Modulation of Starch Digestion and Nutrient Digestibility by Exogenous Fibres and Different Genotypes of Potatoes in Growing Pigs Fed a High-Fat Basal Diet

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ABSTRACT

MODULATION OF STARCH DIGESTION AND NUTRIENT DIGESTIBILITY BY EXOGENOUS FIBRES AND DIFFERENT GENOTYPES OF POTATOES IN GROWING PIGS FED A HIGH-FAT BASAL DIET

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The objectives of this study were to examine effects of dietary supplementations of guar gum and cellulose at 10%, and 8.5% guar gum in combination with six different genotypes of processed dry potatoes at 25.1% on ileal and/or fecal dry matter, starch, amino acid and major bone macro-mineral digestibility, intestinal fermentation and body weight gains in growing pigs fed a high-fat basal diet. Dietary supplementations of guar gum, cellulose and guar gum (8.5%) in combination with each of the six test potatoes decreased ($P < 0.05$) the ileal starch digestibility compared with the negative control diet (**NC**). Out of the six potato genotypes examined, cv 12272-3, cv 96044-3, and cv F05081 were associated with lower ($P < 0.05$) ileal starch digestibility compared with the NC conventional cornstarch. Dietary supplementations of 10% guar gum and guar gum (8.5%) in combination with each of the six test potatoes increased ($P < 0.05$) the distal ileal free glucose recovery compared with the NC. Guar gum supplementation at 10% reduced ($P < 0.05$) the abundance of the phosphorylated mammalian-target-of -Rapamycin (mTOR) in the proximal jejunum compared with the NC. Compared with the NC, the ileal digestibility of Ala, Gly, and Pro were decreased ($P < 0.05$) by the guar gum supplementation, while the digestibility of Gly was reduced ($P < 0.05$) by the cellulose supplementation. The ileal digestibility of several AA, including Ala, Glu, Gly, Leu, Lys, Phe and Pro, were decreased ($P < 0.05$) by the test potatoes plus 8.5% guar gum compared with the NC. Guar gum (10%) also reduced ($P < 0.05$) the apparent fecal P digestibility. Pearson correlation analyses showed that the apparent ileal and fecal Ca and P digestibility were inversely related ($P < 0.05$) to total contents of glycoalkaloids in the potato diets. It can be concluded that dietary supplementations of guar gum and cellulose at 8.5 - 10% significantly reduced ileal starch digestibility. Novel genotypes of potatoes selected for promoting human health should have minimal levels of glycoalkaloids to prevent the potential negative effects of potato glycoalkaloids on intestinal Ca and P absorption and bone health.

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AAFC-ABIP	Agriculture and Agri-Food Canada - Agriculture Bioproducts Innovation Program
AA	Amino Acids
ADG	Average Daily Gain
ADFI	Average Daily Feed Intake
AOAC	Association of Official Analytical Chemists
Ala	Alanine
Asp	Aspartic Acid
Asn	Asparagine
BW	Body Weight
Ca	Calcium
CFIA	Canadian Food Inspection Agency
CP	Crude Protein
CrI	Crystalline Index
cv	Cultivar
Cys	Cysteine
d	day
DE	Digestible Energy
DM	Dry Matter
DMI	Dry Matter Intake
DP	Degree of Polymerization
g	Gravity
GC-MS	Gas Chromatography-Mass Spectrometry
GE	Gross Energy
G:F	Gain to Feed Ratio
Gln	Glutamine
Glu	Glutamic acid
GLUT2	Diffusive Glucose Transporter-2
Gly	Glycine
GL	Glycemic load
GI	Glycemic Index
HPLC	High Performance-Liquid Chromatography
ISO	Isoleucine
K_m	Affinity of enzymes or Transporters
LDL	Low-density Lipoprotein Particles
Leu	Leucine
LSM	Least Square Means

Mg	Magnesium
MGA	Maltase-glucoamylase
ME	Metabolizable Energy
mTOR	Mammalian Target of Rapamycin
NC	Negative Control
NDF	Neutral-detergent Fibre
NE	Net Energy
NSPs	Non-starch Polysaccharides
P	Phosphorus
P	Probability
PC	Positive Control
Phe	Phenylalanine
Pro	Proline
REDD1	Regulated in development and DNA damage response protein-1
REDD2	Regulated in development and DNA damage response protein-2
RDS	Rapidly Digestible Starch
RS	Resistant Starch
SCFAs	Short-chain Fatty Acids
SDS	Slowly Digestible Starch
SE	Standard Errors
SEM	Standard Errors of Means
SGLT1	Na^+-Dependent Glucose Cotransporter-1
Val	Valine
VFA	Volatile Fatty Acids
V_{max}	Maximal Enzyme Activity or Nutrient Transport Rate
TDF	Total Dietary Fibre
wk	Week

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Among food carbohydrates, starch occupies a unique position. It is the major carbohydrate storage material in many higher plants and is considered the second largest natural biopolymer next to cellulose (Sun et al., 2010). Also, starch is an ancient organic nutrient substance that can be traced back to early human civilization. Romans used it to augment their looks as a cosmetic; Egyptians, being more technical, used it as paste to stiffen weaved clothes and as an adhesive; and Chinese were literal about its use to improve paper quality, while Persians and Indians ate it as a dish (Junxia et al., 2001). Current annual production for primary starch sources is estimated to be at 46.1 million tons of corn, 9.1 million tons of cassava, 5.15 million tons of wheat, and 2.45 million tons of potatoes (Röper and Elvers, 2008).

In its native granular form, starch has several applications. Due to its low cost and using chemical and physical modifications, starch has been applied in a wide variety of industrial products including food ingredients, sizing agents for paper, textiles, and starch-based plastics (Ellis et al., 1998). About 43% of produced starch is consumed in the nonfood industries and 57% is used in the food sector (LMC, 2010).

In nonfood applications, the most traditional use of starch has been in the paper industry. The paper industry is the largest consumer of starch, which is estimated at more than 10 million tons per year. Oxidized and enzymatically hydrolyzed starches have wide applications as paper coating, improving the strength and printability of paper (Veen et al., 2002; Lawala et al., 2005). Those starches are also often used in textile and laundry finishing products (Lawala et al., 2005). Recently, biodegradable polymers are an alternative to overcome the problems related to recycling limitations, and the global environment. Furthermore, this material can provide support for a sustainable agriculture. Starch is considered as a potential polymer to be used in biodegradable materials because of its low cost, availability, and production from renewable resources. Starch-based and biodegradable polymers have been used in the production of cars (Bledzki et al., 2006). Corn derived starch can also be used in products such as a biofiller to enhance tire performance, and to reduce the rolling resistance of tires and fuel consumption (Scott, 2002). Also, starch is the basic source of food energy for the majority of the world's human population. In foods, starch contributes to the characteristic properties of food products. Because of the relationship between structure and functionality, starch chemistry and modifying technologies have been studied to obtain structures with specific functionalities. Starch hydrolysis products such as glucose or maltose syrup, maltodextrins and their derivatives by isomerization are used in candy, sweets, chocolate, and cake, dessert, dairy, and pastry products. An increased understanding of starch functionality and how they are affected by other ingredients and processing methods could lead to improvements in the quality of food products.

Statistics from the Heart and Stroke Foundation of Canada (2003) concerning the cardiovascular diseases in Canada is alarming. Cardiovascular diseases include myocardial infarction, ischemic heart, high blood pressure and stroke, which are the underlying cause of death in 1 out

of 3 Canadians. Meanwhile, the number of cardiovascular cases in Canada is going to grow over the next 20 years. Recent studies suggest that the nutritional properties of starch may be of importance for the treatment, as well as the prevention, of cardiovascular diseases commonly found in modern society (Kendall et al., 2004; Johnson et al., 2007; Johnson et al., 2009). In addition, scientists also have strong interest in understanding speed of starch digestion and absorption in foods. These starchy foods show significant differences in their glycemic responses and are defined as high and low glycemic index (**GI**) foods. The glycemic index is defined as the incremental blood glucose area after ingestion of the test product as a percentage of the corresponding area following a reference pure glucose or white bread (Jenkins et al., 1981). Healthy nutrition trends for enhanced consumption on low-GI foods have pushed the growth of resistant starch. Resistant starch (**RS**) causes increased microbial fermentation in the large intestine to produce volatile short-chain fatty acids with similar physiological effects to dietary fibres (Topping et al., 2001). Health benefits of resistant starches have been reviewed by Nugent (2005) and Liu (2007). More discussions about starch in human nutrition and health are provided in Chapter 2.

Pigs have been domesticated for more than ten thousand years and are a major source of human foods (Fan et al., 2008). Digestive physiology of pigs is very close to that of humans. However, the digestive capacity of pigs is much higher than that of human. Therefore, pigs fed on a high-fat and high-animal protein basal diet are recognized as a relevant animal model to examine efficacy of starch components from various food sources such as novel cultivars of potatoes for improving human health as well as to understand biological mechanisms for food and nutrition based disease prevention.

1.2. LITERATURE REVIEW

1.2.1. Definition, Classification and Sources of Starch

Starch has extensive sources in nature including starchy foods such as breads, pasta, cereals, corn, wheat, rice, potatoes, legumes of beans and peas and some vegetables. With respect to nutritional definition, starch has long been regarded as the only quantitatively important digestible polysaccharide (Asp, 1996). According to the dietary carbohydrate classification (Englyst et al., 1992), starches are characterized into three categories based on their different speed of digestion in the small intestine. Rapidly digestible starch (**RDS**) is rapidly and completely digested in the small intestine and RDS is associated with elevated levels of plasma glucose and insulin (Liese et al., 2003). Therefore, it is linked to type-II diabetes and coronary heart diseases (Liu and Manson, 2001). Slowly digestible starch (**SDS**) is completely but slowly digested in the small intestine. It has a moderate influence on the plasma glucose and insulin levels but it is the most desirable form of starch from the nutritional point of view (Lehmann and Robin, 2007). RS is not digested in the small intestine and increasing evidence points to a protective effect of dietary RS fibre on colorectal cancers (Bingham et al., 2003). Chemically speaking, RS belongs to the starch family. However, nutritionally speaking, RS has been regarded as non-viscous soluble fibre (Champ et al., 2003b). According to Singh et al. (2010), RS is defined to be as total starch subtracting RDS and SDS. According to current nutritional classification, there are four categories of RS in foods (Eerlingen and Delcour, 1995;

Rideout et al., 2008b), including RSl - physically inaccessible, located within the plant cell wall, occurring mainly in partially milled cereal grains, seeds or legumes; RS2 - resistant raw starch type, occurring as native starch granules of raw potato, unripe banana, high amylose starch; RS3 - retrograded or crystalline non-granular starch, occurring in cooked and cooled potatoes, bread or corn flakes, retrograded amylose starch; and RS4 - chemically modified starches, occurring in foods containing chemically modified starches or linkage-altered dextrins.

1.2.2. Chemical and Physical Properties of Starch

Starch is a commodity with great nutritional and industrial importance. In view of this importance, its chemical and physical properties are well established. As mentioned earlier, conventional dietary starches are composed of amylose (about 25%) and amylopectin (75%) (Nichols et al., 1998). In some mutant crop species, starch granules may contain nearly 100% amylopectin. In addition to amylose and amylopectin, starch granules also contain some other minor components such as proteins, lipids, and inorganic substances.

Amylose, the minor starch component, consists mainly of α-(1→4) linked D-glucose units. The degree of polymerization (**DP**) of this linear polymer is usually in the range of 500-600 units (Jacobs and Delcour, 1998). However, it is now accepted that this polymer is slightly branched, having occasional α-(1→6) branch points. The molecular weight of amylose has been reported to vary between 10^5 and 10^6 Da (Morrison and Karkalas, 1990). With a linear chemical structure, amylose has the ability to change its conformation. With many hydroxyl groups, there is a high hydrogen-bonding capability with strong internal forces that permit these changes. In natural conditions, starch molecules largely exist in starch granules and relative amylose content and ratio of amylose to amylopectin have been shown linked to the crystalline lamellar organization in potato starch granules (Wikman et al., 2013). An open channel in the center of a helix permits complexing with other molecular species such as iodine and fatty acids. However, the conformation of amylose has been the subject of controversy and has been shown to vary from helical to an interrupted helix, to a random coil. In alkaline solutions (e.g., KOH) and in dimethyl sulfoxide (DMSO), amylose probably has an expanded coil conformation while in water and neutral aqueous potassium chloride solutions it is a random coil with short loose helical segments (Heineck et al., 2008). Amylose can be complexed with lipids when heat energy is supplied. Formation of amylose-lipid complex occurs during heat-moisture treatment for naturally containing lipids (Kugimiya and Donovan, 1981) or when lipids are added to pure amylose that is free of lipids (Biliaderis et al., 1985) or defatted starches (Biliaderis et al., 1986). In food systems, complex formation of amylose with various types of lipids is of interest, because this can affect the functional properties of foods. For example, the following starch complex formations can occur such as decreased swelling, solubilization and thickening power of starch (Galliard and Bowler, 1987), retardation of starch retrogradation and bread firming (Biliaderis and Tonogai, 1991), prevention of stickiness of dried potatoes (Hoover, 2001), and improvement of structural integrity of cereal kernels during cooking (Biliaderis et al., 1993).

Amylopectin is a branched molecule with α-(1→4) linked glucose units in linear chains and α-(1→6) linked branched points (Lineback, 1984). Average polymer molecular weight is at 10^7-10^9 (Aberle et al., 1994). While the average size of unit chains of amylopectin is 20-25, the

amylopectin molecule contains several distributions of chains differing in their chain length being designated as A, B, and C (Shea et al., 1997). It is now widely accepted that linear branched chains with a higher degree of polymerization in amylopectin are the crystalline regions present in the granules. These short chains form a double helical ordered structure and parts of the double helices can pack together in organized arrays in a cluster form (Imberty et al., 1991). Another unique feature of amylopectin is the presence of covalently linked phosphate monoesters and they can be linked to either the C3 or C6 position of the glucose monomers, and occur to a greater extent in starch from tuberous species, especially potato starch (Blennow et al., 2000).

Apart from amylose and amylopectin, smaller amounts of other components such as lipids, proteins, and phosphate groups are present at very low levels in starch granules, and they play an important role in the physicochemical properties of starch (Morrison and Karkalas, 1990). Starch lipids have many implications on properties of starch, e.g., forming complexes with amylose, prevent parts of the amylose from contributing thickening power to gelatinized starch, form undesirable flavors by oxidation of unsaturated lipids and reduce granular swelling (Aprianita, 2010). Blennow et al. (2002) reported that native potato starch contains phosphate monoesters that are mainly located in the amylopectin and that many of the desirable qualities of potato starch such as enhanced paste clarity, high peak consistency, and significant shear thinning and a slow rate of retrogradation are related to its phosphate content. Nitrogen present in the starch is generally considered as protein, but it may also be a part of the starch lipids. The protein content of purified starch is a good indicator of its purity (Jayakody et al., 2007). Alkali extraction is very effective in solubilizing proteins, therefore, careful washing of crude starch with diluted alkali can reduce protein level in purified starch.

1.2.3. Starches in Monogastric Animal and Human Nutrition

The nutritional properties of starch in foods vary considerably, and may be of importance for the treatment, as well as the prevention of several diseases commonly found in our societies, such as type-II diabetes, colorectal cancers, and cardiovascular diseases. Starch in cooked potatoes, most types of bread, and breakfast cereals is rapidly digested and absorbed. In contrast, starch in legumes, pasta, and certain rice or cereal products is slowly digested and absorbed. These starchy foods show significant differences in their glycemic responses and are defined as high and low glycemic index (**GI**) foods. The GI provides a measure of how quickly blood sugar levels rise after eating a starchy food relative to consumption of pure glucose. The GI concept was originally developed by Dr. David J. Jenkins and colleagues in the early 1980's at the University of Toronto (Jenkins et al., 1981). On the other hand, the glycemic load (**GL**) of food is a number that estimates how much the food will raise a person's blood glucose level after eating it (Glycemic Research Institute, 2013). One unit of glycemic load approximates the effect of consuming one gram of glucose (Glycemic Research Institute, 2013). Glycemic load accounts for how much carbohydrates are in the food and how much each gram of carbohydrates in the food raises blood glucose levels (Glycemic Research Institute, 2013). Glycemic load is based on the GI, and is defined as the grams of available carbohydrate in the food times the food's GI and divided by 100 (Glycemic Research Institute, 2013). Although there is an ongoing debate on the clinical implication of GI, it offers a tool to select and classify foods

according to their fate during digestion. Data from a number of medium- to long-term studies in diabetic subjects suggest that low-GI diets improve parameters related to glucose and lipid metabolism. Due to the quantitative importance of starch in the diet, the GI characteristics of the diets tested reflect mainly those of the starchy components (Fontvielle et al., 1992). Reported effects of low-GI diets include reduced glycosylated hemoglobin, fructosamine, and postprandial blood glucose levels, all of which are indicators of improved glycemic control (Miller, 1994). Low-GI diets also reduced serum low-density lipoprotein (**LDL**) cholesterol and triglycerides in diabetes (Wolever et al., 1994). A reduction in blood lipids was also seen in hyperlipidemic patients with diets based on low-GI starchy foods and with the effect being most pronounced in subjects with hypertriglyceridemia (Jenkins et al., 2002). Moreover, a low-GI diet was also shown to reduce 12-h blood glucose profiles and lower insulin secretion in healthy subjects as well as total plasma cholesterol levels (Wolever, 2003), suggesting a protective role against the development of diseases linked to the metabolic syndrome (Mckeown et al., 2004).

In studies conducted mostly in patients with type-2 diabetes, the low-GI starch diets have shown an improvement in glycated hemoglobin (HbA1c) of 10%, compared with high-GI starch foods (Mann et al., 2007). Epidemiological studies suggested that reduced postprandial glucose peaks reduced episodes of hypoglycemia, improved lipid responses, lower concentrations of glycosylated hemoglobin and fructosamine and greater insulin sensitivity were beneficial for diabetic management (Wolever, 2003). Slowly digestible starch intake results in a beneficial metabolic response for these conditions and is recommended for the prevention and management of diabetes (Axelsen et al., 1999; Seal et al., 2003). It was shown that resistant starch (RS) containing foods at breakfast improved carbohydrate metabolism and reduced insulin requirement in insulin-treated type-2 diabetic patients (Golay et al., 1992). During the past 30 years, there has been a steady increase in our knowledge of RS and its health effects. In spite of the differences in clinical methodologies employed and sources of RS used, the substantial body of nutritional and clinical research demonstrates that a broad range of health benefits can be attributed to RS intake. While amylopectin is highly branched, amylose polymers are linear molecules and tend to form semi-crystalline structures via intramolecular hydrogen bonds and have less surface areas (Singh et al., 2010; Zijlstra et al., 2012). Thus, amylose is digested or depolymerized at a lower rate than amylopectin due to decreased accessibility for α-amylase (Batlle et al., 2000; Zijlstra et al., 2012). Dietary starches high in amylose were much less digestible and high in RS, being more readily available for large intestinal fermentation (Zijlstra et al., 2012).

Many of the positive physiological changes afforded by RS appear to be connected to its fermentation in the colon (Morita et al., 2004; Hamer et al., 2008). Fermentation is the process whereby colonic bacteria utilize a dietary substrate to generate metabolic energy for their maintenance and growth. Under the anaerobic conditions of the large intestine (colorectal region in humans or the cecal plus colorectal region in animals), the microflora produce a range of metabolic end-products that include volatile short-chain fatty acids (**SCFAs**), gases (e.g., H_2 and CH_4), and water that are important for the maintenance of a healthy microflora (Toden et al., 2007). The SCFAs include acetate, propionate, and butyrate are available for absorption by the colon for utilization by the individual host or as an energy source for the colonic microflora. The SCFAs directly influence the colonic environment by lowering pH, and improving colonic

function (Duncan et al., 2007). In clinical studies in which diets containing corn and RS were consumed, increased SCFAs particularly butyrate and lower pH, were observed (Van-Munster et al., 1994; Noakes et al., 1996; Jenkins et al., 1998). These observations provide direct indication of microbial RS utilization, and suggest protective outcomes for digestive health. Resistant starch that is fermented is also thought to enhance colonic functions by stimulating colonic blood flow, promoting colonic muscular contraction, which increases intestinal smooth muscle tone and nutrient flow, increases mineral absorption, and promotes colonocyte proliferation (Bird *et al.*, 2000).

Consumption of RS positively influences other markers of digestive health. In clinical studies, RS promotes fecal bulking and laxation (Hylla *et al.*, 1998). The laxative effects of RS are less marked than those observed from wheat bran but RS ingredients can be important contributors to digestive health. With an enriched supply of colonic RS, less favorable bacterial metabolic activity is reduced, minimizing the production of potentially cytotoxic compounds (Rideout et al., 2008b). For example, ammonia, phenol, and secondary bile acids become less prevalent metabolites when an RS-enriched diet is consumed (Van-Munster *et al.*, 1994; Birkett *et al.*, 1996). This may suggest that RS significantly increased fecal bulk in humans. In analogy with the bulking effects noted with fermentable types of dietary starch components, this fecal bulking is probably due to an increased bacterial mass.

RS can be fermented in the large intestine as acetic acid, propionic acid, and butyric acid. Propionate is increasingly being discussed in relation to its beneficial effects on glucose and lipid metabolism (Ximenes et al., 1998). Most of the studies conducted so far have been with dietary supplemental propionate, however, in a recent study, rectal administration of propionate at levels that can be expected to be generated from colonic fermentation reduced total liver cholesterol in hyperinsulinemic rats (Berggren et al., 1996). Meanwhile, the lowering of serum triglyceride levels found by Sacquet et al. (1983) in rats fed amylose cornstarch could only partly be accounted for by increased fecal sterol excretion. Thus, it is possible that the lowering of plasma cholesterol and triglyceride levels noted in rat experiments is mediated by SCFAs produced in the colon. Other types of RS fractions evaluated in this context include cyclodextrins. According to Suzuki and Sato (1985), the addition of cyclodextrins containing seven glucose residues improved parameters related to lipid metabolism in rats.

As described previously, many of the colonic effects of RS are mediated via the production of SCFAs during fermentation. One important role of the large intestine is to salvage water and minerals, which is also SCFA mediated. RS-rich ingredients have been found to be useful for inclusion in foods and preparations designed for alleviating the symptoms of diarrhea. In an Indian study, RS enhanced large bowel salvage of water for people suffering chronic diarrhea from cholera (Ramakrishna et al., 2000).

Apoptosis (controlled cell death) is another biomarker of colonic tissue health that is particularly useful in animal models to simulate the body's responses to the initiation of colorectal cancers by a specific carcinogen and enable observations of the role of the diet in protecting against colorectal cancer. The SCFA butyrate has been associated with a protective role concerning the prevention of colorectal cancer and the consumption of RS has been shown to elevate colonic butyrate concentrations (Rideout et al., 2008b). The ingestion of RS has resulted in

significantly increased levels of apoptosis in a rat model that had been exposed to the colon-specific genotoxin azoxymethane (Le Leu et al., 2002). Feeding RS to these rats increased the apoptotic index by more than 30% in a dose-dependent manner with no effects on cell proliferation in the genotoxin-exposed colonic cells (Le Leu et al., 2003).

RS possibly contributes to the advantageous effects previously assigned solely to conventional dietary fibres. Current knowledge definitely indicates a great challenge for plant breeders and the food industry to optimize the nutritional properties of starch in foods. However, in order to elucidate fully the nutritional potential of differences in RS contents of food, more long-term and large scale animal and human clinical studies are needed with test diets well characterized for RS profile as to address these important health parameters.

1.2.4. Digestive Utilization of Starch in Monogastric Animal and Humans

Digestive utilization of starch in feed & food sources is widely and effectively studied by *in vitro* enzymatic hydrolysis kinetics (Singh et al., 2010; Zijlstra et al., 2012) and by *in vivo* ileal cannulation & portal-vein catheterization in studies with pigs (Zijlstra et al., 2012).

Starch digestion and absorption essentially consist of three phases, including the intraluminal phase, the brush-border phase, and the glucose-absorption phase (Gray, 1992). The digestion of starch is initiated in the oral cavity by salivary α-amylase secreted from the parotid glands. Chewing also disintegrates the food, thus increasing the ratio of surface area to volume in the solid phase and hence enzyme accessibility. Despite the acidic conditions prevailing in the gastric juice, salivary amylase appears to retain some activity when passing through the stomach to the duodenum (Skude and Ihse, 1976). Thus, the relative contribution of salivary amylase to the total amylase activity has been found to be approximately 15% in duodenal aspirates from healthy human subjects (Skude and Ihse, 1976). Another important source of α-amylase is secreted from the exocrine pancreas into the small intestinal lumen. Although slightly different with respect to structure and stability, the substrate specificity of these α-amylases is similar. The end products with amylose as the substrate are exclusively maltose and maltotriose (Gray, 1992). Degradation of amylopectin yields preferably maltose, maltotriose, and α-limit dextrins containing the α-1,6 branch links (Wursh, 1989). Under this context, it should be pointed out that composition of porcine saliva including salivary α-amylase and the general developmental patterns of porcine exocrine pancreatic and the small intestinal mucosal enzymes involved in starch digestion are very similar to these in humans (Yen, 2001), making pigs are a relevant large animal model to study starch digestion for human nutrition and health management.

The degradation products from starch, with maltose dominating, diffuse from the lumen to the brush border of the small intestinal mucosa, where the final digestion to glucose takes place through the action of disaccharidases and oligosaccharidases (sucrase–isomaltase complex, glucoamylase) with isomaltase, maltase-glucoamylase, and α-limit dextrinase activity (Gray, 1992; Gudmand-Hoyer and Skovbjerg 1996). These enzymes are located close to the active D-glucose transport sites, and the glucose released via enzymatic hydrolysis is absorbed across the enterocytes into the interstitial fluid. The glucose is then transported into the portal blood and the liver, enters the systemic circulation, and reaches peripheral tissues through the

regulatory action of insulin (Wright et al., 2011). In infants with low or no pancreatic α-amylase secretion, starch has been shown to evoke a flat blood glucose response (Whitcomb and Lowe, 2007). An optimal efficiency of starch digestion is therefore dependent on the delivery of easily diffusible α-amylolysis products to the small intestinal mucosal surface. However, when the total α-amylase activity was reduced to low levels in rats, the delivery of ingested starch to the hind-gut increased, indicating a comparatively modest reduction in starch digestive capacity (Shih et al., 2007). Resistant starch will enter from the small intestine to the large colon, and is subjected to fermentation by colonic bacteria. Carbohydrates and oligosaccharides are fermented to volatile SCFAs, including acetate, propionate, butyrate and other gases (Cummings et al., 1996).

In the small intestine, glucose is transported across the apical membrane of enterocytes via the Na^+-dependent glucose cotransporter-1 (**SGLT1**, Turk et al., 1996). SGLT 1 has been believed to be the major route for the absorption of dietary glucose across the luminal membrane in pigs (Moran et al., 2010). Increased SGLT1 activity should accelerate intestinal glucose absorption. It has been speculated that the more rapid increase of plasma glucose concentration leads to excessive release of insulin and subsequent stimulation of lipid deposition in fat tissue (Fujita et al., 1998). Conversely, inhibitors of SGLT1 have been shown to counteract obesity (Wagman, 2001). Once in the enterocyte, glucose crosses the basolateral membrane into the interstitial fluid via the facilitative glucose transporter-2 (**GLUT2**). In the classical model of glucose absorption, GLUT2 is located only on the basolateral membrane. More recently, it has been reported that under high luminal glucose concentrations GLUT2 could be resorted onto the apical membrane and represent a major pathway of intestinal sugar absorption in rats (Kellett and Brot-Laroche, 2005). GLUT2 (protein level) was also detected in the small intestinal apical of pigs by Cottrell et al. (2006). Interestingly, as in rats, GLUT2 abundance could be demonstrated in the intestinal apical membrane of pigs, but the functional properties and relevance of GLUT2 for the apical glucose transport were not observed in pigs, other animal species, or humans (Shirazi-Beechey et al., 2011; Wright et al., 2011; Yang et al., 2011).

Given that the starch digestion pathway involves multiple enzyme reactions and glucose transport steps, understanding which of the enzyme reactions & sugar transport steps are relatively more rate-limiting is important to understand how dietary and physiological factors influence *in vivo* starch digestion and their health implications. In neonates, it is known that SGLT1 uptake capacity is very high in conjunction with a high lactase digestive capacity (Yang et al., 2011), and human mucosal maltase-glucoamylase activity is believed to be an alternate pathway for providing limited luminal starch digestion when luminal **a**-amylase endoenzyme activity is minimal because of immaturity of salivary and pancreatic **α**-amylase synthesis (Nichols et al., 1998). These point to the fact that the lack of sufficient **α** -amylase endoenzyme activity is the major limiting factor responsible for poor starch digestion in neonates. In weanling pigs, maintaining high levels of glucose absorption capacity is essential for weaning adaptation and growth performance and dietary supplementation of sweeteners, glucose and lactose are practiced to up-regulate gut SGLT1 expression and maintain a high SGLT1 uptake capacity (Shirazi-Beechey et al., 2011; Lackeyram, 2012). Study by Lackeyram (2012) suggested that although weaning generally increased the expression of mucosal enzymes involved in starch digestion, low mucosal maltase-glucoamylase activity might be the limiting step in starch digestion during the weaning transition. In adult starch digestion, studies with

mice demonstrated that the small intestinal SGLT1 uptake capacity was genetically programmed to not be in very large excess of luminal glucose concentrations in responses to increased starch & sugar loads, and mucosal sugar & starch digestive enzymes and SGLT1 were equally limiting (Weiss et al., 1998). It has been well established that SGLT1-mediated small intestinal apical glucose uptake is a secondary active nutrient transport process coupled with a Na^+ gradient that is maintained largely by the Na^+-K^+-ATP pump at the expense of ATP (Wright et al., 2011). Considering that glucose is major energy nutrient of diets, SGLT1-mediated small intestinal apical glucose uptake costs significant amount of metabolic energy in animals and humans. Weiss et al. (1998) further explained that the biological benefits of minimizing excessive small intestinal SGLT1 uptake capacity was to balance against costs of biosynthetic energy (i.e., ATP) and limited membrane space. Therefore, *in vivo* starch digestion in mature monogastric animals and adult humans is likely regulated at the levels of both starch hydrolytic enzyme activity and the apical SGLT1 uptake activity.

