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Soluble Fiber Dextrins and Pullulans Vary in Extent of Hydrolytic Digestion in Vitro and in Energy Value and Attenuate Glycemic and Insulinemic Responses in Dogs

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The objective of this research was to measure in vitro hydrolytic digestion characteristics, glycemic and insulinemic responses, and true metabolizable energy (TME_n) content of select soluble fiber dextrins (SFDs) and pullulans. The SFDs were derived either from tapioca starch or from corn starch. The pullulans were of low, intermediate, and high molecular weight. Soluble fiber dextrins varied in digestibility, with all substrates resulting in low to intermediate in vitro monosaccharide digestion. Pullulans were nearly completely hydrolyzed after simulated hydrolytic digestion. The glycemic response with dogs varied widely among SFDs, with all but one SFD substrate having lower glycemic response than maltodextrin (Malt). The pullulans all resulted in low glycemic values. Lower relative insulinemic responses (RIR) compared to the Malt control were noted for all SFDs and pullulans. True metabolizable energy (TME_n) values for SFDs obtained using roosters were lower than for Malt, with tapioca-based SFDs having numerically higher values than corn-based SFDs. Pullulans resulted in higher TME_n values than did SFDs. Soluble fiber dextrins and pullulans may be suitable candidates for reduced calorie and glycemic foodstuffs.

KEYWORDS: Soluble fiber dextrins; pullulan; glycemic and insulinemic response; in vitro digestion; true metabolizable energy; gastrointestinal tolerance

INTRODUCTION

In recent years, the demand for functional ingredients that provide specific health benefits has increased (*1*). There are several reasons why consumption of functional foods is increasing, including a concern about the high cost of prescription drugs, a quest for more natural remedies to improve health, engagement in preventative health measures, and an interest in overall health improvement (*2*). The global trend in rising levels of chronic diseases such as obesity, diabetes, heart disease, and cancer is also increasing demand for functional ingredients that can be utilized to control, prevent, or ameliorate these diseases.

Dietary fibers differ widely in chemical and physical properties and exert a variety of physiological and nutritional properties in humans (*3*). Dietary fibers have been shown to promote healthy gut function and other beneficial effects such as laxation, reduction in blood cholesterol concentrations, modulation of blood glucose concentration, and bifidogenic properties (*1*). Substrates possessing properties similar to dietary fiber are being sought for potential incorporation into foodstuffs. This has led to an expansion in the demand for carbohydrates that have functional properties similar to those of dietary fiber, but that may be incorporated more easily into a wider array of solid and liquid food matrices. A class of carbohydrates that may prove to be suitable are low-digestible carbohydrates.

Some carbohydrates are unavailable to digestive enzymes, so are either only partially absorbed or not absorbed in the small intestine, and vary in fermentability upon reaching the large intestine. These low-digestible carbohydrates possess many physiological properties that may provide potential human health benefits including a role in prevention of obesity, diabetes, colon cancer, and irritable bowel syndrome (*4*). Both soluble fiber dextrins (SFD) and pullulans are low-digestible carbohydrates that have physiological attributes resembling dietary fiber and may result in physiological benefits.

Components in starch hydrolysates, such as dextrin, maltodextrin, and corn syrup, may be rendered at least partially indigestible. These products are termed resistant maltodextrins, indigestible dextrins, and soluble fiber dextrins and are produced when a starch source is treated with heat and acid. This treatment causes the starch molecules to undergo hydrolysis and produce short-chain oligosaccharides that randomly rearrange during cooling, forming a highly branched structure (*5*, *6*). During this treatment, the formation of random linkages occurs including the digestible linkages of α 1−4 and α 1−6, and nondigestible linkages such as β 1−4, β 1−6, and α and β 1−3 and 1−2 (*6*, *7*). Due to their structural characteristics, SFDs are only partially hydrolyzed by human digestive enzymes and absorbed in the small intestine (*8*).

The dextrinization process generates a product with a broad molecular weight range (*9*) that can be narrowed by eliminating the higher molecular weight fractions, leading to a dextrin with a reduced viscosity (*9*). Eliminating low molecular weight fractions can make the dextrin sugar-free, improve its digestive tolerance, and reduce hygroscopicity (*9*). The dextrininization process also leaves the product with a discoloration and an off-taste. The heat-treated starch then goes through a purification step with amylases

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to remove undesirable odors and tastes, and this is followed by decolorizing and subsequent demineralization (*9*). This amylase purification step can affect the digestible and indigestible components of the SFD.

Soluble fiber dextrins possess characteristics that allow them to be easily incorporated into a wide variety of foods (*6*, *10*) such as solubility, low viscosity, stability under numerous processing steps, and neutral taste (*6*, *10*). Soluble fiber dextrins also have been shown to have a high digestive tolerance allowing them to be incorporated into foodstuffs at sufficient concentrations to induce beneficial health outcomes (*11*).

Pullulans are naturally occurring fermentation products produced by *Aureobasidium pullulans*, a black yeast found throughout all ecological niches including forest soils, fresh water and seawater, and plant and animal tissues (*12*, *13*). Pullulans are linear polysaccharides consisting of three α-(1−4) linked glucose molecules that are repeatedly polymerized by α-(1−6) linkages on the terminal glucose, resulting in a stair-step structure (*14*, *15*). The stair-step structure resulting from glycosidic linkages in pullulans hinders hydrolysis by enzymes, making them low-digestible carbohydrates (*13*).

Commercially produced pullulans have been available since 1976 from the Hayashibara Company Ltd. (*12*). They are produced by fermentation from a food-grade hydrolyzed starch using a strain of *A. pullulans* that is non-toxin producing (*12*, *14*). Upon completion of fermentation, the fungal biomass, pigments, and other impurities are removed.

