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ARTICLE

# Comparative Methodologies for Measuring Metabolizable Energy of Various Types of Resistant High Amylose Corn Starch

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Energy values of high amylose corn starches high in resistant starch (RS) were determined *in vivo* by two different methodologies. In one study, energy values were determined according to growth relative to glucose-based diets in rats fed diets containing $RS_2$, heat-treated $RS_2$ ($RS_2$−HT), $RS_3$, and amylase predigested versions to isolate the RS component. Net metabolizable energy values ranged from 2.68 to 3.06 kcal/g for the RS starches, and 1.91−2.53 kcal/g for the amylase predigested versions. In a second study, rats were fed a diet containing $RS_2$−HT and the metabolizable energy value was determined by bomb calorimetry. The metabolizable energy value was 2.80 kcal/g, consistent with Study 1. Thus, high amylose corn based RS ingredients and their amylase predigested equivalents have energy values approximately 65−78% and 47−62% of available starch (Atwater factor), respectively, according to the RS type (Garcia, T. A.; McCutcheon, K. L.; Francis, A. R.; Keenan, M. J.; O'Neil, C. E.; Martin, R. J.; Hegsted, M. The effects of resistant starch on gastrointestinal organs and fecal output in rats. *FASEB J.* **2003**, *17*, A335).

**KEYWORDS: High amylose cornstarch; resistant starch; digestible energy; metabolizable energy; rats; net metabolizable energy**

## INTRODUCTION

The energy contribution of different ingredients is of considerable interest to consumers and food product developers. The metabolizable energy content of starch can vary between ingredients, being dependent on its small intestinal digestibility. Resistant starches (RS) are those starches that resist amylase digestion in the small intestine (*1*) and thus potentially contribute a lower energy value. RS ingestion has been associated with health benefits such as reduced colon cancer risk (*2*), improved colon health (*3*), reduced diabetes and cardiovascular disease risk (*3*−*5*), reduced body weight (*4*) and fat (*6*), increased energy expenditure (Zhou, unpublished), and increased GLP-1 and PYY hormones (*7*−*10*). These physiological effects are related to the reduced utilizable energy available from RS; thus it is critical to know the actual energy available from RS.

Typically the Atwater value of 4 kcal/g is used for starches (*11*), however this value does not account for indigestibility, so the value for RS could differ (*11*, *12*). Undigested RS is fermented by bacteria in the large intestine, producing short chain fatty acids, which are subsequently largely absorbed (*13*, *14*). The amount of energy obtained from fermentation of short chain fatty acids depends on the amount and type of short chain fatty acids produced (*15*−*17*), which also depends on the type of RS. Hence energy values for different types of RS should be measured separately. Resistant starch fermentation could potentially contribute up to 12% of the body's energy needs (*18*, *19*).

Standard terminology is used for energy classification and measurement. The energy obtained from the digestion and absorption of a food is defined as digestible energy (DE) and that portion of energy retained within the body is defined as the metabolizable energy (ME) of that food (DE less urinary energy losses) (*11*). The ME minus diet induced thermogenesis, or heat that is released during metabolism, is known as net metabolizable energy (NME). The NME is often determined by indirect calorimetry or by determining ATP yield (*12*). The DE, ME, and NME quantities reported on a per gram basis are known as DEV, MEV, and NMV respectively.

Limited and varied MEV or DEV measurements exist for specific RS sources, particularly commercial RS ingredients. Resistant starch ingredients high in amylose have been available for more than 10 years. These are high in the linear chain form of

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starch, amylose, which is less accessible to digestive enzymes than amylopectin, the branched form of starch. High amylose corn starch typically contains 60−70% amylose and 30−40% amylopectin, whereas completely digestible waxy cornstarch contains 100% amylopectin. Regular retail cornstarch contains approximately 75% amylopectin. High amylose corn starch ingredients are available in the granular (non gelatinized) form as RS type 2 ($RS_2$) and the nongranular (gelatinized) form as RS type 3 ($RS_3$). Resistant starch energy values found, either directly or by factorial methods, range from 1.54 to 3.66 kcal/g, indicating a wide variability (*11*, *15*, *20*−*24*). Thus, there is a need for further direct measurement of energy values for selected RS. In the current study, we used two *in vivo* rat methods and one *in vitro* method to estimate the MEV and NMV of various starches high in RS. The first *in vivo* method was a growth study that measured the NMV of high $RS_2$ and $RS_3$ starch and their amylase predigested versions to isolate the RS component using a method similar to that of Finley et al. that was previously used to investigate the caloric availability of fats and oils (*25*, *26*). The second *in vivo* method compared the MEV for one of the starches from Study 1 ($RS_2$−HT), using bomb calorimetry of diet, feces and urine. In addition, we determined some physiological effects of that starch. Further, we developed a calculation to predict NMV of high RS starches, using NMV of the digestible and resistant portions determined separately by *in vitro* enzymatic hydrolysis.

## MATERIALS AND METHODS

**STUDY 1 - *in vivo* Effect on Growth Curves and *in vitro* Enzymatic Hydrolysis of Starches.** In brief, in this study, rats were fed a baseline diet containing a variety of starches. The weights of the rats were recorded and the change in disemboweled dry body weight compared to those of rats given varying amounts of glucose. Comparison of the change in body weight for the unknown starch compared to that for glucose enabled the calculation of the NMV of each starch. In addition, *in vitro* hydrolysis of starches was carried out to prepare resistant fractions of each starch. These hydrolyzed starch fractions were then also fed to rats as described above to determine their *in vivo* effects on growth curves.