1.2.5. Definition and Classification of Dietary Fibre

The term fibre, or dietary fibre, has many different meanings in the nutrition world. Crude fibre is classically used in animal nutrition but this fibre analysis technique considerably underestimates total dietary fibre contents particularly soluble fibre components (Fan, 2013a). The neutral-detergent fibre (**NDF**) concept was developed by van Soest and associates during 1970s-80s with an emphasis for partitioning plan cell fibre components with implications for lipogensis in dairy cattle and other ruminants (Van Soest et al., 1991). However, NDF essentially represents total insoluble fibre components and does not include any soluble fibre components (Fan, 2013). Total dietary fibre (**TDF**) is initially used to define fibre in human food and nutrition and it is defined to be a mixture of complex organic substances, including hydrophilic compounds such as soluble and insoluble polysaccharides and non digestible oligosaccharides as well as a range of non-swellable, more or less hydrophobic compounds such as cutins, suberins, and lignins as well as resistant starch escaping digestion in the small intestine (e.g., Prosky, 1988). The TDF concept has recently been adopted in swine nutrition (NRC, 2012). The Total dietary fibre is not a precise reference to a chemical component, or components, of the diet, but is essentially a physiological concept as embodied in its original definition (Prosky et al., 1988). The analysis of TDF is widely carried out by using commercial enzyme kits from Megazyme. Champ et al. (2003) and McCleary (2003) concluded that the TDF analysis procedures by using the Megazyme kits largely underestimated non-digestible oligosaccharides and RS as soluble fibre components, resulting in underestimation of TDF content in samples. According to Turner and Lupton (2011), total dietary fibre includes the intrinsic fibre components associated with foods of plant origins, referred to as the "endogenous fibre", as well as "exogenous" sources of pure fibres supplemented into the diets.

Common to accepted definition of fibre is the concept of non-digestible carbohydrates in the small intestine. Furthermore, the TDF and NDF definitions also include non-digestible non-carbohydrate components such as lignins. Non-digestible carbohydrates need to be specifically defined for their physiological functions. If it is the carbohydrates that pass across the ileo-cecal valve, then to define them well requires complex physiological studies in animals and humans. It will include many dietary components, for example, lactose in some adult

human populations, some polycols, some indigestible starches (i.e., RS) and non-starch polysaccharides (**NSPs**). These terms arose out of the early chemistry of NSPs, which showed that the fractional extraction of NSP could be controlled by changing the pH of solutions. These fibre definitions proved to be very useful in the initial understanding of the properties of dietary fibre, allowing a simple division into those that principally had effects on glucose and lipid absorption from the small intestine (soluble fibres) and those that were slowly and incompletely fermented and had more pronounced effects on bowel habit (insoluble fibres) (Champ et al., 2003).

Soluble fibre components have been well recognized in diets and nutrition for animals and humans for their important physiological functions. Viscous soluble fibre components such as guar gum and pectin are documented to effectively reduce blood levels of cholesterol and triglycerides (e.g., Rideout et al., 2008a; Fan, 2013a). Non-viscous soluble fibre components such as inulin, FOS and RS are well recognized prebiotics (Gibson et al., 2004; Fan, 2013a). The separation of soluble and insoluble fractions is very much pH-dependent, making their link to specific physiological properties less certain (Prosky et al., 1988). For instance, some insoluble fibres may be completely fermented and not all soluble fibres have effects on glucose and lipid absorption. Many of the early studies were done with isolated gums or extracts of cell walls, whereas these various forms of fibres exist together mostly in intact cell walls of plants hampering the differentiation of soluble and insoluble fibre functions. On the other hand, with soluble fibres being increasingly recognized for their physiological functions, there is a need to measure and estimate total soluble fibre contents in feed or food samples. Two major approaches are available for analyzing or estimating total soluble fibre contents (Fan, 2013a). The first approach is the direct chemical analysis of total soluble fibre content in samples by using the Megazyme kits. The second approach involved the measurements of TDF and NDF in samples. Since NDF represent total insoluble fibre components, the difference between TDF and NDF is the indirectly estimated total soluble fibre content in samples (Fan, 2013a). As discussed by Champ et al. (2003) and McCleary (2003), the TDF analysis by using the Megazyme kits largely underestimated non-digestible oligosaccharides and RS, resulting underestimation of TDF content in samples. Thus, this indirect method of estimating total soluble fibre contents in samples likely underestimates total soluble fibre contents in samples that are high in non-digestible oligosaccharides and RS.

1.2.6. Effects of Dietary Fibres and Glycoalkaloids on Starch Digestion and Digestibility

Dietary fibres have recently become a focus of discussions in monogastric animal nutrition, because they are poorly utilized and have anti-nutritive properties in diets (e.g., Fan, 2013). The high amounts of soluble arabinoxylans in rye is responsible for the cereals' poor nutritive values to poultry (Campbell et al., 1989) and the concentrations of soluble fibres in wheat are inversely correlated with their apparent starch digestibility in broiler chickens (Annison, 1991). It is believed that the viscous nature of the fibres is the primary cause for their anti-nutritive effect in poultry and swine, and the high gut digesta viscosity decreases rate of diffusion of nutrient substrates and digestive enzymes and hinder effective nutrient and enzyme interactions on the mucosal surface (Ikegami et al., 1990). Soluble NSPs interact with glycocalyx of the intestinal brush border and thicken the rate-limiting unstirred water layer of mucosa, which

reduces the efficiency of nutrient absorption through the intestinal wall (Johnson and Gee, 1981). Elevated levels of insoluble fibres in diets shorten the residence time of digesta in the gut and some argue that this may lead to lower nutrient digestibility. The rationale is that the longer the ingested feed is exposed to digestive processes in the gut, the more complete its digestion will be. As discussed earlier, soluble fibres increase gut digesta viscosity and lower digesta transit retention time, which allows less proliferation of fermentative organisms in the small intestine (Choct et al., 1996). It is believed that when gut digesta viscosity is decreased and nutrient digestion and absorption are enhanced, the indigestible feed materials pass through the gut quickly and insufficient time is available for anaerobic microflora to establish in the upper part of gut.

On the other hand, increases in gut digesta viscosity as affected by increased dietary viscous soluble fibre intake are known to enhance gut digesta viscosity (Chutkan et al., 2012, Zijlstra et al., 2012), potentially inducing gut mucosal osmostress responses. Ortells et al. (2012) demonstrated that osmotic stress in responses to hypertonicity up-regulated the expression of osmostress response genes of the mammalian-target-of-Rapamycin (mTOR)-signaling suppressor proteins (regulated in development and DNA damage responses, **REDD**) REDD1 and REDD2 in mammalian cells. Both REDD1 and REDD2 are established to be the mTOR upper stream inhibitors (Yang et al., 2008). Thus, dietary supplementation of viscous soluble fibres such as guar gum may be mediated via the mTOR-mediated protein translational control mechanism. There is a scarcity of literature reports regarding the effects of dietary supplementation of viscous soluble fibres on mTOR expression in the gut.

In human nutrition, intestinal starch digestion is recognized as the major determinant of glycemic responses (Jenkins et al., 1987). Dietary fibres in attenuation of glycemic responses for nutritional management of type-II diabetes have been carried out in two forms including the dietary incorporation of fibres intrinsic to food ingredients (Aldughpassi et al., 2012; Scazzina et al., 2013) and inclusion of pure exogenous fibres (Brand-Miller et al., 2012; Scazzina et al., 2013). The current literature of these exogenous fibre effects is less consistent especially for less viscous and non-viscous fibre components (Leciere et al., 1993; Rideout et al., 2008a; Chutkan et al., 2012), suggesting that further research is needed to understand these dietary strategies. Furthermore, measurements of starch digestibility, including the classification of RDS, SDS and RS, in starchy foods have been conducted by *in vitro* techniques (Singh et al., 2010). Determination of starch digestibility in starchy foods by *in vitro* techniques is cost-effective and has provided useful information on how various dietary factors influence starch digestion *in vivo* (Singh et al., 2013). Continued efforts in examining factors affecting *in vivo* starch digestibility by using a relevant large animal model such as grower pigs fed a typical high-fat "Western diet" (i.e., with dietary fat contributing to over 30% of dietary gross energy) will facilitate the further development of effective dietary strategies of control of glycemic responses and improvement of prebiotic fibre effects in human nutrition and health management (e.g., Rideout et al., 2008b). Zijlstra et al. (2012) reviewed literature reports of *in vivo* starch digestibility responses to food starch sources that were different in the ratio of amylose and amylopectin. Despite several lines of literature studies regarding effects of non-viscuos and viscous fibres on α-amylase activities (Hansen et al., 1982; Isaksson et al., 1982a, Hansen, 1986; Leng-Peschlow, 1989; Slaughter et al., 2002) and intestinal mucosal enzymes involved in starch digestion such as disaccharidase activities (Thomesn and Tasman-Jones, 1982; Johnson et al.,

1984; Johnson and Gee, 1986; Onning and Asp, 1995; Hannan et al., 2007), there are still limited literature reports of how dietary fibres affect *in vivo* starch digestion (i.e., enzymatic hydrolysis of starch) and starch digestibility (i.e., enzymatic hydrolysis of starch and sugar transport or absorption) under relevant dietary type and conditions (e.g., "Western diets").

On the other hand, conventional potatoes and processed potato products as common staple foods on current food markets for consumers are shown to contribute significantly to highly digestible dietary starch intake, glycemic load (Schwizer et al., 1990; Schulz et al., 2005) and obesity (Mozaffarian et al., 2011). Major types of fibre components identified in potatoes include cellulose, hemicelluloses and pectin, which are primarily distributed in potato cell wall materials (Salvador et al., 2000). Resistant starch has also been identified as a major soluble fibre component in potatoes (Elsmstähl, 2002). Cultivars as well as cooking and processing conditions are shown to affect resistant starch content in potatoes (Mulinacci et al., 2008). Contents of both soluble and insoluble fibre components are affected by potato cultivars (Gumul et al., 2011). Thus, development of new potato cultivars high in non-viscous soluble fibre (i.e., resistant starch) content provides a relatively inexpensive source of human dietary fibre. Bach et al. (2013) identified some new potato cultivars that were very high in resistant starch content by *in vitro* techniques.

Under this context, glycoalkaloids are documented to be a group of anti-nutritive factors in potatoes (Friedman, 2006). Total glycoalkaloids, the sum of α-solanine and α-chaconine, detected within the government regulatory upper limit (200 mg/100 g dry weight or 1 kg of potato tubers, fresh weight by Health Canada and the Canadian Food Inspection Agency, **CFIA**) in consumed potato products were shown to adversely affect mammalian intestinal permeability and could aggravate intestinal inflammatory diseases (Patel et al., 2002). Potato glycoalkaloids are shown to affect mammalian cell membrane integrity and membrane protein structure and functions (Friedman, 2006). However, there is very little information available regarding effects of potato glycoalkaloids on *in vivo* starch digestibility. Thus, there is a need to examine relationships between dietary intake levels of total potato glycoalkaloids and digestibility of nutrients especially starch for novel cultivars of potatoes that potentially provide health benefits.

1.2.7. Effects of Dietary Fibres on Protein Digestion and Amino Acid Digestibility

Digestion of dietary proteins in animals and humans includes enzymatic hydrolysis steps and absorptive uptake of hydrolytic end-products of short peptides and free amino acids (**AA**) across intestinal epithelial membranes via specific nutrient transporters (Cheeseman, 1986; Fan, 2013b). Ileal rather than the fecal crude protein (**CP**) and AA digestibility should be measured in ingredients (Sauer and Ozimek, 1986). The gastrointestinal endogenous CP and AA losses are significant metabolic losses of these nutrients at the whole body level in animals and humans (Nyachoti et al., 1997; Gaudichon et al., 2002). The ileal CP and AA digestibility values measured without corrections for their distal ileal endogenous CP and AA losses are referred to as the apparent ileal CP and AA digestibility values (Nyachoti et al., 1997; Fan, 2013b). True rather than apparent ileal CP and AA digestibility values should be measured in ingredients (Fan and Sauer, 2002; Fan, 2013b). However, distal ileal endogenous CP and AA losses are variable and are specific to dietary and physiological conditions and it is often technically challenging

and expensive to carry out their measurements. Thus, it is not surprising to see some new studies on AA digestibility in studies with pigs are still being reported in their apparent ileal values in the literature.

Dietary fibres have often been observed to cause a decrease in the apparent ileal digestibility of dietary CP and AA in swine. Most natural fibre sources in association with feed ingredeints contain a large number of different fibrous components including soluble and insoluble fibres. Each type of fibre source has its own fibre composition. This means that structural and physical characteristics of fibres associated with feed & food ingredients are specific to each fibre type. In addition, each type of fibre has its own specific interaction with dietary components (Laplace et al., 1989).

According to Sauer and Ozimek (1986), levels and source of dietary fibres are the most important factors influencing the amounts of AA recovered in the distal ileal digesta. Studies by Anderson et al. (1990) showed that the gelling and viscosity properties of viscous water-soluble dietary fibres, in particular, pectins and gums, decreased the digestion and absorption of nutrients by reducing the mixing of intestinal contents, blocking enzyme-substrate interactions, and by forming an unstirred water layer, thereby creating a physical barrier to nutrient absorption. Sauer et al. (1991) reported a decrease in the fecal digestibility values of AA when the fibre Solka-Floc or ground barley straw was included at 10% in a corn starch-based soybean meal diet with little effects on the apparent ieal AA digestibility values. Studies in Mosenthin et al. (1994) showed that exogenous pectin at 7.5% decreased the apparent ileal AA digestibility in growing pigs. Fan and Sauer (2002) observed that there was no dramatic variability in true ileal CP and AA digestibility values among the examined hulled barley cultivar samples, wheareas the distal ileal CP and AA outputs were considerably higher in the barley samples also with a much higher dietary NDF contents ranging from 16-24% on DM basis than the average literature values, which is in agreement with Jansman et al. (2002) that fibre types and levels are one of the major dietary factors affecting the ileal endogenous CP and AA losses and the apparent ileal CP and AA digestibility values. Thus, dietary fibre types and levels may negatively affect the apparent ileal CP and AA digestibility values via hampering enzymatic hydrolysis and enhancing the ieal endogenous CP and AA losses.

Consumption of dietary fibres especially soluble fibre components is recognized for their potential health benefits in human nutrition (Champ et al., 2003). While exogenous isolated fibre components such as wheat bran and guar gum are readily used in food industry, staple foods such as potatoes can be important fibre sources including cellulose, hemicelluloses and pectin, which are primarily distributed in potato cell wall materials (Salvador et al., 2000). Resistant starch has also been identified as a major soluble fibre component in potatoes (Elsmstähl, 2002). Contents of both soluble and insoluble fibre components are affected by potato cultivars (Gumul et al., 2011), thus development of new potato cultivars with high fibre contents may be an effective strategy to enhance dietary fibre consumption for humans. Intermediate dietary fibre levels (e.g., 10%) were shown to be effective in reducing blood cholesterol levels in studies with growing pigs fed a high-fat basal diet (Rideout et al., 2007). A high-fat basal diet with a crude fat at about 20%, contributing to over 30% of dietary GE density, is an important nutritional feature of a typical "Western" diet. Previous studies showed a "protein-sparing" effect by dietay fat supplementation in linearly improving ileal CP and AA

digestibility when dietary oil level was increased from 3 to 12% in young pigs (Li and Sauer, 1994). Therefore, there is need to understand how different sources of intermediate dietary fibre levels (e.g., 10%) influence protein digestion by measuring the apparent ileal AA digestibility responses in growing pigs fed a high-fat basal diet for the purpose of improving human nutrition and health.

1.2.8. Effects of Dietary Fibres and Glycoalkaloids on Mineral Digestion and Digestibility

The addition of dietary fibres has been found to depress mineral digestibility (Brune et al., 1992; Ink, 1988). Some research found that high intake of fiber might impair the absorption of calcium (Branch et al., 1975). It is postulated that dietary fibre binds with polyvalent mineral ions, forming unabsorbable fiber-mineral complexes (Oku et al., 1982). Although dietary fibers are traditionally thought to decrease mineral absorption, animal and human studies have also demonstrated that soluble fermentable fibres appear to increase the absorption of some minerals (Abrams et al., 2005; Holloway et al., 2007). It may increase mineral absorption through increased production of SCFAs with an increase in the villus height, and epithelial cells per crypt, thereby enhancing the secondary active mineral transport activity (Scholz-Ahrens and Schrezemeir, 2002). Coudray et al. (1997) reported soluble fibres have positive effects on intestinal calcium absorption. Drews et al. (1979) found that different types of fibres have different results. Hemicellulose supplementation increased fecal zinc, copper and magnesium excretions (Drews et al., 1979). Cellulose had similar results, while pectin had the least influence on mineral utilization and retention (Drews et al., 1979). Meanwhile, dietary fibres have been shown to bind nutritionally important minerals *in vitro* (Branch et al., 1975). The mineral-binding properties of dietary fibre-containing mineral-complexing or chelating components such as lignins and tannins would be the reason to have an inhibitory effect on mineral digestion (Platt and Clydesdale, 1985).

Despite literature reports of studies in both human subjects and animal models (Coudray et al., 1997; Weaver et al., 2010), the relevance of fibre consumption to the digestive utilization of the macro-minerals important to bone formation and human bone health needs to be addressed for following reasons. Firstly, although calcium (**Ca**), phosphorus (**P**) and magnesium (**Mg**) are the recognized major macro-minerals in bone formation (Allgrove, 2009), effects of dietary fibres on dietary mineral availability have focused much more on Ca and less on P and Mg in both human and animal studies (Coudray et al., 1997; Weaver et al., 2010). Secondly, the majority of the relevant animal studies are conducted with rodents (Hara et al., 1996; Weaver et al., 2010). Bach Knudsen et al. (1994) showed that rats appeared to have a lower capacity to digest fibre polysaccharides than humans. Guilloteau et al. (2010) concluded that pigs are a relevant animal model to study nutritional programming of the gastrointestinal tract development and functions for humans. Thirdly, a relevant typical "Western basal diet" needs to be used in such studies in which not only types of dietary fibres but also adequate levels of dietary fibres should be considered. An intermediate dietary fibre level at about 10% is frequently reported to be effective in improving whole body nutrition and health parameter endpoints (Poksay and Schneeman, 1983; Rideout et al., 2007), thus this dietary fibre level should be used to examine the dietary effect of fibres on the major bone-mineral availability.

Under this context, conventional fibre components identified in potatoes include cellulose, hemicelluloses and pectin, which are primarily distributed in potato cell wall materials (Salvador et al., 2000). Resistant starch has also been identified as a major soluble fibre component in potatoes (Elsmstähl, 2002). Contents of both soluble and insoluble fibre components are affected by potato cultivars (Gumul et al., 2011). Thus, development of new potato cultivars high in non-viscous soluble fibre (i.e., resistant starch) content provides a relatively inexpensive source of human dietary fibre. Bach et al. (2013) identified some new potato cultivars that were very high in resistant starch content by *in vitro* techniques. Thus, there is need to understand how different sources of intermediate dietary fibre levels (e.g., 10%) influence the major bone mineral digestibility responses in growing pigs fed a high-fat basal diet.

Glycoalkaloids are documented to be a group of anti-nutritive factors in potatoes (Friedman, 2006). Total glycoalkaloids detected within the government regulatory upper limit (200 mg/100 g or 1 kg of potato tubers, fresh weight by Health Canada and CFIA) in consumed potato products were shown to negatively affect mammalian intestinal permeability and could aggravate intestinal inflammatory diseases (Patel et al., 2002). Potato glycoalkaloids are capable to affect mammalian cell membrane integrity and membrane protein structure and functions (Friedman, 2006). However, there is very little information available regarding effects of potato glycoalkaloids on *in vivo* mineral digestibility. Therefore, there is a need to examine relationships between dietary intake levels of total potato glycoalkaloids and digestibility of the major bone macro-minerals for novel cultivars of potatoes that potentially provide health benefits for the purpose of improving human nutrition and health.

1.2.9. Research Hypotheses and Objectives

Under the context of this review, it is clear that roles of exogenous soluble and insoluble fibres in influencing starch digestion, digestibility of other important nutrients and BW gains should be further investigated with a more relevant animal model such as growing pigs under typical "Western diet" conditions, which has been well established in our previous studies (Rideout et al., 2007; 2008). As an important staple food, potatoes offer an excellent opportunity for the control of *in vivo* starch digestion and some of the major health concerns associated with starch digestion such as type-II diabetes as well as bowel inflammation and colorectal cancers, since studies have shown that there is a large variability in RS contents among the various cultivar genotypes of potatoes (Bach et al., 2013).

Thus, this thesis research was conducted under the following hypotheses:
1). Dietary supplementations of exogenous soluble and insoluble fibres at intermediate levels (e.g., at about 10% in diets) could effectively reduce *in vivo* starch digestion via different biological mechanisms and these effects can be well measured at the distal ileal level in growing pigs fed a high-fat basal diet.

2). Dietary supplementations of exogenous soluble and insoluble fibres at intermediate levels (e.g., at about 10% in diets) could effectively improve *in vivo* major bone macro-mineral digestibility and improve bone health and these effects can be well measured at the distal ileal level in growing pigs fed a high-fat basal diet.

3). Dietary supplementations of exogenous soluble and insoluble fibres at intermediate levels (e.g., at about 10% in diets) could negatively affect host protein nutrition and this could be effectively quantified by examining responses in the ileal AA digestibility in growing pigs fed a high-fat basal diet.

4). Novel cultivar genotypes of potatoes that are high in resistant starch contents will have a great health-promotion potential and these potatoes can be effectively selected by examining their responses *in vivo* starch digestibility in comparison with conventional cornstarch in growing pigs fed a high-fat basal diet.

Therefore, this thesis research was designed and conducted to address the following major objectives:

1). To examine how dietary supplementations of two exogenous fibres of guar gum and cellulose at 10% as well as guar gum (8.5%) in combination with processed dry potatoes (25.1%) affect ileal starch digestibility, large intestinal fermentation responses and BW gain responses;

2). To reveal potential quantitative relationships between ileal dry matter (DM) and starch digestibility values and the levels of total potato glycoalkaloids in growing pigs fed a high-fat basal diet and to assess potential impacts of potato glycoalkaloids on DM and starch digestibility;

3). To examine effects of dietary supplementations of two conventional fibre components (i.e., guar gum and cellulose) at 10% and 8.5% guar gum plus intrinsic fibres further contributed by six test potato cultivar samples that differed in TDF and soluble fibre content on the apparent ileal AA digestibility in growing pigs fed a high-fat basal diet and to evaluate potential impacts of intermediate levels of dietary fibres and ingesting novel health-promoting potatoes high in RS contents on protein nutrition under typical "Western diet" conditions; and

4). To investigate the effects of dietary inclusion of exogenous fibres of guar gum and cellulose at 10% as well as 8.5% guar gum in combination with six different genotypes of processed dry potatoes on the apparent ileal and fecal Ca, P, and Mg digestibility measured at the pre-cecal and the fecal levels in growing pigs fed the high-fat basal diet.

CHAPTER 2

MODULATION OF ILEAL STARCH DIGESTIBILITY BY EXOGENOUS FIBRES AND DIFFERENT GENOTYPES OF POTATOES IN GROWING PIGS FED A HIGH-FAT BASAL DIET

2.1. ABSTRACT

The roles of exogenous fibres and novel cultivar genotypes of potatoes in influencing *in vivo* starch digestibility for nutritional management of human health issues need to be established. The objectives of this study were to examine effects of dietary supplementations of two exogenous fibres, i.e., guar gum and cellulose, in combination with intrinsic fibres from six cultivar genotypes of potatoes on ileal dry matter and starch digestibility, intestinal fermentation and body weight (**BW**) gains in growing pigs fed a high-fat basal diet. The basal diet was formulated as a zero-fibre negative control (**NC**) to contain 41.5% poultry meal, 4% casein, 15% animal fat-oil blend, 4.31% sucrose, 31% cornstarch, 0.50% salt and 0.40% trace mineral-vitamin premix with crude fat contributing to 37% of the dietary gross energy. The two exogenous fibre diets were formulated by diluting the NC basal diet with 10% guar gum and 10% cellulose at the expense of cornstarch, respectively. Six potato test diets were formulated by diluting the basal diet with 25.1% of one of the six genotypes of cooked and dehydrated potato tuber powder in combination with 8.5% guar gum at the expense of cornstarch to contain about 10% TDF. Titanium oxide was included (0.30%) as a digestibility marker. A total of 90 barrows, with an average initial BW of about 25 kg, were fitted with a simple T-cannula at the distal ileum and fed the diets according to a completely randomized block design with each block lasting 28 d. Dietary supplementations of 10% guar gum, 10% cellulose and 8.5% guar gum in combination with each of six test potatoes at 25.1% (Potatoes diets 1 to 6) considerably increased ($P < 0.05$) the distal ileal starch recovery and decreased ($P < 0.05$) the ileal starch digestibility values compared with the NC diet. Out of the six potato genotypes examined, cv 12272-3, cv 96044-3 and cv F05081 were associated with lower starch digestibility and higher levels of resistant starch contents ($P < 0.05$) compared with NC conventional starch. Albeit of small magnitudes, dietary supplementations of guar gum (10%) and 8.5% guar gum in combination with each of the six test potatoes (Potato diets 1 to 6) at 25.1% increased ($P < 0.05$) the distal ileal free glucose recovery compared with the NC diet. Moreover, 10% guar gum supplementation (PC diet) reduced ($P < 0.05$) the abundance of the phosphorylated mTOR (Ser 2448) in the proximal jejunum compared with the NC diet. Pearson correlation analyses showed a negative linear relationship ($P < 0.05$) between the ileal dry matter digestibility values and the total glycoalkaloid contents in the potato test diets, however, no such a relationship ($P > 0.05$) was observed between the ileal starch digestibility values and the total glycoalkaloid contents in the potato diets. However, dietary supplementations of 10% cellulose, 10% guar gum as well as 8.5% in combination with six individual genotypes of potatoes did not affect ($P > 0.05$) weight gain and gain to feed ratio when feed intakes were controlled according to the NC diet. It can be concluded that dietary supplementations of exogenous viscous fibre guar gum and non-viscous fibre cellulose at 10% significantly reduced ileal starch digestibility with the 10% guar gum negatively affecting glucose absorption likely via the mTOR-signalling pathway. Three novel potato genotypes of cv 12272-3, cv 96044-3 and cv F05081 were shown to have considerably low ileal starch digestibility values and high resistant starch contents, thus having a

potential in serving as healthy staple foods in preventing and managing type-II diabetes, blood dyslipidemia and risks of developing colorectal cancers.

Key Words: starch, dietary fibre, digestibility, growing pigs, potato, resistant starch

2.2. INTRODUCTION

Low fibre and high consumption of digestible starch and sugars, as indicated by high glycemic index and glycemic load, induces chronic postprandial hyperglycemia and is associated with incidence and progression of type-II diabetes mellitus (Vrolix et al., 2008; Mohan et al., 2009; Malik et al., 2010; Koning et al., 2011) and mortality risk of diabetes mellitus (Burger et al., 2012). Under this context, increased consumption of dietary fibres has been well documented to attenuate hyperglycaemic responses (Blackwood et al., 2000; Brennan, 2005; Chutkan et al., 2012). Potatoes and processed potato products as common staple foods contribute significantly to highly digestible dietary starch intake, glycemic load (Schwizer et al., 1990; Schulz et al., 2005) and obesity (Mozaffarian et al., 2011).

Dietary fibre in attenuation of glycemic responses has been carried out in two forms including the dietary incorporation of fibres intrinsic to food ingredients (Aldughpassi et al., 2012; Scazzina et al., 2013) and inclusion of pure exogenous fibres (Brand-Miller et al., 2012; Scazzina et al., 2013). The current literature of these exogenous fibre effects is less consistent especially for less viscous and non-viscous fibre components (Leciere et al., 1993; Rideout et al., 2008a; Chutkan et al., 2012), suggesting that further research is needed to understand these dietary strategies. On the other hand, cultivar genotypes as well as cooking and processing conditions are known to influence fibre contents, including resistant starch and conventional fibre components in potatoes (Mulinacci et al., 2008; Gumul et al., 2011). Bach et al. (2013) identified some new potato cultivars that were very high in resistant starch content. Within this context, glycoalkaloids are documented to be a group of anti-nutritive factors in potatoes (Friedman, 2006). Glycoalkaloids detected within the government regulatory upper limit in consumed potato products were shown to adversely affect mammalian intestinal permeability and could aggravate intestinal inflammatory diseases (Patel et al., 2002). Thus, there is a need to examine relationships between dietary intake of total potato glycoalkaloids and nutrient digestibility especially starch for novel cultivars of potatoes that potentially provide health benefits. The small intestinal enzymatic starch digestion is known to be a major determinant of the glycemic response (Jenkins et al., 1987). Starch that escapes the small intestinal digestion and enters the colon is regarded as resistant starch, exerting protective and prebiotic effects (Kendall et al., 2004). Therefore, there is a need to investigate roles of exogenous fibres and factors associated with potato cultivar genotypes in affecting *in vivo* small intestinal starch digestion and subsequent large intestinal fermentation.

In most human clinic nutrition studies, fibre intake levels are usually expressed as gram of fibre intake per test subject on per day basis or gram of fibre per 1000 kcal (Hannan et al., 2007; Turner and Lupton, 2011; Brand-Miller et al., 2012). In most animal model studies for human nutrition research, fibre levels are usually expressed as a percentage of experimental diets and dietary fibre at about 10% has been reported to be effective in improving nutrition and health

parameter endpoints (Poksay and Schneeman, 1983; Rideout et al., 2007). Thus, the objectives of this study were to i) examine how dietary supplementations of two exogenous fibres of guar gum and cellulose at 10% as well as guar gum (8.5%) in combination with six genotypes of potatoes (25.1%) affect ileal starch digestibility, large intestinal fermentation responses and BW gain responses; and ii) to reveal potential quantitative relationships between ileal dry matter (DM) and starch digestibility values and the levels of total potato glycoalkaloids in growing pigs fed a high-fat basal diet.