The unique linkage pattern of pullulans gives them distinctive characteristics like the capacity to form fibers, compression moldings, and strong oxygen-impermeable films (*15*). Pullulans are stable in aqueous solutions over a wide pH range and dissolve readily in water to form viscous solutions. Upon drying, pullulans form transparent, water-soluble, fat-resistant, odorless, and flavorless films (*15*).

Pullulans possess many characteristics that make them ideal for numerous applications in food and pharmaceutical manufacturing. Pullulans' consistency, dispersibility, and moisture retention characteristics are similar to those of starch, so they are commonly used as a starch replacement in certain foods (*15*, *16*). A popular application for pullulans is for films that are thin, clear, readily dissolved, and highly oxygen-impermeable making them ideal for edible food coatings (*16*). Pharmaceutical uses for pullulan include use in sustained-release formulations, in coatings of pills for strength and shelf life, and as films for oral care products (*16*).

The objective of this study was to evaluate select soluble fiber dextrins and pullulans for physiological properties that could positively impact human health. Properties evaluated included in vitro hydrolytic digestion characteristics, glycemic and insulinemic responses using a dog model, and true metabolizable energy (TME_n) using an avian model.

MATERIALS AND METHODS

Substrates. Carbohydrates studied included six soluble fiber dextrins (SFDs) and three pullulans. Six SFDs were evaluated and were produced using two different starch sources. All SFDs were produced using a standard process of treating starch with heat, acid, and enzymes. Three SFDs were prepared from tapioca starch: tapioca-based SFD1 (T1), tapioca-based SFD2 (T2), and tapioca-based SFD that was hydrogenated (TH). The other three substrates were commercially available SFDs prepared from corn starch: corn-based SFD1 (C1), corn-based SFD2 (C2), and corn-based SFD3 (C3). Tapioca-based SFD1 (T1) and T2 were produced under similar conditions, but with differences in the purification method, and TH underwent a hydrogentation process. Hydrogenation of the tapioca-based SFD involved subjecting the starch solution to hydrogen in the presence of a catalyst (e.g., nickel or platinum (*17*)) in which monosaccharides were converted to alcohols such as sorbitol. The corn-based SFDs varied in total dietary fiber content (AOAC Method 2001.03) with C1 containing 90% fiber, C2, 85% fiber, and C3, 70% fiber, as stated by the manufacturers. Tapioca-based SFDs were prepared by Tate & Lyle (Decatur, IL). Corn-based SFD1 is Fibersol-2 from Matsutani America Inc. (Clinton, IA). Corn-based SFD2 and C3 are Nutriose products (Nutriose FM06 and Nutriose FM10, respectively) from Roquette (Keokuk, IA).

Pullulans evaluated were of varying molecular weights: a low MW pullulan (Pull LMW) (MW 100,000), an intermediate MW pullulan (Pull IMW) (MW 250,000), and a high MW pullulan (Pull HMW) (MW 500,000). Pull LMW and Pull HMW were prepared by Tate & Lyle (Decatur, IL). Pull IMW is produced by Hayashibara Company Ltd. (Okayama, Japan).

Chemical Analyses. Carbohydrates were analyzed for dry matter (DM) and organic matter (OM) according to AOAC (*18*), and for free and hydrolyzed monosaccharide concentrations. Test carbohydrates were hydrolyzed using the procedure described by Hoebler et al. (*19*) where carbohydrates were subjected to hydrolysis with H_2SO_4 acid. Free sugars and hydrolyzed monosaccharides were quantified using a Dionex DX500 high performance liquid chromatography (HPLC) system (Dionex Corporation, Sunnyvale, CA). Standards for quantification included inositol, fucose, arabinose, rhamnose, galactose, xylose, and mannose. 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. (*20*).

In Vitro Hydrolytic Digestion. Approximately 200 mg of each carbohydrate were weighed in triplicate and incubated with 2 mL of a pepsin/hydrochloric acid solution and 2 mL of an enzyme solution consisting of amylogucosidase and α-amylase to simulate gastric and small intestinal digestion (*21*). The samples were analyzed for free released monosaccharides using HPLC (*20*) following the simulated hydrolytic digestion procedure.

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. Differences among means with a *P*-value of less than 0.05 were considered 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 to 29.5 kg), and a mean age of 5 years were used. Dogs were housed individually in 1.2 × 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.

Dogs were orally dosed with 25 g of test carbohydrate (DM basis) in approximately 240 mL of double distilled/deionized water. In order to get carbohydrate sources into solution, water and carbohydrate were mixed using a stir plate. Quantity to be dosed was measured using a disposable 60 cm^3 syringe (without needle), and all 25 g was consumed by the dogs within a 10 min period. During the trial, all dogs were fed the same commercial diet (Iams Weight Control; The Iams Co., Lewisburg, OH). Water was available ad libitum.

Five by five Latin square experimental designs were used to evaluate test substrates. Maltodextrin served as the control, and in every Latin square conducted, the dogs were subjected to four test ingredients and the maltodextrin control. Glycemic tests were 3 h long and spaced 4 days apart. 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 then were 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 serum stored at −20 °C for subsequent analysis of insulin.

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 (*22*). The precision of this testing system for the range of values obtained was 3.4 to 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) (*23*).

The positive incremental area under the curve (AUC), ignoring any areas below baseline, for blood glucose and insulin values was calculated according to the method of Wolever et al. (*24*) 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%.

Data were analyzed by the Mixed Models procedure of SAS (SAS Institute, Cary, NC). 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 to compare only the test ingredients of interest in the numerous Latin squares conducted. A probability of $P < 0.05$ was accepted as being statistically significant.

