

In vitro hydrolytic digestion, glycemic response in dogs, and true metabolizable energy content of soluble corn fibers

M. R. C. de Godoy,^{*1} B. K. Knapp,^{*} C. M. Parsons,^{*} K.S. Swanson,^{*†‡} and George C. Fahey Jr.^{*}

^{*}Department of Animal Sciences; [†]Division of Nutritional Sciences; and

[‡]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana 61801

ABSTRACT: The objective of this research was to measure in vitro hydrolytic digestion, glycemic and insulinemic responses in dogs, and true ME (TME_n) content of select soluble corn fibers (SCF) in roosters. The first generation (G1) SCF included hydrochloric acid-treated corn syrup (G1-CS-HCl), an SCF with an increased total dietary fiber (TDF) content (G1-SCF-HCl), an SCF that was spray-dried (G1-SCF-SD), and a hydrogenated SCF (G1-SCF-hydrog). The second generation (G2) SCF included those prepared using phosphoric acid catalyzation in both a liquid [G2-SCF-phos (Lq)] and powder [G2-SCF-phos (Pw)] form, and SCF that were prepared using hydrochloric acid catalyzation in both a liquid [G2-SCF-HCl (Lq)] and powder [G2-SCF-HCl (Pw)] form. Also, in the G2 set of samples were SCF prepared using the same method, but in 3 separate batches, all of which contained 70% TDF and 15% sugars. Two were in liquid form [G2-SCF-phos+HCl (Lq1)] and [G2-SCF-phos+HCl (Lq2)], and one in powder form

[G2-SCF-phos+HCl (Pw)]. A lower sugar form (80% TDF and 5% sugar) of SCF was also evaluated (G2-SCF-low sugar). Glucose was the major free sugar and bound monosaccharide in all SCF except for G1-SCF-hydrog that had greater concentrations of sorbitol. All SCF had intermediate to low amounts of monosaccharides released as a result of in vitro hydrolytic digestion, with glucose being the primary sugar component released. The G1-SCF were more digestible in vitro (approximately 50%) compared to G2-SCF (approximately 32%). All SCF had attenuated glycemic responses in adult dogs compared to a maltodextrin control ($P < 0.05$). The G2-SCF, on average, had lower glycemic responses and TME_n values in roosters than G1-SCF. All SCF had low free sugar concentrations with varying degrees of resistance to digestion, reduced caloric content, and attenuated glycemic and insulinemic responses in adult dogs. These ingredients are potential candidates for inclusion in reduced calorie and low glycemic canine diets.

Key words: canine, glycemic response, in vitro digestion, soluble corn fibers, true metabolizable energy

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INTRODUCTION

The increased incidence of chronic diseases such as obesity, diabetes, and other metabolic disorders in pet and human populations has led to an increased awareness of how diet, especially dietary fiber and low-digestible carbohydrates, can play a positive role in health. Novel carbohydrates have the potential to not only increase the fiber content of foodstuffs, but also the ability to attenuate postprandial glycemia (Riccardi et al., 2008).

A category of carbohydrate that has the potential functional properties and benefits similar to dietary fiber

is low-digestible carbohydrates. A type of novel, low-digestible carbohydrate is soluble corn fibers (SCF). Soluble corn fibers are obtained by isolating an oligosaccharide-rich component from partially hydrolyzed corn syrup. The different processes utilized to produce these oligosaccharides result in fractions with varying degrees of digestion resistance. Soluble corn fiber is manufactured in a similar manner to type III resistant starch that is often produced using enzymes and retrogradation to increase α -1,6 glycosidic linkages (Thompson, 2000; Haralampu, 2004; Harrison and Hoffman, 2007).

The objective of this study was to determine the sugar composition and the physiological effects of 12 SCF sources as potential ingredients in caloric-restricted and low-glycemic pet foods. Assessments involved the modulation of in vitro hydrolytic digestion, glycemic

¹Corresponding author: mgodoy2@illinois.edu
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and insulinemic responses using a canine model, and true ME (TME_n) using an avian model. The authors hypothesized that the different processing conditions utilized in the production of SCF would increase the concentration of nondigestible carbohydrates and modify the profile and amount of free sugars, resulting in lower hydrolytic digestion, glycemic and insulinemic responses, and TME_n .

MATERIALS AND METHODS

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before animal experimentation.

Substrates

Soluble corn fibers can result from the process of manufacturing oligosaccharides that are digestion resistant or slowly digestible. General steps in the production of SCF may include starch hydrolysis, filtration, isomerization, hydrogenation, catalyst reaction, decolorization, and evaporation (Harrison and Hoffman, 2007).

The SCF evaluated in this study were produced using proprietary processes using different combinations of the general steps mentioned before and were classified into generation 1 (**G1**) and 2 (**G2**) products. The processes utilized in the manufacturing of the G2-SCF were designed to increase the concentration of low-digestible carbohydrates in comparison to G1-SCF. Both generations of SCF relied on the creation of reversion products using different combinations of acid catalysts and enzymes to increase number of glycosidic bonds.

The G1-SCF included 1 made from corn syrup, where HCl only was used as a catalyst (**G1-CS-HCl**); an SCF using the same production methods as for G1-CS-HCl, but that had a greater TDF content (**G1-SCF-HCl**); an SCF prepared using similar production methods, but that was spray dried (**G1-SCF-SD**); and a G1-SCF that went through hydrogenation steps to form sugar alcohols (**G1-SCF-hydrog**). The G2-SCF included a set of 4 carbohydrates where either phosphoric acid or HCl alone were used as catalysts and were produced in either a liquid [**G2-SCF-phos (Lq)** and **G2-SCF HCl (Lq)**] or powder [**G2-SCF phos (Pw)** and **G2-SCF HCl (Pw)**] form. A combination of phosphoric and HCl was used as catalysts for the production of the last 4 SCF in the G2 series. They included an SCF that was evaporated to a greater extent than the SCF in the previous generation, and they were 2 SCF in liquid form [**G2-SCF-phos+HCl (Lq1)** and **G2-SCF-phos+HCl (Lq2)**] and 1 in powder form produced by different batches [**G2-SCF-phos+HCl (Pw)**]. These SCF contained 70% TDF and 15% sugar. Lastly, a lower-sugar (80% TDF and 5% sugar) SCF was produced in this series (**G2-SCF-low sugar**).

Chemical Analyses

Substrates were analyzed for DM (Method 934.01) and OM (Method 942.05) according to AOAC (2000). Free and hydrolyzed monosaccharide concentrations of test carbohydrates were hydrolyzed using the procedure described by Hoebler et al. (1989), where carbohydrates were subjected to hydrolysis with H_2SO_4 . Free sugars and hydrolyzed monosaccharides were quantified (Dionex DX500 HPLC System; Dionex Corporation, Sunnyvale, CA). Maltodextrin (**Malt**; Tate & Lyle, Decatur, IL) was used as a control sample, and pure certified standards for quantification of inositol, fucose, arabinose, rhamnose, galactose, xylose, mannose, glucose, and fructose (Sigma Aldrich Co., St. Louis, MO). Free monosaccharides were injected at a volume of 25 μ L. All assays were conducted using a column (CarboPac PA-1 Column; Dionex Corporation) and guard column (Dionex Corporation) following methods cited by Smiricky et al. (2002). The CV for within and between days were 1 and 2%, respectively.

