JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Physiological Responses to Novel Carbohydrates as Assessed Using Canine and Avian Models

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The objective was to quantify in vitro digestion, true metabolizable energy (TME_n) content, glycemic and insulinemic responses, and gastrointestinal tolerance to fructose (Fruc), maltodextrin (Malt), polydextrose (Poly), pullulan (Pull), resistant starch (RS), sorbitol (Sorb), and xanthan gum (Xan). Limited digestion of RS, Poly, and Xan occurred. Fruc, Malt, and Sorb resulted in the highest ($P < 0.05$) TME_n values, Pull was intermediate, and RS and Poly were lowest. Malt had the highest ($P < 0.05$) area under the curve for glucose and insulin in the glycemic tests. Gastrointestinal tolerance was examined for diets containing carbohydrates at either 100 or 200% of the adequate intake (AI) value for dietary fiber. At 100% and 200% AI, Malt, RS, and Sorb resulted in ideal fecal scores, while Pull and Xan resulted in looser stools and Poly resulted in diarrhea. The carbohydrates studied varied widely in physiological outcomes. Certain carbohydrates could potentially benefit large bowel health.

KEYWORDS: Novel carbohydrates; glycemic and insulinemic response; in vitro digestion; true metabolizable energy; gastrointestinal tolerance

INTRODUCTION

Carbohydrates, along with fat and protein, are the major macronutrients that supply the body with energy. They encompass a broad range of sugars, oligosaccharides, starches, and dietary fibers. Many factors influence the rate of carbohydrate digestion and absorption including physical form and chemical composition. Substrates with physiological properties such as reduced energy value, bifidogenic properties, laxation effects, fecal bulking, and reduced glycemic response are being sought to incorporate into foodstuffs (*1*). This has led to an increase in demand for carbohydrates that have physiological properties similar to those of dietary fibers but that may be incorporated into a wider array of foods more easily.

Low-digestible carbohydrates include polyols, resistant starch (RS), nonstarch polysaccharides, and other oligosaccharides (*2*). They possess many physiological properties that may provide potential human health benefits. Diets containing low-digestible carbohydrates often have lower energy contents due to their decreased rate of small intestinal absorption.

Upon entering the colon, low-digestible carbohydrates are fermented, although fermentation rates will vary depending upon the molecular structure of the carbohydrate. Fermentation yields metabolizable energy for microbial growth and maintenance and other metabolic end products for use by the host (*3*). Short-chain fatty acids (SCFA) provide colonic cells with energy and lower the pH of luminal contents. Both outcomes are beneficial to colonic health. Additional beneficial effects that SCFA have been shown to elicit include stimulation of intestinal Na absorption and anti-inflammatory actions (*4, 5*). Also, SCFAs, specifically butyrate, may possess properties making them important in the prevention of colon cancer (*4, 5*).

With diabetes becoming more of a health concern in the general population, controlling blood glucose in humans is critical for the long-term management of the disease. Incorporating carbohydrates that attenuate the blood glucose response in the diet of people suffering from diabetes could play an important role in management and prevention of the disease. Some carbohydrates are only partially digested in the small intestine, and it is these low-digestible carbohydrates and their slower rate of digestion that result in their ability to decrease the glycemic response. Fructose (Fruc) and sorbitol (Sorb) are not low-digestible carbohydrates but can be utilized in low glycemic products in modest amounts since they do not result in increases in blood glucose concentration due to the manner in which they are absorbed and metabolized. Very few studies have examined the glycemic response of these novel carbohydrates fed alone.

Dogs are suitable models for humans and have several advantages over other animal models. Dogs have a larger body size than rodents that allows for collection of larger amounts of samples, and data collected with dogs are more biologically relevant to humans when evaluating therapeutics (*6*). The dog is commonly used as a model for gastrointestinal function and health since they have a gastrointestinal tract similar to humans as regards the intestinal:body length ratio, possess a

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10.1021/jf801042b CCC: $40.75
Published on Web 08/16/2008

rudimentary cecum, and experience similar motility patterns (*6*). Similarities also are noted in the diet and lifestyle of dogs and humans. Both are omnivorous, eat a diet of similar macronutrient composition, and experience many of the same complex diseases such as obesity, cancers, osteoarthritis, and intestinal diseases (*6*).

As the general public becomes more aware of the potential health benefits associated with ingredients that have a reduced energy content, that are partially fermented in the colon, and that elicit a reduced glycemic and insulinemic response, demand for carbohydrates that possess these characteristics will be increased. Hence, the objective of this study was to evaluate select carbohydrates varying widely in chemical composition and functionality for physiological properties that could impact human health. Properties evaluated included in vitro digestion characteristics, glycemic and insulinemic responses, and gastrointestinal tolerance, all using a dog model, and true metabolizable energy (TME_n) using an avian model.

MATERIALS AND METHODS

Substrates. Carbohydrates studied included Fruc, maltodextrin (Malt), polydextrose (Poly), a low molecular weight (MW 100000) pullulan (Pull), a type-3 RS from high amylose cornstarch, Sorb, and xanthan gum (Xan) (Tate and Lyle, Decatur, IL).

Chemical Analyses. Carbohydrates were analyzed for dry matter (DM) and organic matter (OM) according to AOAC (*7*) and for free and hydrolyzed monosaccharide concentrations. Test carbohydrates were hydrolyzed using the procedure of Hoebler et al. (*8*). Free sugars and hydrolyzed monosaccharides were quantified using a Dionex DX500 high-performance liquid chromatography (HPLC) system (Dionex Corp., Sunnyvale, CA). Standards for quantification included arabinose, fucose, galactose, glucose, inositol, mannose, rhamnose, and xylose. Free monosaccharides were injected at a volume of 25 μL. All assays were conducted using a CarboPac PA-1 column and guard column following methods cited by Smiricky et al. (*9*).

