

Comparison of Two Different In Vivo Models and an In Vitro Model for Caloric Determination of Four Novel Fiber Ingredients

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ABSTRACT: The objective of this study was to compare two in vivo methods using pigs and roosters and an in vitro method for determining the caloric value of four fiber sources [i.e., two resistant starches (RS 60 and RS 75), soluble corn fiber (SCF 70), and pullulan]. Metabolizable energy (ME) in pigs and true metabolizable energy (TME_n) in roosters were determined by using 72 barrows and 24 roosters, respectively. A two-step in vitro procedure was used to quantify monosaccharides released. Results of the two in vivo experiments corresponded well with RS 75 having the least caloric value (7.55 MJ/kg in pigs; 6.19 MJ/kg in roosters) and pullulan having the greatest caloric value (12.21 MJ/kg in pigs; 13.94 MJ/kg in roosters). The caloric values for all the fiber ingredients were less ($P < 0.05$) than in MD both in pigs and in roosters. Despite some limitations, results of the in vitro procedure corresponded well with the in vivo experiments where the concentration of glucose hydrolyzed from RS 60, RS 75, and SCF 70, but not pullulan, was less ($P < 0.05$) than the concentration of glucose hydrolyzed from MD. However, the greatest accuracy was obtained in the in vivo experiments.

KEYWORDS: maltodextrin, metabolizable energy, pullulan, resistant starch, soluble corn fiber

■ INTRODUCTION

There is a positive association between the intake of dietary fiber and human health.¹ The recommended daily intake for total dietary fiber is between 25 and 38 g/d.² However, the average dietary fiber intake in the US is only 12 to 18 g/d.² The need for increasing dietary fiber intake in humans has led to the development of new fiber ingredients with better functionality that can be used in many different food products without altering the organoleptic properties of the food. These novel fiber ingredients include resistant starches (RS), soluble corn fiber (SCF), and pullulan.³

Because dietary fibers are resistant to pancreatic and intestinal enzymes, they generally provide fewer calories than fully digestible carbohydrates. Thus, dietary fibers can also be useful in reducing calories in foods. However, for labeling purposes, the caloric value of the fiber must be determined. The Atwater conversion factors to estimate the caloric value of dietary fiber ingredients has been used, but has also been questioned,⁴ because of the difficulty in quantifying the caloric contribution from fermentation products absorbed in the colon. In vivo measures of metabolizable energy (ME) are, therefore, needed to obtain a reliable caloric value for these novel fiber ingredients. In vivo models using pigs or roosters have been used for caloric estimations of food,^{5–7} but it is not known how results obtained with these models compare with each other. In vitro methods may also be used to estimate the energy contribution from dietary fiber if it can be demonstrated that such procedures can accurately predict in vivo results. The objective of the present work, therefore, was to measure the caloric value of four novel fiber ingredients in two different in vivo models for calorie determination and evaluate how these measures compare with each other and with data from an in vitro digestion method.

■ MATERIALS AND METHODS

Three experiments were conducted. Two of the experiments involved the use of live animals, and the protocols for these experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Dietary Fiber Sources. Two types of RS that contained 60 or 75% total dietary fiber (PROMITOR RS 60 and PROMITOR RS 75, respectively), a source of SCF (PROMITOR SCF 70), and pullulan were used (Table 1). Maltodextrin (MD; Star Dri 10) was also included in the experiment as a control. All ingredients were supplied by Tate & Lyle, Decatur, IL.

Ingredients were analyzed for dry matter (DM), crude protein (CP; $N \times 6.25$), ash, and total dietary fiber and insoluble dietary fiber based on AOAC methods.^{8–11} Ingredients were also analyzed for enzyme hydrolyzed starch¹² using a starch assay kit (STA-20; Sigma; St. Louis, MO). Gross energy (GE) was analyzed by bomb calorimetry (Model 6300, Parr Instruments, Moline, IL) and acid hydrolyzed ether extract was analyzed by hydrolyzing samples with 3N HCl¹³ followed by crude fat extraction using petroleum ether¹⁴ on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN).