The study was approved by the TNO Animal Experimental Committee and was performed at TNO Quality of Life, Zeist, The Netherlands. One hundred-forty 4 week old male rats, Wistar outbred (Crl:(WI)WU BR), were obtained from Charles River Deutschland (Sulzfeld, Germany). Rats, with mean body weights of 92.3 ± 8.3 g, were stratified by weight into 14 groups of 10, individually housed in suspended stainless steel cages, and kept on a 12-h reversed light/dark cycle. Body weights were recorded twice weekly.

*Diets*. The basal diet for the study was an AIN-93G-based diet (*27*)-(**Table 1**). One batch of diet was prepared for the entire study and stored at −18 °C until use. All food and water consumption was recorded. From earlier work, 7 g/d of diet resulted in marginal increases in body weight, so this was considered optimal for constructing a "standard curve" using 7 g basal diet plus glucose fed to six groups of 10 rats. Each group received 0, 1, 2, 3, 4, or 5 g glucose. Another eight groups of ten rats each received 7 g/d of basal diet plus 3 g (dry weight) of one of the eight test starches. Rats were fed their diets for 28 days.

*Test Starches*. All test starches were corn based and varied according to amylose content, RS type and RS content (National Starch, Bridgewater, NJ). These included waxy corn starch (WS, 0% amylose), regular corn starch (CS, 27% amylose), and the high amylose starches (70% amylose) consisting of $RS_2$ (65% RS), heat treated $RS_2$ ($RS_2$−HT, 54% RS), $RS_3$ (54% RS), and their amylase pre digested versions (AT:$RS_2$, AT: $RS_2$−HT, and AT: $RS_3$). RS content was measured according to Englyst et al. (*1*). The $RS_2$−HT used in our studies has a dietary fiber level of 62% (AOAC method 985.29) and shows a granular structure by scanning electron microscopy, gives an X-ray diffraction pattern of the B type, and a $\Delta H$ of 4.73 kcal/g by differential scanning calorimetry. Gel permeation chromatography showed it consists of molecular weights in the 1000−100 000 range, with a peak at about 10 000 (*28*).

**Table 1.** Percentage Composition of the (AIN)-93G[a] Based Diet Used As the Basal Diet in Study 1

| ingredients | % of diet |
|---|---|
| Casein[b] | 30.0 |
| L-Cystine[b] | 0.45 |
| Wheat Starch[b] | 50.45 |
| Cellulose[b] | 5.0 |
| Choline Bitartrate[b] | 0.35 |
| AIN-93G Mineral Mixture[b] | 5.25 |
| AIN-93 Vitamin Mixture[b] | 1.5 |
| Soya Oil[b] | 7.0 |
| Total | 100.0 |

[a] AIN-93G (*27*) [b] Casein was obtained from Havero Hoogwegt, The Netherlands; cystine from Merck, & Co., Inc., Whitehouse Station, NJ; wheat starch, from AVEBE Group, The Netherlands; cellulose from International Filler of Belgium, Begium; choline bitartrate from Fluka Chemical Corporation, Ronkonkoma, NY; and AIN-93G mineral mixture and AIN-93 vitamin mixture were obtained from MP Biochemicals, Santa Ana, CA, who also provided energy values of diet ingredients. Soya oil was from De Oliehoorn, The Netherlands.

*in vitro Study*. Predigestion with pancreatin (Sigma-Aldrich Corporation, St. Louis, MO) for 24 h at 37 °C was conducted to isolate the RS component of the already RS rich starches. These fractions were then subjected to the *in vivo* procedure described above.

*Sacrifice*. All animals were weighed and killed by carbon dioxide administration. The abdomen and the thorax of each animal were opened, the gastrointestinal tract removed, and the contents flushed out with saline. After drying on a filter paper, the empty GI tract was placed back in the rat, and the animal weighed again. Carcasses were frozen at −18 °C and then freeze-dried.

*Body Weight Determination*. Subgroups of six animals of each group of 10 rats were chosen randomly for freeze-drying. The carcasses without GI contents were weighed at least twice and were considered dry when the body weights changed by less than 1% (total drying time 9−12 days). The dry initial body weight of each rat (beginning of the study) was estimated to be 31.7% of the initial wet body weight, which was the mean dry weight of all rats freeze-dried at the conclusion of the study (31.7% ± 1.4%, mean ± SD).

*Estimation of the Glucose Equivalent Amount of Starch*. A linear regression growth curve was obtained for the rats on the glucose-supplemented diets by plotting the mean dry body weight gain against the daily supplemented intake of glucose (0−5 g). Estimations of the equivalent gram amount of glucose for each test starch was made by comparing the dry body weight gain without GI contents after 28 days with the regression curve.

*Net Metabolizable Energy Value (NMV) of Starch*. The NMV of each starch was estimated from the equivalent gram amount of glucose, and expressed as a fraction of the caloric value of glucose, using a value of 3.69 kcal/g for glucose (*29*) and the following equation:

$$\mathrm{NMV} = (\mathrm{DWG} - b) \times 3.69/(mS) \quad (1)$$

where DWG is the dry weight gain, $b$ is the linear regression intercept, 3.69 kcal/g is the energy in kcal/g obtained from glucose, $m$ is the linear regression slope, and $S$ is the average amount of starch ingested in grams.