In Vitro Digestion. Approximately 200 mg of each carbohydrate was weighed in triplicate and incubated with pepsin/hydrochloric acid, amylogucosidase, and α-amylase to simulate gastric and small intestinal digestion (*10*). A set of tubes containing no substrate were used as blanks. The tubes were analyzed for free released monosaccharides using HPLC (*9*) following simulated digestion.

Data were analyzed as a completely randomized design using the Mixed Models procedure of SAS (SAS Institute, Inc., Cary, NC). The statistical model included the fixed effect of substrate. Treatment least-squares means were reported and compared using a Tukey adjustment to ensure the overall protection level. The standard error of the mean (SEM) value was associated with least-squares means as calculated in the Mixed Models procedure. Differences among means with a P value of less than 0.05 were considered significant.

TME_n. Conventional Single Comb White Leghorn roosters (four per carbohydrate evaluated) were utilized in this study. All birds 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. The University of Illinois Institutional Animal Care and Use Committee approved all procedures prior to animal experimentation.

Carbohydrates evaluated were Fruc, Malt, Poly, Pull, RS, and Sorb. Roosters were deprived of feed for 24 h and then crop-intubated with approximately 10−15 g of each carbohydrate using the precision-fed rooster assay (*11*). Each carbohydrate was fed to four roosters. Following crop intubation, excreta (urine and feces) were collected for 48 h on plastic trays placed under each cage. Excreta samples were then lyophilized, weighed, and ground to pass through a 60 mesh screen and analyzed for gross energy (GE) using a bomb calorimeter (Parr Instrument Co., Moline, IL). The nitrogen corrected TME_n values correct for endogenous energy excretion using fasted roosters and were calculated using the following equation:

$$TME_n\ (kcal/g) = (\text{energy intake} - \text{energy excreted by fed birds} + \text{energy excreted by fasted birds})/\text{feed intake}$$

Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Institute, Inc.). Differences among dietary treatments were determined using the least significant difference method. A probability of $P < 0.05$ was accepted as being statistically significant.

Glycemic/Insulinemic Responses. To determine postprandial glycemic and insulinemic responses to the test carbohydrates, five purpose-bred female dogs (Butler Farms, Clyde, NY) with hound bloodlines, a mean initial body weight of 25.1 kg (range, 19.9−29.5 kg), and a mean age of 5 years were used. Dogs were housed individually in 1.2 m × 2.4 m clean floor pens in a climate-controlled room at the animal care facility of the Edward R. Madigan Laboratory on the University of Illinois campus. Dogs were provided with nondestructible toys (hard plastic balls, Nyla bones, etc.). Pens allowed for nose−nose contact between dogs in adjacent runs and visual contact with all dogs in the room. A 16 h light:8 h dark cycle was used. The University of Illinois Institutional Animal Care and Use Committee approved all procedures prior to animal experimentation.

Carbohydrates evaluated included Fruc, Malt (control), Poly, Pull, RS, Sorb, and Xan. Dogs consumed 25 g of carbohydrate (DM basis) in approximately 240 mL of distilled deionized water. To get carbohydrate sources into solution, water and carbohydrate were mixed using a stir plate. The quantity to be dosed was measured using a disposable 60 mL syringe (without needle) and offered to dogs within a 10 min period. Certain carbohydrates (RS, Sorb, and Xan) were not consumed with water but were mixed with a can of white chicken breast meat (276 g) and fed to the dogs. This was done due to the poor solubility of these carbohydrates, which would not allow for the solution to be administered through the 60 mL syringe. All other procedures remained unaltered. During the trial, all dogs were fed the same dry commercial diet (Iams Weight Control; The Iams Co., Lewisburg, OH). Water was available ad libitum.

A series of 5 × 5 Latin square designs were used in which the dogs were subjected to 3 h glycemic tests with Malt serving as the control in each Latin square. Glycemic tests were spaced 4 days apart during the trial. At 1700 h on the evening before each glycemic test, any remaining food was removed, and dogs were food-deprived for 15 h during which time they had access to water. Dogs consumed their allotted treatment after the 15 h of food deprivation.

On the morning of the glycemic test, a blood sample was obtained from dogs before being dosed to serve as the baseline value. Dogs were then dosed with the appropriate carbohydrate, and additional blood samples were taken at 15, 30, 45, 60, 90, 120, 150, and 180 min postprandially. Approximately 3 mL of blood was collected in a syringe via jugular or radial venipuncture. An aliquot of blood was taken immediately for glucose analysis. The remaining blood was centrifuged at 1240*g* for 10 min, and the serum was stored at −20 °C for analysis of insulin at a later date.

Immediately following collection, blood samples were assayed for glucose based on the glucose oxidase method using a Precision-G Blood Glucose Testing System (Medisense, Inc., Bedford, MA). This system measures blood glucose concentrations from the electrical current resulting from electron transfer when the glucose oxidase on the test strip catalyzes the oxidation of glucose to gluconic acid (*12*). The precision of this testing system for the range of values obtained was 3.4−3.7% (coefficient of variation) as reported by the manufacturer. Each glucometer was calibrated prior to each glycemic test according to manufacturer's instructions. Serum was analyzed for insulin using a Rat Insulin Enzyme Immunoassay kit (Cayman Chemical, Ann Arbor, MI) (*13*).