In Vitro Hydrolytic Digestion

Approximately 200 mg of each carbohydrate test sample were weighed in triplicate and incubated with 2 mL of a pepsin/HCl solution and 2 mL of an enzyme solution consisting of amyloglucosidase and α -amylase to simulate gastric and small intestinal digestion (Muir and O'Dea, 1993). The samples were analyzed for free released monosaccharides using HPLC (Smiricky et al., 2002), following the simulated hydrolytic digestion procedure.

Glycemic and Insulinemic Responses

To determine postprandial glycemic and insulinemic responses to the test carbohydrates, 5 purpose-bred female dogs (Butler Farms, Clyde, NY) with hound bloodlines, a mean initial BW of 25.1 ± 4.8 kg and a mean age of 5.6 ± 2.4 yr, 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 (Edward R. Madigan Laboratory, University of Illinois, Urbana, IL). Dogs were provided with nondestructible toys (hard plastic balls, Nyla bones, etc.). Pens allowed for nose-to-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.

Dogs consumed 25 g of carbohydrate (DM basis) in approximately 240 mL of distilled dionized water. To get carbohydrate sources into solution, water and carbohydrate were mixed using a stir plate. Carbohydrate solutions were dosed to the dogs using a disposable 60-mL syringe (without needle) within a 10-min period. During the study, all dogs were fed the same commercial diet (Iams Weight Control; The Iams Co., Lewisburg, OH). Water was available ad libitum.

A 5×5 Latin square design was used to evaluate test substrates. Maltodextrin served as the control, and in each study conducted, the dogs were subjected to 4 test ingredients and the Malt control. Glycemic tests were conducted for a 3-h period with a 4-d recovery period between tests. 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 were dosed 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 were 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 $1240 \times g$ for 10 min at 4°C , and the serum was stored at -20°C for subsequent analysis for insulin.

Immediately after collection, blood samples were assayed for glucose based on the glucose oxidase method (Precision-G Blood Glucose Testing System; Medisense, Inc., Bedford, MA). This system measures blood glucose concentrations from an electric current generated by electron transfer when the glucose oxidase on the test strip catalyzes the oxidation of glucose to gluconic acid (Cass et al., 1984). Each glucometer was calibrated before 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; Wisdom, 1976).

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. (1991) using software (GraphPad Prism 5 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: $[(\text{AUC for test carbohydrate})/(\text{AUC for control})] \times 100\%$.

True ME (TME_n)

Conventional Single Comb White Leghorn roosters were utilized in this study. All roosters were housed individually in cages with raised wire floors. They were kept in an environmentally controlled room and subjected to a 16-h light and 8-h dark photoperiod. Roosters were deprived of feed for 24 h and then crop-intubated with approximately 13 to 26 g of each carbohydrate using the precision-fed rooster assay (Sibbald, 1980; Parsons, 1985). Each carbohydrate was fed to 4 roosters. After 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 analyzed for GE 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 N-corrected true ME (TME_n) values, corrected for endogenous energy, were calculated by the method of Parsons et al. (1982).

Statistical Analysis

In vitro hydrolytic digestion data were analyzed as a completely randomized design using the Mixed Models procedure of SAS (SAS Inst. 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 and insulinemic data were analyzed by the Mixed models procedure of SAS. 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 single degree of freedom 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. The TME_n data were analyzed as a completely randomized design using the GLM procedure of SAS. 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

All SCF had low free sugar concentrations (Table 1). Hydrogenated SCF had the greatest free sugar concentrations of all carbohydrates tested. Glucose was the major free sugar found in the SCF except for G1-SCF-hydrog. For this substrate, a small amount (4.6 mg/g) of free glucose was present, and sugars present in the greatest concentration included fructose, sucrose, and sorbitol.

Hydrolyzed monosaccharide concentrations varied slightly among the G1-SCF. The G1-SCF-HCl, G1-SCF-SD, and G1-SCF-hydrog had increased concentrations of hydrolyzed monosaccharides, while G1-CS-HCl had a lower concentration. Glucose comprised most of the hydrolyzed monosaccharides for all G1-SCF substrates. Minor amounts of mannose were present in G1-SCF-HCl,

Table 1. Free sugar and hydrolyzed monosaccharide concentrations of soluble corn fibers: generation 1 (G1) series

Item	Test carbohydrate ¹			
	G1-CS-HCl	G1-SCF-HCl	G1-SCF-SD	G1-SCF-hydrog
Free sugars, ² mg/g				
Arabinose	0.0	0.4	0.1	5.6
Fructose	0.6	0.0	0.0	36.5
Galactose	0.0	0.0	0.0	8.7
Glucose	67.9	36.7	27.5	4.6
Mannose	0.0	0.5	0.0	0.0
Rhamnose	0.5	0.0	0.5	0.0
Sorbitol	0.0	0.0	0.0	25.2
Sucrose	0.7	1.5	0.8	42.0
Xylose	0.0	0.0	0.0	0.0
Total, ³ mg/g	69.8	39.1	28.9	122.6
Hydrolyzed monosaccharides, ^{2,4} mg/g				
Glucose	709	969	1015	793
Mannose	0.0	5.0	7.2	4.0
Sorbitol	0.0	0.0	0.0	177.3
Total, ³ mg/g	709	974	1023	975

¹G1-CS-HCl = product from hydrochloric acid-catalyzed condensation of corn syrup; G1-SCF-HCl = G-1 soluble corn fiber using hydrochloric acid-catalyzed condensation; G1-SCF-SD = spray-dried version of G1-SDF-HCl; and G1-SCF-hydrog = hydrogenated version of G1-SCF-SD.

²Values expressed on a DM basis.

³Values include water added when starches hydrolyzed into monosaccharides.

⁴Values are corrected for free monosaccharide concentrations.

G1-SCF-SD, and G1-SCF-hydrog. Sugars accounted for more than 90% of the DM in all SCF except for G1-CS-HCl, which had only 71% of the DM as sugars.

Free sugar content (Table 2) varied slightly among G2-SCF with a range of 2 to 14% of the carbohydrate content consisting of free sugars. The G2-SCF-low sugar had the lowest free sugar content at 2.3% and G2-SCF-phos+HCl (Lq1) had the greatest with a 14.1% free sugar concentration. Soluble corn fibers that were treated with phosphoric acid had greater free sugars (approximately 11%) than SCF treated with hydrochloric acid (approximately 6%). Glucose was the major free sugar in all G2-SCF.

Hydrolyzed monosaccharide concentrations were similar among the G2-SCF except for G2-SCF-phos+HCl (Lq1), which had a hydrolyzed monosaccharide concentration of approximately 49%. The hydrolyzed monosaccharide assay destroys fructose so it was measured in the analysis. The G2-SCF-phos+HCl (Lq1) likely contained a major portion of bound fructose. Thus, the inability to measure fructose contributed to the low concentrations of hydrolyzed monosaccharides in this substrate. Most of the hydrolyzed monosaccharide content was from glucose for all 8 G2-SCF. Mannose (1 mg/g) was present only in G2-SCF-phos+HCl (Pw).

