

SELECT NOVEL CARBOHYDRATES AFFECT GLYCEMIC AND INSULINEMIC
RESPONSE, ENERGY VALUE, AND INDICES OF GUT HEALTH AS MEASURED
USING CANINE, AVIAN, RODENT, AND *IN VITRO* MODEL SYSTEMS

BY

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DISSERTATION

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ABSTRACT

The objective of this research was to evaluate select novel, low-digestible carbohydrates – pullulans (Pull), soluble fiber dextrins (SFD), and soluble corn fibers (SCF) - for properties that could positively impact health outcomes. Study 1 measured in vitro hydrolytic digestion characteristics, glycemic and insulinemic responses, and true metabolizable energy (TME_n) content of select SFDs and Pulls. 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 having a 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. Pullulans resulted in higher true metabolizable energy (TME_n) values than did SFDs. Study 2 measured in vitro hydrolytic digestion, glycemic and insulinemic responses, and TME_n content of SCFs (first and second generation products) produced using different methods. All SCFs had intermediate to low amounts of monosaccharides released as a result of in vitro hydrolytic digestion, with glucose being the primary sugar component released. Second generation SCFs, on average, had lower glycemic responses and TME_n values than did first generation SCFs. Study 3 measured in vitro hydrolytic digestion and glycemic and insulinemic responses of select carbohydrate blends, all containing SCF and blended with Pull, sorbitol (Sorb), and (or) fructose (Fruct). The addition of higher amounts of Fruct and Sorb increased the free Fruct and Sorb concentrations of the blends. All SCF blends resulted in low glycemic and insulinemic responses compared to the Malt control. Blends containing Fruct and Sorb were

most effective in attenuating the glycemic and insulinemic responses. Study 4 measured gastrointestinal health outcomes resulting from supplementation of SFD and SCF to rats. Rats were randomly assigned to one of four treatment groups (n=10) for 21 d. An AIN-93G diet with 5% cellulose served as the Control. The 5% cellulose was replaced with either 5% pectin (Pectin, positive control), SFD, or SCF. Consumption of SFD and SCF increased cecal weight but not colon weight. On a per cecum basis, SFD and SCF increased acetate, propionate, and total SCFA concentrations, with no effect on butyrate concentrations, compared to the Control diet. Cecal branched-chain fatty acids (BCFA) concentrations were decreased by SFD and SCF, whereas Pectin increased BCFA concentrations. Supplementation of SFD and SCF did not have an effect on cecal microbial populations compared to the Control diet. Pectin tended to decrease cecal *Escherichia coli* concentrations. Gut histomorphology was positively affected by SFD and SCF. Increased crypt depth, goblet cell numbers, and acidic mucin were observed in both the cecum and colon of rats supplemented with SFD, SCF, and Pectin. These novel, low-digestible carbohydrates appear to have the potential to beneficially impact health through decreased hydrolytic digestion, attenuated glycemic and insulinemic response, decreased energy value, SCFA production, and modulating gut morphology.

To my family, both in Iowa and in Honduras.

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CHAPTER 1: INTRODUCTION

As consumers are becoming more health conscious, the use of foods and ingredients that promote health is increasing. Dietary carbohydrates especially fibrous carbohydrates represent a complex group of food components that have a variety of beneficial physiological and nutritional properties (Crittenden and Playne, 1996; Cummings et al., 1997; Van Loo et al., 1999). There is increasing evidence that dietary fiber has beneficial influences on health; however, most of the population of the United States consumes less than half of the recommended concentration of dietary fiber daily (Anderson et al., 2009). This has led to a demand for the development of novel 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.

One class of carbohydrates, low-digestible carbohydrates, are becoming popular as food ingredients, not only due to their potential to improve both the physical and chemical properties of foods, but also due to the possible health benefits associated with their consumption (they act similarly to dietary fiber; Murphy, 2001). Low-digestible carbohydrates are low molecular weight carbohydrates that resist the hydrolytic activity of human digestive enzymes (Crittenden and Playne, 1996; Roberfroid and Slavin, 2000; Mussatto and Mancilha, 2007). Low-digestible carbohydrates include polyols, resistant starch, fructooligosaccharides, and other oligosaccharides (Marteau and Flourie, 2001).

Factors restricting digestion and absorption of low-digestible carbohydrates are the impaired hydrolysis of constitutive bonds by enzymes and the degree of absorption in the small intestine (Marteau and Flourie, 2001). Possessing a low hydrolytic digestibility makes

these oligosaccharides suitable for use in sweet, low-calorie diet foods, and for consumption by individuals with diabetes because of their ability to attenuate glycemic and insulinemic responses. The management of diabetes is becoming more important as diabetes is a growing health concern in the general population. There is also evidence that low-digestible carbohydrates may affect risk factors associated with diseases such as obesity, non-insulin dependent diabetes mellitus, hyperlipidemia, and hypertension, and cardiovascular disease although the underlying mechanisms are not well understood (Scheppach et al., 2001).

Low-digestible carbohydrates pass into the colon where they are substrates for fermentation by colonic microbiota. Fermentation yields short-chain fatty acids (SCFA) that provide colonic cells with energy and lower the pH of luminal contents. Van Loo et al. (1999) concluded that there was consistent evidence that non-digestible carbohydrates resulted in a fecal bulking effect and a normalization of stool frequency. The stool bulking effect is due to increases in microbial mass from the fermentation of the low-digestible carbohydrates. Low-digestible carbohydrates also have the potential to alter the composition of the colonic microflora, especially by stimulating growth of bifidobacteria and beneficially modulating intestinal morphology. Health benefits associated with bifidogenesis include displacement of detrimental bacteria, strengthening of gut barrier function, and prevention of pathogen invasion (Van Loo et al., 1999; Scheppach et al., 2001).

Low-digestible carbohydrates have effects that beneficially affect host health, so they may be considered as functional foods (defined as ingredients that affect physiological function (s) of the body in a targeted way so as to have a positive effect (s) that may, in due course, justify health claims; Roberfroid, 1996). The advantageous physicochemical and

physiological properties that low-digestible carbohydrates possess make them of interest to food manufacturers, health professionals, and consumers.

The major objectives of the research reported in this thesis were to evaluate select novel, low-digestible carbohydrates - pullulans, soluble fiber dextrins, and soluble corn fibers - for physiological outcomes that could positively impact health indices. Properties evaluated included hydrolytic digestion, glycemic and insulinemic responses, true metabolizable energy content, fermentation characteristics, modulation of microbiota populations, and gut morphological characteristics utilizing in vitro, canine, avian, and rodent models.

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CHAPTER 2: LITERATURE REVIEW

Characteristics of Low-Digestible Carbohydrates

In recent years, the demand for functional foods that contain novel ingredients and provide specific health benefits has increased (Murphy, 2001). The identification of dietary fiber as a promoter of healthy gut function and other health benefits has led to the search for other food components with similar properties (Cummings et al., 2004). Substrates having physiological properties such as reduced energy value, bifidogenic properties, laxation effects, fecal bulking properties, and reduced glycemic response are being sought (Murphy, 2001). This has led to an expansion in the demand for carbohydrates that have functional properties similar to dietary fiber.

Low-digestible carbohydrates represent a heterogeneous group of oligosaccharides that are partially unavailable to digestive enzymes so are either incompletely or not absorbed from the small intestine (Murphy, 2001; Scheppach et al., 2001; Grabitske and Slavin, 2008). These oligosaccharides are low molecular weight carbohydrates containing three to ten sugar moieties (Crittenden and Playne, 1996; Mussatto and Mancilha, 2007). The nondigestibility of these carbohydrates is due to the anomeric C atom of the monosaccharide units having a configuration that makes linkages unable to be hydrolyzed by digestive enzymes. The majority of low-digestible carbohydrates presently available as food ingredients are made from different types of monosaccharides in which the monosaccharide unit is fructose, galactose, glucose, and (or) xylose (Roberfroid and Slavin, 2000; Mussatto and Mancilha, 2007).

In general, food-grade oligosaccharides are not pure products, and differences in production methods can affect chain length, monosaccharide composition, degree of branching, and purity (Crittenden and Playne, 1996; Roberfroid and Slavin, 2000). Industrial production of low-digestible carbohydrates includes extraction of oligosaccharides from natural sources, hydrolysis of polysaccharides, and enzymatic or chemical synthesis from carbohydrate substrates. Enzymatic synthesis of low-digestible carbohydrates can occur through two basic processes. They can be produced from simple sugars, such as sucrose, where enzymatic transglycosylation reactions build an oligosaccharide, or they can be formed by controlled enzymatic hydrolysis of starch (Sako et al., 1999). Production of low-digestible carbohydrates using these processes usually results in a range of oligosaccharides differing in their degree of polymerization and position of glycosidic attachments. Unreacted substrates and monosaccharides also can be present after oligosaccharide formation. Purification methods such as membrane filtration or chromatographic procedures are applied to remove contaminating sugars so as to produce purer oligosaccharides. Low-digestible carbohydrates include polyols, resistant starch, fructooligosaccharides, and other oligosaccharides (Marteau and Flourie, 2001; Murphy, 2001; Scheppach et al., 2001; Grabitske and Slavin, 2008). One of the most widely used and studied low-digestible carbohydrates is inulin-type fructans composed of β -D-fructofuranoses attached by β -2,1 linkages.

Low-digestible carbohydrates possess important physicochemical properties that make them valuable ingredients for incorporation into foodstuffs. They are readily water-soluble and exhibit some sweetness, typically 0.3 – 0.6 times as sweet as sucrose (Roberfroid and Slavin, 2000; Mussatto and Mancilha, 2007). The sweetness depends on

the chemical structure, the degree of polymerization of the oligosaccharides present, and the concentrations of mono- and disaccharides in the mixture (Crittenden and Playne, 1996; Voragen, 1998; Mussatto and Mancilha, 2007). These products often are used as fat replacers and texture modifiers for processed foods. The relatively low sweetness makes low-digestible carbohydrates useful in food production when a bulking agent with reduced sweetness is desirable to enhance other food flavors. In the case of very sweet foods, they may be used as bulking agents in conjunction with artificial sweeteners in order to mask the aftertastes produced by some of the intense sweeteners such as aspartame (Mussatto and Mancilha, 2007).

Compared with mono- and disaccharides, the higher molecular weight of oligosaccharides provides increased viscosity that allows for improved body and mouth feel (Crittenden and Playne, 1996). Low-digestible carbohydrates also can be incorporated into food products to alter the freezing temperature of frozen foods, and to control the intensity of browning due to Maillard reactions in heat-processed foods (Mussatto and Mancilha, 2007). They also provide a high moisture-retaining capacity, prevent excessive drying, and result in a low water activity that helps control microbial contamination (Crittenden and Playne, 1996). The caloric value of low-digestible carbohydrates has been estimated to be 1.5 – 2.0 kcal/g, approximately 40 – 50% of the value for digestible carbohydrates such as sucrose (Sako et al., 1999).

Health Benefits of Low-Digestible Carbohydrates

Evidence for the possible physiological and health benefits associated with low-digestible carbohydrate consumption is substantial and has led to an increased

awareness among consumers (Murphy, 2001). Many benefits of low-digestible carbohydrate consumption exist but of major interest is their effect on energy content, glycemic response, and on fermentation and gastrointestinal health.

Modulation of Bone Health by Increasing Bioavailability of Minerals

There is increasing evidence that low-digestible carbohydrates improve the bioavailability of minerals. Several studies in rats have shown increased Ca, Mg, and Fe absorption following consumption of low-digestible carbohydrates, inulin-type fructans in particular (Younes et al., 1996). Evidence indicates that the increased absorption originates mainly at the level of the large intestine and effectively results in increased bone mineral density (Van Loo et al., 1999). Consumption of low-digestible carbohydrates to increase bone mineral density could have potential use for reducing the risk of osteoporosis.

Modulation of Lipid Metabolism

Supplementation of low-digestible fructans has been reported to lower serum triacylglycerols in rats (Fiordaliso et al., 1995; Delzenne et al., 2002). A review of human studies by Van Loo et al. (1999) found inconsistent results from the supplementation of low-digestible fructans, but indicate that the intake of moderate concentrations of inulin or oligofructose may positively affect lipid metabolism. The hypotriglyceridemia is due mostly to a decrease in the concentration of plasma very-low density lipoproteins (Fiordaliso et al., 1995; Roberfroid and Slavin, 2000). The hypotriacylglycerolemic effect of low-digestible carbohydrate consumption is due to a decrease in hepatic triacylglycerol synthesis (Roberfroid and Delzenne, 1996). This results mainly from a lower rate of lipogenesis that results from a down-regulation of fatty acid synthase, a key hepatic lipogenic enzyme (Kok

et al., 1996; Roberfroid and Delzenne, 1996). Low-digestible carbohydrates may possibly modulate lipid metabolism by increasing the gut peptides, glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (Delzenne et al., 2002). These peptides influence lipid metabolism by promoting insulin-induced glucose uptake and de novo lipogenesis in adipose tissue (Delzenne et al., 2002).

Anti-Carcinogenic Properties

A reduced risk of experimentally induced carcinogenesis has been observed in rats fed low-digestible carbohydrate diets. This anti-carcinogenic effect appears to be related to a reduction in the production of carcinogenic substances by decreasing the amount of pathogenic bacteria in the colon and by increasing cellular immunity (Tungland and Meyer, 2002; Mussatto and Mancilha, 2007). In studies where low-digestible fructans were supplemented and a carcinogenic compound, azoxymethane, administered, a reduction in the numbers of colonic aberrant crypt foci was observed (Rowland et al., 1998). Aberrant crypt foci are early indicators of future risk of tumor development. A comparable effect is induced upon administration of bifidobacteria. Combining a low-digestible carbohydrate simultaneously with bifidobacteria was shown to have a synergistic effect (Gallaher et al., 1996; Rowland et al., 1998).

Increasing butyrate production can potentially contribute to the anti-carcinogenic properties of low-digestible carbohydrates. There is growing evidence that butyric acid has a protective role in the development of colorectal cancer (Murphy, 2001). Butyrate has been shown to arrest cell division through its ability to regulate gene expression (Siavoshian et al., 2000). Growth of normal cells and promotion of DNA repair in damaged cells has

been stimulated by butyrate (Topping et al., 2008). Induced apoptosis in colorectal tumor cell lines and stimulation of immunogenicity in cancer cells has been shown with increased butyrate (Wong et al., 2006). Low-digestible carbohydrates also can decrease ammonia, p-cresol, and indole concentrations. There are fecal metabolites considered as risk factors for colon cancer (Mussatto and Mancilha, 2007).

Glycemic Response and Glycemic Index (GI)

Glycemic response is an indication of how rapidly carbohydrates are digested. The extent to which bioavailable carbohydrates increase postprandial blood glucose is influenced by the rate and extent of carbohydrate digestion and absorption in the gastrointestinal tract (Wolever, 2006). Carbohydrates in the diet include a wide range of different compounds including monosaccharides, disaccharides, oligosaccharides, and polysaccharides that elicit different glycemic responses. Different dietary carbohydrates may influence blood glucose by the nature of the monosaccharide absorbed, the amount of carbohydrate absorbed, and the rate of absorption (Wolever, 2003).

In the small intestine, only monosaccharides can be absorbed; thus, disaccharides, oligosaccharides, and polysaccharides must be hydrolyzed to their monosaccharide units. The majority of the carbohydrate in the diet enters the bloodstream as glucose, which is transported through the body to the tissues (Lunn and Buttriss, 2007). Once in the tissues, glucose has several fates: it can be used for energy, stored as glycogen in the liver or muscles, or converted to fat (Lunn and Buttriss, 2007). The fate of the glucose circulating in the bloodstream is determined by the relative concentrations of insulin. Insulin is released from the β -cells of the pancreas in response to glucose absorption, and triggers glucose uptake into the muscle and liver cells where it is either utilized for energy or stored

as glycogen (Lunn and Buttriss, 2007). The amount of glycogen that the body can store is limited so excess glucose will be converted to fat. Insulin suppresses the metabolic pathways in the liver that synthesize glucose, so provided the body is sensitive to insulin, blood glucose concentrations will fall. 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).

The GI concept is, in many ways, an extension of the dietary fiber hypothesis of Burkitt and Trowell who suggested that dietary fiber may have metabolic benefits related to diabetes, reduction of coronary heart disease risk, and colon cancer (Jenkins et al., 2002; Wolever, 2006). Interest in the GI concept is becoming increasingly widespread as evidence is gathered showing it to be relevant in many areas of human health and performance (Wolever, 2006). A ranking system for carbohydrates, GI was first introduced by Jenkins et al. (1981). The GI is a quantitative assessment of foods based on postprandial blood glucose response expressed as a percentage of the response to an equivalent carbohydrate portion of a reference food (Augustin et al., 2002). The glycemic index is defined as the incremental area under the blood glucose response curve of a 50 g available carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject (Wolever, 2003; Venn and Green, 2007). A common GI classification system for foods is categorizing GI values as low (<55), medium (55-69), or high (>70) (Venn and Green, 2007).

Factors Involved in Determining Glycemic Index

Several factors must be considered in determining the GI of a carbohydrate: amount of carbohydrate tested, method of blood sampling and glucose measurement, and characteristics of the subjects being studied. All can contribute to variation in determining the GI of a test ingredient (Granfeldt et al., 1995; Wolever, 2003; Brouns et al., 2005; Wolever, 2006).

Calculation of Area Under the Curve (AUC)

The AUC is estimated based on measures of blood glucose concentration obtained at various points in time after carbohydrate is ingested. Glucose is usually measured at 15 to 30 minute intervals over 2 to 3 hours after intake of the carbohydrate being tested. Area under the curve can be calculated in several different ways that, depending on how calculated, can lead to major differences in results and interpretation of results of the same data (Wolever, 2003). One of the major considerations in determining AUC is determination of which area to be included in the AUC. The GI is based on the incremental area under the curve (IAUC), defined as the area beneath the curve above the fasting level only (Wolever, 2006). Area beneath the fasting level is ignored. Incremental area under the curve is used because it indicates the amount that the carbohydrate raises blood glucose above the fasting concentration (Wolever, 2006).

Other common methods of calculating the AUC include AUCcut and total AUC (TAUC). AUCcut is calculated in the same way as IAUC, but includes area before the blood glucose concentration drops below the baseline fasting concentration (Wolever, 2006). Total area under the curve is the area under the curve when the blood glucose

concentration is zero and measures the average blood glucose concentration during the period of the test (Wolever, 2003). Total area under the curve for blood glucose will result in a value much greater than that for IAUC because TAUC is determined based on the concentration of fasting glucose (Wolever, 2006).

Amount of Carbohydrate Tested

Given that blood glucose responses differ depending on the amount of carbohydrate consumed, the portion size of food used to determine the GI is critical (Wolever, 2003). The GI is intended to be an index of “blood glucose raising potential” of available or absorbable carbohydrates in food. Only carbohydrate sources assumed to be fully digestible, absorbable, and glycemic are included in the calculation of dosed carbohydrates in the 50 g portion size in the classical GI concept (Brouns et al., 2005). This is difficult in practice since available carbohydrate is difficult to measure accurately and the definitions and methods used to measure dietary carbohydrates vary in different parts of the world (Wolever, 2003).

If a portion size less than 50 g is to be used, then the amount of carbohydrate in the reference food needs to be reduced so that both are supplying equal amounts of available carbohydrate. Test foods were fed at concentrations of 25, 50, and 100 g and the glycemic responses of the foods relative to that of control bread containing the same amount of carbohydrate did not differ significantly (Wolever and Bolognesi, 1996). This suggests that the relative responses of foods are the same at different concentrations of available carbohydrate intake. However, a larger dose of carbohydrate might be preferred as this

study also found that variability of the results increased as the portion size became smaller (Wolever and Bolognesi, 1996).

Method of Blood Sampling and Glucose Measurement

Blood obtained from a vein, artery, or capillary bed can be used to determine glucose concentration. Glucose concentrations also can be measured using whole blood or serum or plasma. The concentration of glucose in venous plasma is less than that in arterial plasma because the glucose is taken up by tissues from the blood as it passes from the arteries (Wolever, 2006). Capillary blood glucose concentrations have been found to be greater than venous when measuring GI of various products so that the AUC was 33-40% lower for venous blood glucose (Granfeldt et al., 1995). The measurements of GI were not affected since both the test and control measurements ended up being affected (Granfeldt et al., 1995). An inter-laboratory study examined the difference in glucose responses in venous plasma versus capillary blood. Measuring glucose responses in venous plasma resulted in greater within-subject variation in GI values than measuring glucose in capillary blood (Wolever et al., 2003), the coefficient of variation of capillary measurements being 23.4 +/- 2.1% compared to venous measurements with a coefficient of variation of 56.8% +/- 4.4% (Wolever et al., 2003). These findings suggest that capillary blood sampling may be the preferred method of determining blood glucose concentrations for GI.

Subject Characteristics

Glycemic index values have been found not to be significantly affected by subject variables such as age, sex, ethnicity, glucose tolerance status, or presence of type 1 or type 2 diabetes, making GI values from these groups comparable to each other (Wolever, 2003).

However, the within-subject variation of glycemic responses is significant and accounts for most of the variation in GI values (Wolever, 2006).

Health Aspects

The GI was originally designed as a food selection guide for people with diabetes (Jenkins et al., 2002). However, GI and glycemic response of carbohydrates have more recently been widely recommended as factors important in chronic diseases including diabetes, obesity, cancer, and heart disease and in the treatment of cardiovascular risk factors, especially dyslipidemia (Jenkins et al., 2002; Wolever, 2003; Han and BeMiller, 2007; Venn and Green, 2007). High blood glucose concentrations create oxidative stress and affect cellular function, lipid oxidation, protein glycosylation, clotting tendency, and inflammatory processes (Brand-Miller, 2007).

A popular application of GI is for body weight management. A low-GI diet is thought to promote weight loss through reduced food intake, reduced fat storage, and increased fat oxidation (Wolever, 2006). Low-GI diets may aid in weight management by increasing food residence time in the gut and may influence the response of such satiety hormones as gastric inhibitory peptide, glucagon-like peptide, and cholecystokinin (Brand-Miller, 2007; Slavin et al., 2009). High-glycemic diets may result in hormonal changes that limit availability of metabolic fuels in the postprandial state and stimulate increased voluntary food intake (Raatz et al., 2005).

Foods with a low-GI or glycemic response have been shown to be most beneficial in the management of diabetes. Epidemiological studies suggest that reduced postprandial glucose peaks, reduced episodes of hypoglycemia, and greater insulin sensitivity are

beneficial for diabetes management (Lehmann and Robin, 2007). Several studies have shown that calorie for calorie, high-GI meals stimulate more insulin secretion than low-GI meals because of relative postprandial hyperglycemia (Ludwig, 2002). High-GI diets also may impair β -cell function through the direct effect of elevated blood glucose and free fatty acid concentrations that high-GI diets are known to cause (Ludwig, 2002).

Energy Content

Evaluation of Energy Content

The increased interest in low-calorie foods has increased the use of low-digestible carbohydrates in foodstuffs and, thus, increased the importance of determining the energy value of these carbohydrates. Particular needs for information in this area are from manufacturers attempting to design foods of lower caloric value and from governing bodies who assign energy values for the purpose of food labeling (Livesey, 1990; Livesey et al., 2000). The use of correct energy values for low-digestible carbohydrates is also essential for consumers.

When evaluating the energy of a diet where the energy value of ingredients is not known, indirect calculations are used to estimate the energy contribution of the individual components. There are several commonly used equations for energy assessment for predicting metabolizable energy such as the Atwater system and British system (Livesey, 1990). The equations include the intakes of protein, fat, and carbohydrate using caloric conversion factors; however, these equations often either overpredict or underpredict the available energy from low-digestible carbohydrates depending on the conversion factor used in the equation (Livesey, 1990). In the past, low-digestible carbohydrates often were

assigned an energy value of 0 kcal/g, but research has indicated that a more appropriate value to be used in calculations would be 2 kcal/g (Livesey, 1990; Livesey et al., 2000). The use of energy conversion factors in determining the caloric value of novel low-digestible carbohydrates may not accurately reflect their energy value and can be misleading.

More accurate energy evaluation is conducted by examining the digestible energy (DE) and metabolizable energy (ME) of diets and individual ingredients. Digestible energy and ME values are used extensively in energy evaluation of ingredients and complete diets. The direct method for determining DE involves measuring the intake of ingredient (s) of a known gross energy (GE) content and measurement of energy in the feces (Livesey et al., 2000). Metabolizable energy is measured similarly to DE, but also takes into account losses of energy in urine. Metabolizable energy can be expressed as either apparent (AME) or true (TME) metabolizable energy, the difference being that TME takes into account endogenous fecal and urinary energy losses (Sibbald, 1976; Miller and Reinecke, 1984). A more advanced system for energy evaluation is net energy (NE). Net energy was developed to provide more accurate estimates of energy that is available to the host for use in maintenance and production (i.e., growth, gestation, lactation, etc.). The main difference between the NE system and the DE and ME systems is that the NE system considers the amount of heat lost during digestion and subsequent deposition of nutrients in protein and adipose tissue (Livesey et al., 2000).

Roosters can be utilized for ME assays to evaluate the energy content of individual ingredients. The rooster can be used to simulate the conditions in the digestive tract of humans for determining the energy content of foodstuffs. Use of roosters allows for a better representation of the digestive process than does *in vitro* assays for determining

metabolizable energy. Also, rooster assays allow for shorter, easier, and more accurate collection of data than using human subjects where total collection of feces and urine can be difficult.

Low-Digestible Carbohydrates as Low-Calorie Ingredients

A major health concern facing today's human and pet animal population is obesity and its related metabolic diseases. This has led to consumer demand for low-calorie ingredients and reduced-calorie versions of many foods without compromise in taste (Stowell, 2006). In response to consumer demand, development and production of new low-calorie food ingredients and additives has increased. The three major categories of low-calorie ingredients include fat substitutes, high-intensity sweeteners, and low-calorie bulking agents.

Low-calorie bulking ingredients are defined as natural or novel food ingredients that provide foods and drinks with such organoleptic properties as body, texture, flavor, mouth-feel, and taste (Annison et al., 1994). Often, low-calorie bulking ingredients are dietary fibers (e.g., pectins, gums, and lignins; Annison et al., 1994; Auerbach et al., 2006). These materials come from a variety of sources and often are complex mixtures of carbohydrates that are chemically not well defined and have limitations as sugar replacements (Annison et al., 1994; Auerbach et al., 2006). This emphasizes the need for novel, high-quality, bulking agents that allow for a greater flexibility when replacing sugar in formulations and that complement the use of high-intensity sweeteners.

Metabolic effects of carbohydrates are dependent on their rate of absorption from the small intestine. The rate of absorption can be reduced by a number of factors including

the molecular structure of the carbohydrate and other inherent properties such as viscosity, gel-forming properties, and water-holding capability (Annison et al., 1994). Diets containing low-digestible carbohydrates will have lower energy content due to their decreased rate of small intestinal carbohydrate absorption. Non- and low-digestible polysaccharides and oligosaccharides are good candidates for use as food ingredients in low-calorie products because they resist digestion in the small intestine and may be partially or totally fermented in the colon. As a result, they have lower caloric content of approximately 1 to 3 kcal/g for low-digestible carbohydrates compared with 4 kcal/g for completely digestible carbohydrates (Sako et al., 1999; Roberfroid and Slavin, 2000; Grabitske and Slavin, 2008).

Gastrointestinal Health and Fermentation

While the physiological effects of fiber depend on numerous variables, the majority of the beneficial effects originate from its fermentative action in the colon. The gastrointestinal tract is heavily populated with bacteria, with the large bowel being the most heavily colonized region. The bacteria are mainly strictly anaerobic. This microbial community is complex in terms of numbers of organisms and their diversity. There are approximately 10^{13} anaerobic bacteria for every gram of intestinal content (Tungland and Meyer, 2002; Mussatto and Mancilha, 2007). Through the use of sequencing of the highly conserved 16S region of ribosomal RNA, it is estimated that there are approximately 800 – 1,000 different bacterial species with over 7,000 strains (O’Keefe, 2008; Qin et al., 2010). Through the process of fermentation, colonic bacteria produce a wide variety of compounds that may affect the gut as well as systemic physiology of the host (Roberfroid and Slavin, 2000). The rate of carbohydrate fermentation is dependent upon factors including degree of

polymerization, glycosidic linkage and degree of branching, bacteria present, and saccharolytic capacity (Voragen, 1998). Due to their chemical structure, low-digestible carbohydrates are substrates that can be fermented only by a limited number of bacteria. Among the groups of bacteria present in the gastrointestinal tract, the bifidobacteria and lactobacilli are those that best utilize oligosaccharides. These microorganisms appear to be beneficial to host health (Bielecka et al., 2002; Mussatto and Mancilha, 2007).

Short-Chain Fatty Acids

Low-digestible carbohydrates are substrates for fermentation by anaerobic colonic microflora since they are partially resistant to hydrolysis and digestion in the stomach and small intestine and eventually enter the colon for fermentation (Wong et al., 2006). Fermentation yields ME for microbial growth and maintenance, and metabolic end-products for use by the host (Wong et al., 2006). The major end-products of fermentation are SCFA, carbon dioxide, methane, hydrogen, and heat (Wong et al., 2006). Short-chain fatty acids are mainly found in the proximal colon where fermentation is most active, and the amount produced is reflective of the supply of carbohydrate in the diet (Nugent, 2005). The major SCFA produced by fermentation are acetate, propionate, and butyrate, although other SCFA such as formate, valerate, and caproate are produced in lesser amounts (MacFarlane and MacFarlane, 2003). Much of the hydrogen produced is converted to methane by bacteria, and both hydrogen and methane are excreted in breath and flatus (Elia and Cummings, 2007). Lactate also is produced from fermentation of oligosaccharides. Both D- and L-lactate are produced, and both are absorbed.