The positive incremental area under the curve (AUC), ignoring any areas below the baseline, for blood glucose and insulin values was calculated according to the method of Wolever et al. (*14*) using GraphPad Prism 4 Software (GraphPad Software, Inc., San Diego, CA). The relative glucose response (RGR) and relative insulinemic response (RIR) of the test carbohydrates were calculated for each individual dog according to the following formula: [(AUC for test carbohydrate)/(AUC for control)] × 100%.

Table 1. Ingredient Composition of Diets Fed to Dogs for Gastrointestinal Tolerance Studies

		test carbohydrate concentration (% of AI)[a]	
ingredient	control diet	100	200
brewers rice	39.47	39.47	32.27
poultry byproduct meal	36.00	36.00	36.00
poultry fat	13.50	13.50	13.50
beet pulp	7.20		
test carbohydrate		7.20	14.40
dried egg	2.25	2.25	2.25
salt	0.65	0.65	0.65
potassium chloride	0.56	0.56	0.56
choline chloride	0.13	0.13	0.13
vitamin premix[b]	0.12	0.12	0.12
mineral premix[c]	0.12	0.12	0.12

[a] AI values for dietary fiber for humans (100 = 14 g/1000 kcal; 200 = 28 g/1000 kcal). [b] Provided per kg of diet: vitamin A, 14970 IU; vitamin D_3, 900 IU; vitamin E, 59.88 IU; vitamin K, 0.60 mg; thiamin, 11.98 mg; riboflavin, 9.58 mg; pantothenic acid, 17.96 mg; niacin, 44.91 mg; pyridoxine, 11.98 mg; biotin, 0.11 mg; folic acid, 0.72 mg; and vitamin B_{12}, 0.02 mg. [c] Provided per kg of diet: Mn (as $MnSO_4$), 12 mg; Fe (as $FeSO_4$), 90 mg; Cu (as $CuSO_4$), 2.4 mg; Zn (as $ZnSO_4$), 120 mg; I (as KI), 1.5 mg; and Se (as Na_2SeO_3), 0.24 mg.

Data were analyzed by the Mixed Models procedure of SAS (SAS Institute). The statistical model included the fixed effect of treatment and the random effects of animal nested within Latin square and test period nested within Latin square. Treatment least-squares means were compared using contrast statements. A probability of $P < 0.05$ was accepted as being statistically significant.

Gastrointestinal Tolerance. Nine purpose-bred female dogs (Marshall Farms, North Rose, NY) with an average age of 13 months and an average starting body weight of 17.5 kg (range, 15.5−19.9 kg) were utilized. Animal care procedures were approved by the University of Illinois Animal Care and Use Committee prior to initiation of the experiment. Dogs were housed individually in kennels (1.2 m × 2.4 m) in a climate-controlled room with a 12 h light:12 h dark cycle at the College of Veterinary Medicine Animal Care Facility. All dogs had free access to water through automatic waterers located in each pen. Each pen contained a raised resting panel with a rubberized coating.

Three dry extruded kibble diets were formulated to contain varying concentrations of each test carbohydrate. Carbohydrates tested were Malt, Poly, Pull, RS, Sorb, and Xan. Diets were poultry meal and brewers rice-based and formulated to provide 0, 18 (7%), or 36 g (14%) of the test carbohydrate daily based on a food intake of 250 g/day. These supplemental concentrations of the test carbohydrates represent the consumption of 100 (14 g/1000 kcal) or 200% (28 g/1000 kcal) of the recommended adequate intake (AI) of dietary fiber for humans (*15*). Beet pulp was provided in the control diet at the same concentration as for the 100% AI diet (14 g/1000 kcal). The ingredient composition of diets fed to dogs is presented in **Table 1**. Dogs were fed once daily, and water was available ad libitum. Diets were manufactured at Kansas State University under the supervision of Pet Food Ingredients and Technologies, Inc. (Topeka, KS).

Dogs were fed once daily, and food refusals were measured. Food intakes were initially 250 g/day; however, this amount proved inadequate to maintain weight in the growing dogs. To prevent weight loss, food intake for all diets was increased first to 300 g/day and then to 350 g/day. Diets were formulated so that 250 g/day provided 100 or 200% AI of dietary fiber; thus, the increase in food intake resulted in dogs consuming more than the recommended AI (~140 or 240% AI). Dogs were allowed free access to water at all times.

A balanced incomplete block design with two blocks of nine dogs each was used for each carbohydrate tested. In each block, dogs were randomly allotted to one of the three diets—control, 100% AI, or 200% AI. It was ensured that no dog received the same diet in both blocks. Each block was conducted over a 10 day period. Days 1−7 were for diet acclimation, followed by an evaluation of tolerance characteristics on days 8−10.

Fecal consistency scores were recorded on days 8−10 of each block for each carbohydrate tested. Feces was scored on a scale from 1 to 5, with 1 being dry, hard pellets; 2 being dry, well-formed stool; 3 being a soft, moist, formed stool; 4 being loose, unformed stool; and 5 being a watery liquid stool that could be poured. Other variables associated with gastrointestinal tolerance such as emesis, poor physical appearance, and abnormal behavior of the dogs also were monitored and recorded.