Quantification of the free sugar and hydrolyzed monosaccharide contents of novel carbohydrates is important if these substrates are incorporated into low glycemic dog foods. Free sugars in carbohydrate substrates are rapidly available for digestion and, thus, have the ability to affect the glycemic response (Englyst et al., 1999). The

Table 2. Free sugar and hydrolyzed monosaccharide concentrations of soluble corn fibers: generation2 (G2) series

Item	Test carbohydrate ¹							
	G2-SCF-phos + HCl (Lq1)	G2-SCF-low sugar	G2-SCF-phos (Lq)	G2-SCF-phos (Pw)	G2-SCF-HCl (Lq)	G2-SCF-HCl (Pw)	G2-SCF-phos + HCl (Lq2)	G2-SCF-phos + HCl (Pw)
Free sugars, ² mg/g								
Arabinose	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fructose	11.9	0.7	7.3	5.1	4.4	4.9	4.4	8.0
Galactose	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.2
Glucose	127.2	21.2	92.0	114.5	54.6	58.3	90.1	53.3
Mannose	0.7	0.0	0.5	0.5	0.4	0.5	0.6	0.7
Rhamnose	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sorbitol	1.0	0.2	0.3	0.3	0.0	0.1	0.7	0.2
Sucrose	0.0	0.8	0.0	0.0	0.0	0.0	0.1	0.0
Total, ³ mg/g	141.9	23.0	100.0	120.3	59.4	63.8	96.0	62.3
Hydrolyzed monosaccharides, ^{2,4} mg/g								
Glucose	490	1,085	981	996	996	1,000	1,027	1,074
Mannose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Total, ³ mg/g	490	1,085	981	996	996	1,000	1,027	1,075

¹G2-SCF-phos + HCl (Lq1) = G2 soluble corn fiber batch 1 [liquid (Lq)]; G2-SCF-low sugar = low sugar version of G2 soluble corn fiber; G2-SCF-phos (Lq) = phosphoric acid-catalyzed condensation of corn syrup L; G2-SCF-phos (Pw) = phosphoric acid-catalyzed condensation of corn syrup powder (Pw); G2-SCF-HCl (Lq) = hydrochloric acid-catalyzed condensation of corn syrup Lq; G2-SCF-HCl (Pw) = hydrochloric acid-catalyzed condensation of corn syrup Pw; G2-SCF-phos + HCl (Lq2) = G2 soluble corn fiber batch 2 (Lq); and G2-SCF-phos + HCl (Pw) = G2 soluble corn fiber batch 3 (Pw).

²Values expressed on a DM basis.

³Values include water added when starches are hydrolyzed into monosaccharides.

⁴Values are corrected for free monosaccharide concentrations.

hydrolyzed monosaccharides comprise the basic units of the test substrate and the fraction of monosaccharides that are potentially available for digestion. Free sugars and hydrolyzed monosaccharides predict the amount and which monosaccharides may potentially affect the glycemic response and TME_n value (Knapp et al., 2008).

In Vitro Hydrolytic Digestion

All of the G1-SCF were found to have intermediate amounts of monosaccharides released, translating to a digestibility value of approximately 50% (Table 3). The G1-SCF-hydrog substrate resulted in the lowest ($P < 0.05$) concentration of released glucose, whereas the greatest glucose release occurred for G1-CS-HCl. The G1-SCF-hydrog resulted in the greatest ($P < 0.05$) concentrations of glucosamine and sorbitol release among the G1-SCF.

Low to intermediate amounts of monosaccharides were released after simulated digestion of all G2-SCF (Table 4). The G2-SCF-low sugar resulted in the lowest ($P < 0.05$) monosaccharide release (approximately 19% digested). The SCF that were prepared by phosphoric acid catalysis resulted in the greatest ($P < 0.05$) monosaccharide release, with G2-SCF-phos (Pw) being greater ($P < 0.05$) than G2-SCF-phos (Lq). Glucose was the major monosaccharide released for all G2-SCF. The G2-SCF-low sugar had the lowest glucose release and G2-SCF-phos (Pw) the greatest ($P < 0.05$). All SCF had low concentrations of fructose (< 20 mg/g) and galactose (< 0.5 mg/g) released during simulated hydrolytic digestion. Sorbitol was released in small amounts for all SCF (< 1.2 mg/g), except G2-SCF-low sugar, which had no released sorbitol. The greatest ($P < 0.05$) concentration of released fructose, galactose, and sorbitol occurred for G2-SCF-phos+HCl (Lq1).

Table 3. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of soluble corn fibers: generation 1(G1) series

Item	Test carbohydrate ¹				SEM ²
	G1-CS-HCl	G1-SCF-HCl	G1-SCF-SD	G1-SCF-hydrog	
Released monosaccharides, ³ mg/g					
Glucosamine	0.0 ^a	0.4 ^b	0.0 ^a	5.5 ^c	0.1
Glucose	515.4 ^c	453.7 ^b	484.2 ^{bc}	395.6 ^a	6.8
Isomaltose	14.4 ^b	20.2 ^c	17.9 ^{bc}	0.0 ^a	0.8
Sorbitol	0.0 ^a	0.0 ^a	0.0 ^a	53.6 ^b	0.5
Total, ⁴ mg/g	529.7 ^c	474.3 ^{ab}	502.0 ^{bc}	454.7 ^a	7.7

^{a-c}Means in the same row with different superscript letters are different ($P < 0.05$); $n = 3$.

¹G1-CS-HCl = product from hydrochloric acid-catalyzed condensation of corn syrup; G1-SCF-HCl = G-1 soluble corn fiber using hydrochloric acid-catalyzed condensation; G1-SCF-SD = spray-dried version of G1-SDF-HCl; and G1-SCF-hydrog = hydrogenated version of G1-SCF-SD.

²Pooled standard error of the mean.

³Values expressed on a DM basis.

⁴Values include water added when starches are hydrolyzed into monosaccharides.

The G1-SCF were approximately 50% digestible and exceeded 32% average digestibility of G2-SCF. The G2-SCF were produced using more stringent processes that likely accounted for the decrease in digestibility. Differences in the acid catalyst used during the production process dramatically affected digestibility of the SCF. Using HCl as a catalyst appeared to create reversion products that were more resistant to enzymes used in hydrolytic digestion.