True Metabolizable Energy (TME_n). Conventional Single Comb White Leghorn roosters 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.

Roosters were deprived of feed for 24 h and then crop-intubated with approximately 13−26 g of each carbohydrate using the precision-fed rooster assay (*25*, *26*). 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 then were lyophilized, weighed, and ground to pass through a 60-mesh screen and analyzed for gross energy using a bomb calorimeter (Parr Instrument Co., Moline, IL). Endogenous corrections for energy were made using roosters that had been fasted for 48 h. The nitrogen-corrected true metabolizable energy (TME_n) values, corrected for endogenous energy, were calculated using the following equation (*27*):

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

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

RESULTS AND DISCUSSION

Free Sugar and Hydrolyzed Monosaccharide Concentrations. Free sugar content of the SFD substrates is presented in **Table 1**. Overall, the SFDs had low free sugar concentrations. Tapioca SFD1, T2, and C2 had the lowest free sugar concentrations, with less than 1% of the substrate being composed of free sugars. The highest free sugar concentration was found for TH, with C1 and C3 having intermediate free sugar concentrations.

For all six substrates, glucose was the free sugar found in the highest concentration. Free glucose was highest in TH which also contained the highest amount of sorbitol. The sorbitol found in TH was likely a result of the hydrogenation process. The corn-based SFDs had small amounts of free fructose, whereas the tapioca-based SFDs did not have any free fructose.

All the SFD substrates had high hydrolyzed monosaccharide concentrations (**Table 1**), with TH having a slightly lower concentration than the rest. The hydrolyzed monosaccharide concentration of T1, C1, C2, and C3 consisted totally of glucose, while a small portion of the hydrolyzed monosaccharide concentration of T2, T3, and C1 consisted of mannose. Only TH resulted in a small amount of sorbitol.

Table 1. Free Sugar and Hydrolyzed Monosaccharide Concentrations of Soluble Fiber Dextrins (SFD)

test carbohydrate[a]	T1	T2	TH	C1	C2	C3
			Free Sugars, mg/g[b]			
arabinose	0.00	0.17	0.00	0.37	0.00	0.00
fructose	0.00	0.00	0.00	0.29	0.80	4.73
galactose	0.00	0.00	0.07	0.00	0.00	0.00
glucose	0.48	0.63	64.46	11.87	2.37	28.20
mannose	0.00	0.00	0.00	0.00	0.20	0.45
rhamnose	0.10	0.00	0.00	0.20	0.00	0.00
sorbitol	0.04	0.10	27.05	0.48	0.00	0.00
sucrose	0.00	0.25	0.00	0.00	0.00	0.00
xylose	0.00	0.00	0.00	0.00	0.00	0.00
total[c]	0.62	1.15	91.59	13.21	3.37	33.38
			Hydrolyzed Monosaccharides, mg/g[b,d]			
galactose	0.00	0.00	0.00	0.00	0.00	0.00
glucose	1,139.67	1,030.29	945.55	1,158.37	1,137.51	1,128.21
mannose	0.00	7.26	5.22	2.09	0.00	0.00
sorbitol	0.00	0.00	9.83	0.00	0.00	0.00
total[c]	1,139.67	1,037.55	960.59	1,160.46	1,137.51	1,128.21

[a] Abbreviations: T1, tapioca-based SFD1; T2, tapioca-based SFD2; TH, tapioca-based SFD hydrogenated; C1, corn-based SFD1; C2, corn-based SFD2; C3, corn-based SFD3. [b] Values are expressed on a dry matter basis. [c] Values include water added when starches are broken down to monosaccharide units. [d] Values are corrected for free monosaccharide concentrations.

Table 2. Free Sugar and Hydrolyzed Monosaccharide Concentrations of Pullulans

test carbohydrate[a]	Pull LMW	Pull IMW	Pull HMW
	Free Sugars, mg/g[b]		
arabinose	0.03	0.00	0.00
fructose	2.62	0.99	0.00
galactose	0.06	0.00	0.00
glucose	4.76	6.79	0.00
mannose	0.55	0.05	0.00
rhamnose	0.00	0.00	0.00
sorbitol	0.06	0.24	0.01
sucrose	0.00	0.00	0.00
xylose	0.09	0.00	0.00
total[c]	8.17	8.07	0.01
	Hydrolyzed Monosaccharides, mg/g[b,d]		
galactose	28.80	0.43	19.38
glucose	840.09	1,112.76	1,081.98
mannose	38.32	0.00	26.60
sorbitol	0.00	0.00	0.00
total[c]	907.21	1,113.19	1,127.96

[a] Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight pullulan. [b] Values are expressed on a dry matter basis. [c] Values include water added when starches are broken down to monosaccharide units. [d] Values are corrected for free monosaccharide concentrations.

All pullulans had very low free sugar concentrations, with Pull HMW having essentially no free sugar present (**Table 2**). Pull LMW and Pull IMW had similar total free sugar concentrations, with glucose being the free sugar present in the highest concentration followed by small amounts of fructose. A very small amount of sorbitol was the only free sugar present in Pull HMW.