2.3. MATERIALS AND METHODS

2.3.1. Animals, Diets and Experimental Design

All the animal experiments were conducted with protocols approved by the University of Guelph Animal Care Committee. The pigs used in this experiment were cared for in accordance with guideline established by the Canadian Council of Animal Care available at (http://www.ccac.ca/en_/standands). Yorkshire growing barrows, with an average initial body weight (**BW**) of 25.0 ± 0.2 kg (n = 90), were housed in an environmentally controlled room (23°C). These pigs were randomly assigned into individual stainless-steel metabolic crates (height, 85 cm; length, 155 cm; width, 90 cm). The animal protein, high-fat basal diet was formulated as a negative control (**NC**) diet that contained zero fibre, 41.5% poultry meal, 4% casein, 15% animal fat-oil blend, 4.31% sucrose, 30.79% cornstarch, 0.50% ionized salt, and 0.40% of the commercial trace mineral and vitamin premix (DSM Nutritional Products Canada, Inc., Ayr, ON, Canada) with crude fat contributing to about 37% of the dietary gross energy (**GE**) (**TABLE 2.1**), which was by adapted from our previous studies (Rideout et al., 2007). Crystalline L-Ala was supplemented to the NC and the two exogenous fibre diets, thus all experimental diets were formulated to be isonitrogenous. Crystalline D,L-Met was supplemented to all the diets to ensure sufficient Met supply by the diets. Sucrose was included in the basal diet, as it was a source of D-fructose in typical "Western diets", which was known to cause numerous diseases and metabolic syndromes (Douard and Ferraris, 2013). The two exogenous fibre diets were formulated by diluting the NC basal diet with 10% guar gum and 10% cellulose, respectively, at the expense of cornstarch. The 10% guar gum supplemented diet was also referred to as the positive control (**PC**). Six other test diets were formulated by including 8.5% guar gum and by further diluting the NC basal diet with 25.1% of one of the six test potato genotype samples of thermo-processed cooking temperature and dehydrated potato tuber powder to contain a target level of about 10% total dietary fibre **(TDF**) at the expense of cornstarch. The six test genotypes of potatoes were variable in TDF and soluble fibre contents, including cv FV12272-3 as Potato diet 1; cv F05035 as Potato diet 2; cv 96044-3 as Potato diet 3; cv WV5475-1 as Potato diet 4; cv Atlantic (a commercial cultivar) as Potato diet 5; and cv F05081 as Potato diet 6 (**TABLE 2.1**). These potato cultivar genotypes were selected by their relatively higher TDF contents compared with other potato cultivars developed by the National Potato Breeding Program at the Agriculture and Agri-Food Canada Potato Research Centre (Fredericton, NB, Canada). Growing conditions and environment affected nutrient composition of potato tubers (Bach et al., 2013). Thus, these six potato cultivars were grown under the same conditions at the University of Guelph Elora Research Station near Elora, ON, Canada. About 2 to 2.5 tons of fresh potatoes were harvested for each of the six potato cultivar-genotype

samples, washed, diced, and thermal-dehydrated at about 200°C for about 30 min (without causing browning effects) via a standard commercial type of belt-drying food processing system at the atmospheric pressure. The dried potato cubes were further ground to be homogenous flours for diet incorporation at the University of Guelph Arkell Research station feed mill. Titanium oxide was included (0.30%) as a digestibility marker. Pigs were fed the experimental diets according to a completely randomized block design for up to 10 blocks in order to obtain 10 replicates for each of the test diets according to our previously established protocols (Rideout et al., 2008a).

2.3.2. Sample Collection and Processing

Pigs in each pen were weighed on d 0 and d 28 in each block. Feed intake by pigs was closely monitored to ensure that daily feed consumption of each of the 8 test diets was similar to the NC control diet and actual feed intake was recorded daily. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and gain to feed ratio (**G:F**) were all calculated.

Each experimental block lasted 4 wk, comprising of the first 7-d period during which the pigs were adapted to the environment and research staff, and were fed their respective diets at 0900 and 1600 h. All test pigs were fed the same amount of test diets close to an *ad libitum* level (3% of their average BW) for the high-fat basal diet, which was established by Rideout et al. (2007). The *ad libitum* feed level for this high-fat diet was much lower than the feed intake level (5-6% of average BW) of grower pigs fed typical corn and soybean meal-based commercial swine diets as compiled by NRC (1998). During the d 8-12 of each experimental block, the pigs were surgically fitted with a T-cannula in the distal ileum for collection of digesta samples (Sauer et al., 1983). During a post-surgical recovery period (d 13-24), the pigs gradually resumed their previous *ad libitum* feeding level from a restricted feed intake at 50 g/d on the 2nd post-surgery. On d 25-28 of the experimental block period, the distal ileal digesta effluent samples were collected from 0800 to 1800 h by attaching a plastic bag containing 5ml of 5% formic acid solution (Fan et al., 1994). The plastic digesta collection bag was inspected at 30-min intervals and changed immediately as needed. Some pigs were lost due to complications during the post-surgical recovery, resulting in miss data for some of the dietary treatment groups.

Upon harvesting the six fresh potatoes from the research farm, representative potatoes were washed and samples were cooked by boiling before freeze-drying. Changes in the weights of the processed potato samples were recorded and used for calculating free water content in these potato samples. At the conclusion of the study, collected ileal digesta samples were freeze-dried. Dried digesta and the test potato samples as well as the test diet samples were ground to be homogeneous in a Wiley mill though a 0.1-mm mesh screen, and were well mixed before weighing out for further analyses.

Proximal jejunal tissues from the NC and PC groups of pigs were collected at the end of the experiments as described by Rideout et al. (2007). The tissue samples were further processed and stored for further molecular endpoint analyses as described by Rideout et al. (2007).

2.3.3. Chemical, Biochemical and Molecular Analyses

DM content of the processed dietary and digesta samples was analyzed according to an Association of Official Analytical Chemists (**AOAC**) (1993) Method. Content of TDF in the test potato samples was analyzed with a commercial kit (Megazyme International Ltd., Co. Wicklow, Ireland) according to the AOAC procedures (Prosky et al., 1988; Lee et al., 1992; Prosky et al., 1992). Neutral-detergent fibre **(NDF**) in the test potato samples was measured according to AOAC (1984) in an Ankom fibre analyzer (Ankom Technology, Macedon, NY). Crude protein (**CP**) content (N × 6.25) in the test potato samples was analyzed according to the combustion method (Dumas Procedure) on a Leco FP-428 nitrogen analyzer (Leco corporation, St. Joseph, MI). Glycoalkaloids in the dehydrated and homogenized test potato samples were measured by high performance-liquid chromatography (**HPLC**) via commercial service at the Alberta Agriculture and Rural development Laboratory. Briefly, α-solanine and α-chaconine levels were analyzed by following the AOAC method 997.13 modified for dry samples (AOAC1997; Iablokov et al., 2010). Glycoalkaloids in these test potatoes, on the fresh basis, were calculated by considering the measured free water content in these potatoes. The digestibility marker titanium oxide content in the diet and digesta samples was analyzed by following the procedures of Leone (1973) and Myers et al. (2004). Resulting absorbance for this analysis was measured at 410 nm by using an epoch microplate-spectrophotometer (BioTek, Winooski, VT).

To quantify free D-glucose content in the diet and digesta samples, about 0.500 g of the samples was weighed out, placed into 17-mL plastic centrifuge tubes and 5.00 mL of aqueous ethanol (80% v/v) was added. The mixture was well mixed and incubated in a water bath at 80-85ºC samples for 30 min. Additional 4.50 mL of distilled and deionized water was added into the 17-mL plastic tubes and the tubes were centrifuged at 2000 x *g* for 10 min. The supernatant had a theoretical volume of 10 mL and aliquots of the supernatant were taken for analyzing free D-glucose content by using a commercial kit (Megazyme International Ltd, Wicklow, Ireland) with the microplate reader. To measure total starch content in the diet, and digesta samples, about 0.100 g of the samples was weighed out and processed for the above procedures to precipitate starch and separate free D-glucose. Then the resulting sample pellet was further treated with Megazyme reagents and enzyme kits to hydrolyze total starch into free D-glucose.

Abundances of the phosphorylated mammalian target of rapamycin (**mTOR**) [mTOR (Ser 2448)] in the proximal jejunum of the PC and the NC diets were conducted by Western blotting (Rideout et al., 2007; Yang, 2009). Same amount of total protein was loaded (i.e. 100 µg/lane) for all tissue samples.

For the analyses of volatile compounds according to Rideout et al. (2004), including VFA and their derivatives of phenols and indoles, pulverized frozen digesta & fecal samples (2 g) were extracted with 100% methanol (6 mL), homogenized with a Power Gen homogenizer (700D, Fisher Scientific, Pittsburgh, PA, USA) at 10,000 rpm for 2 min and centrifuged at 800 × *g* for 20 min. Decanol (0.1244 s/tube) was added at the beginning of the extraction as an internal standard for quantification. The supernatant was treated with a suitable amount of anhydrous Na_2SO_4 to remove moisture, cleaned up with 0.45-um syringe filters and analyzed by gas chromatography-mass spectrometry (**GC-MS**) with a 6890 GC coupled with a Hewlett-Packard

5973 N mass selective detector (Agilent Technologies, Inc., Wilmington, DE) (Rideout et al., 2004).

2.3.4. Calculations and Statistical Analyses

Distal ileal outputs of free D-glucose and starch were calculated according to equation (Fan and Sauer, 1997).

$$C_d = C_b \times (M_d/M_p)$$

Where C_d represents distal ileal D-glucose or total starch output expressed on the basis dry matter diet intake (**DMI**) (grams per kilogram DMI); C_b is ileal digesta content of D-glucose and total starch (grams per kilogram DM feces); M_d is the digestibility marker content in the diets (%, on DM basis); and M_p is the digestibility marker content in the ileal digesta (%, on DM basis).

The apparent ileal DM and starch digestibility values in the diets were calculated by the marker method (Fan et al., 1994). The apparent ileal digestibility values of DM and starch in the experimental diets were calculated according to the equation as follows:

$$D_D = 100\% - [(I_D \times S_I)/(S_D \times I_I)] \times 100\%$$

where D_D = apparent ileal starch digestibility in the assay diet (percentage), I_D = marker content in the assay diet (%, on an air-dry basis), S_I = DM or starch content in ileal digesta (%, on an air-dry basis), S_D = DM or starch content in the assay diet (%, on an-air-dry basis), and I_I = marker content in ileal digesta (%, on an air-dry basis).

The expriement was carried out according to a completely randomized block design with 10 blocks and a total of ninty pigs by using the following model:

$$y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}$$

Where μ is the general mean, T_i is dietary treatment effect, P_j is the block effect, and ε_{ij} is the experimental error. Dietary treatment was included as a fixed effect and block was regarded as a random effect with individual pigs being randomly allocated to their test pigs within each of the study blocks. Data were subjected to ANOVA and F tests according to the Proc mixed model of SAS (SAS Institute, Cary, NC) for a completely randomized block design. Due to losing some test pigs and missing endpoint values, least square means (**LSM**) were calculated for the major observation endpoints. Multiple comparisons among the dietary treatment LSM were conducted by using the Tukey-Krammer's test. The reason that the Tukey's test was chosen as a suitable test in comparison with other multiple comparsion tests such as the Duncan's test was due to the fact the Tukey's test is much stringent and it compares the means of every treatment to the means of every other treatment, that is, it applies simultaneously to the set of all pairwise comparisons. The difference in the abundances of phosphorylated mTOR between the PC 10%-guar gum diet and the NC diet was compared by using the Dunnett's test. Data were expressed as LSM ± SE. Differences were considered to be significant for $P < 0.05$.

2.4. RESULTS

All commercial dietary ingredients including the two exogenous fibres were used in diet formulations on an air-dry basis. The test potatoes were thermal processed to be on air-dry basis. Thus, all experimental diets were formulated on an air-dry basis, being consistent with the dietary DM content ranging from 92.3 (Potato 2 diet) to 95.3% (NC diet). Correspondingly, bound water content in the diets ranged from 4.7 (NC diet) to 7.3% (Potato 2 diet) (**TABLE 2.1**). The contents of total glycoalkaloids, including α-solanine and α-chaconine, in the six test potatoes (Potatoes 1 to 6) were 5.88, 6.63, 11.88, 7.31, 7.64 and 10.14 mg/100 g samples, respectively, on their fresh basis. Total glycoalkaloid contents in the potato diets ranged from 6.38 to 12.12 mg/100 g diets on as-fed or air-dry basis. The PC and the Cellulose diets were formulated to contain 10% TDF by using guar gum and cellulose, respectively, whereas TDF in the six potato test diets was at about 11%, which was composed of 8.5% exogenous guar gum plus intrinsic fibres contributed by the corresponding test potatoes. Total starch content in the diets ranged from 12.6 (Potato 3 diet) to 22.7% (NC diet) on an air-dry basis. The calculated dietary energy density, including GE, DE, ME and NE, changed little likely due to relevant changes in dietary CP and starch contents among the diets with crude fat contributing to about 37-40% of total dietary GE.

Endpoints of growth performances of the experimental pigs are summarized in **TABLE 2.2**. There were no differences ($P > 0.05$) in the initial and final BW of the pigs among the diets. There also were no differences ($P > 0.05$) in the other major endpoints of the growth performances including ADFI, ADG and G:F.

The apparent ileal DM and starch digestibility values are compared and summarized in **TABLE 2.3**. There were differences ($P < 0.05$) in the ileal DM digestibility values between the NC and each of the other test diets with each of the test diets being considerably lower in the DM digestibility compared with the NC diet. While dietary free glucose contents were very low, ranging from 0.02 to 0.05%, on the DM basis, there were no differences ($P > 0.05$) in the free glucose contents among diets. Free glucose content (% DM basis) in the distal ileal digesta was lower ($P < 0.05$) in the NC diet than in the Potato 2 diet. However, no such difference ($P > 0.05$) was detected between the NC diet and each of the other test diets. When expressed as % of dietary total starch content, 10% cellulose supplementation did not affect ($P > 0.05$) the distal ileal free glucose recovery in comparison with NC diet. Albeit of small magnitudes, dietary supplementations of guar gum (10%) and 8.5% guar gum in combination with each of the six test potatoes (Potato diets 1 to 6) at 25.1% increased ($P < 0.05$) the distal ileal free glucose recovery compared with the NC diet. When expressed as % of dietary total starch content, dietary supplementations of 10% guar gum, 10% cellulose and 8.5% guar gum in combination with each of six test potatoes at 25.1% (Potatoes diets 1 to 6) considerably increased ($P < 0.05$) the distal ileal recovery of starch. The distal ileal starch recovery was much higher ($P < 0.05$) in the dietary supplementation with 10% cellulose than with 10% guar gum. Furthermore, 8.5% guar gum in combination with individual test potatoes further dramatically increased ($P < 0.05$) the distal ileal starch recovery compared with the PC or the 10% cellulose diet. The ileal starch digestibility values were considerably lower ($P < 0.05$) in the 8.5% guar gum in combination with the test potato diets than in the NC or the 10% guar gum or the 10% cellulose supplemented

diet. Pearson correlation analyses showed a negative linear relationship ($P < 0.05$) between the apparent ileal DM digestibility values and the total glycoalkaloid contents in the potato test diets, however, no such a relationship ($P > 0.05$) was observed between the ileal starch digestibility values and the total glycoalkaloid contents in the potato diets **(Figure 2.1A and 2.1B)**.

To further reveal the link between the small intestinal mTOR abundances and distal ileal free glucose recovery & glucose absorption via SGLT1 as affected by consumption of 10% guar gum, changes in the phosphorylated mTOR abundances in the proximal jejunum were examined in the growing pigs fed both the NC and PC diets. As shown in **Figure 2.2**, 10% guar gum supplementation (PC diet) reduced ($P < 0.05$) the abundance of the phosphorylated mTOR (Ser 2448) in the proximal jejunum compared with the NC diet.

Cecal digesta concentrations (mg/g DM cecal digesta) and cecal digesta outputs (mg/ kg DM diet intake) of major volatile compounds, as affected by dietary treatments, are summarized and compared in **TABLES 2.4** and **2.5**. Cecal concentration of butyrate in the pigs fed the Potato 3 diet was much higher ($P < 0.05$) than that of the pigs fed the NC diet (**TABLE 2.4**). No differences ($P > 0.05$) in the other major volatile compound endpoints were observed in the cecal digesta. Effects of diets on the distal ileal digesta concentrations (mg/g DM ileal digesta) and the distal ileal outputs (mg/kg DM diet intake) of the major volatile compounds are summarized and compared in **TABLES 2.6** and **2.7**. Furthermore, responses in the fecal concentrations (mg/g DM feces) and the fecal outputs (mg/kg DM diet intake) of the major volatile compounds are summarized and compared in **TABLES 2.8** and **2.9**. There were generally no differences ($P > 0.05$) in the examined major volatile compound endpoints in the distal ileal digesta and feces among the diets.

2.5. DISCUSSION

The major objective of this study was to examine effects of intermediate levels (10-11%) dietary fibres supplemented as exogenous fibres and contributed by six different genotypes of potatoes on the ileal starch digestibility in growing pigs fed a high-fat basal diet. The total dietary fibre levels of 19.27 – 21.48 g/1000 kcal GE as shown in **TABLE 2.1** in this study were higher than the recommended dietary fibre intake level of 14 g/1000 kcal in adult humans by about 46% for maintaining a healthy cardiac system (Turner and Lupton, 2011). The grower pigs fed a typical high-fat and high-animal protein "Western diet" model, which was adapted from our previous study for hypercholesterolemia in growing pigs as a relevant large animal model (Rideout et al., 2007). Starch digestion *in vivo* and starch digestibility can be effectively determined by using the distal ileal cannulated pig model (Zijlstra et al., 2012). Poultry meal by-products include viscera, heads, feet, and other meat waste. Poultry meal by-products, although in their physical forms and nature are not suitable for human consumption, can serve as less expensive sources of animal proteins to conduct human nutrition and health research with our study model of growing pigs fed a typical “Western diet”. Also, the rendering of poultry dead stock generates by-products that are rich in protein, crude fat, minerals and vitamins and constitute a potentially valuable feed material for use in animal diets (Urlings et al., 1993). Research has shown poultry meal by-products are highly digestible protein sources as a replacement to spray-dried animal plasma protein products (Zier et al., 2004). Meanwhile, conventional cornstarch is also highly digestible. It is well established that the *in vivo* starch digestion pathway is consisted of

enzymatic hydrolyses of starch by amylolytic enzymes and absorptive transport of D-glucose across the enterocyte apical and the basolateral membranes (Gray, 1992). In order to understand and differentiate how dietary supplemented exogenous fibres and 8.5% guar gum in combination with different genotypes of potatoes influenced *in vivo* starch digestion via potentially affecting enzymatic starch hydrolysis and the digestive end-product glucose absorption. Efforts were made to measure the distal ileal free glucose and starch recoveries by measuring both free glucose and starch contents in the distal ileal digesta in combination with using a digestibility marker in this study. Considering the experimental variability (i.e. SE values), the distal ileal free glucose (0.10 ± 0.14%) and starch recovery (1.6 ± 2.2%) values along with the ileal starch digestibility (98.6 ± 2.2%) results in the NC diet suggest that the conventional cornstarch was completely digested in the growing pigs in this study. These results are consistent with our previous study by Rideout et al. (2008). Effects of dietary supplementations of isolated viscous and non-viscous fibres on nutrient digestibility have been mostly focused on DM, energy and CP and/or amino acids with fewer studies on starch digestion in the pig in the literature (Zijlstra et al., 2102). Results of the PC and the Cellulose diets in comparison with the NC diet from this study suggest that dietary supplementation of an intermediate level (10%) of both viscous fibre guar gum and non-viscous fibre cellulose significantly reduced starch digestibility by about 11-16%. Furthermore, there was no significant difference between the viscous fibre guar gum and the non-viscous fibre cellulose in decreasing *in vivo* starch digestibility. However, our previous study by Rideout et al. (2008) did not observe significant effects of the exogenous fibres on *in vivo* starch digestibility in pigs. This discrepancy was likely due to experimental variability as indicated by SE values. The decreases in distal ileal starch recovery rates in the Rideout et al. (2008) study, as affected by the exogenous fibres, were associated with a much larger variability (SE of 3.90%) due to a smaller number of test pigs used in comparison with SE of 1.9-2.0% of this study. Therefore, it can be concluded that dietary supplementations of both viscous exogenous fibres of guar gum and cellulose at an intermediate level of 10% could effectively reduce *in vivo* starch digestibility by about 11-16% and potentially contribute to the attenuation of dietary glycemic load and large bowel health.

On the other hand, the six potato diets of this study were designed to examine the combined effects of intermediate levels of TDF (at 10-11%), as contributed by 8.5% exogenous guar gum plus additional intrinsic fibres from the six different test genotypes of potatoes, as well as the different genotypes of potatoes in supplying different sources of potato starch on the ileal starch digestibility values in potatoes. Thus, by comparing with the NC, PC and Cellulose diets, variability in the ileal starch digestibility among the six testing potato genotypes could be effectively examined to select health-promotion potato genotypes that could be low in starch digestibility. Indeed, the ileal starch digestibility values were considerably lower in all of the six potato diets compared with NC, PC and the Cellulose diets. Furthermore, there were significant differences in the ileal starch digestibility values among the six test potato genotypes, ranging from 38.7 (Potato-1 diet) to 64.3% (Potato-4 diet). The *in vivo* starch digestibility results of this study suggest that potato genotypes of cv FV12272-3, cv 96044-3 and cv F05081 that were correspondingly represented by Potato diets 1, 3 and 6 were the novel healthy potato cultivars, being much lower in rapidly digestible starch values but much higher in resistant starch contents compared with three other potato genotypes. These results of this study are in general agreement with a recent study by Bach et al. (2013) in showing that potato genotype cv 96044-3

had a high resistant starch content, being a potentially healthy potato cultivar. Out of the six potato genotypes examined, cv FV12272-3, cv 96044-3 and cv F05081 were considerately low in the ileal starch digestibility. These three potato selections are not commercially available at the present, thus commercialization of these potato cultivars can greatly contribute to the health management of chronic diseases such as type-II diabetes and bowel inflammation.

It is important to understand the biological mechanisms associated with the exogenous fibre effects on decreasing *in vivo* ileal starch and DM digestibility demonstrated in this study. The significant reduction in the ileal starch digestibility, as measured from the cellulose-supplemented diet in this study, was resulted from using a commercial product of Solka-Floc®. The Solka-Floc® cellulose has a high crystalline index (**CrI**, 0.4 - 0.7), similar to other commercial cellulose sources such as filter paper (Watman # 1, CrI at 0.45), and avicel (CrI, 0.5 - 0.6) (Zhang et al., 2006). Thus, Solka-Floc® cellulose is a non-amorphous and insoluble fibre. To the best of our knowledge, this is the first study in showing that crystalline cellulose could effectively reduce *in vivo* starch digestion under the high-fat feeding. Since the small intestinal starch digestion is the major determinant of glycemic responses (Jenkins et al., 1987), our cellulose effects on starch digestion of this study challenge the present view and concept that non-viscous fibres do not exhibit the health effect of glycemic control (Chutkan et al., 2012). Further studies should be conducted to examine effects of more practical non-viscous fibre sources such as wheat bran on *in vivo* starch digestion. Effects of non-viscous fibres such as Solka-Floc® cellulose on glycemic responses should also be investigated. The significant decreases in the ileal starch digestibility in response to the intermediate levels (8.5 - 10%) of guar gum supplementations were determined in the PC diet and the six potato diets. These results are consistent with the literature reports that pure viscous soluble fibres are effective to dampen glycemic responses (Chutkan et al., 2012; Zijlstra et al., 2012). The depression effects of exogenous non-viscous and viscous fibres on *in vivo* starch digestion may be explained by the following biological mechanisms. Firstly, all types of fibres and non-starch polysaccharides (NSP), in general, reduce the exocrine pancreatic α-amylase activity, thus negatively affecting *in vivo* starch digestion. Several lines of literature suggest that both non-viscuos and viscous fibres decreased α-amylase activities *in vitro* (Hansen et al., 1982; Isaksson et al., 1982a, Hansen, 1986; Leng-Peschlow, 1989). Isaksson et al (1982b) showed that inhibitory fibre effects on α-amylase activity seemed to be more pronounced when exerted in human duodenal juice than in regular buffers. Slaughter et al. (2002) specifically demonstrated that the inhibitory effects of NSP or fibres on α-amylase activities were a non-competitive nature but were not time-dependent and were effective in a low concentration (0.5% or 3.3 uM) via forming a fibre-α-amylase complex, thereby reducing the maximal α-amylase activities ($\boldsymbol{V_{max}}$) without affecting α-amylase catalytic affinity ($\boldsymbol{K_m}$). Fewer *in vivo* studies were conducted and the literature reports tended to suggest that fibres had little direct effects on α-amylase activities in pancreas (Poksay and Schneeman, 1983; Calvert et al., 1985; Dukehart et al., 1989). Future studies should be conducted to elucidate effects of exogenous fibres on the pancreatic α-amylase gene expression including the *in vivo* α-amylase biosynthesis by using stable isotopic tracers, giving the challenge of measuring accurate total pancreatic α-amylase secretion responses. Under this context, mucosal phase of starch digestion is essential. Maltase-glucoamylase (**MGA**) can directly hydrolyze starch, however, little literature information is available regarding effects of exogenous dietary fibres on gut MGA expression. Several gut mucosal disaccharidases are essential to the terminal digestion of

maltose that is derived from the upper stream steps of starch digestion, including sucrase, isomaltase and maltase, while sucrase also typically hydrolyzes sucrose (Gray, 1992). Hannan et al. (2007) showed that soluble fibres inhibited intestinal disaccharidase activities. However, inconsistent results exist regarding the effects of exogenous dietary fibres on gut disaccharidase activities (Thomesn and Tasman-Jones, 1982; Johnson et al., 1984; Johnson and Gee, 1986; Onning and Asp, 1995). The discrepancy in the gut mucosal hydrolase activity responses, as affected by exogenous fibres, is also likely due to the fact the assay results were usually expressed as per unit of wet tissue weight or tissue protein content. It is well established that exogenous fibres enhance intestinal mucosa cell proliferation and hypertrophic differentiation (Calvert et al., 1985; Stangias and Pearce, 1985; Johnson and Gee, 1986; Fan et al., 2006). Thus, intestinal hydrolase activity response endpoints, as affected by exogenous fibres, should be expressed on per unit of cellular DNA or per unit of isolated apical membrane protein content for meaningful comparison (Fan et al., 2001; Fan et al., 2002). Secondly, exogenous fibres can reduce and tend to delay food digesta retention time in the small and the large intestines, which allow less time for starch and enzyme interactions and glucose absorption, contributing to decreased starch digestibility. Blackwood et al. (2000) and Zijlstra et al. (2012) proposed that viscous NSP could reduce gastric emptying and cause slower food digesta passage or transit time through the bowel. However, original research concluded by Tadesse (1986) and Johansen et al. (1996) showed that dietary fibre components had little effects on gastric emptying. Several studies demonstrated that both insoluble and soluble fibres increased intestinal digesta passage rates (Stanogis and Pearce, 1985; Cherbut et al., 1990; Fahey et al., 1990). Johansen and Knudsen (1994) reported that oat bran high in viscous β-glucan but not cellulose decreased digesta mean transit time through the proximal jejunum in pigs. The much lower ileal DM digestibility values in the two exogenous fibre diets (PC and the cellulose diets) were also resulted from the inclusions of these fibres in the diets at 10% that were less digestible compared with conventional cornstarch in the NC diet. Therefore, it can be concluded that the decreased ileal starch digestibility values in responses to the exogenous fibres in this study were likely resulted from noncompetitive inhibitory effects of these fibre components on pancreatic α-amylase activity and on food digesta retention time in the intestinal tract.

Viscous fibres, to a much small extent, negatively affect the small intestinal apical glucose transport activity, thus contributing to decreased *in vivo* starch digestion. Glucose transport activities across the intestinal apical and basolateral membranes are important final biochemical steps of the *in vivo* starch digestion pathway. As compared in **TABLE 2.3**, albeit of small magnitudes, dietary supplementation of viscous fibre guar gum but not the non-viscous fibre cellulose significantly increased the intestinal luminal free glucose content in the distal ileal digesta, thus reducing glucose absorption at a level equivalent to about 1% of the dietary starch intake in the PC and the six potato diets. Our results are consistent with the literature reports that dietary supplementations of soluble fibres but not insoluble fibre cellulose inhibited glucose transport and absorption across the small intestinal epithelia (Johnson et al., 1984; Rainbird et al., 1984; Hannan et al., 2007). Our previous studies by Yang et al. (2011) showed that the major gut apical glucose transporter SGLT1 expression along the crypt-villus axis was regulated at the level of translational control involving the mTOR-signaling pathway. Interestingly, compared with NC diet, total proximal jejunal phosphorylated mTOR abundance was significantly reduced by 10% guar gum supplementation in the PC diet. Ortells et al. (2012) demonstrated that osmotic stress in responses to hypertonicity up-regulated the expression of osmostress response

genes of the mTOR-signaling suppressor proteins (regulated in development and DNA damage responses, **REDD**) REDD1 and REDD2 in mammalian cells. Both REDD1 and REDD2 are established to be the mTOR upper stream inhibitors (Yang et al., 2008). It is known that dietary inclusions of viscous fibres including guar gum effectively enhance digesta viscosity (Chutkan et al., 2012, Zijlstra et al., 2012), inevitably inducing gut mucosal osmostress responses. Hence, the significant decrease in the proximal jejunal total phosphorylated mTOR abundance, as affected by the 10% guar gum supplementation in the PC diet, was likely mediated via an up-regulation of the REDD1 and REDD2 genes, which needs to be further confirmed in future studies. In turn, these would result in a reduced gut mucosal apical SGLT1 abundance via increased luminal digesta viscosity and mucosal osmostress response genes REDD1 and REDD2 in negatively affecting mTOR-mediated protein translational control of enterocytic SGLT1 synthesis. However, the reduction in the jejunal phosphorylated mTOR abundance in responses to the guar gum inclusion could not lead to decreased mucosal sucrase-isomaltase and maltase-glucoamylase expression or enzyme activity, otherwise, total free glucose output from the distal ileum would have been lower rather than being higher due to a projected decrease in hydrolytic release of glucose. Thus, viscous fibre guar gum in depressing the ileal starch digestion was partially mediated through reducing free glucose absorption to a small magnitude, which was likely regulated through decreasing the major entercotytic glucose transporter SGLT1 synthesis via the mTOR-signaling pathway.