Resistance to hydrolytic digestion may be explained by the molecular structure of the carbohydrate. The glycosidic linkages that bind monosaccharides affect carbohydrate resistance to enzymatic digestion. Enzyme resistance is mainly due to the presence of α -1,6 glycosidic linkages

Table 4. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of soluble corn fibers: generation 2 (G2) series

Item	Test carbohydrate ¹								SEM ²
	G2-SCF-phos + HCl (Lq1)	G2-SCF-low sugar	G2-SCF-phos (Lq)	G2-SCF-phos (Pw)	G2-SCF-HCl (Lq)	G2-SCF-HCl (Pw)	G2-SCF-phos + HCl (Lq2)	G2-SCF-phos + HCl (Pw)	
Released monosaccharides, ³ mg/g									
Fructose	20.2 ^f	10.5 ^a	16.6 ^c	12.8 ^{bc}	13.4 ^c	12.1 ^b	14.9 ^d	17.4 ^c	0.3
Galactose	0.5 ^f	0.1 ^a	0.1 ^{ab}	0.1 ^{abc}	0.2 ^{cde}	0.2 ^{bcd}	0.3 ^{de}	0.3 ^e	0.0
Glucose	264.3 ^{cd}	177.1 ^a	383.4 ^f	513.8 ^g	251.3 ^c	276.7 ^d	349.3 ^e	231.6 ^b	3.7
Sorbitol	1.2 ^f	0.0 ^a	0.3 ^c	0.2 ^c	0.1 ^b	0.2 ^b	0.7 ^e	0.4 ^d	0.0
Total, ⁴ mg/g	286.1 ^c	187.7 ^a	400.3 ^c	526.9 ^f	265.0 ^b	289.2 ^c	365.2 ^d	249.7 ^b	3.6

^{a-g}Means in the same row with different superscript letters are different ($P < 0.05$); $n = 3$.

¹G2-SCF-phos + HCl (Lq1) = G2 soluble corn fiber batch 1 [liquid (Lq)]; G2-SCF-low sugar = low sugar version of G2 soluble corn fiber; G2-SCF-phos (Lq) = phosphoric acid-catalyzed condensation of corn syrup L; G2-SCF-phos (Pw) = phosphoric acid-catalyzed condensation of corn syrup powder (Pw); G2-SCF-HCl (Lq) = hydrochloric acid-catalyzed condensation of corn syrup Lq; G2-SCF-HCl (Pw) = hydrochloric acid-catalyzed condensation of corn syrup Pw; G2-SCF-phos+HCl (Lq2) = G2 soluble corn fiber batch 2 (Lq); and G2-SCF-phos+HCl (Pw) = G2 soluble corn fiber batch 3 (Pw).

²Pooled standard error of the mean.

³Values expressed on a DM basis.

⁴Values include water added when starches are hydrolyzed into monosaccharides.

Table 5. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble corn fibers: generation 1 (G1) series

Item	Test carbohydrate ¹				SEM ²	
	Malt	G1-CS-HCl	G1-SCF-HCl	G1-SCF-SD		
AUC for glucose, mmol/L	157.2 ^c	121.2 ^b	98.2 ^b	98.9 ^b	34.0 ^a	18.2
RGR, %	100.0 ^c	80.9 ^b	60.5 ^b	62.8 ^b	14.9 ^a	10.2
AUC for insulin, nmol/L	10.8 ^c	4.8 ^b	4.3 ^b	4.2 ^b	0.9 ^a	1.6
RIR, %	100.0 ^c	58.3 ^b	43.8 ^b	48.6 ^b	10.7 ^a	9.0

^{a-c}Means in the same row with different superscript letters are different ($P < 0.05$). $n = 5$.

¹Malt = maltodextrin; G1-CS-HCl = product from hydrochloric acid-catalyzed condensation of corn syrup; G1-SCF-HCl = G1 soluble corn fiber using hydrochloric acid-catalyzed condensation; G1-SCF-SD = spray-dried version of G1-SDF-HCl; and G1-SCF-hydrog = hydrogenated version of G1-SCF-SD.

²Pooled standard error of the mean.

and inaccessibility of the α -1,4 linkages (Kendall et al., 2008). During the production of the SCF, different acid and enzyme combinations were utilized to form glycosidic linkages, predominantly α -1,6 linkages, among monosaccharides in the corn syrup to produce a more digestion-resistant carbohydrate. Kendall et al. (2008) evaluated the in vitro digestibility of an SCF that was similar to G2-SCF-phos+HCl (Lq1). The SCF was calculated to be 14.5% digestible, which is less than the 28% digestibility that was measured in the current study. Sinaud et al. (2002) evaluated a novel low-digestible carbohydrate produced using a starch in the presence of an acidic catalyst like the SCF. The low-digestible carbohydrate had an in vitro digestibility of 39.8%. A type III resistant starch evaluated by Brouns et al. (2007) had an in vitro digestion of 40.5%. Both if the low-digestible carbohydrates evaluated in the previous studies had in vitro digestion values greater than the average 32% in vitro digestibility of the G2-SCF. Overall, the production processes implemented for G2-SCF were very successful in producing low-digestible carbohydrates.

Glycemic and Insulinemic Responses

Maltodextrin was used as a control for each glycemic response test because it is highly digestible, rapidly absorbed, and produces a constant glycemic response (Wolf et al., 2003). The carbohydrates were not all evaluated in the same trial, so Malt was used in each trial as a control to calculate a relative response to the test carbohydrate in all periods. Glucose AUC for Malt was greater ($P < 0.05$) than the AUC values for all G1-SCF (Table 5). Intermediate AUC values resulted for G1-CS-HCl, G1-SCF-HCl, and G1-SCF-SD. The G1-SCF-hydrog resulted in the lowest ($P < 0.05$) AUC value.

Because Malt served as the control to which all test carbohydrates were compared, it was assigned an RGR value of 100. The RGR is an appropriate method for interpretation of glycemic response because carbohydrates were run in a series of tests and were not all evaluated in the same period. Relative glycemic responses are calculated from AUC values and directly related; thus, test

carbohydrates with increased AUC values will have correspondingly increased RGR values (Table 5). The RGR values for G1-SCF followed the same pattern as AUC values. Maltodextrin had the greatest RGR ($P < 0.05$), followed by intermediate values for G1-CS-HCl, G1-SCF-HCl, and G1-SCF-SD, whereas G1-SCF-hydrog resulted in the lowest RGR values ($P < 0.05$).

A similar pattern in blood glucose response resulted from digestion of G1-CS-HCl, G1-SCF-HCl, and G1-SCF-SD (Fig. 1). All 3 carbohydrates resulted in an intermediate peak at 30 min, which preceded a blunted response in blood glucose concentrations throughout the remainder of the glycemic response test. The G1-SCF-hydrog peaked minimally at 30 min and remained at or below basal blood glucose concentration throughout the glycemic response test. These blood glucose patterns were consistent with sugar composition data. Although G1-SCF-hydrog had a greater free sugar concentration than G1-CS-HCl, G1-SCF-HCl, and G1-SCF-SD, the increased concentrations of sorbitol and fructose are likely explanations for the lack of a glycemic response (de Godoy et al., 2013). Free sugar and released monosaccharide data show that G1-CS-HCl, G1-SCF-HCl, and G1-SCF-SD have glucose available for digestion, as reflected in the intermediate blood glucose responses.

Maltodextrin had a greater ($P < 0.05$) insulin AUC and RIR value than for all G1-SCF (Table 5). The G1-CS-HCl, G1-SCF-HCl, and G1-SCF-SD all resulted in intermediate RIR responses, approximately 50% of that of Malt. The G1-SCF-hydrog resulted in the lowest ($P < 0.05$) insulin response among the SCF.