Diet samples were collected from each of the dietary treatments and were ground using a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2 mm screen and dry ice in preparation for chemical analyses. Diet samples were analyzed for DM and OM according to AOAC (*7*). Crude protein (CP) was determined according to AOAC (*16*) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corp., St. Joseph, MI). Fat concentrations were measured by acid hydrolysis (*17*) followed by ether extraction (*18*). The GE content was measured by use of a bomb calorimeter (model 1261, Parr Instrument Co., Moline, IL). The total dietary fiber (TDF) concentration was determined according to Prosky et al. (*19*). All ingredients were analyzed in duplicate with a 5% error allowed between duplicates; otherwise, analyses were repeated.

Fecal score data were analyzed as a balanced incomplete block design using the GLIMMIX procedure of SAS (SAS Inst. Inc.). The statistical model included the fixed effect of treatment and the random effects of block and dog nested within block. A probability of $P < 0.05$ was accepted as statistically significant. Probabilities between 0.05 and 0.10 were considered a statistical trend.

Table 2. Free Sugar Concentrations of Select Carbohydrates

free sugars	carbohydrate[a]						
(mg/g)[b]	Fruc	Malt	Poly	Pull	RS	Sorb	Xan
arabinose	0.00	0.01	0.83	0.03	0.00	0.00	0.00
fructose	990.09	0.00	1.39	2.62	0.00	0.22	0.00
galactose	0.00	0.00	1.27	0.06	0.00	0.00	0.00
glucose	0.00	17.53	31.43	4.76	0.13	0.50	0.00
mannose	0.00	0.00	0.34	0.55	0.00	0.00	0.00
rhamnose	0.00	0.00	0.80	0.00	0.00	0.00	0.00
sorbitol	0.00	0.00	17.01	0.06	0.05	1116.53	0.36
sucrose	0.00	0.00	4.94	0.00	0.00	2.32	0.00
xylose	0.00	0.00	0.90	0.09	0.00	0.00	0.00
total (mg/g)	990.09	17.54	58.91	8.17	0.18	1119.57	0.36

[a] Values are expressed on a DM basis. [b] Values include water that is added when starches are broken down to monosaccharide units.

Table 3. Hydrolyzed Monosaccharide Concentrations of Select Carbohydrates Corrected for Free Monosaccharide Concentrations

hydrolyzed monosaccharides	carbohydrate						
(mg/g)[a]	Fruc	Malt	Poly	Pull	RS	Sorb	Xan
galactose	0.00	0.00	0.00	28.80	0.00	0.00	0.00
glucose	0.00	1148.21	980.51	840.99	1072.87	5.61	313.90
mannose	0.00	0.00	0.00	38.32	0.00	0.00	198.51
sorbitol	0.00	0.00	75.64	0.00	0.00	0.00	0.00
total[b]	0.00	1148.21	1056.15	907.21	1072.87	5.61	512.41

[a] Values are expressed on a DM basis. [b] Values include water that is added when starches are broken down to monosaccharide units.

RESULTS AND DISCUSSION

Free Sugar and Hydrolyzed Monosaccharide Concentrations. Free sugar contents (**Table 2**) varied greatly among the seven carbohydrates evaluated. Most of the DM contents of Fruc and Sorb existed as free sugars with considerably less free sugars present in the other carbohydrates tested. RS and Xan had the lowest free sugar concentrations of the test carbohydrates evaluated.

Malt, Poly, and RS had the highest hydrolyzed monosaccharide concentrations (**Table 3**). Glucose was the main monosaccharide of all carbohydrates measured. The hydrolyzed monosaccharide concentration of Malt and RS consisted totally of glucose, while a small portion of the hydrolyzed monosaccharide

Table 4. Monosaccharides (Including Free Monosaccharides) Released after Simulated Digestion of Select Carbohydrates[a]

	carbohydrate							
released monosaccharides (mg/g)[b]	Fruc	Malt	Poly	Pull	RS	Sorb	Xan	SEM[d]
fructose	846.01 b	0.00 a	2.95 a	0.00 a	0.00 a	0.36 a	0.00 a	3.01
galactose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
glucosamine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
glucose	0.00 a	1053.21 d	137.71 b	1041.62 d	377.28 c	4.12 a	15.52 a	18.34
isomaltose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
sorbitol	0.00 a	0.00a	21.05 a	0.00 a	0.00 a	1057.42 b	0.00 a	21.71
sucrose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
total (mg/g)[c]	846.01 d	1053.21 e	161.71 b	1041.62 e	377.28 c	1061.90 e	15.52 a	28.37

[a] Means in the same row with different letters are different ($P < 0.05$). [b] Values are expressed on a DM basis. [c] Values include the addition of water added when starches are broken down to monosaccharide units. [d] Pooled SEM.

concentration of Poly was Sorb. Pull also had a relatively high hydrolyzed monosaccharide concentration with glucose, galactose, and mannose represented. Sorb contained only a small quantity of hydrolyzed glucose.

Evaluation of the free sugar and hydrolyzed monosaccharide content of carbohydrates allows for a better understanding of their potential for being incorporated into foodstuffs. Knowing the free sugar content is important for carbohydrates that will be incorporated into foodstuffs meant to have a low glycemic response since the free sugar concentration can greatly affect the glycemic response. The hydrolyzed monosaccharide concentration describes the building blocks of carbohydrate polymers and the fraction potentially available for digestion.