*Statistical Methods*. Animals were stratified by weight into 14 groups of 10 so that body weights were similar between the groups. The study determined dry body weight gains in 6 rats for each starch. Body weight gains were compared using one-way analysis of covariance followed by Dunnett's multiple comparison tests. Linear regression analysis was used to determine the amount of starch ingested, as glucose equivalents in grams, based on the standard curve derived from feeding varying levels of glucose (0−6 g) versus dry body weight gain. One way ANOVA was used to determine the significance of NMVs calculated for the starches and individual comparisons between two starches using one way ANOVA. All statistics were analyzed using BMDP statistical software (Statistical Solutions, Ltd., Cork, Ireland). Significance was $p < 0.05$.

**STUDY 2-Bomb Calorimetry for *in vivo* Estimation of MEV.** This *in vivo* study utilized bomb calorimetry of food, feces, and urine to calculate the MEV of resistant starch. This study was approved by the

Institutional Animal Care and Use Committee (IACUC) of Louisiana State University. Eighteen four-week old male Sprague−Dawley rats (Harlan, Indianapolis, IN) with a mean weight of 94.4 ± 5.1 g (SD) were housed initially in individual stainless steel cages with wire mesh bottoms allowing free access to food and water. The cages were kept in a room with a controlled environment at 22 °C and 60% humidity and on a 12-h light/dark cycle. Rats were stratified by weight, then randomly assigned to one of three treatment groups ($n$ = 6/group): baseline (BL), control (C), and $RS_2$−HT (RS). After one week acclimation, the C and RS groups were placed into plastic metabolism cages (Lab Products, Maywood, NJ) for a one week adaptation, while BL rats remained in wire mesh cages. All rats received the control diet for the first two weeks and then the experimental diets were fed to the C and RS rats for a further 6 weeks. Metabolic data were collected for the final two weeks (weeks 7−8).

*Diets*. Diets were a modified AIN-93G diet (*27*) for growing rats. RS diets contained 34% RS and 12% WS; control diets contained 46% WS and no RS. Otherwise the diets were identical. The two corn based starches were waxy cornstarch (WS) (Cerestar, Hammond, IN) and $RS_2$−HT.

*Sample Collection*. Feces were collected from each C and RS rat in metabolism cages daily at 0800 h during weeks 3−8. Urine samples were collected during weeks 7−8, into 50 mL tubes containing one mL of 10% (w/v) HCl to reduce nitrogen losses. Urine and fecal samples were pooled for individual rats. Food intake was calculated daily and body weights were measured three times per week.

The BL rats were euthanized unfasted at week two, whereas C and RS rats were euthanized unfasted at week 8. Each rat was anesthetized by 2.5% (v/v) isoflurane inhalation and euthanized by exsanguination via cardiac puncture. The gastrointestinal (GI) tract of each rat was excised and emptied organs were returned to the carcass for whole body energy measurements. The pH of the cecal contents was measured using pH indicator strips (EM Science, Gibbstown, NJ).

*Bomb Calorimetry*. Diet, fecal, and urine samples were freeze-dried (Thermo Savant, Holbrook, NY). The carcasses, containing the cleaned GI tracts and adipose tissues, were homogenized with distilled water (Pro Scientific, Inc., Monroe, CT) and freeze-dried. Energy contents of the diets, starch, feces, urine, and carcasses were determined by bomb calorimetry (Model 1722 Bomb Calorimeter, Parr Instrument Company, Moline, IL). Digestible energy (DE) and metabolizable energy (ME) of the diets were determined using the equations of Miller (*30*):

$$\text{DE} = \text{Gross energy intake} - \text{gross fecal energy} \quad (2)$$

$$\text{ME} = \text{Gross energy intake} - (\text{gross fecal energy} + \text{gross urine energy}) \quad (3)$$

The DE and ME were used to determine the DEV and the MEV of the diets (DEV = DE/gram of diet; MEV = ME/gram of diet).

The DEV and MEV of the starches were calculated by the method of Livesey (*31*):

$$\text{DEV} = \Delta H_{c,s} - \{\{[(E_{tf}/M_{td}] - [(E_{cf} - E_{if})/M_{cd}]\}/(M_s/M_{td})\} \quad (4)$$

The heat of combustion ($\Delta H_{c,s}$) was measured as kcal per gram of the $RS_2$−HT cornstarch . In this case, the test group is the RS group. The gross energy of the test group feces ($E_{tf}$) and the control group feces ($E_{cf}$) were measured in kcal, which was calculated as the heat of combustion multiplied by the mass of each collection. The gross energy lost to the feces from the replaced energy source ($E_{if}$) was estimated to be 0.00, according to the method of Livesey (*31*). The intake masses of the test diet ($M_{td}$), the control diet ($M_{cd}$), and the test substance ($M_s$) were measured in grams.

$$\text{MEV} = \Delta H_{c,s} - \{\{[(E_{tf}+E_{tu})/M_{td}] - [(E_{cf}+E_{cu} - E_{if} - E_{iu})/M_{cd}]\}/(M_s/M_{td})\} \quad (5)$$

Gross energy of the urine from the test group ($E_{tu}$) and the control group ($E_{cu}$), as well as test group feces ($E_{tf}$) and control group feces ($E_{cf}$) were measured in kcal, which were calculated as the heat of combustion multiplied by the mass of each collection. Gross energy lost to the urine from the replaced energy source (WS) ($E_{iu}$), and gross energy lost to the feces from the replaced energy source (WS) ($E_{if}$) were estimated to be 0.00 according to the method of Livesey (*31*).

The heat of combustion (kcal/g) and gross energy value (GEV, gross energy gained per gram) of each carcass were used to compare energy retention of BL, C, and RS rats. The net energy gained from the end of week 2 to the end of week 8 was determined by subtracting the heats of combustion of the BL carcasses from those of the C and RS rat carcasses.