The generation of SCFA from fermentation has several effects on colonic function and health. Short-chain fatty acids are rapidly absorbed and provide colonic cells with

substrate for energy production, with butyrate being the preferred energy source for the colonic epithelial cells (Wong et al., 2006; Elia and Cummings, 2007). Propionate passes to the liver where it is taken up and metabolized aerobically to glucose (Topping and Clifton, 2001; Elia and Cummings, 2007). Acetate is the major SCFA produced in all types of fermentation. The molar ratio of acetate:propionate:butyrate is approximately 60:20:20 (Elia and Cummings, 2007). Acetate is rapidly absorbed, stimulating sodium absorption, and passes to the liver and into the peripheral tissues to be oxidized by skeletal and cardiac muscles as an energy source (Topping and Clifton, 2001).

Along with providing fuels for maintaining normal colonic function, SCFA can regulate colonocyte gene expression, induce arrest of cell division and apoptosis, and can exert trophic effects on colonic epithelium (Mentschel and Claus, 2003). It has been reported that butyrate can inhibit inflammation responses by down-regulating the activity of the transcription factor nuclear factor kappa β (NF- κ β) (Segain et al., 2000). Nuclear factor kappa β is a major regulator of inflammation and many immune responses. The ability of butyrate to affect NF- κ β expression and activity may have beneficial effects on colonic function, particularly in inflammatory bowel disease (Nugent, 2005).

Low-digestible carbohydrates increase SCFA production that leads to a lower colonic pH and potential modification of the metabolism of bile acids. A decrease in colonic pH might reduce the risk of developing colonic cancer because an inverse correlation between stool pH and colon cancer risk has been observed (Bouhnik et al., 2004). A lowered pH also inhibits the transformation of primary to secondary bile acids. Secondary bile acids are cytotoxic to colonic cells and may promote the formation of tumors (Young and Le Leu, 2004). Lower pH is thought to control the overgrowth of potentially

pathogenic bacteria, limit absorption of potentially cytotoxic agents, and lower the risk of infectious diarrhea (Topping et al., 2008).

Microbiota Modulation

Microbiota are present throughout the gastrointestinal tract, but the majority are located in the large intestine and consist of a wide variety of bacterial genera, species, and strains that are thought to be either beneficial (e.g., bifidobacteria and lactobacilli) or detrimental (e.g., *Clostridium* spp.) to host health (Bouhnik et al., 2004). Diet can have an impact on many microbiota-associated activities and, thus, may impact host health.

Microbial populations present in the gut are able to produce a range of compounds through the process of fermentation. These compounds can have both positive and negative effects on gut physiology. Competition for nutrients, production of antimicrobial compounds, lowering of intestinal pH, and stimulation of the immune system all may play a role in providing an efficient barrier for invading pathogens and all are affected by the microbial population present (Macfarlane, 2008; Adam-Perrot, 2009). Evidence is emerging supporting the concept that the maintenance of a healthy gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel disease, and colon cancer (Puupponen-Pimia et al., 2002).

Bifidobacteria are thought to stimulate the immune system, produce B vitamins, inhibit pathogen growth, reduce blood ammonia and blood cholesterol concentrations, and help restore the normal flora after antibiotic therapy (Gibson and Roberfroid, 1995; Wang, 2009).

Lactobacilli may aid digestion of lactose in lactose-intolerant individuals, reduce constipation and infantile diarrhea, help resist infections such as salmonella, and help to relieve irritable bowel syndrome (Manning and Gibson, 2004; Wang, 2009).

Selectively fermented ingredients that allow specific changes, both in the composition and (or) activity of the gastrointestinal microbiota that confer benefits upon host health, are termed prebiotics (Gibson et al., 2004). They provide benefits by producing SCFA that lower the pH of the contents of the large intestine. A reduced pH is beneficial by creating an environment that stimulates the growth of certain bacteria like bifidobacteria and lactobacilli and by attenuating growth of harmful bacteria such as *Clostridium perfringens* (Topping and Clifton, 2001).

The genera, bifidobacteria and lactobacilli, are considered beneficial because they do not contain any strains known to be pathogenic, and they are primarily carbohydrate-fermenting bacteria, unlike other groups such as bacteroides and clostridia that are also proteolytic and amino acid-fermenting (Guarner, 2007). The products of protein breakdown and amino acid fermentation, which include ammonia, phenols, indoles, thiols, amines, and sulfides, are not beneficial to gut and host health, and actually can be detrimental.

Bifidobacteria and lactobacilli also play a significant role in the maintenance of colonization resistance and have potential health benefits in strengthening the gut barrier against infection (Gibson et al, 2005). Viruses, protozoa, fungi, and bacterial can all cause acute gastroenteritis. The gut microbiota produce SCFA that lower gut pH to levels below those at which such pathogens can effectively compete. Also, many lactobacilli and bifidobacterial species are able to excrete natural antibiotics (Manning and Gibson, 2004). Prebiotic bacteria may be able to stimulate both non-specific host defense mechanisms and specific immune responses. The result often is increased phagocytic activity and (or)

elevated concentrations of immunological molecules such as secretory IgA, which may negatively affect pathogens such as salmonella and rotovirus (Manning and Gibson, 2004).

The effective bifidogenic doses vary among the different low-digestible carbohydrate types. Many studies have been performed using daily doses ranging from 3–15 g of the low-digestible carbohydrates for 1-3 weeks, and most oligosaccharides have been demonstrated to increase bifidobacteria numbers in the colon at these doses (Crittenden and Playne, 1996; Roberfroid and Slavin, 2000). Inulin-type fructans have been extensively studied, and their bifidogenic effect has been shown at doses as low as 4 g/day, but there is some consensus that larger doses, preferentially 8-15 g/day, would be needed to increase bifidobacteria and lactobacilli cell numbers in the colon (Bouhnik et al., 2004; Manning and Gibson, 2004; Roberfroid, 2005). Bifidogenic effects noted at lower doses of inulin-type fructans have been attributed to the host baseline bifidobacteria level being low (Mussatto and Mancilha, 2007).

Gut Morphology

Dietary fiber ingestion is associated with changes in the structure of the gastrointestinal tract. Changes include alterations in length and weight and modifications in the morphology of the mucosa such as higher villi, deeper crypts, increased number of goblet cells, and a thicker mucus layer on the colonic epithelium (Sigelo et al., 1984; Kim, 2002). These modifications may impact barrier function and elements of immune function of the large intestine since it encompasses a significant portion of the human immune system (Tungland and Meyer, 2002). The fermentation of low-digestible carbohydrates may be important in modulating the barrier function by increasing the number of bifidobacteria and lactobacilli in the epithelial mucosa of the colon (Guarner, 2007).

Ingestion of inulin-type fructans and other low-digestible carbohydrates have resulted in increased cecum weights when supplemented in diets of rats (Levrat et al., 1991; Campbell et al., 1997; Lu et al., 2000; Kim, 2002). Length of the small intestine also can be increased through dietary supplementation of low-digestible carbohydrates (Chun et al., 1989). Low-digestible carbohydrates also have been reported to increase villus height throughout the small intestinal tract of rats and piglets (Chun et al., 1989; Kim, 2002; Xu et al., 2002; Van Nevel et al., 2003; Rossi et al., 2008). Along with increased villus height, another beneficial morphological effect of low-digestible carbohydrate supplementation is increased crypt depth (Jin et al., 1994; Kleessen et al., 2003). The crypts contain intestinal stem cells, the principal site of cell proliferation in the intestinal mucosa, and increased depth may be associated with increased rate of turnover of intestinal mucosal cells.

Low-digestible carbohydrates can affect the mucosal architecture by affecting goblet cell numbers and mucin types secreted. Mucins are high molecular mass glycoproteins that are synthesized and secreted by the goblet cells. They form a gel-like layer on the mucosal surface and provide ideal conditions for the colonization of residential microbiota (Forstner, 1978; Kleessen et al., 2003). Mucins are assumed to provide lubrication and protection of the underlying epithelium against potentially injurious chemicals, enzymes, bacteria, and dietary constituents. The amount and composition of the mucus layer reflects an equilibrium between mucus secretion on the one hand and its erosion and degradation by bacteria on the other (Deplancke and Gaskins, 2001).

Mucins are classified into neutral and acidic subtypes. Acidic mucins are further classified as sulfated (sulfomucins) or non-sulfated (sialomucins) groups. It is suggested that acidic mucins protect against bacterial translocation because sulfated mucins in

particular appear to be less degradable by bacterial glycosidases and host proteases (Deplancke and Gaskins, 2001). Increases in goblet cell numbers have been reported in rats fed dietary fiber, including inulin-type fructans (Satchithanandam et al., 1990; Meslin et al., 1993; Fontaine et al., 1996; Schmidt-Wittig et al., 1996; Kim, 2002). Rat studies with diets supplemented with inulin-type fructans have been shown to modulate mucins in the intestinal tract by increasing acidic mucins, especially the protective sulfomucins (Fontaine et al., 1996; Delzenne, 2003; Kleessen et al., 2003). Alterations in the mucosal architecture and amounts of sulfomucins and sialomucins could have important effects on the gut mucosal barrier and health maintenance of the gut.

Health Detriments of Low-Digestible Carbohydrates

Low-digestible carbohydrates offer many potential benefits, but these benefits can be offset by tolerance issues when consumed. Low-digestible carbohydrates are generally well tolerated but since they may increase the rate of fermentation in the colon, they may result in abdominal pain, excessive flatus, and diarrhea (Livesey, 2001; Marteau and Flourie, 2001; Grabitske and Salvin, 2008). Due to differences in degree of absorption and fermentation, and to differences in osmotic effects, there is variation in tolerance of low-digestible carbohydrates. Also, there is inter-individual variability in tolerance, perhaps due to differences in absorption capacities, motility patterns, and colonic responses among individuals (Marteau and Flourie, 2001).

Undesirable outcomes often are caused by altered osmotic responses that unabsorbed, low-digestible carbohydrates exert in the intestinal lumen. This osmotic effect increases water flow rate and may induce borborygmi, abdominal pain, and diarrhea (Marteau and Flourie, 2001). Diarrhea resulting from low-digestible carbohydrate

consumption may be associated with the fermentation process. The rapid fermentation of low-digestible carbohydrates can yield lactate and SCFA at a faster rate than can be absorbed, thus temporarily inducing diarrhea (Livesey, 2001). When low-digestible carbohydrates are ingested, they also may lead to an elevated water load entering the colon. This occurs because additional water may be drawn into the small intestine to maintain osmolality (Livesey, 2001).

When low-digestible carbohydrates enter the colon, they are subjected to anaerobic fermentation that leads to gaseous end-products, carbon dioxide, hydrogen, and methane (Livesey, 2001). High rates of gas production may cause discomfort and pain in some people. It is not clear whether individuals can adapt to the higher rates of gas production that low-digestible carbohydrates cause (Livesey, 2001).

Besides tolerance issues, there is some research that inulin-type fructans can negatively affect the epithelial barrier in the gut by increasing intestinal permeability. Ten Bruggencate et al. (2005) found that inulin supplementation of rats on a low-calcium diet increased translocation of salmonella in infection studies. Also, inulin supplementation was associated with increased fecal water cytotoxicity, increased excretion of nitrates, and increased excretion of mucins after the rats were inoculated with salmonella (Ten Bruggencate et al., 2003). Supplementing inulin to humans showed signs of mucosal irritation through increased mucin excretion, but adverse effects on resistance to intestinal infections were not observed as in rats (Ten Bruggencate et al., 2006). The rapid fermentation of low-digestible carbohydrates can result in overproduction of organic acids which may lead to epithelial injury and increased intestinal permeability; however, the

mechanisms responsible for this adverse effect of inulin-type fructan consumption is unclear (Ten Bruggencate et al., 2005; Guarner, 2007)

Novel Low-Digestible Carbohydrates

Pullulan

Pullulans are naturally occurring, fungal polysaccharides produced by *Aureobasidium pullulans*, a black yeast found throughout all ecological niches including forest soils, fresh and sea water, and plant and animal tissues (Wolf et al., 2003; Shingel, 2004). 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 (Leathers, 2003; Shingel, 2004). The stair-step structure resulting from glycosidic linkages in pullulans hinders hydrolysis by enzymes, making them low-digestible carbohydrates (Wolf et al., 2003).

Commercially produced pullulan has been available since 1976 from the Hayashibara Company Ltd., Okayama, Japan (Shingel, 2004). It is produced by fermentation of a food-grade hydrolyzed starch using a strain of *A. pullulans* that is non-toxin producing (Kimoto et al., 1997; Shingel, 2004). Upon completion of fermentation, the fungal biomass, pigments, and other impurities are removed. 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 (Leathers, 2003).

The unique linkage pattern of pullulans gives them distinctive characteristics like the capacity to form fibers, compression moldings, and strong oxygen-impermeable films

(Leathers, 2003). 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 (Leathers, 2003).

Pullulans possess many characteristics that make them ideal for numerous applications in food and pharmaceutical manufacturing. Their consistency, dispersibility, and moisture retention characteristics are similar to those of starch, so they are commonly used as a starch replacement in certain foods (Singh et al., 2008). 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 (Singh et al., 2008). 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 (Singh et al., 2008).

Pullulan also has been evaluated for its potential to attenuate glycemic response. Spears et al. (2005) evaluated two pullulans in dogs, one high molecular weight (MW 100,000) and one low molecular weight (MW 6,300). Authors reported that, although not statistically significant, the low-MW pullulan had a numerically lower glycemic response for the first 60 min postprandial compared to maltodextrin. Wolf et al. (2003) evaluated the glycemic response of a low-MW pullulan (MW 100,000) in humans and found that it reduced ($P<0.01$) the glucose AUC by 50% compared to maltodextrin.

Soluble Fiber Dextrin

Components in starch hydrolysates, such as dextrans, maltodextrin, and corn syrup, may be rendered at least partially indigestible. These products are termed resistant maltodextrins, indigestible dextrans, or soluble fiber dextrans (SFD) 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 re-arrange during cooling, forming a highly branched structure (Laurentin and Edwards, 2004; Lefranc-Millot et al., 2009). During this treatment, formation of random linkages occurs including the digestible α -1-4 linkages and the non-digestible linkages such as β -1-4, α and β -1-6, α and β -1-3, 1-2 (Laurentin et al., 2003). Due to their structural characteristics, SFD are only partially hydrolyzed by human digestive enzymes and absorbed in the small intestine (van den Heuvel et al., 2005).

The dextrinization process generates compounds with a broad molecular weight range that can be narrowed by eliminating the higher molecular weight fractions, leading to a dextrin with a reduced viscosity (Joshi and Neves, 2006). Eliminating low molecular weight fractions can make the dextrin sugar-free, improve its digestive tolerance, and reduce hygroscopicity (Joshi and Neves, 2006). The dextrinization process also leaves the product with a discoloration and an off-taste. The heat-treated starch then goes through a purification step with amylases to remove undesirable odors and tastes, and this is followed by decolorizing and demineralization (Joshi and Neves, 2006). The amylase purification step also can slightly increase digestibility of the SFD.

Soluble fiber dextrins possess characteristics that allow them to be easily incorporated into a wide variety of foods (Ohkuma and Wakabayashi, 2001) such as high solubility, low viscosity, stability under numerous processing steps, and neutral taste (Ohkuma and Wakabayashi, 2001). 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 (Pasman et al., 2006).

Slavin et al. (2009) reported in a review that wheat dextrin had a GI of 25% and insulin response of 13% compared with dextrose when evaluated in healthy human subjects. Kendall et al. (2008) evaluated the glycemic response in humans of 25 g of a SFD that was incorporated into a beverage. Authors reported an AUC of 50.1 mmol/L for SFD that was lower compared to the glucose control (103.7 mmol/L). The same trend was noted for the insulin response.

Fibersol-2[®] is a commercially available SFD that has been found to have a caloric value of 1.0-1.5 kcal/g (Ohkuma and Wakabayashi, 1998). Tsuji and Gordon (1998) measured the caloric value of Fibersol-2[®] in rats and calculated a caloric value of 1.2 kcal/g. Goda et al. (2006) measured net metabolizable energy of Fibersol-2[®] in humans using indirect calorimetry and calculated a caloric value of 1.5 kcal/g.

Nutriose[®] FB is a commercially available low-digestible carbohydrate produced from wheat starch. Caloric values of 2 and 1.7 kcal/g have been reported for Nutriose[®] FB (Auerbach et al., 2006; Lefranc-Millot et al., 2009). The degree of resistance to digestion of modified starches depends not only on structural differences, but also on starch source and manufacturing processes used.

Lefranc-Millot et al. (2009) reported that supplementation of 15 g per day of a wheat dextrin (Nutriose[®]) over two weeks decreased fecal pH and significantly reduced *Clostridium perfringens* from 5.9 to 5.6 log CFU/g in human feces. Fasting et al. (2008) evaluated the consumption of 7.5 and 15 g/d of Fibersol-2[®] in 38 humans. Over the 3 wk period, Fibersol-2[®] supplementation tended to increase the molar proportion of butyrate produced (16.5 %) and bifidobacteria populations (10.4 log CFU/g dry feces) with the 15

g/d treatment over baseline values (molar butyrate proportion 14.1%; 9.62 log CFU/g dry feces).

Soluble Corn Fiber

Another novel, low-digestible carbohydrate is soluble corn fiber (SCF) obtained by isolating an oligosaccharide-rich fraction from partially hydrolyzed corn syrup. During corn starch hydrolysis, an aqueous stream comprised of dextrose, fructose, and a mixture of oligosaccharides is formed (Harrison and Hoffman, 2007). This stream undergoes several additional processes including nanofiltration and use of enzymes to create a compound with increased glycosidic α -1,6 and other non-digestible bonds (Harrison and Hoffman, 2007). These processing methods yield an oligosaccharide with approximately 72% total dietary fiber (AOAC method 2001.03; Adam-Perrot et al., 2009). Soluble corn fibers function like glucose syrup and are incorporated easily into many food formulations (Adam-Perrot et al, 2009). They may be added to cereal bars, breakfast cereals, cookies, biscuits, beverages, yogurts, ice creams, sauces, confections, and processed meats.

Increased dietary fiber intake often is associated with adverse gastrointestinal symptoms; thus, the type of dietary fiber should be taken into consideration before being incorporated into the diet. Stewart et al. (2010) found SCF to be well-tolerated when supplemented at 12 g/day. Low scores in bloating, cramping, flatulence, and borborygmi were observed (Stewart et al., 2010). Sanders et al. (2008) also reported SCF to be well tolerated by healthy humans at daily dosages of 5, 15, and 25 g.

Soluble corn fibers may possess beneficial glycemic properties due to their increased glycosidic bonds and ability to resist hydrolytic digestion and be fermented. Kendall et al.

(2008) evaluated the *in vitro* digestibility of a SCF and it was calculated to be 14.5%.

Kendall et al. (2008) also evaluated the glycemic response in humans of 25 g of SCF incorporated in a beverage. Authors reported an AUC of 28.5 mmol/L for SCF which was lower compared to the glucose control (103.7 mmol/L). The insulin response of SCF was only 13% of the glucose control (Kendall et al., 2008).

A significant portion of SCF will enter the colon and have the potential to be fermented by the microbiota. Maathuis et al. (2009) evaluated the prebiotic activity of SCF using a validated dynamic computer-controlled *in vitro* model of the human proximal large intestine (TIM-2). The fiber was first pre-digested using an *in vitro* hydrolytic digestion assay and then inoculated with intestinal inoculum in TIM-2 (Maathuis et al., 2009). Soluble corn fiber and other low-digestible carbohydrates tested resulted in higher SCFA production (168.8 mmol) compared with a cellulose control (94.6 mmol) after 72 hours (Maathuis et al., 2009). Soluble corn fiber was noted to increase bifidobacteria, but not lactobacilli concentrations, as measured using a DNA microarray chip (Maathuis et al., 2009).

Soluble corn fiber has been reported to benefit bone health in rats. Supplementation of SCF was shown to increase whole body bone mineral content and density, cortical thickness and area, and peak breaking strength of the distal femur compared with a cellulose control and several other low-digestible carbohydrates (Weaver et al., 2010). Immunity modulation properties of SCF also have been evaluated using rodent models. Bassaganya-Riera et al. (2010) used interleukin-10-deficient mice as a model of inflammatory bowel disease and found supplementation of SCF to have preventive effects as regards spontaneous colitis and ileitis and ameliorated disease activity. Soluble corn fiber decreased

cytokine IFN- γ production by cells from the Peyer's patches (Bassaganya-Riera et al., 2010). Soluble corn fiber, therefore, may have the potential to minimize gut inflammation.

Summary

With increased awareness of the potential health benefits associated with dietary fibers, the demand for carbohydrates with functional properties similar to dietary fiber is growing. Low-digestible carbohydrates with properties similar to dietary fiber are of interest because they can be incorporated into a variety of foodstuffs more easily. Beneficial properties associated with low-digestible carbohydrates include diminished hydrolytic digestion, attenuated glycemic and insulinemic responses, reduced energy content, fermentation to produce SCFA, the ability to stimulate the growth of beneficial bacteria in the colon, and beneficial modulation of intestinal morphology. A group of novel low-digestible carbohydrates, including pullulan, soluble fiber dextrin, and soluble corn fiber, will be evaluated for these characteristics. The information gained from this research will provide insight into the physiological characteristics of select carbohydrates that could positively impact human health.

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CHAPTER 3: 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

ABSTRACT: 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.

INTRODUCTION

In recent years, the demand for functional ingredients that provide specific health benefits has increased (Murphy, 2001). 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 (Burdock et al., 2006). 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 (van Loo et al., 1999). 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 (Murphy, 2001). 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 (Scheppach et al., 2001). 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 dextrans, and soluble fiber dextrans 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 (Laurentin and Edwards, 2004; Lefranc-Millot et al., 2009). 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, α and β 1 - 3, 1 - 2. (Laurentin et al., 2003; Lefranc-Millot et al., 2009). Due to their structural characteristics, SFDs are only partially hydrolyzed by human digestive enzymes and absorbed in the small intestine (van den Heuvel et al., 2005).

The dextrinization process generates a product with a broad molecular weight range (Joshi, 2006) that can be narrowed by eliminating the higher molecular weight fractions, leading to a dextrin with a reduced viscosity (Joshi, 2006). Eliminating low molecular weight fractions can make the dextrin sugar-free, improve its digestive tolerance, and reduce hygroscopicity (Joshi, 2006). The dextrinization process also leaves the product with a discoloration and an off-taste. The heat-treated starch then goes through a purification step with amylases to remove undesirable odors and tastes, and this is followed by decolorizing and subsequent demineralization (Joshi, 2006). This amylase purification step can affect the digestible and indigestible components of the SFD.

Soluble fiber dextrans possess characteristics that allow them to be easily incorporated into a wide variety of foods (Ohkuma et al., 2001; Lefranc-Millot et al., 2009) such as solubility, low viscosity, stability under numerous processing steps, and neutral taste

(Ohkuma et al., 2001; Lefranc-Millot et al., 2009). Soluble fiber dextrans 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 (Pasman et al., 2006).

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 (Wolf et al., 2003; Shingel, 2004).

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 (Kimoto et al., 1997; Leathers, 2003). The stair-step structure resulting from glycosidic linkages in pullulans hinders hydrolysis by enzymes, making them low-digestible carbohydrates (Wolf et al., 2003).

Commercially produced pullulans have been available since 1976 from the Hayashibara Company Ltd. (Shingel, 2004). They are produced by fermentation from a food-grade hydrolyzed starch using a strain of *A. pullulans* that is non-toxin producing (Kimoto et al., 1997; Shingel, 2004). 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 (Leathers, 2003). 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 (Leathers, 2003).

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 (Leathers, 2003; Singh et al., 2008). 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 (Singh et al., 2008). 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 (Singh et al., 2008).

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 hydrogenation 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 (Boyers, 1959)) 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). IMW pullulan is produced by Hayashibara Company Ltd. (Okayama, Japan).

Chemical Analyses:

Carbohydrates were analyzed for dry matter (DM) and organic matter (OM) according to AOAC (2000), and for free and hydrolyzed monosaccharide concentrations. Test carbohydrates were hydrolyzed using the procedure described by Hoebler et al. (Hoebler et al., 1989) where carbohydrates were subjected to hydrolysis with H₂SO₄ 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. (Smiricky et al., 2002).

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 (Muir and O'Dea et al., 1993). The samples were analyzed for free released monosaccharides using HPLC (Smiricky et al., 2002) 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 ± 4.8 kg, and a mean age of 5.6 ± 2.4 years were used. Dogs were housed individually in 1.2 x 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 cc 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 1,240g 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 (Cass et al., 1984). 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) (Wisdom, 1976).

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

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 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.

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 (Sibbald, 1980; Parsons, 1985). 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 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 (Parsons et al., 1992):

TME_n (kcal/g) =

$$\frac{\text{Energy intake (kcal)} - \text{energy excreted by fed birds (kcal)} + \text{energy excreted by fasted birds (kcal)}}{\text{Feed intake (g)}}$$

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 3-1. 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 3-1), 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.

All pullulans had very low free sugar concentrations, with Pull HMW having essentially no free sugar present (Table 3-2). 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 3-2). All three pullulans had 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 (Spears et al., 2005). Commercial 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 3-3). 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 3-4. 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.

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., 2009). The results indicated an average small intestinal digestibility of 15% with a range of 8.7% to 19% (Lefranc-Millot et al., 2009). 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 (Laurentin and Edwards, 2004) found that the portions of SFDs that escaped digestion were extensively fermented. A variety of starch sources can be used to form indigestible dextrans such as those from corn, potato, wheat, lentil, cassava, and tapioca. The enzyme-resistant fractions of indigestible dextrans may vary significantly with the botanical source of the starch used (Laurentin et al., 2003).

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 (Laurentin et al., 2003). 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. (2003) 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. (2007) also reported on the digestibility of two pullulans (MW 63,000 and MW 100,000). Hydrolytic digestion was determined to be ~ 55% (Spears et al., 2007), 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 3-1, and the corresponding values for AUC (mmol/L) and relative glycemic response (RGR) are presented in Table 3-5. 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 glyceemic 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 glyceemic 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 glyceemic response since sorbitol does not elicit a glyceemic 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, 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% (Donazzolo et al., 2003; Lefranc-Millot, 2008). 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) (Kendall et al., 2008).

The changes in serum insulin for the SFD substrates are presented in Figure 3-2, and the corresponding values for AUC (pmol/L) and relative insulinemic response (RIR) are presented in Table 3-5. 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 (Donazzolo et al., 2003; Lefranc-Millot, 2008). A tapioca-based SFD resulted in a somewhat similar RIR in humans as T2 in this experiment (40.2 and 50.5, respectively) (Kendall et al., 2008). 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 (Slavin and Green, 2007; Bellisle, 2008).

The changes in plasma glucose for the pullulans are presented in Figure 3-3, and the corresponding values for AUC (mmol/L) and relative glyceic response (RGR) are presented in Table 3-6. 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. (2005) 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 glyceic response for the first 60 min postprandial compared to Malt.

Wolf et al. (2003) evaluated the glyceic 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 3-4, and the corresponding values for AUC (pmol/L) and relative insulinemic response (RIR) are presented in Table 3-6. 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 glyceic 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 glyceic responses where no large, sharp peaks resulted and, thus, no sharp increases in insulin were noted. Spears et al. (2005) 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. (Spears et al., 2005) 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, 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 3-7). 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 (avg 1.7 kcal/g) as a wheat-based Nutriose SFD (1.7 kcal/g) evaluated in men (Vermorel et al.,

2004). 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 (Goda et al., 2006). 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.

True metabolizable energy data for the pullulans are presented in Table 3-8. 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 measurements. 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 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 carbohydrates 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 (Slavin et al., 2009). 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 digestibility. 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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Table 3-1. Free sugar and hydrolyzed monosaccharide concentrations of soluble fiber dextrins

Test carbohydrate ¹	T1	T2	TH	C1	C2	C3
Free sugars, mg/g ²						
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, mg/g ³	0.62	1.15	91.59	13.21	3.37	33.38
Hydrolyzed monosaccharides, mg/g ^{2,4}						
Galactose	0.00	0.00	0.00	0.00	0.00	0.00

Table 3-1 (con't.)

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, mg/g ³	1,139.67	1,037.55	960.59	1,160.46	1,137.51	1,128.21

¹ 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.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units

⁴ Values are correcte for free monosaccharide concentrations.

Table 3-2. Free sugar and hydrolyzed monosaccharide concentrations of pullulans

Test carbohydrate ¹	Pull LMW	Pull IMW	Pull HMW
Free sugars, mg/g ²			
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, mg/g ³	8.17	8.07	0.01
Hydrolyzed monosaccharides, mg/g ^{2,4}			
Galactose	28.80	0.43	19.38
Glucose	840.09	1,112.76	1,081.98

Table 3-2 (con't.)

Mannose	38.32	0.00	26.60
Sorbitol	0.00	0.00	0.00
Total, mg/g ³	907.21	1,113.19	1,127.96

¹ Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

⁴ Values are corrected for free monosaccharide concentrations.

Table 3-3. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of soluble fiber dextrins

Released monosaccharides, mg/g ²	Test carbohydrate ¹						SEM
	T1	T2	TH	C1	C2	C3	
Fructose	0.00 ^a	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, mg/g ³	427.35 ^c	457.46 ^c	630.92 ^d	164.81 ^a	228.47 ^{ab}	284.77 ^b	15.30

¹Abbreviations: 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.

² Values are expressed on a dry matter basis.

³ Values include addition of water added when starches are broken down to monosaccharide units.

^{abcd} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 3-4. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of pullulans

Released monosaccharides, mg/g ²	Test carbohydrate ¹			SEM
	Pull LMW	Pull IMW	Pull HMW	
Fructose	0.00 ^a	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, mg/g ³	1,041.62 ^b	1,048.21 ^b	866.16 ^a	27.12

¹ Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight.

² Values are expressed on a dry matter basis.

³ Values include water that is added when starches are broken down to monosaccharide units.

^{ab} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 3-5. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble fiber dextrins.

Item	Carbohydrate ¹							SEM ²
	T1	T2	TH	C1	C2	C3	Malt	
AUC for glucose, mmol/L	91.38 ^{ab}	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

¹ 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.

² Pooled standard error of the mean.

^{abcd} Means in the same row with different superscript letters are different (P < 0.05).

Table 3-6. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of Pullulans.