We next want to understand what factors were likely responsible for the significant differences in the ileal starch digestibility among the six test potatoes. It is conceivable that the much lower ileal starch digestibility values in the potato test diet compared with the NC and the PC diets were the combined effects of fibres and other intrinsic factors associated with the test potato genotypes. By correcting or removing the quantitative negative effects of guar gum on starch digestibility determined from the PC diet by 12%, the ileal starch digestibility values in the six test potatoes excluding the exogenous fibre guar gum effect were calculated by the difference according Fan and Sauer (1995). The ileal starch digestibility values in the test potatoes were calculated to be 50.71 ± 1.72% for cv FV12272-3 from the Potato 1 diet, 61.80 ± 2.71% for cv F05035 from the Potato 2 diet, 52.47 ± 1.17% for cv 96044-3 from the Potato 3 diet, 75.95 ± 3.30% for cv WV5475-1 from the Potato 4 diet, 66.43 ± 2.99% for cv Atlantic from the Potato 5 diet, and 51.45 ± 2.19% for cv F05081 from the Potato 6 diet, respectively. Correspondingly, estimated resistant starch contents without the negative exogenous fibre effects in these test potatoes were estimated to be 49.29 ± 1.72% for cv FV 12272-3, 38.24 ± 2.71% for cv F05035%, 47.53 ± 1.17% for cv 96044-3, 24.05 ± 3.30% for cv WV5475-1, 33.57 ± 2.99% for cv Atlantic, and 48.46 ± 2.19% for cv F05081. There are limited literature reports of ileal starch digestibility values of potatoes in pigs and humans. Since intestinal starch digestion is the major determinant of glycemic responses (Jenkins et al., 1987), glycemic index in potatoes reported in humans in the literature can also reflect starch digestion in the commercially available potatoes. Glycemic index was 71% for Irish potatoes (Ramdath et al., 2004). Glycemic index was 72 and 77%, respectively, for California white and US Russet potatoes consumed (Fernandes et al, 2005). Cooling after cooking affected potato GI values (Kinnean et al., 2011). GI values for boiled red potatoes consumed cold and boiled red potatoes consumed were 56 and 89%, respectively (Fernandes et al., 2005). The test potato of cv Atlantic represented a commercial potato. Potatoes cv F05053 and cv WV5475-1 were associated with high ileal starch digestibility values (62-76%), whereas cv FV12272-3, cv F05035 and cv 05081

were associated with low ileal starch digestibility values (51-53%). Thus, these three potato genotypes with low ileal starch digestibility values have the potential to be developed as low-glycemic response health-promotion potatoes. Future studies should be conducted to examine how cooking and food processing influence ileal starch digestibility in the pig model and glycemic response associated with these three novel healthy potatoes in human clinical nutrition trials.

On the other hand, it is interesting to observe that the resistant starch contents in the test potatoes estimated from our *in vivo* starch digestion studies in the pig are much lower than the chemically analyzed resistant starch contents from the same test potatoes harvested in Elora in 2009 as reported by Bach et al. (2013). For example, resistant starch contents were 49% for cv FV12272-3, 48% for cv 96044-3, and 49% for cv F05081, respectively, estimated in this *in vivo* study, in comparison with 78% for cv FV 12272-3, 80% for cv 96044-3 and 77% for cv F05081, respectively, via chemical analyses in the study by Bach et al. (2013), resulting a discrepancy of about 28-32% in resistant starch contents in these three potato cultivars between the *in vivo* and the *in vitro* methods. This observation on estimating resistant starch content is consistent with the report by Rideout et al. (2008b) who also showed that *in vitro* chemical analyses considerably overestimate resistant starch contents in samples. The current chemical analyses of resistant starch content in samples according to AOAC method 2002.02 by using the Megazyme kits likely overestimate the true values of resistant starch for the following two reasons. Firstly, the *in vitro* resistant starch chemical analysis procedures do not include pre-treatment steps of chemical gelatinization and liquefaction of starches in samples in terms of destroying the semi-crystalline structure of raw starch in food samples similar to cooking and *in vivo* gastric acid actions. Secondly, apart from using the α-amylase, the use of a single genetically engineered microbial amyloglucosidase to mimic *in vivo* mucosal phase of multiple-enzyme terminal starch digestion is an over-simplification. Champ et al. (2003) and McCleary (2003) concluded that the TDF analysis procedures by using the Megazyme kits largely underestimated non-digestible oligosaccharides and RS as soluble fibre components. All these are likely responsible for reported and our observed underestimation of resistant starch contents. Future research should be conducted to further improve the present resistant starch chemical analysis procedures.

Under this context, it is clear that different resistant starch contents were largely responsible for the variable ileal starch digestibility values among the test potato cultivar genotypes. Capriles et al. (2008) reported that resistant starch content was proportional to starch granule size in potatoes. Wikaman et al. (2013) further demonstrated that starch granule size was dependent on both amylose content and degree of starch branches in potatoes. Thus, variability in starch granule size, as affected by amylose content and degree of starch branches, was likely the major intrinsic factor responsible for the differences in the ileal starch digestibility and resistant starch contents among the six potato cultivar genotypes in this study. Total glycoalkaloids, including α-solanine and α-chaconine, in the six test potatoes (Potatoes 1 to 6) were from 5.88 to 11.88 mg/100 g samples, on their fresh tuber bases, were within the regulatory upper limit of 200 mg/100 g of fresh potato tubers by Health Canada and CFIA. However, previous animal model studies shown that potato glycoalkaloid content within the government regulatory safety limit caused bowel inflammation (Patel et al., 2002). Although Pearson correlation analyses showed a significant negative linear relationship between the ileal DM digestibility values and the total

potato glycoalkaloid contents, there was no such a significant relationship between the ileal starch digestibility values and the total potato glycoalkaloid contents in these test potatoes from this study. Potential contributions of variable glycoalkaloid contents to the variability of the ileal starch digestibility values among the test potatoes may be eliminated. Therefore, it can be concluded that differences in the ileal starch digestibility values among the test potatoes of this study were likely attributed to by intrinsic differences in starch granule size with amylose content and degree of starch branches as the main influencing factors, which is to be further clarified in future studies.

TDF contents for the test potato diets were at about 11%, on as-fed basis. Based on the ileal starch digestibility values, the *in vivo* resistant starch contents in the six potato test diets of this study ranged from 36 (potato 4) to 61% (Potato 1 and Potato 6 diets). Clearly, the TDF analysis procedures (AOAC 991.43 and AOAC 985.29) by using the Megazyme kits did not include much of the resistant starch as a fibre component in these potato test diets in this study. Our results are in agreement with the conclusion made by Champ et al. (2003) and by McCleary (2003) that the current TDF analysis procedures largely underestimate non-digestible oligosaccharides and resistant starch as soluble fibre components. However, it is interesting to note that the dietary treatments did not significantly influence BW gain and gain to feed conversion ratio in this study, suggesting very little potential effects of these dietary treatments on obesity or BW gain control. These results may be explained by the following two major reasons. Firstly, feed intake levels of pigs in this study were largely controlled under pair-fed condition by referencing the NC diet (net energy, **NE**, of 12.10 for the NC vs. NE of 11.1 – 11.8 MJ/kg for the other test diets, on as-fed basis). Although studies by Livesey et al. (1995) suggested that crystalline cellulose provided as Solka-Floc® was very poorly fermentable in rats, Bach Knudsen et al. (1994) demonstrated that rodent models underestimate fibre fermentations in humans. Rideout et al. (2008) demonstrated that up to 62% Solka-Floc® cellulose was fermented at the end of the distal ileum, and additional 20% cellulose was fermented by microbes in the large intestine in growing pigs. Apparently, the inhibitory effects of the cellulose on starch digestion shown in this study would have occurred in the upper small intestine. Several lines of evidence also support the concept that microbial cellulases and hemicellulases are expressed in the distal small intestine and the large intestine, enabling effective fibre fermentation in growing-finishing pigs (Pond, 1987; Varel and Yen, 1997; Wang et al., 2012). Soluble fibres such as sugar beet pulp and inulin were effectively fermented in the gut in providing NE for maintenance in humans (Castiglia-Delavaud et al., 1998). Rideout et al. (2008) showed that resistant starches included in diets and recovered from the distal ileum were completely fermented in large intestine, while about 50% of guar gum included in the diet was fermented in the large intestine. The Potato diet 3 that contained cv 96044-3 of this study showed significantly higher cecal butyrate content compared with the NC diet. However, there were generally no significant differences in the examined short-chain fatty acid contents and outputs among the diets largely due to very large SE values associated with endpoints, reflecting very large individual pig variability in the VFA endpoints. Nevertheless, exogenous fibres in enhancing volatile short-chain fatty acid production in the large intestine have been well established in the literature (Stanogias and Pearce, 1985; Rideout et al., 2008). Under this context, it should be pointed out that apart from being potentially low-glycemic responses for preventing and managing type-II diabetes, the three novel and healthy potato genotypes, i.e., cv 12272-3, cv 96044-3 and cv F05081, were also associated with high levels of resistant starch

contents compared with current commercial potatoes on markets. It has been well documented that resistant starches are a group of soluble fibre prebiotics (Gibson et al., 2004). Resistant starches are shown to have additional health benefits such as reducing postprandial insulin levels (Kendall et al., 2004), lowering the risk of developing colorectal cancer with butyrate being specifically reconized for its anti-carcinogenic functionality (Champ et al., 2003; Kendall et al., 2004) and depressing blood dyslipidemia (Younes et al., 1995). Thus, it can be concluded that although exogenous fibres and these three novel high-resistant starch potato cultivars may be limited in their efficacy for control of weight gains, they may have additional health benefits through providing resistant starch as prebiotics especially potato genotype cv 96044-3.

In summary, dietary supplementations of exogenous fibres at 10% reduced ileal starch digestibility and glucose absorption involving the mTOR-signaling pathway. The three novel potato genotypes of cv 12272-3, cv 96044-3 and cv F05081 were shown to have considerably low ileal starch digestibility values and high resistant starch contents. Thus these three novel genotypes of potatoes have a potential in serving as healthy staple foods in preventing and managing type-II diabetes, blood dyslipidemia and risks of developing colorectal cancers in particular with the potato genotype of cv cv 96044-3.

Table 2. 1 Composition (%) of experimental diets (on as-fed basis) fed to the growing pigs

Items	Experimental diets[1]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
Poultry meal	41.50	41.50	41.50	41.50	41.50	41.50	41.50	41.50	41.50
Casein	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Animal fat-oil blend	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
DL-methionine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
L-Alanine[2]	2.81	2.81	2.81	0.00	0.00	0.00	0.00	0.00	0.00
Sucrose	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31
Cornstarch	30.79	20.79	20.79	0.00	0.00	0.00	0.00	0.00	0.00
Solka-Floc®[3]	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
Guar gum[4]	0.00	10.00	0.00	8.50	8.50	8.50	8.50	8.50	8.50
Potato-1	0.00	0.00	0.00	25.10	0.00	0.00	0.00	0.00	0.00
Potato-2	0.00	0.00	0.00	0.00	25.10	0.00	0.00	0.00	0.00
Potato-3	0.00	0.00	0.00	0.00	0.00	25.10	0.00	0.00	0.00
Potato-4	0.00	0.00	0.00	0.00	0.00	0.00	25.10	0.00	0.00
Potato-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.10	0.00
Potato-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.10
Min-Vit premix[5]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

Iodized salt[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Titanium oxide[7]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Nutrient contents (on as-fed or air-dry basis)									
Dry matter[8]	95.26	92.64	94.40	93.03	92.25	92.99	93.07	92.75	94.49
Total starch[9]	22.70	19.79	18.81	15.42	14.59	12.58	14.42	14.69	14.10
Crude fat[10]	20.26	20.26	20.26	20.67	20.67	20.67	20.67	20.67	20.67
Crude protein[11]	33.14	33.14	33.14	33.38	33.13	33.34	33.05	33.14	32.78
Glycoalkaloids[12]	0.00	0.00	0.00	6.38	7.73	12.12	8.68	8.84	11.47
Total dietary fibre[13]	0.00	10.00	10.00	11.15	11.13	10.90	10.82	10.46	10.99
NDF[14]	0.00	0.00	10.00	1.32	1.29	1.47	1.39	0.76	1.10
Total soluble fibre[15]	0.00	10.00	0.00	9.84	9.84	9.44	9.43	9.70	9.89
GE[16]	21.66	21.70	21.70	21.10	21.10	21.10	21.10	21.10	21.10
DE[17]	17.80	17.21	17.38	16.62	16.62	16.62	16.62	16.62	16.62
ME[18]	15.93	15.35	15.52	14.72	14.72	14.72	14.72	14.72	14.72
NE[19]	12.10	11.64	11.76	11.08	11.10	11.10	11.08	11.09	11.06
GE from Fat[20]	37.22	37.15	37.15	38.98	39.98	38.98	38.98	38.98	38.98

Note: [1]NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as the basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[2]Evonik Industries AG, Hanau-Wolfgang, Germany.
[3]Solka-Floc®, pure cellulose commercially available from International Fibre Corporation (North Tonawanda, NY).
[4]Guar gum, food grade with an estimated average molecular weight of about 1.5 million and granulation of medium coarse commercially available from Nealanders International Inc. (Mississauga, ON, Canada).
[5]The vitamin and trace mineral premix supplied the following vitamins and trace minerals (IU or mg/kg diet): vitamin A, 8000 IU; vitamin D_3, 800 IU; vitamin E, 32 IU; vitamin K, 2 mg; vitamin $B_{12,}$ 0.02 mg; D-biotin, 0.16 mg; thiamine, 1.2 mg; riboflavin, 4 mg; D-pantothenic acid, 12 mg; pyridoxine, 1.2 mg; niacin, 20 mg; folic acid, 1.6 mg; choline, 400 mg; iron, 80 mg; copper, 12 mg; manganese, 16 mg; zinc, 84 mg; iodine, 0.4 mg; and selenium, 0.24 mg.
[6]Supplied by the Windsor Salt Co. (Toronto, ON, Canada). Composition (g/kg): NaCl, 965.0; ZnO, 40.0; $FeCO_3$, 1.6; MnO, 1.2; CuO, 0.33; $Ca(IO_3)_2$, 0.07; and CaO, 0.04.
[7]Nutrient digestibility marker purchased from Fisher Scientific (Ottawa, ON, Canada).
[8]Analyzed dietary dry matter content, *%*.
[9]Analyzed dietary total starch content, *%*.
[10]Crude fat content in diets, *%*, calculated by using crude fat contents in the concerned ingredients compiled by NRC (1998); and crude fat content in cooked and dried potatoes reviewed by Whittemore (1977).
[11]Crude protein content in diets, *%*, calculated by using crude protein contents in the concerned ingredients compiled in NRC (1998); and the analyzed crude protein contents in the cooked and dried test potato samples.
[12]Total glycoalkaloid content including α-chaconine and α-solanine in the diets, mg/100 g diet, calculated total dietary content based on the analyzed constituent contents in the cooked and dried test potato samples.
[13]Total dietaryfibre (TDF) content in diets, *%*, calculated dietary content based on the analyzed TDF contents in the cooked and dried test potato samples and the TDF content in Solka-Floc® and guar gum being considered to be 100%.
[14]NDF, dietary neutral-detergent fibre content in diets, *%*, calculated contents based on the analyzed NDF contents in the cooked and dried test potato samples and the NDF content considered to be 100% in Solka-Floc® and 0% in guar gum, respectively.
[15]Total soluble fibre content in diets, *%*, calculated content = total dietary fibre (TDF, *%*) – neutral-detergent fibre (NDF, *%*).
[16]Calculated by using GE in ingredients cited in NRC (1998); GE from cooked and dried potatoes reviewed by Whittemore (1977); GE in poultry meal reported by Pesti et al. (1986). GE of fibre = 17.8 MJ/kg; GE of crude protein = 23.8 MJ/kg; and GE of crude fat = 39.8 MJ/kg as summarized by Brouwer (1965); and GE of starch = 17.4 MJ/kg; GE of protein = 23.6 MJ/kg; GE of Met = 21.2 MJ/kg; and GE of Ala = 22.8 MJ/kg as summarized by Livesey(1984).
[17]Calculated by using DE in ingredients cited in NRC (1998); DE from cooked and dried potatoes as reviewed by Whittemore (1977); and GE in poultry meals reported by Pesti et al. (1986).
[18]ME, metabolizable energy content in diets, *MJ/kg*, calculated by using ME content in the concerned ingredients compiled in NRC (1998); ME from cooked and dried test potatoes obtained by multiplying by a correction factor of 0.96 according to NRC (1998) with DE in cooked and dried potatoes reviewed by Whittemore (1977); and ME content in poultry meals reported by Pesti et al. (1986).
[19]Calculated NE content in diets, MJ/Kg, by using the equation 7 from Noblet et al. (1994). NE = 0.870 * ME - 442 (units, Kcal/kg, on DM basis).
[20]Percentage contribution of GE from crude fat to total dietary GE, *%*, calculated by using GE of crude fat = 39.8 MJ/kg, as summarized by Brouwer (1965).

Table 2. 2 Responses[1] in the body weight gain, feed intake and feed conversion efficiency in the growing pigs fed the experimental diets

	Experimental diets[2]								
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
n[3]	7	9	10	10	7	10	8		8
Initial BW[4]	25.7±0.7	24.8±0.6	25.3±0.6	24.7±0.6	25.6±0.7	25.6±0.6	25.4±0.6	24.6±0.6	23.8±0.6
Final BW[4]	35.6±0.7	34.8±0.6	35.7±0.6	35.8±0.6	37.0±0.7	36.3±0.6	35.1±0.7	36.3±0.6	35.9±0.7
ADFI[5]	0.91±0.06	0.87±0.05	0.98±0.05	0.93±0.05	0.98±0.06	0.97±0.05	0.87±0.05	0.98±0.05	1.05±0.05
ADG[6]	0.42±0.03	0.36±0.02	0.37±0.02	0.40±0.02	0.41±0.03	0.38±0.02	0.35±0.03	0.42±0.02	0.43±0.03
G:F[7]	0.47±0.02	0.41±0.02	0.38±0.02	0.43±0.02	0.42±0.02	0.39±0.02	0.40±0.02	.43±0.02	0.42±0.02

Note: [1]Values are least square means ± SE of the estimates.
[2]NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as the basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.
[3]Number of observations.
[4]BW, body weight (kg).
[5]ADFI, average daily feed intake (kg/pig·day).
[6]ADG, average daily gain (kg/pig·day).
[7]G:F, gain (G) to feed (F) ratio.

Table 2. 3 Responses in the apparent ileal dry matter (DM) and starch digestibility values[1] in the experimental diets fed to the growing pigs

	Experimental diets[2]								
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (CV96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
n[3]	7	9	10	10	7	10	6	9	8
Dietary DM[4]	95.3	92.6	94.4	93.0	92.3	93.0	93.1	92.8	94.5
Ileal DM[4]	94.1±0.7^{ab}	93.6±0.6^{a}	94.7±0.7^{ab}	94.4±0.6^{ab}	93.4±0.7^{a}	94.9±0.6^{ab}	96.4±0.7^{b}	94.4±0.6^{ab}	94.1±0.7^{ab}
Ileal DM digestibilty[5]	79.4±3.0$^{\S\P a}$	48.3±2.6^{*b}	52.6±2.8^{*b}	50.9±2.5^{*b}	54.0±3.0^{*b}	45.2±2.5^{*b}	51.4±3.2^{*b}	52.8±2.6^{*b}	46.2±2.8^{*b}
Dietary free Glc[6]	0.04	0.03	0.03	0.03	0.03	0.02	0.05	0.03	0.04
Ileal free Glc[6]	0.14±0.05^{a}	0.32±0.04^{*ab}	0.19±0.04^{ab}	0.26±0.04^{ab}	0.39±0.05^{*b}	0.26±0.04^{ab}	0.24±0.05^{ab}	0.22±0.04^{ab}	0.24±0.04^{ab}
Ileal free Glc output[7]	0.27±0.27$^{\S\P a}$	1.64±0.24$^{*\P b}$	0.88±0.25$^{\S *b}$	1.28±0.22^{*b}	1.81±0.27^{*b}	1.43±0.22^{*b}	1.06±0.29^{*b}	0.99±0.23$^{*\S b}$	1.28±0.25^{*b}
Ileal free Glc recovered[8]	0.02±0.02$^{\S a}$	0.15±0.02^{*b}	0.08±0.02$^{\S ab}$	0.12±0.02^{*b}	0.16±0.02^{*b}	0.13±0.02^{*b}	0.10±0.03^{b}	0.09±0.02^{b}	0.12±0.02^{*b}
Ileal free Glc recovery[9]	0.10±0.14$^{\S\P a}$	0.71±0.12^{*b}	0.40±0.13^{ab}	0.70±0.12^{*b}	1.03±0.14$^{*\S b}$	1.00±0.12$^{*\P b}$	0.62±0.15^{*b}	0.55±0.12^{*b}	0.80±0.13^{*b}
Dietary starch[10]	23.62	20.42	19.75	16.56	15.73	13.22	15.55	16.08	14.52
Ileal starch[10]	1.93±0.75$^{\S\P a}$	3.71±0.66$^{*\P b}$	7.14±0.70$^{*\S c}$	21.20±0.63$^{*\S\P d}$	17.44±0.75$^{*\S\P e}$	14.85±0.63$^{*\S\P f}$	11.88±0.81$^{*\S\P g}$	15.92±0.66$^{*\S\P f}$	16.39±0.70$^{*\S\P f}$
Ileal starch output[11]	3.91±3.50$^{\S\P a}$	18.58±3.11$^{*\P b}$	33.43±3.28$^{*\S c}$	101.50±2.94$^{*\S\P d}$	78.99±3.50$^{*\S\P e}$	78.70±2.94$^{*\S\P e}$	55.79±3.78$^{*\S\P f}$	73.06±3.09$^{*\S\P e}$	87.80±3.28$^{*\S\P g}$
Ileal starch recovered[12]	0.39±0.35$^{\S\P a}$	1.86±0.31$^{*\P b}$	3.34±0.33$^{*\S c}$	10.15±0.29$^{*\S\P d}$	7.90±0.35$^{*\S\P e\P}$	7.87±0.29$^{*\S\P e}$	5.58±0.38$^{*\S\P f}$	7.31±0.31$^{*\S\P e}$	8.78±0.33$^{*\S\P g}$

Ileal starch recovery[13]	1.6±2.2^{§¶a}	9.0±1.9^{*¶b}	16.8±2.0^{*§c}	61.3±1.8^{*§¶d}	50.0±2.2^{*§¶e}	59.5±1.8^{*§¶d}	35.8±2.3^{*§¶f}	45.4±1.9^{*§¶e}	60.5±2.0^{*§¶d}
Ileal starch digestibility[14]	98. 6±2.2^{§¶a}	87. 6±2.0^{*b}	83.3±2.0^{*b}	38.7±1.8^{*§¶c}	49.9±2.2^{*§¶d}	40.5±1.8^{*§¶c}	64.3±2.3^{*§¶e}	54.7±1.9^{*§¶d}	39.3±2.0^{*§¶c}

Note: [1]Values are least square means ± SE of the estimates.

[2]NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as the basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]Number of observations.

[4]DM content, %, in the dietary samples or in the ileal digesta samples.

[5]The apparent ileal DM digestibility in the corresponding experimental diets, %.

[6]Free glucose (Glc) content in the dietary samples or in the distal ileal digesta samples,%, on DM basis.

[7]Free glucose (Glc) output in the distal ileal digesta expressed as g/kg DM diet

[8]Free glucose (Glc) recovered in the distal ileal digesta expressed as % of diet, on DM basis.

[9]Free glucose (Glc) recovery in the distal ileal digesta expressed as % of dietary total starch content, on DM basis.

[10]Total starch content in dietary samples or in the distal ileal digesta samples,%, on DM basis.

[11]Total starch output in the distal ileal digesta expressed as g/kg DM diet.

[12]Total starch recovered in the distal ileal digesta expressed as % of diet, on DM basis.

[13]Total starch recovery in the distal ileal digesta expressed as % of dietary total starch content, on DM basis.

[14]The apparent ileal starch digestibility in the corresponding experimental diets, %.

a,b,c,d,e,f,gMeans that diets with different superscript letters differ ($P < 0.05$) from each other as analyzed by the Tukey-Kramer's tests.

[*]Difference from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

[§]Difference from the PC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

[¶]Difference from the Cellulose group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

Table 2. 4 Responses in the cecal concentrations[1] (mg/g DM cecal digesta) of major volatile compounds in the growing pigs fed the experimental diets

	Experimental diets[2]									
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)	Root MSE[3]
n[4]	3	5	5	5	5	6	5	6	5	
Acetic acid	25.82	36.40	26.55	25.19	22.53	40.65	26.32	31.14	23.46	15.71
Butyric acid	5.08[a]	12.16[ab]	12.16[ab]	8.16[ab]	13.84[ab]	17.77[b*]	14.07[ab]	15.46[ab*]	15.44[ab]	5.10
Propionic acid	19.05	35.76	26.55	20.13	32.67	28.74	36.52	30.51	22.80	11.26
Isobutyric acid	1.10	1.40	1.72	1.27	2.15	1.49	1.07	1.58	0.74	0.72
2-Methylbutyric acid	1.40	1.74	1.99	1.52	2.96	1.71	1.65	2.11	0.68	1.22
Isovaleric acid	2.25	2.96	3.96	2.54	4.57	3.99	2.74	4.23	1.74	1.93
Valeric acid	5.10	9.82	6.98	5.73	10.81	9.33	9.56	9.67	7.98	3.80
Hexanoic acid	6.14	7.96	5.84	5.17	9.03	6.09	9.11	9.69	5.17	4.64
P-Cresol	0.13	0.21	0.20	0.29	0.31	0.29	0.12	0.20	0.26	0.14
4-Ethylphenol	0.0036	0.0108	0.0201	0.0044	0.0094	0.0197	0.0093	0.0116	0.0048	0.0138
Indole	0.0364	0.0213	0.0676	0.0565	0.0434	0.0291	0.0131	0.0362	0.0243	0.0321
Skatole	0.0848	0.1503	0.1298	0.1830	0.2023	0.2210	0.1084	0.1612	0.1702	0.0939
Total VFA[5]	66.34	107.44	84.98	69.70	98.82	109.76	101.31	104.37	78.73	31.52

Note: [1]Values are least square means.

[2]Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]The pooled root mean square of error (MSE). The SEM for corresponding dietary treatments would equal the root MSE/√n, and n is number of observations of corresponding dietary treatments.

[4]Number of observations.

[5]Total VFA is sum of all of the analyzed volatile compounds.

*Values are different from the NC group ($P < 0.05$) as analyzed with the Dunnett-Hsu's test.

[a,b]Means that diets with different superscript letters differ ($P < 0.05$) from each other as analyzed by the Tukey-Kramer's tests.

Table 2. 5 Responses in the cecal output[1] (mg/kg DM diet intake) of major volatile compounds in the growing pigs fed the experimental diets

	Experimental diets[2]									
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)	Root MSE[3]
n[4]	3	5	5	5	5	6	5	6	5	
Acetic acid	6607.31	11003.00	5136.85	5188.24	9968.40	9151.05	10305.00	5428.40	5838.21	5843.97
Butyric acid	1512.79	3493.27	2177.32	1752.40	3359.28	3714.51	3392.40	2907.43	2971.52	1393.45
Propionic acid	5379.83	9429.17	5293.53	4166.92	10573.00	5782.99	11934.00	6750.19	4483.56	4112.15
Isobutyric acid	316.67	412.24	395.64	273.51	526.49	348.90	220.11	353.53	197.83	272.59
2-Methylbutyric	401.82	525.63	404.41	360.33	787.95	358.78	366.90	514.65	338.67	463.87
Isovaleric acid	650.12	859.54	781.03	585.19	1127.91	768.54	587.47	878.09	584.31	682.85
Valeric acid	1448.97	2538.63	1468.20	1290.02	2748.18	1909.54	2187.35	2532.42	1880.80	1525.09
Hexanoic acid	1671.24	2445.51	1530.70	1291.47	2613.69	1416.34	2548.56	3000.12	1725.15	2165.35
P-Cresol	25.86	47.64	34.25	64.53	60.72	56.94	23.51	31.14	47.72	24.47
4-Ethylphenol	1.38	3.67	2.32	1.22	1.30	2.33	1.55	0.90	3.34	2.66
Indole	7.55	5.47	18.87	10.15	6.14	8.56	3.08	4.29	5.09	10.21
Skatole	17.33	34.88	19.40	41.43	36.80	44.57	19.09	26.50	32.79	16.67
Total VFA[5]	18007.00	30654.00	17134.00	14965.00	31691.00	23451.00	31528.00	22365.00	18017.00	13166.62

Note: [1] Values are least square means.

[2]Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]The pooled root mean square of error (MSE). The SEM for corresponding dietary treatments would equal the root MSE/√n, and n is number of observations of corresponding dietary treatments.

[4]Number of observations.

[5]Total VFA is sum of all of the analyzed volatile compounds.

Table 2. 6 Responses in the ileal concentrations[1] (mg/g DM ileal digesta) of major volatile compounds in the growing pigs fed the experimental diets

Experimental diets[2]										
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)	Root MSE[3]
n[4]	3	5	5	6	5	6	5	6	5	
Acetic acid	18.36	15.29	13.44	25.75	15.15	28.14	12.26	15.11	14.77	9.49
Butyric acid	5.57	6.32	2.87	5.38	5.31	6.59	3.39	4.98	4.54	3.15
Propionic acid	6.11	11.02	7.42	18.45	8.96	21.92	8.46	9.16	10.00	10.88
Isobutyric acid	0.41	0.80	0.44	0.63	0.45	0.33	0.45	0.54	0.43	0.27
2-Methylbutyric acid	0.93	1.58	1.32	1.24	1.60	0.83	1.12	1.28	0.84	0.84
Isovaleric acid	3.40	4.26	2.24	2.18	3.43	2.17	2.10	2.72	1.96	1.98
Valeric acid	3.09	4.65	2.63	3.30	3.23	2.62	2.70	2.97	1.89	1.63
Hexanoic acid	5.08	5.83	3.29	3.91	3.97	2.33	3.48	3.55	2.34	2.63
P-Cresol	0.03	0.04	0.04	0.07	0.06	0.04	0.03	0.03	0.01	0.04
4-Ethylphenol	0.0157	0.0320	0.0193	0.0082	0.0439	0.0133	0.0109	0.0155	0.0071	0.0291
Indole	0.0424	0.0648	0.0303	0.1062	0.1174	0.0825	0.0957	0.0505	0.0789	0.0643
Skatole	0.0038	0.0155	0.0080	0.0383	0.0181	0.0115	0.0120	0.0088	0.0043	0.0261
Total VFA[5]	42.45	49.42	33.32	60.82	42.42	64.92	34.27	40.31	36.84	22.58

Note: [1]Values are least square means.