Except for G1-SCF-hydrog, the G1-SCF induced moderate peaks in insulin at the beginning of the response test before dropping to basal concentrations (Fig. 2). The peaks in insulin had similar pattern to that of the blood glucose, both peaking around 15 to 30 min after dosing. The G1-SCF-hydrog resulted in negligible insulin response, which is expected because the hydrogenation process leads to increased sorbitol, which does not elicit a glycemic or insulinemic response (de Godoy et al., 2013).

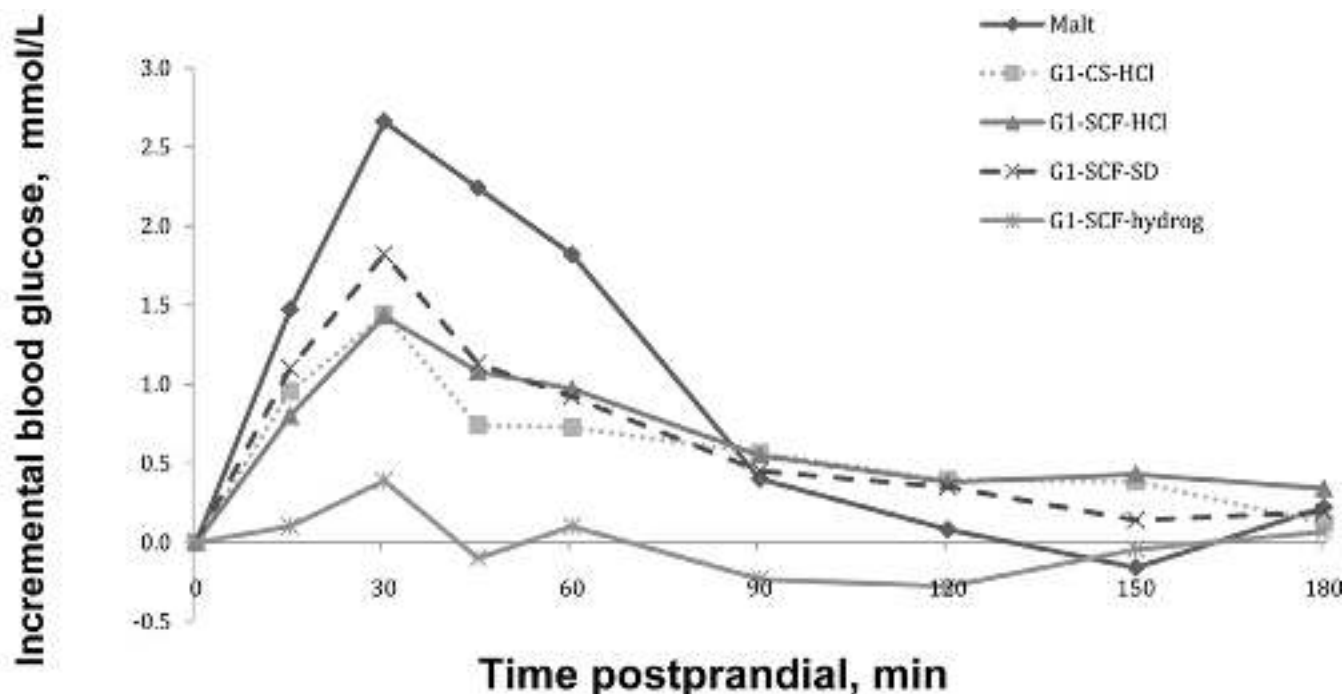


Figure 1. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble corn fibers (SCF): generation 1 (G1) series ($n = 5$). Malt = maltodextrin; G1-CS-HCl = product from hydrochloric acid-catalyzed condensation of corn syrup; G1-SCF-HCl = G1 soluble corn fiber using hydrochloric acid-catalyzed condensation; G1-SCF-SD = spray-dried version of G1-SDF-HCl; and G1-SCF-hydrog = hydrogenated version of G1-SCF-SD. Pooled SEM for carbohydrates were 0.14 (Mal), 0.32 (G1-CS-HCl), 0.34 (G1-SCF-HCl), 0.30 (G1-SCF-SD), and 0.27 (G1-SCF-hydrog).

Maltodextrin had the greatest ($P < 0.05$) glucose AUC when compared with all of the G2-SCF (Table 6). The G2-SCF-phos (Pw) resulted in the greatest glucose AUC ($P < 0.05$) among the SCF. All other G2-SCF had intermediate AUC values. The RGR data followed the same pattern, with Malt resulting in the greatest ($P < 0.05$) value and the SCF resulting in intermediate values (average RGR of 44%). Kendall et al. (2008) evaluated the glycemic response in humans of a SCF that was similar to G2-SCF-phos+HCl (Lq1) and incorporated into a beverage. Authors reported an AUC of 28.5 mmol/L, which was similar to the AUC (39.1 mmol/L) found in the current study. A RGR value of 58.5% was reported for a type III resistant starch (Brouns et al., 2007), which is similar to some of the SCF evaluated.

The majority of the G2-SCF resulted in similar blood glucose response patterns except for G2-SCF-phos (Pw) and G2-SCF-phos (Lq; Fig. 3). The SCF that had similar glycemic responses induced a blood glucose pattern of small blunted peaks during the first 60 min, then decreased to near basal concentrations for the remainder of the test. The greatest glycemic response among G2-SCF occurred for G2-SCF-phos (Pw), which had an increased peak in blood glucose concentration at 45 min, then a steady drop to baseline concentrations. This greater glycemic response corresponded to increased free sugar and released monosaccharide values for G2-SCF-phos (Pw). The G2-SCF-phos (Lq) also had increased free sugar concentrations and released monosaccharides from hydrolytic digestion, but produced the second lowest RGR values. The lower RGR

is due to the blood glucose pattern elicited by G2-SCF-phos (Lq). The later resulted in a peak of blood glucose at the beginning of the glycemic response test, but then decreased to below basal concentrations for the remainder of the test.

Area under the curve for insulin was greatest ($P < 0.05$) for Malt when compared with all of the G2-SCF (Table 6). These SCF had low-to-intermediate RIR values with an average RIR of 35.5%, which is lower than the average RIR of the G1-SCF (RIR = 50.0%, excluding G1-SCF-hydrog). Yamada et al. (2005) evaluated the glycemic and insulinemic responses of a resistant starch produced using heat and de-branching enzymes. Lowered insulin, but not glucose, responses were observed after consumption of bread containing 6 g of the resistant starch during a 2-h test (Yamada et al., 2005). Brouns et al. (2007) reported the insulinemic response of a type III resistant starch to have a RIR of 24.8%, which is similar to the average RIR for the G2-SCF (RIR = 35.5%).