Item	Test carbohydrate ¹				SEM ²
	Pull LMW	Pull IMW	Pull HMW	Malt	
AUC for glucose, mmol/L	60.18 ^a	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

¹Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight pullulan; Malt, maltodextrin.

² Pooled standard error of the mean.

^{ab} Means in the same row with different superscript letters are different (P<0.05).

Table 3-7. True metabolizable energy (TME_n) values for soluble fiber dextrins

Item	Test carbohydrate ¹							SEM ²
	T1	T2	TH	C1	C2	C3	Malt	
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}	2.30 ^c	2.57 ^c	1.23 ^a	1.66 ^{ab}	1.65 ^{ab}	4.06 ^d	0.12

¹ 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.

² Pooled standard error of the mean.

^{abcd} Means in the same row with different superscript letters are different (P < 0.05).

Table 3-8. True metabolizable energy (TME_n) values for pullulans

Item	Test carbohydrate ¹				SEM ²
	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	3.95 ^b	NM	4.06 ^b	0.17

¹ Abbreviations: Pull LMW, low molecular weight pullulan; Pull IMW, intermediate molecular weight pullulan; Pull HMW, high molecular weight pullulan; Malt, maltodextrin; NM, not measured.

² Pooled standard error of the mean.

^{ab} Means in the same row with different superscript letters are different (P<0.05).

Figure 3-1. 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: 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).

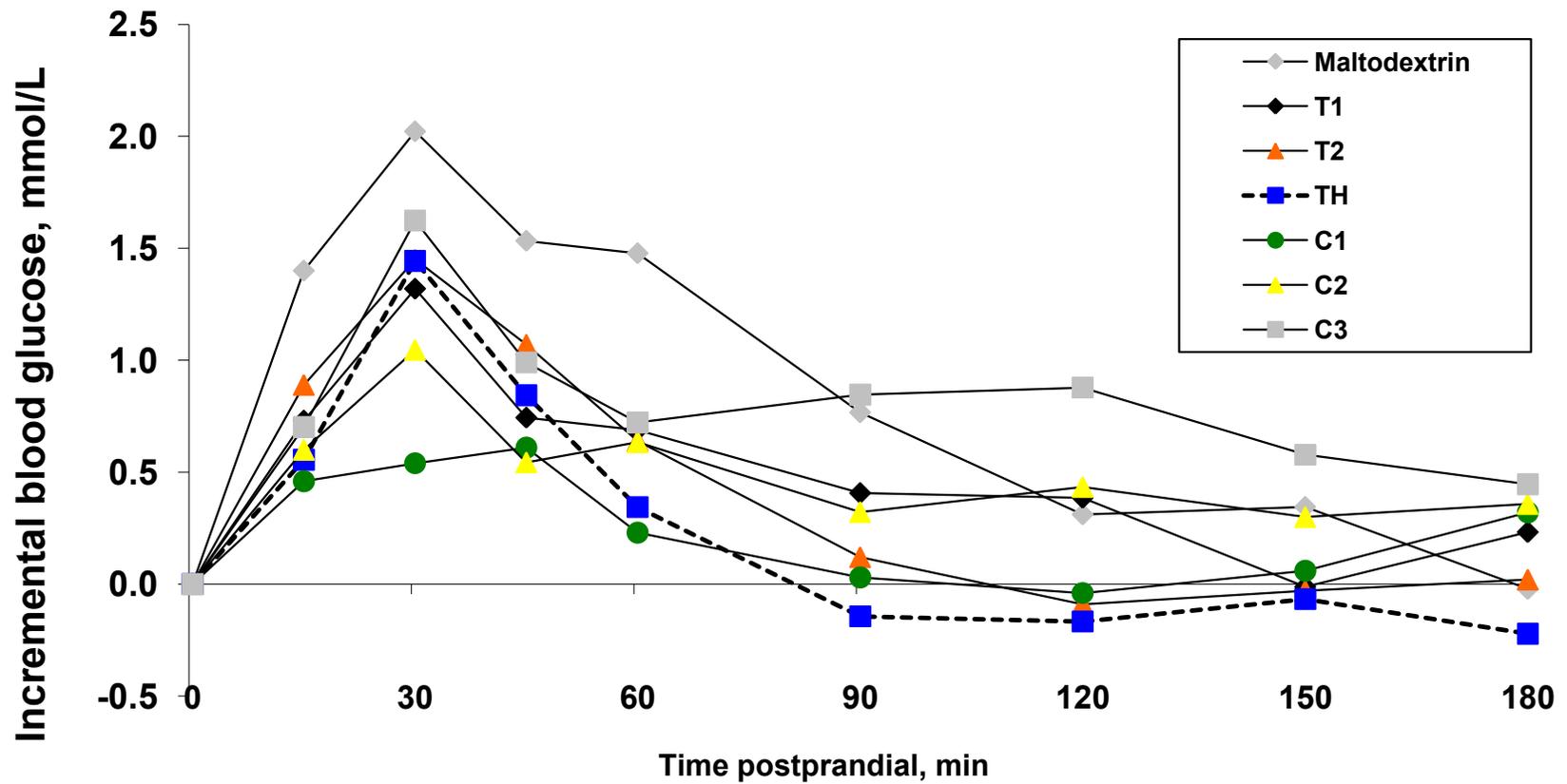


Figure 3-2. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble fiber dextrins. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (17.27), tapioca-based SFD1 (T1) (28.33), tapioca-based SFD2 (T2) (27.33), tapioca-based SFD hydrogenated (TH) (27.33), corn-based SFD1 (C1) (27.33), corn-based SFD2 (C2) (27.21), corn-based SFD3 (C3) (27.21).

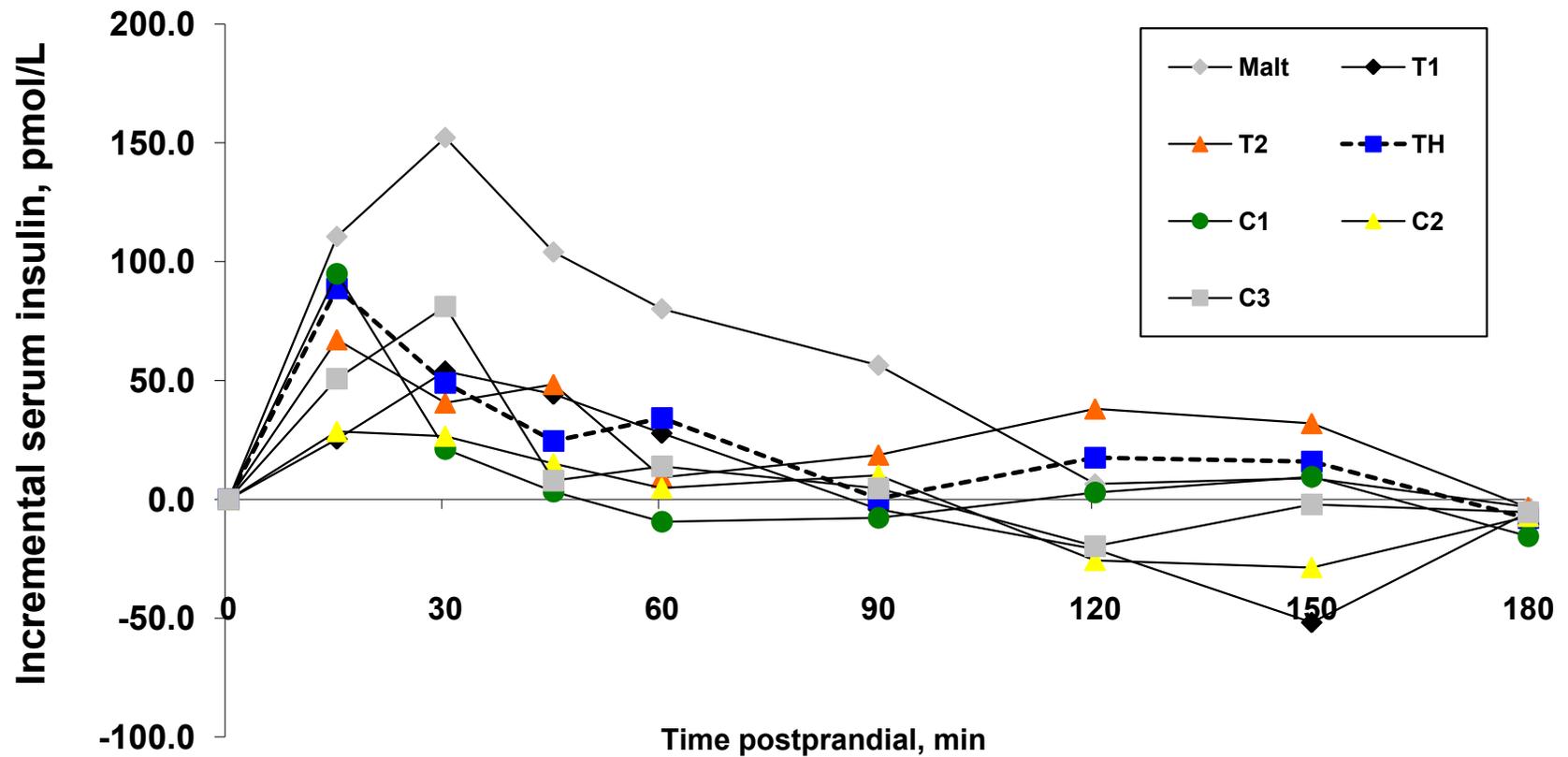


Figure 3-3. Incremental change from baseline in blood glucose response for dogs consuming 25 g of pullulans.

Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (0.20), low molecular weight pullulan (Pull LMW) (0.30), intermediate molecular weight pullulan (Pull IMW) (0.31), high molecular weight pullulan (Pull HMW) (0.33).

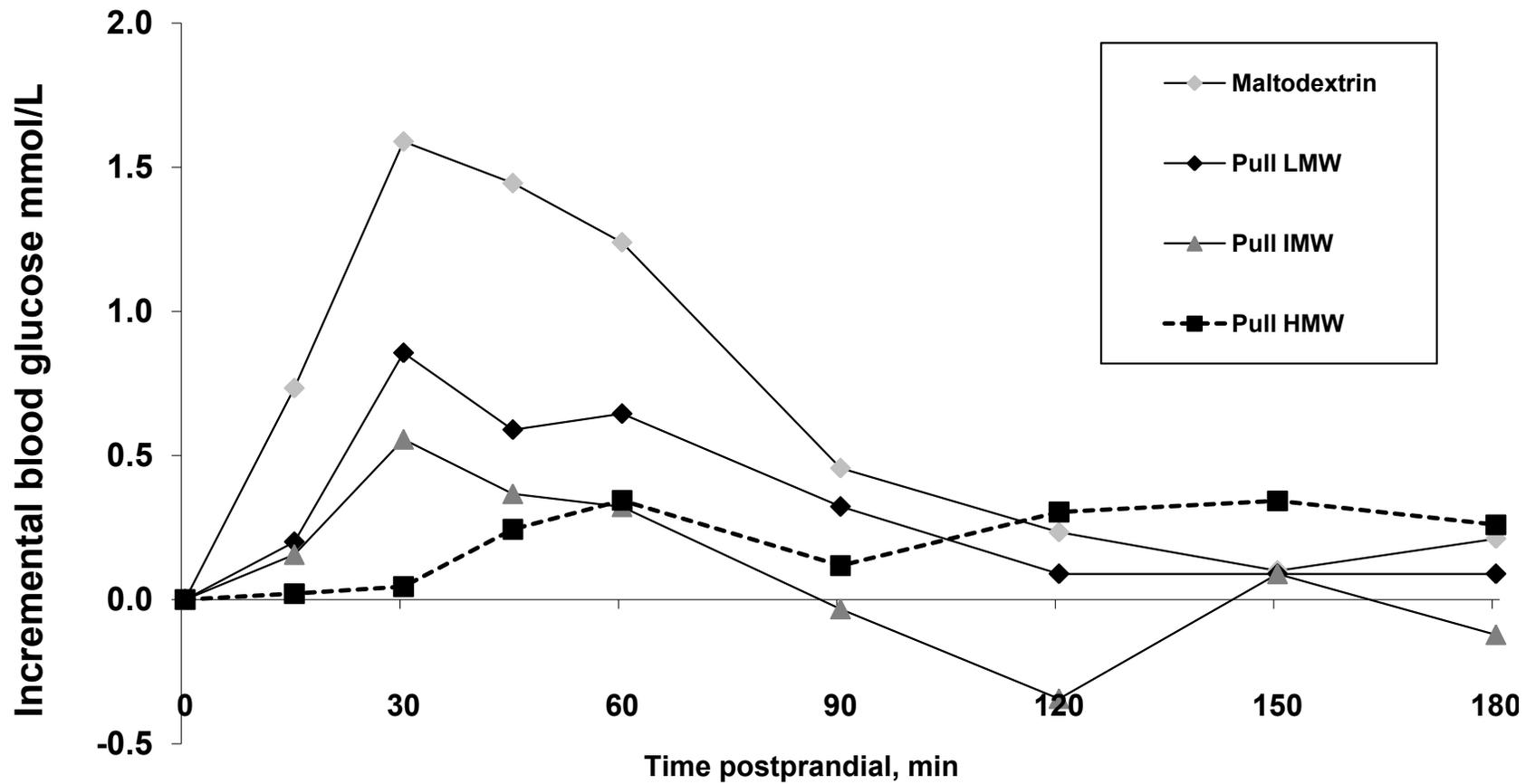
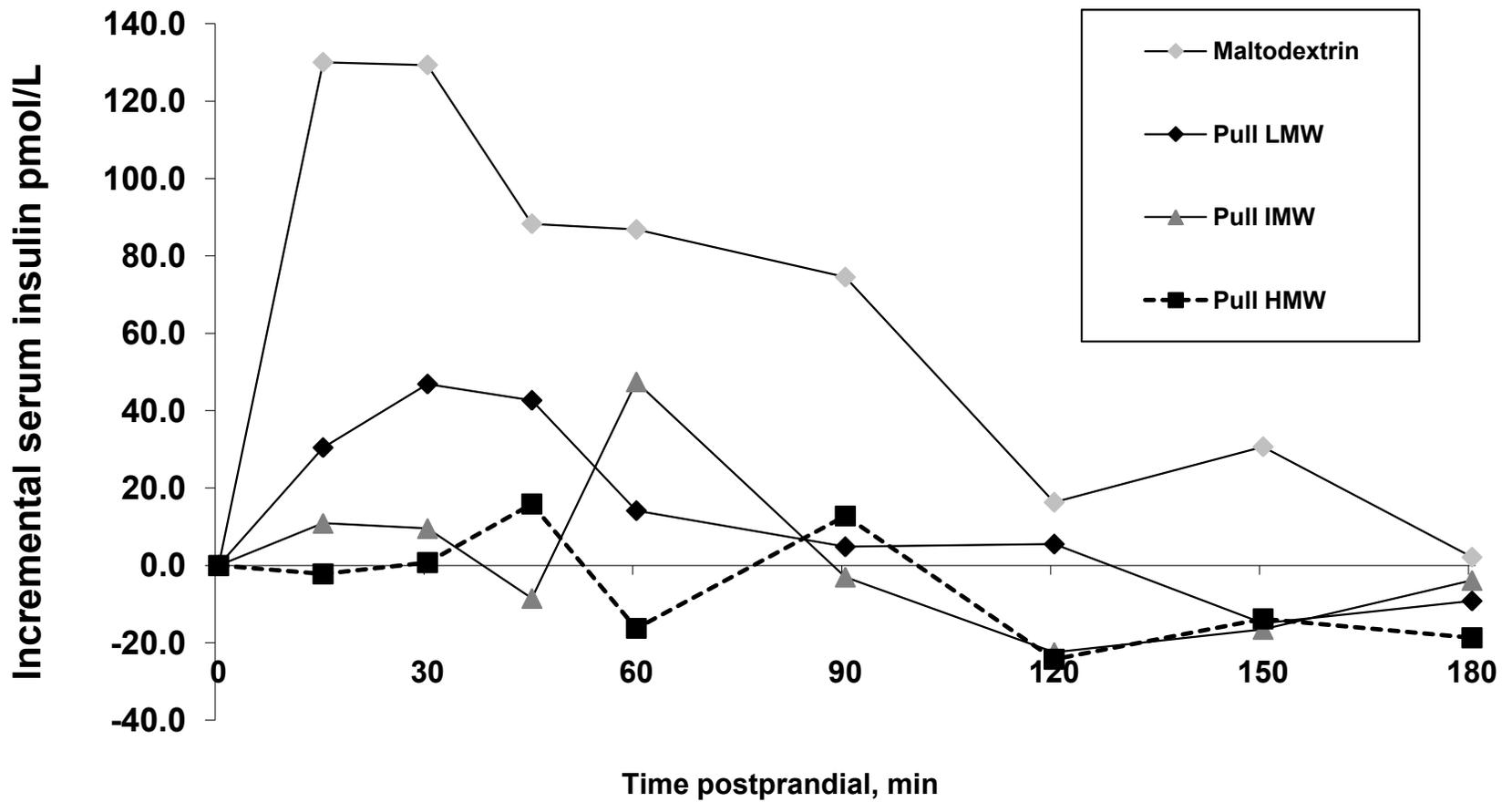


Figure 3-4. Incremental change from baseline in serum insulin response for dogs consuming 25 g of pullulans.

Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (19.34), low molecular weight pullulan (Pull LMW) (26.35), intermediate molecular weight pullulan (Pull IMW) (27.76), high molecular weight pullulan (Pull HMW) (32.74).



CHAPTER 4: *IN VITRO* HYDROLYTIC DIGESTION, GLYCEMIC RESPONSE IN DOGS, AND TRUE METABOLIZABLE ENERGY CONTENT OF SOLUBLE CORN FIBERS

ABSTRACT: The objective of this research was to measure *in vitro* hydrolytic digestion, glycemic and insulinemic responses, and true metabolizable energy (TME_n) content of select soluble corn fibers (SCFs). The first generation SCFs included hydrochloric acid- treated corn syrup (HCl corn syrup); a SCF with an increased total dietary fiber (TDF) content (SCF gen 1); a SCF that was spray-dried (SCF spray-dried); and a hydrogenated SCF (SCF hydrogenated). The second generation SCFs included those prepared using phosphoric acid catalyzation in both a liquid (SCF phos (l)) and powder (SCF phos (p)) form, and SCFs that were prepared using hydrochloric acid catalyzation in both a liquid (SCF HCl (l)) and powder (SCF HCl (p)) form. Also, in the second generation set of samples were SCFs prepared using the same method but in three separate batches. All contained 70% TDF and 15% sugars. Two were in liquid form (SCF 2A and SCF 2B), and one in powder form (SCF 2C). A lower sugar form (80% TDF and 5% sugar) SCF also was evaluated (SCF low sugar). Glucose was the major free sugar and bound monosaccharide in all SCFs except for SCF hydrogenated that had higher concentrations of sorbitol. All SCFs had intermediate to low amounts of monosaccharides released as a result of *in vitro* hydrolytic digestion, with glucose being the primary sugar component released. First generation SCFs were more digestible *in vitro* (~50%) compared to second generation SCFs (~ 32%). All SCFs had attenuated glycemic responses in dogs compared to a maltodextrin (Malt) control. Second generation SCFs, on average, had lower glycemic responses and

TME_n values in roosters than first generation SCFs. All SCFs had low free sugar concentrations with varying degrees of resistance to digestion, reduced caloric content, and attenuated glyceimic and insulinemic responses. These ingredients are potential candidates for inclusion in reduced calorie and low glyceimic foodstuffs.

INTRODUCTION

The increasing incidence of chronic diseases such as obesity, diabetes, heart disease, cancers, and other metabolic disorders in today's society is leading to an increased awareness of how diet, especially dietary fiber and low-digestible carbohydrates, can play a positive role in health maintenance. As consumers become more aware of the potential health benefits associated with ingredients that have reduced energy content, that are partially fermented in the colon, and that elicit reduced glyceimic and insulinemic responses, demand for these ingredients will increase. A growing body of research indicates that dietary fiber may reduce the risk for developing several diseases including coronary heart disease, hypertension, diabetes, obesity, and some gastrointestinal disorders (Anderson et al., 2009). However, most of the population of the United States consumes less than half of the recommended levels of dietary fiber daily (Anderson et al., 2009). This has led to a demand for the development of novel carbohydrates that have functional properties similar to dietary fiber, but that may be incorporated more easily into a wider array of solid and liquid food matrices.

Novel carbohydrates have the potential to not only increase the fiber content of foodstuffs, but also the ability to positively influence postprandial glycemia (Riccardi et al., 2008). Low glyceimic diets have been shown to help control postprandial glucose and

insulin concentrations in diabetics (Livesey et al., 2008; Riccardi et al., 2008). Decreasing the glycemic response also may have a role in improving risk factors associated with heart disease and diabetes (Livesey et al., 2008; Riccardi et al., 2008).

A category of carbohydrate that has the potential to have functional properties and benefits similar to dietary fiber is low-digestible carbohydrates. Low-digestible carbohydrates represent a heterogeneous group of compounds that are partially unavailable to digestive enzymes so are either incompletely or not absorbed from the small intestine (Murphy, 2001; Scheppach et al., 2001; Grabitske et al., 2008). These low-digestible carbohydrates pass into the colon where they are totally or partially fermented by colonic microflora. Low-digestible carbohydrates include polyols, resistant starch, non-starch polysaccharides, fructooligosaccharides, and other oligosaccharides (Marteau and Flourie, 2001; Murphy, 2001; Scheppach et al., 2001; Grabitske et al., 2008). Factors restricting digestion and absorption of low-digestible carbohydrates are the impaired hydrolysis of constitutive bonds by enzymes and the degree of absorption in the small intestine (Marteau and Flourie, 2001). Once low-digestible carbohydrates reach the colon, they are totally or partially fermented to short-chain fatty acids (SCFA) and gases.

A type of novel, low-digestible carbohydrate is soluble corn fiber (SCF). Soluble corn fibers are obtained by isolating an oligosaccharide-rich component from partially hydrolyzed corn syrup. During corn starch hydrolysis, an aqueous stream comprised of dextrose, fructose, and a mixture of oligosaccharides is formed (Harrison and Hoffman, 2007). This stream undergoes several additional processes including nanofiltration and use of enzymes to create products with increased glycosidic α -1,6 bonds (Harrison and Hoffman, 2007). 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 glycosidic linkages. (Haralampu, 2000; Thompson, 2000).

Evaluation of a variety of physiological responses to these carbohydrates is beneficial as it allows for a better understanding of the potential functional benefits that select carbohydrates possess. The objective of the study was to evaluate SCFs for physiological properties that could positively impact human health to include sugar composition, in vitro hydrolytic digestion, glycemic and insulinemic responses using a dog model, and true metabolizable energy (TME_n) using an avian model.

MATERIALS AND METHODS

Substrates:

Soluble corn fibers resulted from the process of manufacturing oligosaccharides that are digestion-resistant or slowly digestible. The first step in the production of SCFs was to isolate an oligosaccharide-rich stream. During cornstarch hydrolysis, an aqueous stream comprised of dextrose, fructose, and a mixture of oligosaccharides results (Harrison and Hoffman, 2007). The aqueous solution is nanofiltered, resulting in a monosaccharide and an oligosaccharide-rich stream. This latter stream is comprised of at least 50% and as much as 90% by weight (dry solids basis) of oligosaccharides, but still may have up to 50% dextrose and fructose present.

Once oligosaccharide-rich syrup has been formed, it can be treated with an isomerization enzyme such that some of the dextrose present in the syrup is converted to fructose, producing an isomerized oligosaccharide-rich stream (Harrison and Hoffman,

2007). The oligosaccharide-rich syrup may go through another membrane filtering step to remove monosaccharides from the syrup. The syrup also can be hydrogenated, converting some of the monosaccharides to alcohols. Lastly, in yet another process, the syrup may be subjected to a catalyst(s) that creates reversion products where the residual monosaccharides in the syrup become covalently bonded to oligosaccharides or other monosaccharides present in the syrup, thus increasing glycosidic α -1,6 bond formation in the structure (Harrison and Hoffman, 2007).

The last steps in the production process include decolorization of the syrup and evaporation and drying to produce a powder. The different processes utilized to produce these oligosaccharides result in fractions with varying degrees of resistance to digestion.

All the SCFs evaluated were produced using the basic steps outlined above, but procedures were modified to produce a variety of SCFs. The SCFs evaluated can be classified into first generation and second generation products. Both generations of SCFs rely on the creation of reversion products using different combinations of acid catalysts and enzymes to increase number of glycosidic bonds.

The first generation of SCFs included: one made from corn syrup where hydrochloric acid only was used as a catalyst (HCl corn syrup); a SCF using the same production methods as for HCl corn syrup but that had a higher TDF content (SCF gen 1); a SCF prepared using similar production methods but that was spray dried (SCF spray-dried); and a SCF gen 1 that went through hydrogenation steps to form sugar alcohols (SCF hydrogenated).

A new process then was developed and eight second generation SCFs were produced. The second generation SCFs included a set of four carbohydrates where either phosphoric acid or hydrochloric acid alone were used as catalysts and that were produced in either a liquid (SCF phos (l) and SCF HCl (l)) or powder (SCF phos (p) and SCF HCl (p)) form. A combination of phosphoric and hydrochloric acids was used as catalysts for the production of the last four SCFs in the second generation series. They included a SCF that was evaporated to a greater extent than the SCFs in the previous generation and that contained 70% TDF and 15% sugar (SCF 2 B1). Two other SCFs were produced using the same exact conditions but were made in different batches, one in liquid form (SCF 2 B2) and the other in powder form (SCF 2 B3). Lastly, a lower sugar (80% TDF and 5% sugar) SCF was produced in this series (SCF low sugar).

Chemical Analyses:

Carbohydrates were analyzed for dry matter (DM) and organic matter (OM) according to AOAC (2000), and for free and hydrolyzed monosaccharide concentrations. Test carbohydrates were hydrolyzed using the procedure described by Hoebler et al. (1989) where carbohydrates were subjected to hydrolysis with H₂SO₄ 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 μ L. All assays were conducted using a CarboPac PA-1 column and guard column following methods cited by Smiricky et al. (2002).

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 amyloglucosidase and α -amylase to simulate gastric and small intestinal digestion (Muir et al., 1993). The samples were analyzed for free released monosaccharides using HPLC (Smiricky et al., 2002) 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 ± 4.8 kg, and a mean age of 5.6 ± 2.4 years were used. Dogs were housed individually in 1.2 x 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 non-destructible 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 consumed 25 g of carbohydrate (DM basis) in approximately 240 mL dd 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 cc syringe (without needle) and offered to 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 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 1,240 x 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 (Cass et al., 1984). 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) (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 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: $[(\text{AUC for test carbohydrate}) / (\text{AUC for control})] \times 100\%$.

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 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.

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 (Sibbald, 1980; Parsons, 1985). 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 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 (Parsons et al., 1992):

TME_n (kcal/g) =

$$\frac{\text{Energy intake (kcal)} - \text{energy excreted by fed birds (kcal)} + \text{energy excreted by fasted birds (kcal)}}{\text{Feed intake (g)}}$$

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 and hydrolyzed monosaccharide concentrations for the first generation SCFs are presented in Table 4-1. All SCFs had low free sugar concentrations. Hydrogenated SCF had the highest free sugar concentrations of all carbohydrates tested. Glucose was the major free sugar found in the SCFs except for SCF hydrogenated. For this substrate, little free glucose was present and sugars present in the highest concentration included fructose, sucrose, and sorbitol.

Hydrolyzed monosaccharide concentrations (Table 4-1) varied slightly among the first generation SCFs. First generation SCF, SCF spray-dried, and SCF hydrogenated had high concentrations of hydrolyzed monosaccharides, while HCL corn syrup has a lower concentration. Most of the hydrolyzed monosaccharides were glucose for all first generation SCF substrates. Minor amounts of mannose were present in SCF gen 1, SCF spray-dried, and SCF hydrogenated. An intermediate amount of sorbitol was present in SCF hydrogenated. Sugars accounted for nearly all the dry matter in all SCFs except for HCl corn syrup where only 71% of the dry matter was accounted for.

Free sugar content (Table 4-2) varied slightly among second generation SCFs with a range of 2 to 14% of the carbohydrate content consisting of free sugars. Low sugar SCF has the lowest free sugar content at 2.3% and generation 2 SCF had the highest with a 14.1% free sugar concentration. Soluble corn fibers that were treated with phosphoric acid had higher free sugars (~11 %) than SCFs treated with hydrochloric acid (~ 6%). Glucose was the major free sugar in all second generation SCFs.

Hydrolyzed monosaccharide concentrations (Table 4-2) were similar among the second generation SCFs except for SCF 2 B1 that had a considerably lower hydrolyzed

monosaccharide concentration (~49%). The hydrolyzed monosaccharide assay destroys fructose so it not accounted for in the sugar analysis. It is likely that SCF 2 B1 contained a major portion of bound fructose and, thus, its absence contributes to the low concentrations of hydrolyzed monosaccharides in this substrate. Most of the hydrolyzed monosaccharide content was from glucose for all eight second generation SCFs. A small amount of mannose was present in SCF 2 B3.

Quantification of the free sugar and hydrolyzed monosaccharide contents of novel carbohydrates is important if these substrates are to be considered for incorporation into foodstuffs. Analyzing the free sugar content is important for carbohydrates that may be included in low glycemic foodstuffs. Free sugars in carbohydrate substrates are rapidly available for digestion and, thus, have the ability to affect the glycemic response. The hydrolyzed monosaccharides constitute the building blocks of the test substrate and the fraction of monosaccharides that are potentially available for digestion. Free sugars and hydrolyzed monosaccharides provide an indication of how much and what monosaccharides may potentially affect the glycemic response and TME value.

In Vitro Hydrolytic Digestion:

Monosaccharides released as a result of simulated hydrolytic digestion of the first generation SCFs are presented in Table 4-3. All SCFs were found to have intermediate amounts of monosaccharides released, translating to a digestibility value of approximately 50%. The SCF hydrogenated substrate resulted in the lowest ($P < 0.05$) concentration of released glucose whereas the greatest glucose release occurred for HCl corn syrup.