[2]Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]The pooled root mean square of error (MSE). The SEM for corresponding dietary treatments would equal the root MSE/√n, and n is number of observations of corresponding dietary treatments.

[4]Number of observations.

[5]Total VFA is sum of all of the analyzed volatile compounds.

Table 2. 7 Responses in the distal ileal output[1] (mg/kg DM diet intake) of major volatile compounds in the growing pigs fed the experimental diets

	Experimental diets[2]									
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)	Root MSE[3]
n[4]	3	5	5	6	5	6	5	6	5	
Acetic acid	2456.12	2500.70	2797.23	4974.69	2153.38	3691.55	1723.03	3185.69	3993.79	1633.23
Butyric acid	728.17	869.78	551.83	1213.22	884.48	961.40	624.39	1030.98	1021.37	491.32
Propionic acid	1093.54	1943.41	1453.28	3703.58	1302.30	2413.39	1642.45	1844.09	2059.60	1334.99
Isobutyric acid	97.52	156.92	103.36	186.51	76.38	66.22	102.07	127.90	111.29	81.18
2-Methylbutyric	226.70	366.34	269.85	395.96	226.07	158.61	295.73	303.59	224.69	180.05
Isovaleric acid	543.19	733.20	422.28	606.64	483.43	338.36	454.63	572.18	461.42	272.72
Valeric acid	688.13	1179.72	604.25	1012.14	549.31	466.33	688.95	727.99	506.16	457.55
Hexanoic acid	1097.02	2017.12	807.96	1361.15	744.49	556.67	1014.08	1018.00	684.96	880.40
P-Cresol	3.92	6.07	5.68	9.24	6.72	3.97	4.44	4.60	3.62	4.26
4-Ethylphenol	1.79	3.32	1.87	1.27	4.38	1.35	1.57	1.99	1.90	2.37
Indole	5.03	14.82	6.35	19.17	19.23	17.32	19.73	9.30	25.94	13.69
Skatole	0.59	1.92	1.11	4.53	1.81	1.24	1.62	1.48	1.61	2.70
Total VFA[5]	6964.45	9789.00	7031.84	13454.00	6458.20	8652.53	6583.69	8810.44	9026.42	3758.37

Note: [1]Values are least square means.

[2] Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]The pooled root mean square of error (MSE). The SEM for corresponding dietary treatments would equal the root MSE/√n, and n is number of observations of corresponding dietary treatments.

[4]Number of observations.

[5]Total VFA is sum of all of the analyzed volatile compounds.

Table 2. 8 Responses in the fecal concentrations[1] (mg/g DM feces) of major volatile compounds in the growing pigs fed the experimental diets

	Experimental diets[2]									
Item	NC (no fibre)	PC (guar gum)	Cellulose (10%)	ɔtato 1 'V12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)	Root MSE[3]
n[4]	3	5	5	6	5	6	5	6	5	
Acetic acid	7.09	10.32	9.73	7.64	7.40	9.45	10.59	6.52	6.91	3.16
Butyric acid	3.20	3.98	4.78	2.54	4.00	4.27	5.90	2.80	5.31	2.37
Propionic acid	6.38	9.21	8.30	5.82	7.62	9.41	9.60	5.92	8.10	2.97
Isobutyric acid	1.40	1.30	1.46	0.90	1.12	1.19	1.29	1.01	1.03	0.39
2-Methylbutyric acid	1.42	1.19	1.30	0.91	1.20	1.19	1.40	1.03	0.96	0.41
Isovaleric acid	2.35	1.82	1.98	1.39	1.78	1.85	1.98	1.57	1.64	0.64
Valeric acid	3.22	3.27	3.18	2.32	3.54	3.84	3.73	2.29	3.22	1.47
Hexanoic acid	1.22	1.18	0.95	1.56	2.47	2.50	2.39	0.99	1.70	1.40
P-Cresol	0.67	0.51	0.54	0.48	0.58	0.55	0.48	0.52	0.53	0.15
4-Ethylphenol	0.011	0.003	0.0029	0.0030	0.0014*	0.0023*	0.0040	0.0036	0.0023*	0.0041
Indole	0.0453	0.0419	0.0516	0.0417	0.0356	0.0412	0.0384	0.0545	0.0356	0.3193
Skatole	0.41	0.31	0.37	0.30	0.36	0.33	0.35	0.31	0.38	0.11
Total VFA[5]	26.15	32.21	31.63	23.09	29.18	33.69	36.96	22.13	28.86	10.71

Note: [1]Values are least square means.

[2] Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]The pooled root mean square of error (MSE). The SEM for corresponding dietary treatments would equal the root MSE/√n, and n is number of observations of corresponding dietary treatments.

[4]Number of observations.

[5]Total VFA is sum of all of the analyzed volatile compounds.

*Values are different from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's test.

Table 2. 9 Reponses in the fecal output[1] (mg/kg DM diet intake) of major volatile compounds in the growing pigs fed the experimental diets

Item	Experimental diets[2]									
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)	Root MSE[3]
n[4]	3	5	5	6	5	6	5	6	5	
Acetic acid	855.45	1671.93	1592.11	1196.92	1132.88	1608.59	1530.65	948.54	1086.30	527.00
Butyric acid	390.39	679.08	792.87	400.62	634.74	718.98	849.64	417.70	821.15	400.08
Propionic acid	823.92	1515.40	1349.55	911.58	1203.03	1581.30	1371.31	889.10	1253.45	516.24
Isobutyric acid	172.18	203.80	228.79	151.79	173.01	211.16	179.13	144.84	158.08	59.59
2-Methylbutyric	181.88	185.05	203.11	158.56	188.54	207.68	196.09	136.28	145.32	65.15
Isovaleric acid	288.48	273.64	311.62	239.84	275.88	312.95	275.08	205.74	239.40	98.68
Valeric acid	421.57	547.84	503.74	371.55	568.62	645.83	531.03	338.47	510.25	275.62
Hexanoic acid	195.14	201.23	153.05	241.09	416.01	414.12	361.85	139.61	278.46	236.58
P-Cresol	84.37	82.13	85.89	93.70	89.49	108.98	66.48	75.21	84.37	42.37
4-Ethylphenol	1.74	0.51	0.49	0.57	0.20*	0.35*	0.53	0.33*	0.34*	0.63
Indole	6.07	6.59	8.11	8.30	5.40	8.24	5.14	7.89	5.60	3.63
Skatole	51.67	51.29	59.54	60.69	55.68	64.00	49.41	45.91	59.35	30.63
Total VFA[4]	3313.43	5270.87	5127.75	3671.95	4595.63	5700.61	5297.71	3220.27	4494.26	1867.64

Note: [1] Values are least square means.

[2] Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]The pooled root mean square of error (MSE). The SEM for corresponding dietary treatments would equal the root MSE/√n, and n is number of observations of corresponding dietary treatments.

[4]Number of observations.

[5]Total VFA is sum of all of the analyzed volatile compounds.

*Values are different from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's test.

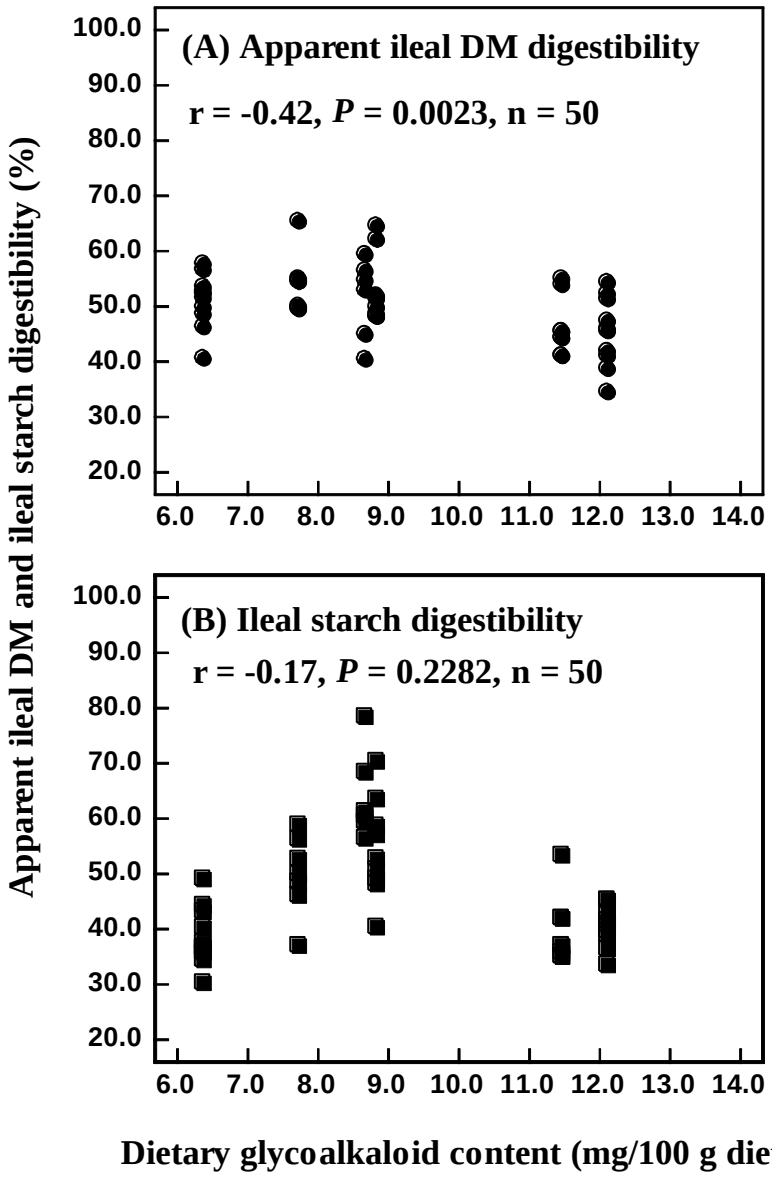

Figure 2.1. Pearson correlations between **(A)** the apparent ileal dry matter (DM), and **(B)** the ileal starch digestibility values (%) and total glycoalkaloid contents (mg/100 g diet, on an air-dry basis) in the six potato test diets for the growing pigs fed a high-fat basal diet.

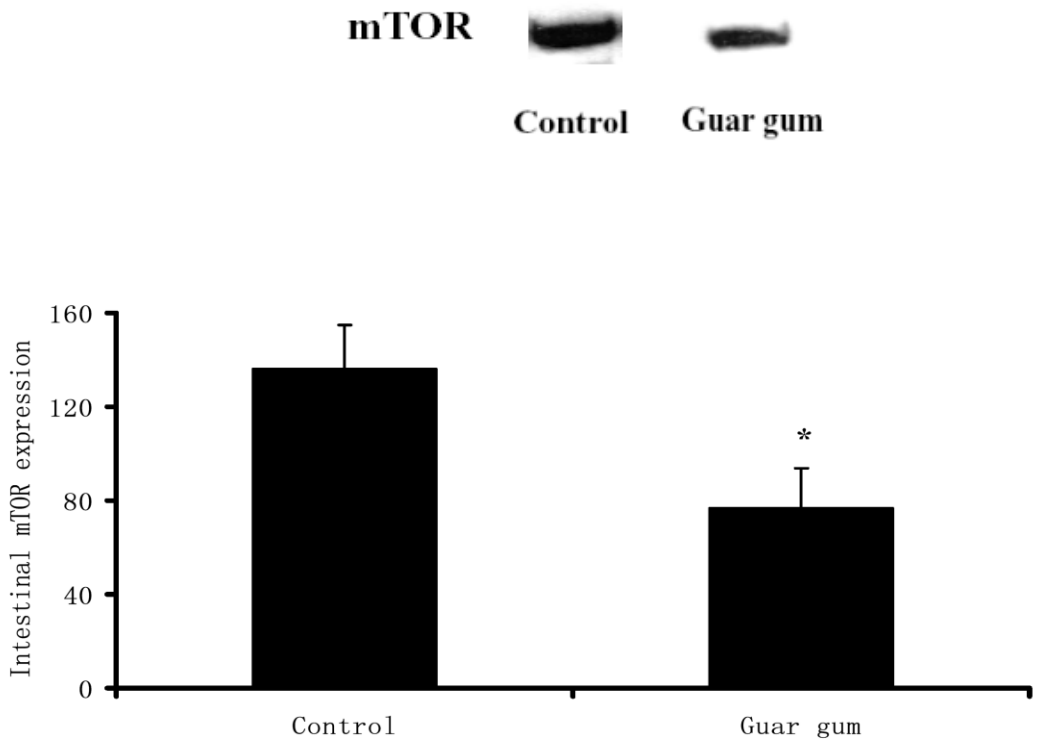

Figure 2.2. Effects of dietary supplementation of 10% guar gum (the PC diet vs. the NC diet) on expression (in arbitrary units) of phosphorylated mammalian target of rapamycin (**mTOR**) [mTOR (Ser 2448)] in the proximal jejnum of growing pigs fed a high-fat basal diet. Values are represented as mean ± SE (n = 7 and 5 for control and the guar gum group, respectively). *Indicates a difference ($P < 0.05$) between the 10%-PC guar gum group and the NC control compared by using the Dunnett's test.

CHAPTER 3

RESPONSES OF DIETARY APPARENT ILEAL AMINO ACID DIGESTIBILITY TO CONSUMPTION OF CONVENTIONAL FIBRES AND DIFFERENT GENOTYPES OF POTATOES IN GROWING PIGS FED A HIGH-FAT BASAL DIET[1]

3.1. ABSTRACT

While dietary fibre components are well recognized for nutritional management of human health issues, fibre is also known to be one of the dietary factors potentially affecting digestive utilization of dietary proteins. As a staple food, potato may be a significant dietary fibre source. The objective of this study was to examine effects of dietary supplementation of two exogenous conventional fibre components (i.e., guar gum and cellulose) in combination with intrinsic fibres from six potato genotype samples that differed in total dietary fibre (**TDF**) and soluble fibre content on the apparent ileal amino acid (**AA**) digestibility in growing pigs fed a high-fat basal diet. The basal diet was formulated as a zero-fibre negative control (NC) to contain 41.5% poultry meal, 4% casein, 15% animal fat-oil blend, 4.31% sucrose, 31% cornstarch, 0.50% salt and 0.40% trace mineral-vitamin premix with crude fat contributing to 37% of the dietary gross energy. The two exogenous fibre diets were formulated by respectively diluting the NC basal diet with 10% guar gum and 10% cellulose at the expense of cornstarch. Six other test diets were formulated by diluting the basal diet with 25.1% of one of the six genotypes of cooked and dehydrated potato tuber powder to contain about 10% TDF in combination with 8.5% guar gum at the expense of cornstarch. Titanium oxide was included (0.30%) as a digestibility marker. A total of 81 barrows, with an average initial body weight of about 25 kg, were fitted with a simple T-cannula in the distal ileum and fed the diets according to a completely randomized block design with each block lasting 28 d. Total AA content in samples was analyzed by gas chromatography-mass spectrometry. Compared with the NC, the ileal digestibility values of Ala, Gly, and Pro were decreased ($P < 0.05$) by 10% of guar gum supplementation, while the digestibility of Gly was reduced ($P < 0.05$) by 10% of cellulose supplementation. The ileal digestibility of several AA, including Ala, Glu, Gly, Leu, Lys, Phe and Pro, were decreased ($P < 0.05$) by the test potatoes plus 8.5% guar gum compared with the NC. Our results suggest that dietary inclusion of fibres at about 10% from guar gum, cellulose and intrinsic fibres contributed by potatoes may adversely affect the apparent ileal digestibility of some AA in growing pigs fed a high-fat basal diet.

Key words: amino acids, dietary fibre, digestibility, growing pigs, potato genotypes

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3.2. INTRODUCTION

The potato has a long history of being valued as a staple food, contributing carbohydrate calories to diets. Nowadays, potatoes play a critical role in fighting hunger problems in many countries due to a tremendous yield per unit arable land compared with many other food crops (Nah and Chau, 2010). Almost all the potato crops harvested are sold on the fresh vegetable market, or are further processed into fries and chips, potato starch and other products. There are growing human health concerns regarding obesity mainly in industrial countries, which may be attributed to over consumption of foods containing potatoes and processed potato products currently available on the market characterized as being rich in their highly digestible starch but relatively low in fibre content (Mozaffarian et al., 2011).

Consumption of dietary fibre especially soluble fibre components is recognized for their potential health benefits (Champ et al., 2003), for example, to attenuate hyperglycemic responses (Blackwood et al., 2000; Brennan, 2005; Chutkan et al., 2012). Major types of fibre components identified in potatoes include cellulose, hemicelluloses and pectin, which are primarily distributed in potato cell wall materials (Salvador et al., 2000). Resistant starch has also been identified as a major soluble fibre component in potatoes (Elsmstähl, 2002). Cultivar genotypes as well as cooking and processing conditions are known to affect resistant starch content in potatoes (Mulinacci et al., 2008). Contents of both soluble and insoluble fibre components are affected by potato cultivars (Gumul et al., 2011). Thus, development of new potato cultivars high in non-viscous soluble fibre (i.e., resistant starch) content provides a relatively inexpensive source of human dietary fibre. Resistant starch is an effective prebiotic (Gibson et al., 2004). Long-term ingestion of resistant potato starch was shown to enhance butyrate production in the large intestine in rats (Le Blay et al., 1999). Meanwhile, potato resistant starch is supplemented as a prebiotic in weanling pig nutrition (Bhandari et al., 2009), and as a modulator of hindgut fermentation to control odor and off-flavor of pork in swine production (Lösel et al., 2006).

On the other hand, fibres especially viscous soluble fibre components are also known to be one of the dietary antinutritive factors potentially affecting digestive utilization of dietary proteins (e.g., Mosenthin et al., 1994). Potato cultivars that are high in fibre content may also be associated with low protein and amino acid (**AA**) digestibility, which is referred to as resistant proteins (Morita et al., 1999; 2004). Plant sources of resistant proteins are shown to elucidate beneficial effects on the gut by further enhancing microbial butyrate production (Morita et al., 1999; 2004). However, several lines of evidence also indicate that some key potential pathogenic bacterial species are predominantly protein-fermenters (Bauer et al., 2006). Dietary CP and AA are the major precursors for biogenesis of volatile off-flavor and odor-causing compounds (Fan, 2013b). The effects of isolated exogenous fibre components on dietary nutrient utilization were examined in growing pigs fed a high-fat basal diet (Rideout et al., 2008b). Under this context the objective of this study was to examine effects of dietary supplementation of two conventional fibre components (i.e., guar gum and cellulose) and intrinsic fibres further contributed by six test potato cultivar samples that differed in TDF and soluble fibre content on the apparent ileal AA digestibility in growing pigs fed a high-fat basal diet.

3.3. MATERIALS AND METHODS

3.3.1. Animals, Diets and Experimental Design

All the animal experiments were conducted with protocols approved by the University of Guelph Animal Care Committee. The pigs used in this experiment were cared for in accordance with guideline established by the Canadian Council of Animal Care. Yorkshire growing barrows, with initial body weight (**BW**) of 25.0 ± 0.2 kg (n = 81), were housed in an environmentally controlled room (23°C). These barrows were randomly assigned into individual stainless-steel metabolic crates (height, 85 cm; length, 155 cm; width, 90 cm). The basal diet was formulated as the negative control (**NC**) diet that contained no fibre, 41.5% poultry meal, 4% casein, 15% animal fat-oil blend, 4.31% sucrose, 30.79% cornstarch, 0.50% ionized salt, and 0.40% of the commercial trace mineral and vitamin premix (DSM Nutritional Products Canada, Inc., Ayr, ON, Canada) with crude fat contributing to about 37% of the dietary gross energy (**GE**) (**TABLE 3.1**). The two exogenous fibre diets were formulated by diluting the NC basal diet with 10% guar gum and 10% cellulose, respectively, at the expense of cornstarch. The 10% guar gum supplemented diet was also referred to as the positive control (**PC**). Six other test diets were formulated by including 8.5% guar gum and by further diluting the NC basal diet with 25.1% of one of the six test genotypes of cooked and dehydrated potato tuber powder to contain a target level of about 10% total dietary fibre **(TDF**) at the expense of cornstarch. The six test genotypes of potatoes were variable in TDF and soluble fibre contents, including FV12272-3 as Potato diet 1; F05035 as Potato diet 2; CV96044-3 as Potato diet 3; WV5475-1 as Potato diet 4; Atlantic (a commercial cultivar) as Potato diet 5; and F05081 as Potato diet 6 (**TABLE 3.1**). These potato cultivars were selected by their relatively higher TDF contents compared with other potato cultivars developed by the National Potato Breeding Program at the Agriculture and Agri-Food Canada Potato Research Centre (Fredericton, NB, Canada). These six genotypes of potatoes were grown under the same conditions at the University of Guelph Elora Research Station near Elora, ON, Canada. About 2 to 2.5 tons of fresh potatoes were harvested for each of the six genotypes of potato samples, washed, diced, and thermal-dehydrated (without causing browning effects, as described in details in Chapter 2) before further grinding for diet incorporation at the University of Guelph Arkell Research station feed mill. Crystalline L-Ala was included in the NC, PC and cellullose diets to ensure that the test diets were isonitrogenous. Titanium oxide was included (0.30%) as a digestibility marker. Pigs were fed the experimental diets according to a completely randomized block design for up to 9 blocks in order to obtain 9 replicates for each of the test diets according to our previously established protocols (Rideout et al., 2008).

3.3.2. Sample Collection and Processing

Each experimental block lasted 4 wk, comprising of the first 7-d period during which the pigs were adapted to the environment and research staff, and were fed their respective diets at 0900 and 1600 h at close to an *ad libitum* intake level with a feeding regime as described in detail in Chapter 2. During the d 8-12 of each experimental block, the pigs were surgically fitted with a

distal ileal T-cannula for collection of digesta samples (Sauer et al., 1983). During a post-surgical recovery period (d 13-24), the pigs gradually resumed their previous *ad libitum* feeding level from a restricted feed intake at 50 g/d on the 2nd post-surgery. On d 25-28 of the experimental block period, the distal ileal digsta effluent samples were collected from 0800 to 1800 h by attaching a plastic bag containing 5 ml of 5% formic acid solution (Fan et al., 1994). The plastic digesta collection bag was inspected at 30-min intervals and changed immediately as needed.

Upon harvesting the six test fresh potatoes from the research farm, representative potatoes were washed and samples were cooked by boiling before freeze-drying. Changes in the weights of the processed potato samples were recorded for calculating free water content in these potato samples. At the conclusion of the study, collected ileal digesta samples were freeze-dried. Dried digesta and the test potato samples as well as the test diet samples were ground to be homogeneous in a Wiley mill though a 1-mm mesh screen, and well mixed before weighing out for further analyses.

3.3.3. Chemical Analyses

Dry matter (**DM**) content in the processed dietary and digesta samples was analysed according to an Association of Official Analytical Chemists (**AOAC**) (1993) Method. Content of TDF in the test potato samples were analysed with a commercial kit (Megazyme International Ltd., Co. Wicklow, Ireland) according to the AOAC procedures (Prosky et al., 1988; Lee et al., 1992; Prosky et al., 1992). Neutral-detergent fibre **(NDF**) in the test potato samples was measured according to AOAC (1984) in an Ankom fibre analyzer (Ankom Technology, Macedon, NY). Crude protein (**CP**) content (N ×6.25) in the test potato samples was analyzed according to the combustion method (Dumas Procedure) on a Leco FP-428 nitrogen analyzer (Leco corporation, St. Joseph, MI). Glycoalkaloids in the homogenized test potato samples were measured by HPLC via commercial service at the Alberta Agriculture and Rural development Laboratory. Briefly, α-solanine and α-chaconine levels were analyzed by following the AOAC method 997.13 modified for dry samples (AOAC 1997; Iablokov et al., 2010). Glycoalkaloids in these test potatoes, on the fresh basis, were calculated by considering the measured free water content in these potatoes. The digestibility marker titanium oxide content in the diet and digesta samples was analyzed by following the procedure of Leone (1973) and Myers et al. (2004). Resulting absorbance for this analysis was measured at 410 nm by using an epoch microplate-spectrophotometer (BioTek, Winooski, VT).

For AA analysis, about 0.1 g of processed diet and digesta samples was hydrolyzed in duplicate in 3 ml of 6 mol/L HCL at 110°C for 24 h in screw-capped pyrex tubes purged with nitrogen gas to remove air from the headspace. Following the hydrolysis, the hydrolysed samples were centrifuged (2000 × *g* at 4°C for 20-min) and the supernatant was further cleaned on a cation-exchange column (Dowex 50 W × 8 H^+ - form; Bio-Rad) as previously described (Bregendahl et al., 2004). L-Norleucine was included as an internal standard for calculating amino acid concentrations in samples. Amino acids were analyzed as the n-propyl heptafluorobutyrate derivative by GC-MS (6890 GC – 5973 MS; Agilent Technologies, Canada Inc., Mississauga, ON, Canada) through monitoring the ion fragments of 91 (phenylalanine) 226

(glycine) 240 (alanine) 252 (glutamine + glutamate) 266 (proline) 268 (valine), 282 (norleucine, isoleucine, and leucine) 280 (lysine) 284 (asparagine + aspartate) (Bregendahl et al., 2004).

3.3.4. Calculations and Statistical Analyses

The apparent ileal AA digestibility values in the diets were calculated by the marker method (Fan et al., 1994). The apparent ileal AA digestibility values in the experimental diets were calculated according to the equation as follows:

$$D_D = 100\% - [(I_D \times A_I)/(A_D \times I_I)] \times 100\%$$

where D_D = apparent ileal AA digestibility in the assay diet (percentage), I_D = marker content in the assay diet (%, on an air-dry basis), A_I = AA content in ileal digesta (%, on an air-dry basis), A_D = AA content in the assay diet (%, on an-air-dry basis), and I_I = marker content in ileal digesta (%, on an air-dry basis).

The expriement was carried out according to a completely randomized block design with 10 blocks and a total of ninty pigs by using the following model:

$$y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}$$

Where μ is the general mean, T_i is dietary treatment effect, P_j is the block effect, and ε_{ij} is the experimental error. Dietary treatment was included as a fixed effect and block was regarded as a random effect with individual pigs being randomly allocated to their test pigs within each of the study blocks. Data were subjected to ANOVA and F tests according to the Proc mixed model of SAS (SAS Institute, Cary, NC) for a completely randomized block design. Due to losing some test pigs and missing endpoint values, least square means (**LSM**) were calculated for the major observation endpoints. Multiple comparisons among the dietary treatment LSM were conducted by using the Tukey-Krammer's test. The reason that the Tukey's test was chosen as a suitable test in comparison with other multiple comparsion tests such as the Duncan's test was due to the fact the Tukey's test is much stringent and it compares the means of every treatment to the means of every other treatment, that is, it applies simultaneously to the set of all pairwise comparisons. Data were expressed as LSM ± SE. Differences were considered to be significant for $P < 0.05$.

3.4. RESULTS

Free water content in the six test potatoes (potatoes 1 to 6) was determined as 76.86, 78.48, 75.41, 78.87, 78.30 and 77.81%, respectively. The content of total glycoalkaloids, including a-solanine and a-chaconine, in the cooked, dehydrated and homogenized six test potato samples (potatoes 1 to 6) was analyzed to be 25.40, 30.80, 48.30, 34.60, 35.20, and 45.70 mg/100 g samples, respectively, on an air-dry basis. Thus, content of total glycoalkaloids in the six test fresh potato samples (potatoes 1 to 6) was calculated to be at 5.88, 6.63, 11.88, 7.31, 7.64 and 10.14 mg/100 g samples, respectively, on a fresh basis.

On the other hand, CP content in the processed potato samples (potatoes 1 to 6) was determined be 11.95, 10.98, 11.81, 10.65, 10.99, and 9.85%, respectively, on the air-dry basis. The CP content of the experimental diets was formulated to be at about 33% (on air-dry basis). Thus, the

six test potatoes contributed about 33% to their corresponding diets in terms of total dietary CP content (**TABLE 3.1**). Dietary AA contents, as analyzed by GC-MS, were summarized in **TABLE 3.2**

The effects of exogenous fibres and 8.5% guar gum in combination with potato supplementations on the apparent ileal digestibility of AA measured in the diets are summarized and compared in **TABLE 3.3**. When compared with the NC, the ileal digestibility values of Ala, Gly, and Pro were decreased ($P < 0.05$) by 10% guar gum (PC), while the digestibility of Gly was also reduced ($P < 0.05$) by 10% cellulose supplementation. Potato supplementations in combination with 8.5% guar gum also decreased ($P < 0.05$) the ileal digestibility values of several AA compared with the NC diet likely due to the combined effects of 8.5% guar gum supplementation and intrinsic fibres contributed by the corresponding test potatoes. However, there were generally no significant differences in the ileal AA digestibility values between each of the potato test diets and the PC diet or the cellulose diet.

3.5. DISCUSSION

The primary objective of this study was to examine the effects of intermediate dietary levels of two exogenous fibre components and 8.5% guar gum plus additional intrinsic fibres further contributed by the six test genotypes of potatoes on the apparent ileal digestibility of AA in growing pigs fed a typical high-fat and high-animal protein "Western diet". As observed by Bregendahl et al. (2004), it was not possible to quantify a complete AA profile by the GC-MS available to our laboratory. Thus, several essential and non-essential AA endpoints were not measured in this study. Furthermore, it should be pointed out that there is very a large variability in the analyzed AA contents and their ieal digestibility values, for example, in Gly within each of the test diets, as reflected by their large SE values associated with the ileal digestibility values, in this study by using a GC-MS system in comparison with the previous studies (Fan et al., 1994; Fan and Sauer, 1995) that were conducted under similar animal research protocols by using a HPLC system for AA analyses. While GC-MS is advantageous in analyzing stable isotopic tracer AA enrichments *in vivo* protein synthesis studies (Bregendahl et al., 2004), it may be limited for quantitative AA analyses in studies such as an ileal AA digestibility study for the following factors intrinsic to a GC-MS system. Firstly, organic chemical derivatization of AA is essential for GC-MS analyses of AA and this process may be sensitive to laboratory conditions and various biological sample matrix backgrounds. Secondly, because of different structures, different AA may be derivatized with different efficacy, thus affecting the precision and accuracy of their analyses. Thirdly, chemically derivatized AA are volatile and evaporation of these volatile compounds during sample preparation and analyses are likely to be inevitable, becoming a major contributing factor to additional experimental errors. On the other hand, it is also noteworthy that dietary contents of the measured essential AA were much higher than the levels recommended for growing pigs by the NRC (1998). This is due to the fact that the basal diet of this study is specifically designed as a typical high-fat and high-animal protein "Western diet" by adapting from our previous study for studying human nutrition and metabolic disease concerns with growing pigs as a relevant large animal model (Rideout et al., 2007).