The G2-SCF, except the phosphoric acid-catalyzed SCF, had similar serum insulin patterns where insulin peaked at 15 min of the test and decreased to basal concentrations (Fig. 4). The greater RIR values for G2-SCF-phos (Pw) and G2-SCF-phos (Lq) results from an increased insulin response during the first 60 min of the response test corresponding to an increased peak in blood glucose concentrations during this time frame. On average, SCF resulting from a hydrochloric acid catalyst had lower glycemic and insulinemic responses (RGR = 41%, and RIR = 28%) compared to SCF from a phosphoric acid catalyst

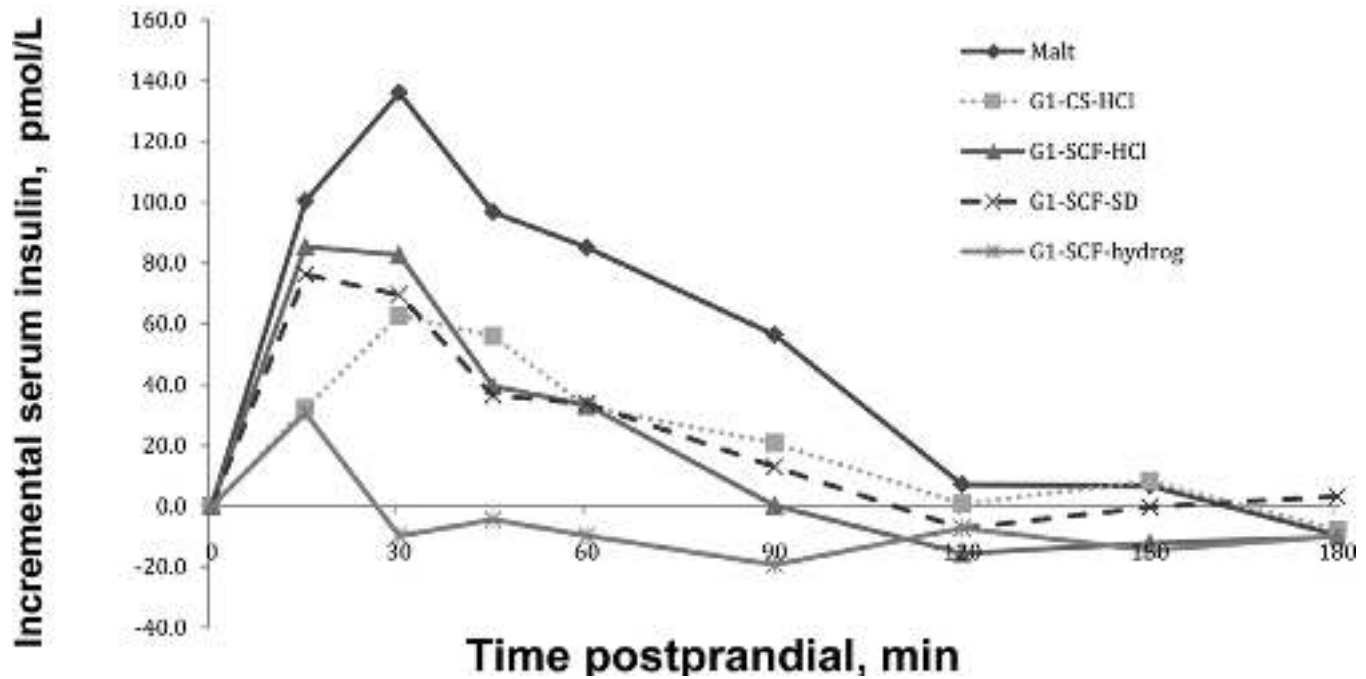


Figure 2. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble corn fibers: generation 1 (G1) series ($n = 5$). Malt = maltodextrin; G1-CS-HCl = product from hydrochloric acid-catalyzed condensation of corn syrup; G1-SCF-HCl = G1 soluble corn fiber using hydrochloric acid-catalyzed condensation; G1-SCF-SD = spray-dried version of G1-SDF-HCl; and G1-SCF-hydrog = hydrogenated version of G1-SCF-SD. Pooled SEM for carbohydrates were 13.36 (Malt), 25.38 (G1-CS-HCl), 25.38 (G1-SCF-HCl), 23.21 (G1-SCF-SD), and 25.38 (G1-SCF-hydrog).

(RGR = 51%, and RIR = 50%). Hydrochloric acid may form more digestion-resistant glycosidic bonds than phosphoric acid when used as a catalyst under these conditions.

All SCF from both generations induced glycemic and insulinemic responses in dogs. The G2-SCF generally resulted in lower responses than the G1-SCF did. The increased resistance to digestibility of these carbohydrates may have been due to increased glycosidic linkage formation resulting from different production methods. Reduced blood glucose and insulin responses have several beneficial health effects, especially in diabetic patients (American Diabetes Association, 2007; Livesey et al., 2008; Riccardi et al., 2008) with similar benefits presumed for dogs. Postprandial glucose and

insulin responses can be reduced by decreasing available carbohydrate intake, which can be achieved by replacing available carbohydrates with low-digestible carbohydrates (Wolever, 2003). Several of these SCF substrates may have utility in that regard. Additionally, all SCF tested were well tolerated by the dogs, with no gastrointestinal distress being observed.

True Metabolizable Energy (TME_n)

An increased demand for reduced calorie foodstuffs has stimulated interest in production of low-calorie sweeteners and bulking agents. A good method for evaluating the caloric content of ingredients is the TME_n assay with

Table 6. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble corn fibers: generation 2 (G2) series

Item	Test carbohydrate ¹									SEM ²
	Malt	G2-SCF-phos + HCl (Lq1)	G2-SCF-low sugar	G2-SCF-phos (Lq)	G2-SCF-phos (Pw)	G2-SCF-HCl (Lq)	G2-SCF-HCl (Pw)	G2-SCF-phos + HCl (Lq2)	G2-SCF-phos + HCl (Pw)	
AUC for glucose, mmol/L	157.25 ^d	39.10 ^a	75.32 ^b	59.25 ^{ab}	108.52 ^c	71.31 ^{ab}	71.49 ^{ab}	70.03 ^{ab}	57.42 ^{ab}	18.30
RGR, %	100.00 ^d	24.49 ^a	50.15 ^{bc}	33.33 ^{ab}	68.35 ^c	40.82 ^{ab}	40.22 ^{ab}	50.31 ^{bc}	47.86 ^{abc}	8.10
AUC for insulin, nmol/L	10.8 ^b	4.9 ^a	4.1 ^a	6.4 ^a	7.0 ^a	3.7 ^a	3.0 ^a	3.6 ^a	4.1 ^a	1.65
RIR, %	100.0 ^c	26.2 ^a	21.2 ^a	43.0 ^{ab}	56.5 ^b	31.0 ^{ab}	24.7 ^a	36.6 ^{ab}	44.5 ^{ab}	8.26

^{a-d}Means in the same row with different superscript letters are different ($P < 0.05$); $n = 5$.

¹Malt = maltodextrin; G2-SCF-phos + HCl (Lq1) = G2 soluble corn fiber batch 1 [liquid (Lq)]; G2-SCF-low sugar = low sugar version of G2 soluble corn fiber; G2-SCF-phos (Lq) = phosphoric acid-catalyzed condensation of corn syrup Lq; G2-SCF-phos (Pw) = phosphoric acid-catalyzed condensation of corn syrup powder (Pw); G2-SCF-HCl (Lq) = hydrochloric acid-catalyzed condensation of corn syrup Lq; G2-SCF-HCl (Pw) = hydrochloric acid-catalyzed condensation of corn syrup Pw; G2-SCF-phos + HCl (Lq2) = G2 soluble corn fiber batch 2 (Lq); and G2-SCF-phos + HCl = G2 soluble corn fiber batch 3 (Pw).