Sorb and Fruc had the highest concentrations of free sugars, and both are known to be easily absorbed by the body; however, because neither contains any significant amounts of glucose, they would be expected to result in very low glycemic and insulinemic responses. Malt had little free sugar concentrations but high concentrations of glucose in the hydrolyzed monosaccharide form that is readily digestible. Poly, Pull, RS, and Xan had low concentrations of free sugars but high concentrations of hydrolyzed monosaccharides, especially glucose. While these carbohydrates had few free sugars, the high concentrations of glucose present in the hydrolyzed form mean that some glucose would likely be available during digestion, leading to low or intermediate glycemic responses.

In Vitro Digestion. Monosaccharides released from the two stage in vitro procedure (simulated hydrolytic digestion) are presented in **Table 4**. Glucose was released after simulated digestion of all carbohydrates except Fruc. Glucose concentrations were numerically highest for Malt and Pull. Intermediate amounts of glucose release were noted for Poly and RS, while very little glucose was released from Sorb and Xan. Substantial concentrations of free Sorb were released from Sorb, while minor amounts were released from Poly. Free Fruc release was highest for Fruc, followed by release of small amounts of Fruc from Poly and Sorb.

High concentrations of released monosaccharides measured after simulated digestion indicate that the carbohydrate was highly digestible as was the case for Malt and Pull. Because Fruc and Sorb are available as free sugars, they also result in high concentrations of released monosaccharides and are readily absorbed. Carbohydrates with low concentrations of released monosaccharides have a low digestibility as was noted for Poly, RS, and Xan. Carbohydrates that are highly digestible would result in little residue left for fermentation, while those with low digestion would have substantial residue left to be potentially fermented to SCFA in the colon.

Normally, a fermentation stage of this assay is conducted. In the fermentation stage, residues remaining after simulated digestion are fermented with a diluted fecal inoculum. However, when several of these substrates are subjected to fermentation, the data are meaningless as the monosaccharides resulting from digestion cannot be separated from the carbohydrate residue not digested, thus resulting in the entire compound (rather than just the undigested residue) being fermented to SCFA. Inflated SCFA values are the result of this exercise.

Table 5. TME_n of Select Carbohydrates[a]

	carbohydrate						
item	Fruc	Malt	Poly	Pull	RS	Sorb	SEM[b]
amount dosed (g DMB)	14.81	14.27	14.23	7.03	13.53	14.81	
TME_n (kcal/g)	3.99 c	4.06 c	1.74 a	3.33 b	1.89 a	3.84 c	0.12

[a] Means in the same row with different letters are different ($P < 0.05$). [b] Pooled SEM.

Some dietary carbohydrates are resistant to digestion by mammalian enzymes due to their molecular structure and types of glycosidic linkages between the monomeric constituents. Such carbohydrates are substrates for the microbiota of the large intestine and are more or less extensively fermented. Poly, Pull, RS, and Xan are all resistant to hydrolytic digestion to varying degrees. As compared to Poly, RS, and Pull, Xan was the most resistant to digestion. Sunvold et al. (*20*) found Xan to be a poorly fermentable substrate, and this corresponds to studies showing Xan to be an effective laxative resulting in increased stool output (*21*). These carbohydrates vary in the amount digested as compared with the amount fermented.

TME_n. Incorporating reduced calorie ingredients into foodstuffs is becoming more prevalent as health complications associated with obesity are on the rise and consumers are becoming more health conscience. This is increasing the demand for low-calorie sweeteners and bulking agents. One method for evaluating the caloric content of ingredients is the use of the TME_n assay. This assay uses the rooster to simulate the conditions in the digestive tract of humans for determining the energy content of feedstuffs. Use of the roosters allows for a better representation of the digestive process than do in vitro assays for determining metabolizable energy. Also, the TME_n assay allows for a shorter, easier, and more accurate collection of data than using human subjects where total collection of feces and urine can be difficult.

The TME_n assay was conducted on six of the test carbohydrates: Fruct, Malt, Poly, Pull, RS, and Sorb. Fruc, Malt, and Sorb resulted in the highest ($P < 0.05$) TME_n values (**Table 5**). Pull resulted in an intermediate value (3.33 kcal/g). RS and Poly resulted in the lowest ($P < 0.05$) TME_n values (1.89 and 1.74 kcal/g, respectively).

Metabolizable energy of carbohydrates varies due to the degree to which they are digested and absorbed. Another

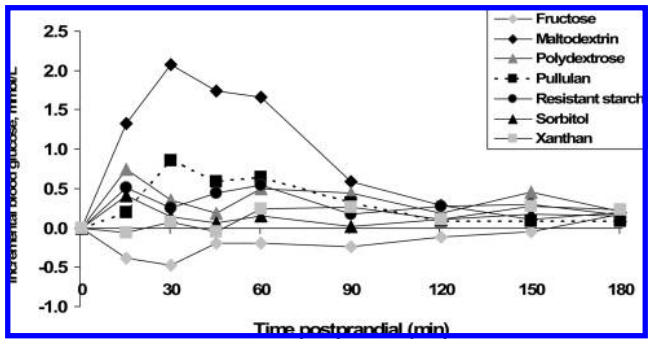

Figure 1. Incremental change from baseline in blood glucose response for dogs consuming 25 g of select carbohydrates. Pooled SEM values for carbohydrates are as follows: Fruc, 0.27; Malt, 0.19; Poly, 0.27; Pull, 0.27; RS, 0.27; Sorb, 0.27; and Xan, 0.29.

consideration is the fact that low-digestible carbohydrates provide an amount of energy dependent upon their fermentability. Because Malt is highly digestible and absorbed in the small intestine, it resulted in a high TME_n value. Fruc and Sorb are free monosaccharides so they are absorbed easily into the bloodstream, accounting for their high caloric values. While Sorb is absorbed more slowly than Fruc into the bloodstream, it is fermented in the colon and this results in its higher energy value. Although Fruc is not a reduced calorie ingredient, it is an effective replacement for typical sugars like sucrose since it is sweeter, so less is needed to achieve the same level of sweetness (*22*).