Hydrolyzed monosaccharide concentrations varied slightly among the pullulan substrates (**Table 2**). All three pullulans had

Table 3. Monosaccharides (Including Free Monosaccharides) Released after Simulated Hydrolytic Digestion of Soluble Fiber Dextrins

	test carbohydrate[a]						
released monosaccharides, mg/g[b]	T1	T2	TH	C1	C2	C3	SEM
fructose	0.00 a[c]	0.00 a	0.00 a	0.00 a	0.80 b	5.57 c	0.05
galactose	0.00 a	0.00 a	0.00 a	0.00 a	0.03 b	0.07 c	0.01
glucosamine	0.00	0.00	0.00	0.00	0.00	0.00	0.00
glucose	427.35 c	457.46 c	588.19 d	164.81 a	227.63 ab	279.13 b	20.57
isomaltose	0.00 a	0.00 a	8.83 b	0.00 a	0.00 a	0.00 a	0.32
sorbitol	0.00 a	0.00 a	33.90 b	0.00 a	0.00 a	0.00 a	0.59
sucrose	0.00	0.00	0.00	0.00	0.00	0.00	0.00
total[d]	427.35 c	457.46 c	630.92 d	164.81 a	228.47 ab	284.77 b	15.30

[a] Abbreviations: T1, tapioca-based SFD1; T2, tapioca-based SFD2; TH, tapioca-based SFD hydrogenated; C1, corn-based SFD1; C2, corn-based SFD2; C3, corn-based SFD3. [b] Values are expressed on a dry matter basis. [c] Means in the same row with different letters are different ($P < 0.05$). [d] Values include addition of water added when starches are broken down to monosaccharide units.

high concentrations of hydrolyzed monosaccharides, with Pull LMW having a slightly lower concentration. The majority of the hydrolyzed monosaccharide content was from glucose for all three pullulan substrates. Small amounts of galactose and mannose were present in Pull LMW and Pull HMW.

For a complete understanding of novel carbohydrate potential for incorporation into foodstuffs, quantification of the free sugar and hydrolyzed monosaccharide contents of the test ingredient is essential. Knowledge of the free sugar content is important for carbohydrates that will be incorporated into foodstuffs meant to have a low glycemic response and reduced calorie content because these free sugars are readily digested and absorbed. The hydrolyzed monosaccharides constitute the building blocks of carbohydrate polymers and the fraction potentially available for digestion, and that also could affect the glycemic response and caloric content. Soluble fiber dextrins had low free sugar concentrations but were high in bound glucose concentrations. Depending on the linkages contained in the SFDs, branches composed of glucose units may be readily available to enzymes and have impacts on their digestibility, glycemic response, and energy values.

Like the SFD substrates, pullulans had very low free sugar content but high hydrolyzed monosaccharide content, mainly glucose. Interestingly, Pull IMW and Pull HMW had higher hydrolyzed monosaccharide content than that of Pull LMW. It has been reported that lowering the molecular weight of pullulan makes it more available for enzymatic digestion (*28*). Commerical pullulans are commonly produced using sucrose or cornstarch and, thus, can contain fructose and other sugar impurities. Some fructose is destroyed during the hydrolyzed monosaccharide assay and, along with sugar alcohols, are not included in the hydrolyzed monosaccharide value. Even though a portion of fructose is destroyed during the hydrolyzed monosaccharide assay, Pull LMW contained high amounts of fructose. This accounts for the lower hydrolyzed monosaccharide content of Pull LMW. Since these pullulans are manufactured by different companies, production and purification methods likely differ and this could account for differences in sugar composition and impurities found in the samples.

In Vitro Digestion. All SFDs had low to intermediate amounts of monosaccharides (mainly glucose) released (**Table 3**). Released glucose concentrations were higher for the tapioca-based SFDs than for the corn-based SFDs. The highest concentration of released monosaccharides occurred for TH (~63% of DM), while C1 had the lowest concentration of released sugars (~16% of DM).

Monosaccharides released from the simulated hydrolytic digestion of pullulans are presented in **Table 4**. High amounts of glucose were released after simulated digestion of all pullulans. Both Pull LMW and Pull HMW released only glucose, while Pull IMW had small amounts of fructose released after simulated digestion. Pull HMW had the lowest amount of released monosaccharide (~87% of DM) while Pull LMW and Pull IMW were completely digestible.

Table 4. Monosaccharides (Including Free Monosaccharides) Released after Simulated Hydrolytic Digestion of Pullulans

	test carbohydrate[a]			
released monosaccharides, mg/g[b]	Pull LMW	Pull IMW	Pull HMW	SEM
fructose	0.00 a[c]	6.97 b	0.00 a	0.13
galactose	0.00	0.00	0.00	0.00
glucosamine	0.00	0.00	0.00	0.00
glucose	1,041.62 b	1,041.24 b	866.16 a	27.11
isomaltose	0.00	0.00	0.00	0.00
sorbitol	0.00	0.00	0.00	0.00
sucrose	0.00	0.00	0.00	0.00
total[d]	1,041.62 b	1,048.21 b	866.16 a	27.12

[a] Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight pullulan. [b] Values are expressed on a dry matter basis. [c] Means in the same row with different letters are different ($P < 0.05$). [d] Values include water added when starches are broken down to monosaccharide units.

Carbohydrates with low concentrations of released monosaccharides have low digestibility as was noted for the SFD substrates, with the corn-based SFDs being approximately 20% digestible and the tapioca-based SFDs 50% digestible. The digestibility of a SFD derived from wheat (Nutriose) was evaluated using three different methods (in vitro, TNO intestinal model, and intestinal infusion in rats) (Lefranc-Millot et al. (*6*)). The results indicated an average small intestinal digestibility of 15% with a range of 8.7% to 19% (*6*). The two Nutriose SFD (C2 and C3) products evaluated in this study resulted in a higher in vitro digestibility (average of 25%). Carbohydrates that are highly digestible would result in little residue left for fermentation; however, these carbohydrates that have a lower digestion would contribute substantial substrate for potential fermentation in the colon.