²Pooled standard error of the mean.

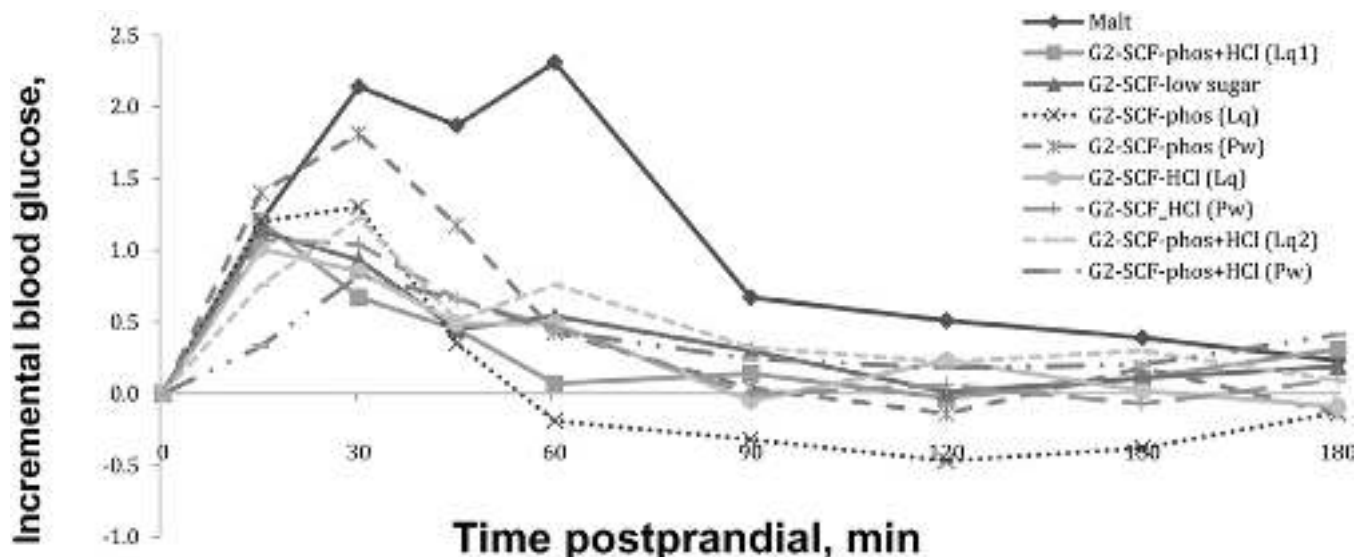


Figure 3. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble corn fibers: generation 2 (G2) series ($n = 5$). Malt = maltodextrin; G2-SCF-phos + HCl (Lq1) = G2 soluble corn fiber batch 1 [liquid (Lq)]; G2-SCF-low sugar = low sugar version of G2 soluble corn fiber; G2-SCF-phos (Lq) = phosphoric acid-catalyzed condensation of corn syrup Lq; G2-SCF-phos (Pw) = phosphoric acid-catalyzed condensation of corn syrup powder (Pw); G2-SCF-HCl (Lq) = hydrochloric acid-catalyzed condensation of corn syrup Lq; G2-SCF-HCl (Pw) = hydrochloric acid-catalyzed condensation of corn syrup (Pw); G2-SCF-phos + HCl (Lq2) = G2 soluble corn fiber batch 2 (Lq); and G2-SCF-phos + HCl = G2 soluble corn fiber batch 3 (Pw). Pooled SEM for carbohydrates were 0.19 (Malt), 0.28 [G2-SCF-phos+HCl (Lq1)], 0.24 (G2-SCF-low sugar), 0.33 [G2-SCF-phos (Lq)], 0.32 [G2-SCF-phos (Pw)], 0.30 [G2-SCF-HCl (Lq)], 0.32 [G2-SCF-HCl (Pw)], 0.29 [G2-SCF-phos+HCl (Lq2)], and 0.29 [SCF-phos+HCl (Pw)].

roosters. This in vivo animal model assay allows for exposure of test substrates to actual digestive processes.

All of the G1-SCF had a lower ($P < 0.05$) TME_n value compared to the Malt control (Table 7). Among the SCF, G1-SCF-hydrog had the greatest ($P < 0.05$) ME, G1-SCF-HCl and G1-SCF-SD had intermediate values, and G1-CS-HCl had the lowest ($P < 0.05$) TME_n value. The G1-SCF-hydrog had the greatest content (12%) of free sugars (sucrose, sorbitol, and fructose) readily available for digestion, which may have contributed to its greater energy value.

Maltodextrin had a greater ($P < 0.05$) TME_n value compared to all G2-SCF (Table 8). Lower and similar TME_n values were found for the majority of the G2-SCF, with G2-SCF-phos (Pw) being statistically greater than all except for G2-SCF-phos+HCl (Lq1). The greater TME_n value of G2-SCF-phos (Pw) supports the free sugar, released monosaccharide, and glycemic response data that show G2-SCF-phos (Pw) to be more digestible than the other SCF. A low-digestible carbohydrate produced using acid catalysts was evaluated by Sinaud et al. (2002) and resulted in a ME value of 3.37 kcal/g, which

is greater than the SCF evaluated here, indicating that these production methods for SCF were more successful in creating a less digestible ingredient.

The TME_n assay was useful for evaluating the ME content of novel carbohydrates alone, without the interferences from dietary matrix components. This is important information when developing food products. These substrates, especially the G2-SCF, had low caloric content, making them potential candidates for inclusion in low-calorie foods.

In summary, the SCF evaluated varied widely in their sugar composition. All SCF exhibited varying degrees of resistance to hydrolytic digestion and, consequently, had attenuated glycemic responses and lower caloric content than the Malt control. The G2-SCF series, on average, had lower digestibilities, glycemic responses, and TME_n values than the SCF from the G1-SCF series. The variation noted among carbohydrates in physiological responses was likely due to the individual carbohydrate molecular structure and bonding pattern and was greatly influenced by the production methods used. For example, the hydrogenation process of SCF in the G1-

Table 7. True ME (TME_n) values for soluble corn fibers: generation 1 (G1) series

Item	Test carbohydrate ¹					SEM ²
	Malt	G1-CS-HCl	G1-SCF-HCl	G1-SCF-SD	G1-SCF-hydrog	
Substrate dose, g DM	14.3	26.7	13.3	14.6	14.7	–
TME_n , kcal/g	4.1 ^d	1.9 ^a	2.4 ^b	2.3 ^b	3.0 ^c	0.10

^{a-d}Means in the same row with different superscript letters are different ($P < 0.05$); $n = 4$.

¹Malt = maltodextrin; G1-CS-HCl = product from hydrochloric acid-catalyzed condensation of corn syrup; G1-SCF-HCl = G1soluble corn fiber using hydrochloric acid-catalyzed condensation; G1-SCF-SD = spray-dried version of G1-SDF-HCl; and G1-SCF-hydrog = hydrogenated version of G1-SCF-SD.

²Pooled standard error of the mean.