Hydrogenated SCF resulted in the highest ($P<0.05$) concentrations of glucosamine and sorbitol release among the first generation SCFs.

Monosaccharides released as a result of simulated hydrolytic digestion of the second generation SCFs are presented in Table 4-4. Low to intermediate amounts of monosaccharides were released after simulated digestion of all second generation SCFs. Low sugar SCF resulted in the lowest ($P<0.05$) monosaccharide release (~19% digested). The SCFs that were prepared by phosphoric acid catalyzation resulted in the highest ($P<0.05$) monosaccharide release, with SCF phos (p) being higher ($P<0.05$) than SCF phos (l). Glucose was the major monosaccharide released for all second generation SCFs. Low sugar SCF had the lowest glucose release and SCF phos (p) the highest ($P<0.05$). All SCFs had low concentrations of fructose and galactose release during simulated hydrolytic digestion. Sorbitol was released in small amounts for all SCFs except SCF low sugar, which had no released sorbitol. The highest ($P<0.05$) concentration of released fructose, galactose, and sorbitol occurred for second generation SCF 2 B1.

First generation SCFs were approximately 50% digestible, higher than the 32% average digestibility of second generation SCFs. Second generation SCFs were produced using more stringent processes that likely accounted for the decrease in digestibility. Differences in the acid catalyst used during the production process affected digestibility of the SCFs dramatically. 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 linkages that hold the monosaccharides together can make the

carbohydrate resistant to enzymatic digestion. Enzyme resistance is due mainly to the presence of α -1,6 glycosidic linkages and inaccessibility of the α -1,4 linkages (Kendall et al., 2008). During the production of the SCFs, different acid and enzyme combinations were utilized to form glycosidic linkages, predominantly α -1,6 linkages, among monosaccharides in the corn syrup in order to produce a more digestion-resistant carbohydrate. Kendall et al. (2008) evaluated the *in vitro* digestibility of a SCF that was similar to SCF 2 B1. The SCF was calculated to be 14.5% digestible (13) which is lower 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 SCFs. 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 of the low-digestible carbohydrates evaluated above resulted in *in vitro* digestion values greater than the average 32.0% *in vitro* digestibility of the second generation SCFs. Overall, the production processes implemented for second generation SCFs were very successful in producing low-digestible carbohydrates.

Glycemic and Insulinemic Responses:

Incremental AUC data for glucose for the first generation SCFs are presented in Figure 4-1, and the corresponding values for AUC and relative glycemic response (RGR) are presented in Table 4-5. Maltodextrin was used as a control for every set of glycemic response tests because it is highly digestible and rapidly absorbed, resulting in a consistently high glycemic response. The carbohydrates were not all evaluated in the same period, so Malt was used in every period to serve as a control to calculate a relative response to the test carbohydrate in all periods. Area under the curve for Malt was higher ($P < 0.05$) than the

AUC values for all first generation SCFs. Intermediate AUC values resulted for HCl corn syrup, SCF gen 1, and SCF spray-dried. Hydrogenated SCF resulted in the lowest ($P < 0.05$) AUC value.

Since Malt served as the control to which all test carbohydrates were compared, it was assigned a RGR value of 100. The RGR is a better value 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, so test carbohydrates with high AUC values will have correspondingly high RGR values. The RGR values for first generation SCFs followed the same pattern as AUC values. Maltodextrin had the highest RGR, followed by intermediate values for HCl corn syrup, SCF gen 1, and SCF spray-dried. Hydrogenated SCF resulted in the lowest RGR values.

A similar pattern in blood glucose response resulted from digestion of HCl corn syrup, SCF gen 1, and SCF spray-dried. All three carbohydrates resulted in an intermediate peak at 30 min followed by a blunted response in blood glucose concentrations throughout the remainder of the glycemic response test. Hydrogenated SCF peaked minimally at 30 min and remained at or below basal blood glucose concentration throughout the glycemic response test. These blood glucose patterns correspond well to sugar composition data. While SCF hydrogenated had a higher free sugar concentration than HCl corn syrup, SCF gen 1, and SCF spray-dried, it was due to higher concentrations of sorbitol and fructose that do not elicit a glycemic response. Free sugar and released monosaccharide data show that HCl corn syrup, SCF gen 1, and SCF spray-dried have glucose available for digestion, and this was reflected in the intermediate blood glucose responses.

Incremental AUC data for insulin for the first generation SCFs are presented in Figure 4-2, and the corresponding values for AUC and RGR are presented in Table 4-5. Malt had a higher ($P<0.05$) AUC and RIR value than for all first generation SCFs. Hydrochloric acid-catalyzed corn syrup, SCF gen 1, and SCF spray-dried all resulted in intermediate RIR responses, approximately 50% of that of Malt. Hydrogenated SCF resulted in the lowest ($P<0.05$) insulin response among the SCFs.

Except for SCF hydrogenated, the first generation SCFs induced moderate peaks in insulin at the beginning of the response test before dropping to basal concentrations. The peaks in insulin result from increased blood glucose concentrations elicited by the SCFs. Hydrogenated SCF resulted in a very low insulin response which is expected since the hydrogenation process leads to increased sorbitol, which does not elicit a glycemic or insulinemic response.

Incremental AUC data for glucose for the second generation SCFs are presented in Figure 4-3, and the corresponding values for AUC and RGR are presented in Table 4-6. Maltodextrin had the highest ($P<0.05$) AUC, with all SCFs resulting in lower AUCs for blood glucose. The powdered form of phosphoric-acid catalyzed SCF resulted in the highest AUC ($P<0.05$) among the SCFs. All other second generation SCFs had intermediate AUC values. The RGR data followed the same pattern, with Malt resulting in the highest ($P<0.05$) value and the SCFs resulting in intermediate values (avg RGR of 44). Kendall et al. (2008) evaluated the glycemic response in humans of a SCF that was similar to SCF 2 B1 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 SCFs evaluated.

The majority of the second generation SCFs resulted in similar blood glucose response patterns except for phosphoric acid-catalyzed SCFs. The SCFs that had similar glycemic responses showed a blood glucose pattern of small blunted peaks during the first 60 min, then decreases to near basal concentrations for the remainder of the test. The highest glycemic response among second generation SCFs occurred for SCF phos (p) that had a high peak in blood glucose concentration at 45 min, then a steady drop to baseline concentrations. This higher glycemic response corresponded to higher free sugar and released monosaccharide values for SCF phos (p). While SCF phos (l) also had higher free sugars and released monosaccharides from hydrolytic digestion, it resulted in one of the numerically lowest RGR values. The lower RGR is due to the blood glucose pattern elicited by SCF phos (l). The phosphoric acid-catalyzed SCF (l) peaked in blood glucose at the beginning of the glycemic response test but then decreased to below basal concentrations for the remainder of the test.

Incremental AUC data for insulin for the second generation SCFs are presented in Figure 4-4, and the corresponding values for AUC and RGR are presented in Table 4-6. Area under the curve was highest ($P < 0.05$) for Malt, with all other SCFs having similar but lower values. The second generation values had low to intermediate RIR values with an average RIR of 35.5 which is lower than the average RIR of the first generation SCFs (RIR 50.0, excluding SCF hydrogenated). 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 resulted upon 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 second generation SCFs (RIR 35.5).

The second generation SCFs, except the phosphoric acid catalyzed SCFs, had similar serum insulin patterns where insulin peaked at 15 min of the test and decreased to basal concentrations. The higher RIR values for SCF phos (p) and SCF phos (l) results from a larger insulin response during the first 60 min of the response test, which corresponds to a higher peak in blood glucose concentrations during this time frame. On average, SCFs resulting from a hydrochloric acid catalyst had lower glycemic and insulinemic responses (RGR 41, RIR 28) compared to SCFs from a phosphoric acid catalyst (RGR 51, RIR 50). Hydrochloric acid may form more digestion-resistant glycosidic bonds than phosphoric acid when used as a catalyst under these conditions.

All SCFs from both generations had reduced glycemic and insulinemic responses. Second generation SCFs generally resulted in lower responses than did first generation SCFs. The increased resistance to digestibility of these carbohydrates was likely because of increased glycosidic linkage formation resulting from different production methods. Reduced blood glucose and insulin responses have been shown to have several beneficial health effects, especially in diabetic patients (American Diabetes Association, 2007; Livesey et al., 2008; Riccardi et al., 2008). 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.

True Metabolizable Energy (TME_n):

An increasing demand for reduced calorie foodstuffs is increasing the trend for production of low-calorie sweeteners and bulking agents. A method for evaluating the caloric content of ingredients is the true metabolizable energy (TME_n) assay with roosters. This in vivo animal model assay allows for exposure of test substrates to actual digestive processes.

True metabolizable energy (TME_n) data for the first generation SCFs are presented in Table 4-7. All SCFs had a lower (P<0.05) TME_n value compared to the Malt control. Among the SCFs, SCF hydrogenated had the highest (P<0.05) metabolizable energy, SCF gen 1 and SCF spray-dried had intermediate values, and HCl corn syrup had the lowest (P<0.05) TME_n value. Hydrogenated SCF had the highest content (12%) of free sugars (sucrose, sorbitol, and fructose) readily available for digestion and leading to its higher energy value.

Maltodextrin had a higher (P<0.05) TME_n value compared to all second generation SCFs (Table 4-8). Lower and similar TME_n values were found for the majority of the second generation SCFs, SCF phos (p) being statistically higher than all but SCF 2 B1. The higher TME_n value of SCF phos (p) corroborates free sugar, released monosaccharide, and glycemic response data that show SCF phos (p) to be more digestible than the other SCFs. A low-digestible carbohydrate produced using acid catalysts was evaluated by Sinaud et al. (2002) and resulted in a metabolizable energy value of 3.37 kcal/g which is higher than the SCFs evaluated here, indicating that these production methods for SCFs were more successful in creating a less digestible ingredient.

The TME_n assay is useful for evaluating the metabolizable energy content of novel carbohydrates alone, without the interferences from dietary matrix components. This is important information when developing food products. These substrates, especially the second generation SCFs, were shown to have low caloric content, making them potential candidates for inclusion in low calorie foodstuffs.

In conclusion, the SCFs evaluated varied widely in their sugar composition and physiological responses. Overall, all SCFs exhibited varying degrees of resistance to hydrolytic digestion and, consequently, had attenuated glycemic responses and lower caloric content than the Malt control. The second generation series of SCFs, on average, had lower digestibilities, glycemic responses, and TME_n values than did the SCFs from the first generation series. The variation noted among carbohydrates in physiological responses was due largely to the individual carbohydrate molecular structure and bonding pattern and was influenced greatly by the production methods used. Along with the beneficial characteristics evaluated above, it is important for ingredients to be well tolerated. Stewart et al. (2010) found a SCF similar to SCF 2A to be well-tolerated when supplemented at 12 g/d. Low scores in bloating, cramping, flatulence, and stomach noises were observed when supplementing SCF (Stewart et al, 2010). Sanders et al. (2008) also reported SCF to be well tolerated at doses of 5, 15, and 25 g. The beneficial physiological characteristics observed would make SCFs candidate ingredients for incorporation into reduced glycemic and caloric foodstuffs.

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Table 4-1. Free sugar and hydrolyzed monosaccharide concentrations of soluble corn fibers: 1st generation series

Item	Test carbohydrate ¹			
	HCl corn syrup	SCF gen 1	SCF spray-dried	SCF hydrogenated
Free sugars, mg/g ²				
Arabinose	0.03	0.44	0.05	5.55
Fructose	0.58	0.00	0.00	36.49
Galactose	0.00	0.00	0.00	8.73
Glucose	67.93	36.72	27.47	4.58
Mannose	0.00	0.49	0.00	0.00
Rhamnose	0.49	0.00	0.53	0.00
Sorbitol	0.02	0.04	0.02	25.21
Sucrose	0.72	1.45	0.78	42.03
Xylose	0.00	0.00	0.00	0.03
Total, mg/g ³	69.77	39.14	28.86	122.62
Hydrolyzed monosaccharides, mg/g ^{2,4}				
Glucose	709.01	968.64	1,015.31	793.26

Table 4-1 (con't.)

Mannose	0.00	5.03	7.16	3.96
Sorbitol	0.00	0.00	0.00	177.27
Total, mg/g ³	709.01	973.67	1,022.48	974.48

¹ Abbreviations: HCl corn syrup, product from hydrochloric acid-catalyzed condensation of corn syrup; SCF gen 1, generation 1 soluble corn fiber; SCF spray-dried, spray-dried version of generation 1 soluble corn fiber; SCF hydrogenated, hydrogenated version of SCF spray-dried.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

⁴ Values are corrected for free monosaccharide concentrations.

Table 4-2. Free sugar and hydrolyzed monosaccharide concentrations of soluble corn fibers: 2nd generation series

Item	Test carbohydrate ¹							
	SCF 2 B1	SCF low sugar	SCF phos (l)	SCF phos (p)	SCF HCl (l)	SCF HCl (p)	SCF 2 B2	SCF 2 B3
Free sugars, mg/g ²								
Arabinose	0.46	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Fructose	11.93	0.70	7.25	5.06	4.39	4.87	4.40	7.96
Galactose	0.09	0.00	0.00	0.00	0.00	0.00	0.11	0.15
Glucose	127.19	21.22	92.01	114.48	54.56	58.32	90.10	53.32
Mannose	0.72	0.00	0.45	0.46	0.41	0.46	0.59	0.65
Rhamnose	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorbitol	1.00	0.17	0.32	0.28	0.00	0.11	0.73	0.22
Sucrose	0.00	0.82	0.00	0.00	0.00	0.00	0.08	0.00
Total, mg/g ³	141.87	22.95	100.03	120.28	59.36	63.76	96.00	62.30

Table 4-2 (con't.)

Hydrolyzed monosaccharides, mg/g ^{2,4}								
Glucose	489.51	1,084.72	980.87	996.08	996.07	999.81	1,026.52	1,073.63
Mannose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.01
Total, mg/g ³	489.51	1,084.72	980.87	996.08	996.07	999.81	1,026.52	1,074.64

¹ Abbreviations: SCF 2 B1, generation 2 soluble corn fiber batch 1; SCF low sugar, low sugar version of generation 2 soluble corn fiber; SCF phos (l), phosphoric acid-catalyzed condensation of corn syrup liquid ; SCF phos (p), phosphoric acid-catalyzed condensation of corn syrup powder; SCF HCl (l), hydrochloric acid-catalyzed condensation of corn syrup liquid; SCF HCl (p), hydrochloric acid-catalyzed condensation of corn syrup powder; SCF 2 B2, generation 2 soluble corn fiber batch 2; SCF 2 B3 generation 2 soluble corn fiber batch 3.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

⁴ Values are corrected for free monosaccharide concentrations.

Table 4-3. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of soluble corn fibers: 1st generation series

Released monosaccharides, mg/g ²	Test carbohydrate ¹				SEM
	HCL corn syrup	SCF gen 1	SCF spray-dried	SCF hydrogenated	
Glucosamine	0.00 ^a	0.36 ^b	0.00 ^a	5.52 ^c	0.05
Glucose	515.36 ^c	453.74 ^b	484.19 ^{bc}	395.56 ^a	6.82
Isomaltose	14.36 ^b	20.23 ^c	17.85 ^{bc}	0.00 ^a	0.81
Sorbitol	0.00 ^a	0.00 ^a	0.00 ^a	53.64 ^b	0.50
Total, mg/g ³	529.72 ^c	474.33 ^{ab}	502.04 ^{bc}	454.72 ^a	7.65

¹ Abbreviations: HCL corn syrup, product from hydrochloric acid-catalyzed condensation of corn syrup; SCF gen 1, generation 1 soluble corn fiber; SCF spray-dried, spray-dried version of generation 1 soluble corn fiber; SCF hydrogenated, hydrogenated version of SCF spray-dried.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

^{abc} Means in the same row with different superscript letters are different (P<0.05).

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

Released monosaccharides, mg/g ²	Test carbohydrate ¹								SEM
	SCF 2 B1	SCF low sugar	SCF phos (l)	SCF phos (p)	SCF HCl (l)	SCF HCl (p)	SCF 2 B2	SCF 2 B3	
Fructose	20.15 ^f	10.53 ^a	16.56 ^e	12.78 ^{bc}	13.43 ^c	12.13 ^b	14.93 ^d	17.35 ^e	0.30
Galactose	0.52 ^f	0.05 ^a	0.10 ^{ab}	0.13 ^{abc}	0.22 ^{cde}	0.20 ^{bcd}	0.29 ^{de}	0.30 ^e	0.02
Glucose	264.27 ^{cd}	177.09 ^a	383.43 ^f	513.77 ^g	251.27 ^c	276.72 ^d	349.31 ^e	231.62 ^b	3.68
Sorbitol	1.16 ^f	0.00 ^a	0.25 ^c	0.24 ^c	0.13 ^b	0.17 ^b	0.69 ^e	0.40 ^d	0.01
Total, mg/g ³	286.10 ^c	187.66 ^a	400.34 ^e	526.93 ^f	265.04 ^b	289.21 ^c	365.21 ^d	249.68 ^b	3.63

¹ Abbreviations: SCF 2 B1, generation 2 soluble corn fiber batch 1; SCF low sugar, low sugar version of generation 2 soluble corn fiber; SCF phos (l), phosphoric acid-catalyzed condensation of corn syrup liquid ; SCF phos (p), phosphoric acid-catalyzed

condensation of corn syrup powder; SCF HCl (l), hydrochloric acid-catalyzed condensation of corn syrup liquid; SCF HCl (p), hydrochloric acid-catalyzed condensation of corn syrup powder; SCF 2 B2, generation 2 soluble corn fiber batch 2; SCF 2 B3 generation 2 soluble corn fiber batch 3.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

^{abcdefg} Means in the same row with different superscript letters are different (P<0.05).

Table 4-5. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble corn fibers: 1st generation series

Item	Test carbohydrate ¹					SEM ²
	Malt	HCL corn syrup	SCF gen 1	SCF spray-dried	SCF hydrogenated	
AUC for glucose, mmol/L	157.16 ^c	121.15 ^b	98.20 ^b	98.90 ^b	34.04 ^a	18.15
RGR, %	100.00 ^c	80.94 ^b	60.46 ^b	62.79 ^b	14.85 ^a	10.20
AUC for insulin, pmol/L	10,165.00 ^c	4,758.15 ^b	4,328.04 ^b	4,241.59 ^b	935.61 ^a	1,640.88
RIR, %	100.00 ^c	58.33 ^b	43.83 ^b	48.55 ^b	10.66 ^a	8.96

¹ Abbreviations: Malt, maltodextrin; HCl corn syrup, product from hydrochloric acid-catalyzed condensation of corn syrup; SCF gen 1, generation 1 soluble corn fiber; SCF spray-dried, spray-dried version of generation 1 soluble corn fiber; SCF

hydrogenated, hydrogenated version of SCF spray-dried.

² Pooled standard error of the mean.

^{abc} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 4-6. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble corn fibers: 2nd generation series

Item	Test carbohydrate ¹									SEM ²
	Malt	SCF 2 B1	SCF low sug	SCF phos (l)	SCF phos (p)	SCF HCl (l)	SCF HCl (p)	SCF 2 B2	SCF 2 B3	
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, pmol/L	10,814.00 ^d	4,942.85 ^a	4,137.45 ^a	6,409.85 ^a	7,011.85 ^a	3,735.45 ^a	3,031.43 ^a	3,641.70 ^a	4,095.10 ^a	1,654.49
RIR, %	100.00 ^c	26.16 ^a	21.22 ^a	43.04 ^{ab}	56.52 ^b	31.03 ^{ab}	24.74 ^a	36.57 ^{ab}	44.47 ^{ab}	8.26

¹ Abbreviations: SCF 2 B1, generation 2 soluble corn fiber batch 1; SCF low sugar, low sugar version of generation 2 soluble corn fiber; SCF phos (l), phosphoric acid-catalyzed condensation of corn syrup liquid ; SCF phos (p), phosphoric acid-catalyzed

condensation of corn syrup powder; SCF HCl (l), hydrochloric acid-catalyzed condensation of corn syrup liquid; SCF HCl (p), hydrochloric acid-catalyzed condensation of corn syrup powder; SCF 2 B2, generation 2 soluble corn fiber batch 2; SCF 2 B3 generation 2 soluble corn fiber batch 3.

² Pooled standard error of the mean.

^{abcd} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 4-7. True metabolizable energy (TME_n) values for soluble corn fibers: 1st generation series

Item	Test carbohydrate ¹					SEM ²
	Malt	HCL corn syrup	SCF gen 1	SCF spray-dried	SCF hydrogenated	
Grams dosed, dry matter basis	14.3	26.7	13.3	14.6	14.7	
TME _n , kcal/g	4.06 ^d	1.91 ^a	2.35 ^b	2.30 ^b	3.03 ^c	0.10

¹ Abbreviations: HCL corn syrup, product from hydrochloric acid-catalyzed condensation of corn syrup; SCF gen 1, generation 1 soluble corn fiber; SCF spray-dried, spray-dried version of generation 1 soluble corn fiber; SCF hydrogenated, hydrogenated version of SCF spray-dried.

² Pooled standard error of the mean.

^{abcd} Means in the same row with different superscript letters are different (P<0.05).

Table 4-8. True metabolizable energy (TME_n) values for soluble corn fibers: 2nd generation series.

Item	Test carbohydrate ¹									SEM
	Malt	SCF 2 B1	SCF low sug	SCF phos (l)	SCF phos (p)	SCF HCl (l)	SCF HCl (p)	SCF 2 B2	SCF 2 B3	
Grams dosed, dry matter basis	14.3	26.8	27.0	25.6	26.9	25.4	27.9	23.9	23.7	
TME _n , kcal/g	4.06 ^d	1.96 ^{bc}	1.58 ^{ab}	1.75 ^b	2.30 ^c	1.82 ^b	1.76 ^b	1.85 ^b	1.31 ^a	0.10

¹ Abbreviations: SCF 2 B1, generation 2 soluble corn fiber batch 1; SCF low sugar, low sugar version of generation 2 soluble corn fiber; SCF phos (l), phosphoric acid-catalyzed condensation of corn syrup liquid ; SCF phos (p), phosphoric acid-catalyzed condensation of corn syrup powder; SCF HCl (l), hydrochloric acid-catalyzed condensation of corn syrup liquid; SCF HCl (p), hydrochloric acid-catalyzed condensation of corn syrup powder; SCF 2 B2, generation 2 soluble corn fiber batch 2; SCF 2 B3 generation 2 soluble corn fiber batch 3.

² Pooled standard error of the mean.

^{abcd} Means in the same row with different superscript letters are different (P<0.05).

Figure 4-1. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble corn fibers: 1st generation series. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (0.14), HCl corn syrup (0.32), SCF gen 1(0.34), SCF spray-dried (0.30), and SCF hydrogenated (0.27). Abbreviations: HCl corn syrup, product from hydrochloric acid-catalyzed condensation of corn syrup; SCF gen 1, generation 1 soluble corn fiber; SCF spray-dried, spray-dried version of generation 1 soluble corn fiber; SCF hydrogenated, hydrogenated version of SCF spray-dried.

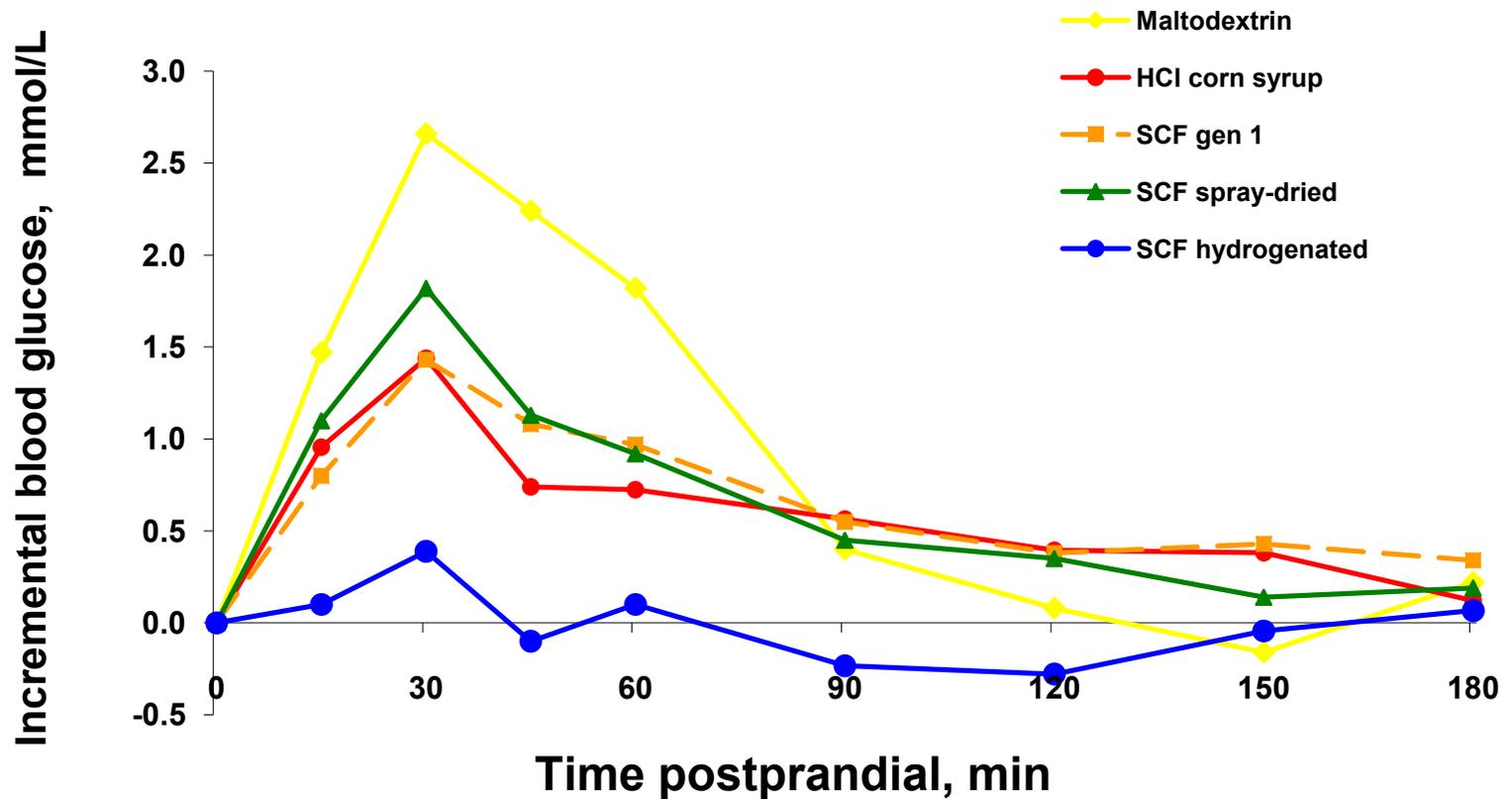


Figure 4-2. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble corn fibers: 1st generation series. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (13.36), HCl corn syrup (25.38), SCF gen 1(25.38), SCF spray-dried (23.21), and SCF hydrogenated (25.38). Abbreviations: HCl corn syrup, product from hydrochloric acid-catalyzed condensation of corn syrup; SCF gen 1, generation 1 soluble corn fiber; SCF spray-dried, spray-dried version of generation 1 soluble corn fiber; SCF hydrogenated, hydrogenated version of SCF spray-dried.

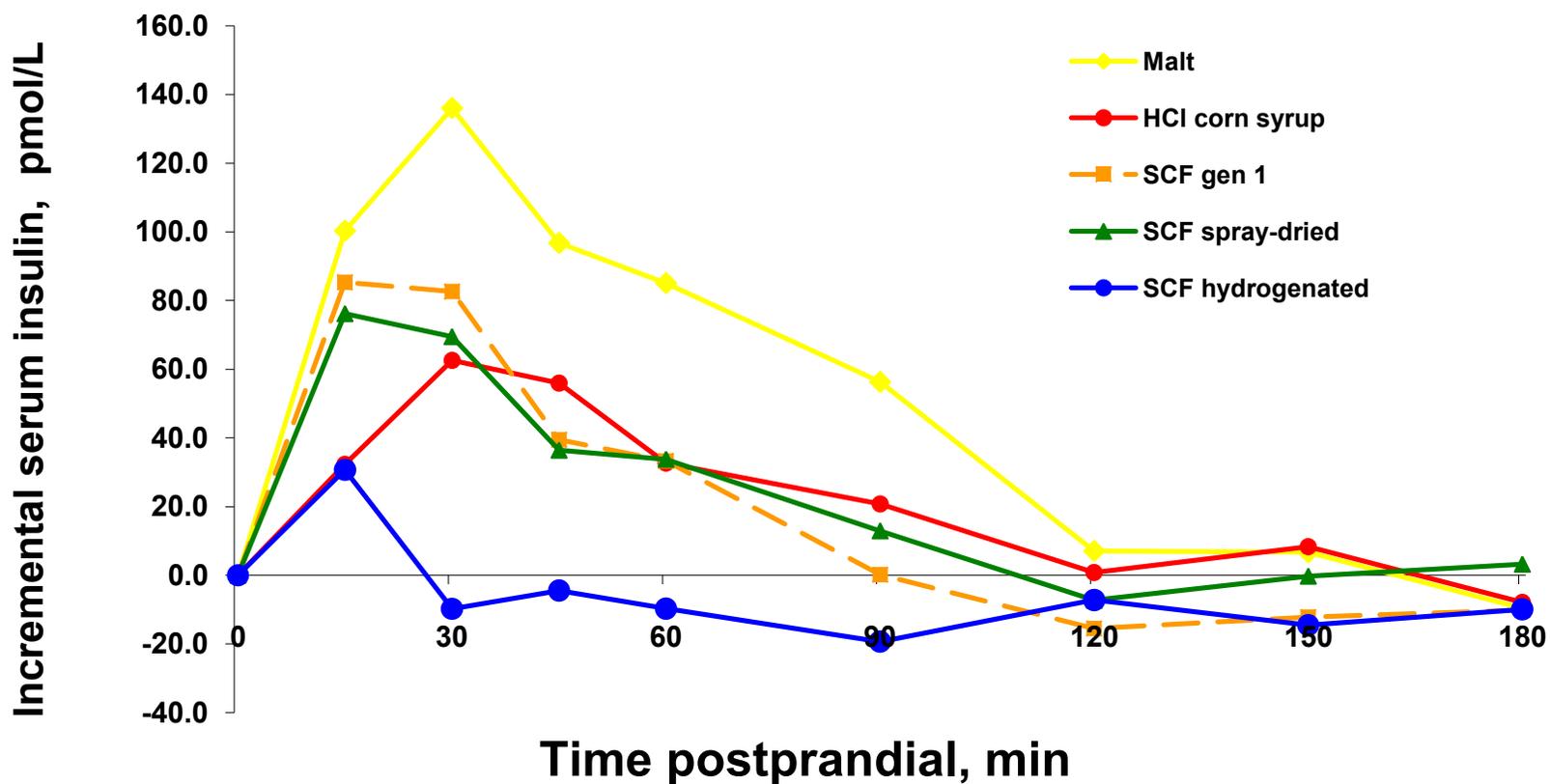


Figure 4-3. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble corn fibers: 2nd generation series. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (0.19), SCF 2A (0.28), SCF low sug (0.24), SCF phos (l) (0.33), SCF phos (p) (0.32), SCF HCl (l) (0.30), SCF HCl (p) (0.32), SCF 2B (l) (0.29), and SCF 2B (p) (0.29). Abbreviations: SCF 2 B1, generation 2 soluble corn fiber batch 1; SCF low sugar, low sugar version of generation 2 soluble corn fiber; SCF phos (l), phosphoric acid-catalyzed condensation of corn syrup liquid ; SCF phos (p), phosphoric acid-catalyzed condensation of corn syrup powder; SCF HCl (l), hydrochloric acid-catalyzed condensation of corn syrup liquid; SCF HCl (p), hydrochloric acid-catalyzed condensation of corn syrup powder; SCF 2 B2, generation 2 soluble corn fiber batch 2; SCF 2 B3 generation 2 soluble corn fiber batch 3.