Pull, RS, and Poly are low-digestible carbohydrates, thus resulting in lower TME_n values. The Pull evaluated is low MW and, thus, is more digestible than higher MW Pull, resulting in an intermediate TME_n value. Both the RS and the Poly resulted in lower TME_n values than Pull, indicating that they are more resistant to digestion. Pull, RS, and Poly are likely fermented to some extent, accounting for some of their caloric value. These three carbohydrates were shown to have reduced caloric value, especially RS and Poly, making them suitable ingredients for low-calorie foodstuffs.

Few data exist regarding the metabolizable energy content of novel carbohydrates fed alone. In those instances where data exist, carbohydrates are part of a diet matrix. The TME_n assay is useful in that the carbohydrate alone can be studied without interferences from dietary matrix components. This is important information when developing food products.

Glycemic and Insulinemic Responses. Incremental AUC data for glucose for the test carbohydrates are presented in **Figure 1**, and the corresponding values for AUC (mmol/L) and RGR are presented in **Table 6**. Malt was used as a control in every set of glycemic response tests because it is highly digestible and rapidly absorbed, resulting in a consistently high glycemic response. For the glycemic tests where carbohydrates had to be mixed with chicken breast meat due to their poor solubility, the AUC for Malt (120 mmol/L) was similar to the average (161 mmol/L) for other glycemic tests. This indicates that the chicken breast meat did not affect the glycemic response of the carbohydrates being evaluated. AUC for Malt was higher ($P < 0.05$) than for the remainder of the test carbohydrates. After Malt, the carbohydrates with the highest AUC values were Poly, Pull, and RS. The lowest AUC values were noted for Fruc, Sorb, and Xan. The AUC for Fruc was lower ($P < 0.05$) than for Malt or Poly but was not lower ($P > 0.05$) than for Pull, RS, Sorb, or Xan. There was a trend ($P < 0.10$) for the AUC for Fruc to be lower than for Pull and for Sorb to be lower than for Poly.

Because Malt served as the control to which all test carbohydrates were compared, it has an assigned RGR value of 100. Relative glycemic responses are related directly to AUC, so test carbohydrates with high AUC values will have correspondingly high RGR values. The RGR is a better value for interpretation of glycemic response because carbohydrates were run in a series of tests to determine their glycemic response and were not all evaluated in the same period. This is the reason Malt is used in every period as a control to calculate a relative response to the test carbohydrates in any particular period. After Malt, the carbohydrates with the highest RGR values were Poly, Pull, and RS. Poly and Pull resulted in RGR values about 50% that of Malt. RS resulted in a numerically lower response of about 25% that of Malt. Fruc, Sorb, and Xan had the lowest RGR values that were approximately 13% that of Malt.

Although Fruc and Sorb are free monosaccharides, they are absorbed without increasing blood glucose concentrations, resulting in the lowest AUC and RGR values of the test carbohydrates evaluated. Sorb had a numerically higher AUC than Fruc. This may be due to the small amount of glucose associated with Sorb (**Tables 2** and **3**) and that Fruc was able to attenuate blood glucose concentrations more than Sorb.

The remaining carbohydrates, Poly, Pull, RS, and Xan, all demonstrated varying degrees of resistance to digestion in the small intestine. All of them had an attenuated postprandial glycemic response that resulted in AUC and RGR values that were lower ($P < 0.05$) than for the Malt control. Xan resulted in the lowest glycemic response, with a RGR value comparable to that of Fruc and Sorb. Blood glucose values were close to baseline for the first hour and did not reach peak until 64 min of the glycemic test. The time to reach peak value for Xan was numerically longer than for all of the other carbohydrates tested except for Fruc, making it one of the most slowly digested carbohydrates in the group. Pull and RS had moderate RGR, indicating that they were partially digested. Both reached peak blood glucose concentrations around 30 min of the glycemic test. Of the carbohydrates demonstrating digestive resistance, Poly had the highest numerical glycemic response, but the value was still lower ($P < 0.05$) than for Malt.

Literature on the glycemic response of pure carbohydrate sources is limited. Most glycemic response tests are conducted with carbohydrate sources present in a foodstuff or mixed meal. A study examining physiological responses of Poly in humans found a decrease ($P < 0.05$) from baseline (RGR, 100) after ingestion of 12 g of Poly (RGR, 88) (*23*). Jenkins et al. (*24*) evaluated a type 3 RS and found no significant differences in RGR of RS as compared with a low-fiber control. Our studies indicated that RS moderately increased blood glucose, so depending on the glycemic nature of the low-fiber control used in the study by Jenkins et al. (*24*), it was not surprising that RS had a similar RGR as compared to the control.

Spears et al. (*25*) evaluated two Pulls in dogs, one high MW and one low MW, with the low MW one being similar to the Pull evaluated in the present experiment. It was reported that although not statistically significant, the low MW Pull had a lower glycemic response for the first 60 min postprandial as compared to Malt. Wolf et al. (*26*) evaluated the glycemic response of Pull in humans and found that it reduced ($P < 0.01$) the glucose AUC by 50% as compared to Malt. This result is similar to the decrease observed in the present experiment where Pull reduced the glucose AUC by approximately 60% as compared to the Malt control.

