energy value is predicted as kcal. ME is the metabolizable energy value calculated, P, F, and C, represent gram (g) amount of protein, fat, and carbohydrate, respectively contributed by the food (Livesey, 1991b). This method overestimates the metabolizable energy value when the RS content is high in a food or complete diet.
(Livesey, 1995). Although, with 26 grams of unavailable carbohydrate included in the
diet, this calculation has been found to only slightly overestimate the metabolizable
energy value (Livesey, 1990).

Generally, a 20% error can occur when calculating the metabolizable energy
value of a food that contains RS or any other type of unavailable carbohydrate with the
factorial method when using the Atwater Factors (Miller & Judd, 1984). Previous
empirical calculations may have a four to six percent overestimation or a four to 12%
underestimation of the metabolizable energy value (Livesey, 1991a). For this reason, it is
necessary for researchers to use specific energy values that have been analyzed using
bomb calorimetry for the most precise values.

In 1989, Livesey published three empirical models that calculate effectively the
metabolizable energy of unavailable carbohydrate with a minimal level of measurement
errors. Only one of the three procedures was determined to provide the smallest error in
measuring the metabolizable energy value of a test substance. For example, the value of
the RS component in a food would be calculated using the Livesey formula:

\[
ME = \Delta H_{c,s} - \left( \frac{(E_{tf} + E_{tu} / M_{td})- ((E_{cf} + E_{cu} - E_{if} - E_{iu})/ M_{cd})}{(M_{s}/ M_{td})} \right),
\]

where all energy values are noted in kJ (1 kJ= 4.184 kcal), \(\Delta H_{c,s}\) is the heat of combustion
(kJ/g) of the test substance, \(E_{tf}\) is the gross energy (kJ) of the test feces, \(E_{iu}\) is the gross
energy of the urine from the test diet, \(E_{cf}\) is the gross energy of the control diet feces, \(E_{cu}\)
is the gross energy of urine from the control diet, \(E_{if}\) is the gross energy of the feces
which is lost due to the test diet, \(E_{iu}\) is the gross energy of the urine which is lost due to
the test diet, \(M_{td}\) is the basal portion (g) of the test diet, \(M_{cd}\) is the basal portion of the
control diet, and \(M_{s}\) is the basal portion of the test substance. The dry mass weight of the
diet, feces, and urine are used within the equation (Livesey, 1989).
The best method to determine correctly the metabolizable energy value of a diet is to use the ‘True Value Method’ when one is able to obtain gross energy values of dietary intake, feces, and urine of one consuming the diet, (Miller & Judd, 1984):

\[ ME = \text{Gross intake energy} - \text{gross fecal energy} - \text{gross urine energy}, \]

where the energy values can be recorded in kcal or kJ. This method is the major backbone of the factorial and empirical model energy value equations.

**Measurement of Digestible Energy**

Digestible energy values of RS diets are significant given that not all research studies include energy analysis of urine. When urine energy is not incorporated into the calculation, nitrogen-containing urea is not accounted for in the total energy excreted (Livesey, 1990). The metabolizable energy value of the diet would be overestimated. However, the loss of energy due to urine has been found to be small. When urine is not measured, studies involve only the gross energy value of diet intake and feces. The digestible energy value of a diet can be estimated by using the Atwater factors in a factorial calculation:

\[ DE = 5.25P + 9F + 4C, \]

where \( DE \) is the digestible energy value (kcal), \( P, F, \) and \( C \) represents the gram (g) amount of protein, fat, and available carbohydrate within the diet. This calculation does not take into account the 1.25 kcal per gram of protein lost in urine excretion, as did the metabolizable energy calculation of the Atwater factors or the presence of unavailable carbohydrate (Livesey, 1990).

An empirical model calculation for determining the digestible energy value of RS or any test substance within a diet was also published:

\[ DE = \Delta H_{c,s} - (((E_{cf} / M_{td}) - (E_{cf} - E_{tf} / M_{cd})) / (M_s / M_{td})), \]
where all energy values are noted in kJ. \( \Delta H_{c,s} \) is the heat of combustion (kJ/g) of the test substance, \( E_{sf} \) is the gross energy (kJ) of the test feces, \( E_{cf} \) is the gross energy of the control diet feces, \( E_{lf} \) is the gross energy of the feces lost from the test diet, \( M_{td} \) is the basal portion (g) of the test diet, \( M_{cd} \) is the basal portion of the control diet, and \( M_s \) is the basal portion of the test substance. The dry mass weight of the diet intake and feces are used within the equation. This equation, similar to the previous metabolizable energy calculation, was determined to greatly minimize experimental error (Livesey, 1989).

The digestible energy, as with metabolizable energy, is calculated efficiently with the ‘True Value’ Equation when the dietary intake and feces is collected and analyzed (Miller & Judd, 1984):

\[
DE = \text{Gross intake energy} - \text{gross fecal energy},
\]

where energy values can be recorded as kcal or kJ.

**Metabolizable Energy of RS**

In general, 70% of RS is fermented into SCFA and absorbed within the large intestine. One gram of unavailable carbohydrate has been reported to provide 1.7 kcal of energy from the production of SCFA (Livesey, 1995). Thirty percent of the energy produced from fermentation appears in the feces (Livesey, 1991b). Thus, a high percentage of SCFA produced by colonic bacteria are absorbed in the large intestine for energy and not excreted in the feces. Approximately 50% of the gross energy of non-digestible carbohydrate can be made available to humans for energy via fermentation (Livesey, 1995). Eighty to 90% of the gross energy of highly digestible standard starch contributes to its metabolizable energy value through normal digestion and absorption in the small intestine (Haralampu, 2000; Vonk et al., 2000).
There is a wide range for the metabolizable energy value for all non-digestible carbohydrate, which includes RS, fiber, and oligosaccharides. The greatest range is -5 to +3 kcal per gram of unavailable carbohydrate, which includes RS (Livesey, 1991a; Miller & Judd, 1984). The most common range is 1.5 to 2.5 kcal (Food and Nutrition Board, 2000; Livesey et al., 1990; Johnson, Livesey, Gee, Brown, & Wortley, 1990). The value of standard starch is closer to four kcal per gram, signifying a difference in digestion and absorption between RS and standard starch (Livesey, 1991a). Studies have generalized a digestible energy value of two kcal per gram for unavailable carbohydrate when intake is up to 70 grams daily (Behall & Howe, 1996; Livesey, 1991b; Livesey, 1995).

Through empirical methods, Behall & Howe determined a metabolizable energy value of 2.8 kcal per gram of RS$_2$ in human participants, however this value was not significantly different from the metabolizable energy value of the standard starch. The value determined was less than the standard value of four kcal per gram for digestible carbohydrate (Behall & Howe, 1996). Similarly, Livesey used an empirical method to measure the digestible energy value of the RS to be 3.66 and 2.96 kcal per gram in corn and pea RS sources, respectively. In another study by Behall and Howe, the digestible energy value of RS was less compared to the digestible starch, which was 3.94 kcal per gram (1990).

Stoichiometric calculations were used to determine the metabolizable energy value of RS, measuring the SCFA production. A range of 2.1 to 2.3 kcal/g was calculated (Mathers, 1992). Roberfroid used a factorial method to calculate the metabolizable energy of non-digestible carbohydrate, RS included, which undergoes fermentation within the colon. He determined that approximately 1.5 kcal per gram of carbohydrate would be metabolized within the human body (1999).
Summary

To understand the physiologic role of Hi-Maize® RS in humans and animals, it is necessary to determine the true value of digestible and metabolizable energy value of this component. Since 1910, researchers have been more successful in pinpointing the exact metabolizable energy values of composite foods. More research is needed to determine the metabolizable energy value of RS, to investigate the energy values of different food sources of RS (corn, pea, potato, and legumes), and to determine if RS truly has a significantly less metabolizable energy value compared to a digestible starch.

Resistant starch has been observed to avoid digestion and absorption in the small intestine and to ferment in the cecum or large intestine. In many studies, this has been observed to improve overall health, in both humans and animals. The limitations in our knowledge have been the result of much variation observed since studies have used various categories and sources of RS. There have also been differences seen between humans and animals, which complicate the reliability of associating results from animal studies to human results.