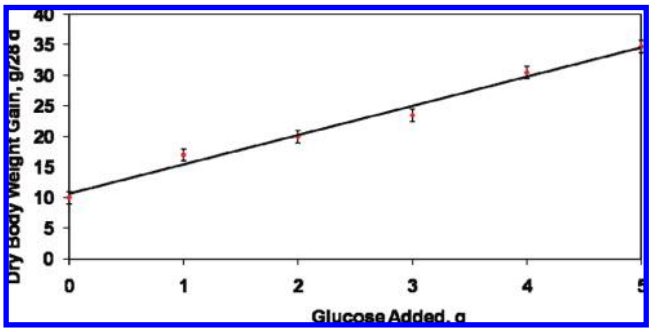


**Figure 1.** Growth response to added glucose to basal (AIN)-93G diet in Study 1.

**Table 2.** Corresponding Amount of Glucose (g), and NMV of Starches in Study 1

| starch | corresponding amt. of glucose (g)[a] | mean NMV ± SD kcal/g[b] |
|---|---|---|
| WS | 3.61 | $4.3 \pm 0.3^{a}$ |
| CS | 3.89 | $4.6 \pm 0.6^{a}$ |
| $RS_2$ | 2.57 | $3.1 \pm 0.8^{b}$ |
| $RS_2$-HT | 2.28 | $2.8 \pm 0.6^{bc}$ |
| $RS_3$ | 2.24 | $2.7 \pm 0.5^{bc}$ |
| AT: $RS_2$ | 2.10 | $2.5 \pm 0.6^{bcde}$ |
| AT:$RS_2$−HT | 1.76 | $2.2 \pm 0.5^{cde}$ |
| AT:$RS_3$ | 1.57 | $1.9 \pm 0.6^{e}$ |

[a] The corresponding amounts of glucose and the NMV for each of the starches were calculated from the linear regression line obtained from adding glucose (0−5 g) to the basal (AIN)-93G diet using the energy for glucose of 3.69 kcal/g. Glucose = (mean dry weight body gain − 10.819)/4.8057. [b] One way ANOVA of all starches was significantly different at $P < 0.0001$. Values with different letter superscript are significantly different at $P < 0.05$.

*Statistical Methods*. Animals were allocated into three groups of 6 rats so that body weights were similar between the three groups, BL, C, or RS. The data from study 2 were analyzed using SPSS Version 12.0 (SPSS Inc., Chicago, IL) or SAS Version 9 (SAS Institute, Inc., Cary NC). Analysis of variance with F protected LSD (3 group comparison) was used to analyze the differences between BL, C, and RS group dependent variables. Results are expressed as the mean ± SD. Significance was $p < 0.05$.

## RESULTS

**STUDY 1.** All rats gained weight during the study. Those rats in the glucose groups showed a dose-related increase in body weight gain relative to glucose ($r^2$ range 0.991−0.998). On average, rats maintained on a diet supplemented with 5 g of glucose gained 27.6 ± 1.0 g per week (similar to that of previous studies, unpublished). The carcass moisture content of starch-fed rats ranged from 63.2 to 70.7%, mean 68.2 ± 1.3% (SD).

*Estimation of NMV for Starch*. Comparison of dry body weight gain ($y$ axis) versus 0−5 g glucose intake ($x$ axis) resulted in a linear regression equation of $y = 4.8057x + 10.819$ ($r^2 = 0.9851$) (**Figure 1**). By relating this equation to the dry body weight gain for each starch and the NMV for glucose, the NMVs of the test starches were estimated to range between 1.9 and 4.6 kcal/g, with values for $RS_2$, $RS_2$−HT and $RS_3$ ranging between 2.7 and 3.1 kcal/g, and amylase-predigested RS ranging between 1.9 and 2.5 kcal/g (**Table 2**). Significant differences were found between all the resistant starches and CS or WS. Although differences between each RS and their respective amylase pretreated RS was only significant in $RS_3$, it approached significance in $RS_2$−HT ($p = 0.0893$).

**Table 3.** Body Weight, Diet Intake, and Fecal Excretion (Mean ± SD) for Rats Fed the Control or RS Diets in Study 2[a]

| | control | RS |
|---|---|---|
| Body Weight (g) | | |
| Initial | 96 ± 5.0 | 94 ± 4.5 |
| Weeks 7−8 | 382 ± 29.3 | 383 ± 23.0 |
| Average Cumulative Diet Intake (g) | 673 ± 41.6 | 676 ± 25.3 |
| Average Cumulative Feces Wet Weight (g) | 82 ± 7.7* | 196 g ± 14.8 |

[a] Each Control/RS mean pair in row with superscript (*) is significantly different, $P < 0.0001$.

*Use of in vitro Digestibility Data to Calculate NMV*. The $RS_2$, $RS_2$−HT, and $RS_3$ starch ingredients contained 65, 54, and 54% RS respectively, that is, the ingredients also contained 35, 46, and 46% digestible starch, respectively. By allocating the NMV of WS to the digestible component, and the NMV of each amylase-predigested starch to the RS component, the NMV for the high RS starch ingredients was calculated. Mathematically, this is expressed as:

$$\text{NMV(high RS starch)} = (\%\text{RS}) \times \text{NMV(AT : RS)} + (\%\text{digested starch or } 100 - \%\text{RS}) \times \text{NMV(Waxy Starch)} \quad (6)$$

Results are shown in **Table 2** (bottom 3 entries). The calculated vs measured NMV for $RS_2$ was 3.2 vs 3.1 kcal/g, 3.2 vs 2.8 kcal/g for $RS_2$−HT, and 3.0 vs 2.7 kcal/g for $RS_3$. Thus there was close agreement between measured NMV and a calculated value by applying the NMV of starch components to *in vitro* digestibility data.