Exp. 1. Pig Experiment. Experiment 1 was designed to measure ME and apparent total tract disappearance (ATTD) of carbohydrates and of GE in MD, RS 60, RS 75, SCF 70, and pullulan when fed to growing pigs. A total of 72 growing barrows (initial body weight: 22.0 ± 1.2 kg) that were the offspring of line 337 boars mated to C-22 females (Pig Improvement Company, Hendersonville, TN) were individually housed in metabolism cages that were equipped with a feeder and a nipple drinker. Cages were also equipped with screens and funnels to allow for total, but separate, collection of feed refusals,

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Table 1. Analyzed Energy and Nutrient Composition of Maltodextrin, Resistant Starch 60, Resistant Starch 75, Soluble Corn Fiber 70, and Pullulan, As-Fed Basis^a

item	ingredient				
	maltodextrin	resistant starch 60	resistant starch 75	soluble corn fiber 70	pullulan
GE, MJ/kg	16.38	15.64	16.04	15.73	16.10
DM, %	97.41	90.07	92.99	93.92	94.80
CP, %	0.74	0.68	0.70	0.44	0.44
ash, %	0.19	0.04	0.10	0.10	0.04
acid hydrolyzed ether extract, %	ND	ND	ND	ND	ND
total carbohydrates, ^b %	96.48	89.35	92.19	93.38	94.32
starch, %	88.95	29.32	27.78	10.96	20.61
total dietary fiber	NA	62.7	80.1	68.5	74.9
insoluble dietary fiber	NA	59.2	78.0	3.9	74.0
soluble dietary fiber ^c	NA	3.5	2.1	64.6	0.90

^aGE, gross energy; DM: dry matter; CP, crude protein ($N \times 6.25$); ND, not detected; NA, not analyzed. Acid hydrolyzed ether extract was analyzed but none was detected. ^bTotal carbohydrates = DM - (CP + ash + acid hydrolyzed ether extract). ^cSoluble dietary fiber = Total dietary fiber - Insoluble dietary fiber.

Table 2. Ingredient Composition of Experimental Diets, As-Fed Basis, Exp. 1

ingredient, %	diet					
	basal diet	maltodextrin	resistant starch 60	resistant starch 75	soluble corn fiber 70	pullulan
ground corn	53.88	48.48	48.48	48.48	48.48	48.48
soybean meal	21.50	19.35	19.35	19.35	19.35	19.35
sucrose	15.00	13.50	13.50	13.50	13.50	13.50
casein	5.00	4.50	4.50	4.50	4.50	4.50
soybean oil	2.00	1.80	1.80	1.80	1.80	1.80
vitamin-mineral premix ^a	0.33	0.30	0.30	0.30	0.30	0.30
ground limestone	0.95	0.86	0.86	0.86	0.86	0.86
dicalcium phosphate	0.90	0.81	0.81	0.81	0.81	0.81
salt	0.44	0.40	0.40	0.40	0.40	0.40
maltodextrin		10.00				
resistant starch 60			10.00			
resistant starch 75				10.00		
soluble corn fiber 70					10.00	
pullulan						10.00
total	100.00	100.00	100.00	100.00	100.00	100.00

^aThe vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete test diets: Vitamin A as retinyl acetate, 3828 μg ; vitamin D₃ as cholecalciferol, 55.1 μg ; vitamin E as DL- α tocopheryl acetate, 66 μg ; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B₁₂, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

urine, and feces. Pigs were randomly allotted to receive one of six diets. Therefore, each diet had 12 replicates.

Six diets were formulated (Table 2). The basal diet was a corn–soybean meal diet that also contained casein, soybean oil, vitamins, and minerals. The remaining five diets were prepared by mixing 90% of the total diet and 10% of MD or each of the four novel fiber ingredients.

The daily feed allowance was calculated as 2.5 times the estimated maintenance requirement for energy (i.e., 0.44 MJ of ME/kg of BW^{0.75}).¹⁵ The daily feed was divided into two equal meals that were provided at 0800 and 1500 h. Water was available at all times. Pigs were fed their assigned diets during a 14-day period.