References


THE METABOLIZABLE ENERGY VALUE AND PHYSIOLOGIC EFFECTS OF 
HI-MAIZE® RESISTANT STARCH IN MALE RATS

Introduction

Carbohydrates are classified into two categories: digestible and non-digestible. 
Digestible carbohydrates consist of simple sugars and complex carbohydrates. Digestible 
starches can be either rapidly or slowly digested within the small intestine, releasing 
glucose for absorption. For example, rapidly digested starches include freshly cooked 
starchy foods. Slowly digested starches include most raw cereals (Englyst, Kingman, & 
Cummings, 1992). Non-digestible carbohydrates consist of complex carbohydrates such 
as fiber and some starches. Non-digestible starch is termed resistant starch (RS) because 
it resists the action of amylase enzymes in the small intestine. In the Western diet, RS 
would be found in, but not limited to, partly milled grains, legumes, and high amylose 
cornstarch. These non-digestible carbohydrates have been found to resist normal 
digestion and absorption within the gastrointestinal tract (Englyst, Kingman, & 
Cummings, 1992; Cummings, Beatty, Kingman, Bingham, & Englyst, 1996; Vitapole, 
2001).

One of the differences between digestible and non-digestible starch is the ratio of 
amylopectin to amylose molecules comprising the total starch content. Amylopectin is a 
starch molecule that can be easily digested and absorbed into the gastrointestinal tract. 
Amylose is less available than amylopectin for digestion and absorption because the 
glucose bonds are not easily accessible to digestible enzymes (Haralampu, 2000). 
Generally, dietary RS cornstarch contains 60 to 70% amylose and 30 to 40% amylopectin 
(Behall & Howe, 1996; Bird, Brown, & Topping, 2000; Haralampu, 1998).
Since RS is not digested in the stomach or absorbed in the small intestine RS may deliver less energy to the consumer (Cummings et al., 1996; Haralampu, 2000). Colonic bacteria of the large intestine and cecum ferment the undigested starch to produce short chain fatty acids (SCFA) (Bird et al., 2000; Bjork, Nyman, Pedersen, Siljestrom, Asp, & Eggum, 1986). The absorption of SCFA, as available energy to the body, salvages some of the energy lost from the non-digestible RS (Behall & Howe, 1996; Phillips, Muir, Birkett, Lu, Jones, O’Dea, et al., 1995). The amount of energy received from the production of SCFA varies depending on the amount and type of SCFA produced (Behall & Howe, 1996; Mathers, 1992). Up to 12% of the body’s energy needs can come from fermentation of RS (Behall & Howe, 1996; Cummings et al., 1996). However, not all RS is fermented since an increased amount of starch in the feces has been found in subjects consuming a diet containing RS compared to those on a consuming standard starch (Haralampu, 1998).

Previously, the energy values of carbohydrates, as well as proteins and fats have been determined by bomb calorimetry to be four, four, and nine kilocalories (kcal) per gram, respectively. These are called the Atwater Factors of 1910 (Moe, 1994). These values assume that the energy of the carbohydrate is fully digested and absorbed. Since RS carbohydrates are not readily digested and absorbed, the calculated value of four kcal per gram for carbohydrate is not likely to be correct (Livesey, 1991; Moe, 1994).

The amount of energy produced from the digestion and absorption of a dietary food source and retained within the body is defined as the metabolizable energy value of that food (Moe, 1994). Resistant cornstarch has been analyzed for metabolizable energy through bomb calorimetry in the past, but the value of 2.8 kcal per gram 70% amylose RS in male participants, was not significantly different from standard digestible starch.
(Behall & Howe, 1996). It is still believed that the metabolizable energy value is less for RS compared to digestible starch. Roberfroid used a factorial method to calculate approximately 1.5 kcal per gram of non-digestible carbohydrate would be metabolized within the human body when fermented in the colon (1999). This study was designed to calculate the digestible and metabolizable energy value of Hi-Maize® RS, a specific brand of 60% amylose cornstarch and to evaluate the physiological effects of Hi-Maize® RS in young growing male rats.

**Materials and Methods**

Eighteen, four-week old male Sprague-Dawley rats with a mean weight of 94.4 ± 5.1 grams were housed initially in individual stainless steel cages with wire mesh bottoms. All rats were kept on a 12-hour light/dark regimen (0700 hours light/ 1900 hours dark) with free access to food and water. The room was controlled at 22°C, with 60% humidity. The rats were stratified by weight and then assigned randomly to one of the three treatment groups: baseline (n=6) and control (n=6), fed a non-resistant starch diet; control (n=6); resistant starch (RS) (n=6), fed a RS diet after Week 2.

At the beginning of Week 2, the control and RS rats were placed in plastic metabolism cages (Lab Products, Maywood, NJ) for a one-week acclimation period to the cages before data collection was initiated. Baseline rats remained in wire mesh cages. All rats were fed the control diet for the first two weeks.

After the adaptation periods, the study was composed of three two-week periods (metabolic periods) through week 3 to week 8 (Table 1) where data of the control and RS groups were recorded. The baseline group was sacrificed prior to the beginning of the metabolic periods. The baseline group was included in this study to show differences in energy accumulation of the control and RS group carcasses throughout the three periods.
Diets

The two diets were modified from the standard American Institute of Nutrition (AIN)-93G diet for growing rats (Table 2). Two cornstarch sources were used for the diets, 100% amylopectin cornstarch (Cerestar, Hammond, IN) and Hi-Maize® RS (Penford, Plover, WI) which consisted of 60% amylose and 40% amylopectin. Control and baseline rats were fed the AIN-93G diet with 100% amylopectin starch. Resistant starch rats were fed the AIN-93G diet with 20% amylose (RS) and 26% amylopectin. The baseline, control, and RS rats were provided with 25 g diet daily.

Collection Procedure

Feces and urine were collected from each rat in a metabolism cage daily at 0800 hours. Each urine collection tube contained one ml of 10% HCl, which was added to
reduce nitrogen loss in the urine (Ozelci, Romas, & Leveille, 1997). For each metabolic period, all data collections and samples were pooled for each rat. Food intake for control and RS rats was recorded daily. Body weight, in grams, was measured and recorded three times a week for all rats.

Table 2. Modified American Institute of Nutrition (AIN)-93G Diet for Growing Rodents.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>RS Diet&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Control Diet&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Amylopectin Cornstarch&lt;sup&gt;3&lt;/sup&gt;</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>60% Amylose/40% Amylopectin Cornstarch&lt;sup&gt;4&lt;/sup&gt;</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>Casein/Gelatin&lt;sup&gt;5&lt;/sup&gt;</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose&lt;sup&gt;5&lt;/sup&gt;</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral Mix&lt;sup&gt;1,5&lt;/sup&gt;</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin Mix&lt;sup&gt;1,5&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline Bitartrate&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Cystine&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Soybean Oil&lt;sup&gt;5&lt;/sup&gt;</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>BHT&lt;sup&gt;5,6&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Total

<sup>1</sup>AIN-93G (Reeves, Nielsen, & Fahey, 1993)
<sup>2</sup>All values are in percent (%) of diet.
<sup>3</sup>High Amylopectin starch. (Cerestar, Hammond, IN)
<sup>4</sup>High amylose starch, Hi-Maize® (Penford, Plover, WI).
<sup>5</sup>Sucrose was attained from Thrify Maid (Sun Mateo, CA). Casein, Cellulose, Mineral Mix AIN-93G, Vitamin Mix AIN-93, Choline Bitartrate, L-Cystine, and BHT were attained from Dyets (Bethesda, PA). Soybean oil (Aster) was attained from Deep South Products.
<sup>6</sup>(BHT) Butylated hydroxytoluene

**Sacrifice**

The baseline rats were weighed and sacrificed at Week 3. The control and RS rats were weighed and sacrificed after Metabolic Period 3 (Week 9). Rats were anesthetized by isoflurane inhalation, 2.5%, administered to each rat via a nose cone. Under continued anesthesia, each rat was sacrificed by exsanguination through cardiac puncture. The gastrointestinal tract of each rat was excised. Weights of full and empty gastrointestinal tract organs (stomach, small intestine, cecum, and large intestine) were recorded. The
cleaned gastrointestinal tract of the rat was replaced in the carcass. pH indicator color strips measured the acidic level of the cecal contents (EM Science, Gibbstown, NJ). Adipose tissue from the abdomen, epididymis, and perirenal area was excised and weighed separately then replaced in the carcass.

**Analytical Procedure**

Diets and feces collections were dried in a freeze dryer (Modylo D, Thermo Savant, Holbrook, NY). Only urine collections for the third period were freeze dried, due to time constraints. The carcasses were homogenized with distilled water (Pro 250, Pro Scientific, Inc., Monroe, CT), and dried. Energy content of the diets, feces, urine, and carcasses was determined by bomb calorimetry (Parr 1722 Bomb Calorimeter, Moline, IL). Gross energy measurements from the bomb calorimeter were used to determine the digestible and metabolizable energy of the control and RS diet, and the control and RS component.