Due to molecular structure and type of glycosidic linkages among monomeric units, some dietary carbohydrates are able to resist digestion by mammalian enzymes better than others. Based on the released monosaccharide data from the simulated hydrolytic digestion experiment, the SFDs appear to resist digestion and, thus, are potential substrates for fermentation by the microbiota in the large intestine. A comparison of SFDs from different starch sources by Laurentin and Edwards (*5*) found that the portions of SFDs that escaped digestion were extensively fermented. A variety of starch sources can be used to form indigestible dextrins such as those from corn, potato, wheat, lentil, cassava, and tapioca. The enzyme-resistant fractions of indigestible dextrins may vary significantly with the botanical source of the starch used (*7*).

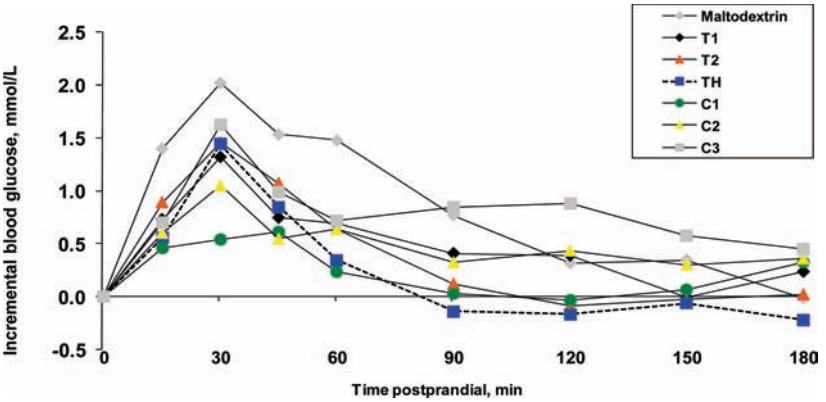

Figure 1. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble fiber dextrins. Pooled standard error of the mean (SEM) values for carbohydrates are as follows: maltodextrin (0.17), tapioca-based SFD1 (T1) (0.32), tapioca-based SFD2 (T2) (0.31), tapioca-based SFD hydrogenated (TH) (0.31), corn-based SFD1 (C1) (0.31), corn-based SFD2 (C2) (0.30), and corn-based SFD3 (C3) (0.30).

Table 5. Incremental Area under the Curve (AUC) for Glucose and Insulin, and Relative Glycemic Response (RGR) and Relative Insulinemic Response (RIR) of Soluble Fiber Dextrins

	carbohydrate[a]							
item	T1	T2	TH	C1	C2	C3	Malt	SEM[b]
AUC for glucose, mmol/L	91.38 ab[c]	80.11 ab	63.75 a	46.12 a	82.60 ab	132.86 bc	155.52 c	20.96
RGR, %	52.85 ab	50.43 ab	44.37 a	27.22 a	76.47 bc	90.58 cd	100.00 d	10.20
AUC for insulin, pmol/L	2,115.82 a	5,353.38 a	5,007.46 a	3,774.38 a	2,206.00 a	3,162.38 a	10,561.00 b	1,156.42
RIR, %	18.44 a	50.52 bc	57.68 c	37.97 abc	20.33 a	30.13 ab	100 d	6.94

[a] Abbreviations: T1, tapioca-based SFD1; T2, tapioca-based SFD2; TH, tapioca-based SFD hydrogenated; C1, corn-based SFD1; C2, corn-based SFD2; C3, corn-based SFD3; Malt, maltodextrin. [b] Pooled standard error of the mean. [c] Means in the same row with different letters are different ($P < 0.05$).

The different starch sources in this experiment varied significantly from each other with the SFDs from cornstarch having a lower ($P < 0.05$) digestibility than the SFDs from tapioca starch. It also has been documented that dextrinization increases in vitro indigestibility of the starch and that, as the degree of dextrinization increases, the extent of hydrolysis decreases (*5*, *7*). It may be the case that the corn-based SFDs had a higher degree of dextrinization, more branching, or a higher concentration of nondigestible linkages compared to the SFDs derived from tapioca, making them less susceptible to digestive enzymes.

High concentrations of monosaccharides released after simulated hydrolytic digestion indicate that the carbohydrate is highly digestible, as was the case for the pullulan substrates. However, other research conducted with pullulans indicates otherwise. Wolf et al. (*13*) found pullulan (MW 100,000) to be extensively hydrolyzed in vitro, but this hydrolysis occurred slowly over time. They concluded that pullulan was slowly digested and would result in a low glycemic response. Spears et al. (*29*) also reported on the digestibility of two pullulans (MW 63,000 and MW 100,000). Hydrolytic digestion was determined to be ~55% (*29*), indicating that the pullulans were not completely digested in the small intestine and portions were available to be fermented in the large intestine.

Glycemic and Insulinemic Responses. The change in plasma glucose for the SFDs is presented in **Figure 1**, and the corresponding values for AUC (mmol/L) and relative glycemic response (RGR) are presented in **Table 5**. Maltodextrin 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. Area under the curve for Malt was statistically higher than for the SFDs except for C3. The SFDs with the lowest AUC were C1 and TH, with T1, T2, and C2 having intermediate AUC values.

Since Malt served as the control to which all test carbohydrates were compared, it was assigned an 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 useful value for interpretation and comparison of glycemic responses among test substrates, particularly in this case where the carbohydrates were run in a series of tests and were not all evaluated in the same period. This is the reason Malt was used in every period as a control to calculate a relative response to the test carbohydrate in any particular period. The RGR values followed a similar pattern as AUC values for the SFDs. Corn-based SFD 3 and C2 had the numerically highest RGR values, with C3 being statistically similar to Malt. All three tapioca-based SFDs had similar intermediate RGR values, with a response averaging ~50% of the Malt response. The lowest RGR response was observed for C1, with a RGR value of 27%.