Pig body weight was recorded at the beginning of the experiment and the amount of feed provided each day was also recorded. Pigs were allowed 7 day to adapt to the experimental diet followed by 5 day of total collection of unconsumed feed and urine, and feces was collected according to the marker to marker approach.¹⁶ Two grams of chromic oxide were added to the diet in the morning meal on day 8, and 2 g of ferric oxide were added to the morning meal on day 13. Fecal collection started upon appearance of chromic oxide in the feces and ceased upon appearance of ferric oxide in the feces. Feces were

collected as soon as voided and stored at $-20\text{ }^{\circ}\text{C}$. Urine collection started on day 8 at 0800 h and ceased on day 13 at 0800 h. Preweighed urine buckets were placed under the metabolism cages to allow for total collection. Buckets were weighed and emptied every morning and a preservative of 50 mL 6N HCl were added to the buckets each time they were emptied. Twenty percent of the daily urine collections were stored at $-20\text{ }^{\circ}\text{C}$.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and a subsample was collected, lyophilized, and subsequently analyzed. Fecal samples were dried at $57\text{ }^{\circ}\text{C}$ in a forced air oven and finely ground before a subsample was collected for chemical analysis. Diets and fecal samples were analyzed for DM, CP, ash, and acid hydrolyzed ether extract as described for the ingredients. Diets, fecal samples, and lyophilized urine samples were also analyzed for GE as described for the ingredients.

The concentration of total carbohydrates was calculated by subtracting the concentration of CP, acid hydrolyzed ether extract, and ash from the concentration of DM in ingredient, diets, or fecal samples. The ME of each diet was calculated by subtracting the GE excreted in the feces and urine from total GE intake.¹⁶ Using the

Table 3. Analyzed Concentration of Energy and Nutrients in Experimental Diets, As-Fed Basis, Exp. 1^a

item	diet					
	basal diet	maltodextrin	resistant starch 60	resistant starch 75	soluble corn fiber 70	pullulan
GE, MJ/kg	17.18	16.99	16.89	16.90	16.63	16.78
DM, %	88.91	89.53	88.82	88.89	88.64	89.03
CP, %	19.60	17.18	17.53	17.53	17.75	16.37
ash, %	4.28	3.79	3.68	3.65	3.72	3.83
acid hydrolyzed ether extract, %	4.72	4.28	4.35	4.42	4.61	4.56
total carbohydrate, %	60.30	64.28	63.26	63.28	62.57	64.27
starch, %	33.47	41.45	33.45	33.54	33.57	34.52

^aGE, gross energy; DM, dry matter; CP, crude protein. Total carbohydrates = DM - (CP + ash + acid hydrolyzed ether extract).

Table 4. Daily Energy and Carbohydrate Balance in Diets or Ingredients Fed to Growing Pigs, As-Fed Basis, Exp. 1^a

item	diet						SEM	P-value
	basal	maltodextrin	resistant starch 60	resistant starch 75	soluble corn fiber 70	pullulan		
Diets								
ATTD of GE, %	89.0 ^c	89.5 ^c	85.8 ^e	85.8 ^e	87.6 ^d	88.9 ^c	0.4	0.001
ATTD of total carbohydrate, ^b %	93.9 ^c	94.8 ^c	90.1 ^e	90.1 ^e	92.6 ^d	94.5 ^c	0.4	0.001
Ingredients								
ATTD of GE, %		94.1 ^c	57.3 ^e	57.2 ^e	75.3 ^d	87.9 ^c	4.6	0.001
ATTD of total carbohydrate, ^b %		103.4 ^c	56.4 ^e	55.6 ^e	80.5 ^d	99.7 ^c	0.4	0.001

^aData are least-squares means of 12 observations per treatment. ATTD, apparent total tract disappearance; GE, gross energy; ME, metabolizable energy. ^bTotal carbohydrates = Dry matter - (CP + ash + acid hydrolyzed ether extract). ^{c,d,e}Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

difference approach, the ME contribution of the basal diet was subtracted from the ME of the treatment diets to calculate the ME from the test ingredients. By dividing this value by 0.10, the ME of the test ingredients was calculated.¹⁶ By correcting the ME of the test ingredients for their respective DM concentration, the ME of each ingredient was calculated on a DM basis.

The ATTD of GE in the diets was calculated by dividing the difference between the concentration of the GE consumed and the concentration of the GE excreted in the feces by the concentration of GE consumed.¹⁶ The ATTD of total carbohydrate in the diets were obtained using the same calculations as the ATTD of GE except that the GE was replaced with total carbohydrates.