G. Livesey (1989) established a calculation for determining the digestible and metabolizable energy of a RS component within a diet:

\[
DEV = \Delta H_{c,s} - \left\{ \left\{ \frac{(E_{tf} / M_{td}) - (E_{cf} - E_{if} / M_{cd})}{M_s / M_{td}} \right\} \right.
\]

\[
MEV = \Delta H_{c,s} - \left\{ \left\{ \frac{(E_{tf} + E_{tu}) / M_{td}) - ((E_{cf} + E_{cu} - E_{if} - E_{iu} / M_{cd})}{M_s / M_{td}} \right\} \right.
\]

in which the digestible and metabolizable energy value is abbreviated DEV and MEV, respectively. For the DEV and MEV equations, the heat of combustion (\(\Delta H_{c,s}\)) is measured as kJ per gram of the Hi-Maize® RS. The gross energy of the test group feces (\(E_{tf}\)), control group feces (\(E_{cf}\)), control diet (\(E_{cd}\)), gross energy lost to the feces from the replaced energy source (\(E_{if}\)), and the test substance (\(E_s\)) is measured in kJ, which is calculated as the heat of combustion multiplied by the mass of each collection. The basal portion of the test diet (\(M_{td}\)), basal portion of the control diet (\(M_{cd}\)), and basal portion of
the test substance (Mₜ) are measured in grams. For the MEV equation, gross energy of
the urine from the test group (Eₜᵤ), urine from the control group (Eₙᵤ), and gross energy
lost to the urine from the replaced energy source (Eᵣᵤ), is measured in kJ.

Analysis over each period for the RS group digestible and metabolizable energy
values could not be accomplished because the equation does not allow the calculation of
each rat’s energy value of the diet. The equation involves grouping all data from the RS
group and all data from the control group. Analysis of each period (1-3) would not be
accurate for the digestible energy value. Analysis of the metabolizable energy value
could not be completed because only the third period was calculated.

The digestible and metabolizable energies of the diets were calculated with the
‘True Value’ Equation (Miller & Judd, 1984):

\[ DE = \text{Gross intake energy} - \text{gross fecal energy}, \]
\[ ME = \text{Gross intake energy} - (\text{gross fecal energy} + \text{gross urine energy}). \]

The difference of the digestible and metabolizable energy values from the total gross
intake energy was calculated as a percent and multiplied by the total gross intake energy.
The gross energy value of the carcasses was used to evaluate the amount of energy
retained within the control group compared to the RS group.

The heat of combustion and total gross energy values for the baseline, control,
and RS groups were compared to see the energy retained within the tissues of the rat.
The amount of gross energy gained from the beginning of the first metabolic period
throughout the end of the third period was measured by comparing the baseline carcass
energy values to the values of the RS and control groups. The average gross energy
value for the baseline group was subtracted from each individual rat in the RS and control
group providing the value of gross energy gained.
Statistical Methods

The data were analyzed using the Statistical Package for Social Sciences, SPSS Student Version 11.0 for Windows (SPSS Inc., Chicago, IL). Analysis of Variance (ANOVA) was used to analyze the differences between baseline, control, and RS group dependent variables. ANOVA was used to determine the significance of probability, $p \leq 0.05$. T-tests were used to compare differences between variables for the control and RS rat groups. Repeated Measures ANOVA determined any time-treatment or time effect among the three periods for each dependent variable.

Results

Health Properties Affected by Diet Treatment

Body weight and diet intake of the rats were not affected by diet treatment (Table 3). As expected, body weight increased over the three study periods showing a time effect for both diet groups ($p = 0.001$). Intake increased over time only for the RS-fed rats ($p < 0.001$).

Fecal excretion was greater in the RS group compared to the control ($p < 0.001$) with a time-treatment interaction compared to the control group ($p = 0.015$) (Table 3). There was an increased ratio of feces excretion to diet intake for the RS ($0.61 \pm 0.07, 0.54 \pm 0.02, 0.56 \pm 0.06$) compared to the control group ($0.22 \pm 0.01, 0.24 \pm 0.01, 0.26 \pm 0.02; p < 0.001$) for Periods 1, 2, and 3 respectively. There was a time-treatment interaction for the ratio of feces excretion to diet intake ($p = 0.02$). Percent moisture content of the feces remained relatively constant within each group. The average value for all periods combined was significantly greater for the RS group ($33.27\% \pm 2.94$) compared to the control ($11.71\% \pm 1.59; p < 0.001$). Urinary excretion was not affected by diet or period for either group.
### Table 3. Body Weight, Diet Intake, and Fecal Excretion per Period (Mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>RS Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>285 ± 15.0a</td>
<td>280 ± 10.8a</td>
</tr>
<tr>
<td>Period 2</td>
<td>339 ± 23.6b</td>
<td>335 ± 19.2b</td>
</tr>
<tr>
<td>Period 3</td>
<td>382 ± 23.0c</td>
<td>383 ± 29.3c</td>
</tr>
<tr>
<td><strong>Diet Intake (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>221 ± 8.1</td>
<td>216 ± 15.0a</td>
</tr>
<tr>
<td>Period 2</td>
<td>225 ± 14.5</td>
<td>224 ± 11.8</td>
</tr>
<tr>
<td>Period 3</td>
<td>227 ± 20.6</td>
<td>236 ± 8.4b</td>
</tr>
<tr>
<td><strong>Feces Wet Weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>26 g ± 1.5**</td>
<td>70 g ± 7.5d</td>
</tr>
<tr>
<td>Period 2</td>
<td>27 g ± 2.6**</td>
<td>60 g ± 3.8e</td>
</tr>
<tr>
<td>Period 3</td>
<td>29 g ± 3.8**</td>
<td>66 g ± 6.7</td>
</tr>
</tbody>
</table>

Each RS/Control mean pair in row with a *superscript is significantly different, * p < 0.01, ** p < 0.001. Each column value with a different letter is significantly different, (a, b, c; p < 0.001), (d, e; p < 0.01).

At sacrifice, total gastrointestinal tract weight, including its contents, was greater for the RS group (28.4 g ± 3.6) compared to the control group (16.5 g ± 1.3; p < 0.001).

The cecum and large intestine weighed more for the RS group than the control group (p < 0.001) (Table 4). The contents of the cecum (p < 0.001) and large intestine (p < 0.001) of the RS group were greater than those of the control group. The pH of the cecal contents of the RS group was more acidic (pH 6.1 ± 0.31) than the control group (pH 8.0 ± 0.14; p < 0.001).

The RS group showed a decrease in abdominal fat mass compared to the control group (p = 0.04) although the total weight of adipose tissue from the abdomen, epididymis, and perirenal area was not different between diet groups (Table 4). The
percentage of abdominal fat to body weight of the RS group (1.26% ± 0.32) was less than the control group (1.68% ± 0.27; p = 0.03).

Table 4. Gastrointestinal Tract Organ and Content Weight, and Abdominal Area Adipose Tissue Weight (Mean ± SD).

<table>
<thead>
<tr>
<th>Gastrointestinal tract organ and content weight</th>
<th>Organ Weight (g)</th>
<th>Control Group</th>
<th>RS Group</th>
<th>Control Group</th>
<th>RS Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>1.41 ± 0.1</td>
<td>1.42 ± 0.1</td>
<td>1.76 ± 0.6</td>
<td>2.54 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Small Intestine</td>
<td>6.00 ± 0.6</td>
<td>6.27 ± 1.0</td>
<td>1.15 ± 0.6</td>
<td>1.60 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>0.69 ± 0.1**</td>
<td>1.78 ± 0.4</td>
<td>2.61 ± 0.4**</td>
<td>10.17 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Large Intestine</td>
<td>1.14 ± 0.1**</td>
<td>1.50 ± 0.1</td>
<td>1.20 ± 0.5**</td>
<td>2.65 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abdominal Area Adipose Tissue Accumulation</th>
<th>Adipose Weight (g)</th>
<th>Control Group</th>
<th>RS Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen</td>
<td>6.45 g ± 1.3*</td>
<td>4.81 g ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Epididymis</td>
<td>2.64 g ± 0.6</td>
<td>2.58 g ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Perirenal area</td>
<td>1.60 g ± 0.4</td>
<td>1.24 g ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Total Adipose</td>
<td>10.69 ± 2.1</td>
<td>8.63 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>

Each RS/Control mean pair in row with superscript is significantly different, * p < 0.05, ** p < 0.001.