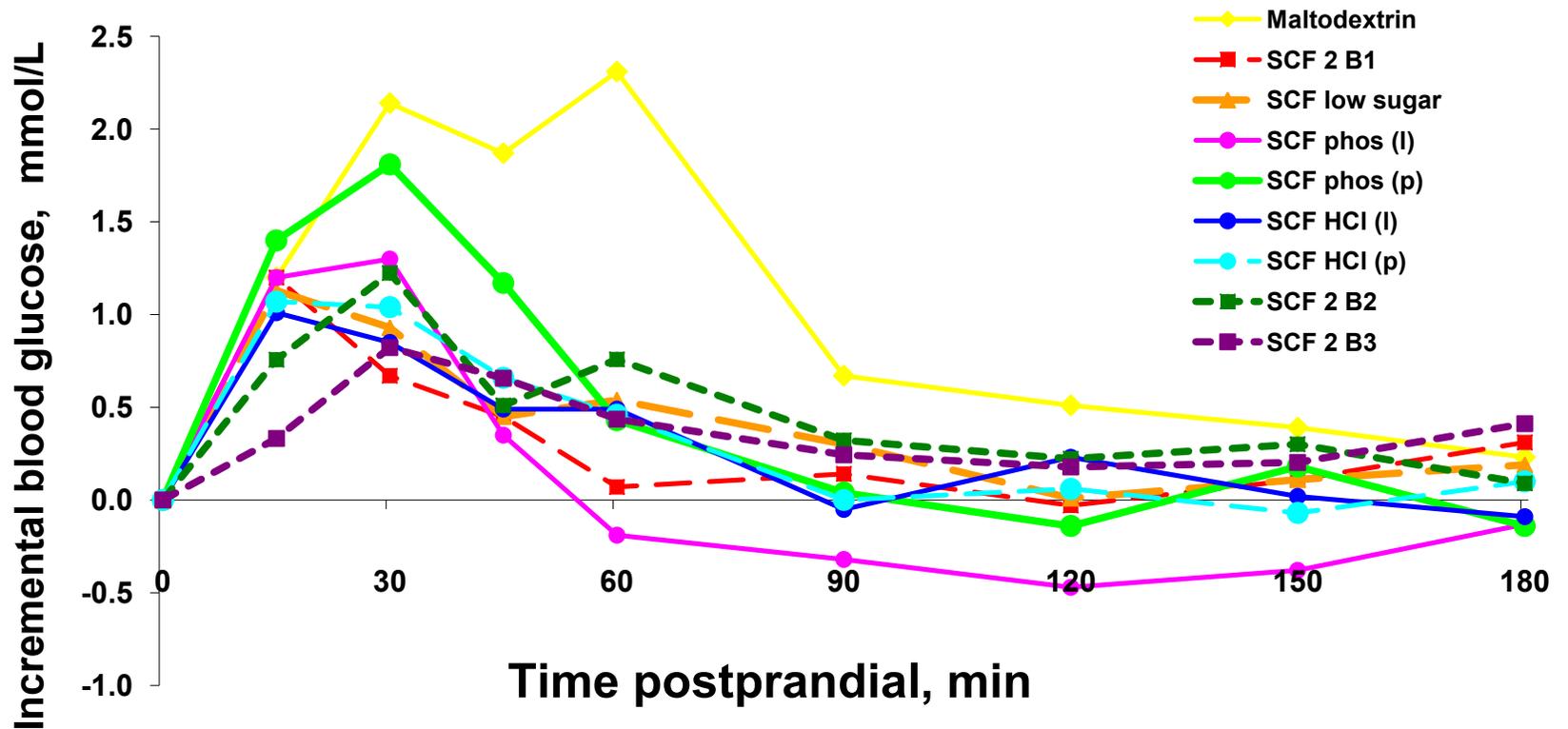
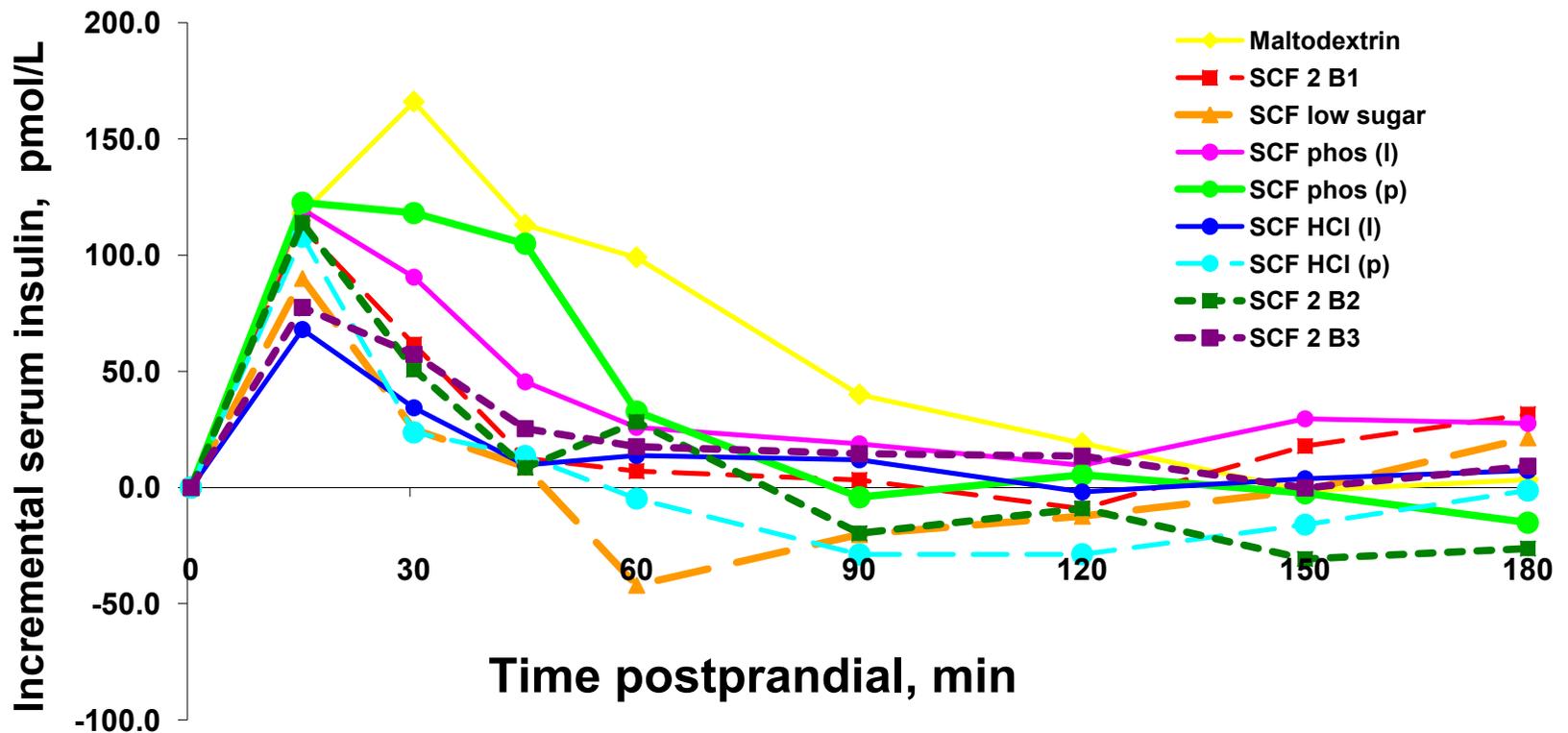


Figure 4-4. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble corn fibers: 2nd generation series. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (13.36), SCF 2A (23.21), SCF low sug (23.21), SCF phos (l) (23.21), SCF phos (p) (23.21), SCF HCl (l) (23.21), SCF HCl (p) (23.21), SCF 2B (l) (23.21), and SCF 2B (p) (23.21). Abbreviations: SCF 2 B1, generation 2 soluble corn fiber batch 1; SCF low sugar, low sugar version of generation 2 soluble corn fiber; SCF phos (l), phosphoric acid-catalyzed condensation of corn syrup liquid ; SCF phos (p), phosphoric acid-catalyzed condensation of corn syrup powder; SCF HCl (l), hydrochloric acid-catalyzed condensation of corn syrup liquid; SCF HCl (p), hydrochloric acid-catalyzed condensation of corn syrup powder; SCF 2 B2, generation 2 soluble corn fiber batch 2; SCF 2 B3 generation 2 soluble corn fiber batch 3.



**CHAPTER 5: BLENDING OF SOLUBLE CORN FIBER WITH PULLULAN,
SORBITOL, OR FRUCTOSE ATTENUATES GLYCEMIC AND INSULINEMIC
RESPONSES IN THE DOG AND AFFECTS HYDROLYTIC DIGESTION *IN VITRO***

ABSTRACT: The objective of these experiments was to measure in vitro hydrolytic digestion and glycemic and insulinemic responses of select carbohydrate blends, all containing the novel carbohydrate, soluble corn fiber (SCF). Two SCFs that varied in their method of production were used to formulate the carbohydrate blends. One set of blends contained a SCF that was spray-dried (SCFsd), then blended with different amounts of either pullulan (Pull), sorbitol (Sorb), or fructose (Fruct). The other set of blends contained a SCF produced using a different production method (SCF), then blended with different ratios of Pull, Sorb, and Fruct (SCF:Pull:Sorb:Fruct blends). All blends had low to intermediate free sugar concentrations except those with higher percentages of Fruct and Sorb. The addition of higher amounts of Fruct and Sorb increased the free Fruct and Sorb concentrations of the blends. Glucose was the primary bound monosaccharide in the blends. The SCFsd blends had intermediate to high amounts of monosaccharides released as a result of in vitro hydrolytic digestion. The SCFsd:Pull blends were more digestible *in vitro* (~91%) than SCFsd:Fruct or SCFsd:Sorb. Total released monosaccharides were higher in SCFsd blends containing either 50% Fruct or Sorb, but the combination resulted in lower concentrations of glucose released. The SCF:Pull:Sorb:Fruct blends also had intermediate to high released monosaccharides as a result of in vitro hydrolytic digestion. The 30:30:20:20 SCF:Pull:Sorb:Fruct blend was the most digestible (~85%), but also released the highest concentrations of Fruct and Sorb. All SCF blends resulted in low glycemic and insulinemic

responses compared to the maltodextrin (Malt) control. Blends containing Fruct and Sorb were most effective in attenuating the glyceamic and insulinemic responses. The blending of SCFs with Pull, Fruct, and Sorb resulted in mixtures varying in degree of resistance to digestion, but all blends were successful in attenuating postprandial glucose and insulin concentrations. These blends may prove beneficial as components of low glyceamic foodstuffs.

INTRODUCTION

Metabolic disorders such as type 2 diabetes, obesity, hypertension, cardiovascular disease, and cancer are major global health problems today. In the United States alone, diabetes is estimated to affect 7% of the population (Blonde, 2007). Controlling postprandial glyceamia has been shown to be of great importance in the management of diabetes (American Diabetes Association, 2009). Low glyceamic diets have been shown to help control postprandial glucose and insulin concentrations in diabetics (Livesey et al., 2008; Riccardi et al., 2008). Decreasing the glyceamic response also may play a role in improving risk factors associated with heart disease and diabetes (Livesey et al., 2008; Riccardi et al., 2008). This has led to a demand for the development of novel carbohydrates that have the ability to attenuate glyceamic and insulinemic responses and that may be incorporated easily into a wide array of food matrices.

A class of carbohydrates that has the potential to improve glyceamic control is low-digestible carbohydrates. Low-digestible carbohydrates represent a heterogeneous group of compounds that are partially unavailable to digestive enzymes so are either incompletely or not absorbed from the small intestine (Murphy, 2001; Scheppach et al., 2001; Grabitske et

al., 2008). Low-digestible carbohydrates include polyols, resistant starch, fructooligosaccharides, and other oligosaccharides (Marteau and Flourie, 2001; Murphy, 2001; Scheppach et al., 2001; Grabitske et al., 2008). Factors restricting digestion and absorption of low-digestible carbohydrates are the impaired hydrolysis of constitutive bonds by enzymes and the degree of absorption in the small intestine (Marteau and Flourie, 2001).

Soluble corn fibers (SCF) are types of novel, low-digestible carbohydrates. The different processes used to produce SCFs result in fractions with varying degrees of digestion resistance due to increased α -1,6 glycosidic bonds formed during their production. Research conducted on various forms of SCF found them to have low free sugar concentrations, increased resistance to in vitro hydrolytic digestion, and low true metabolizable energy values (Knapp et al. unpublished data).

Pullulan is a naturally occurring fermentation product produced by *Aureobasidium pullulans*, a black yeast found widely throughout nature (Wolf et al., 2003; Shingel, 2004). 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 (Kimoto et al., 1997; Leathers, 2003). The stair-step structure resulting from glycosidic linkages in Pull hinders hydrolysis by enzymes, making them low-digestible carbohydrates (Wolf et al., 2003).

Fructose is a naturally occurring sugar and is the sweetest of all the natural sugars. Fructose is a six-carbon monosaccharide similar to that of glucose. Fructose differs from glucose by the presence of a keto group attached to carbon 2 of the molecule, while glucose has an aldehyde group at carbon 1. Fructose exhibits a synergy with other sweeteners

present in a formulation and allows for a product to have a higher total level of sweetness without increasing the total level of sweetener (Hanover and White, 1993). Energy reduction, simple-sugar reduction, and flavor profile enhancement are three popular formulation trends that fructose can impact favorably (Hanover and White, 1993). Fructose also is widely used in food products for diabetics as it is rapidly removed from the portal blood by the liver and enters the peripheral circulation in only small amounts. The active hepatic enzyme system extracts fructose into the liver. This process is insulin-independent (White and Osberger, 2001). Sorbitol is a sugar found in many natural sources including fruits such as plums, cherries, apples, and numerous other plants.

Sorbitol is a six-carbon alcohol approximately 60% as sweet as sucrose and is soluble in water (Le and Mulderrig, 2001). Sorbitol is slowly absorbed into the body from the gastrointestinal tract and is metabolized by the liver, largely as fructose. Sorbitol is absorbed and metabolized in the liver by a pathway located entirely in the cytoplasmic compartment (Le and Mulderrig, 2001). Ingestion of sorbitol does not usually result in an immediate demand for extra insulin and the initial steps in the metabolism of sorbitol in the liver, uptake by the liver cells, and conversion to glucose are independent of insulin (Le and Mulderrig, 2001).

Evaluation of glycemic and insulinemic responses to individual carbohydrates is beneficial as it allows for a precise understanding of their potential effects without the interference of other dietary components. Soluble corn fibers have a lower digestibility and attenuated glycemic and insulinemic responses (Knapp et al. unpublished data), but blending SCFs with other carbohydrates that resist digestion and (or) do not elicit a glycemic response, such as Pull, Fruct, and Sorb, have the potential to further attenuate

glycemic and insulinemic responses. As the concentrations of these carbohydrate increase in the blend, glycemic and insulinemic responses would be expected to drop in order to create ideal low-glycemic carbohydrate blends. The objective of the study was to evaluate select blends of SCFs with Pull, Fruct, and Sorb for in vitro hydrolytic digestion as well as glycemic and insulinemic responses using a dog model.

MATERIALS AND METHODS

Substrates:

Carbohydrate blends incorporating SCFs were evaluated. The production of SCFs involves isolating an oligosaccharide-rich fraction from corn syrup. During cornstarch hydrolysis, an aqueous stream comprised of dextrose, fructose, and a mixture of oligosaccharides results (Harrison and Hoffman, 2007). The aqueous solution is nanofiltered, resulting in a monosaccharide and an oligosaccharide-rich stream. Once oligosaccharide-rich syrup has been formed, it can be treated with an isomerization enzyme such that some of the dextrose present in the syrup is converted to fructose, producing an isomerized oligosaccharide-rich stream (Harrison and Hoffman, 2007). The syrup is subjected to a catalyst(s) that creates reversion products where the residual monosaccharides in the syrup become covalently bonded to oligosaccharides or other monosaccharides present in the syrup, thus increasing α -1,6 glycosidic bond formation in the structure (Harrison and Hoffman, 2007). The last steps in the production process include decolorization of the syrup and evaporation and drying to produce a powder.

Two sets of blends containing different SCFs were evaluated. The SCFs used in the blends evaluated were produced using the basic steps outlined above, but differed in specific

production steps. One set included a SCF that was spray-dried (SCFsd). The other included a SCF that was produced using a process involving longer evaporation times than occurred for SCFsd (SCF). The carbohydrates blended with SCFsd and SCF included fructose (Fruct), sorbitol (Sorb), and pullulan (Pull).

Seven blends were formulated using SCFsd with different percentages of Pull, Fruct, or Sorb. The SCFsd blends included four with varying percentages of Fruct: SCFsd with 5% Fruct (95:5 SCFsd:Fruct), SCFsd with 15% Fruct (85:15 SCFsd:Fruct), SCFsd with 30% Fruct (70:30 SCFsd:Fruct), and SCFsd with 50% Fruct (50:50 SCFsd:Fruct). Two blends included SCFsd and Pull: SCFsd with 30% Pull (70:30 SCFsd:Pull) and SCFsd with 50% Pull (50:50 SCFsd:Pull). One blend was SCFsd with 50% Sorb (50:50 SCFsd:Sorb).

The second set of blends consisted of four blends using SCF with different ratios of Pull, Sorb, and Fruct: SCF blended with 10% Pull, 10% Sorb, and 10% Fruct (70:10:10:10 SCF:Pull:Sorb:Fruct), SCF blended with 20% Pull, 10% Sorb, and 10% Fruct (60:20:10:10 SCF:Pull:Sorb:Fruct), SCF blended with 30% Pull, 10% Sorb, and 10% Fruct (50:30:10:10 SCF:Pull:Sorb:Fruct), and SCF blended with 30% Pull, 20% Sorb, and 20% Fruct (30:30:20:20 SCF:Pull:Sorb:Fruct). The Pull used in the blends had a molecular weight (MW) of 250,000 and was produced by Hayashibara Company Ltd. (Okayama, Japan). Soluble corn fibers, sorbitol, and fructose were produced by Tate and Lyle (Decatur, IL). All blends were formulated and prepared by Tate & Lyle (Decatur, IL).

Chemical Analyses:

Carbohydrates were analyzed for dry matter (DM) and organic matter (OM) according to AOAC (2000), and for free and hydrolyzed monosaccharide concentrations.

Test carbohydrates were hydrolyzed using the procedure described by Hoebler et al. (1989) where carbohydrates were subjected to hydrolysis with H₂SO₄. 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 μ L . All assays were conducted using a CarboPac PA-1 column and guard column following methods cited by Smiricky et al. (2002).

In Vitro Hydrolytic Digestion:

Approximately 200 mg of each carbohydrate or carbohydrate blend were weighed in triplicate and incubated with 2 mL of a pepsin/hydrochloric acid solution and 2 mL of an enzyme solution consisting of amyloglucosidase and α -amylase to simulate gastric and small intestinal digestion (Muir et al., 1993). The samples were analyzed for free released monosaccharides using HPLC (Smiricky et al., 2002) 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 ± 4.8 kg, and a mean age of 5.6 ± 2.4 years were used. Dogs were housed individually in 1.2 x 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 non-destructible 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 consumed 25 g of carbohydrate (DM basis) in approximately 240 mL dd 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 cc syringe (without needle) and offered to 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 Malt 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 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 $1,240 \times g$ for 10 min and serum stored at $-20\text{ }^{\circ}\text{C}$ 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 (Cass et al., 1984). 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) (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 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: $[(\text{AUC for test carbohydrate}) / (\text{AUC for control})] \times 100\%$.

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 effect 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.

RESULTS AND DISCUSSION

Free Sugar and Hydrolyzed Monosaccharide Concentrations:

Free sugar and hydrolyzed monosaccharide concentrations for the SCFsd blends are presented in Table 5-1. Free sugar concentrations varied among the blends, ranging from approximately 1 to 50%. The two Pull blends (70:30 SCFsd:Pull and 50:50 SCFsd:Pull) had the lowest free sugar concentrations of all the blends tested and were similar in free sugar concentrations to SCFsd alone. The blends with 50% Fruct and 50% Sorb had the highest free sugar concentrations (43-53%) The SCFsd blends with 5, 15, and 30% Fruct had 11-23% free sugar concentrations.

Fructose and glucose were the major free sugars found in SCFsd blends with the exception of the 50:50 SCFsd:Sorb blend where sorbitol was the primary free sugar present. Glucose was the primary free sugar for substrates 70:30 SCFsd:Pull, 50:50 SCFsd:Pull, and SCFsd, but the concentrations were low (~2.3%). In blends where Fruct was added to

SCFsd, Fruct was the free sugar found in highest concentration, and concentrations increased in proportion to the percentage of Fruct added.

Hydrolyzed monosaccharide concentrations (Table 5-1) varied slightly among the SCFsd blends. The majority of the blends were completely hydrolyzed to their monosaccharide components. The blends with higher percentages of Fruct or Sorb had lower hydrolyzed monosaccharide concentrations due to their higher free sugar concentrations. Glucose accounted for most of the hydrolyzed monosaccharides for SCFsd and all the SCFsd blends.

Free sugar content (Table 5-2) was similar among the SCF:Pull:Sorb:Fruct blends except the 30:30:20:20 SCF:Pull:Sorb:Fruct blend that had the highest Fruct, Sorb, and total free sugar concentrations. Free Fruct and Sorb increased and glucose decreased with addition of Sorb and Fruct to SCF.

Hydrolyzed monosaccharide concentrations (Table 5-2) for the SCF:Pull:Sorb:Fruct blends followed similar trends to the SCFsd blends where higher percentages of Fruct and Sorb resulted in lower hydrolyzed monosaccharide concentrations. Soluble corn fiber had a low hydrolyzed monosaccharide concentration along with a low free sugar concentration. It is likely that SCF contained a major portion of bound Fruct that would account for the low hydrolyzed monosaccharide concentration. The hydrolyzed monosaccharide assay destroys fructose so it not included in the total hydrolyzed monosaccharide value. The main monosaccharide found in SCF and all the blends was glucose. Minor amounts of Sorb were present in three of the SCF:Pull:Sorb:Fruct blends.

Free sugar and hydrolyzed monosaccharide data are important in an evaluation of substrates that may be included in glycemic foodstuffs. The free sugars found in these substrates will be rapidly digested upon consumption and, thus, may have a major impact on the postprandial glycemic response. Overall, the free sugar concentrations found in the individual SCFsd and SCF substrates were low, but varied. Soluble corn fiber spray-dried had a lower free sugar concentration compared to SCF (2.8 vs 14.2%). Glucose was the main free sugar found in both SCFsd and SCF, but at different concentrations (2.7 vs 12.7%, respectively). Fructose was present in SCF, whereas no free fructose was found in SCFsd.

The 50:50 SCFsd:Pull blend resulted in a higher glucose and total free sugar concentration compared to the 70:30 SCFsd:Pull blend due to the small amounts of free glucose associated with Pull. The addition of Pull to the SCF:Pull:Sorb:Fruct blends resulted in lower free glucose concentrations compared to SCF alone. As the percentage of Pull increased and replaced SCF in the SCF:Pull:Sorb:Fruct blends, glucose concentrations decreased. Overall, addition of Pull, even at high concentrations, did not significantly increase free sugar values. Pullulan has been reported to contain very low free sugar concentrations (Knapp et al., 2010).

Increased amounts of Fruct and Sorb decreased the free glucose concentrations in the SCFsd blends while increasing free sugar concentrations of Fruct and Sorb. The increased Fruct and Sorb concentrations resulted in increased total free sugar concentrations. This same trend was noted for the SCF:Pull:Sorb:Fruct blends. Increased concentrations of Fruct and Sorb (from 10 to 20%) decreased free glucose while increasing free Fruct and Sorb. The Fruct and Sorb added to the blends were available as free sugars and could have a major impact on glycemic response.

The hydrolyzed monosaccharides constitute the building blocks of carbohydrate polymers and provide an indication of what sugars are potentially available for digestion. The hydrolyzed monosaccharide concentration (especially glucose) of SCF was lower compared to SCFsd. Data indicate that SCF has a much greater concentration of bound Fruct than SCFsd. The bound Fruct could have a major impact on the glycemic and insulinemic responses of these substrates. This difference in carbohydrate content is likely due to production method used.

The SCFsd:Pull blends are high in bound glucose. The SCFsd:Fruct and SCFsd:Sorb blends are lower in hydrolyzed monosaccharide concentrations due to higher concentrations of free Fruct and Sorb. The different concentrations of Pull in the SCF:Pull:Sorb:Fruct blends had little impact on hydrolyzed monosaccharide values. Addition of Pull at 10, 20, or 30% resulted in similarly high hydrolyzed monosaccharide values. As noted for the SCFsd blends, higher concentrations of Sorb and Fruct decreased hydrolyzed monosaccharide values. Depending on their digestibility, glucose monomers may become available and have an impact on the glycemic response.

In Vitro Hydrolytic Digestion:

Monosaccharides released as a result of simulated hydrolytic digestion of the SCFsd blends are presented in Table 5-3. Soluble corn fiber spray-dried had an approximate monosaccharide digestibility value of 50%. The blends with the lowest percentages of Fruct added (95:5 SCFsd and 85:15 SCFsd:Fruct) had similar monosaccharide digestibility values as SCFsd. The blends containing 30 and 50% Fruct or Sorb had higher ($P < 0.05$)

monosaccharide digestibility values (~ 75%). The Pull blends, 70:30 SCFsd:Pull and 50:50 SCFsd:Pull, had similar but higher ($P<0.05$) digestibility values (~ 91%).

Glucose was the sugar released in the greatest concentration except for the blends that had 50% Fruct or 50% Sorb added. Released glucose concentrations were increased ($P<0.05$) by addition of 30 or 50% Pull to SCFsd and had the highest ($P<0.05$) glucose concentrations among all blends. Fructose was found to be released in all the blends. As the percentage of Fruct was increased in the blends, the concentration of Fruct released increased, with the 50:50 SCFsd:Fruct blend having the highest ($P<0.05$) amount of Fruct released. The 50:50 SCFsd:Sorb was the only blend with a significant amount of Sorb released (~43% of released sugars); all other blends had little to no sorbitol released. Isomaltose was released in low concentrations in SCFsd, 85:15 SCFsd:Fruct, and 50:50 SCFsd:Fruct.

Data reporting released monosaccharide values from simulated hydrolytic digestion of the SCF:Pull:Sorb:Fruct blends are presented in Table 5-4. Soluble corn fiber had the lowest ($P<0.05$) amount of monosaccharides released after simulated digestion (~29%). The 30:30:20:20 SCF:Pull:Sorb:Fruct blend had the highest ($P<0.05$) concentration of released monosaccharides (~85%). The remaining blends were intermediate (~60%) in digestibility. Total released monosaccharide concentrations increased as the percentage of SCF decreased in the blends.

The major monosaccharide released from SCF and all the SCF:Pull:Sorb:Fruct blends was glucose. Soluble corn fiber had the lowest ($P<0.05$) concentration of glucose released. The concentrations of glucose released increased as the percentage of Pull

increased in the blends. Released Fruct was similar among blends except for the 30:30:20:20 SCF:Pull:Sorb:Fruct blend that had the highest ($P<0.05$) concentration of released Fruct. This same trend was noted for the concentrations of released Sorb.

The SCFs used in both sets of blends demonstrated a resistance to hydrolytic digestion, with SCF having an increased resistance compared to SCFsd (29 vs 50% digestible, respectively). Kendall et al. (2008) evaluated a SCF similar to the ones used in the blends and reported the in vitro digestibility was 14.5% which is lower than either of the SCFs tested in the current study. Differences in production methods likely affected digestibility of the different SCFs evaluated. The ability of the SCFs to resist hydrolytic digestion was due, in part, to the linkages found in their molecular structure. The presence of α -1,6 glycosidic linkages and inaccessibility of the α -1,4 linkages increase digestive enzymatic resistance in SCFs (Kendall et al., 2008).