**STUDY 2.** *Physiological Measures*. Body weight increased significantly with time ($p < 0.0001$) for both the C and RS diet groups, but was not affected by diet (**Table 3**). Food intake was not significantly different between the groups, however fecal excretion was greater in the RS group ($P < 0.0001$) (**Table 3**).

For the RS group, cecal contents were more acidic (pH 6.1 ± 0.31 vs 8.0 ± 0.14, $p < 0.0001$), empty ceca weight was greater (1.78 ± 0.38 g vs 0.69 ± 0.07 g, $p < 0.0001$), and cecal contents were greater (10.17 ± 2.62 g vs 2.61 ± 0.42 g, $P < 0.0001$)(data not shown).

*Energy Values of Feces and Urine*. In weeks 7−8, the gross energy (total kcal) of fecal combustion and fecal heat of combustion (kcal/g) were both greater for the RS than the C group ($P < 0.0001$, $P < 0.05$, **Table 4**). The gross energy of urine was similar for the RS (41.4 ± 2.3 calories) and C groups (41.1 ± 2.6 calories) (data not shown).

*Energy Values of the Diet*. The cumulative gross energy consumed over the entire study was similar for the RS (3.27 ± 0.19 Mcal) and C (3.35 ± 0.12 Mcal) groups (data not shown). Likewise, there was no difference in gross energy of food consumed during weeks 7−8 (**Table 4**).

The DE (kcal) of diet consumed was similar for the RS group compared to the C group in weeks 7−8, however the DEV (kcal/g) was significantly lower for the RS group ($P < 0.0001$).

The MEV of the complete diet was lower for the RS diet (4.25 ± 0.1 kcal/g) than the C diet (4.47 ± 0.02 kcal/g, $P < 0.01$). As expected, the MEV for C diet (4.47 ± 0.02 kcal/g) was lower than the DEV (4.64 ± 0.02 kcal/g, $P < 0.0001$) and the gross energy value (4.88 ± 0.00 kcal/g, $P < 0.0001$) (data not shown).

*Energy Value of $RS_2$−HT*. The heat of combustion of $RS_2$−HT was 3.70 ± 0.12 kcal/g and the heat of combustion of the WS starch was 3.61 ± 0.01 kcal/g. Using eq 4, the calculated DEV for $RS_2$−HT was 2.8 kcal/g. The MEV for $RS_2$−HT using eq 5 was 2.8 kcal/g for RS, the same value found for DEV.

**Table 4.** Energy Values of Feces and Diet for Weeks 7−8 (Mean ± SD) in Rats Fed the Control or RS Diets in Study 2

| parameter | control | RS |
|---|---|---|
| Heat of Combustion of Fecal Excretion (kcal/g) | 1.75 ± 0.11[a] | 1.94 ± 0.21 |
| Gross Energy of Fecal Excretion (kcal) | 51 ± 7.2[b] | 128 ± 24 |
| Gross Energy of Diet Consumption (kcal) | 1103 ± 100 | 1172 ± 41 |
| DE of Diet Consumption (kcal) | 1052 ± 94 | 1045 ± 39 |
| DEV of the Diet (kcal/g) | 4.64 ± 0.01[b] | 4.42 ± 0.01 |
| ME of the Diets (kcal) | 1011 ± 94 | 1003 ± 38 |
| MEV of Diets (kcal/g) | 4.47 ± 0.02[c] | 4.25 ± 0.10 |

Each RS/Control mean pair in row with superscript (*, **, or ***) is significantly different, [a] RS/Control mean pair in row is significantly different, $P < 0.05$. [b] RS/Control mean pair in row is significantly different, $P < 0.0001$. [c] RS/Control mean pair in row is significantly different, $P < 0.01$.

**Table 5.** Gross Energy Values for the Carcasses (Mean ± SD) after the Two Week Baseline or after Fed the Control or RS Diets for Six Additional Weeks in Study 2[a]

| parameter | baseline | control | RS |
|---|---|---|---|
| Heat of Combustion (kcal/g) | 1.87 ± 0.45[a] | 2.72 ± 0.63[b] | 2.23 ± 0.17[ab] |
| Total Gross Energy (kcal) | 364 ± 101[a] | 1005 ± 226[b] | 793 ± 72[c] |
| Gross Energy Gained During Study (kcal) | | 641 ± 226 | 428 ± 72 |

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

*Energy Values of the Carcasses*. The total gross energy of the BL carcasses was lower than the RS and C carcasses ($P < 0.001$, **Table 5**); the total gross energy of the RS carcasses was lower than the C carcasses ($P < 0.05$).

Total abdominal cavity fat (the sum of retroperitoneal, epididymal, and perirenal fat) was significantly lower in the RS group (8.6 ± 1.7 g vs 10.7 ± 2.1 g, $P < 0.05$). There was a positive correlation between carcass gross energy and total abdominal fat across all groups ($r = 0.863$; $p < 0.0001$), but not within individual treatment groups. Moisture content of the carcasses was not different for the RS group (65 ± 5%) compared to the C and BL groups (57 ± 11 and 69 ± 7%, respectively), however, the C group was significantly different from the BL group ($P < 0.05$) (data not shown).