**Energy Values of Feces, Urine, and Diets**

The average fecal heat of combustion (kcal/g) for the RS group for periods 2 and 3 was greater than the average of the control group feces (p < 0.001) (Table 5). The gross energy (kcal) of fecal excretion of each period for the RS group was greater than the control group values (p < 0.001). There was a time-treatment interaction for fecal gross energy (p < 0.001). The gross energy (kcal) of the urine excretion for Period 3 did not differ between the RS group (41.4 kcal ± 2.33) and the control group (41.1 kcal ± 2.58).

The gross energy (kcal) of the total diet consumed for the RS and control group did not differ between diet treatments (Table 5). There was a time effect showing a gradual increase in consumption of gross energy (kcal) for both groups, but the RS group
had a greater increase in diet consumption over the control group (p < 0.05). There was a
slightly significant time-treatment interaction for consumption of gross energy (p = 0.05).
Rats fed the RS diet showed a slightly greater gross energy value (kcal/g) than the control
diet value by the end of the study (p< 0.05) (Table 6).

Table 5. Fecal, Gross, and Digestible Energy Values for Each Period (Mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heat of Combustion of Fecal Excretion (kcal/g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>3.36 ± 0.24a</td>
<td>3.30 ± 0.22a</td>
</tr>
<tr>
<td>Period 2</td>
<td>3.21 ± 0.16*</td>
<td>3.40 ± 0.24a</td>
</tr>
<tr>
<td>Period 3</td>
<td>3.04 ± 0.13b**</td>
<td>4.03 ± 0.24b</td>
</tr>
<tr>
<td><strong>Gross Energy of Fecal excretion (kcal)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>361 ± 33**</td>
<td>965 ± 95a</td>
</tr>
<tr>
<td>Period 2</td>
<td>361 ± 36**</td>
<td>860 ± 70b</td>
</tr>
<tr>
<td>Period 3</td>
<td>371 ± 59**</td>
<td>1115 ± 139c</td>
</tr>
<tr>
<td><strong>Gross Energy of Diet Consumed (kcal)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>1075 ± 40</td>
<td>1071 ± 74a</td>
</tr>
<tr>
<td>Period 2</td>
<td>1096 ± 71</td>
<td>1109 ± 59</td>
</tr>
<tr>
<td>Period 3</td>
<td>1103 ± 100</td>
<td>1173 ± 42b</td>
</tr>
<tr>
<td><strong>Digestible Energy of Diet Consumed (kcal)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>989 ± 38**</td>
<td>840 ± 55</td>
</tr>
<tr>
<td>Period 2</td>
<td>1009 ± 62**</td>
<td>903 ± 60</td>
</tr>
<tr>
<td>Period 3</td>
<td>1014 ± 88**</td>
<td>906 ± 48</td>
</tr>
<tr>
<td><strong>Digestible Energy of the Diet (kcal/g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>4.48 ± 0.04**</td>
<td>3.89 ±0.05a</td>
</tr>
<tr>
<td>Period 2</td>
<td>4.48 ± 0.01**</td>
<td>4.04 ± 0.09b</td>
</tr>
<tr>
<td>Period 3</td>
<td>4.48 ± 0.03**</td>
<td>3.83 ± 0.14a</td>
</tr>
</tbody>
</table>

Each RS/Control mean pair in row with superscript is significantly different, * p < 0.05, ** p < 0.005.
Each column value with a different letter is significantly different, (p < 0.05).
Table 6. Energy Values (kcal/g) for Diets at the End of Third Period (Mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>RS Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross Energy (kcal/g)</td>
<td>4.87 ± 0.80*</td>
<td>4.96 ± 0.17a</td>
</tr>
<tr>
<td>Digestible Energy (kcal/g)</td>
<td>4.48 ± 0.03**</td>
<td>3.83 ± 0.14bd</td>
</tr>
<tr>
<td>Metabolizable Energy (kcal/g)</td>
<td>4.29 ± 0.03**</td>
<td>3.66 ± 0.14ce</td>
</tr>
</tbody>
</table>

Each RS/Control mean pair in row with superscript is significantly different, * p < 0.05, ** p < 0.005. Each column value with a different letter is significantly different, (a, b, c; p < 0.001), (d, e; p < 0.05).

The digestible energy (kcal) of the total diet consumed and utilized for all three periods was less for the RS group compared to the control group (p < 0.001) (Table 5). There was a time-treatment interaction (p = 0.002). The digestible energy value (kcal/g) of the RS diet was less than the control diet for all periods (p < 0.001). In the second period, the digestible energy value in the RS group increased compared to the first and third periods (p < 0.001). A constant digestible energy value of the diet for the control group was observed for all three periods. There was a 79% ± 2.6 average decrease from gross energy to digestible energy value (kcal/g) for the RS diet compared to a 92% ± 0.6 average decrease of the control group for all three periods (p < 0.001).

The metabolizable energy value (kcal/g) of the RS diet in the third period was significantly less than the value of the control diet (p < 0.005) (Table 6). The percent of RS diet metabolized was 73.75% ± 2.80 compared to an 88.24% ± 0.60 decrease of the control diet (p < 0.001). There was a 4.57% ± 0.28 difference in percent of RS diet digested compared to percent metabolized, which was not significantly different compared to the 4.08% ± 0.48 difference for the control group. The difference between the digestible and the metabolizable energy value for either diet was insignificant.

Calculating the metabolizable energy (kcal) of the total diet consumed and utilized, for both groups, showed that the RS group metabolized less dietary energy compared to the control group for each of the three metabolic periods (p = 0.004) (Table
7). Food efficiency (weight gained, g/diet intake, g) of the RS group (0.43 g/g ± 0.05) was not different from the control group (0.43 g/g ± 0.04) for the third metabolic period.

Table 7. Metabolizable EnergyΦ (kcal) of the Diets (Mean ± SD).

<table>
<thead>
<tr>
<th>Metabolized energy of Diet Consumed (kcal)</th>
<th>Control</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>948 ± 36**</td>
<td>801 ± 52</td>
</tr>
<tr>
<td>Period 2</td>
<td>968 ± 60**</td>
<td>861 ± 57</td>
</tr>
<tr>
<td>Period 3</td>
<td>973 ± 88**</td>
<td>865 ± 47</td>
</tr>
</tbody>
</table>

Each RS/Control mean pair in row with superscript is significantly different, ** p < 0.005, Φ The metabolizable energy value from period three was used to calculate the metabolized energy intake for the first and second periods.

Energy Values of Hi-Maize® RS

The heat of combustion (kcal/g) of the Hi-Maize® cornstarch portion of the RS diet contained 3.71 ± 0.11 kcal/g. The heat of combustion for the 100% amylopectin cornstarch of the control diet was 3.60 ± 0.09 kcal/g. The calculated digestible energy values (kcal/g) for the Hi-Maize® RS was 1.72 kcal/g, 2.13 kcal/g, and 1.55 kcal/g, respectively for Periods 1, 2, and 3. The calculated metabolizable energy value was 1.55 kcal/g for Period 3.

Energy Values of the Carcass

The heat of combustion (kcal/g) values of the RS carcasses were not significantly different from either baseline or control (Table 8). The baseline group, as expected, had the lowest gross energy (kcal) of the carcass groups at sacrifice (p < 0.001). The control group had the greatest gross energy at sacrifice (p = 0.03), with RS group in-between. From Period 1 through Period 3, the RS group gained less carcass energy (p = 0.05) compared to the control group. The control group gained approximately 213 kcal more than the RS group from Period 1 through Period 3. The energy efficiency ratio of the
diets (energy gained, kcal/total energy metabolized, kcal) for the RS group (0.496 kcal/kcal ± 0.03) was less than the ratio for the control group (0.663 kcal/kcal ± 0.06; p < 0.001).

Table 8. Gross Energy Values within the Carcass (Mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Control</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat of Combustion (kcal/g)</td>
<td>1.87 ± 0.45a</td>
<td>2.72 ± 0.63b</td>
<td>2.23 ± 0.17ab</td>
</tr>
<tr>
<td>Total Gross Energy at Sacrifice (kcal)</td>
<td>364 ± 101a</td>
<td>1005 ± 226b</td>
<td>793 ± 72c</td>
</tr>
<tr>
<td>Gross Energy Gained During Study (kcal)</td>
<td>641 ± 226</td>
<td>428 ± 72</td>
<td></td>
</tr>
</tbody>
</table>

Each row value with a different letter is significantly different, p < 0.05.

There was a positive correlation observed between the gross energy and total abdominal area fat of all groups (r = 0.84; p ≤ 0.01). For each individual treatment group, baseline, control, and RS groups, results show no correlation for carcass gross energy to fat. Relating to the energy composition of the carcasses, percent moisture content of the carcasses in the RS group (65% ± 4.5) was not significantly different from the control group (57% ± 11.0) or the baseline group (69% ± 7.0). The control group had lower percent moisture content compared to the baseline (p = 0.03).