Varying degrees of resistance to digestion in the small intestine were noted for the SFD substrates. Low amounts of released monosaccharides were noted after simulated digestion of C1, and this corresponded to a low RGR for this test carbohydrate. A similarly low RGR was noted for TH, even though it had the highest ($P < 0.05$) amount of monosaccharide released after simulated hydrolytic digestion of all SFDs tested. However, TH had a portion of its free sugar and hydrolyzed monosaccharide content composed of sorbitol that would result in a blunting of the glycemic response since sorbitol does not elicit a glycemic response. Both C1 and TH resulted in a similar pattern of blood glucose response to carbohydrate ingestion: a peak in blood glucose at 30 min followed by steadily decreasing blood glucose concentrations and, by 90 min, blood glucose concentrations near or below baseline values.

Higher RGRs were noted for C2 and C3 compared to T1 and T2, even though C2 and C3 resulted in lower ($P < 0.05$) amounts of monosaccharides released after simulated digestion. The high glycemic responses noted for C2 and C3 are due, in part, to their pattern of blood glucose response during digestion. Peaks in blood glucose concentration during the first 45 min of the glycemic response test were larger for T1 and T2 compared to C2 and C3,

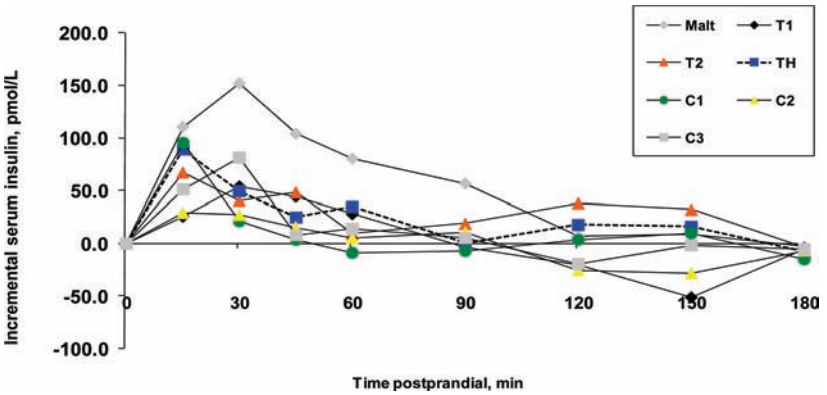

Figure 2. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble fiber dextrins. Pooled standard error of the mean (SEM) values for carbohydrates are as follows: maltodextrin (17.27), tapioca-based SFD1 (T1) (28.33), tapioca-based SFD2 (T2) (27.33), tapioca-based SFD hydrogenated (TH) (27.33), corn-based SFD1 (C1) (27.33), corn-based SFD2 (C2) (27.21), and corn-based SFD3 (C3) (27.21).

but for the remainder of the test, T1 and T2 resulted in an attenuated glycemic response. Higher RGRs were noted for C2 and C3 due, in part, to the fact that they had sustained blood glucose values after 90 min whereas the remainder of the SFDs resulted in glucose values that were closer to baseline.

The relative glycemic response (average 84%) of the Nutriose SFDs (C2 and C3) evaluated were higher than what has been reported for a wheat Nutriose SFD in humans with a lower RGR of 25% (*30*, *31*). A tapioca-based SFD similar to T1 and T2 was evaluated in humans and resulted in a similar RGR value as what was found using the canine model (48.2 and 51.6, respectively) (*32*).

The changes in serum insulin for the SFD substrates are presented in **Figure 2**, and the corresponding values for AUC (pmol/L) and relative insulinemic response (RIR) are presented in **Table 5**. All SFD substrates resulted in similar and lower ($P < 0.05$) AUC responses for insulin compared to the Malt control. All SFD substrates had a lower ($P < 0.05$) RIR value compared to the Malt control. Among the SFD substrates, T1, C1, C2, and C3 resulted in similar RIR responses with a response averaging ~26% of the Malt response. Tapioca-based SFD2 (T2) and TH resulted in the numerically highest RIR values among the substrates with a response averaging ~50% of Malt.

All SFDs had small peaks in insulin concentrations at 15, 30, and 45 min time points, but these peaks were all considerably lower in comparison to the Malt response. At 60 and 90 min after dosing, the insulin values for the SFDs had returned to baseline and stayed close to baseline for the remainder of the test. The higher values for T2 and TH compared to the rest of the SFD substrates are a result of a low blunted increase in serum insulin during later time points (120 and 150 min) of the glycemic response test.

The insulin responses of the SFDs are reflective of their glycemic responses. The SFDs all consisted of a portion of readily digestible starch that resulted in moderate peaks in blood glucose concentrations during the first 30 min of the glycemic response test. The increases in blood glucose concentrations induced insulin secretion and resulted in small peaks in insulin during the first 60 min of the test. Due to the nature of the glycemic curve, even SFDs with high RGR values (C2 and C3) resulted in low RIR values (average 25%). The higher RGR values result from a blunted response that lasted throughout the entire test with no sharp increases in blood glucose concentrations. These results are in comparison to a RIR of 13% reported for a wheat-based SFD in humans (*30*, *31*). A tapioca-based SFD resulted in a somewhat similar RIR in humans as T2 in this experiment (40.2 and 50.5, respectively) (*32*). The attenuated blood glucose peaks do not induce high insulin secretion resulting in low insulin response values for the SFDs. It has been suggested that the low insulinemic responses after ingesting low-digestible carbohydrates could contribute to a better feeling of satiety (*33*, *34*).