## DISCUSSION

Resistant starch has unique technical and physiological properties, which make its use of considerable interest for foods and supplements targeting prevention and treatment of long-term disorders such as obesity and diabetes. The unique physiological properties appear to be linked to RS indigestibility and subsequent fermentation. For example, RS fermentation generates short chain fatty acids that in turn increase concentrations of important gut hormones such as PYY and GLP-1 (*7*−*10*). The current studies were conducted because only limited metabolizable energy data for various high amylose corn starches are available to support diet preparations for research studies on these properties. Thus, the goal of this research was to generate and validate energy values for different high RS starches.

The first study used dry body weight gain to calculate NMV. This method has been validated for lipids (*25*, *26*). The second study used bomb calorimetry of food, feces, and urine to calculate the MEV (*31*). One high RS starch ($RS_2$−HT) was used in both studies to compare and validate the utility of either method. Values obtained for this starch were consistent between the two methods 2.8 kcal/g in Study 2 and 2.8 kcal/g in Study 1. Thus the two methods validate and complement each other.

Any method used to measure energy value has inherent limitations. The Study 2 method was accurate at calculating the

gross energy content of diet, feces, and urine, but could overestimate MEV due to unmeasured energy lost through gas formation and heat. Smith, et al., (*32*) reported that rats consuming fermentable nonstarch polysaccharides lost 18% of fermentable energy through volatile gases and heat, and Poppitt et al. (*33*) used whole body calorimetry to show that the contribution of hydrogen and methane gas to energy lost was small when people consumed nonstarch polysaccharides. However, the similarity in energy values obtained for $RS_2$−HT in Study 1 and Study 2 validates that gaseous and heat of fermentation losses are small for RS and do not detract significantly from the MEV. In addition, this method was suitable for measuring both MEV and DEV, as both values were identical due to negligible urine energy excretion. Typically, urinary losses of energy from unavailable carbohydrates are insignificant (*34*).

The method used in Study 1 was not limited by energy lost due to the above reasons, but was based on three assumptions: (1) that growth due to ingestion of glucose is a valid estimator of energy obtained from starch intake; (2) that the moisture content of rats at 4 weeks was similar to that of rats at 8 weeks, allowing us to estimate the dry weight of the rats at baseline; and (3) that using the weight of intact animals for initial weights versus the weights of rats at the end of the study without GI contents approximates weight gain after changing from a low residue to a high RS diet. We verified the accuracy of the second assumption in Study 2 by determining that the percent dry weight of 4-week old male Sprague−Dawley rats was very similar to that of 8-week old Sprague−Dawley rats and also the 8-week old male Wistar rats in Study 1. The third assumption is acceptable due to the increased ceca weight and ceca content weight observed for RS vs C rats in Study 2. As a separate issue, the NMVs that we obtained for waxy starch and corn starch were $4.3 \pm 0.3$ kcal/g and $4.6 \pm 0.6$ kcal/g, respectively. These values are higher than the general Atwater factor of 4.0 kcal/g for starch. The disparity may be the result of experimental error caused by decreased water content in rats (approximately 67.8% for waxy starch and 66.8% for corn starch) consuming these starches compared with RS (approximately 68.5%). In addition, it is worth noting that the Atwater factor is an average value for dietary starch and is not representative of all individual starches (*11*). **Table 6** shows the NMVs obtained in Study 1 along with the values calculated using eq 6 for the average factor determined for starch of 4.5 kcal/g and 4.0 kcal/g. The data tend to show that using the value of 4.0 kcal/g gives values much closer to those found experimentally ($y = 0.351x + 1.977$; $r = 0.7983$) than does a factor of 4.5 kcal/g ($y = 0.210x + 2.578$; $r = 0.5878$).

Several studies have reported the MEV or DEV for RS and similar unavailable carbohydrates using various methodologies. These values encompass a broad range (1.5−3.7 kcal/g). Based on these previous results, the average value for the DEV for all unavailable carbohydrate is approximately 2.5 kcal/g, which is well below the gross energy utilized within the body for fully digestible carbohydrate (*23*, *35*). Energy values established in the past have varied due to the type of RS tested, the dietary source, and test model. The average value of 2.8 kcal/g for the $RS_2$−HT cornstarch reported in this study agrees with that found by Behall and Howe (*15*), who similarly used high amylose corn $RS_2$.

Increased large intestinal and cecal fermentation agrees with most studies, which show that dietary RS increases fecal matter in both humans and animals (*23*, *36*−*38*). The $RS_2$−HT starch resulted in a 3-fold increase in cecal and large intestinal contents over the control group (data not shown), nearly doubled the large intestinal and cecal masses, and lowered cecal content pH for these rats. The fermentation of RS not only signals distention, possibly to handle the increased bacterial mass and fermentation products, but RS fermentation to short chain fatty acids has also been associated with increased intestinal peptide YY and proglucagon gene expression and blood levels in RS rats (*7*, *8*, *10*). At the end of the study, the caloric and metabolizable energy intakes were similar to that of the control group. These data show that the RS group makes up for the lower energy density of their diet by increasing food intake so that energy equilibrates to levels of those from the ingestion of fully digestible carbohydrate. Notwithstanding, however, RS fed animals were significantly smaller in body size and had less total abdominal cavity fat and abdominal fat than C rats, which agrees with previous studies in our laboratory (*7*, *8*). These differences may be attributable to the acute effects of lower energy density, and/or to potential energy expended in GI proliferation, remodeling, and handling the burden of increased fermentation, or possibility due to altered energy expenditure (*24*). Further studies will be required to elucidate whether the animals eventually recover from this deficit. This decrease in abdominal fat, and possibly total body fat, may have been a limitation of the results obtained in the weight gain study (Study 1) when comparing RS treated animals to control animals. Since the control animals had more fat mass and RS more lean mass, the measurement of weight gain may be confounded. We have evidence that the fat reducing effect of RS may be due to increased energy expenditure (Zhou, unpublished), again confounding our results. This effect is difficult to measure and is probably small over the short-term. We believe that the identical results obtained for RS2-HT in Study 1 and Study 2 support our results.