**Discussion**

The metabolizable energy value of Hi-Maize® RS was determined to be 1.55 kcal/g after a six-week adaptation to the RS diet. The digestible energy value of the Hi-Maize® cornstarch was basically the same as the metabolizable energy value due to negligible energy excretion from urine. The metabolizable energy value of the RS diet (20% RS) was 0.63 kcal/g less than the digestible starch diet. This study suggests that a
diet containing RS will provide less energy to the body compared to a digestible starch diet.

Several studies have reported a metabolizable or digestible energy value for RS or similar unavailable carbohydrate (Table 9). Previous studies either measured the energy balance of the energy intake to output through bomb calorimetry (Behall & Howe, 1996) or determined the energy value through specific calculations or general estimations (Mathers, 1992; Roberfroid, 1999). The digestible energy value that many researchers generalize for all unavailable carbohydrate is 2 kcal/g. This value is approximately 50% of the gross energy utilized within the body (Livesey, 1990; Livesey, 1995).

Energy values established in the past have varied due to the type of RS tested (RS₁-RS₄), the dietary source consumed (corn, pea, potato, or bean), or whether it was a human or animal study. Most metabolizable and digestible energy values reported from other studies have been calculated to be greater than 1.55 kcal/g for Hi-Maize® RS₂ reported in this study. In a rat study, Livesey and associates found a difference in digestible energy values for two RS₃ sources, 3.66 kcal/g and 2.96 kcal/g, for corn and pea, respectively (1990). The main difference from the present study was that the study used RS₃, retrograded starch, not RS₂, ungelatinized starch. Behall and Howe determined that the metabolizable energy value of RS₂ from a corn source was 2.8 kcal/g, but this value was not different from the metabolizable energy value of the digestible cornstarch diet (1996). The main differences between the Behall and Howe study compared to the present study was human verses rat study and the use of 70% amylose cornstarch for the RS diet instead of 60% amylose cornstarch. Only one study provided evidence for a value similar to that of the present study (Roberfroid, 1999). Roberfroid used a factorial method calculation to determine the metabolizable energy value of all fermentable
unavailable carbohydrates to obtain 1.5 kcal/g. The method accounts for fermentable substrate, excretion of feces, bacterial mass, loss of carbon atoms due to fermentation, and the efficiency of SCFA compared to glucose.

Table 9. Previously Reported Energy Values (kcal/g).

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Type of Carbohydrate</th>
<th>Type of Energy</th>
<th>Energy Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atwater, 1910†</td>
<td>All carbohydrate</td>
<td>Metabolizable</td>
<td>4 kcal/g</td>
</tr>
<tr>
<td>Behall &amp; Howe, 1996*</td>
<td>Resistant corn starch (RS₂)</td>
<td>Metabolizable</td>
<td>2.8 kcal/g</td>
</tr>
<tr>
<td>Livesey et al., 1990**</td>
<td>Resistant corn starch (RS₃)</td>
<td>Partial digestible</td>
<td>3.66 kcal/g</td>
</tr>
<tr>
<td>Mathers, 1992***</td>
<td>Resistant pea starch (RS₃)</td>
<td>Partial digestible</td>
<td>2.96 kcal/g</td>
</tr>
<tr>
<td></td>
<td>Resistant haricot bean starch (RS₃)</td>
<td>Metabolizable</td>
<td>2.1 to 2.3 kcal/g</td>
</tr>
<tr>
<td>Livesey, 1995***</td>
<td>Unavailable carbohydrate</td>
<td>Metabolizable</td>
<td>2 kcal/g</td>
</tr>
<tr>
<td>Roberfroid, 1999***</td>
<td>Unavailable carbohydrate</td>
<td>Metabolizable</td>
<td>1.5 kcal/g</td>
</tr>
</tbody>
</table>

†Livesey, 1991. Metabolizable = Gross energy intake – (fecal + urinary energy); Partial digestible = Gross energy intake – fecal energy; * Human Study; ** Rat Study; *** Calculation.

Most studies agree that dietary RS increases fecal matter in both humans and animals (Ferguson et al., 2000; Heijnen et al., 1996; Hylla et al., 1998; Livesey, Davies, Brown, Faulks, & Southon, 1990). Calculations determining the metabolizable energy values presume the feces to contain only undigested diet and products of fermentation that are not absorbed. Consumption of a digestible starch diet generally produces less than one percent starch in the feces (Ferguson et al., 2000), but as high as ten percent with consumption of a RS diet (Cummings et al., 1996). Conversely, a study by Mathers observed no RS in the feces even with dietary consumption of RS₃ at very high concentrations (1992). The increase in fecal mass is also due to the proliferation of beneficial bacteria in the colon (Hylla et al., 1998). This can be measured by an increase in fecal nitrogen (Cummings et al., 1996). Therefore, possible under-estimation of the metabolizable energy value may occur if the energy from the bacterial mass is mistaken to be from undigested RS.
The digestible energy value differences between the periods for the RS diet may be the result of adaptation to fermentation of dietary Hi-Maize® RS in the cecum and large intestine. Malabsorption of RS was obvious in the first period, whereas in the second period, the rate of fermentation appeared to increase. Hence, the increased SCFA absorption decreased total fecal mass excretion causing the digestible energy value of the RS diet to increase. By the end of the third period, fermentation of the RS diet became more efficient compared to the first and second periods. The products of fermentation increased, as did fecal bulk due to fermentation, causing the digestible energy value of the RS diet to decrease. A two-week study measuring the digestion and absorption of RS3 in the cecum of rats showed no sign of an adaptation period to either of two diets containing RS (corn or pea) (Faulks, Roe, & Livesey, 1992). Two weeks may be too short for any physiologic changes within the animal to take place.

There was no association between the consumption of RS and body weight. The energy intake (kcal) was less for the RS group compared to the control group, while diet intake (g) was the same. The rats had minimal compensation for the decreased energy density of the RS diet by increasing their food consumption. This suggests that RS may provide satiation with decreased energy intake, which may suppress overeating of excess calories. This satiety factor may also be due to the increase in fermentation of the RS in the colon, which provides energy from the SCFA but also may expand the abdomen due to the gas production providing a full feeling. Previous research did not suggest that RS3 could prevent obesity (Livesey et al., 1990). The rats consuming a RS diet had similar diet intake (g), weight gain, and epididymal fat compared to the control rats consuming a digestible starch diet.
The energy efficiency value for the RS group was less than that of the control group. The RS group gained less carcass energy for the amount of the metabolized diet. This shows that the RS group expended or wasted more energy compared to the control group. Many reasons could be possible for this result. The RS group may have had an increase in metabolic rate, which led to the burning of more calories by not exerting more effort. Another possibility is that the RS group could have been more physically active than the control group, though this is unlikely because the groups were in the same small cages. The amount of gas production could be an additional reason for the difference in energy. The consumption of RS produces methane and hydrogen from bacterial fermentation, which allows energy to be released as the gases (Heijnen et al., 1996; Hylla et al., 1998).

Although Livesey and associates (1990) found no effect of RS3 on epididymal fat pads, the present study provides evidence that consumption of RS2 can reduce adiposity. The RS group had less abdominal fat and increased lean body tissue, which is very important for preventing coronary heart disease and diabetes. In the RS group, the increase observed in lean mass was due to the enlarged cecum and large intestine and was confirmed by the greater moisture content of the RS carcasses compared to the control carcasses. These results are supported by the findings of Livesey and associates (1990). Many studies support the role that RS increases lean tissue mass where fermentation occurs (De Schrijver et al., 1999; Faulks et al., 1989; Livesey et al., 1990). Since the RS group did not differ in body weight but had less adipose tissue than the control group, the increased weight of the lean tissue contributed to making the RS group weight comparable to the control group.
Evidence of increased moisture of the feces and decreased pH within the cecum supports previous studies findings that consumption of a RS diet promotes colon health in humans and animals. Contrary to most studies, moisture content of the feces was greater for the RS group showing that the RS diet possessed some water-holding properties (Ferguson et al., 2000; Livesey et al., 1990). The increase in water content may have been due to the assumed decrease in transit time, which decreases water reabsorption. The transit time was assumed to be decreased for the RS group due to the greater amount of feces excreted over a 24-hour period compared to the control group. The less time the feces stay within the colon and the greater the moisture content of the feces are beneficial criteria for a healthy environment (Ferguson et al., 2000). Generally, a decreased transit time is not observed in humans or animals consuming RS, since it is relatively insoluble (Ferguson et al., 2000; Hylla et al., 1998; Livesey et al., 1990). The observed decrease in pH confirms an increase in SCFA within the cecum and large intestine. At high levels, butyrate has been suggested to protect against colorectal cancer (Ahmed, Segal, & Hassan, 2000; Ferguson et al., 2000). Propionate has been found to reduce serum and hepatic cholesterol levels when highly concentrated in the blood in rats (Cheng & Lai, 2000) but not for humans (Heijnen et al., 1996). Before making any conclusions determining the type and amount of SCFA produced specifically from Hi-Maize® RS, more research must be completed to determine whether consumption of Hi-Maize® RS can protect against cancer and reduce cholesterol levels in rats and humans.