Incremental AUC data for insulin for the test carbohydrates are presented in **Figure 2**, and the corresponding values for

Table 6. Incremental AUC for Glucose and Insulin and RGR and RIR of Select Carbohydrates[a]

	carbohydrate							
item	Fruc	Malt	Poly	Pull	RS	Sorb	Xan	SEM[b]
AUC for glucose (mmol/L)	8.67 a	145.21 c	70.58 b	54.19 ab	50.16 ab	25.08 ab	25.14 ab	18.77
RGR	13.25 a	100.00 c	47.27 b	41.21 b	24.18 ab	10.88 a	12.27 a	7.76
AUC for insulin (pmol/L)	957.93 a	9597.05 b	1321.75 a	2416.73 a	1147.56 a	1206.20 a	892.77 a	2076.20
RIR	2.46 a	100.00 c	8.00 ab	27.67 b	26.32 ab	16.00 ab	6.21 ab	7.94

[a] Means in the same row with different letters are different ($P < 0.05$). [b] Pooled SEM.

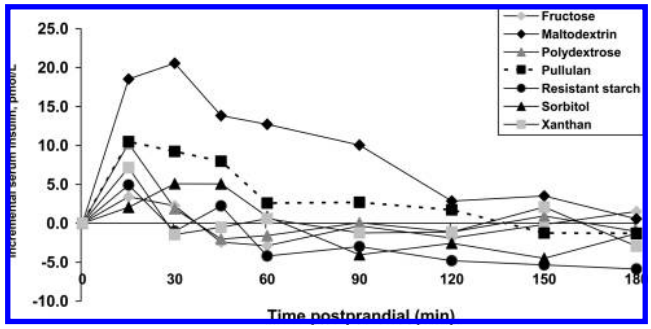

Figure 2. Incremental change from baseline in serum insulin response for dogs consuming 25 g of select carbohydrates. Pooled SEM values for select carbohydrates are as follows: Fruc, 4.27; Malt, 2.88; Poly, 4.27; Pull, 4.27; RS, 4.81; Sorb, 4.28; and Xan, 4.38.

AUC (pmol/L) and RIR are presented in **Table 6**. Malt resulted in the highest ($P < 0.05$) AUC for insulin. All other test carbohydrates had similar and lower ($P < 0.05$) AUC values. The RIR values indicated that while some test carbohydrates had moderately large increases in blood glucose, all of the carbohydrates tested resulted in lower ($P < 0.05$) insulin production as compared to Malt. It is not surprising that Fruc resulted in the numerically lowest RIR since it does not elicit a glycemic response. Poly, Sorb, and Xan had similar lower RIR values. While Poly had a relatively high RGR of 47.27, it did not elicit nearly as high of an RIR (8.0). While not as great of a difference, this also was noted for Pull where the RIR was not as high as the RGR. RS resulted in an intermediate RIR value where two small peaks of insulin resulted within the first 60 min of the glycemic test.

Poly, Pull, and Xan had RIR values lower than RGR values. This difference is due to the pattern of blood glucose response to carbohydrate ingestion (**Figure 1**). Poly resulted in a small, sharp peak of insulin at the beginning that progressed as a blunted curve above baseline concentrations throughout the 3 h glycemic test. The small increase in blood glucose concentration at the beginning of the glycemic test likely resulted from the free sugar content of Poly and resulted in a similar peak in insulin before dropping below or near baseline values. Xan also had a lower RIR value as compared to the RGR value. It resulted in a glycemic response where blood glucose values were below or near baseline throughout the entire glycemic test so little insulin stimulation occurred except for a small peak within the first 15 min of the glycemic response. Pull resulted in an increased blood glucose concentration but in a pattern such that the glucose curve was blunted throughout the entire glycemic test. This long blunted response was because Pull is slowly digestible (*26*) and glucose was slowly released throughout the glycemic test. There was no sudden, sharp increase in blood glucose at the beginning of the glycemic test, so there also was not as large of an insulin stimulation as was the case for a carbohydrate that resulted in a sharp, sudden increase in blood glucose.

Table 7. Intake and Fecal Score[a] Characteristics of Dogs Fed Diets Containing Select Carbohydrates[b]

item	average food intake (g/day)	test carbohydrate concentration (% of AI)[c]			
		control	100	200	SEM[d]
Malt	246	2.8 a	3.0 b	2.7 a	0.11
Poly	291	2.9 a	4.2 b	4.6 c	0.08
Pull	334	3.0 a	3.8 b	4.7 c	0.09
RS	335	2.5 ab	2.5 b	2.3 a	0.30
Sorb	338	2.7 a	2.8 a	3.3 b	0.16
Xan	339	2.7 a	3.4 b	3.9 c	0.17

[a] Scores based on the following scale: 1 = dry, hard pellets; 2 = dry, well-formed stool; 3 = soft, moist, formed stool; 4 = unformed stool; and 5 = watery liquid that can be poured. [b] Means in the same row with different letters are different ($P < 0.05$). [c] AI of dietary fiber for humans. [d] Pooled SEM.