**Table 6.** Comparison of NMVs Obtained Using Equation 6 with NMVs of (4.0 kcal/g) and (4.48 kcal/g) for Starch vs NMVs Obtained in Study 1 Experimentally[a]

| Starch | using 4.0 kcal/g for starch | using 4.5 kcal/g for starch | obtained in Study 1 |
|---|---|---|---|
| $RS_2$ | 3.0 | 3.2 | 3.1 |
| $RS_2$−HT | 3.0 | 3.2 | 2.8 |
| $RS_3$ | 2.9 | 3.1 | 2.6 |

[a] Values are expressed in kcal/g.

It is very difficult to determine an "exact" DEV for a food product. Food energy values represent average values in people or animals. Such a value was needed for RS in general, and in particular, for our studies with $RS_2$−HT corn starch. As such, we recommend using the MEV and/or NMV found from both our studies of 2.8 kcal/g for $RS_2$−HT from high amylose corn starch because it is more likely to represent a value obtained during a state of adaptation. When using amylase-predigested RS, or starch containing more than 55% RS an even lower energy value could be recommended.

In summary, RS exhibits unique properties as a dietary constituent, attributable directly to the reduction in utilizable energy. The addition of this food to the diet may have enormous potential as a weight management agent by diluting the metabolizable energy of the diet. This approach is in line with the DRI Committee on Energy and Macronutrients' recommendation, that more research be conducted on the use of dilution of dietary energy density to address the major problem of obesity in the US (*39*).

## ABBREVIATIONS USED

AT, amylase treated; BL, baseline; C, control; GI, gastrointestinal; G, Glucose; CS, cornstarch; DE, digestible energy (kcal); DEV, digestible energy value (kcal/g); DWG, dry weight gain; GE, gross energy (kcal); GEV, gross energy value (kcal/g); HT, heat treated; ME, metabolizable energy (kcal); MEV, metabolizable energy value (kcal/g); NMV, net MEV (kcal/g); NS, not

significant; RS, resistant starch; $RS_1-RS_4$ resistant starch forms 1−4; WS, waxy starch.


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## LITERATURE CITED


(1) Englyst, H. N.; Kingman S. M.; Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **1992**, *46* (Suppl 2), S33−S50.

(2) Brown, I. L. Applications and uses of resistant starch. *J. AOAC Int.* **2004**, *87*, 727–732.

(3) Young, G. P.; LeLeu, R. K. Resistant starch and colorectal neoplasia. *J. AOAC Int.* **2004**, *87*, 775–786.

(4) Kendall, C. W. C.; Emam, A.; Augustin, L. S. A.; Jenkins, D. J. A. Resistant starch and health. *J. AOAC Int.* **2004**, *87*, 769–774.

(5) Higgins, J. A. Resistant starch: metabolic effects and potential health benefits. *J. AOAC Int.* **2004**, *87*, 761–768.

(6) Tapsell, L. C. Diet and metabolic syndrome: where does resistant starch fit in? *J. AOAC Int.* **2004**, *87*, 756–760.

(7) Keenan, M. J.; Zhou, J.; McCutcheon, K. L.; Raggio, A. M.; Bateman, H. G.; Todd, E.; Jones, C. K.; Tulley, R. T.; Melton, S.; Martin, R. J.; Hegsted, M. Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity* **2006**, *14*, 1523–34.

(8) Zhou, J.; Hegsted, M.; McCutcheon, K. L.; Keenan, M. J.; Xi, X.; Raggio, A. M.; Martin, R. J. Peptide YY and proglucagon mRNA expression patterns and regulation in the gut. *Obesity* **2006**, *14*, 683–9.

(9) Zhou, J.; Martin, R. J.; Tulley, R. T.; Raggio, A. M.; McCutcheon, K. L.; Shen, L.; Danna, S. C.; Tripathy, S.; Hegsted, M.; Keenan, M. J. Dietary resistant starch up-regulates total GLP-1 and PYY in a sustained daylong manner through fermentation in rodents. *Am. J. Physiol. Endocrinol. Metab.* **2008**, *295*, E1160–E1166.

(10) Shen, L.; Keenan, M. J.; Martin, R. J.; Tulley, R. T.; Reggio, A. M.; McCutcheon, K. L.; Zhou, J. Dietary resistant starch increases hypothalamic POMC expression in rats. *Obesity* **2009**, *17*, 40–45.

(11) Moe, P. W. Future directions for energy requirements and food energy values. *J. Nutr.* **1994**, *124*, 1738S–1743S.

(12) Livesey, G. A perspective on food energy standards for nutrition labeling. *Br. J. Nutr.* **2001**, *85*, 271–287.

(13) Bird, A. R.; Brown, I. L.; Topping, D. L. Starches, resistant starches, the gut microflora and human health. *Curr. Issues Intest. Microbiol.* **2000**, *1*, 25–37.

(14) Bjorck, I.; Nyman, M.; Pedersen, B.; Siljestrom, M.; Asp, N. G.; Eggum, B. O. On the digestibility of starch in wheat bread-studies in vitro and in vivo. *J. Cereal Sci.* **1986**, *4*, 1–11.