This study confirmed that dietary Hi-Maize® RS provides less metabolizable energy to the body compared to digestible cornstarch. The digestible energy value is suitable for determining the amount of dietary energy rats absorb and utilize within the body. The metabolizable energy value is similar to the digestible energy value in rats.
Less energy was digested and absorbed from a RS diet, but the RS group did not compensate this loss by increasing the intake. Food intake was minimally increased over the three periods for the RS group; however the digestible energy of the total intake was constantly less than the control group. This suggests that colonic fermentation of RS may produce satiety at a level of energy intake that is significantly less than that of a digestible starch. This also suggests that a diet containing RS may reduce the health risk of obesity, with a decrease in abdominal fat composition and an increase in lean mass. Future studies will be able to confirm the amount of dietary energy from Hi-Maize® RS absorbed and metabolized in humans. This study’s findings are important to understanding the physiologic role of RS within the body.

References


CONCLUSION

The purpose of this study was to determine the metabolizable energy value of Hi-Maize® RS and to observe physiologic differences in rats consuming a high-RS diet compared to rats consuming a standard digestible starch diet.

As hypothesized, the Hi-Maize® RS cornstarch and the RS diet had lower digestible and metabolizable energy values compared to the gross energy value of the cornstarch and diet itself. This shows that the consumed gross energy of Hi-Maize® RS is not completely digested and absorbed. The digestible and metabolizable values of the RS diet and the Hi-Maize® RS component were similar suggesting the digestible energy value is suitable for measuring the energy utilized within the body in rats.

This study also confirmed the hypothesis that the RS diet would increase fermentation within the cecum and large intestine. There was a decrease in pH and an increase in cecal and large intestine contents observed for the RS group compared to the control.

Hi-Maize® RS was hypothesized to have health benefits. The total body weight of the RS group was similar to the control group for each period, but by the end of the study, abdominal adipose tissue decreased while lean tissue mass increased. This was supported by the increased water composition of the carcass and the increase in cecal and large intestinal weight compared to the control group. Since abdominal fat in humans is a risk factor for a number of chronic diseases, any dietary treatment that can specifically decrease abdominal fat has potential to improve health.

As assumed, the study observed a possible adaptation to the RS diet over the six-week period. There was a definite difference in fecal excretion for the three periods of
the RS group. Hence the digestible energy value for the RS diet and Hi-Maize® component itself was different for each metabolic period. Adaptive changes in fecal excretion only occurred in the RS group since there was no difference seen with the group consuming the digestible starch diet. This observation suggests that for optimal efficiency of the Hi-Maize® RS diet, there must be time allowed for the body to adjust to the malabsorption of RS in the small intestine and increase in fermentation of RS in the cecum and large intestine.

This study confirmed the hypothesis that less energy would be retained in the RS carcasses compared to the digestible starch carcasses. The total gross energy at sacrifice was less for the RS group compared to the control group. Less energy was gained per RS intake (kcal) metabolized compared to the digestible starch intake. Despite no differences in final body weight, the energy difference was apparently from an increase in lean tissue for the RS group compared to an increase in adipose tissue for the control group.

Overall, this study has shown that the metabolizable energy from Hi-Maize® RS is 1.55 kcal/g. Thus, RS addition to the diet can decrease the total energy consumed. In addition, the RS diet was found to increase the lean body tissue while decreasing total adipose tissue within the body. Therefore, consumption of Hi-Maize® RS may be a useful tool in reducing obesity, but there is need for further studies before RS should be recommended to a great extent in human diets.
APPENDIX A

DIET MIXING PROCEDURE

The two diets were modified from the standard American Institute of Nutrition (AIN)-93G diet specialized for growing rats (Reeves, Nielsen, & Fahey, 1993). The control and baseline rats were fed the AIN-93G with the high amylopectin starch (Cerestar, Hammond, IN). The RS rats were fed the AIN-93G diet with the Hi-Maize® RS (Penford, Plover, WI) as the amylose starch source. The composition of the diets is presented in Table 1. The preparation of the AIN-93G diet included preparing an AIN-93G macronutrient mix (Dyets, Bethesda, PA) and an AIN-93G micronutrient mix (Dyets, Bethesda, PA). The ingredients of the macronutrient mix were measured and added to a 20-quart stainless steel mixing bowl: 0.6 kilograms (kg) sucrose, 1.20 kg casein, 2.76 kg starch, and 0.3 kg fiber. The bowl was covered with a plastic bag to reduce spillage. The ingredients were mixed at low speed for ten minutes in the Hobart A200FD Industrial mixer (Hobart Mfg., Troy, OH). The sides of the bowl were scraped to assure even and complete mixing. The mixing continued for an additional five minutes. Butylated hydroxytouluene (BHT) was added to soybean oil in a ratio of 0.9g: 1.25 gallons. The mixture of BHT and soybean oil, 0.84 kg, was measured and set aside. The micronutrient mix was prepared by adding 0.21 kg AIN-93G mineral mix (Dyets, Bethlehem, PA), 0.06 kg vitamin mix (Dyets, Bethlehem, PA), 0.015 kg choline bitartrate (Dyets, Bethlehem, PA), 0.018 kg L-cystine (Dyets, Bethlehem, PA), and a small unmeasured amount of macronutrient mix to a small mixing bowl and mixing on low speed for five minutes. The micronutrient mix was then added to the macronutrient mix in the larger bowl using a wire mesh sieve. After mixing the macro- and micro- nutrient
Dry mixtures for ten minutes, the sides of the bowl were scraped and the diet was mixed for another ten minutes. The soybean oil with the BHT mixture was added to the dry mixture and mixed for five minutes. The side of the bowl was scraped. The diet was mixed another ten minutes for even distribution. The diet was stored in Rubbermaid containers or Ziploc plastic storage bags. The storage container was marked with the type of diet produced, the date, and the batch number. The prepared diets were kept in a freezer until they were used for feeding the rats.

Table 1. Composition for Experimental Diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>RS Diet</th>
<th>Control Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Amylopectin Cornstarch³</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>60% Amylose/40% Amylopectin Cornstarch⁴</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>Casein/Gelatin⁵</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose⁵</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose⁵</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral Mix¹,5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin Mix¹,5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline Bitartrate⁵</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Cystine⁵</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Soybean Oil⁵</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>BHT⁵,6</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Total

¹AIN-93G (Reeves, Nielsen, & Fahey, 1993)
²All values are in percent (%) of diet.
³High amylopectin starch. (Cerestar, Hammond, IN).
⁴High amylose, Hi-Maize® starch (Penford, Plover, WI).
⁵Sucrose was attained from Thrifty Maid (Sun Mateo, CA). Casein, Cellulose, Mineral Mix AIN-93G, Vitamin Mix AIN-93, Choline Bitartrate, L-Cystine, and BHT were attained from Dyets (Bethesda, PA). Soybean oil used was an Astor Product attained from Deep South Products (Fitzgerald, GA).
⁶(BHT) Butylated hydroxytoluene
# APPENDIX B

## TIMELINE OF PROCEDURES

<table>
<thead>
<tr>
<th>Adaptation Period 1</th>
<th>Adaptation Period 2</th>
<th>ME 1 Period</th>
<th>ME 2 Period</th>
<th>ME 3 Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n=6)</td>
<td>Sacrifice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless Steel Cages</td>
<td>Control diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats (n=18)</td>
<td>Control (n=6)</td>
<td>Control diet</td>
<td>Control diet</td>
<td>Control diet</td>
</tr>
<tr>
<td>Stainless Steel Cages</td>
<td>Metabolism Cages</td>
<td>Control diet</td>
<td>Control diet</td>
<td>Control diet</td>
</tr>
<tr>
<td>Control diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant Starch (n=6)</td>
<td>20% RS diet</td>
<td>20% RS diet</td>
<td>20% RS diet</td>
<td>20% RS diet</td>
</tr>
<tr>
<td>Metabolism Cages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"Week"
APPENDIX C

DATA COLLECTION

The metabolic cages contained two pre-weighed 50 ml Corning centrifuge collection tubes (Corning, Inc., Acton, MA) per cage, which collected the feces and urine separately. The collections of feces and urine started daily at 0800 hours. The slide was rinsed with five ml of distilled water each morning. The collection tubes were removed and capped until prepared for weighing and cleaning. Clean, pre-weighed collection tubes replaced the full tubes collected for the previous 24 hours. Each clean tube for collection of urine contained 1 ml of 10% HCl (1HCl: 9 distilled water), which was added to reduce nitrogen loss in the urine.