In typical swine nutrition research diets, dietary insoluble fibre NDF content can range 5-15% (Fan and Sauer, 2002). Dietary insoluble fibre NDF content can reach over 20% when high-fibre feed ingredients such as hulled-barley grains are used as the major energy feeds (Fan and Sauer, 2002). Although TDF concept has been recently introduced to swine nutrition (NRC, 2012), TDF and soluble fibre contents are rarely reported in swine nutrition studies in the present literature. In clinical human nutrition studies, TDF levels are usually expressed as gram of fibre intake per test subject per d (Rideout et al., 2008a). However, in most reported animal model studies for human nutrition and research, test fibre levels are usually expressed as % of test diets (Rideout et al., 2008a). Dietary fibre level of about 10% TDF is an intermediate level and is frequently reported to be effective in improving whole body health parameters such as blood cholesterol levels (Rideout et al., 2007; Rideout et al., 2008b). Thus, we have used the dietary TDF level targeted at about 10% for conducting this study.

Dietary supplementation of 10% guar gum considerably reduced ileal digestibility of some of the measured essential AA in this study. For example, in comparison with NC diet, the ileal Leu and Phe digestibility values were decreased by about 30 and 32 percentage units, respectively. However, these differences were not statistically significant due to the fact the large SE values were associated with these measurements. Nevertheless, the ileal digestibility of Ala, Gly and Pro was significantly decreased by 10% guar gum supplementation. In a similar pattern, dietary supplementation of 10% cellulose reduced most of the examined ieal AA digestibility values. However, most of these differences were not statistically significant due to relatively large SE associated with the endpoint measurements. The ileal digestibility of Gly was significantly reduced by 10% cellulose supplementation. The six test potato diets (potato 1 to potato 6) were designed to include 8.5% guar gum with additional intrinsic fibres contributed by the individual test cultivars of potatoes as staple foods for reaching the target intermediate TDF level at about 10%. The six test potato diets actually contained about 11% TDF higher than the NC diet and the cellulose diet by about 1% TDF. Potato diet 5 was slightly lower in TDF content due to the fact this was a conventional potato cultivar (Atlantic) that is currently available on the market. The other five test potatoes were relatively high in TDF content and were screened out as potentially new healthy high-fibre potato cultivars. In general, compared with the NC diet, dietary supplementations of the six test potatoes in combination with 8.5% guar gum decreased the apparent ileal AA digestibility values similar to the patterns observed with 10% guar gum and 10% cellulose supplementations. Compared with the endpoints in the NC diet, the decreases in the ileal digestibility values were only observed in some AA and were not consistent for the same AA among the test potatoes. Glycoalkaloids are a group of anti-nutritive factors that are known to adversely affect the structure and functions of gut mucosa (e.g., Iablokov et al., 2010). Although glycoalkaloids were detected in the six test potatoes in this study, their content (5.88-11.88 mg/100 g samples) on their fresh basis was far below the current cut-off safety limit of 20 mg/100 g fresh potato samples established by Health Canada and CFIA (e.g., Iablockov et al., 2010). Thus, the presence of the glycoalkaloids was not likely responsible for the reduction in the ileal AA digestibility in the six test potato diets compared with the NC diet. It should also be pointed out that potatoes contributed to about 33% of the total dietary CP contents in these potato test diets, thus intrinsic differences in protein quality and AA digestibility between the potato protein in potato diets and the additional proteins from poultry

meal and casein in the NC basal diet might have contributed to these differences in the concerned ileal AA digestibility values, as compared in **TABLE 3.3**.

These results of the ileal AA digestibility, as affected by exogenous fibres in combination with potato supplementations, are in general agreement with the adverse effect of fibre on the apparent ileal AA digestibility reported in the literature (Hove and King, 1979; Keim and Kies, 1979; Mosenthin et al., 1994; Lenis et al., 1996). However, Lien et al., (1996) did not observe changes in true ileal CP and AA digestibility values and ileal mucin output in human subjects with ileostomies when these subjects ingested multiple levels of soy fibre. As have been recognized as "abrasive" dietary components, dietary fibres can decrease the apparent ileal CP and AA digestibility values through enhancing the distal ileal endogenous CP and AA losses via increasing gut mucosal sloughing loss (Fan et al., 2006). Jakob et al. (2000) showed that dietary supplementation of 2% potato fibre tended to increase the exocrine pancreatic secretion in growing pigs. The basal distal ileal endogenous CP and AA losses are defined to be the estimated basal levels of these nutrient losses when pigs are fed semi-purified or purified diets that contains no abrasive components such as fibres and other anti-nutritive factors including tannins and legume protease inhibitors (Jansman et al., 2002; Stein et al., 2007). For example, as summarized by Jansman et al. (2002), the basal ileal endogenous outputs of CP, Leu and Lys were 11.82, 0.49 and 0.40 g/kg DM intake, respectively, in growing pigs. In comparison, the ileal endogenous outputs of CP, Leu and Lys in association with diets containing 5-15% insoluble fibre NDF were 14.71±1.11, 0.58±0.04 and 0.45 g/kg DM intake, respectively, in growing pigs (Fan and Sauer, 2002). Furthermore, the ileal endogenous outputs of CP, Leu and Lys in association with hulled-barley grain diets containing 16.8-23.8% insoluble fibre NDF contents were 35.1±3.0, 2.56±0.20 and 1.46±0.20 g/kg DM intake, respectively, in growing pigs (Fan and Sauer, 2002). Thus, dietary supplementations of the exogenous fibres and intrinsic fibres contributed by these test potatoes overall decreased the apparent ileal AA digestibility in the test diets and these effects were likely due to the enhanced the distal ileal outputs of the concerned endogenous AA by the fibres in this study.

On the other hand, it is interesting to note that the ileal AA digestibility values of the NC diet are generally very high, further suggesting that the basal dietary proteins are highly digestible. Inclusion of milk protein casein in the diet is at 4% and CP content of casein is approximately 89% (NRC, 1998). Although casein is known to very highly digestible, casein contribution to the NC diet is about 10%. Thus, poultry meal is the primary protein source of the NC diet and the measured ileal AA digestibility values of the NC diet reflect the ileal AA digestibility of the poultry meal by-product used in this study. In comparison, the apparent ileal AA digestibility values reported in poultry by-products in the literature are relatively low. For example, the apparent ileal digestibility values of Leu and Lys in the NC diet comprising primarily of poultry meal of this study were 86.3±8.5 and 90.2±8.3%, respectively. Knabe et al. (1989) reported that the apparent ileal digestibility values of Leu and Lys in poultry-by-product meal were 82.0±0.4 and 86.0±0.3%, respectively. NRC (1998) summarized the apparent ileal digestibility values of Leu and Lys in rendered poultry by-product meal were 78 and 78, respectively. Two factors might have contributed to this discrepancy in the ileal AA digestibility value ranges. Firstly, the poultry meal by-product used in this study was likely of much higher quality. Secondly, as mentioned earlier, the basal diet was designd to be a typical high-fat and high-animal protein "Western diet" with dietary CP and AA levels being much higher than NRC

recommended essential AA requirement levels for the growing pigs. Fan et al. (1994) demonstrated that the apparent ileal CP and AA digestibility values were dramatically affected by CP and AA levels in diets before these apparent digestibility values reached their corresponding plateau values. The plateau apparent ileal CP and AA digestibility values were close to their corresponding true ileal digestibility values because the ileal endogenous CP and AA contributing effects are minimal at their plateau measurements (Fan et al., 1995). Thus, the apparent ileal AA digestibility values of the NC diet were likely a reflection of their plateau apparent values and were close to their corresponding true digestibility values. These results further suggest that the basal NC diet is a relevant high-fat and high-animal protein "Western diet" with highly digestible dietary CP and AA for studying human nutrition and metabolic diseases in studies with the growing pig model.

In conclusion, dietary supplementations of intermediate levels of exogenous fibres and intrinsic fibres, as contributed from six different genotypes of potatoes as staple foods, might negatively affect the apparent ileal digestibility values of some AA in growing pigs fed the high-fat and high-animal protein basal diet. Our results suggest that the poultry meal by-product protein and AA used in our model "Western diet" are highly digestible.

Table 3. 1 Composition (%) of the experimental diets (on as-fed basis) fed to the growing pigs

	Experimental diets[1]								
Items	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (CV12272-3)	Potato 2 (F05035)	Potato 3 (CV96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
Poultry meal	41.50	41.50	41.50	41.50	41.50	41.50	41.50	41.50	41.50
Casein	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Animal fat-oil blend	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
DL-methionine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
L-Alanine[2]	2.81	2.81	2.81	0.00	0.00	0.00	0.00	0.00	0.00
Sucrose	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31
Cornstarch	30.79	20.79	20.79	0.00	0.00	0.00	0.00	0.00	0.00
Solka-Floc®[3]	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
Guar gum[4]	0.00	10.00	0.00	8.50	8.50	8.50	8.50	8.50	8.50
Potato-1	0.00	0.00	0.00	25.10	0.00	0.00	0.00	0.00	0.00
Potato-2	0.00	0.00	0.00	0.00	25.10	0.00	0.00	0.00	0.00
Potato-3	0.00	0.00	0.00	0.00	0.00	25.10	0.00	0.00	0.00
Potato-4	0.00	0.00	0.00	0.00	0.00	0.00	25.10	0.00	0.00
Potato-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.10	0.00
Potato-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.10
Min-Vit premix[5]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Iodized salt[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Titanium oxide[7]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Nutrient contents (on air-dry basis):									
Dry matter[8]	95.26	92.64	94.40	93.03	92.25	92.99	93.07	92.75	94.49
Crude fat[9]	20.26	20.26	20.26	20.67	20.67	20.67	20.67	20.67	20.67
Crude protein[10]	33.14	33.14	33.14	33.38	33.13	33.34	33.05	33.14	32.78
Glycoalkaloids[11]	0.00	0.00	0.00	6.38	7.73	12.12	8.68	8.84	11.47
Total dietary fibre[12]	0.00	10.00	10.00	11.15	11.13	10.90	10.82	10.46	10.99
NDF[13]	0.00	0.00	10.00	1.32	1.29	1.47	1.39	0.76	1.10
Total soluble fibre[14]	0.00	10.00	0.00	9.84	9.84	9.44	9.43	9.70	9.89
DE[15]	17.80	17.21	17.38	16.62	16.62	16.62	16.62	16.62	16.62
ME[16]	15.93	15.35	15.52	14.72	14.72	14.72	14.72	14.72	14.72
NE[17]	12.10	11.64	11.76	11.08	11.10	11.08	11.08	11.09	11.06
GE from Fat[18]	37.22	37.15	37.15	38.98	38.98	38.98	38.98	38.98	38.98

Note: [1] NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar CV96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as the basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[2]Evonik Industries AG, Hanau-Wolfgang, Germany.

[3]Solka-Floc®, pure cellulose commercially available from International Fibre Corporation (North Tonawanda, NY).

[4]Guar gum, food grade with an estimated average molecular weight of about 1.5 million and granulation of medium coarse commercially available from Nealanders International Inc. (Mississauga, ON, Canada).

[5]The vitamin and trace mineral premix supplied the following vitamins and trace minerals (IU or mg/kg diet): vitamin A, 8000 IU; vitamin D_3, 800 IU; vitamin E, 32 IU; vitamin K, 2 mg; vitamin $B_{12,}$ 0.02 mg; D-biotin, 0.16 mg; thiamine, 1.2 mg; riboflavin, 4 mg; D-pantothenic acid, 12 mg; pyridoxine, 1.2 mg; niacin, 20 mg; folic acid, 1.6 mg; choline, 400 mg; iron, 80 mg; copper, 12 mg; manganese, 16 mg; zinc, 84 mg; iodine, 0.4 mg and selenium, 0.24 mg.

[6]Supplied by the Windsor Salt Co. (Toronto, ON, Canada). Composition (g/kg): NaCl, 965.0; ZnO, 40.0; $FeCO_3$, 1.6; MnO, 1.2; CuO, 0.33; $Ca(IO_3)_2$, 0.07; and CaO, 0.04.

[7]Nutrient digestibility marker purchased from Fisher Scientific (Ottawa, ON, Canada).

[8]Analyzed dietary dry matter content, *%*.
[9]Crude fat content in diets, *%*, calculated by using crude fat contents in the concerned ingredients compiled by NRC (1998); and crude fat content in cooked and dried potatoes reviewed by Whittemore (1977).
[10]Crude protein content in diets, *%*, calculated by using crude protein contents in the concerned ingredients compiled in NRC (1998); and the analyzed crude protein contents in the cooked and dried test potato samples.
[11]Total glycoalkaloid content, including α-chaconine and α-solanine in diets, mg/100 g diet, calculated total dietary content based on the analyzed constituent contents in the cooked and dried test potato samples.
[12]Total dietary fibre (TDF) content in diets, *%*; calculated dietary content based on the analyzed TDF contents in the cooked and dried test potato samples and the TDF content in Solka-Floc® and guar gum being considered to be 100%.
[13]NDF, dietary neutral-detergent fibre content in diets, *%*; calculated contents based on the analyzed NDF contents in the cooked and dried test potato samples and the NDF content assumed to be 100% in Solka-Floc® and 0% in guar gum, respectively.
[14]Total soluble fibre content in diets, *%*, calculated content = total dietary fibre (TDF, *%*) – neutral-detergent fibre (NDF, *%*).
[15]DE, digestible energy in diets, *MJ/kg*; calculated by using DE content in the concerned ingredients compiled in NRC (1998); DE in cooked and dried potatoes reviewed by Whittemore (1977); and DE in poultry meals reported by Pesti et al. (1986).
[16]ME, metabolizable energy content in diets, *MJ/kg*, calculated by using ME content in the concerned ingredients compiled in NRC (1998); ME from cooked and dried test potatoes obtained by multiplying a correction factor of 0.96 according to NRC (1998) with DE in cooked and dried potatoes reviewed by Whittemore (1977); and ME content in poultry meals reported by Pesti et al. (1986).
[17]NE, net energy content in diets, *MJ/kg*; calculated according to the formula of NE (MJ/kg) = 0.00364 * ME (MJ/kg) – 1.8493, reported by Noblet et al. (1994) with corrections to be on the air-dry basis.
[18] Calculated by using GE of crude fat = 39.8 *MJ/kg* summarized by Brouwer (1965).

Table 3. 2 Analyzed total amino acid content[1] in the experimental diets fed to the growing pigs

Items	Experimental diets[2]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (CV96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
Essential AA									
Leu	2.08	2.16	1.80	2.24	2.02	1.54	2.24	2.21	2.37
Lys	1.75	1.55	1.81	1.41	1.48	2.17	2.13	2.161	1.87
Iso	1.65	1.73	1.70	1.69	1.60	1.86	1.89	1.82	1.86
Phe	1.05	0.95	1.33	1.04	1.01	1.18	1.05	0.98	1.06
Val	1.44	1.28	1.54	2.03	1.06	1.92	1.75	1.65	1.41
Nonessential AA									
Ala	4.28	4.08	4.24	4.68	5.12	4.77	4.42	4.68	4.44
Asp+Asn	2.04	2.18	2.53	2.39	2.27	2.87	2.28	2.45	2.73
Gln+Glu	2.71	2.63	2.78	2.84	1.24	2.37	1.83	2.45	2.57
Gly	1.89	1.25	0.98	2.03	0.84	3.51	2.59	1.92	1.07
Pro	1.99	1.91	2.11	2.13	2.05	1.53	2.30	2.06	2.74

Note: [1] Expressed as % on dry matter basis.

[2] Refer to Table 1 for details of the diet formulations. NC, the negative control diet (NC) without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar CV96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as the basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

Table 3. 3 Responses in the apparent ileal AA digestibility values[1] in the experimental diets fed to the growing pigs

Items	Experimental diets[2]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (CV96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
n[3]	7	9	8	10	7	10	8	9	8
Essential AA									
Leu	86.3±8.5	55.5±7.3	58.8±7.3	51.3±6.5	55.3±6.9	49.0±6.5*	63.5±7.3	46.5±6.5*	52.1±8.5
Lys	90.2±8.3^{a}	76.8±7.3^{ab}	82.9±7.3^{ab}	66.9±6.7^{b*}	75.4±7.0^{ab}	77.0±6.7^{ab}	74.5±7.3^{ab}	70.8±6.7^{b*}	78.5±8.0^{ab}
Iso	80.3±5.6	73.1±4.8	80.2±4.8	74.3±4.3	72.5±4.5	73.2±4.3	82.2±4.8	79.0±4.3	84.0±5.6
Phe	89.4±9.4^{a}	56.9±5.8^{ab}	77.9±8.5^{a}	51.3±8.0^{*b}	58.5±8.2^{ab}	52.8±8.0^{b*}	63.9±8.5^{ab}	48.9±8.0^{b*}	51.1±9.4^{b*}
Val	81.5±5.7	72.6±5.0	78.8±5.0	77.7±4.5	67.7±4.6	74.6±4.5	81.3±5.0	76.2±4.5	81.6±5.7
Nonessential AA									
Ala	95.7±1.9	90.2±1.8*	92.4±1.8	90.3±1.7*	92.9±1.7	89.4±1.7*	91.4±1.8	90.5±1.7*	90.1±1.9*
Asp+Asn	85.5±4.0	82.0±3.6	84.4±3.6	79.9±3.3	84.3±3.5	83.0±3.3	83.7±3.6	83.6±3.3	83.8±4.0
Gln+Glu	88.1±7.0^{a}	75.1±6.4^{ab}	80.7±6.4^{ab}	71.4±6.0^{ab}	49.5±6.1^{c*}	64.0±6.0^{bc*}	64.5±6.4^{bc*}	70.2±6.0^{ab*}	71.9±7.0^{ab}
Gly	90.6±6.2^{a}	62.1±5.5^{cd*}	47.9±5.5^{d*}	76.7±5.1^{ab}	55.9±5.3^{d*}	83.1±5.1^{ab}	83.9±5.5^{ab}	75.3±5.1^{abc}	73.4±6.2^{abc}
Pro	98.1±5.9^{a}	73.3±5.2^{b*}	82.1±5.2^{ab}	79.7±4.9^{ab*}	83.1±5.0^{ab}	73.8±4.9^{b*}	80.1±5.2^{ab*}	76.5±4.9^{b*}	86.7±5.1^{ab}

Note: [1] Values (%) are least square mean ± SE of the estimates.

[2] Refer to Table 1 for details of the diet formulations. NC, the negative control diets without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar CV96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3] Number of observations.

[a,b,c,d]Means diets with different superscript letters differ ($P < 0.05$) among the diets as analyzed by the Tukey-Kramer's tests.

*Difference from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

CHAPTER 4

MODULATION OF CALCIUM, PHOSPHORUS AND MAGNESIUM DIGESTIBILITY BY EXOGENOUS FIBRES AND DIFFERENT GENOTYPES OF POTATOES IN GROWING PIGS FED A HIGH-FAT BASAL DIET

4.1. ABSTRACT

The roles of exogenous fibres and novel genotypes of potatoes high in resistant starch in affecting major bone macro-mineral absorption need to be elucidated. The objectives of this study were to examine effects dietary supplementations of two exogenous fibres of guar gum and cellulose and guar gum in combination with thermo-processed six genotypes of potatoes on the apparent ileal and fecal Ca, P and Mg digestibility values in growing pigs fed a high-fat basal diet. The basal diet was formulated as a zero-fibre negative control (NC) to contain 42% poultry meal, 4% casein, 15% animal fat-oil blend, 4% sucrose, 31% cornstarch, 0.5% salt and 0.4% trace mineral-vitamin premix with crude fat contributing to 37% of the dietary gross energy. The two exogenous fibre diets were formulated by diluting the NC basal diet with 10% guar gum and 10% cellulose at the expense of cornstarch, respectively. Six other potato test diets were formulated by diluting the basal diet with 25.1% of one of the six different genotypes of potatoes to contain about 10% total dietary fibre in combination with 8.5% guar gum at the expense of cornstarch. Titanium oxide was included in the diets (0.3%) as a digestibility marker. A total of 90 growing barrows, with an average initial body weight of 25 kg, were surgically fitted with a simple T-cannula in the distal ileum and fed the diets according to a completely randomized block design with each block lasting 28 d. Dietary supplementations of guar gum and cellulose at 10% did not affect ($P > 0.05$) the apparent fecal Ca and Mg digestibility, whereas guar gum at 10% reduced ($P < 0.05$) the apparent fecal P digestibility. Pearson correlation analyses showed that the apparent ileal and fecal Ca and P digestibility values were inversely related ($P < 0.05$) to total contents of glycoalkaloids in the potato diets. In conclusion, exogenous fibres of guar gum and cellulose at 10% did not affect the apparent fecal Ca and Mg digestibility values. However, the guar gum supplementation at 10% significantly decreased the apparent fecal P digestibility values in growing pigs fed a high-fat basal diet. The presence of variable levels of potato glycoalkaloids within the government regulatory safety limit was negatively linked to dietary Ca and P digestibility. Thus, novel genotypes of potatoes selected for promoting human health should have minimal levels of glycoalkaloids to prevent the potential adverse effects of potato glycoalkaloids on intestinal Ca and P absorption and bone health.

Key words: bone macro-mineral digestibility, fibres, growing pigs, potato genotyes

4.2. INTRODUCTION

Low fibre and high intake of digestible starch and sugar-based, animal protein-rich high-fat "Western diets", as indicated by high glycemic index and glycemic load, induces chronic postpradial hyperglycemia and is associated with the incidence and progression of type-II diabetes mellitus (Mckeown et al., 2004; Vrolix et al., 2008; Mohan et al., 2009), mortality risk of diabetes mellitus (Burger et al., 2012), obesity (Ford and Frost, 2010; Mozaffarian et al.,

2011), cardiovascular disease risk factors (Amano et al., 2004) and cancers (Bruce et al., 2000; Fedirko et al., 2013). Under this context, potatoes and processed potato products as common staple foods contribute significantly to highly digestible dietary starch, glycemic load and some of the major health concerns (Schweizer et al., 1990; Schulz et al., 2005; Mazaffarian et al., 2011). Increased consumption of dietary fibres, especially soluble fibres, has been well documented to attenuate the aforementioned metabolic syndromes and fatal diseases (Blackwood et al., 2000; Chutkan et al., 2012).

Despite literature reports of studies in both human subjects and animal models (Coudray et al., 1997; Weaver et al., 2010), the relevance of fibre consumption to the digestive utilization of the macro-minerals important to bone formation and human bone health needs to be addressed for following uncertainties. Firstly, although calcium (**Ca**), phosphorus (**P**) and magnesium (**Mg**) are the recognized major macro-minerals in bone formation (Allgrove, 2009), effects of dietary fibres on dietary mineral availability have focused much more on Ca and less on P and Mg in both human and animal studies (Coudray et al., 1997; Weaver et al., 2010). Secondly, majority of the relevant animal studies are conducted with rodents (Hara et al., 1996; Weaver et al., 2010). Bach Knudsen et al. (1994) showed that rats appeared to have a lower capacity to digest fibre polysaccharides than humans. Guilloteau et al. (2010) concluded that pigs are a relevant animal model to study nutritional programming of the gastrointestinal tract development and functions for humans. Thirdly, a relevant typical Western basal diet needs to be used in such studies in which not only types dietary fibres but also adequate levels of dietary fibres should be considered. In clinical human nutrition studies, fibre intake levels are usually expressed as gram of fiber intake per test subject on per unit of body weight per day basis (Hannan et al., 2007; Rideout et al., 2008a; Brand-Miller et al., 2012). However, in most reported animal model studies for human nutrition research, test fibre levels are usually expressed as % of test diets (Rideout et al., 2008a). An intermediate dietary fibre level at about 10% is frequently reported to be effective in improving whole body nutrition and health parameter endpoints (Poksay and Schneeman, 1983; Rideout et al., 2007), thus this dietary fibre level should be used to examine the dietary effect of fibres on mineral availability.

Resistant starch has emerged to be an important non-viscous soluble fibre with well-documented prebiotic effects (Gibson et al., 2004; Scott et al., 2011). Apart from the traditional fibre components identified in potatoes including cellulose, hemicellulose and pectin that are primarily distributed in potato cell wall materials (Salvador et al., 2000), resistant starch has also been identified as a major soluble fibre component in potatoes (Elsmstähl, 2002). Cultivar genotypes as well as cooking and processing conditions are shown to affect resistant starch and the conventional fibre components in potatoes (Mulinacci et al., 2008; Gumul et al., 2011). Bath et al. (2013) have recently identified some new potato genotypes that were high in resistant starch content. On the other hand, glycoalkaloids are well documented to be a group of anti-nutritive factors in potatoes (Friedman, 2006). Contents of glycoalkaloids normally detected within the government regulatory level in consumed potato products were shown to adversely affect the mammalian intestinal permeability and can aggravate inflammatory bowel diseases (Patel et al., 2002). Thus, how normally allowed variable contents of potato glycoalkaloids affect mineral digestibility when novel cultivars of potatoes are included in diets to provide extra dietary fibre components needs to be investigated.

We have developed a high animal protein and high-fat basal diet that has no fibre and contains digestible starch and D-fructose supplied as sucrose in studies with growing pigs (Rideout et al., 2007). Using this basal diet and pigs as a relevant animal model, we have examined how various dietary fibre components influenced cholesterol metabolism, nutrient utilization and colonic adaptive immune responses (Rideout et al., 2007, 2008b; Fan et al., 2012). Therefore, the objectives of this study were to investigate the effects of dietary inclusion of exogenous fibres of guar gum and cellulose at 10% as well as 8.5% guar gum in combination with six different genotypes of potatoes on the apparent ileal and fecal Ca, P, and Mg digestibility in growing pigs fed the high-fat basal diet measured at the pre-cecal and the fecal levels.

4.3. MATERIALS AND METHODS

4.3.1. Animals, Diets and Experimental Design

All the animal experiments were conducted with protocols approved by the University of Guelph Animal Care Committee. The pigs used in this experiment were cared for in accordance with guideline established by the Canadian Council of Animal Care. Yorkshire growing barrows, with initial body weight (**BW**) of 25.0 ± 0.2 kg (n = 90), were housed in an environmentally controlled room (23°C). The barrows were randomly assigned to individual stainless-steel metabolic crates (height, 85 cm; length, 155 cm; width, 90 cm). The high animal protein and high-fat "Western basal diet" was formulated as a negative control (**NC**) diet that contained no fibre, 41.5% poultry meal, 4% casein, 15% animal fat-oil blend, 4.31% sucrose, 30.79% cornstarch, 0.50% ionized salt, and 0.40% of the commercial trace mineral and vitamin premix (DSM Nutritional Products Canada, Inc., Ayr, ON, Canada) with crude fat contributing to about 37% of the dietary gross energy (**GE**) (**TABLE 4.1**), which was adapted from our previous studies (Rideout et al., 2007). The reason for inclusion of sucrose in the basal diet is that sucrose is a common source of D-fructose in typical "Western" diets. D-Fructose has been recognized as a signalling nutrient and is associated with the development of numerous diseases and metabolic syndromes such as obesity, renal and cardiomyocyte function, hypertension and vitamin D activation (Douard and Ferraris, 2013). Crystalline L-Ala was supplemented to the NC and the two exogenous fibre diets, thus all experimental diets were formulated to be isonitrogenous. Crystalline D,L-Met was supplemented to all the diets to ensure sufficient Met supply by the diets. The two exogenous fibre diets were formulated by diluting the NC basal diet with 10% guar gum and 10% cellulose, respectively, at the expense of cornstarch. The 10% guar gum supplemented diet was also referred to as the positive control (**PC**). Six other test diets were formulated by including 8.5% guar gum and by further diluting the NC basal diet with 25.1% of one of the six test genotypes of cooked and dehydrated potato tuber powder to contain a target level of about 10% total dietary fibre **(TDF**) at the expense of cornstarch. The six test genotypes of potatoes were variable in TDF and soluble fibre contents, including FV12272-3 as Potato diet 1; F05035 as Potato diet 2; CV96044-3 as Potato diet 3; WV5475-1 as Potato diet 4; Atlantic (a commercial cultivar) as Potato diet 5; and F05081 as Potato diet 6 (**TABLE 4.1**). These potato genotypes were selected by their relatively higher TDF contents compared with other potato cultivars developed by the National Potato Breeding Program at the Agriculture and Agri-Food Canada Potato Research Centre (Fredericton, NB, Canada). These six genotypes of potatoes were grown under the same conditions at the University of Guelph Elora Research

Station near Elora, ON, Canada. About 2 to 2.5 tons of fresh potatoes were harvested for each of the six potato cultivar-genotype samples, washed, diced, and thermal-dehydrated at about 200°C for about 30 min (without causing browning effects) via a standard commercial type of belt-drying food processing system at the atmospheric pressure. The dried potatoe cubes were further ground to be homogenous flours for diet incorporation at the University of Guelph Arkell Research station feed mill. Titanium oxide was included (0.30%) as a digestibility marker. Pigs were fed the experimental diets according to a completely randomized block design for up to 10 blocks in order to obtain 10 replicates for each of the test diets according to our previously established protocols (Rideout et al., 2008a).

4.3.2. Sample Collection and Processing

Each experimental block lasted 4 wk, comprising of the first 7-d period during which the pigs were adapted to the environment and research staff. The pigs were fed the same amount of their respective diets at 0900 and 1600 h at close to an *ad libitum* level (3% of their average BW) for the high-fat basal diet, which was established by Rideout et al. (2007). The *ad libitum* feed level for this high-fat diet was much lower than the feed intake level (5-6% of average BW) of grower pigs fed typical corn and soybean meal-based commercial swine diets as compiled by NRC (1998). During the d 8-12 of each experimental block the pigs were surgically fitted with a distal ileal T-cannula (Sauer et al., 1983). During a post-surgical recovery period (d 13-24), the pigs gradually resumed their previous *ad libitum* feeding level from a restricted feed intake at 50 g/d on the 2nd post-surgery. On d 25-28 of the experimental block period, representative fresh fecal samples were collected and the distal ileal digesta effluent samples were collected from 0800 to 1800 h by attaching a plastic bag containing 5 ml of 5% formic acid solution (Fan et al., 1994). The plastic digesta collection bag was inspected at 30-min intervals and changed immediately as needed. However, some pigs were lost largely due to complications after the recovery surgical procedures, resulting in missing data for some of the dietary groups.