Blending Pull with either of the SCFs resulted in increased released monosaccharide concentrations, especially glucose. In both sets of blends, the higher the percentage of Pull in the blend, the higher the digestibility compared to SCF alone. These data indicate that Pull was digestible by hydrolytic enzymes and resulted in glucose release. Knapp et al. (2010) and Wolf et al. (2003) found pullulans of different molecular weights to be extensively hydrolyzed in vitro. However, Wolf et al. (2003) found the hydrolysis to occur slowly over time and concluded that Pull was a slowly digestible carbohydrate with the potential to positively impact glycemic response.

Fructose and Sorb blended with the SCFs increased total released monosaccharide concentrations after hydrolytic digestion, especially when added at 30 or 50%

concentrations. This increase occurred because the Fruct and Sorb added are completely available as free sugars. However, the concentration of released glucose decreased as Fruct or Sorb concentration in the blend increased.

Glycemic and Insulinemic Responses:

Incremental AUC data for glucose for the SCFsd blends are presented in Figure 5-1, and the corresponding values for AUC and relative glycemic response (RGR) are presented in Table 5-5. Maltodextrin was used as a control for every set of glycemic response tests because it is highly digestible and rapidly absorbed, resulting in a consistently high glycemic response. The carbohydrates were not all evaluated in the same period, so Malt was used in every period to serve as a control to calculate a relative response to the test carbohydrate in all periods.

Maltodextrin served as the control to which all test carbohydrates were compared and was assigned a RGR value of 100. Relative glycemic response values are calculated from AUC values and are directly related, so test carbohydrates with high AUC values will likely have correspondingly high RGR values. The RGR values allow for an easier, more accurate interpretation of the glycemic response as it is a percentage of the Malt response and because carbohydrates were run in a series of tests and not all evaluated in the same period.

All the SCFsd blends had lower ($P<0.05$) AUC and RGR values than did Malt. Only the blends with 30 and 50% Fruct or sorbitol were able to lower ($P<0.05$) the AUC compared to SCFsd. All other blends resulted in similar AUCs compared to SCFsd. The RGR value of SCFsd was 63%, and all blends except the 70:30 SCFsd:Pull blend resulted in

lower ($P<0.05$) RGR values. Blends with 30 or 50% Pull had lower ($P<0.05$) RGRs (avg RGR value of 38) compared to both SCFsd and Malt. A similar RGR (avg RGR value of 33) resulted from the addition of only 5 or 15% Fruct in the blends. The lowest ($P<0.05$) RGR values resulted from the 70:30 SCFsd:Fruct, 50:50 SCFsd:Fruct, and 50:50 SCFsd:Sorb blends. The average RGR of these blends was only 4.8% compared to that of Malt.

The majority of the SCFsd blends resulted in similar blood glucose response patterns. The blends resulted in small to intermediate peaks during the first 30 min of the glycemic response test. All of these peaks were greatly attenuated compared to the high peak produced by the Malt control. Most of the SCFsd blends also showed attenuated peaks during the first 30 min compared SCFsd. After 30 min into the glycemic response test, the blends decreased to near basal blood glucose concentrations for the remainder of the test. The 70:30 SCFsd:Fruct, 50:50 SCFsd:Fruct, and 50:50 SCFsd:Sorb blends had blood glucose patterns different from the other blends. These blends did not result in any major peaks and attenuated the glycemic response to basal or below basal levels throughout the entire glycemic test.

Incremental AUC data for insulin for the SCFsd blends are presented in Figure 5-2, and the corresponding values for AUC and RIR are presented in Table 5-5. Maltodextrin had higher ($P<0.05$) AUC and RIR values than did SCFsd and all the SCFsd blends. Trends in the insulinemic response were similar to those observed in the glycemic response for the SCFsd blends. All the SCFsd blends had similar AUC values to SCFsd. The RIR value of SCFsd was approximately 49 and was similar to all SCFsd blends except the SCFsd blends

with 30 or 50% Fruct and Sorb. These blends had lower ($P<0.05$) RIR values than did SCFsd, with an average RIR value of 10.

Serum insulin patterns for SCFsd and the SCFsd blends were similar to blood glucose patterns. All the SCFsd blends resulted in an attenuated peak insulin response compared to Malt. The 70:30 SCFsd:Fruct, 50:50 SCFsd:Fruct, and 50:50 SCFsd:Sorb blends resulted in concentrations near or below basal insulin concentrations throughout the entire response test. The remaining SCFsd blends all resulted in small peaks during the first 30 min of the response test that dropped rapidly to near basal concentrations for the remainder of the test. The attenuated insulin response of the SCFsd blends resulted from their blunted glycemic response.

Incremental AUC data for glucose for the SCF:Pull:Sorb:Fruct blends are presented in Figure 5-3, and the corresponding values for AUC and RGR are presented in Table 5-6. Maltodextrin had the highest ($P<0.05$) AUC, with SCF and all SCF:Pull:Sorb:Fruct blends resulting in similar but low AUCs for blood glucose. Numerically, the 60:20:10:10 SCF:Pull:Sorb:Fruct and 30:30:20:20 SCF:Pull:Sorb:Fruct treatments resulted in the lowest RGR values (avg RGR value of 6), but were not significantly lower than for SCF (RGR value of 24).

The SCF:Pull:Sorb:Fruct blends resulted in similar blood glucose patterns. All the blends had a small blunted peak at 15 min of the glycemic response test that was attenuated compared with the peak resulting from either Malt or SCF. After the short blunted peak at 15 min, the SCF:Pull:Sorb:Fruct blends resulted in near or below basal concentrations until 150 min into the response test. At this point, all the blends except the 30:30:20:20

SCF:Pull:Sorb:Fruct began to show increasing blood glucose concentrations. The increased concentrations of free Sorb and Fruct in the 30:30:20:20 SCF:Pull:Sorb:Fruct blend resulted in below basal blood glucose concentrations from 45 min into the response test.

Incremental AUC data for insulin for the SCF:Pull:Sorb:Fruct blends are presented in Figure 5-4, and the corresponding values for AUC and RIR are presented in Table 5-6. Malt had a higher ($P < 0.05$) AUC and RIR value than did SCF and all the SCF:Pull:Sorb:Fruct blends. The SCF:Pull:Sorb:Fruct blends resulted in low but similar RIR values (avg RIR value of 20) that were not different from the SCF value. These low insulin response values corresponded to the lower glycemic responses elicited by the SCF:Pull:Sorb:Fruct blends.

Serum insulin patterns were similar among the SCF:Pull:Sorb:Fruct blends. All the blends, except 50:30:10:10 SCF:Pull:Sorb:Fruct, had a small insulin peak at 15 min of the test that then decreased to basal concentrations. The 50:30:10:10 SCF:Pull:Sorb:Fruct blend had a similar small peak, but at 30 min of the response test, before decreasing to basal concentrations. Even though the AUC and RIR values were similar for SCF and the SCF:Pull:Sorb:Fruct blends, the blends showed an attenuated peak in serum insulin at the beginning of the response test compared to SCF.

The SCFs used in both sets of blends partially resisted hydrolytic digestion and attenuated blood glucose concentrations upon consumption. Soluble corn fiber had a lower glycemic response than did SCFsd. Sugar composition and hydrolytic digestion data found SCF to be comprised of less glucose and more fructose and less digestible than SCFsd. These factors likely contributed to SCFs ability to attenuate blood glucose concentrations

more than SCFsd. Kendall et al. (2008) evaluated the glycemic response in humans of SCF incorporated into a beverage. Authors reported an AUC of 28.5 mmol/L which was similar to the AUC (39.1 mmol/L) of SCF reported in the current study. Along with low free sugar concentrations, the reduced glycemic response obtained with SCFs is due to the increased resistance to digestibility from increased glycosidic linkage formation resulting from SCF production methods.

Even though in vitro hydrolytic digestion studies have shown Pull to be nearly completely digested, this novel carbohydrate can attenuate the glycemic response due to its property of being slowly digestible. Wolf et al. (2003) evaluated the glycemic response of a Pull (MW 100,000) in humans and found that it lowered ($P<0.01$) the AUC for glucose by 50% compared to Malt. Knapp et al. (2010) evaluated a set of pullulans of different MWs in dogs and found lower ($P<0.05$) RGR and RIR values compared to Malt. Also, these pullulans successfully blunted the glucose and insulin curves throughout the response test. The addition of Pull reduced the glycemic response compared to Malt at all concentrations, but only 50:50 SCFsd:Pull resulted in a lowering of the glycemic response compared to SCFsd alone.

The addition of Fruct and Sorb in the blends had the greatest impact on glycemic and insulinemic responses. Fructose and Sorb do not acutely raise blood glucose, nor stimulate insulin secretion. Even at concentrations as low as 5% of the blend, Fruct reduced glycemic and insulinemic responses. Several studies in humans and rats have shown that small doses of Fruct are effective in decreasing the glycemic response when supplemented with glucose (Moore et al., 2000; Wolf et al., 2002). Possible reasons for the decreased glycemic response caused by Fruct include fructose-induced malabsorption of carbohydrate or

enhancement of blood glucose uptake by the liver due to the ability of Fruct to stimulate hepatic glucokinase activity (Heacock et al., 2002; Shiota et al., 2002; Segal et al., 2007). This attenuation in blood glucose and serum insulin responses was greatest when Fruct and Sorb were added to the blends at 30 and 50% concentrations. However, consumption of large doses of Fruct and Sorb may have adverse effects as they are incompletely absorbed in the small intestine and their malabsorption can lead to abdominal pains and development of diarrhea (Fernandez-Banares et al., 2009). Consumption of high concentrations of Fruct also have been attributed to other adverse effects such as hypertriglyceridemia and hypercholesterolemia (Wolf et al., 2002; Segal et al., 2007).

In summary, carbohydrate blends tested in this study varied in sugar composition and in degree of resistance to simulated hydrolytic digestion. However, even considering these differences, all SCF blends attenuated glycemic and insulinemic responses. Attenuation of glycemic and insulinemic responses was achieved using a slowly digestible carbohydrate like Pull or sugars such as Fruct and Sorb that do not elicit a glycemic response. Reduced blood glucose and insulin responses have been shown to have several beneficial health effects, especially in diabetic patients (American Diabetes Association, 2007; Livesey et al., 2008; Riccardi et al., 2008). These SCF blends could be utilized to lower postprandial glucose and insulin responses by replacing available carbohydrates in foods with low-digestible carbohydrates (Wolever, 2003).

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Table 5-1. Free sugar and hydrolyzed monosaccharide concentrations of soluble corn fiber spray-dried blends

Item	Test carbohydrate ¹							
	SCFsd	95:5 SCFsd:Fruct	85:15 SCFsd:Fruct	70:30 SCFsd:Fruct	50:50 SCFsd: Fruct	70:30 SCFsd:Pull	50:50 SCFsd:Pull	50:50 SCFsd:Sorb
Free sugars, mg/g ²								
Arabinose	0.05	0.26	0.14	0.16	0.08	0.00	0.21	0.00
Fructose	0.00	62.30	171.47	210.44	415.68	0.91	0.23	0.51
Glucose	27.47	42.60	37.37	18.17	10.98	12.57	30.76	18.25
Mannose	0.00	0.05	0.17	0.00	0.00	0.08	0.11	0.00
Rhamnose	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorbitol	0.02	0.62	0.53	0.02	0.05	0.42	0.68	508.54
Sucrose	0.78	0.39	0.74	0.00	0.00	0.00	0.27	0.25
Total, mg/g ³	28.86	106.22	213.42	228.79	426.79	13.98	32.26	527.55

Table 5-1 (con't.)

Hydrolyzed monosaccharides, mg/g ^{2,4}								
Glucose	1,015.31	1,070.98	957.50	814.04	526.54	1,130.19	1,115.24	595.03
Mannose	7.16	0.00	0.00	4.89	6.62	0.00	0.00	0.00
Sorbitol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total, mg/g ³	1,022.48	1,070.98	957.50	818.93	533.16	1,130.19	1,115.24	595.03

¹ Abbreviations: SCFsd, spray-dried soluble corn fiber; 95:5 SCFsd:Fruct, blend of 95% SCFsd:5% fructose; 85:15 SCFsd:Fruct, blend of 85% SCFsd:15% fructose; 70:30 SCFsd:Fruct, blend of 70% SCFsd:30% fructose; 50:50 SCFsd:Fruct, blend of 50% SCFsd:50% fructose; 70:30 SCFsd:Pull, blend of 70%SCFsd:30% pullulan; 50:50 SCFsd:Pull, blend of 50% SCFsd:50% pullulan; 50:50 SCFsd:Sorb, blend of 50% SCFsd:50% sorbitol.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

⁴ Values are corrected for free monosaccharide concentrations.

Table 5-2. Free sugar and hydrolyzed monosaccharide concentrations of soluble corn fiber:pullulan:sorbitol:fructose blends

Item	Test carbohydrate ¹				
	SCF	70:10:10:10 SCF:Pull:Sorb:Fruct	60:20:10:10 SCF:Pull:Sorb:Fruct	50:30:10:10 SCF:Pull:Sorb:Fruct	30:30:20:20 SCF:Pull:Sorb:Fruct
Free sugars, mg/g ²					
Arabinose	0.46	0.00	0.00	0.00	0.00
Fructose	11.93	99.89	84.45	101.50	192.05
Galactose	0.09	0.00	0.05	0.00	0.00
Glucose	127.19	42.92	36.63	31.69	20.61
Mannose	0.72	0.00	0.32	0.00	0.00
Rhamnose	0.49	0.00	0.00	0.00	0.00
Sorbitol	1.00	78.52	79.39	87.03	155.82
Sucrose	0.00	0.00	0.00	0.00	0.00
Total, mg/g ³	141.87	221.33	200.84	220.22	368.48
Hydrolyzed monosaccharides, mg/g ^{2,4}					
Glucose	489.51	833.10	855.60	820.40	501.71

Table 5-2 (con't.)

Sorbitol	0.00	23.41	19.60	0.00	8.26
Total, mg/g ³	489.51	856.52	875.20	820.40	509.97

¹ Abbreviations: SCF, soluble corn fiber; 70:10:10:10 SCF:Pull:Sorb:Fruct, blend of 70% SCF:10% pullulan:10% sorbitol:10% fructose; 60:20:10:10 SCF:Pull:Sorb:Fruct, blend of 60% SCF:20% pullulan:10% sorbitol:10% fructose; 50:30:10:10 SCF:Pull:Sorb:Fruct, blend of 50% SCF:30% pullulan:10% sorb:10% fructose; 30:30:20:20 SCF:Pull:Sorb:Fruct, blend of 30% SCF:30% pullulan:20% sorbitol:20% fructose.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

⁴ Values are corrected for free monosaccharide concentrations.

Table 5-3. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of soluble corn fiber spray-dried blends

Released monosaccharides, mg/g ²	Test carbohydrate ¹								SEM
	SCFsd	95:5 SCFsd:Fruct	85:15 SCFsd:Fruct	70:30 SCFsd:Fruct	50:50 SCFsd:Fruct	70:30 SCFsd:Pull	50:50 SCFsd:Pull	50:50 SCFsd:Sorb	
Fructose	0.00 ^a	49.83 ^a	138.71 ^b	273.12 ^c	420.36 ^d	7.90 ^a	7.12 ^a	7.19 ^a	11.26
Glucose	484.19 ^d	483.37 ^d	422.61 ^c	454.77 ^{cd}	351.55 ^b	927.86 ^f	882.80 ^e	298.68 ^a	10.25
Isomaltose	17.85 ^d	0.00 ^a	0.00 ^a	12.92 ^c	5.20 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.57
Sorbitol	0.00 ^a	0.41 ^a	0.53 ^a	0.00 ^a	0.00 ^a	0.33 ^a	0.68 ^a	430.90 ^b	2.78
Total ³	502.04 ^a	533.61 ^a	561.86 ^a	740.81 ^{bc}	775.38 ^c	936.09 ^d	890.60 ^d	736.77 ^{bc}	13.94

¹ Abbreviations: SCFsd, spray-dried soluble corn fiber; 95:5 SCFsd:Fruct, blend of 95% SCFsd:5% fructose; 85:15

SCFsd:Fruct, blend of 85% SCFsd:15% fructose; 70:30 SCFsd:Fruct, blend of 70% SCFsd:30% fructose; 50:50 SCFsd:Fruct, blend of 50% SCFsd:50% fructose; 70:30 SCFsd:Pull, blend of 70%SCFsd:30% pullulan; 50:50 SCFsd:Pull, blend of 50% SCFsd:50% pullulan; 50:50 SCFsd:Sorb, blend of 50% SCFsd:50% sorbitol.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

^{abcd} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 5-4. Monosaccharides (including free monosaccharides) released after simulated hydrolytic digestion of soluble corn fiber:pullulan:sorbitol:fructose blends

Released monosaccharides, mg/g ²	Test carbohydrate ¹					SEM
	SCF	70:10:10:10 SCF:Pull:Sorb:Fruct	60:20:10:10 SCF:Pull:Sorb:Fruct	50:30:10:10 SCF:Pull:Sorb:Fruct	30:30:20:20 SCF:Pull:Sorb:Fruct	
Fructose	20.15 ^a	121.07 ^c	118.86 ^{bc}	110.11 ^b	234.83 ^d	2.19
Galactose	0.52 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.03
Glucose	264.27 ^a	317.58 ^b	394.43 ^c	489.27 ^e	413.64 ^d	3.80
Sorbitol	1.16 ^a	100.10 ^c	98.41 ^c	79.06 ^b	198.05 ^d	3.51
Total ³	286.10 ^a	538.76 ^b	611.69 ^c	652.16 ^c	846.52 ^d	13.68

¹ Abbreviations: SCF, soluble corn fiber; 70:10:10:10 SCF:Pull:Sorb:Fruct, blend of 70% SCF:10% pullulan:10% sorbitol:10% fructose; 60:20:10:10 SCF:Pull:Sorb:Fruct, blend of 60% SCF:20% pullulan:10% sorbitol:10% fructose; 50:30:10:10 SCF:Pull:Sorb:Fruct, blend of 50% SCF:30% pullulan:10% sorb:10% fructose; 30:30:20:20 SCF:Pull:Sorb:Fruct, blend of 30% SCF:30% pullulan:20% sorbitol:20% fructose.

² Values are expressed on a dry matter basis.

³ Values include water added when starches are broken down to monosaccharide units.

^{abc} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 5-5. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble corn fibers spray-dried blends

Item	Test carbohydrate ¹									SEM ²
	Malt	SCFsd	95:5 SCFsd: Fruct	85:15 SCFsd: Fruct	70:30 SCFsd: Fruct	50:50 SCFsd: Fruct	70:30 SCFsd:Pull	50:50 SCFsd:Pull	50:50 SCFsd: Sorb	
AUC for glucose, mmol/L	154.87 ^c	98.90 ^b	50.68 ^{ab}	49.56 ^{ab}	13.22 ^a	7.56 ^a	80.36 ^b	49.22 ^{ab}	4.79 ^a	16.14
RGR, %	100.00 ^d	62.79 ^c	34.90 ^b	30.27 ^b	6.69 ^a	2.06 ^a	44.85 ^{bc}	31.29 ^b	5.79 ^a	7.41
AUC for insulin, pmol/L	10,354.00 ^b	4,241.59 ^a	4,025.76 ^a	2,444.32 ^a	1,460.10 ^a	818.41 ^a	3,238.80 ^a	4,201.47 ^a	927.28 ^a	1,610.24
RIR, %	100.00 ^c	48.55 ^b	34.45 ^{ab}	29.37 ^{ab}	13.56 ^a	7.73 ^a	28.25 ^{ab}	45.23 ^b	9.46 ^a	9.52

¹ Abbreviations: SCFsd, spray-dried soluble corn fiber; 95:5 SCFsd:Fruct, blend of 95% SCFsd:5% fructose; 85:15

SCFsd:Fruct, blend of 85% SCFsd:15% fructose; 70:30 SCFsd:Fruct, blend of 70% SCFsd:30% fructose; 50:50 SCFsd:Fruct, blend of 50% SCFsd:50% fructose; 70:30 SCFsd:Pull, blend of 70%SCFsd:30% pullulan; 50:50 SCFsd:Pull, blend of 50% SCFsd:50% pullulan; 50:50 SCFsd:Sorb, blend of 50% SCFsd:50% sorbitol.

² Pooled standard error of the mean.

^{abcd} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 5-6. Incremental area under the curve (AUC) for glucose and insulin, and relative glycemic response (RGR) and relative insulinemic response (RIR) of soluble corn fiber:pullulan:sorbitol:fructose blends

Item	Test carbohydrate ¹						SEM ²
	Malt	SCF	70:10:10:10 SCF:Pull:Sorb :Fruct	60:20:10:10 SCF:Pull:Sorb :Fruct	50:30:10:10 SCF:Pull:Sorb :Fruct	30:30:20:20 SCF:Pull:Sorb :Fruct	
AUC for glucose, mmol/L	210.40 ^b	39.10 ^a	59.79 ^a	8.83 ^a	23.84 ^a	10.54 ^a	17.24
RGR, %	100.00 ^c	24.49 ^{ab}	38.64 ^b	5.19 ^a	25.76 ^{ab}	7.05 ^a	7.92
AUC for insulin, pmol/L	13,279.00 ^b	4,942.85 ^a	2,286.83 ^a	2,593.40 ^a	2,743.60 ^a	1,605.94 ^a	1,411.56
RIR, %	100.00 ^b	26.16 ^a	23.98 ^a	23.65 ^a	24.28 ^a	9.32 ^a	7.70

¹Abbreviations: SCF, soluble corn fiber; 70:10:10:10 SCF:Pull:Sorb:Fruct, blend of 70% SCF:10% pullulan:10% sorbitol:10% fructose; 60:20:10:10 SCF:Pull:Sorb:Fruct, blend of 60% SCF:20% pullulan:10% sorbitol:10% fructose; 50:30:10:10 SCF:Pull:Sorb:Fruct, blend of 50% SCF:30% pullulan:10% sorb:10% fructose; 30:30:20:20 SCF:Pull:Sorb:Fruct, blend of 30% SCF:30% pullulan:20% sorbitol:20% fructose.

² Pooled standard error of the mean.

^{ab} Means in the same row with different superscript letters are different ($P < 0.05$).

Figure 5-1. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble corn fiber spray-dried blends. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (0.48), SCFsd (0.51), 95:5 SCFsd:Fruct (0.53), 85:15 SCFsd:Fruct (0.53), 70:30 SCFsd:Fruct (0.54), 50:50 SCFsd:Fruct (0.54), 70:30 SCFsd:Pull (0.53), 50:50 SCFsd:Pull (0.53), 50:50 SCFsd:Sorb (0.53). Abbreviations: SCFsd, spray-dried soluble corn fiber; 95:5 SCFsd:Fruct, blend of 95% SCFsd:5% fructose; 85:15 SCFsd:Fruct, blend of 85% SCFsd:15% fructose; 70:30 SCFsd:Fruct, blend of 70% SCFsd:30% fructose; 50:50 SCFsd:Fruct, blend of 50% SCFsd:50% fructose; 70:30 SCFsd:Pull, blend of 70%SCFsd:30% pullulan; 50:50 SCFsd:Pull, blend of 50% SCFsd:50% pullulan; 50:50 SCFsd:Sorb, blend of 50% SCFsd:50% sorbitol.

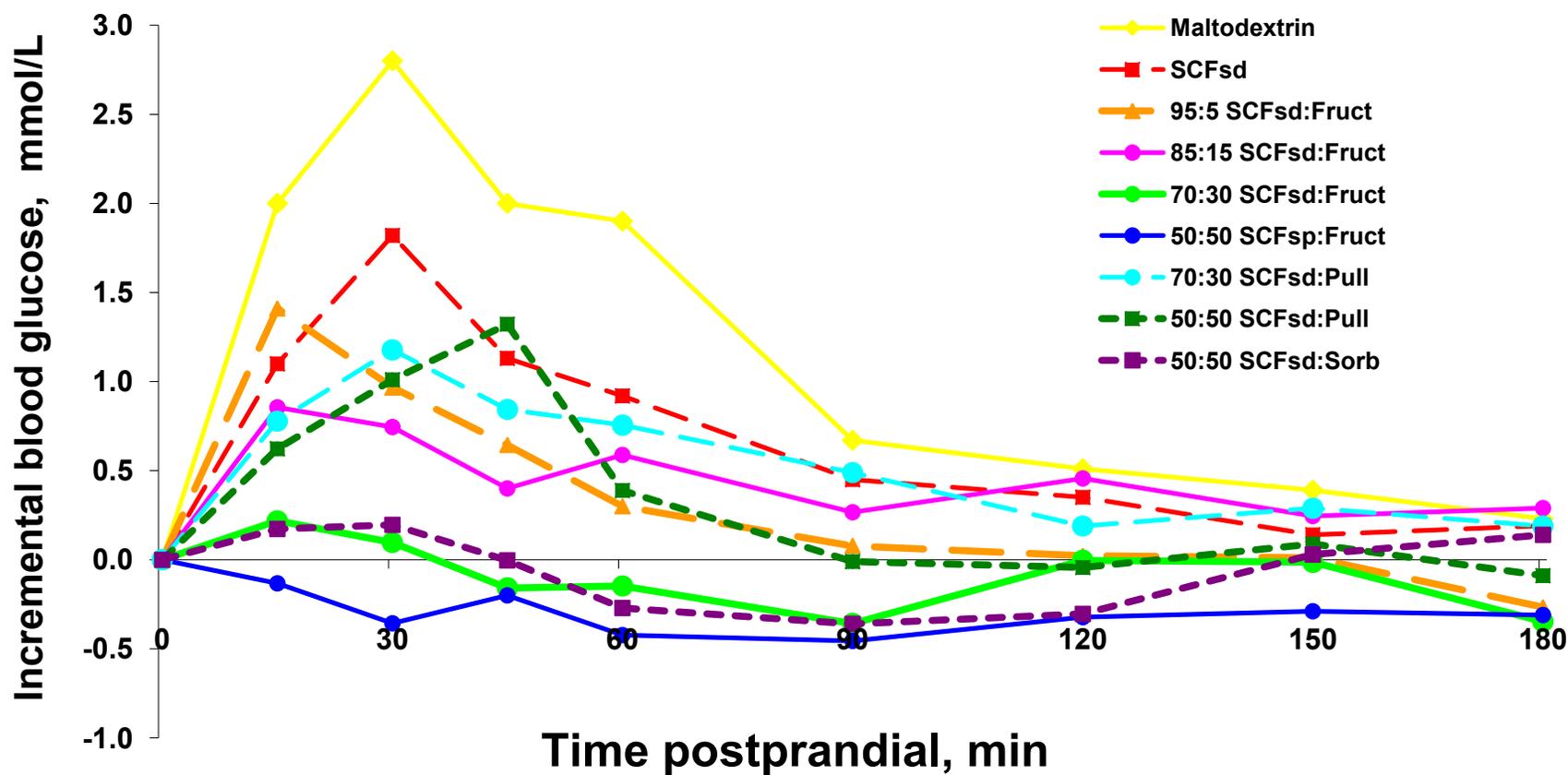


Figure 5-2. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble corn fiber spray-dried blends. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (13.64), SCFsd (24.46) 95:5 SCFsd:Fruct (25.60), 85:15 SCFsd:Fruct (23.00), 70:30 SCFsd:Fruct (23.00), 50:50 SCFsd:Fruct (23.62), 70:30 SCFsd:Pull (23.62), 50:50 SCFsd:Pull (23.62), 50:50 SCFsd:Sorb (23.62). Abbreviations: SCFsd, spray-dried soluble corn fiber; 95:5 SCFsd:Fruct, blend of 95% SCFsd:5% fructose; 85:15 SCFsd:Fruct, blend of 85% SCFsd:15% fructose; 70:30 SCFsd:Fruct, blend of 70% SCFsd:30% fructose; 50:50 SCFsd:Fruct, blend of 50% SCFsd:50% fructose; 70:30 SCFsd:Pull, blend of 70%SCFsd:30% pullulan; 50:50 SCFsd:Pull, blend of 50% SCFsd:50% pullulan; 50:50 SCFsd:Sorb, blend of 50% SCFsd:50% sorbitol.