Cage cleaning was set to a daily rotating schedule that gave each rat a clean cage once per week. The used funnels and slides were replaced with clean funnels and slides. Fifty ml of distilled water was used to rinse the rat hair and the food spillage from the funnel and slide, which was collected into two tubes. Every other week, the rack of cages were completely changed and washed in the RW4250 Cage Washer (Basil Equipment Corporation, Wilson, OH).

The fecal contents for the control and RS rats were weighed and cleaned of the rat hair and spillage daily. The cleaning process involved emptying all of the contents of the collection tubes onto a weigh boat lined with disposable napkins. The feces were rinsed with distilled water to dissolve any remaining starch (spillage) and to remove any rat hair. The cleaned feces were dried for 30 minutes, after which any remaining rat hair on the feces was removed with tweezers and Kimwipes. The dry, cleaned feces were poured back into the rinsed and dried collection tube for weighing. The spillage weight of the
diet from the feces was recorded for each rat. The feces were emptied into an eight-
ounce glass ointment jar and stored in a freezer at -20°C until the analysis of the energy
content could be performed.

The urine was collected in marked 50 mL Corning centrifuge tube, labeled Tube
one, and weighed. Parafilm covered each tube after removal from the metabolic cages.
All the collection tubes of urine for the control and RS rats were placed into the J6B
Centrifuge (Beckman, Palo Alto, CA) to remove any spilled diet (spillage) in the
collection tube of urine. The tubes were centrifuged at 3000 rpm for 15 minutes at 20°C.
The centrifuged urine was poured into an empty collection tube, labeled Tube 2, leaving
the spillage in Tube 1. Five ml of distilled water was added to Tube 1 to dilute the
spillage. Tube 1 was stirred, parafilmed, and centrifuged a second time for 15 minutes at
3000 rpm at 20°C. The additional urine in Tube 1 was combined with Tube 2. The urine
in Tube 2 was weighed, recorded, and stored in the freezer at -20°C until used in the
energy analysis. The precipitate (i.e., centrifuged spillage) in Tube 1 was resuspended in
five ml of distilled water and filtered in pre-weighed #2 Brew Rite coffee filters
(Rockland Industries, Sheboygan, WI).

Spillage was retrieved from cleaning the funnel from the metabolism cage. The
cage was rinsed with 50 ml distilled water and centrifuged at 3000 rpm for 15 minutes,
decanted, and filtered in the same filters mentioned previously. The filters dried within
24 hours. The spillage weight was calculated from weighing the filter. The total spillage
weight, from the cage and urine and feces collection tubes, was subtracted from the
specific diet intake of each rat.
APPENDIX D

PREPARATION FOR ENERGY ANALYSIS

After each two-week period (ME1, ME2, ME3), the collections of the feces and the samples of diets used were placed in the ModuloD Freeze Dryer (Thermo Savant, Holbrook, NY) to remove all moisture. The frozen samples, including the jars, were weighed and placed in the freeze dryer when the temperature reached –45°C. The jars were allowed to continue to dry until the weight of the samples remained the same between drying periods. The dried samples were ground using a mortar and pestle to completely mix the contents. The ground samples were stored in a Sampla desiccator (Samplec, Japan) until they were analyzed of their energy content.

In order to prepare a sample of each rat carcass, the individually bagged frozen rats were autoclaved in an Amsco Autoclave (Continental Equipment Co., Lawrence, KS) for 30 minutes at a 120°C liquid cycle. The rats were cut into small pieces with a cleaver to reduce the homogenization time. The pieces were placed into the homogenizer cylinder. Distilled water was added to cover the pieces. All materials used were rinsed with distilled water to clean off anything that remained. The rinsing water was added to the same homogenizer cylinder. A Pro 250 Homogenizer, model number 91-01250, (Pro Scientific, Inc., Monroe, CT), with a one-inch diameter blender blade, homogenized the carcass pieces for two to four minutes at 600 rpm. The homogenized mixture was transferred into a Windmere commercial blender (Applica Consumer Products Inc., Miami Lakes, FL). The homogenizer cylinder was rinsed with distilled water to retrieve all of the mixture into the blender. The blended mixture was then poured into a pre-weighed bucket to measure the weight of the liquefied carcass. An aliquot was taken
from the total homogenized liquid while a hand-held mixer stirred the liquid. The mixture was poured into a pre-weighed Qorpack eight-ounce ointment jar (Fisher Scientific, Houston, TX), and weighed. The remaining homogenate was stored in a large glass jar and frozen at –20°C. The aliquot was freeze-dried, ground with a mortar and pestle, and stored until the energy content analysis.

Two one-gram aliquots of dried feces, urine, and carcass were pressed into a pellet using a Pellet Press (Parr Instruments Co., Moline, IL). Only the urine collected during the ME 3 period was dried and pelleted. Six pellets were made for each diet for the energy analysis. Four pellets were made for the 100% amylopectin starch and the Hi-Maize® RS each. The pellets were stored in a desiccator until the analysis of the energy could be performed.

A 1722 Bomb Calorimeter (Parr, Moline, IL) was used to analyze the heat of combustion for all the samples. Each pellet’s weight was recorded. The temperature of the water bath reached 35°C. A ten cm fuse wire was connected to the bomb lid touching the pellet sample only. After the lid was placed on the bomb, oxygen, at 450 psi (pounds per square inch), was sent into the bomb creating pressure. The bomb was placed in a 2 L (liter) bucket of water from the water bath. The stainless steel bucket (Parr, Moline, IL) was placed inside the bomb jacket. The bomb identification number, sample identification number, and weight of the sample were entered into the computer. After the bomb fired, the temperature change of the water bath was measured. A print out of the results provided the heat of combustion for the sample. The computed value of the gross heat was used in a calculation of the metabolizable energy value of the starch diets.
APPENDIX E

METABOLIZABLE ENERGY ANALYSIS

The heat of combustion values, mass amounts, and the gross energy (heat of combustion multiplied by the mass) from feces, urine, and intake were used in a formula for determining the digestible and metabolizable energy of the diets and the Hi-Maize® RS. The primary equation to calculate the digestible energy of the treatment starch ingredient was determined by G. Livesey in 1989:

\[
\text{DEV} = \Delta H_{c,s} - \frac{\{(E_{tf} / M_{td}) - ((E_{cf} - E_{if}) / M_{cd})\}}{(M_{s} / M_{td})},
\]

where the digestible energy value is DEV. The heat of combustion (\(\Delta H_{c,s}\)) is measured as kJ per gram of the Hi-Maize® starch. The gross energy of the test group feces (\(E_{tf}\)), gross energy of the control group feces (\(E_{cf}\)), gross energy of the control diet (\(E_{cd}\)), gross energy lost to the feces from the replaced energy source (\(E_{if}\)), and the test substance (\(E_{s}\)) is measured in kJ, which is calculated as the heat of combustion multiplied by the mass of each collection. The basal portion of the test diet (\(M_{td}\)), basal portion of the control diet (\(M_{cd}\)), and basal portion of the test substance (\(M_{s}\)) are measured in grams.

The equation to calculate the metabolizable energy of the treatment starch ingredient was also determined by G. Livesey in 1989:

\[
\text{MEV} = \Delta H_{c,s} - \frac{\{((E_{tf} + E_{tu}) / M_{td}) - ((E_{cf} + E_{cu} - E_{if} - E_{iu}) / M_{cd})\}}{(M_{s} / M_{td})},
\]

in which the metabolizable energy value is MEV. The heat of combustion (\(\Delta H_{c,s}\)) is measured as kJ per gram of the Hi-Maize® starch. The gross energy of the test group feces (\(E_{tf}\)), gross energy of the urine from the test group (\(E_{tu}\)), gross energy of the control group feces (\(E_{cf}\)), gross energy of urine from the control group (\(E_{cu}\)), gross energy lost to the feces from the replaced energy source (\(E_{if}\)), and gross energy lost to the urine from
the replaced energy source \((E_{iu})\), is measured in kJ. The basal portion of the test diet \((M_{td})\), basal portion of the control diet \((M_{cd})\), and basal portion of the test substance \((M_s)\) are measured in grams.