Upon harvesting the six test fresh potatoes from the research farm, representative potatoes were washed and samples were cooked by boiling before freeze-drying. Changes in the weights of the processed potato samples were recorded for calculating free water content in these potato samples. At the conclusion of the study, collected fecal and ileal digesta samples were freeze-dried. Dried fecal and ileal digesta and the test potato samples as well as the test diet samples were ground to be homogeneous in a Wiley mill though a 0.1-mm mesh screen. The ground samples were stored in sealed zip block plastic bags in a cold room (4°C) and were well mixed before weighing out for further analyses.

4.3.3. Chemical Analyses

Dry matter (**DM**) content in the processed dietary, digesta and fecal samples was analysed according to an Association of Official Analytical Chemists (**AOAC**) (1993) Method. Content of TDF in the test potato samples was analysed with a commercial kit (Megazyme International Ltd. Co. Wicklow, Ireland) according to the AOAC procedures (Prosky et al., 1988; Lee et al., 1992; Prosky et al., 1992). Neutral-detergent fibre **(NDF**) content in the test potato samples

was measured according to AOAC (1984) in an Ankom fibre analyzer (Ankom Technology, Macedon, NY). Crude protein (**CP**) content (N ×6.25) in the test potato samples was analyzed according to the combustion method (Dumas Procedure) on a Leco FP-428 nitrogen analyzer (Leco corporation, St. Joseph, MI). Glycoalkaloids in the homogenized test potato samples were mesasured by HPLC via commercial service at Alberta Agriculture and Rural development Laboratory. Briefly, α-solanine and α-chaconine levels were analyzed by following the AOAC method 997.13 modified for dry samples (AOAC 1997; Iablokov et al., 2010). Glycoalkaloids in these test potatoes, on the fresh basis, were calculated by considering the measured free water content in these potatoes. The digestibility marker titanium oxide content in the diet, digesta and fecal samples was analyzed by following the procedure of Leone (1973) and Myers et al. (2004). Resulting absorbance for this analysis was measured at 410 nm by using an epoch microplate-spectrophotometer (BioTek, Winooski, VT). For Ca, P, and Mg analyses, samples about 0.2 g of the diets, digesta and feces were ashed in pyrex glass test tubes at 550°C for 12 h in a muffle furnace, then solubilized with 3 ml 6 M HCL and severeal drops of concentrated HNO_3 on a heating block and resulting ash was incubated at 110°C overnight. Solubilized minerals were quantitatively transferred into 50-ml volumetric flask and supernatant samples were sampled and stored for further analyses. Total inorganic phosphorus in the sample solutions was analyzed by spectrophotometric analysis (Fan et al., 2001). Total Ca and Mg contents in the sample solution were determined with further appropriate sample dilutions by using an atomic absorption spectrometer (SpectrAA-10/20; Varian, Mulgrave, Australia). The wavelengths for Ca (422.7 nm), Mg (202.6 nm) P (355.2 nm) were used with bandwidths of 0.5, 0.5 and 0.2 nm for Ca, Mg, P respectively.

4.3.4. Calculations and Statistical Analyses

The apparent ileal and fecal mineral digestibility values in the diets were calculated by the marker method (Fan et al., 1994). The apparent ileal and fecal mineral digestibility values in the experimental diets were calculated according to the equation as follows:

$$D_D = 100\% - [(I_D \times M_I)/(M_D \times I_I)] \times 100\%$$

where D_D = apparent ileal and fecal mineral digestibility in the assay diet (percentage), I_D = marker content in the assay diet (%, on an air-dry basis), S_I = mineral content in ileal digesta (%, on an air-dry basis), S_D = mineral content in the assay diet (%, on an-air-dry basis), and I_I = marker content in ileal digesta (%, on an air-dry basis).

The expriement was carried out according to a completely randomized block design with 10 blocks and a total of ninty pigs by using the following model:

$$y_{ij} = \mu + T_i + B_j + \varepsilon_{ij}$$

Where μ is the general mean, T_i is dietary treatment effect, P_j is the block effect, and ε_{ij} is the experimental error. Dietary treatment was included as a fixed effect and block was regarded as a random effect with individual pigs being randomly allocated to their test pigs within each of the study blocks. Data were subjected to ANOVA and F tests according to the Proc mixed model of SAS (SAS Institute, Cary, NC) for a completely randomized block design. Due to losing some test pigs and missing endpoint values, least square means (**LSM**) were calculated for the major observation endpoints. Multiple comparisons among the dietary treatment LSM were conducted by using the Tukey-Krammer's test. The reason that the Tukey's test was chosen

as a suitable test in comparison with other multiple comparsion tests such as the Duncan's test was due to the fact the Tukey's test is much stringent and it compares the means of every treatment to the means of every other treatment, that is, it applies simultaneously to the set of all pairwise comparisons. Data were expressed as LSM ± SE. Differences were considered to be significant for $P < 0.05$.

4.4. RESULTS

As shown in **TABLE 4.1**, except the test potato samples, all other ingredients in the experimental diets were commercially available and had no free H_2O content. The test potatoes were thermo-processed, dehydrated to be on an air-dry basis and ground to be homogenous before diet mixing. DM content in the experimental diets was analyzed to range from 92 to 95%, further suggesting that all the diets were essentially on an air-dry basis. Calculated crude fat content was at 20.3% (on as-fed basis) for the NC and the exogenous fibre-supplemented diets but was a little higher at 20.7% (on as-fed basis) for the six potato test diets due to the fact that the test potatoes included at 25.1% in the diets contributed to additional crude fat to the test diets (**TABLE 4.1**). Thus, contribution of crude fat to dietary GE was at 37.2% compared with 39.0% in the six test potato diets. Commercially available L-Ala was supplemented to the NC and the exogenous fibre-supplemented diets, thus all experimental diets were formulated to be isonitrogenous. The PC and the Cellulose diets were formulated to contain 10% TDF by using guar gum and cellulose, respectively, whereas TDF in the six test potato diets was at about 11% due to extra TDF contributed from the respective potatoes. Based on the analyzed free H_2O content in the six fresh test potatoes and the content of total glycoalkaloids, including α-solanine and α-chaconine, in the cooked, dehydrated and homogenized six test potato samples, total glycoalkaloids in the six test potato samples (potatoes 1 to 6) were calculated to be at 5.88, 6.63,11.88, 7.31, 7.64, and 10.14 mg/100 g samples, respectively, on their fresh basis. Furthermore, the total glycoalkaloids in the six test potato diets (potato diets 1 to 6) were calculated to be at 6.38, 7.73, 12.12, 8.68, 8.84, and 11.47 mg/100 g diets, respectively (**TABLE 4.1**). Total dietary Ca content was similar among the diets, ranging from 1.5 to 1.7%, on as-fed basis. Total dietary P content was also similar among the diets, varying from 0.9 to 1.0%, on as-fed basis. Dietary total Ca to total P ratio was also very similar among the diets, ranging from 1.5 to 1.8. Furthermore, total dietary Mg content was similar among the diets, varying from 0.8 to 1.0%, on as-fed basis (**TABLE 4.1**).

As shown in **TABLE 4.2**, the apparent ileal Ca digestibility was decreased ($P < 0.05$) by 10% guar gum supplementation in the PC diet compared with the NC diet. However, the increase in the apparent fecal Ca digestibility, as affected by 10% guar gum supplementation in the PC diet, was not significant ($P > 0.05$) in comparison with the NC diet. Meanwhile, the apparent ileal and fecal Ca digestibility values were not affected ($P > 0.05$) by 10% of cellulose supplementation compared with the NC diet. Although the apparent ileal Ca digestibility was lower ($P < 0.05$) in the NC diet compared with PC. There were no differences ($P > 0.05$) in the apparent fecal Ca digestibility between the NC and the PC diets. The apparent ileal and fecal Ca digestibility values were lower ($P < 0.05$) in the Potato 3 diet in comparison with the NC diet, as examined by the Dunnett-Hsu's test and the Tukey-Kramer's test. The apparent ileal Ca digestibility was lower ($P < 0.05$) in the Potato 1, Potato 5 and Potato 6 diets than in the NC diet compared by using the Dunnett-Hsu's test, however, these differences were not significant

($P > 0.05$) when compared by using the Tukey-Kramer's test. These discrepancies were likely due to different levels of SE values associated with the different types of comparisons. There were differences ($P < 0.05$) in the apparent ileal and fecal Ca digestibility values among some of the six experimental potato diets compared by the Tukey-Kramer's test. To reveal potential effects of variable levels of total glycoalkaloids on the apparent ileal and fecal Ca digestibility values in the test potato diets, the analyses of Pearson correlations between the apparent ileal and fecal Ca digestibility values and the contents of total dietary glycoalkaloids (mg/100 g diet, on as-fed basis) were conducted. There were negative correlations ($P < 0.05$) between the apparent ileal Ca digestibility (r = -0.28; $P = 0.048$) and the apparent fecal Ca digestibility values (r = -0.41; $P = 0.003$) and contents of total dietary glycoalkaloids, respectively (**Figure 4.1A and B**).

The apparent ileal and fecal P digestibility values were reduced ($P < 0.05$) by 10% guar gum supplementation in the PC diet in comparison with the NC diet, as compared by the Dunnett-Hsu's test and the Tukey-Kramer's test (**TABLE 4.3**). There were no differences ($P > 0.05$) in the apparent ileal and fecal P digestibility values between the 10% cellulose supplementation diet and the NC diet when compared by the Tukey-Kramer's test. However, the apparent ileal and fecal P digestibility values were also lower ($P < 0.05$) in the 10% guar gum supplementation diet than in the 10% cellulose supplementation diet when compared by using the Dunnett-Hsu's test and the Tukey-Kramer's test. The apparent ileal and fecal P digestibility values were lower ($P < 0.05$) in the six potato diets than in the NC and/or the 10% cellulose diets when compared by using the Dunnett-Hsu's test and the Tukey-Kramer's test. Although there were no differences ($P > 0.05$) in the apparent ileal P digestibility values among the six potato diets, there were differences ($P < 0.05$) in the apparent fecal P digestibility values among some of the test potato diets when examined by using the Tukey-Kramer's test. The Pearson correlation analyses showed negative correlations ($P < 0.05$) between the apparent ileal P digestibility (r = -0.42; $P = 0.002$) and the apparent fecal P digestibility values (r = -0.50; $P = 0.001$) and contents of the total dietary glycoalkaloids, respectively (**Figure 4.2A and B**).

There were no differences ($P>0.05$) in the apparent ileal and fecal Mg digestibility values between the PC and the NC diets, between the Cellulose (10%) and the NC diets, and between the PC and the Cellulose diets (**TABLE 4.4**). There were also no differences ($P >0.05$) in the apparent ileal Mg digestibility values among all the test diets when examined by the Tukey-Kramer's test. The apparent fecal Mg digestibility of the Potato 4 diet was lower diet in the NC ($P < 0.05$) than in the 10% cellulose diet when examined by the Dunnett-Hsu's and/or the Tukey-Kramer's tests. There were no differences ($P > 0.05$) in the apparent ileal and fecal Mg digestibility values among the potato diets when these were examined by the Tukey-Kramer's test. The Pearson correlation analyses did not show any correlations ($P > 0.05$) between the apparent ileal Mg digestibility (r = -0.12; $P = 0.403$) and the apparent fecal Mg digestibility values (r = -0.18; $P = 0.204$) and contents of the total dietary glycoalkaloids, respectively (**Figure 4.3A and B**).

4.5. DISCUSSION

The primary objectives of this study were to examine the effects of two types of exogenous fibres and different genotypes of potatoes on Ca, P and Mg digestibility in growing pigs fed to a typical "Western" high-fat basal diet. Due to the presence of the gastrointestinal endogenous nutrient outputs, the apparent ileal and fecal nutrient digestibility values are dramatically affected by their dietary content before reaching their corresponding plateau apparent digestibility values (Fan et al., 1994; Fan et al., 2001). The rendered poultry by-product meal included a large proportion of bones and was rich in mineral content. Thus, contents (% as-fed basis) of all test dietary Ca (1.5-1.7%), P (0.9-1.0%) and Mg (0.8-1.0%) were simiar among the diets and were primarily originated from the poultry meal and were much higher than the correspondingly recommended requirements (0.60% Ca, 0.50% P and 0.04% Mg) in commercial swine diets for growing pigs by NRC (1998, 2012). The apparent ileal and fecal Ca, P and Mg digestibility values measured in this study likely reflected their plateau apparent digestibility values, reflecting their true digestibility values. More recently, inadequate dietary Ca to P ratio (0.5:1 to 0.7:1) has been recognized as an important factor in affecting bone formation and health and bone mineral utilization (Kemi et al., 2010). NRC (1998) recommended total Ca to available P ratio of 2:1 to 3:1 for pigs. Total Ca to total P ratio values (1.5:1 to 1.8:1) did not vary much among the diets, which unlikely affected the apparent ileal and fecal mineral digestibility values among the test diets. On the other hand, it is not surprising to observe the high apparent ileal and fecal P digestibility values (79-82%) in the NC diet (**TABLE 4.3**), as majority of the P originated from the rendered poultry-by product meal including bone components. However, the apparent ileal and fecal digestibility values of both Ca (56 - 60%) and Mg (45 - 61%) were much lower than the corresponding P digestibility values in the NC diet (**TABLE 4.2** and **4.4**) with the poultry meal being the predominant dietary Ca and Mg source. It is intriguing to observe that dietary Mg contents (0.84 - 1.04%, on as-fed basis) were many folds of the NRC (1998) recommended Mg requirement level (0.04%, on as-fed basis) in growing pigs. Mg content in poultry meal is at 0.18% (on as-fed basis), as compiled by NRC (1998). Apparently, Mg content in poultry meal used in our study was extremely high and we did not find experimental mistakes in analyzing Mg in our study samples. It is now well established that the transient receptor potential (TRP) family ion channels are important gatekeepers shared by divalent cations of Ca^{2+} and Mg^{2+} in their intestinal absorption (Dimke et al., 2011). Thus very high dietary Ca and Mg contents might have competitively inhibited their respective transport via the shared TRP family ion channels, resulting their relatively low digestibility.

Dietary supplementation of 10% guar gum (PC diet) significantly reduced the apparent ileal Ca digestibility, while 10% cellulose did not affect the apparent ileal Ca digestibility. This was likely due to the fact that 10% guar gum supplementation might have increased the gastrointestinal endogenous Ca loss. Previous studies demonstrated that viscous fibre pectin included at 8% in diets increased the endogenous Ca loss without affecting true Ca digestibility in pigs (Fan et al., 2003). Furthermore, both exogenous fibres at 10% did not significantly affect the apparent fecal Ca digestibility values. These results of this study were consistent with the observations by Rideout et al. (2008b). However, these results of this study are not in agreement with earlier studies by Partridge (1978) and Demigné et al. (1989). Partridge (1978) showed that dietary inclusion of cellulose at 9% vs. 3% significantly increased the apparent fecal Ca digestibility in pigs. Demigné et al. (1989) showed that dietary supplementations of 10%

viscous soluble fibre pectin and 10% non-viscous soluble fibre lactulose and resistant amylose cornstarch significantly increased free Ca^{2+} pools and absorptive Ca^{2+} influx from cecum in the rat. Hara et al. (1996) showed that dietary supplementation of 5% guar gum increased Ca absorption primarily via the cecum site in the rat. Younes et al. (2001) showed that 10% inulin and 15% resistant starch significantly increased intestinal Ca absorption in the rat. Coudray et al. (1997) showed that ingestion of non-viscous soluble fibre inulin significantly increased apparent Ca absorption but ingestion of partially soluble viscous fibre sugar beet pulp did not affect Ca absorption in healthy young men. In the study by Rideout et al. (2008b), amylose resistant cornstarch did not affect the apparent fecal Ca digestibility, however, granular potato resistant starch reduced the fecal Ca digestibility. Whereas retrograded resistant cornstarch improved the fecal Ca digestibility in the pig (Rideout et al., 2008b). The apparent fecal Ca digestibility, however, granular potato resistant starch reduced the fecal Ca digestibility whereas retrograded cornstarch improved the fecal Ca digestibility in pig. This discrepancy is consistent with what Weaver (2005) reviewed that many, but not all, experimental animal studies reported increased mineral absorption by feeding non-digestible oligosaccharides and resistant starch as soluble fibres.

On the other hand, the potato diet 1 to 6 examined the effects of 8.5% guar gum in combination with different cultivar genotypes of cooked potatoes as a supplemental staple food in providing the target level of TDF between 10-11% on the apparent ileal and fecal Ca digestibility values. Except for the potato diet 3, there were no significant differences in Ca digestibility values between the other potato diets and the NC or the PC diet (**TABLE 4.2**). Clearly, fibres were not responsible for the significantly lower ileal and fecal Ca digestibility values in the potato diet 3 compared with the NC or the PC diet. It has been well documented that Ca contents in potatoes are very low (Whittemore et al., 1975). Thus, contributions of Ca from the test potatoes were unlikely responsible for the much lower Ca digestibility values in the potato 3 diet and the significant differences in the fecal Ca digestibility values between some of the test potato diets (i.e., Potato 1 vs. Potato 3 diets). Chymotrypsin inhibitor activity was reported to be high in raw potatoes (Whittemore et al., 1975), and this could negatively affect protein digestion and indirectly reduce mineral absorption. However, cooking is known to remove all chymotrypsin inhibitor activity (Whittemore et al., 1975). All the test potatoes used in this study were effectively thermo-processed and presumably had no chymotrypsin inhibitor activity. Total glycoalkaloids, including α-solanine and α-chaconine, in the six test potatoes (Potatoes 1 to 6) were from 5.88 to 11.88 mg/100 g samples, on their fresh tuber basis in this study, were within the regulatory upper limit of 200 mg/100 g of fresh potato tubers by Health Canada and CFIA. Noticeably, potato glycoalkaloids within the government regulation limit were shown to adversely affect intestinal permeability and aggravate inflammatory bowel diseases in the rodent model (Patel et al., 2002). The significant inverse correlations between the ileal and fecal Ca digestibility values associated with the six test potato diets and contents of the total glycoalkaloids suggest that contents of glycoalkaloids negatively affected the ileal and the fecal Ca digestibility values in this study. To the best of our knowledge, this is the first *in vivo* study in showing that potato glycoalkaloids were linked to reduced Ca digestibility. Therefore, it can be summarized that exogenous fibres of guar gum and cellulose at 10% had little effects on Ca digestibility, whereas potato glycoalkaloids could adversely affect Ca digestibility as demonstrated with growing pigs fed a typical high-fat "Western diet".

Dietary supplementation of 10% guar gum (PC diet) significantly reduced the apparent ileal and fecal P digestibility, while 10% cellulose did not affect the ileal and fecal P digestibility in growing pigs fed the high-fat basal diet. This was likely due to the fact that endogenous fecal P loss is a significant component of the metabolic P loss (Fan et al., 2001). Guar gum supplementation at 10% might have significantly enhanced the endogenous P loss at the distal ileal and the fecal levels, whereas 10% cellulose supplementation was not abrasively sufficient to induce a similar extent of the endogenous P loss in these pigs. In support of this notion, our previous studies showed that viscous soluble fibre pectin included at 8% in a diet increased the fecal endogenous P loss (Fan et al., 2004). Rideout et al. (2008b) showed that 10% guar gum significantly reduced the apparent ileal but did not affect the apparent fecal P digestibility values in the pig likely due to larger experimental errors (i.e., SE) and fewer study pigs used in their study. On the other hand, Rideout et al. (2008b) did not observe any negative efforts of 10% cellulose on the ileal and fecal Pi digestibility. In contrast, a much early study by Partridge (1978) showed that dietary inclusion of cellulose at 9% vs. 3% significantly increased the apparent fecal Pi digestibility in pigs. Furthermore, the potato diets 1 to 6 examined the effect of 8.5% guar gum supplementation in combination with different cultivar genotypes of cooked potatoes as a supplemental staple food in providing the target level of TDF at about 10% on the apparent ileal and fecal P digestibility. Although the ileal and fecal P digestibility values were all significantly lower in the potato diets than in the NC diet, there were no significant differences between most of the potato diets and the PC diet except the potato-3 diet. These results suggest that the decreased P digestibility values associated with the potato diets were, in part, resulted from the 8.5% guar gum supplementation. Other factors were likely further responsible for the much lower fecal P digestibility observed in the Potato-3 diet. Rideout et al. (2008b) showed that granular potato resistant starch reduced fecal P digestibility. Bath et al. (2013) have recently shown that the genotype cv 96044-3, also as a new test potato cultivar in the Potato-3 diet in this study, had the highest resistant starch content among the potato genotype samples they examined. Giving the relatively low P contents in potatoes that were well documented (Whittemore et al., 1975) and the negligible P contributions from the test potatoes to the diets, intrinsic differences in P such as total P content and phytate-P content were unlikely to be responsible for the variability in the apparent P digestibility values among the potato diets of this study. The significant negative correlations between the ileal and fecal P digestibility values associated with the potato diets and contents of the total glycoalkaloids also indicate that presence of glycoalkaloids within the government regulatory limit in the diets was negatively linked to the P digestibility.

Dietary supplementations of guar gum (PC diet) and cellulose at 10% did not significantly affect the apparent ileal and fecal Mg digestibility values in this study. Mg has been increasingly recognized as an important macro-mineral for bone formation and health (Tucker, 2009). Our results of the exogenous fibre effects on Mg digestibility in this study are not in agreement with two previous studies. Partridge (1978) showed that 9% vs. 3% exogenous fibres significantly enhanced the apparent fecal Mg digestibility values in the pig. Demigné et al. (1989) demonstrated that the exogenous viscous fibre pectin along with three other sources of non-viscous fibres significantly increased absorptive influxes of Mg in the rat. Knudsen et al. (1996) using stable isotope tracer Mg in studies with human subjects showed that the gastrointestinal endogenous Mg loss was a significant component and was affected by fibre intake. Coudray et al. (2004) reviewed that exogenous fibre effects on Mg absorption were not

consistent in animal studies, whereas numbers of reported human subject studies were limited. Nevertheless, non-viscous soluble fibre components, including inulin, resistant starch and resistant maltodextrin, were shown effective in improving intestinal Mg absorption in rats (Younes et al., 2001; Miyazato et al., 2010). On the other hand, the effects of 8.5% guar gum combined with 25% individual test potatoes on Mg digestibility were examined in the potato diets 1 to 6. There were no significant differences between each of the test potato diets and the 10% guar gum PC diet. However, the fecal Mg digestibility values of the potato diet 3, 4 and 6 were significantly lower than that of the NC diet as compared with the Tukey's and/or the Dunnett-Hsu's tests. The discrepancy between the statistical test methods was likely due to the differences of the SE values associated with the endpoint means. Clearly, other intrinsic factors were responsible for causing the much lower fecal Mg digestibility values in the potato diets 3, 4 and 6. Potatoes are well documented to be very low in total P and Mg contents (Whittemore et al., 1975), thus differences in phytate and Mg contents, as contributed from the test cultivar genotype of potatoes, unlikely played a role in affecting the low Mg digestibility values. Pearson correlation analyses did not suggest that potato glycoalkaloids played a negative role in affecting Mg absorption in the potato diets. Paradoxically, Ca^{2+} and Mg^{2+} are sharing the same TRP family ion channels in their intestinal absorption (Dimke et al., 2011). Biological mechanisms of how potato glycoalkaloids differentially influence intestinal Ca^{2+} and Mg^{2+} absorption need to be elucidated. Previous studies showed that granular potato resistant starch and phosphate-esterified potato starch reduced mineral digestibility (Rideout et al., 2008b; Mineo et al., 2009). Bath et al. (2013) showed very high and variable resistant starch contents among the cultivar genotypes that were also used in this study. Intrinsic differences in the resistant starch contents and their chemical and physical properties might have further contributed to the lower fecal Mg digestibility values seen in some of the test potato diets.

In summary, exogenous guar gum and cellulose supplemented at 10% did not affect the apparent fecal Ca and Mg digestibility values, whereas 10% guar gum supplementation significantly reduced the apparent fecal P digestibility in growing pigs fed the high-fat basal diet. The presence of variable levels of potato glycoalkaloids within the government regulatory safety limit was negatively linked to intestinal Ca and Pi absorption. Thus, novel genotypes of potatoes to be selected for promoting human health should have minimal levels of glycoalkaloids to minimize their potential adverse effects on Ca and P absorption and bone health.

Table 4. 1 Composition (%) of the experimental diets (on as-fed basis) fed to the growing pigs

Item	Experimental diets[1]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 ((FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
Poultry meal	41.50	41.50	41.50	41.50	41.50	41.50	41.50	41.50	41.50
Casein	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Animal fat-oil blend	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
DL-methionine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
L-Alanine[2]	2.81	2.81	2.81	0.00	0.00	0.00	0.00	0.00	0.00
Sucrose	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31
Cornstarch	30.79	20.79	20.79	0.00	0.00	0.00	0.00	0.00	0.00
Solka-Floc®[3]	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
Guar gum[4]	0.00	10.00	0.00	8.50	8.50	8.50	8.50	8.50	8.50
Potato-1	0.00	0.00	0.00	25.10	0.00	0.00	0.00	0.00	0.00
Potato-2	0.00	0.00	0.00	0.00	25.10	0.00	0.00	0.00	0.00
Potato-3	0.00	0.00	0.00	0.00	0.00	25.10	0.00	0.00	0.00
Potato-4	0.00	0.00	0.00	0.00	0.00	0.00	25.10	0.00	0.00
Potato-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.10	0.00
Potato-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.10
Min-Vit premix[5]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Iodized salt[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Titanium oxide[7]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Nutrient contents (on as-fed or air-dry basis)									
Dry matter[8]	95.26	92.64	94.40	93.03	92.25	92.99	93.07	92.75	94.49
Crude fat[9]	20.26	20.26	20.26	20.67	20.67	20.67	20.67	20.67	20.67
GE from crude fat[10]	37.22	37.15	37.15	38.98	38.98	38.98	38.98	38.98	38.98
Crude protein[11]	33.14	33.14	33.14	33.38	33.13	33.34	33.05	33.14	32.78
Glycoalkaloids[12]	0.00	0.00	0.00	6.38	7.73	12.12	8.68	8.84	11.47
Total dietary fibre[13]	0.00	10.00	10.00	11.15	11.13	10.90	10.82	10.46	10.99
NDF[14]	0.00	0.00	10.00	1.32	1.29	1.47	1.39	0.76	1.10
Total soluble fibre[15]	0.00	10.00	0.00	9.84	9.84	9.44	9.43	9.70	9.89
ME[16]	15.93	15.35	15.52	14.72	14.72	14.72	14.72	14.72	14.72
Total Ca[17]	1.52	1.52	1.54	1.55	1.57	1.65	1.48	1.57	1.55

Total P[18]	0.95	0.92	0.93	0.85	0.96	0.93	0.97	1.02	0.94
Ca:P ratio	1.60	1.65	1.66	1.82	1.64	1.77	1.53	1.54	1.65
Total Mg[19]	1.02	0.84	0.88	1.04	1.01	0.97	0.87	0.97	1.03

Note: [1]NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as the basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[2]Evonik Industries AG, Hanau-Wolfgang, Germany.

[3]Solka-Floc®, pure cellulose commercially available from International Fibre Corporation (North Tonawanda, NY).

[4]Guar gum, food grade with an estimated average molecular weight of about 1.5 million and granulation of medium coarse commercially available from Nealanders International Inc. (Mississauga, ON, Canada).

[5]The vitamin and trace mineral premix supplied the following vitamins and trace minerals (IU or mg/kg diet): vitamin A, 8000 IU; vitamin D_3, 800 IU; vitamin E, 32 IU; vitamin K, 2 mg; vitamin $B_{12,}$ 0.02 mg; D-biotin, 0.16 mg; thiamine, 1.2 mg; riboflavin, 4 mg; D-pantothenic acid, 12 mg; pyridoxine, 1.2 mg; niacin, 20 mg; folic acid, 1.6 mg; choline, 400 mg; iron, 80 mg; copper, 12 mg; manganese, 16 mg; zinc, 84 mg; iodine, 0.4 mg; and selenium, 0.24 mg.

[6]Supplied by the Windsor Salt Co. (Toronto, ON, Canada). Composition (g/kg): NaCl, 965.0; ZnO, 40.0; $FeCO_3$, 1.6; MnO, 1.2; CuO, 0.33; $Ca(IO_3)_2$, 0.07; and CaO, 0.04.

[7]Nutrient digestibility marker purchased from Fisher Scientific (Ottawa, ON, Canada).

[8]Analyzed dietary dry matter content, *%*.

[9]Crude fat content in diets, *%*, calculated by using crude fat contents in the concerned ingredients compiled by NRC (1998); and crude fat content in cooked and dried potatoes reviewed by Whittemore (1977).

[10]Percentage contribution of GE from crude fat to total dietary GE, *%*, calculated by using GE of crude fat = 39.8 *MJ/kg* summarized by Brouwer (1965).

[11]Crude protein content in diets, *%*, calculated by using crude protein contents in the concerned ingredients compiled in NRC (1998); and the analyzed crude protein contents in the cooked and dried test potato samples.

[12]Total glycoalkaloid content including α-chaconine and α-solanine in diets, mg/100 g diet, calculated total dietary content based on the analyzed constituent contents in the cooked and dried test potato samples.

[13]Total dietary fibre (TDF) content in diets, *%*, calculated dietary content based on the analyzed TDF contents in the cooked and dried test potato samples and the TDF content in Solka-Floc® and guar gum being considered to be 100%.

[14]NDF, dietary neutral-detergent fibre content in diets, *%*, calculated contents based on the analyzed NDF contents in the cooked and dried test potato samples and the NDF content considered to be 100% in Solka-Floc® and 0% in guar gum, respectively.

[15]Total soluble fibre content in diets, *%*, calculated content = total dietary fibre (TDF, *%*) – neutral-detergent fibre (NDF, *%*).