The ATTD of GE in the ingredients was calculated using eq 1:

$$\% \text{ATTD of GE} = \frac{(\text{GE intake}_{\text{ing}} - \text{GE output}_{\text{ing}}) / \text{GE intake}_{\text{ing}}}{\times 100} \quad (1)$$

where GE intake_{ing} was the GE intake contributed by the fiber ingredient and calculated as the difference between GE intake of the treatment diet and the GE intake contributed by the basal diet to the treatment diet. The GE output_{ing} was the GE of the fecal output contributed by the fiber ingredient and was calculated as the difference between the GE fecal output of the treatment diet and the GE fecal output contributed by the basal diet in the treatment diet. The ATTD of total carbohydrates in the fiber ingredients were obtained using the same calculations as the ATTD of GE except that GE was replaced with total carbohydrates.

Exp. 2. Rooster Experiment. Experiment 2 was designed to measure the nitrogen-corrected true metabolizable energy (TME_n) of the same fiber ingredients as used in experiment 1, when fed to roosters. Twenty Single Comb White Leghorn roosters were housed individually in cages with raised wire floors. They were kept in an environmentally controlled room and subjected to a 16 h light and 8 h dark photoperiod. Water was available at all times. Four roosters were randomly allotted to each of the carbohydrate sources in a completely randomized design. Roosters were deprived of feed for 24 h and then crop-intubated with the carbohydrate sources using the precision-fed rooster assay.¹⁷ Roosters that were fed pullulan were given 6 to 8 g of pullulan due to the relatively high intrinsic viscosity of pullulan, but roosters fed MD, RS 60, RS 75, or SCF 70 were fed 10 to 22 g of each

carbohydrate source. Four additional roosters were used to measure basal endogenous losses of GE and nitrogen and these roosters were fasted for 48 h. Excreta (urine and feces) were collected from all roosters for 48 h.⁶ Excreta samples were lyophilized, weighed, and ground to pass through a 60-mesh screen and analyzed for GE. The TME_n values for each carbohydrate source were calculated based on the equation reported previously.⁶

Exp. 3. In Vitro Experiment. Experiment 3 was designed to measure the amount of monosaccharides released during a two-step in vitro assay.^{7,18} Each ingredient was incubated with pepsin and hydrochloric acid to simulate gastric digestion and in the second step, samples were incubated with an enzyme mixture composed of α -amylase and amyloglucosidase to simulate small intestinal digestion. Monosaccharides that were released during the in vitro assay were quantified using high performance liquid chromatography (Dionex DX500; Dionex Corp., Sunnyvale, CA). Chromatographic peaks were compared to appropriate standards and were conducted using a CarboPac PA-1 column and guard column.¹⁹ The caloric value of the fiber ingredients were estimated by multiplying the concentration of total monosaccharides by 16.75 MJ/kg.

Statistical Analysis. Data for Exp. 1, 2, and 3 were analyzed using the PROC MIXED (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure was used to verify the normality of the variances. Outliers were determined as values that deviated from the treatment mean by more than 1.5 times the interquartile range.²⁰ An ANOVA was conducted with diet as the main effect. Differences among treatment means were separated using the LSMEANS statement and the PDIF option of PROC MIXED. The animal was the experimental unit for all analyses in Exp. 1 and 2 and the ingredient was the experimental unit for Exp. 3. An α -value of 0.05 was used to assess significance among means.

RESULTS

Composition of Ingredients and Diets. Concentrations of DM was between 90.07 and 97.41%, and the combined concentration of CP, acid hydrolyzed ether extract, and ash were less than 1% in all the novel fiber sources and in MD (Table 1). As a result, the concentration of total carbohydrates was between 89.35 and 96.48% in all ingredients (as-fed basis).