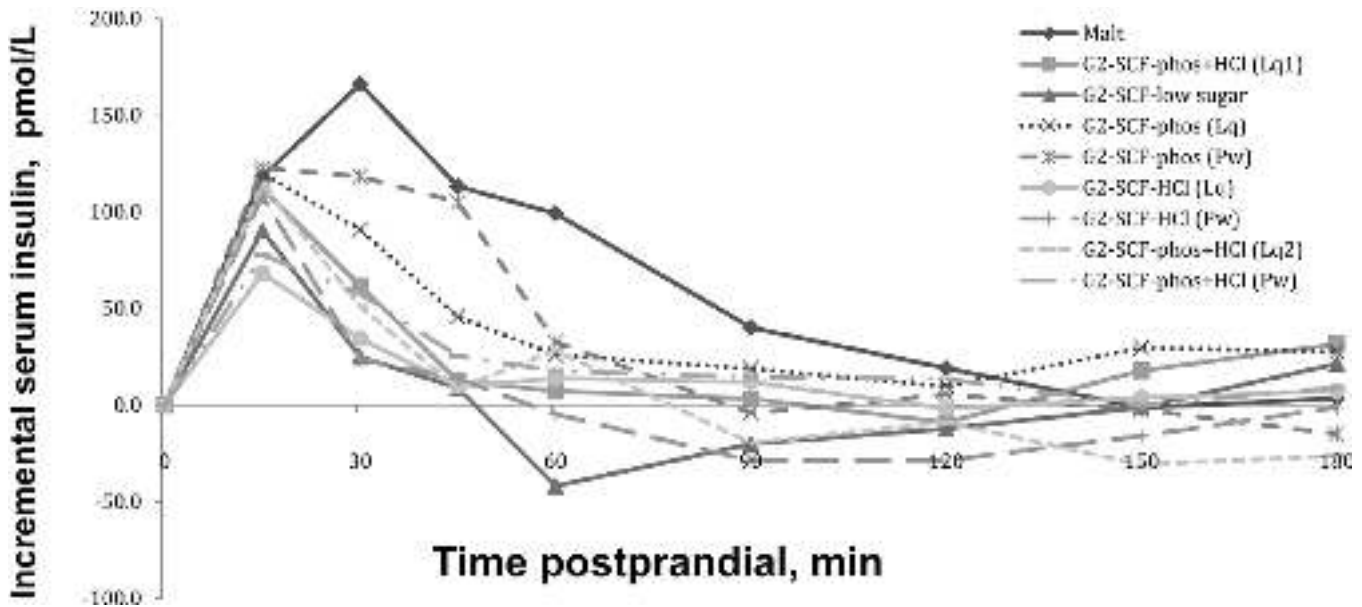


Figure 4. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble corn fibers: generation 2 (G2) series ($n = 5$). Malt = maltodextrin; G2-SCF-phos + HCl (Lq1) = G2 soluble corn fiber batch 1 [liquid (Lq)]; G2-SCF-low sugar = low sugar version of G2 soluble corn fiber; G2-SCF-phos (Lq) = phosphoric acid-catalyzed condensation of corn syrup Lq; G2-SCF-phos (Pw) = phosphoric acid-catalyzed condensation of corn syrup powder (Pw); G2-SCF-HCl (Lq) = hydrochloric acid-catalyzed condensation of corn syrup Lq; G2-SCF-HCl (Pw) = hydrochloric acid-catalyzed condensation of corn syrup (Pw); G2-SCF-phos + HCl (Lq2) = G2 soluble corn fiber batch 2 (Lq); and G2-SCF-phos + HCl = G2 soluble corn fiber batch 3 (Pw). Pooled SEM for carbohydrates were 13.36 (Malt), 23.21 [G2-SCF-phos+HCl (Lq1)], 23.21 (G2-SCF-low sugar), 23.21 [G2-SCF-phos (Lq)], 23.21 [G2-SCF-phos (Pw)], 23.21 [G2-SCF-HCl (Lq)], 23.21 [G2-SCF-HCl (Pw)], 23.21 [G2-SCF-phos+HCl (Lq2)], and 23.21 [SCF-phos+HCl (Pw)].

SCF series resulted in lower glycemic response because of an increased concentration of sorbitol, while the hydrochloric acid treatment used in the G2-SCF series was more effective than the phosphoric acid treatment in attenuating the glycemic response by decreasing the concentration of free sugars. Along with the beneficial characteristics discussed before, the animal must tolerate ingredients. In this study, dogs had no adverse effects to consumption of 25 g of SCF. Similarly, in humans, dietary supplementation of SCF similar to G2-SCF-phos+HCl (Lq1) was well-tolerated when supplemented at 12 g/d with low scores in bloating, cramping, flatulence, and stomach noises reported (Stewart et al., 2010). Another study also reported SCF to be well tolerated at doses of 5, 15, and 25 g (Sanders et al., 2008). Inclusion of SCF in a canine diet matrix at the 7% inclusion level was well tolerated by adult dogs, showing no detrimen-

tal effects on nutrient digestibility, food intake, and fecal characteristics (Guevara et al., 2008). The beneficial physiological characteristics observed would make SCF candidate ingredients for incorporation into reduced glycemic and caloric canine diets. Future research should investigate the effect of inclusion of these SCF in diet matrixes and the impact of further processing (e.g., extrusion, canning) in the ability of SCF to attenuate glycemic responses in adult dogs.

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Table 8. True ME (TME_n) values for soluble corn fibers: generation 2 (G2) series

Item	Test carbohydrate ¹									
	Malt	G2-SCF-phos + HCl (Lq1)	G2-SCF-low sugar	G2-SCF-phos (Lq)	G2-SCF-phos (Pw)	G2-SCF-HCl (Lq)	G2-SCF-HCl (Pw)	G2-SCF-phos + HCl (Lq2)	G2-SCF-phos + HCl (Pw)	SEM ²
Substrate dose, g DM	14.3	26.8	27.0	25.6	26.9	25.4	27.9	23.9	23.7	–
TME_n , kcal/g	4.1 ^d	2.0 ^{bc}	1.5 ^{ab}	1.8 ^b	2.3 ^c	1.8 ^b	1.8 ^b	1.9 ^b	1.3 ^a	0.10

^{a-d}Means in the same row with different superscript letters are different ($P < 0.05$); $n = 4$.

¹Malt = maltodextrin; G2-SCF-phos + HCl (Lq1) = G2 soluble corn fiber batch 1 [liquid (Lq)]; G2-SCF-low sugar = low sugar version of G2 soluble corn fiber; G2-SCF-phos (Lq) = phosphoric acid-catalyzed condensation of corn syrup Lq; G2-SCF-phos (Pw) = phosphoric acid-catalyzed condensation of corn syrup powder (Pw); G2-SCF-HCl (Lq) = hydrochloric acid-catalyzed condensation of corn syrup Lq; G2-SCF-HCl (Pw) = hydrochloric acid-catalyzed condensation of corn syrup Pw; G2-SCF-phos + HCl (Lq2) = G2 soluble corn fiber batch 2 (Lq); and G2-SCF-phos + HCl = G2 soluble corn fiber batch 3 (Pw).

²Pooled standard error of the mean.

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