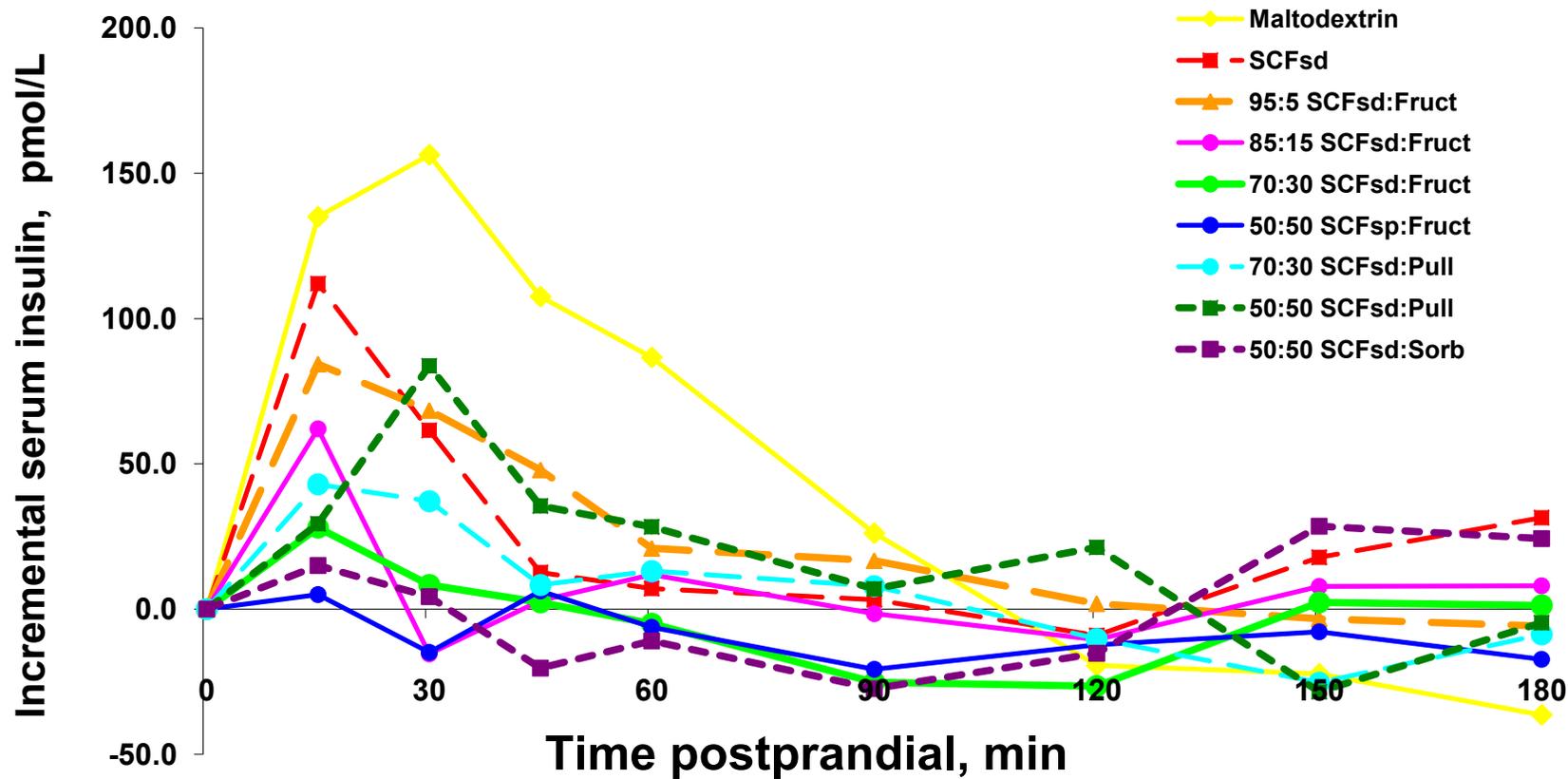


Figure 5-3. Incremental change from baseline in blood glucose response for dogs consuming 25 g of soluble corn fiber:pullulan:sorbitol:fructos blends. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (0.82), SCF (0.53), 70:10:10:10 SCF:Pull:Sorb:Fruct (0.82), 60:20:10:10 SCF:Pull:Sorb:Fruct (0.82), 50:30:10:10 SCF:Pull:Sorb:Fruct (0.82), 30:30:20:20 SCF:Pull:Sorb:Fruct (0.82). Abbreviations: SCF, soluble corn fiber; 70:10:10:10 SCF:Pull:Sorb:Fruct, blend of 70% SCF:10% pullulan:10% sorbitol:10% fructose; 60:20:10:10 SCF:Pull:Sorb:Fruct, blend of 60% SCF:20% pullulan:10% sorbitol:10% fructose; 50:30:10:10 SCF:Pull:Sorb:Fruct, blend of 50% SCF:30% pullulan:10% sorb:10% fructose; 30:30:20:20 SCF:Pull:Sorb:Fruct, blend of 30% SCF:30% pullulan:20% sorbitol:20% fructose.

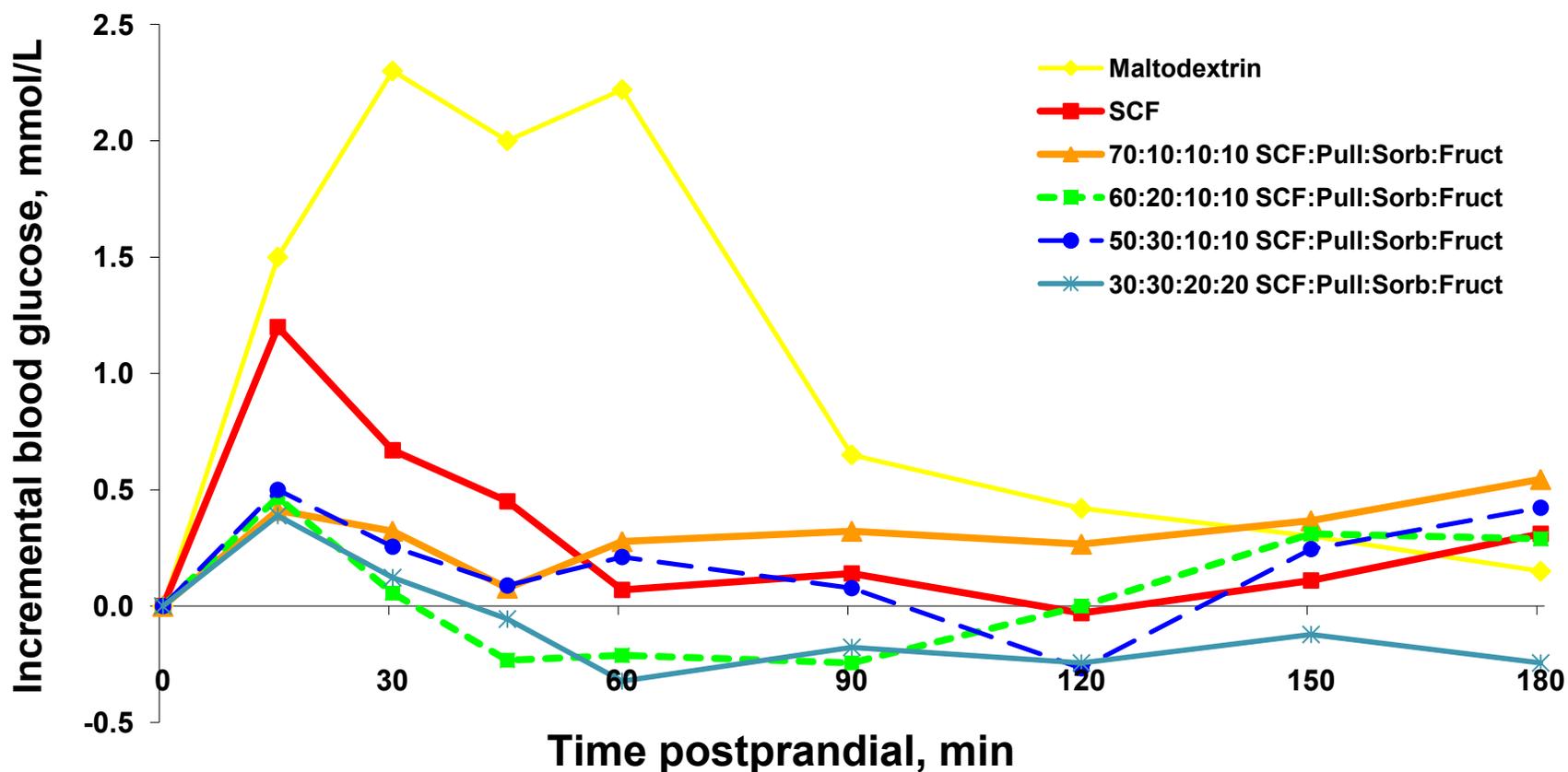
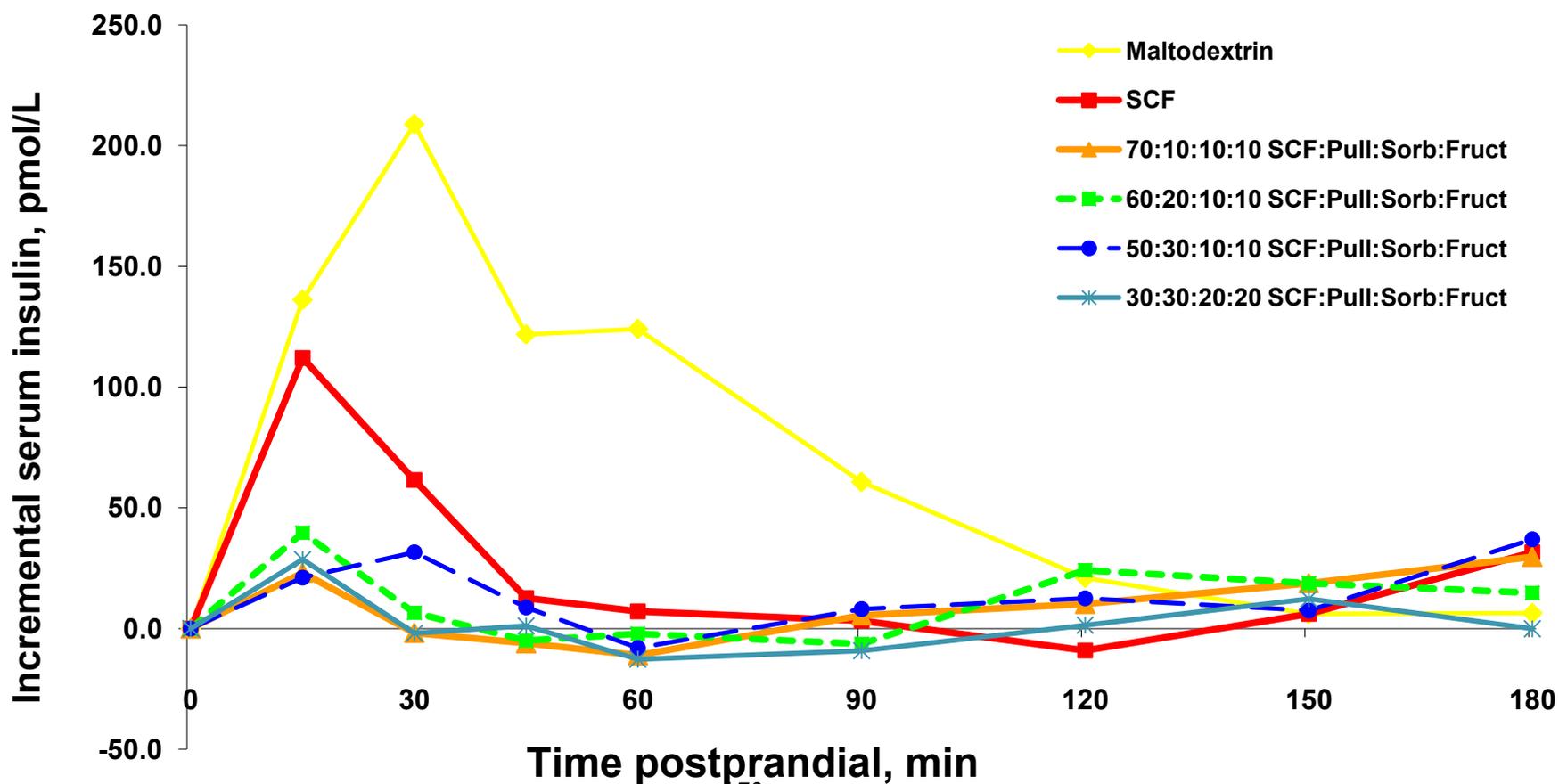


Figure 5-4. Incremental change from baseline in serum insulin response for dogs consuming 25 g of soluble corn fiber:pullulan:sorbitol:fructos blends. Pooled standard error of the mean (SEM) values for carbohydrates are: Maltodextrin (23.62), SCF (25.21), 70:10:10:10 SCF:Pull:Sorb:Fruct (23.62), 60:20:10:10 SCF:Pull:Sorb:Fruct (23.62), 50:30:10:10 SCF:Pull:Sorb:Fruct (23.62), 30:30:20:20 SCF:Pull:Sorb:Fruct (23.62). Abbreviations: SCF, soluble corn fiber; 70:10:10:10 SCF:Pull:Sorb:Fruct, blend of 70% SCF:10% pullulan:10% sorbitol:10% fructose; 60:20:10:10 SCF:Pull:Sorb:Fruct, blend of 60% SCF:20% pullulan:10% sorbitol:10% fructose; 50:30:10:10 SCF:Pull:Sorb:Fruct, blend of 50% SCF:30% pullulan:10% sorb:10% fructose; 30:30:20:20 SCF:Pull:Sorb:Fruct, blend of 30% SCF:30% pullulan:20% sorbitol:20% fructose.



**CHAPTER 6: SOLUBLE FIBER DEXTRIN AND SOLUBLE CORN FIBER
SUPPLEMENTATION MODIFY INDICES OF GASTROINTESTINAL HEALTH IN
THE RAT**

ABSTRACT: The objective of this study was to evaluate gastrointestinal health outcomes resulting from supplementation of novel, low-digestible carbohydrates to rats. After a 7 d acclimation period, rats were randomly assigned to one of four treatment groups (n=10) for 21 d. An AIN-93G diet with 5% cellulose served as the Control. The 5% cellulose was replaced with either 5% pectin (Pectin, positive control), soluble fiber dextrin (SFD), or soluble corn fiber (SCF). Rats fed the Pectin diet had a higher average daily food intake, but no differences in final body weights or rates of gain among treatments were observed. Consumption of SFD and SCF increased ($P<0.05$) cecal weight but not colon weight. No differences were observed in short-chain fatty acid (SCFA) or branched-chain fatty acid (BCFA) concentrations ($\mu\text{mol/g}$) in the colon of rats fed either SFD or SCF. Pectin resulted in increased ($P<0.05$) BCFA in the colon. On a per cecum basis, SFD and SCF increased ($P<0.05$) acetate, propionate, and total SCFA concentrations, with no effect on butyrate concentrations, compared to the Control diet. Cecal BCFA concentrations were decreased ($P<0.05$) by SFD and SCF, whereas Pectin increased ($P<0.05$) BCFA concentrations. Supplementation of SFD and SCF did not have an effect on cecal microbial populations compared to the Control diet. Pectin tended to decrease ($P<0.10$) *Escherichia coli* concentrations. Gut histomorphology was positively affected by SFD and SCF. Increased ($P<0.05$) crypt depth, goblet cell numbers, and acidic mucin were observed in both the cecum and colon of rats supplemented with SFD, SCF, and Pectin. These novel,

low-digestible carbohydrates appear to be beneficial in modulating indices of gastrointestinal health when supplemented in the diet of rats.

INTRODUCTION

Dietary fiber as a promoter of healthy gut function and other health benefits is well recognized (Cummings et al., 1997). However, most of the population of the United States consumes less than half of the recommended concentrations of dietary fiber daily (Anderson et al., 2009). This has led to a demand for the development of novel 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.

One class of carbohydrates, low-digestible carbohydrates, are becoming popular as food ingredients, not only due to their potential to improve both the physical and chemical properties of foods, but also due to possible health benefits associated with their consumption that are similar in nature to those of dietary fiber (Murphy, 2001). Low-digestible carbohydrates are low molecular weight carbohydrates that resist hydrolytic activity of human digestive enzymes (Crittenden and Playne, 1996; Roberfroid and Slavin, 2000; Mussatto and Mancilha, 2007). They pass into the colon where they are substrates for complete or partial fermentation by colonic microbiota. Fermentation results in short-chain fatty acids (SCFA) that provide colonic cells with energy and lower the pH of luminal contents, stimulating a healthy environment for beneficial bacteria. Low-digestible carbohydrates also may beneficially impact the morphology of the gastrointestinal tract, especially through modulation of the mucosal layer. This layer is primarily composed of mucin glycoproteins synthesized and secreted by goblet cells that serve as a protective

barrier for the epithelial cells (Ito et al., 2009). Modulation of the mucosal layer may positively or detrimentally affect this barrier and, thus, the health of the gastrointestinal tract.

Two novel, low-digestible carbohydrates of interest are soluble fiber dextrin (SFD) and soluble corn fiber (SCF). Soluble fiber dextrin is an indigestible dextrin produced when corn starch is treated with heat and acid, and SCF is produced by isolating an oligosaccharide-rich fraction from corn syrup. Both of these novel, low-digestible carbohydrates are produced in such a way that branching and the number of α -1,6 glycosidic bonds are increased (Laurentin et al., 2003; Harrison and Hoffman, 2007). Soluble fiber dextrin and SCF have been reported to have a decreased *in vitro* hydrolytic digestion. Also, they attenuate glycemic and insulinemic responses and have reduced energy values (Knapp et al., 2010; Knapp et al., unpublished data). However, little research exists regarding these novel, low-digestible carbohydrates on indices of gut health.

The objective of this study was to determine the effects of supplementation of SFD and SCF on select indices of gut health. This was determined by measuring pH, SCFA concentrations, and microbial populations in the cecum and (or) colon of rats. Cecum and colon mass and crypt and goblet cell measurements also were taken to determine the impact of these low-digestible carbohydrates on gut morphology.

MATERIALS AND METHODS

Animals:

Forty male Sprague Dawley rats (average initial weight, 174 ± 11 g) were purchased from Harlan Laboratories Inc. (Indianapolis, IN). Rats were housed individually in stainless

steel wire-bottom cages in a temperature and humidity controlled facility with 12 hour light and dark cycles. Prior to the experiment, rats were fed for 7 d on an AIN-93G diet (Reeves et al., 1993). Rats were given free access to water. All animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before initiation of the experiment.

Experimental design and treatments:

Rats were randomly assigned to one of four dietary treatments (10 rats/treatment) after the adaptation period of 7 d. Rats were given free access to pelleted diets. Four dietary treatments were utilized in this study: a control diet that was the AIN-93G diet with 5% cellulose (Control), a positive control that consisted of the AIN-93G diet with 5% pectin (high-methoxy pectin, TIC Gums, White Marsh, MD) substituted for cellulose (Pectin), a treatment that consisted of the AIN-93G diet with 5% soluble fiber dextrin (Nutriose, Roquette, Keokuk, IA) substituted for cellulose (SFD), and a treatment that consisted of the AIN-93G diet with 5% soluble corn fiber (Promitor, Tate & Lyle, Decatur, IL) substituted for cellulose (SCF). All diets were prepared by Research Diets Inc. (New Brunswick, NJ). The ingredient composition of the diets is listed in Table 6-1. The duration of the study was 21 d. Food intake was determined daily and body weights were measured weekly.

Sample collection:

On day 21, rats were euthanized by placement in a CO₂ chamber. A ventral midline incision then was made and the cecum and colon were removed. Immediately after removal, cecum and colon with contents were weighed to determine total weight. pH of cecal and colonic contents was taken using a Beckman pH meter and electrode (Beckman

Instruments, Inc., Fullerton, CA.) Aliquots of cecal and colon contents then were taken for DM, SCFA, and microbiota analysis. The SCFA aliquots were acidified with 5 mL 2N HCl before storing at -20°C. The aliquot for microbial analysis was sealed in a sterile cryovial, snap frozen in liquid nitrogen, and stored at -80°C. No colonic contents were collected for microbiota analysis due to insufficient amounts of colonic digesta.

Following removal of the appropriate samples, the tissues were cleaned with water, blotted dry, and weighed to determine empty cecum and colon weights. Total cecal and colonic contents were calculated as total tissue weight with contents minus empty tissue weight. Cecal and colonic tissue from rats was collected and fixed in phosphate buffered formalin for histomorphological analysis.

Chemical analysis:

Diet samples were analyzed for dry matter (DM), organic matter (OM) (AOAC 2000), Leco N (AOAC 2000), acid hydrolyzed fat (AHF; Budde 1952; AACC 1983), and gross energy (GE) (Parr Instrument Co., Moline, IL; Parr Instrument Manuals). Diet samples also were analyzed for total dietary fiber (TDF) content (Prosky et al., 1992). All procedures were performed in duplicate. To maintain quality control during chemical analysis, the error between duplicate samples was determined and, if it exceeded 5%, the assay was repeated. Fresh cecal and colonic contents were analyzed for DM and pH (as indicated above), and SCFA using gas chromatography (Erwin et al., 1961). Briefly, acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations were determined on the supernatant of acidified cecal and colonic contents using a Hewlett-Packard 5890A Series II gas chromatograph (Palo Alto, CA) and a glass column packed

with 10% SP-1200/1% H₃PO₄ on 80/100+ mesh Chromosorb WAW (Supelco, Bellefonte, PA).

Microbial analysis:

Microbial populations were analyzed using methods described by Middelbos et al. (2007) with minor modifications. Cecal DNA was extracted from freshly collected samples that had been stored at -80°C until analysis, using the repeated bead beater method described by Yu and Morrison (2004) followed by a QIAamp DNA stool mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE). *Escherichia coli*, the *Bifidobacterium* genus, and the *Lactobacillus* genus were quantified using quantitative polymerase chain reaction (qPCR) specific primers. Amplification was performed for each bacterial group within each sample according to the procedures of Deplancke et al. (2002). For amplification, 10 µL final volume containing 5 µL of 2 × SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers of the bacteria of interest, and 5 ng of extracted cecal DNA were used. Pure cultures of each bacterium were used to create serial dilutions in triplicate of the targeted bacterial genus to obtain standard curves. Bacterial DNA was extracted from each dilution and amplified along with cecal DNA samples using a Taqman ABI PRISM 7900HT Sequence Detection System (Applied BioSystems). Colony forming units (cfu) of each standard curve serial dilution was determined previously by plating on specific agars. *E. coli* was grown on Luria-Bertani medium, *Lactobacillus* on Difco Lactobacilli MRS broth (Becton, Dickenson, and Co., Sparks, MD), and *Bifidobacterium* on Difco Reinforced Clostridial Medium (Becton, Dickenson, and Co.). Cycle threshold values were plotted

against the standard curves for quantification (cfu/g cecal contents) of the targeted bacterial DNA from cecal samples.

Cecal and colonic histomorphology:

Cecal and colonic sections from each rat were embedded in a paraffin block, sliced into 5 μm thick sections using a microtome, and stained. One set of slides was stained with alcian blue (AB) and periodic acid Schiff and counterstained with hematoxylin for determining crypt depth, goblet cell numbers, and mucin (acidic and neutral) components. Another set of slides was stained with high iron diamine (HID) and AB to determine sulfated and sialylated mucins. Slides were prepared and stained at the University of Illinois Department of Veterinary Biosciences Histology Laboratory. Crypt depth, goblet cell counts, and mucin composition measurements were attempted on a minimum of 15 crypts per section. Digital images of tissues and measurements were taken using Axiovision LE software and an AxioCam MRc5 (Zeiss, Oberkochen, Germany).

Statistical analyses:

Data were analyzed as a completely randomized design using the Mixed Models procedure of SAS (SAS Institute, Inc., Cary, NC). The model contained the fixed effect of diet and the random effect of rat. Differences among treatments were determined using a Fisher-protected least significant difference test with a Tukey adjustment to control for experiment-wise error. Reported pooled standard errors of the mean (SEM) were determined according to the Mixed Models procedure of SAS. Significant differences were accepted at a probability of $P < 0.05$ and a probability of $P < 0.10$ was considered a trend.

RESULTS AND DISCUSSION

Diet composition:

The chemical composition of the experimental diets is presented in Table 6-2.

Dietary treatments were similar in DM, OM, CP, AHF, and GE composition. Total dietary fiber concentrations were lower for the SFD and SCF diets because the particular TDF method used does not fully quantify low-molecular weight dietary fibers like SFD and SCF. The complete carbohydrate composition of SFD and SCF is presented in Knapp et al. (2010) and Knapp et al. (unpublished data).

Body weight and food intake:

Daily food intake values, final body weights, and rate of gain are presented in Table 6-3. Initial body weights of the rats were similar among the groups (avg 178.44 g) and after 21 d on the experimental diets, the final body weights and rate of gain did not differ significantly. Daily food intake was approximately 16.5 g/d, with rats fed the Pectin diet having a higher ($P < 0.05$) daily food intake. Rats consuming the SCF diet developed diarrhea soon after starting the treatment, but did not significantly decrease their food intake or lose weight. Consumption of the Pectin and SFD diets also resulted in looser stools by the end of the study, but not to the extent experienced by rats fed the SCF diet. Weaver et al. (2010) supplemented SCF and SFD to rats at 10% of the diet and found that they also developed loose stools. The test carbohydrates then were reduced to 5% dietary concentration and loose stools persisted as was the case in the current study. Low-digestible carbohydrates such as SCF and SFD can have tolerance problems such as diarrhea when

consumed for a period of time (Livesey, 2001; Marteau and Flourie, 2001; Grabitske and Salvin, 2008).

Short-chain fatty acids:

Fermentative end-product concentrations in cecal and colonic contents are presented in Tables 6-4 and 6-5, respectively. Pectin resulted in increased ($P<0.05$) acetate concentrations in cecal contents compared to the other treatments. Propionate concentrations were highest ($P<0.05$) for SFD, with SCF and Pectin having lower ($P<0.05$) but similar concentrations. Cecal concentrations of butyrate were lowest ($P<0.05$) for the SFD and SCF diets. Pectin supplementation resulted in the highest ($P<0.05$) total cecal SCFA concentration among treatments. Supplementation of SFD and SCF resulted in similar total SCFA concentrations as the Control.

Colonic SCFA concentrations (Table 6-5) were lower compared to those in cecal contents. Acetate and total SCFA concentrations were higher ($P<0.05$) for the Pectin diet compared to the SCF diet. Soluble fiber dextrin resulted in higher ($P<0.05$) propionate concentrations compared to the Control diet. Similarly to cecal SCFA, butyrate concentrations were higher ($P<0.05$) for the Pectin and Control diets compared to the SFD and SCF diets.

The lack of difference between the SFD and SCF diets as regards cecal SCFA compared to the Control diet may be due to the increased cecal volume of rats consuming the SFD and SCF diets, thus leading to a dilution effect for SCFA in the cecal contents. When cecal SCFA were expressed as $\mu\text{mol}/\text{cecum}$ (Table 6-4), SCFA were altered considerably due to SFD and SCF supplementation. Acetate concentration increased

($P < 0.05$) in the SCF, SFD, and Pectin diets compared to the Control, with Pectin having the highest ($P < 0.05$) concentration. Similar to acetate, propionate and total SCFA concentrations in cecal contents were increased ($P < 0.05$) with supplementation of Pectin, SFD, and SCF. Butyrate concentrations for SFD and SCF treatments were not increased compared with the Control treatment, even when expressed on a per cecum basis.

Colonic SCFA were expressed as $\mu\text{mol}/\text{colon}$ (Table 6-5). Small but similar amounts of colonic contents were found for all dietary treatments; thus, few differences among treatments were observed. The Control diet resulted in higher ($P < 0.05$) butyrate concentrations compared to the SFD and SCF diets.

Neither of the novel, low-digestible carbohydrates were butyrogenic. Weaver et al. (2010) found a similar response to SCF and SFD in cecal SCFA concentrations in rats. The supplemented SCF and SFD did not increase butyrate concentrations compared to a cellulose control when supplemented at 4% of the diet. Stewart et al. (2010) found that supplementation of 12 g/d SFD and SCF to human subjects resulted in no differences in fecal SCFA concentrations compared with a maltodextrin control. Soluble corn fiber has been supplemented at 21 g/d to human subjects and, similar to results with rats, fecal butyrate concentrations were not increased compared with the non-fiber control (Vester Boler et al., unpublished data).

Cecal isobutyrate, valerate, and total BCFA concentrations were lower ($P < 0.05$) with supplementation of both SFD and SCF compared to either the Control or Pectin treatments. Isovalerate concentrations were lower ($P < 0.05$) for the SFD and SCF diets compared to

Pectin. Pectin resulted in the highest ($P<0.05$) cecal concentrations of all BCFA except isovalerate for all dietary treatments.

Concentrations of BCFA were expressed on a per cecum basis (Table 6-5). No differences among treatments were noted in isobutyrate concentration. Isovalerate concentrations were higher ($P<0.05$) for the SFD and SCF diets compared to the Control, but not when compared to the Pectin diet. Pectin resulted in the highest ($P<0.05$) concentrations of valerate and total BCFA.

Concentrations of BCFA were lower in the colon compared to concentrations in the cecum. Pectin resulted in higher ($P<0.05$) concentrations of isobutyrate than did SFD. Isovalerate concentrations were higher ($P<0.05$) for Pectin compared to the Control and the SCF diets. Pectin resulted in the highest ($P<0.05$) concentrations of valerate and total BCFA, and the Control, SFD, and SCF diets resulted in lower ($P<0.05$) but similar concentrations.

Concentrations of BCFA on a per colon basis are presented in Table 6-6. No differences in isovalerate concentration were noted among dietary treatments. As was noted when concentrations were expressed on a DM basis, Pectin tended to result in higher concentrations of BCFA compared to SFD and SCF, but not when compared to the Control diet. Higher concentrations for the Control diet were noted for BCFA when expressed on a per colon basis because rats fed this diet had more colonic contents than did rats fed the other treatments.

In general, colonic BCFA concentrations for the Control rats were similar to those for rats fed SFD and SCF. However, as regards cecal BCFA concentrations, the Control

tended to result in higher concentrations than did the SFD and SCF diets. Overall, for both cecal and colonic BCFA, Pectin tended to have higher values than did the other dietary treatments. Pectin increases the viscosity of digesta which could decrease crude protein digestion, resulting in higher quantities of protein reaching the cecum and colon where they would be fermented, thus producing BCFA (Brunsgaard et al., 1995; Burazewska et al., 2007)

Large bowel weight and pH:

Total weight, empty weight, and pH values of the cecum and colon are presented in Table 6-6. Total weight of the cecum was dramatically increased ($P<0.05$) as a result of consumption of SFD and SCF. However, this effect was not noted in the colon where all treatments resulted in a similar total colon weight. In some rats fed Pectin, SFD, and SCF diets, the colon was empty upon removal. Empty cecal weight was increased ($P<0.05$) compared with the Control as a result of Pectin, SFD, and SCF consumption, with values for the latter two fibers being higher than that for Pectin. Empty colonic weight was unaffected by diet. Cecal and colonic pH values were lowered ($P<0.05$) by the SFD and SCF treatments.

Weaver et al. (2010) also found that supplementation with SCF, SFD, and other novel fibers increased cecum weight compared to cellulose. In that study, supplementation of 4% SCF and SFD resulted in a cecum weight of 5.58 g, similar to what was found in the current study (cecal weight of 6.15 g and 6.72 g, respectively). Other research has demonstrated that ingestion of low-digestible carbohydrates resulted in increased cecum weights of rats (Levrat et al., 1991; Campbell et al., 1997; Lu et al., 2000; Kim, 2002). The

increased cecal weight likely is due to increased epithelial cell proliferation from the trophic effects of SCFA (Frankel et al., 1994). The major differences in organ weights were noted only for the cecum and not the colon. This is probably due to the fact that the major site of fermentation for rodents is the cecum and not the colon as in humans. The decreased cecal pH is probably due to increased SCFA production at that site.