Table 5. Energy Concentration in Maltodextrin, Resistant Starch 60, Resistant Starch 75, Soluble Corn Fiber 70, and Pullulan Measured in Pigs, Roosters, and the Two-Step In Vitro Procedure

item	ingredient					SEM	P-value
	maltodextrin	resistant starch 60	resistant starch 75	soluble corn fiber 70	pullulan		
Pigs ^e							
ME of ingredients, MJ/kg DM	14.37 ^a	8.85 ^c	7.55 ^c	7.63 ^c	12.22 ^b	1.06	0.001
Roosters ^f							
TME _n of ingredients, MJ/kg DM	16.99 ^a	10.54 ^c	6.19 ^d	8.56 ^c	13.94 ^b	0.06	0.001
In Vitro ^g							
caloric value of ingredients, MJ/kg DM	17.63 ^a	5.05 ^b	2.28 ^c	4.79 ^b	17.43 ^a	0.37	0.001

^{a,b,c,d}Values within a row lacking a common superscript differ ($P \leq 0.05$). ^eME, metabolizable energy. ^fTME_n, nitrogen-corrected true metabolizable energy. ^gCalories are estimated by multiplying the total monosaccharides by 16.75 MJ/kg.

Table 6. Concentration of Monosaccharides (mg/g) Released after Gastric and Intestinal In Vitro Digestion in Maltodextrin, Resistant Starch 60, Resistant Starch 75, Soluble Corn Fiber 70, and Pullulan, DM Basis, Exp. 3

item	ingredients					SEM	P-value
	maltodextrin ^a	resistant starch 60	resistant starch 75	soluble corn fiber 60	pullulan ^a		
sorbitol	0	0	0	1.16	0	0.02	0.001
galactose	0	0.05 ^c	0.02 ^c	0.52 ^b	0	0.02	0.001
glucose	1053.21 ^b	301.34 ^c	136.16 ^d	264.27 ^c	1041.62 ^b	22.04	0.001
fructose	0	0	0	20.15	0	0.31	0.001
total	1053.21 ^b	301.39 ^c	136.17 ^d	286.10 ^c	1041.62 ^b	22.04	0.001

^aData for maltodextrin and pullulan were previously published. ^{b,c,d}Values within a row lacking a common superscript differ ($P \leq 0.05$).

The GE of the MD ingredient was 16.38 MJ/kg and the GE of RS 60, RS 75, SCF 70, and pullulan was between 15.64 and 16.10 MJ/kg. Starch concentration in MD was 88.95%, whereas the concentration of starch in the other carbohydrates was between 10.96 and 29.32%. Pullulan, RS 60, and RS 75 were mainly composed of ethanol insoluble dietary fibers, whereas SCF 70 was mainly composed of ethanol soluble dietary fiber.

Exp. 1. Pig Experiment. Inclusion of 10% MD, RS 60, RS 75, SCF 70, or pullulan to the basal diet reduced the concentration of CP, acid hydrolyzed ether extract, and ash (Table 3). Gross energy was also reduced in the diets containing MD, RS 60, RS 75, SCF 70, or pullulan compared with the basal diet. The ATTD of GE in the pullulan diet did not differ from the MD or basal diet, but the ATTD of GE in the RS 60, RS 75, or SCF 70 diets was less ($P < 0.05$) than the ATTD of GE in the MD or basal diets (Table 4). The ATTD of carbohydrates in the pullulan diet was not different from the ATTD of carbohydrates in the basal or MD diet, but the ATTD of carbohydrates in the RS 60, RS 75, or SCF 70 diets was less ($P < 0.05$) than in the basal or MD diets. The ATTD of GE and carbohydrates from the RS 60, RS 75, or SCF 70 ingredients was less ($P < 0.05$) than the ATTD of GE and carbohydrates of the MD ingredient, whereas the ATTD of GE and carbohydrates from pullulan did not differ from the ATTD of GE and carbohydrates of the MD ingredient.

The ME of MD (14.37 MJ/kg DM) was greater ($P < 0.05$) than the ME of all other ingredients (Table 5), and the ME of pullulan (12.22 MJ/kg DM) was greater ($P < 0.05$) than the ME of RS 60 (8.85 MJ/kg), RS 75 (7.55 MJ/kg DM), and SCF 70 (7.63 MJ/kg DM). However, no difference in ME among RS 60, RS 75, and SCF 70 was observed.