The changes in plasma glucose for the pullulans are presented in **Figure 3**, and the corresponding values for AUC (mmol/L) and relative glycemic response (RGR) are presented in **Table 6**. Maltodextrin had the highest ($P < 0.05$) AUC, with all pullulans resulting in lower but statistically similar AUCs for blood glucose. The RGR data followed the same pattern.

Spears et al. (*28*) evaluated two pullulans in dogs, one of MW 6,300 and the other of MW 100,000. Authors reported that, although not statistically significant, the 6,300 MW Pull had a lower glycemic response for the first 60 min postprandial compared to Malt. Wolf et al. (*13*) evaluated the glycemic response of a MW 100,000 Pull in humans and found that it reduced ($P < 0.01$) the glucose AUC by 50% compared to Malt. This result is similar to the decrease observed in the present experiment where Pull LMW reduced the glucose AUC by approximately 60% compared to the Malt control.

The data for change in serum insulin for the pullulans are presented in **Figure 4**, and the corresponding values for AUC (pmol/L) and relative insulinemic response (RIR) are presented in **Table 6**. All pullulan substrates resulted in lower ($P < 0.05$) and similar AUC and RIR values compared to Malt. A low blunted curve during the first 60 min of the test was noted for Pull LMW, which mirrors the glycemic response and accounts for Pull LMW having a numerically higher RGR and RIR among the Pull substrates. In general, the pullulans resulted in insulin response values that stayed close to baseline values throughout the entire response test. These responses mirror the glycemic responses where no large, sharp peaks resulted and, thus, no sharp increases in insulin were noted. Spears et al. (*28*) also evaluated insulin responses in dogs fed pullulans of 6,300 MW and 100,000 MW and found the 100,000 MW Pull to significantly reduce serum insulin, but not the 6,300 MW Pull. However, the 6,300 MW Pull used by Spears et al. (*28*) was a much smaller compound than that used in this current study (100,000 MW), explaining in part why the latter was capable of significantly reducing both glycemic and insulinemic responses compared to Malt.

Pullulans resulted in low glycemic and insulinemic responses, with an average response value of ~24% for both RGR and RIR. Numerically, Pull LMW had the highest glycemic response with a RGR value ~41% of that of the Malt control. The lower MW of this pullulan could make it more readily available for digestion. The overall glycemic curve patterns of the pullulans showed low blunted curves throughout the entire glycemic test, indicating that the pullulans are slowly digestible.

True Metabolizable Energy (TME_n). As health complications associated with obesity are a growing problem in today's society,

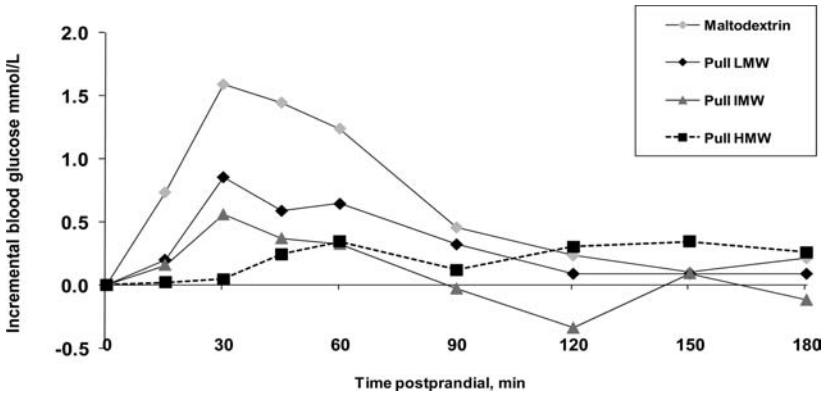

Figure 3. Incremental change from baseline in blood glucose response for dogs consuming 25 g of pullulans. Pooled standard error of the mean (SEM) values for carbohydrates are as follows: maltodextrin (0.20), low molecular weight pullulan (Pull LMW) (0.30), intermediate molecular weight pullulan (Pull IMW) (0.31), and high molecular weight pullulan (Pull HMW) (0.33).

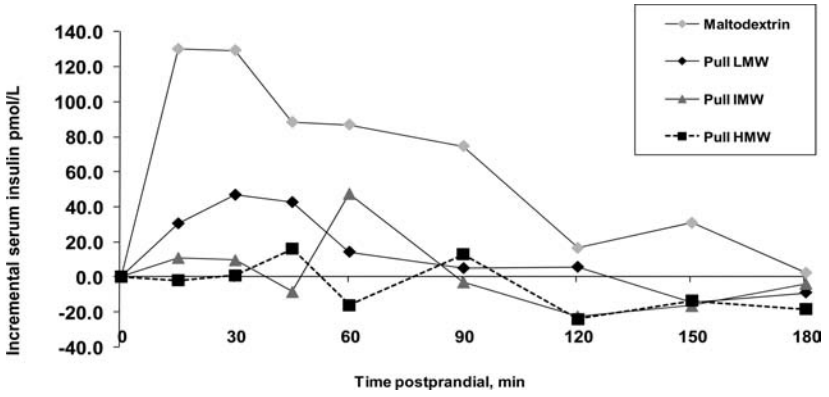

Figure 4. Incremental change from baseline in serum insulin response for dogs consuming 25 g of pullulans. Pooled standard error of the mean (SEM) values for carbohydrates are as follows: maltodextrin (19.34), low molecular weight pullulan (Pull LMW) (26.35), intermediate molecular weight pullulan (Pull IMW) (27.76), and high molecular weight pullulan (Pull HMW) (32.74).