(15) Behall, K. M.; Howe, J. C. Resistant starch as energy. *J. Am. Coll. Nutr.* **1996**, *15*, 248–254.

(16) Phillips, J.; Muir, J. G.; Birkett, A.; Lu, Z. X.; Jones, G. P.; O'Dea, K.; Young, G. P. Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *Am. J. Clin. Nutr.* **1995**, *62*, 121–130.

(17) Mathers, J. C. Energy value of resistant starch. *Eur. J. Clin. Nutr.* **1992**, *46* (Suppl. 2), S129−S130.

(18) Cummings, J. H.; Beatty, E. R.; Kingman, S. M.; Bingham, S. A.; Englyst, H. N. Digestion and physiological properties of resistant starch in the human large bowel. *Br. J. Nutr.* **1996**, *75*, 733–747.

(19) Behall, K. M.; Howe, J. C. Contribution of fiber and resistant starch to metabolizable energy. *Am. J. Clin. Nutr.* **1995**, *62* (Suppl), 1158S−1160S.

(20) Aust, L.; Dongowski, G.; Frenz, U.; Taufel, A.; Noack, R. Estimation of available energy of dietary fibres by indirect calorimetry in rats. *Eur. J. Nutr.* **2001**, *40*, 23–29.

(21) Goda, T.; Kajiya, Y.; Suruga, K.; Tagami, H.; Livesey, G. Availability, fermentability, and energy value of resistant maltodextrin: modeling of short-term indirect calorimetric measurements in healthy adults. *Am. J. Clin. Nutr.* **2006**, *83*, 1321–1330.

(22) Kinoyama, M.; Nitta, H.; Hatase, Y.; Watanabe, A. Quantititative assessment of effective energy of resistant cornstarch using $^{13}CO_2$, $H_2$, and $CH_4$ breath tests. *J. Health Sci.* **2007**, *53*, 475–480.

(23) Livesey, G.; Davies, I. R.; Brown, J. C.; Faulks, R. M.; Southon, S. Energy balance and energy values of α-amylase (EC 3.2.1.1)-resistant maize and pea (Pisum sativum) starches in the rat. *Br. J. Nutr.* **1990**, *63*, 467–480.

(24) Tagliabue, A.; Raben, A.; Heijnen, M. L.; Deurenberg, P.; Pasquali, E.; Astrup, A. The effect of raw potato starch on energy expenditure and substrate oxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 1070–1075.

(25) Finley, J. W.; Gilbert, A. L.; Klemann, L. P.; Sourby, J. C.; Ayres, P. H.; Appleton, S. Growth method for estimating the caloric availability of fats and oils. *J. Agric. Food Chem.* **1994**, *42*, 489–494.

(26) Finley, J. W.; Klemann, L. P.; Leveille, G. A.; Otterburn, M. S.; Walchak, C. G. Calorie availability of SALATRIM in rats and humans. *J. Agric. Food Chem.* **1994**, *42*, 495–499.

(27) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C.Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.

(28) Shi, Y. C.; Jeffcoat, R. Structural Features of Resistant Starch. In *Advanced Dietary Fibre Technology*; McCleary, B. V., Leon Prosky, L., Eds.; Blackwell Science, Ltd.: Oxford, 2001; 430−439.

(29) Davidson, S.; Passmmore, R.; Brock, J. F.; Truswell, A. S. Energy. In *Human Nutrition and Dietetics*; Churchill Livingstone: Edinburgh, 1979; pp 12−25.

(30) Miller, D. S.; Judd, P. A. The metabolisable energy value of foods. *J. Sci. Food Agric.* **1984**, *35*, 111–116.

(31) Livesey, G. Procedure for calculating the digestible and metabolisable energy values of food components making a small contribution to dietary intake. *J. Sci. Food Agric.* **1989**, *48*, 475–481.

(32) Smith, T.; Brown, J. C.; Livesey, G. Energy balance and thermogenesis in rats consuming nonstarch polysaccharides of various fermentabilities. *Am. J. Clin. Nutr.* **1998**, *68*, 802–19.

(33) Poppitt, S. D.; Livesey, G.; Elia, M. Energy expenditure and net substrate utilization in men ingesting usual and high amounts of nonstarch polysaccharide. *Am. J. Clin. Nutr.* **1998**, *68*, 820–6.

(34) Livesey, G. Determinants of energy density with conventional foods and artificial feeds. *Proc. Nutr. Soc.* **1991**, *50*, 371–382.

(35) Livesey, G. Metabolizable energy of macronutrients. *Am. J. Clin. Nutr.* **1995**, *62* (suppl), 1135S-1142S.

(36) Ferguson, L. R.; Tasman-Jones, C.; Englyst, H.; Harris, P. J. Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. *Nutr. Cancer* **2000**, *36*, 230–237.

(37) Heijnen, M. L. A.; van Amelsvoort, J. M. M.; Deurenberg, P.; Beynen, A. C. Neither raw nor retrograded resistant starch lowers fasting serum cholesterol concentrations in healthy normolipidemic subjects. *Am. J. Clin. Nutr.* **1996**, *64*, 312–318.

(38) Hylla, S.; Gostner, A.; Dusel, G.; Anger, H.; Bartram, H. P.; Christl, S.; Kasper, H.; Scheppach, W. Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. *Am. J. Clin. Nutr.* **1998**, *67*, 136–142.

(39) Food and Nutrition Board. *Dietary reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids* (*Macronutrients*); The National Academies Press: Washington, D.C., 2002.


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