Gastrointestinal Tolerance. All diets had similar DM (93%) and OM (92%) concentrations (data not shown). CP was approximately 30%, acid hydrolyzed fat approximately 19%, and GE content approximately 5.2 kcal/g for all diets evaluated. The TDF varied among diets. The Malt and Sorb diets resulted in the lowest TDF values (2.1 and 3.7%, respectively), as expected, since neither Malt nor Sorb are dietary fibers. For the 100% AI diets for Pull, RS, and Xan, all TDF values were similar to the control diet (8.2% TDF) with an approximate TDF concentration of 8%. The 200% AI diets for Pull, RS, and Xan had higher TDF values (~13%). Given that diets were formulated based on 250 g/day food intake, 8% TDF translates to 20 g and 13% TDF to 32.5 g of dietary fiber per day. These values are close to the projected 18 and 36 g/day quantities the diets were formulated to provide to ensure 100 and 200% AI amounts of dietary fiber for humans.

Food intake (**Table 7**) did not differ ($P > 0.05$) between the control and 100 and 200% treatments for any test carbohydrate evaluated. Average values are presented for the six carbohydrates. Any decrease in food intake was associated with certain dogs but not with diet. Most dogs readily ingested the diets at both concentrations and rarely was any diet refused.

Malt resulted in similar fecal scores between control (Con) and 200% AI treatments. The Malt 100% treatment resulted in a higher ($P < 0.05$) fecal score of 3.0. Malt would not be expected to greatly affect fecal score since it is a highly digestible carbohydrate.

Poly, Pull, and Xan feeding resulted in fecal scores that increased ($P < 0.05$) as the AI level increased. Also, values were higher ($P < 0.05$) than fecal scores for dogs on Con. Spears et al. (*25*) reported that a low MW Pull resulted in a fecal score of 3.3, close to the value for the 100% AI treatment in this experiment. RS resulted in similar fecal scores among treatments. Fecal scores for Sorb at 200% AI were higher ($P < 0.05$) than for Con and 100% AI treatments.

When examining fecal score data among experiments, the Con elicited a consistent response throughout (2.5−3.0). These values are considered ideal. For the 100% AI treatments, Poly,

Pull, and, to a lesser extent, Xan, resulted in softening of the stool. Malt, RS, and Sorb had little effect. The same pattern was evident for the 200% AI treatments, with dogs fed Poly and Pull experiencing symptoms of diarrhea. Surprisingly, RS, Sorb, and Xan resulted in no such effect. Food intake, behavior patterns, and appearance of the dogs were never considered abnormal for any of the treatments imposed. Rapid carbohydrate fermentation and elevated water loading of the colon no doubt occurred when Poly and Pull were fed, especially at the 200% AI level. These processes have been noted for low-digestible carbohydrates fed to humans (*27*).

Other studies have shown adverse gastrointestinal effects of some of the novel carbohydrates evaluated. As reviewed by Flood et al. (*28*), Poly caused flatus and diarrhea when humans consumed over 90 g/day (1.3 g/kg BW/day). In the present study, Poly caused diarrhea in dogs fed 21 and 42 g/day (1.0 and 2.1 g/kg BW/day). Daly et al. (*16*) reported that consumption of 15 g/day of Xan by humans resulted in a laxative effect with increased stool output, frequency of defecation, and flatulence. Dogs consuming up to approximately 50 g/day Xan in the current study had loose stools but tolerated the dose well. A type 3 RS consumed at 17.4 or 19.0 g/day (0.27 and 0.29 g/kg BW/day) was reported to have a modest laxative effect in humans (*29*), whereas dogs consuming daily doses of 25 or 50 g/day (1.25 and 2.5 g/kg BW/day) experienced no laxative effects.

Although no adverse effect of Sorb was found in the dogs consuming either the 100 or 200% AI diets, McRorie et al. (*30*) found that consumption of 40 g/day Sorb by humans resulted in loose, liquid stools and abdominal cramping. In contrast, dogs were able to tolerate a similar daily dose (~50 g/day) of Sorb well without experiencing diarrhea. Although the dogs did not experience diarrhea on the 200% AI Sorb diet, it did result in a looser stool, indicating a mild laxative effect of the Sorb. Gastrointestinal distress after ingestion of incompletely absorbed carbohydrates may be prone to great variability among subjects depending on dosage, concentration, time spent consuming, and also differences in absorption capacity of the subject (*31*). The lack of diarrhea as a result of Sorb consumption by the dogs could be due to a dilution effect within the diet. The dogs were allowed to eat the diet containing Sorb at their leisure throughout the day and were not given a large dose at a single time.

In summary, carbohydrates that are readily digestible should result in high concentrations of monosaccharide release after simulated digestion and would be expected to have a high glycemic response, a high energy content, and no gastrointestinal tract tolerance issues. Malt followed this pattern. Fruc and Sorb also followed this pattern except for a glycemic response that was attenuated due to the nature of their absorptive mechanism. Poly and Pull had moderate amounts of free and hydrolytically released glucose that resulted in a glycemic response, but the portion that was not digested was highly fermentable, resulting in tolerance issues. Xan had low concentrations of free and hydrolytically released sugars, resulted in a low glycemic response, and resulted in a laxative effect in the dog. The RS followed a pattern similar to that of Xan except that no tolerance issues were identified. In conclusion, data indicate that novel carbohydrates vary in digestion capacity, energy content, glycemic and insulinemic responses, and gastrointestinal tolerance. Variation in responses is due largely to the individual carbohydrate molecular structure and bonding pattern. Evaluation of a variety of physiological responses allows for a better understanding of the potential functional benefits that select carbohydrates possess. As the demand for functional ingredients providing health benefits increases, it is important to have this information to make wise decisions about potential inclusion of novel carbohydrates in both liquid and solid food matrices.

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Received for review April 2, 2008. Revised manuscript received July 3, 2008. Accepted July 21, 2008.

JF801042B