Microbial concentrations:

Cecal microbial data are presented in Table 6-7. Supplementation of SFD or SCF did not beneficially modulate microbial populations in the rat cecum. Cecal concentrations of *Bifidobacterium* spp. and *Lactobacillus* spp. were not increased compared with the Control treatment. Also, decreased concentrations of *Escherichia coli* were not observed with supplementation of SFD and SCF. The only dietary treatment with an effect on cecal microbial concentrations was Pectin that tended ($P < 0.10$) to decrease *Escherichia coli* concentrations with no effect on *Bifidobacterium* spp or *Lactobacillus* spp concentrations.

Soluble corn fiber has been shown to affect microbial concentrations *in vitro*. Maathuis et al. (2009) reported a 2-fold increase in *Bifidobacterium* spp. using SCF in a validated dynamic computer-controlled *in vitro* model of the human proximal large intestine (TIM-2). A bifidogenic response also was found in a human *in vivo* study where healthy men were supplemented with 21 g/d of SCF (Vester Boler et al., unpublished data). This dose of SCF was found to increase ($P < 0.05$) fecal concentrations of *Bifidobacterium* spp. compared with the non-fiber control (from 6.9 log₁₀ cfu/g to 8.2 log₁₀ cfu/g), but did not have any effect on *Lactobacillus* spp. or *Escherichia coli* populations. Pasman et al. (2006)

found that neither 30 nor 45 g/d of SFD increased *Lactobacillus* spp in feces compared with a maltodextrin control in a human study.

Histomorphology:

Histomorphology data collected on cecum and colon of rats are presented in Table 6-8. Crypt depth in both the cecum and colon was increased ($P<0.05$) compared with the Control treatment with supplementation of Pectin, SFD, and SCF. A similar pattern for goblet cell number was noted. In both cecum and colon, supplementation of Pectin, SFD, and SCF increased ($P<0.05$) goblet cell numbers compared to the Control treatment.

The majority of goblet cells found in both cecum and colon crypts was found to be comprised of acidic mucin, and increased ($P<0.05$) acidic mucins were found in rats fed the Pectin, SFD, and SCF diets. These were found concentrated towards the bottom of the crypts. No goblet cells composed of only neutral mucin were observed in the cecal or colonic crypts for any treatment; however, goblet cells comprised of a mixture of both acidic and neutral mucins were observed. These cells stained purple, indicating that both types of mucins were present (Filipe, 1979). No differences between cecum and colon or among dietary treatments were observed for mixed goblet cells.

Acidic mucin can be classified as sulfomucins or sialomucins. Both types of acidic mucins were found in both the cecal and colonic crypts. In the cecum, no differences among dietary treatments were observed. However, for the colonic crypts, diets supplemented with Pectin, SFD, and SCF had higher numbers of sulfomucins compared with the Control treatment.

Increased crypt depth as a result of dietary supplementation of low-digestible carbohydrates is a beneficial morphological effect. The crypts contain intestinal stem cells, the principal site of cell proliferation in the intestinal mucosa, and increased depth is associated with increased rate of turnover of intestinal mucosal cells (Jin et al., 1994; Kleessen et al., 2003). Several studies have shown that pectin and other dietary fibers increase crypt depth throughout the intestinal tract (Jacobs, 1983; Lupton and Kurtz, 1993; Kleessen et al., 2003). However, pectin has been reported to simultaneously increase crypt depth and decrease villus height of the small intestine (Jacobs, 1983).

The increase in goblet cells per crypt may have a positive impact on gut health by increasing the thickness of the mucous layer of the large bowel. Other studies have reported increased goblet cell numbers in rats fed fermentable fibers including fructans and galactooligosaccharides (Satchithanandam et al., 1990; Meslin et al., 1993; Fontaine et al., 1996; Schmidt-Wittig et al., 1996; Kim, 2002). Acidification of large intestinal contents is postulated to stimulate mucus synthesis and secretion (Meslin et al., 1999) and could perhaps explain the increased numbers of goblet cells with the dietary treatments tested in this experiment. It has been suggested that acidic mucins protect against bacterial translocation because sulfated mucins (sulfomucins) in particular appear to be less degradable by bacterial glycosidases and host proteases (Deplancke and Gaskins, 2001). Rats fed diets supplemented with low-digestible, inulin-type fructans have been shown to modulate mucins in the intestinal tract by increasing acidic mucins, especially the protective sulfomucins (Fontaine et al., 1996; Delzenne, 2003; Kleessen et al., 2003). Alterations in the mucosal architecture and amounts of sulfomucins and sialomucins could have important effects on the gut mucosal barrier and health maintenance of the gut.

In summary, SFD and SCF both resulted in extensive fermentation in the cecum of rats and had positive results on indices of gut health. Dietary supplementation at the 5% level of the diet resulted in tolerance issues (diarrhea) for the Pectin, SFD, and SCF treatments, but this did not affect food intake, body weight, or rate of gain. Pectin resulted in higher cecal SCFA concentrations than did SFD or SCF, except for propionate concentrations where SFD resulted in higher concentrations. However, on a per cecum basis, SFD and SCF resulted in total SCFA concentrations more similar to those of Pectin. Pectin and SFD treatments tended to have higher colonic SCFA concentrations compared to SCF, except for butyrate. The trend for lower butyrate production from the SFD and SCF treatments was not noted for both cecal and colonic contents, when expressed on a DM basis and a total contents basis. In general, Pectin resulted in higher concentrations of BCFA in cecal and colonic contents compared to SFD and SCF. The only dietary treatment that had an effect on cecal microbial concentrations was Pectin that tended to decrease *Escherichia coli* concentrations. Even though SFD and SCF did not result in increased butyrate concentration as did Pectin, they nevertheless resulted in increased total SCFA concentrations that had positive effects on cecal and colonic histomorphology.

In conclusion, the fermentative properties of SFD and SCF have the potential to beneficially impact large bowel health. Both of these low-digestible carbohydrates increased cecal weight, increased cecal and colonic crypt depths, and had a positive effect on goblet cells and mucin composition. Even though SFD and SCF do not appear to be butyrogenic or bifidogenic in the rat, sufficient positive outcomes were noted so as to make SFD and SCF carbohydrates of interest as regards modulation of indices of gut health.

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Table 6-1. Ingredient composition of diets containing select dietary fibers and fed to rats

Ingredient	Control	Pectin	SFD ¹	SCF ²
-----% of diet-----				
Casein	20.00	20.00	20.00	20.00
L-Cystine	0.30	0.30	0.30	0.30
Cornstarch	39.75	39.75	39.75	39.75
Maltodextrin	13.20	13.20	13.20	13.20
Sucrose	10.00	10.00	10.00	10.00
Cellulose	5.00	0.00	0.00	0.00
Pectin	0.00	5.00	0.00	0.00
SFD	0.00	0.00	5.00	0.00
SCF	0.00	0.00	0.00	5.00
Soybean oil	7.00	7.00	7.00	7.00
t-Butylhydroquinone	0.0014	0.0014	0.0014	0.0014
Mineral mix ³	3.50	3.50	3.50	3.50
Vitamin mix ⁴	1.00	1.00	1.00	1.00
Choline bitartrate	0.25	0.25	0.25	0.25
Dye	0.005	0.005	0.005	0.005

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Mineral mix = AIN-93G-MX. Mineral (g/kg): Calcium carbonate, 357.00; Potassium phosphate, 196.00; Potassium citrate, 70.78; Sodium chloride, 74.00; Potassium sulfate, 46.60; Magnesium oxide, 24.00; Ferric citrate, 6.06; Zinc carbonate, 1.65; Sodium

meta-silicate, 1.45; Manganous carbonate, 0.63; Cupric carbonate, 0.30; Chromium potassium sulfate, 0.28; Boric acid, 0.08; Sodium fluoride, 0.06; Nickel carbonate, 0.03; Lithium chloride, 0.02; Sodium selenate, 0.01; Potassium iodate, 0.01; Ammonium paramolybdate, 0.008; Ammonium vanadate, 0.007; Powdered sucrose, 221.03.

⁴ Vitamin mix = AIN-93G-VX. Vitamin (mg/kg) (except as noted): Nicotinic acid, 3.00; Ca pantothenate, 1.60; Pyridoxine, 0.70; Thiamin, 0.60; Riboflavin, 0.60; Folic acid, 0.20; Biotin, 0.02; Vitamin B₁₂, 2.50; Vitamin E (500 IU/g), 15.00; Vitamin A (500,000 IU/g), 0.80; Vitamin D₃ (400,000 IU/g), 0.25; Vitamin K, 0.08; Powdered sucrose, 974.65.

Table 6-2. Chemical composition of diets containing select dietary fibers and fed to rats

Item	Treatment			
	Control	Pectin	SFD ¹	SCF ²
Dry matter (DM)	90.59	90.36	89.08	89.55
	-----% DM basis-----			
Organic matter	97.38	97.32	97.38	97.38
Crude protein	18.69	19.51	19.32	19.21
Total dietary fiber	5.88	5.30	2.01	2.19
Acid hydrolyzed fat	6.91	6.92	7.01	6.99
Gross energy, kcal/g	4.73	4.71	4.74	4.73

¹ Soluble fiber dextrin.

² Soluble corn fiber.

Table 6-3. Daily food intake, rate of weight gain, and final body weights of rats fed select dietary fibers

Item	Treatment				SEM ³
	Control	Pectin	SFD ¹	SCF ²	
Food intake, g/d	16.5 ^a	17.4 ^b	16.1 ^a	15.9 ^a	0.26
Initial body weight, g	178.21	178.80	179.33	177.42	2.09
Final body weight, g	314.6	323.8	327.4	318.5	6.01
Rate of gain, g/d	4.56	4.86	4.98	4.51	0.22

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Pooled SEM.

^{ab} Means in the same row with different superscript letters are different (P<0.05).

Table 6-4. Concentrations of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) in cecal contents of rats fed select dietary fibers

Item	Treatment				SEM ³
	Control	Pectin	SFD ¹	SCF ²	
Cecal SCFA, $\mu\text{mol/g}^4$					
Acetate	192.34 ^a	460.93 ^b	206.77 ^a	171.64 ^a	20.46
Propionate	53.03 ^a	88.05 ^b	113.54 ^c	90.39 ^b	6.33
Butyrate	52.80 ^b	60.16 ^b	13.59 ^a	13.97 ^a	5.15
Total SCFA	298.17 ^a	609.15 ^b	333.91 ^a	276.01 ^a	26.17
Cecal SCFA, $\mu\text{mol/cecum}$					
Acetate	452.33 ^a	1,260.25 ^c	1,052.11 ^{bc}	827.32 ^b	71.32
Propionate	115.18 ^a	234.37 ^b	584.95 ^d	422.51 ^c	23.89
Butyrate	99.79 ^a	163.12 ^b	70.12 ^a	67.75 ^a	14.09
Total SCFA	628.52 ^a	1,662.95 ^b	1,719.61 ^b	1,330.36 ^b	93.81
Cecal BCFA, $\mu\text{mol/g}^4$					
Isobutyrate	4.53 ^b	5.29 ^b	2.73 ^a	2.19 ^a	0.44
Isovalerate	4.57 ^{ab}	5.63 ^b	3.99 ^a	3.53 ^a	0.35

Table 6-4 (con't.)

Valerate	4.98 ^b	8.05 ^c	1.47 ^a	1.88 ^a	0.41
Total BCFA	14.07 ^b	18.97 ^c	8.20 ^a	7.60 ^a	1.04
Cecal BCFA, $\mu\text{mol}/\text{cecum}$					
Isobutyrate	10.14	14.45	14.07	10.55	1.80
Isovalerate	10.15 ^a	15.37 ^{ab}	20.55 ^b	16.99 ^b	1.37
Valerate	11.34 ^a	21.97 ^b	7.59 ^a	9.08 ^a	1.56
Total BCFA	31.62 ^a	51.78 ^b	42.23 ^{ab}	36.63 ^{ab}	4.10

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Pooled SEM.

⁴ Values are expressed on a dry matter basis.

^{abcd} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 6-5. Concentrations of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) in colonic contents of rats fed select dietary fibers

Item	Treatment				SEM ³
	Control	Pectin	SFD ¹	SCF ²	
Colonic SCFA, $\mu\text{mol/g}^4$					
Acetate	118.11 ^{ab}	214.90 ^b	100.65 ^{ab}	81.68 ^a	29.94
Propionate	29.66 ^a	50.78 ^{ab}	73.94 ^b	34.11 ^{ab}	11.09
Butyrate	30.32 ^b	43.56 ^b	6.69 ^a	6.33 ^a	5.86
Total SCFA	178.79 ^{ab}	309.09 ^b	180.89 ^{ab}	121.74 ^a	42.70
Colonic SCFA, $\mu\text{mol/colon}$					
Acetate	112.91	132.73	42.70	46.14	28.93
Propionate	27.19	29.19	30.16	19.40	5.95
Butyrate	29.78 ^b	25.27 ^{ab}	2.74 ^a	3.61 ^a	6.76
Total SCFA	169.87	181.11	75.78	70.33	41.66
Colonic BCFA, $\mu\text{mol/g}^4$					
Isobutyrate	2.30 ^{ab}	3.32 ^b	1.21 ^a	1.57 ^{ab}	0.45
Isovalerate	2.26 ^a	4.54 ^b	2.95 ^{ab}	2.19 ^a	0.54

Table 6-5 (con't.)

Valerate	2.35 ^a	4.91 ^b	0.86 ^a	1.25 ^a	0.55
Total BCFA	6.89 ^a	12.78 ^b	5.01 ^a	5.03 ^a	1.43
Colonic BCFA, $\mu\text{mol}/\text{colon}$					
Isobutyrate	2.18 ^c	1.92 ^{bc}	0.49 ^a	0.89 ^{ab}	0.35
Isovalerate	2.11	2.62	1.19	1.24	0.47
Valerate	2.21 ^b	2.85 ^b	0.35 ^a	0.71 ^a	0.38
Total BCFA	6.47 ^{bc}	7.40 ^c	2.05 ^a	2.86 ^{ab}	1.26

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Pooled SEM.

⁴ Values are expressed on a dry matter basis.

^{abc} Means in the same row with different superscript letters are different ($P < 0.05$).

Table 6-6. Cecal and colonic total and empty weights, and pH values in rats fed select dietary fibers

Item	Treatment				SEM ³
	Control	Pectin	SFD ¹	SCF ²	
Total weight, g					
Cecum	3.12 ^a	3.96 ^a	6.73 ^b	6.15 ^b	0.38
Colon	1.81	1.46	1.28	1.49	0.16
Empty weight, g					
Cecum	0.94 ^a	1.23 ^b	1.58 ^c	1.33 ^c	0.08
Colon	0.87	0.88	0.87	0.92	0.07
Cecal pH	6.85 ^b	6.68 ^b	5.88 ^a	6.00 ^a	0.10
Colon pH	6.90 ^b	6.88 ^b	6.16 ^a	6.06 ^a	0.14

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Pooled SEM.

^{abc} Means in the same row with different superscript letters are different (P<0.05).

Table 6-7. Cecal concentrations of microbiota for rats fed select dietary fibers

Item	Treatment				SEM ³
	Control	Pectin	SFD ¹	SCF ²	
	-----log10 cfu/g cecal DM-----				
<i>Bifidobacterium</i> spp.	9.14	9.99	9.70	9.81	0.52
<i>Escherichia coli</i>	11.37 ^a	10.86 ^b	11.58 ^a	11.62 ^a	0.32
<i>Lactobacillus</i> spp.	11.59	12.20	11.33	11.52	0.33

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Pooled SEM.

^{ab} Means in the same row with different superscript letters tend to be different (P<0.10).

Table 6-8. Effect of select dietary fibers on cecum and colon histomorphology of rats

Item	Treatment				SEM ³
	Control	Pectin	SFD ¹	SCF ²	
Cecum					
Crypt depth, μm	164.58 ^a	210.61 ^b	208.81 ^b	201.48 ^b	5.46
Goblet cells (n) per crypt					
Total	12.6 ^a	20.1 ^b	19.8 ^b	19.3 ^b	1.48
Acidic mucin	7.1 ^a	14.2 ^b	13.9 ^b	12.5 ^b	1.22
Mixed (acidic/neutral)	5.7	7.5	6.9	6.9	0.41
Mucin(n) per crypt					
Sulfomucins	6.8	8.2	7.8	8.1	0.68
Sialomucins	5.9	6.2	6.7	6.0	0.44
Colon					
Crypt depth, μm	216.45 ^a	257.54 ^b	245.43 ^b	242.71 ^b	7.94
Goblet cells (n) per crypt					
Total	15.8 ^a	25.0 ^b	23.9 ^b	24.3 ^b	1.36
Acidic mucin	11.5 ^a	21.4 ^b	19.4 ^b	20.1 ^b	1.28
Mixed (acidic/neutral)	3.8	4.5	5.2	4.9	0.32

Table 6-8 (con't.)

Mucin(n) per crypt					
Sulfomucins	6.6 ^a	13.3 ^b	10.5 ^b	10.9 ^b	1.00
Sialomucins	10.7	10.2	9.9	10.2	0.92

¹ Soluble fiber dextrin.

² Soluble corn fiber.

³ Pooled SEM.

^{ab} Means in the same row with different superscript letters are different (P<0.05).

CHAPTER 7: SUMMARY

Dietary fiber represents a complex group of carbohydrates that have a variety of beneficial physiological and nutritional properties (Crittenden and Playne, 1996; Cummings et al., 1997; Van Loo et al., 1999). Evidence that dietary fiber consumption may positively affect risk factors associated with diseases such as obesity, non-insulin dependent diabetes mellitus, hyperlipidemia, hypertension, and cardiovascular disease continues to grow (Scheppach et al., 2001). This has led to a demand for the development of novel 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. Novel carbohydrates usually are relatively pure compared to natural fibers such as brans and those in whole grains.

A class of carbohydrate that is becoming popular as a proxy for commodity dietary fibers is low-digestible carbohydrates. These are a heterogeneous group of oligosaccharides that are partially unavailable to digestive enzymes so are either incompletely or not at all absorbed from the small intestine (Murphy, 2001; Scheppach et al., 2001; Grabitske and Slavin, 2008). The relative non-digestibility of these carbohydrates is due to the anomeric C atom of the monosaccharide unit having a configuration that makes linkages unable to be hydrolyzed by digestive enzymes. Low-digestible carbohydrates pass into the colon where they are substrates for either complete or partial fermentation by the colonic microbiota.

Evidence for the potential physiological and health benefits associated with low-digestible carbohydrate consumption is substantial and has led to an increased awareness among consumers (Murphy, 2001). Many benefits of low-digestible carbohydrate consumption exist including positive effects on mineral bioavailability and lipid metabolism in addition to their

anti-carcinogenic properties, but of major interest is their effect on energy content, glycemic response, fermentation characteristics, and indices of gastrointestinal tract health.

The objective of this research was to evaluate select novel, low-digestible carbohydrates - pullulans (Pull), soluble fiber dextrins (SFD), and soluble corn fibers (SCF) - for properties that could positively impact health outcomes. Monosaccharide composition (free, bound), simulated hydrolytic digestion, glycemic and insulinemic responses, true metabolizable energy content, fermentation characteristics, microbiota populations, and gut morphological characteristics were evaluated using *in vitro*, canine, avian, and rodent models.

The objective of the first study was to determine *in vitro* hydrolytic digestion characteristics, glycemic and insulinemic responses using a dog model, and true metabolizable energy (TME_n) content using an avian model, of select SFDs and Pulls. Carbohydrates studied included six soluble fiber dextrins (SFDs) and three pullulans. Soluble fiber dextrins were evaluated and were produced using two different starch sources, corn and tapioca. Pullulans evaluated were of varying molecular weight.

All SFDs and Pulls had low free sugar and high hydrolyzed monosaccharide concentrations, with glucose being the primary free and bound sugar present in all substrates. Hydrolytic digestibility values were higher for the tapioca-based SFDs (50%) than for the corn-based SFDs (20%). All Pulls were found to be nearly completely digested to glucose. Overall, the SFDs exhibited varying degrees of resistance to digestion, thus resulting in attenuated glycemic responses compared to maltodextrin (Malt). The corn-based SFDs had relative glycemic responses (RGR) ranging from 27 to 100%. All three tapioca-based SFDs had similar RGR values averaging ~50% of the Malt control. Despite the differences in RGR, all SFDs

lowered the relative insulinemic response (RIR) compared to Malt. Pullulans resulted in low glycemic and insulinemic responses, with an average response value of ~24% for both RGR and RIR. Soluble fiber dextrins had lowered TME_n values compared to Malt, with tapioca-based SFDs being numerically higher than for the corn-based SFDs. Pullulans resulted in higher TME_n values, similar to the 4.0 kcal/g value for Malt.

The SFDs varied in sugar composition and physiological responses, and demonstrated varying degrees of resistance to digestion. 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. Perhaps the corn-based SFDs had a higher degree of dextrinization, more branching, or a higher concentration of non-digestible linkages compared to the SFDs derived from tapioca, making them less susceptible to digestive enzymes. Having a decreased hydrolytic digestibility enabled the SFDs to attenuate the glycemic and insulinemic responses, making them suitable candidates for reduced glycemic and low-calorie foodstuffs. A portion of SFDs pass into the colon and perhaps promote health due to their fermentative properties.

Even though the Pulls were nearly completely hydrolyzed after simulated digestion, they resulted in a significantly blunted glycemic curve, corroborating results showing that Pulls are slowly digestible carbohydrates. Other research also has found Pulls to be extensively hydrolyzed in vitro, with hydrolysis occurring slowly over time, thus making them slowly digestible carbohydrates (Wolf et al., 2003). Pullulans could be ideal candidates for incorporation into foodstuffs for diabetics as they result in low glycemic responses without eliciting large peaks in blood glucose or insulin.

The objective of the second study was to determine *in vitro* hydrolytic digestion characteristics, glycemic and insulinemic responses using a dog model, and TME_n content using an avian model of select soluble corn fibers (SCF). Soluble corn fibers were obtained by isolating an oligosaccharide-rich component from partially hydrolyzed corn syrup. During corn-starch hydrolysis, an aqueous stream comprised of dextrose, fructose, and a mixture of oligosaccharides is formed (Harrison and Hoffman, 2007). This stream undergoes several additional processes including nanofiltration and use of enzymes to create a product with increased α -1,6 glycosidic bonds (Harrison and Hoffman, 2007). The last steps in the production process include decolorization of the syrup and evaporation and drying to produce a powder. All the SCFs evaluated were produced using the basic steps outlined above, but procedures were modified to produce a variety of SCFs. The SCFs evaluated were classified into first generation and second generation products. Both generations of SCFs rely on the creation of reversion products using different combinations of acid catalysts and enzymes to increase the number of glycosidic bonds.

Soluble corn fibers from both generations of product had low free sugar concentrations and were hydrolyzed completely to glucose. One SCF had a substantial portion of bound fructose. The first generation SCFs were found to have a digestibility value of approximately 50% whereas second generation SCFs had digestibility values averaging 32%. Differences in the acid catalyst used during the production process affected digestibility of the SCFs dramatically. Using hydrochloric acid as a catalyst appeared to create reversion products that were more resistant to the enzymes used in simulated hydrolytic digestion compared to reversion products made with phosphoric acid. All SCFs from both generations resulted in reduced glycemic and insulinemic responses. Second generation SCFs resulted in generally lower responses than did

first generation SCFs. Second generation SCFs resulted in lower (avg 1.80 kcal/g) TME_n values than did first generation SCFs (avg 2.40 kcal/g).

Overall, the production processes implemented for second generation SCFs were very successful in producing low-digestible carbohydrates. The second generation series of SCFs, on average, had lower digestibilities, glycemic responses, and TME_n values than did the SCFs from the first generation series. The increased resistance to digestibility of these carbohydrates was likely due to increased glycosidic bond formation resulting from different production methods.

The objective of the third study was to measure *in vitro* hydrolytic digestion and glycemic and insulinemic responses of select carbohydrate blends containing the novel carbohydrate, SCF. Two SCFs produced using different production methods were used in the formulation of these blends, SCF spray-dried (SCFsd) and SCF. In the previous study, both SCFs were found to have a lower hydrolytic digestion, but nonetheless elicited a glycemic response (SCFsd, 63%; SCF, 25%). Further attenuation of glycemic and insulinemic responses was achieved by blending SCF with carbohydrates that did not elicit blood glucose responses upon consumption such as Pull, sorbitol (Sorb), and fructose (Fruct). One set of blends contained SCFsd blended with different concentrations of either Pull, Sorb, or Fruct (SCFsd blends). The other set of blends contained SCF blended with different ratios of Pull, Sorb, and Fruct (SCF:Pull:Sorb:Fruct blends).

All blends had low to intermediate free sugar concentrations except those with higher percentages of Fruct and Sorb. The addition of Pull, even at high percentages, did not have a large impact on free sugar concentrations for either set of blends. However, addition of Pull increased the hydrolyzed monosaccharide concentrations of the blends, especially by increasing

the concentration of glucose. Addition of higher amounts of Fruct and Sorb increased free Fruct and Sorb concentrations of the blends. Blends with higher percentages of Fruct or Sorb had lower hydrolyzed monosaccharide concentrations due to their higher free sugar concentrations. Blends of SCFsd and Pull resulted in high digestibility values (~91%). The digestibility values of SCFsd blends increased with added percentages of Fruct or Sorb; however, concentrations of released glucose decreased with higher percentages of Fruct or Sorb. Total released monosaccharide concentrations increased as the percentage of SCF decreased in the SCF:Pull:Sorb:Fruct blend. The concentrations of glucose released increased as the percentage of Pull increased in the blends. As noted with the SCFsd blends, increased Fruct and Sorb in the SCF:Pull:Sorb:Fruct resulted in higher total released monosaccharides, with higher concentrations of Fruct and Sorb released. All SCF blends resulted in low glycemic and insulinemic responses that were attenuated compared to Malt. Blends containing Fruct and Sorb were most effective in attenuating glycemic and insulinemic responses.

The SCFs used in both sets of blends partially resisted hydrolytic digestion and attenuated blood glucose concentrations upon consumption. Soluble corn fiber had a lower glycemic response than SCFsd, likely due to increased number of digestion-resistant bonds and bound fructose. Blending these SCFs with select carbohydrates was generally successful in further attenuating glycemic and insulinemic responses. Even though in vitro hydrolytic digestion studies have shown Pull to be nearly completely digested, Pull attenuated the glycemic response due to its property of being slowly digestible. Even at concentrations as low as 5% of the blend, Fruct reduced glycemic and insulinemic responses because Fruct and Sorb do not acutely raise blood glucose or stimulate insulin secretion. These SCF blends could be utilized to lower postprandial glucose and insulin responses by replacing available carbohydrates in foods

with low-digestible carbohydrates, and be of potential help to diabetic patients in blood glucose management.

The objective of the fourth study was to evaluate gastrointestinal health outcomes resulting from supplementation of the novel, low-digestible carbohydrates, SFD and SCF, to rats. Previous studies showed that SFD and SCF had decreased hydrolytic digestion *in vitro*, attenuated glycemic and insulinemic responses, and lower energy values. These data point to the fact that SFD and SCF contain a fraction that resists digestion and passes to the colon for potential fermentation. Indices of gastrointestinal health included digesta pH, short-chain fatty acid (SCFA) concentrations, microbial populations, large bowel mass, and histomorphology measurements in cecum and (or) colon. Cellulose was included as a poorly fermentable negative control treatment and pectin as a highly fermentable positive control treatment.

Consumption of SFD and SCF resulted in increased cecal weight but not colon weight. Decreased cecal and colonic pH values also resulted from dietary supplementation of SFD and SCF. Cecal and colonic SCFA concentrations were not increased by SFD and SCF supplementation when expressed on a per gram digesta DM basis. However, SFD- and SCF-fed rats had more cecal contents so when analyzed on a per cecum basis, increased cecal SCFA concentrations compared to the cellulose control resulted for both SFD and SCF. While acetate and propionate increased with SFD and SCF supplementation, neither resulted in increased butyrate concentration compared to the cellulose control. In both the cecum and colon, SFD and SCF promoted less branched-chain fatty acid concentrations compared to pectin. Treatment had little effect on microbiota concentrations in cecal contents. No differences were noted in *Bifidobacterium* spp. or *Lactobacillus* spp. among treatments. The only difference noted was for pectin that tended to promote lower *Escherichia coli* concentrations. Soluble fiber dextrin and

SCF, in addition to pectin, were able to modulate cecal and colonic histomorphology. These fermentable carbohydrates increased crypt depth and the number of goblet cells per crypt compared with the cellulose control. Increased acidic mucins per crypt resulted from supplementation of SFD, SCF, and pectin. Only in the colon did supplementation of SFD, SCF, and pectin increase sulfomucins per crypt.

This study showed that SFD and SCF are, indeed, low-digestible, partially fermentable carbohydrates that positively impact gastrointestinal health outcomes. Supplementation of these low-digestible carbohydrates resulted in increased SCFA concentrations, but not butyrate. This also has been observed in other studies with SCF in particular. Unlike other studies using *in vitro* methodologies or human subjects, SFD and SCF did not result in increased gut microbiota concentrations. Perhaps longer-term studies or more dramatic changes in diet are necessary to elicit microbiota alterations. Since both SFD and SCF are somewhat digestible, perhaps larger dosages, too, are needed to demonstrate changes in microbial populations. Although SFD and SCF were not butyrogenic or bifidogenic in the rat, their fermentative capacity had a positive effect on cecal and colonic morphology by increasing crypt depth, goblet cell numbers, and acidic mucins. These outcomes positively impact gut health by increasing the mucous thickness and beneficially impacting the gut mucosal barrier.

In conclusion, the research presented here clarifies that select novel carbohydrates are low-digestible, partially fermentable, and have the potential to beneficially impact health in several ways. This research offers insight into the importance of evaluating monosaccharide composition and how it can impact outcomes such as hydrolytic digestion, glycemic and insulinemic responses, and energy values. The data presented in this dissertation provide valuable information for food formulators and consumers wishing to incorporate ingredients with

advantageous physiological properties into foodstuffs and diets. Future research is necessary to determine the impact of these supplemental low-digestible carbohydrates on health outcome in disease states using diabetic or immune-challenged models.

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