Table 6. Incremental Area under the Curve (AUC) for Glucose and Insulin, and Relative Glycemic Response (RGR) and Relative Insulinemic Response (RIR) of Pullulans

	test carbohydrate[a]				
item	Pull LMW	Pull IMW	Pull HMW	Malt	SEM[b]
AUC for glucose, mmol/L	60.18 a[c]	14.73 a	44.10 a	153.76 b	20.81
RGR, %	40.79 a	13.33 a	19.05 a	100.00 b	10.13
AUC for insulin, pmol/L	2,800.34 a	2,613.04 a	1,167.08 a	11,978.00 b	2,335.66
RIR, %	38.10 a	18.27 a	15.29 a	100.00 b	6.84

[a] Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight pullulan; Malt, maltodextrin. [b] Pooled standard error of the mean. [c] Means in the same row with different letters are different ($P < 0.05$).

there is an increasing trend for producing reduced calorie foodstuffs. This is increasing the demand for low-calorie sweeteners and bulking agents. One method for evaluating the energy value of ingredients is use of the true metabolizable energy (TME_n) assay with roosters. This in vivo animal model assay allows for a better representation of the digestive process than do in vitro assays for determining metabolizable energy.

Maltodextrin had a higher ($P < 0.05$) TME_n value compared to all SFDs (**Table 7**). Similar TME_n values were found for T1, T2, and TH. Also, C1, C2, and C3 had similar values, with the tapioca-based SFDs being numerically higher than the corn-based SFDs. The corn-based Nutriose SFDs (C2 and C3) resulted in exactly the same energy value (average 1.7 kcal/g) as a wheat-based Nutriose SFD (1.7 kcal/g) evaluated in men (*35*). Fibersol-2 has been evaluated for its energy content in humans and has been reported to have an energy value of 1.5 kcal/g (*36*). This is similar to the energy value of 1.2 kcal/g found when evaluating Fibersol-2 (C1) using the precision-fed rooster assay. Metabolizable energy of carbohydrates varies due to the degree to which they are digested and absorbed; thus, the results of the TME_n assay corroborate well with the in vitro hydrolytic digestion results.

Table 7. True Metabolizable Energy (TME_n) Values for Soluble Fiber Dextrins

	test carbohydrate[a]							
item	T1	T2	TH	C1	C2	C3	Malt	SEM[b]
amount dosed, g DM basis	13.47	20.29	26.07	25.68	23.58	17.80	14.27	
TME_n, kcal/g	2.19 bc[c]	2.30 c	2.57 c	1.23 a	1.66 ab	1.65 ab	4.06 d	0.12

[a] Abbreviations: T1, tapioca-based SFD1; T2, tapioca-based SFD2; TH, tapioca-based SFD hydrogenated; C1, corn-based SFD1; C2, corn-based SFD2; C3, corn-based SFD3; Malt, maltodextrin. [b] Pooled standard error of the mean. [c] Means in the same row with different letters are different ($P < 0.05$).

True metabolizable energy data for the pullulans are presented in **Table 8**. Energy data were not collected for Pull HMW because, due to its physical properties, sufficient substrate could not be provided to the roosters for accurate measurementss. Low molecular weight pullulan resulted in the lowest ($P < 0.05$) TME_n value (3.33 kcal/g) with Pull IMW and Malt having similar values (3.95 and 4.06 kcal/g). While the glycemic assay shows pullulans

Table 8. True Metabolizable Energy (TME_n) Values for Pullulans

item	test carbohydrate[a]				SEM[b]
	Pull LMW	Pull IMW	Pull HMW	Malt	
amount dosed, g DM basis	14.23	14.40	NM	14.27	
TME_n, kcal/g	3.33 a[c]	3.95 b	NM	4.06 b	0.17

[a] Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight pullulan; Malt, maltodextrin. [b] Pooled standard error of the mean. [c] Means in the same row with different letters are different ($P < 0.05$).

to have significantly lower responses than Malt, indicative of resistance to digestion, that test lasts for 3 h only. The hydrolytic in vitro digestion and TME_n results indicate that the LMW and IMW pullulan substrates are not resistant to digestion but are, instead, slowly digestible carbohydrates.

Few data exist regarding metabolizable energy content of novel carbohydrates fed alone. In those instances where these carbohydates are evaluated, the 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.

In summary, the SFDs evaluated varied in sugar composition and physiological responses. The corn-based SFDs resulted in a lower content of released monosaccharides and lower energy content compared to the tapioca- based SFDs after simulated hydrolytic digestion, but this did not correspond to lower glycemic responses as was the case for C2 and C3. Overall, the SFDs showed varying degrees of resistance to digestion, thus resulting in attenuated glycemic responses compared to Malt, making them suitable candidates for reduced glycemic and low-calorie foodstuffs. Low-digestible carbohydrates also may promote health benefits due to their potential for colonic fermentation. Some positive effects that have been observed with low-digestible carbohydrates such as SFDs are decreases in colon pH, production of short-chain fatty acids, increased absorption of minerals, positive impacts on sugar and fat metabolism, and an increase of energy expenditure (*37*). Even though the pullulans were almost completely hydrolyzed after simulated digestion, they resulted in significantly attenuated glycemic responses, indicating that pullulans are slowly digestible carbohydrates. Pullulans could be ideal candidates for incorporation into foodstuffs for diabetics as they result in low glycemic response without eliciting large peaks in blood glucose or insulin. Higher MW pullulans also would be beneficial in products meant to help sustain low blood glucose concentrations over time since they are more slowly digested compared to lower MW pullulans. The differences in physiological responses among carbohydrates can be contributed, in part, to individual carbohydrate molecular structure and bonding pattern and how they affect digestibilty. Evaluation of a variety of physiological responses is beneficial as it allows for a more complete understanding of the potential functional benefits that select carbohydrates possess.

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