Exp. 2. Rooster Experiment. The TME_n of MD (16.99 MJ/kg DM) was greater ($P < 0.05$) than the TME_n of all novel fiber ingredients (Table 5). Pullulan had the greatest ($P < 0.05$) TME_n (13.94 MJ/kg) among the fiber ingredients followed by RS 60 (10.54 MJ/kg) and SCF 70 (8.56 MJ/kg), but the TME_n

of RS 75 (6.19 MJ/kg) was less ($P < 0.05$) than in any other ingredient.

Exp. 3. In Vitro Experiment. The caloric values of MD and pullulan were greater ($P < 0.05$) than the values for other fiber ingredients, and RS 60 and SCF 70 had greater ($P < 0.05$) caloric values than RS 75 (Table 5). Glucose was the predominant monosaccharide that was released after in vitro incubation of the novel carbohydrates, although sorbitol, galactose, and fructose also were released from SCF 70 (Table 6). The concentration of total monosaccharides hydrolyzed from pullulan (1,041.62 mg/g DM) was similar to the concentration of total monosaccharides hydrolyzed from MD (1,053.21 mg/g DM), but greater ($P < 0.05$) than the monosaccharides hydrolyzed from the other fiber ingredients. Resistant starch 75 released the least ($P < 0.05$) quantity of total monosaccharides after in vitro hydrolysis (136.17 mg/g DM).

DISCUSSION

Values for TDF in diets and ingredients that are analyzed by AOAC procedure 991.43 may underestimate the total amount of TDF because the soluble low-molecular weight carbohydrates are not quantified using this procedure. When the procedure is applied to new sources of carbohydrates, as we did in this case, it is not known how accurate the procedure is. We therefore also calculated the quantities of carbohydrates in the diets by differences, and we could then compare these values to the analyzed values. For MD, RS 60 and pullulan, the calculated and analyzed values were close, indicating that the analyzed values likely were accurate. However, for RS 75 and SCF 70, greater differences between analyzed and calculated values were observed, which indicates that for these ingredients, the TDF procedure is less accurate. The reason there are differences among ingredients is most likely that the ingredients are produced using different procedures and different raw materials.

Results of these experiments indicate that ME values obtained in pigs are relatively similar to TME_n values obtained with roosters. Results of the in vitro procedure indicated the same trend in caloric values as the in vivo measurements. As expected, results of all three experiments indicate that the caloric value of MD is greater than the value of the test fiber ingredients. Maltodextrin is a product of enzymatic or acidic hydrolysis of starch derived from cereal grains.²¹ It is composed primarily of digestible carbohydrates, and is, therefore, often used as a full calorie control (16.75 MJ/kg) in experiments that aim at studying the caloric value of dietary fiber ingredients.^{6,22,23} The ME for MD obtained in pigs was slightly less than the TME_n value obtained in roosters. However, results of a previous experiment indicated that the TME_n of MD obtained in roosters is 15.45 MJ/kg,²⁴ which is close to the value obtained from pigs in this experiment. No comparable value for ME is available for swine and to our knowledge, this is the first reported value for ME of MD by pigs. The in vitro study also yielded a caloric value for MD that was close to the expected value, which is not surprising considering that MD is expected to be completely digested in the small intestine.

Measuring the caloric value of dietary fiber ingredients presents a challenge to in vitro systems, because it is hypothesized that metabolites of dietary fiber fermentation may contribute up to 10% of the energy value from dietary fibers.²⁵ However, the in vitro results obtained in this experiment for the fiber ingredients were less than the in vivo data indicating that in vivo results for dietary fiber are more accurate. However, the ranking of the fiber ingredients from least to greatest caloric value was the same for all three procedures.

The two-step in vitro procedure is limited by providing an estimate of calories obtained only from small intestinal digestion. This value may be somewhat underestimated because more diversified set of starch-hydrolyzing enzymes is present in the small intestine of monogastric animals as opposed to that used in the in vitro procedure, that is, α -amylase and amyloglucosidase. Nevertheless, for glucose based dietary fiber ingredients such as those in this experiment, the in vitro procedure captured the majority of small intestinal digestion as evidenced by the full caloric value obtained for MD. The RS 60 and RS 75 used in this experiment are type 3 RS that are retrograded starch and have highly crystalline structures that makes them resistant to enzymatic digestion.²⁶ This resistance to digestion was reflected in the small amount of glucose that was released in the in vitro assay from RS 60 and RS 75. However, resistant starches are known to be highly fermentable²⁷ and may, therefore, provide additional calories through fermentation. The caloric contribution from large intestinal fermentation, along with the enzymatic limitations of the in vitro method, explain why the ME and TME_n values for the two RS fiber ingredients were greater than the in vitro assay.