The digestible and metabolizable energies of the diets were calculated with the ‘True Value’ Equation (Miller & Judd, 1984):

\[
DE = \text{Gross intake energy} - \text{gross fecal energy},
\]

\[
ME = \text{Gross intake energy} - \text{gross fecal energy} - \text{gross urine energy}.
\]

The difference of the digestible and metabolizable energy values from the total gross intake energy was calculated as a percent and multiplied by the total gross intake energy. The gross energy value of the carcasses was used to evaluate the amount of energy retained within the control group compared to the RS group.
APPENDIX F

GROSS ENERGY GAIN ANALYSIS

The gain of gross energy for the RS and control group during the study was calculated by subtracting the average gross energy (kcal) retained of the baseline rats from the gross energy retained of the individual rats in each of the groups. The mean gross energy of each group was then analyzed for statistical significance. The gross energy gained for each group was used to compare the effects the diet treatments had on increasing energy storage within the groups throughout the six-week period.
APPENDIX G

STATISTICAL ANALYSIS

The study was analyzed using the Statistical Package for Social Sciences, SPSS Student Version 11.0 for Windows (SPSS Inc., Chicago, IL). Analysis of Variance (ANOVA) was used to determine the significance, set at $P \leq 0.05$. ANOVA was used to analyze the differences between baseline, control, and RS group dependent variables. T-tests were used to compare differences between data variables for the control and RS rat groups. The comparison between the ME 1, 2, and 3 periods was analyzed for each dependent variable using an ANOVA for Repeated Measures to measure the time-treatment effect.
APPENDIX H

IACUC PROTOCOL

LOUISIANA STATE UNIVERSITY
AND AGRICULTURAL AND MECHANICAL COLLEGE
Institutional Animal Care and Use Committee
Division of Laboratory Animal Medicine

Animal Welfare Assurance # A3611-01
License # 72-3
Multiple Assurance # M1128

April 18, 2002

Dr. Maren Hegsted
Department of Human Ecology

Dear Dr. Hegsted:

Protocol #01-086, entitled “The Effects of Low Versus High Glycemic Index (GI) Starch on Weight Gain and Fat Accumulation” lists you as the Principal Investigator.

I am happy to inform you that your amendment to the above protocol was approved at the regularly scheduled meeting of the IACUC on April 11, 2002. This approval authorizes: 1) measuring and recording food intake and body weight daily; 2) inclusion of effects of two GI diets on hunger signals and brain recognition of glucose levels, eating behavior under stress, and satiety; and 3) request for 30 additional rats.

Thank you.

Sincerely,

Claire Advokat, Ph.D.
Acting Chair

jdb
Date: April 3, 2002

From: Maren Hegsted  
Human Ecology

To: Phil Elzer, Chair  
IACUC

Re: Protocol 01-086 Amendment 2

Please approve the following amendment to protocol number 01-086 "The effects of low versus high glycemic index (GI) starch on weight gain and fat accumulation".

1) We will need to measure and record food intake and body weight data on a daily basis. To prevent contamination, we request that our stainless steel cart and scale remain in the room. We will provide a plastic covering to enclose the cart and contents when not in use.

2) We want to expand our measurements to include the effect of the two different GI diets on hunger signals and brain recognition of glucose levels. This will involve measuring Glut-2 RNA expression in brain samples. The hypothalamus and brain stem must be removed from animals immediately after death without interference from anesthesia. For this reason we request permission to euthanize un-anesthetized rats by decapitation. This is the method of euthanasia routinely used in Dr. Martin's laboratory at PBRC. Jun Zhou, Dr. Martin's research associate, will be responsible for the decapitations. The animals will be brought in shoeboxes with microisolator lids from the animal room in the Vivarium to the Nutrition lab for sacrifice. They will be isolated in a separate room from the staging area for the decapitation.

3) We would also like to investigate the effects of the two different GI diets on eating behavior under stress. Dr. Rick Hawkins, has agreed to demonstrate a reproducible, mild but acute stress induced by a hemostat tail pinch. In his experience, this produces a short term feeding response in the stressed animals. We would like to investigate the appropriateness of this procedure with our animal model and diet system. Dr. Hawkins and his graduate student, Sara Uzelac, would be the ones evaluating this response in our test animals.

4) We would also like to examine the effects of the two different GI diets on satiety. This would involve evaluating the appropriateness of gavage feeding to provide a known amount of diet to the animals followed by measured ad lib feeding at different timed intervals. Thus, we need permission to gavage feed some of the animals. Dr. Martin has extensive training in gavage feeding and will train others to use this technique.

5) We need to measure the metabolizable energy content of the low and high GI diets. This will require an additional 30 Sprague-Dawley rats since we cannot use these rats for any other measurements. 10 rats will be killed at the beginning to measure whole body energy content. The remaining 20 rats will be assigned to either the low or high GI diets and placed in metabolism cages for the accurate measurement of all food intake and collection of urinary and fecal output.
After the rats have gained 100 g in body weight they will be killed and body energy content measured. The rats for this study will be killed by carbon dioxide inhalation.

6) In reiteration, the three additional persons who will be working with the animals on this protocol are Jon Zhou, Mike Hawkins, and Sara Uzelac. They are already on other animal research protocols and have all taken the Rules and Regulations class in 1999. All three have extensive animal experience in the procedures described in the amendment.
Dr. Maren Hegstad  
Department of Human Ecology  

Dear Dr. Hegstad:  

Protocol #01-086, entitled "The Effects of Low versus High Glycemic Index (GI) Starch on Weight Gain and Fat Accumulation" lists you as the Principal Investigator.  

I am happy to inform you that your protocol was approved by the IACUC during our regularly scheduled meeting held on November 8, 2001. This approval is valid for 3 years and authorizes the use of 300 rats.  

In accordance with federal regulations, all personnel conducting animal-based research must receive training in the rules and regulations of animal use, and proper handling methods for the species involved. To meet this requirement all personnel, including yourself, involved with this research project must attend a rules and regulations class. Exemption from participation in the wet-lab, based on previous experience, may be obtained by written request. Dr. Martin has six months to satisfy this requirement. This is the only reminder you will receive concerning this.  

When ordering animals for this project, please provide a copy of this letter to DLAM along with your order. This will help keep better track of the animals being used by various investigators. Thank you!  

Sincerely,  

Philip Effes, Ph.D.  
Chairman  

LSU Department of Animal Science  

November 14, 2001
LSU PROTOCOL FOR ANIMAL CARE AND USE

Instructions for Submission: MUST BE TYPED! (Use additional sheets if necessary and attach to this form or use word processor and add lines). SUBMIT ORIGINAL plus 12 COPIES to the IACUC Office (Rm. 1502 School of Veterinary Medicine).

PROTOCOL NUMBER:  
APPROVAL DATE: 

SECTION 1: Principal Investigator

<table>
<thead>
<tr>
<th>Name</th>
<th>Office Phone: 573-1518</th>
<th>Home Phone: 769-3067</th>
<th>Email address: <a href="mailto:mhegsted@lsu.edu">mhegsted@lsu.edu</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maran Hegsted</td>
<td>Office Phone: 573-2284</td>
<td></td>
<td>Email address: <a href="mailto:rimartin@lsu.edu">rimartin@lsu.edu</a></td>
</tr>
<tr>
<td>Roy Martin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION 2: Project Title (Enter the name of your project/course number in the block below)

The effects of low versus high glycemic index (GI) starch on weight gain and fat accumulation.

SECTION 3:

Animal Species

<table>
<thead>
<tr>
<th>Species: Rat</th>
<th>Strain: Wistar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zucker</td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley</td>
</tr>
</tbody>
</table>
VITA

Tanya Garcia was born on June 4, 1979, in New Orleans, Louisiana. Living all her life in Kenner, Louisiana, she graduated Lutheran High School in Metairie, Louisiana, in May of 1997. Tanya began Louisiana State University in Baton Rouge, in August of 1997. In her second semester freshman year, she decided to pursue a major in dietetics in the School of Human Ecology. She received her Bachelor of Science degree in dietetics from Louisiana State University in December of 2001. She will receive her Master of Science degree in human nutrition and food also from Louisiana State University in December of 2003. In August of 2003, Tanya will move back to Kenner and begin a nine-month dietetic internship program with Touro Infirmary in New Orleans. Following the internship, Tanya plans to obtain a dietitian position in a New Orleans area hospital and get married in July of 2004.