[16]ME, metabolizable energy content in diets, *MJ/kg*, calculated by using ME content in the concerned ingredients compiled in NRC (1998); ME from cooked and dried test potatoes obtained by multiplying by a correction factor of 0.96 according to NRC (1998) with DE in cooked and dried potatoes reviewed by Whittemore (1977); and ME content in poultry meals reported by Pesti et al. (1986).

[17]Analyzed total calcium (Ca) content in diets, *%*, on as-fed basis.

[18]Analyzed total phosphorus (P) content in diets, *%*, on as-fed basis.

[19]Analyzed total magnesium (Mg) content in diets, *%*, on as-fed basis.

Table 4. 2 Responses in the apparent ileal and fecal calcium (Ca) digestibility[1] (%) in the exprimental diets fed to the growing pigs

Item	Experimental diets[2]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
n[3]	7	9	8	10	7	10	8	9	8
Ileal DM[4]	94.1±0.7^{ab}	93.6±0.7^{a}	94.7±0.7^{ab}	94.4±0.6^{ab}	93.4±0.7^{a}	94.9±0.6^{ab}	96.4±0.6^{b}	94.4±0.7^{ab}	94.1±0.6^{ab}
Fecal DM[4]	94.9±0.6^{ab}	96.5±0.6^{b}	94.9±0.6^{ab}	94.7±0.6^{ab§}	94.5±0.6^{ab§}	94.8±0.6^{ab§}	94.7±0.6^{ab§}	94.5±0.6^{a§}	94.5±0.6^{ab§}
Dietary Ca[5]	1.59	1.64	1.63	1.67	1.70	1.78	1.58	1.69	1.64
Ileal Ca[5]	3.04±0.18§¶	1.66±0.16*	1.56±0.17*	2.06±0.15*	1.88±0.18*	2.11±0.15*	1.70±0.17*	1.84±0.16*	1.76±0.17
Fecal Ca[5]	6.83±0.26§¶	3.09±0.23*	3.70±0.24*	3.79±0.22	4.39±0.26	4.89±0.22*§	3.76±0.24*	3.72±0.23*	3.96±0.24
Ileal Ca digestibilty[6]	60.2±3.8^{b}	50.5±3.3^{a}	56.8±3.5^{b}	48.1±3.1^{ab}*	48.8±3.8^{ab}	39.8±3.1^{a}*¶	48.1±3.5^{ab}	47.6±3.3^{ab}*	45.5±3.5^{ab}*
Fecal Ca digestbility[6]	55.7±2.8^{b}	59.2±2.4^{b}	53.3±2.6^{ab}	53.7±2.3^{b}	52.1± 2.8^{ab}	42.7±2.3^{a§}	52.3±2.6^{ab}	53.3±2.6^{ab}	49.3±2.6^{ab§}

Note: [1] Values are least square means ± SE of the estimates.

[2] Refer to Table 1 for details of the diet formulations. NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]Number of observations.

[4]Analyzed DM content in ileal digesta or feces.

[5]Analyzed total Ca content in diets, ileal digesta or feces, %, on DM basis.

[6]The apparent ileal or fecal Ca digestibility, %.

[a,b]Means that diets with different superscript letters differ ($P < 0.05$) among the diets as analyzed by the Tukey-Kramer's tests.

*Difference from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

[§]Difference from the PC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

[¶]Difference from the Cellulose group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

Table 4. 3 Responses in the apparent ileal and fecal phosphorus (P) digestibility[1] (%) in the experimental diets fed to the growing pigs

Item	Experimental diet[2]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
n[3]	7	9	8	10	7	10	8	9	8
Dietary P[4]	0.99	0.99	0.99	0.91	1.04	1.00	1.04	1.03	1.00
Ileal P[4]	$0.94\pm0.05^{b¶}$	$0.80\pm0.05^{b¶}$	$0.55\pm0.06^{a*§}$	0.75 ± 0.04^{ab}	0.80 ± 0.05^{ab}	$0.83\pm0.04^{b¶}$	0.76 ± 0.06^{ab}	$0.83\pm0.05^{b¶}$	$0.70\pm0.06^{ab}*$
Fecal P[4]	$1.62\pm0.10^{bc¶}$	$1.53\pm0.08^{bc¶}$	$1.02\pm0.09^{a*§}$	$1.26\pm0.08^{ab*}$	$1.45\pm0.10^{abc¶}$	$1.80\pm0.08^{c¶}$	$1.60\pm0.09^{bc¶}$	$1.50\pm0.08^{bc¶}$	1.35 ± 0.09^{ab}
Ileal P digestibilty[5]	$79.3\pm2.0^{c§}$	$58.8\pm1.8^{ab*¶}$	$75.3\pm1.9^{c§}$	$63.0\pm1.7^{b*¶}$	$62.5\pm2.0^{b*¶}$	$51.3\pm1.7^{b*§¶}$	$61.6\pm1.9^{b*¶}$	$64.1\pm1.8^{b*¶}$	$62.3\pm1.9^{b*¶}$
Fecal P digestibilty[5]	$82.2\pm1.9^{d§}$	$65.3\pm1.7^{b*¶}$	$77.0\pm1.8^{cd*§}$	$70.7\pm1.6^{bc*¶}$	$71.1\pm1.9^{bc*}$	$57.1\pm1.6^{a*§¶}$	$66.6\pm1.8^{b*¶}$	$67.8\pm1.7^{b*¶}$	$69.3\pm1.8^{b*¶}$

Note: [1]Values are least square means ± SE of the estimates.

[2]Refer to Table 1 for details of the diet formulations. NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.

[3]Number of observations.

[4]Total P content in diets, ileal, digesta or feces, %, on DM basis.

[5]The apparent ileal or fecal P digestibility, %.

[a,b,c,d]Means that diets with different superscript letters differ ($P < 0.05$) from each other as analyzed by the Tukey-Kramer's tests.

*Difference from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

[§]Difference from the PC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

[¶] Difference from the Cellulose group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

Table 4. 4 Responses in the apparent ileal and fecal magnesium (Mg) digestibility[1] (%) in the experimental diets fed to the growing pigs

Item	Experimental diet[2]								
	NC (no fibre)	PC (guar gum)	Cellulose (10%)	Potato 1 (FV12272-3)	Potato 2 (F05035)	Potato 3 (96044-3)	Potato 4 (WV5475-1)	Potato 5 (Atlantic)	Potato 6 (F05081)
n[3]	7	9	8	10	7	10	8	9	8
Dietary Mg[4]	1.07	0.90	0.93	1.11	1.09	1.05	0.93	1.05	1.09
Ileal Mg[4]	2.94±0.12^{d§¶}	1.02±0.10^{a¶}	2.25±0.1^{abc*}	1.49±0.09^{abc*§}	1.76±0.12^{cd§¶}	1.28±0.09^{abc¶}	1.19±0.11^{ab¶}	1.32±0.10^{abc*}	1.29±0.11^{abc}
Fecal Mg[4]	4.12±0.16^{b§}	1.94±0.14^{a*}	2.49±0.15^{ab§}	2.65±0.14^{b§}	2.89±0.16^{b§}	2.63±0.16^{b§}	2.63±0.15^{b§}	2.50±0.14^{b§}	2.82±0.15^{b§}
Ileal Mg digestibilty[5]	44.5±3.7	47.4±3.2	40.6±3.4	40.3±3.0	35.5±3.7	35.0±3.0§	35.9±3.4	41.2±3.2	39.6±3.4
Fecal Mg digestibilty[5]	61.2±3.5^{bc}	52.7±3.1^{abc}	57.5±3.3^{bc}	50.8±2.9^{abc}	51.2±3.5^{abc}	47.2±2.9^{abc*}	42.4±3.3^{a*¶}	49.9±3.1^{abc}	44.5±3.3^{ab*¶}

Note: [1]Values are least square means ± SE of the estimates.
[2]Refer to Table 1 for details of the diet formulations. NC, the negative control diet without fibre; PC, the positive control diet with 10% guar gum; Cellulose, the cellulose-supplemented diet with 10% cellulose from Solka-Floc® at the expense of cornstarch by using the NC as the basal diet; Potato 1, test potato cultivar FV12272-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 2, test potato cultivar F05035 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 3, test potato cultivar 96044-3 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 4, test potato cultivar WV5475-1 supplemented at the expense of cornstarch by using the NC as the basal diet; Potato 5, test potato cultivar Atlantic supplemented at the expense of cornstarch by using the NC as basal diet; and Potato 6, test potato cultivar F05081 supplemented at the expense of cornstarch by using the NC as the basal diet.
[3]Number of observations.
[4]Total Mg content in diets, ileal digesta or feces, %, on DM basis.
[5]The apparent ileal or fecalMg digestibility, %.
[a,b,c,d]Means that diets with different superscript letters differ ($P < 0.05$) from each other as analyzed by the Tukey-Kramer's tests.
*Difference from the NC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.
[§]Difference from the PC group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.
[¶] Difference from the Cellulose group ($P < 0.05$) as analyzed by the Dunnett-Hsu's tests.

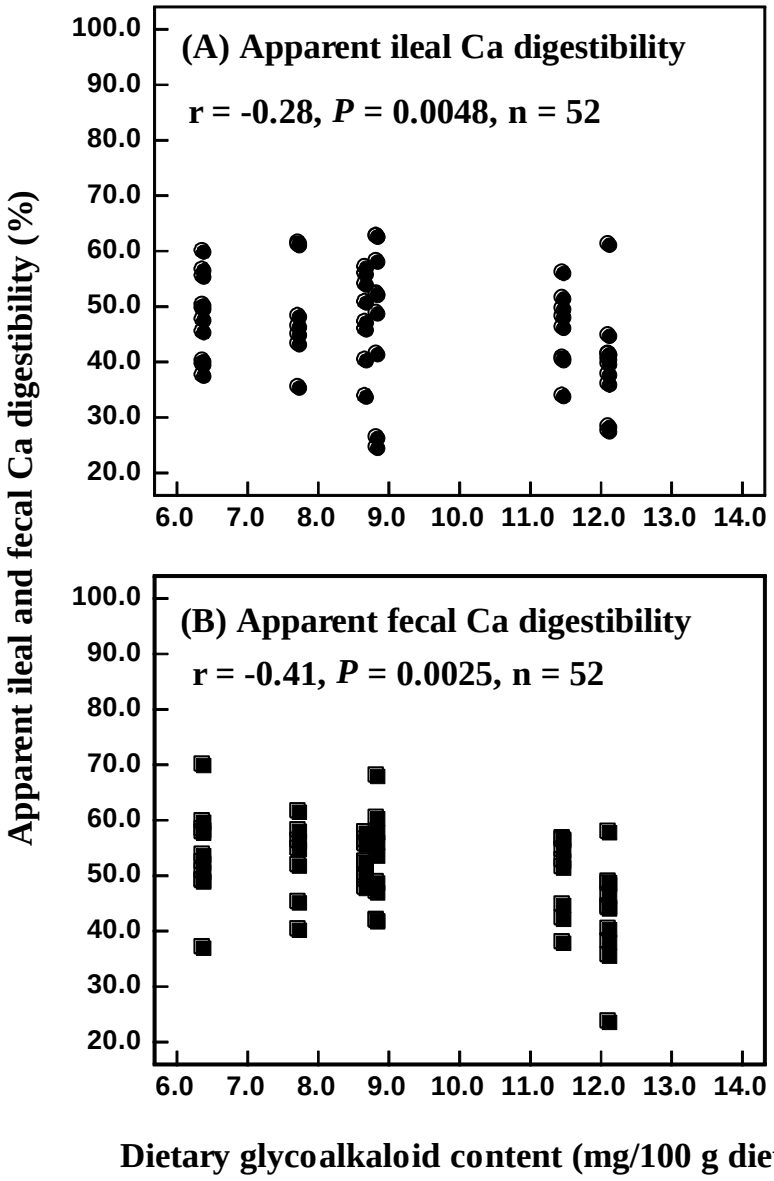

Figure 4. 1 Pearson correlations between **(A)** the apparent ileal Ca (calcium), and **(B)** the apparent fecal Ca digestibility values (%) and total glycoalkaloid contents (mg/100 g diet, on an air-dry basis) in the six potato test diets for the growing pigs fed a high-fat basal diet.

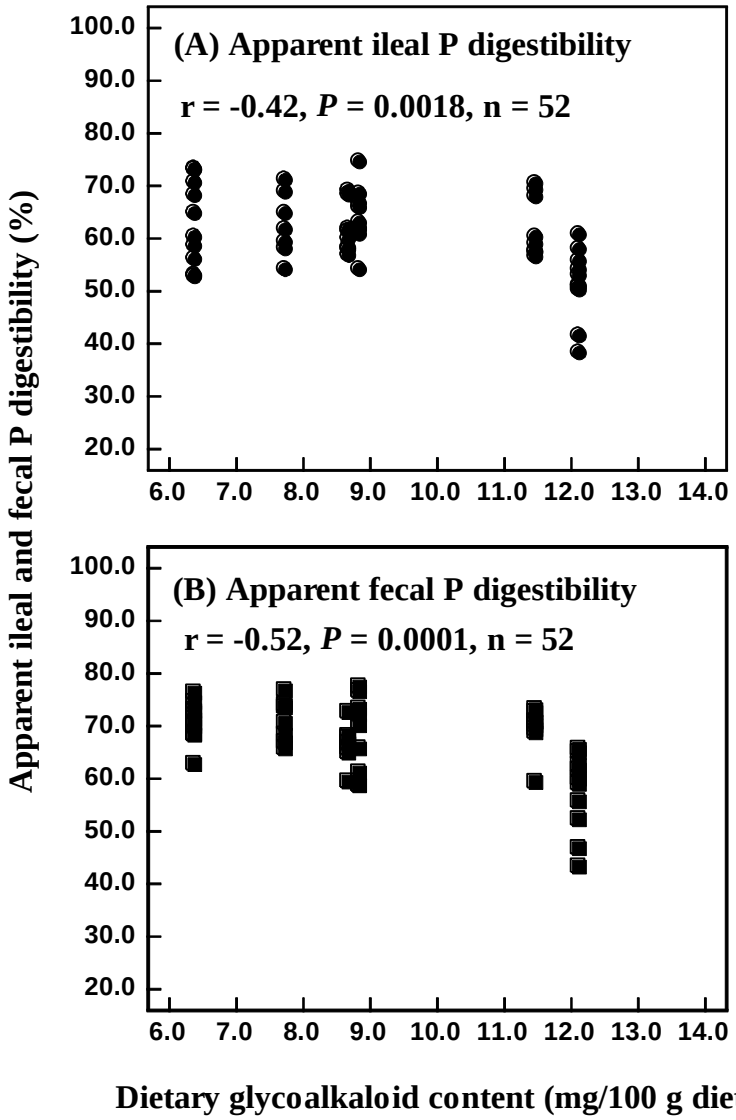

Figure 4. 2 Pearson correlations between **(A)** the apparent ileal phosphorus (P), and **(B)** the apparent fecal P digestibility values (%) and total glycoalkaloid contents (mg/100 g diet, on an air-dry basis) in the six potato test diets for the growing pigs fed a high-fat basal diet.

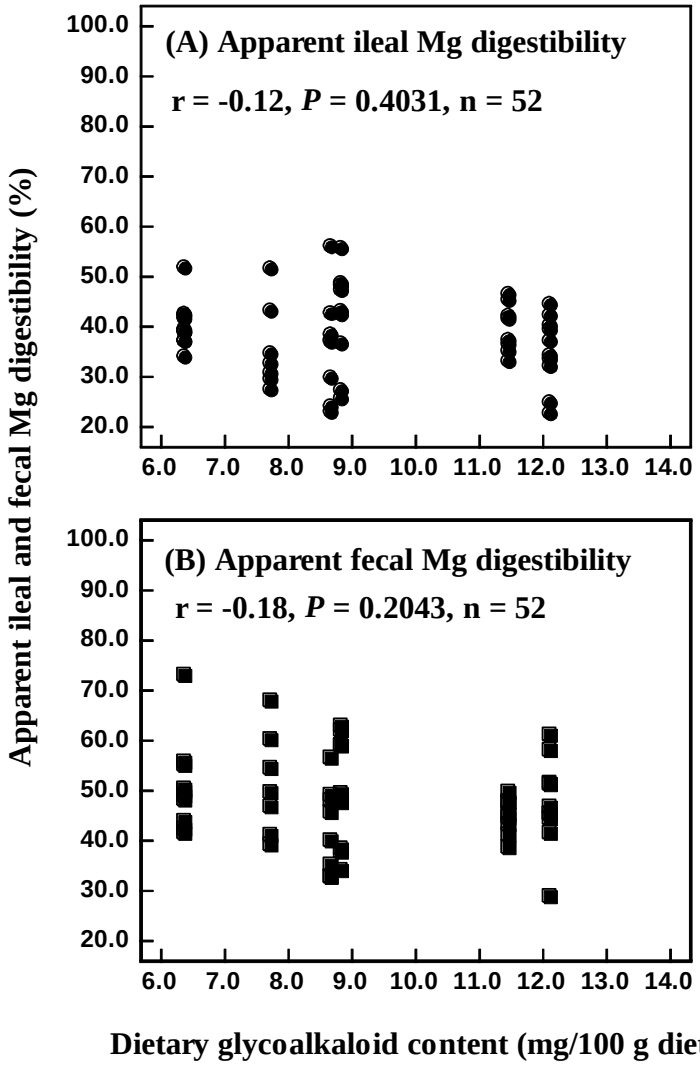

Figure 4. 3 Pearson correlations between **(A)** the apparent ileal magnesium (Mg), and **(B)** the apparent fecal Mg digestibility values (%) and total glycoalkaloid contents (mg/100 g diet, on an air-dry basis) in the six potato test diets for the growing pigs fed a high-fat basal diet.

CHAPTER 5

GENERAL CONCLUSIONS AND DISCUSSIONS

Numerous previous research has shown that exogenous dietary fibre is generally accepted as having protective effects against a range of diseases predominant in Western countries and in the newly economic emerging countries, including type-II diabetes, colorectal cancers, coronary heart diseases, obesity, and diverticular diseases. Increased fibre consumption has been associated with lowering total serum cholesterol and LDL cholesterol, modifying the glycemic and insulinemic responses and protecting the large intestine from inflammatory diseases (Blackwood et al., 2000; Brennan, 2005; Chutkan et al., 2012). Sources of human diets are very heterogenous. Because human diets are mostly in fresh status and contain large amounts of free water, dietary fire intake is usually expressed as g of fibres per human and per day and as g of fibres per 1000 kcal dietary gross energy (Turner and Lupton, 2011). The total dietary fibre levels of 19.27 – 21.48 g/1000 kcal GE were used in this study, as were shown in **TABLE 2.1.** Although these total dietary fibre levels in this study were higher than the recommended dietary fibre intake level of 14 g/1000 kcal (cited by Turner and Lupton, 2011) in adult humans for maintaining a healthy cardiac system by about 46%, these total dietary fibre levels used in the grower pig model in this study were relevant to realistic adult human nutrition.

The potato has been widely accepted throughout the world as a staple food and is available in many forms to many consumers. Consumers tend to believe that potatoes are high in available energy compared with other carbohydrate sources such as rice. Beyond the nutritional properties of a quality staple food, potato may have a role to play in human health over a lifetime of consumption. Commercially available potatoes have been implicated in contribution to diabetes and obesity because of its high glycemic index. Potato chips and French fries that are currently processed from commercially available potatoes have been considered to be the major contributors to obesity by human clinical nutritionists, as these products contain high levels of fat and rapidly digestible starch (Mozaffarian et al., 2011). In novel genotypes of potatoes, the major starch component that escapes digestion and absorption and in small intestine as dietary fibre is resistant starch (Bach et al., 2013). They have aroused significant interest in recent years due to the prebiotic effects of potato RS in providing potential health benefits including inhibition of intestinal infection and reduction in colorectal cancer risks.

5.1. GENERAL CONCLUSIONS

The following novel and conceptual findings have been observed from this thesis study:

1). Dietary supplementations of exogenous viscous fibre guar gum and non-viscous fibre cellulose at 10% significantly reduced ileal starch digestibility and our results showed that this was largely due to negatively affecting *in vivo* starch digestion - (**Chapter 2**).

2). Albeit of small magnitudes, 8.5-10% guar gum negatively affected *in vivo* starch digestibility by decreasing glucose absorption and this was directly shown by increased distal ileal output of free D-glucose under the dietary inclusions of 8.5-10% guar gum. Moreover, the depression effect of guar gum at 8.5-10% on glucose absorption was likely mediated via the mTOR-signaling pathway, since 10% guar gum supplementation significantly reduced the abundance of the phosphorylated mTOR (Ser 2448) in the proximal jejunum - (**Chapter 2**).

3). Out of a little over 100 different genotypes of potato samples being screened based upon their TDF contents under the AAFC-ABIP Biopotato Network program, six different genotypes of potatoes were selected for use in this *in vivo* study. The cv Atlantic was used as a control and the other five genotypes of potatoes were test genotypes. Three novel potato genotypes of cv 12272-3, cv 96044-3 and cv F05081 were shown to have considerably lower ileal starch digestibility values and higher resistant starch contents in comparison with the conventional cornstarch control of the NC diet, thus having a potential in serving as healthy staple foods in preventing and managing type-II diabetes, blood dyslipidemia and risks of developing colorectal cancers - (**Chapter 2**).

4). Although total glycoalkaloid contents in the potato test diets were within the federal government regulatory upper limit, Pearson correlation analyses showed a significant negative linear relationship between the ileal DM digestibility values and the total glycoalkaloid contents in the potato test diets, suggesting the presence of potato glycoalkaloids might have general negative effects on nutrient digestibility of the diets - (**Chapter 2**).

5). When feed intake was controlled to be at equal levels, dietary supplementations of the exogenous fibres and the novel low-glycemic genotypes of potatoes that were higher in RS contents were not effective for control of weight gains and had little effects on feed conversion efficiency - (**Chapter 2**).

6). The presence of variable levels of potato glycoalkaloids within the government regulatory safety limit was negatively linked to dietary Ca and P digestibility. Thus, novel cultivar genotypes of potatoes selected and their further processed potato products for promoting human health should have minimal levels of glycoalkaloids to prevent the potential adverse effects of potato glycoalkaloids on Ca and P absorption and bone health - (**Chapter 4**).

Meanwhile, the following findings that have observed from this thesis study are conformational in nature:

1). Conventional cornstarch was completely digested in growing pigs fed a high-fat basal diet - (**Chapter 2**).

2). Resistant starch contents estimated by chemical analyses by using a commercial assay kit considerably overestimate RS contents in starchy food samples compared with RS estimates obtained with *in vivo* ileal starch digestibility study in growing pigs fed a high-fat basal diet - (**Chapter 2**).

3). Our results showed that the poultry meal by-product and casein-based high-fat basal diet was animal protein in nature and was highly digestible in dietary protein and AA, as shown with the NC diet - (**Chapter 3**).

4). Our results suggest that dietary inclusion of fibres at about 10% from guar gum, cellulose and intrinsic fibres contributed by potatoes may adversely affect the apparent ileal digestibility values of some AA - (**Chapter 3**).

5). Our results showed that macro-minerals such as Ca, P and Mg in poultry meal by-product and casein-based high-fat basal diet were very digestible in dietary, as these were shown with the NC diet - (**Chapter 4**).

6). The dietary supplementation of soluble viscous fibre guar gum at 10% significantly decreased the apparent fecal P digestibility values in growing pigs fed a high-fat basal diet - (**Chapter 4**).

5.2. GENERAL DISCUSSION

The observation of dietary supplementations of exogenous non-viscous fibre cellulose at 10% significantly reduced ileal starch digestibility largely due to negatively affecting *in vivo* starch digestion in growing pigs fed a high-fat basal diet from this thesis research from Chapter 2 was novel and significant. Considering that exogenous non-viscous fibres are more abundantly available compared with soluble viscous fibres, dietary supplementations of exogenous non-viscous fibres would be economical and practical, for example, to prevent and manage type-II diabetes. However, this observation was not in a general agreement with current literature reports and understanding including a previous study from our own lab group (Rideout et al, 2008; Chutkan et al., 2012). Thus, there is a need to further explore the effects of dietary supplementations of exogenous non-viscous fibres on *in vivo* starch digestion and glycemic responses in both relevant animal model studies human clinical studies. Furthermore, sources and dietary inclusion levels of exogenous non-viscous fibres may also affect their interactions and potential inhibitory effects on alpha-amylase activity, thus *in vivo* starch digestibility and glycemic responses. Another consideration is that there is a relatively large variability or SE associated with *in vivo* ileal starch digestibility measurements, as demonstrated in Chapter 2 of this thesis research and a previous study by Rideout et al. (2008). Therefore, future studies

should be conducted to examine effects of graded levels of exogenous non-viscous fibres from various food sources on the *in vivo* ileal starch digestibility and glycemic responses in growing pigs fed a high-fat basal diet with more replicates (e.g., n = 12 – 15 for each test diet). Once the inhibitory effects of effective doses of exogenous non-viscous fibres on *in vivo* ileal starch digestibility are further confirmed, more human clinical glycemic response study may need to be conducted with effective sources and doses of exogenous non-viscous fibres.

These findings show that the three novel potato genotypes of cv 12272-3, cv 96044-3 and cv F05081 had considerably lower ileal starch digestibility values and higher resistant starch contents in comparison with the conventional cornstarch control of the NC diet. These results are in agreement with a recent study by Bach et al. (2013) by using RS chemical analyses for screening a similar batch of potato cultivar samples in showing that cv 96044-3 was a novel potato cultivar being high in RS content. An interesting observation was that our *in vivo* starch digestibility study was likely of more discriminative or selective in screening out differences of starch digestibility and RS contents among the tested potato cultivar samples. Thus, these results and this comparison between this thesis study in Chapter 2 and the study by Bach et al. (2013) would suggest that the animal model and the basal diet used in this study is effective and sensitive to select or rank starchy foods that are intrinsically different in *in vivo* starch digestibility and RS contents.

While the small intestinal enzymatic starch digestion is known to be a major determinant of the glycemic response (Jenkins et al., 1987), quantitative relationships between glycemic responses and the small intestinal or ileal starch digestibility values have not been established. The *in vitro* assay of RDS and SDS contents in starchy foods can, in principle, provide some indication of GI values, as RDS gives indication of rate of starch digestion. Bach et al. (2013) determined RDS, SDS and RS in the similar batches of potato cultivar tuber samples by using *in vitro* assays and showed that RDS contents as % of total starch in the examined potatoes were within 10%, suggesting that these potatoes are associated with low GI values. This study (Chapter 2) and previous studies by Champ et al. (2003), McCleary (2003) and Rideout et al. (2008b) showed that current *in vitro* enzyme-based assays likely overestimate RS contents, thus leading to underestimation of RDS and SDS contents in starchy food samples including potatoes. Research efforts are to me made to further refine the present *in vitro* enzyme-based assays to be more closely related to *in vivo* measurements. Nevertheless, *in vivo* starch digestibility study in combination with improved *in vitro* analyses of RDS and SDS will help provide much more useful inside into the implications of starchy foods to both human and animal health

These results show that the presence of variable levels of potato glycoalkaloids within the government regulatory safety limit was negatively linked to dietary DM, Ca and P digestibility, and could be practically important. Glycoalkaloids are well documented as a group of

anti-nutritive factors in potatoes (Friedman, 2006). Although contents of glycoalkaloids normally detected within the government regulatory level in consumed potato products were shown to adversely affect the mammalian intestinal permeability and could aggravate inflammatory bowel diseases in a previous study (Patel et al., 2002), our results reported in Chapters 2 and 4, to the best our of knowledge, are the first report regarding the potential negative effects of potato glycoalkaloids on nutrient digestibility. However, significant correlations do not necessarily reveal real causal relationship for the two variables. Future study should be conducted to examine direct effects of dietary potato glycoalkaloids within the government regulatory level on bone mineral digestibility, bowel health and bone mineralization.

Finally, the results that 10% guar gum supplementation significantly reduced the abundance of the phosphorylated mTOR (Ser 2448) in the proximal jejunum reported in Chapter 2 is scientifically interesting and important. To the best our of knowledge, this is the first report and observation in showing that a soluble viscous fibre guar gum supplementation significantly decreased the expression of a master intracellular regular of mTOR in the small intestine. Fibres especially viscous soluble fibre components are also known to be one of the dietary anti-nutritive factors potentially affecting digestive utilization of dietary proteins (e.g., Mosenthin et al., 1994). Several lines of evidence suggest that dietary supplementations of soluble fibres, especially viscous soluble fibres, but not insoluble fibre cellulose, inhibited glucose transport and absorption across the small intestinal epithelia (Johnson et al., 1984; Rainbird et al., 1984; Hannan et al., 2007). Thus, these mTOR abundance data from this thesis research (Chapter 2) have provided important cellular mechanisms that mTOR-signalling pathway may be involved in regulation of gut functions in responses to soluble fibre intake levels in diets. However, our current level of data regarding the effects of 10% guar gum on mTOR abundances from this thesis research is also limited for the two following reasons. Firstly, the relationship between levels of guar gum intake and changes in gut luminal digesta and gut mucosal viscosity are not established. Secondly, corresponding changes in SGLT1 activity and SGLT1 protein synthesis in the jejunum in responses to levels of guar gum intake and potential changes in gut luminal digesta and gut mucosal viscosity are not established. Future studies should be conducted to examine these aspects.

On the other hand, the findings of 8.5-10% guar gum in decreasing glucose absorption and increasing the distal ileal output of free D-glucose from this thesis study provided direct evidence in support the notion that dietary inclusion of viscous fibre of guar gum at 8.5-10% might have decreased small intestinal glucose transport presumably via SGLT1 in growing pigs fed the high-fat bsal diet. These results have two implications. Firstly, the small intestinal SGLT1 uptake capacity in the adult gut such as in mature grower pigs and adult humans are not likely programmed to be in large excess, as demonstrated in adult mice in previous studies (Weiss et al., 1998). Secondly, dietary supplementations of intermediate levels of viscous soluble fibres

such as guar gum can potentially reduce this SGLT1 uptake capacity and *in vivo* starch & sugar digestibility in adult humans and growing-finishing pigs with a mature gut. Changes in the small intestinal SGLT1 uptake capacity, as affected by dietary supplementation of an intermediate level of viscous fibres, can be biochemically dectected by measuring their enterocytic apical maximal SGLT1 transport activity (V_{max}) responses as well as through analysis of SGLT1 abundances on the apical membrane via Western blotting analysis (e.g., Yang et al., 2011), which needs to be explored in future studies.

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