The predominance of α -1,6 glucosidic linkages and the presence of some α -1,2 and α -1,3 glucosidic linkages in SCF 70 also makes SCF 70 resistant to digestion.²⁶ However, the lack of a crystalline structure in SCF 70 is likely the reason why the results of the in vitro method were closer to the caloric value obtained in the in vivo experiments than results for RS 75.

Pullulan differs from RS 60, RS 75, and SCF 70 in that it is a product of fermentation by *Aureobasidium pullulans*. Although pullulan has a relatively low concentration of starch compared with MD, the chemical composition and some of the glucosidic linkages of pullulan are similar to that of starch.^{7,28} However,

the presence of α -1,3,²⁹ branched β -1,3,³⁰ and α -1,6 glucosidic linkages may provide steric hindrances³¹ that may reduce the susceptibility of pullulan to enzyme digestion. Pullulan has also been observed to be fermented in models of large intestine fermentation^{32,33} and to a much greater extent than any of the other fibers included in this experiment. Even in the current pig experiment, the ATTD of GE and carbohydrates from pullulan did not differ from maltodextrin, indicating that pullulan is almost completely digested and fermented. This may explain why caloric values were observed to be greater for pullulan than for the other fibers. However, results of the in vitro experiment indicate that most calories obtained from pullulan are absorbed in the small intestine.

It was expected that the caloric value of all fibers is less than that of MD, but greater than zero and the results supported this hypothesis. Both insoluble fiber (RS 60 and RS 75) and soluble fibers (SCF 70 and pullulan) have ME values between 7.55 and 12.21 MJ/kg and a TME_n between 6.19 and 13.94 MJ/kg. Thus, soluble and insoluble fibers positively contribute to the energy status of the animal as well as the human. Currently, the caloric value assigned to dietary fiber is based on Atwater conversion factors.³⁴ In the U.S., insoluble dietary fiber is assigned a caloric value of 0 MJ/kg, whereas soluble dietary fiber is assigned a caloric value similar to that of digestible carbohydrates (16.75 MJ/kg).³⁴ However, results of this experiment indicate that using the Atwater conversion factors of 0 and 16.75 MJ/kg for insoluble and soluble dietary fibers, respectively, is not an accurate basis for estimating the caloric value of dietary fiber ingredients because the use of these values may overestimate or underestimate the caloric value of a specific source of fiber. Recent Canadian and European regulations have considered this and have recommended that all dietary fibers be assigned a caloric value of 8.37 MJ/kg.³⁵ Considering the results of this experiment, this appears a reasonable estimate for calories from dietary fiber ingredients. However, for greatest accuracy, in vivo measurement of ME or TME_n should be preferred for labeling purposes and as a guide for correct usage in "low calorie" diets or preparations.

In conclusion, two different in vivo procedures for determining caloric values of dietary fibers correspond well to each other. Each of the four novel fiber ingredients tested had caloric values ranging from 6.19 to 13.94 MJ/kg, and these values were less than the caloric value for MD. Data from the in vitro experiment were less accurate than the in vivo results. Additional work to determine sites of digestion and estimation of calories from fermentation are required to increase the understanding of the caloric contribution of dietary fiber to the caloric status of individuals.

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Author Contributions

Sarah Cervantes-Pahm performed the pig experiment and wrote most of the manuscript. Brenda K. Knapp, Carl M. Parsons, and George C. Fahey Jr. performed the rooster and the in vitro experiments. Beob G. Kim contributed to the calculations and statistical analyses. Yanhong Liu consolidated the experiments and provided valuable input in writing the manuscript. Hans H. Stein supervised the pig project and writing of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATION USED

ATTD, apparent total tract disappearance; CP, crude protein ($N \times 6.25$); DM, dry matter; GE, gross energy; MD, maltodextrin; ME, metabolizable energy; RS, resistant starch; SCF, soluble corn fiber; TMEn, nitrogen-corrected true metabolizable energy

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