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The role of functional properties of dietary fiber in the control of nutrient flow and intestinal health in pigs

by

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Dedicated to my kids

Nimra & Ayush

Abstract

Viscosity and fermentability are considered important properties of dietary fiber. Viscosity increases digesta viscosity and reduces digesta passage rate, nutrient digestion and absorption whereas fermentability increases fermentation into short chain fatty acids (SCFA). The objectives were to enhance the understanding of these properties of dietary fiber and their effects on digestive physiology using surgical models in pigs.

In study 1, three catheterized pigs fed 3 diets containing 0, 3, or 6% oat β-glucan concentrate (BG0, BG3, and BG6) in a repeated 3 × 3 Latin square. Pigs fed BG6 had a lower net glucose flux. Pigs fed BG6 tended to have lower portal C-peptide without lowered insulin. Pigs fed BG6 had lower portal glucose dependent insulinotropic peptide (GIP) and glucagon like peptide-1 (GLP-1), which in turn were correlated (R^2 = 0.81 and 0.88, respectively) with portal glucose. Pigs fed BG3 and BG6 had a higher net SCFA flux, indicating increased fermentation.

In study 2 and 3, ileal cannulated grower pigs were fed semi-purified diets supplemented with 5% purified non-starch polysaccharides (NSP) in a 2 (low and high viscous) × 2 (low, and high fermentable) factorial arrangement using cellulose (CEL), carboxymethylcellulose (CMC), and low and high viscous oat β-glucan (LBG and HBG, respectively). The CMC, LBG and HBG induced high ileal digesta viscosity coinciding with high nutrient digestibility; in contrast, CEL had lowest viscosity and nutrient digestibility. The CEL, LBG and HBG increased fecal SCFA. Linear discriminate analysis of NSP and TRFLP profiles and 16S

rRNA gene copy numbers of bacterial groups revealed that CMC resulted in distinctive bacterial communities. The gene copy number of butyryl-CoA CoA transferase was higher than for butyrate kinase, indicating that this pathway is dominant for butyrate production in pigs.

In study 4, similar dietary treatments were fed to catheterized pigs. High viscous and fermentable HBG increased net butyrate flux and insulin and GLP-1 production but did not affect net glucose flux.

Thus, the viscosity and fermentability are important functional properties of dietary fiber that better explained the physiological effects on kinetics of digestion, absorption, hormones responses, fermentation and thus nutrient flow and intestinal health.

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List of Abbreviations

AA	Amino acids
ADF	Acid detergent fiber
ADG	Average daily gain
AID	Apparent ileal digestibility
ANOVA	Analysis of variance
AOAC	American association of cereals chemists
ATTD	Apparent total tract digestibility
BG	β-glucan
BG3	3% β-glucan supplemented diet
BG6	6% β-glucan supplemented diet
BG0	0% β-glucan supplemented diet
BNC	Bayonet neill-concelman
BW	Body weight
CCAC	Canadian council on animal care
CEL	Cellulose
CEL-HF	Cellulose high fermentable
CEL-LF	Cellulose low fermentable
CMC	Carboxymethylcellulose
CP	Crude protein
CV	Coefficient of variance
DE	Digestible energy
DM	Dry matter

DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EE	Ether extract
GC	Gas chromatography
GE	Gross energy
GIP	Glucose- dependent insulinotropic peptide
GLC	Gas liquid chromatography
GLP-1	Glucagon-like peptide- 1
HBG	High viscous beta glucan
HSD	Honestly significant difference
IU	International unit
IV	Intravenous
LA	Lactic acid
LBG	Low viscous β-glucan
LC	Liquid chromatography
MJ	Mega joule
mRNA	Messenger ribonucleic acid
MRT	Mean retention time
MW	Molecular weight
N	Nitrogen
NDF	Neutral detergent fiber
NRC	National research council
NSP	Non starch polysaccharide

PC	Principal component
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RDP	Ribosomal database project
RIA	Radioimmunoassay
rpm	Rotations per minute
rRNA	Ribosomal ribonucleic acid
RS	Resistant starch
SC	Subcutaneous
SCFA	Short-chain fatty acid
SEM	Standard error of mean
TDF	Total Dietary fiber
TDMAC	Trido decyl methylammonium complex heparinate
TRF	Terminal restriction fragments
TRFLP	Terminal restriction fragment length polymorphism

Chapter 1. The role of cereal β-glucans in the control of nutrient flow and intestinal health. A review

1.1 Introduction

β-Glucans are non-starch polysaccharides (NSP) consisting of D-glucose units that can be isolated from wide variety of prokaryotic organisms such as algae, fungi, yeast, lichen, and cereals grains (Zekovic et al., 2005). The structure of glucan consists of linear (1→3) β-linked chains that are common among these organisms along with (1→4) β-units in cereal glucan (**Figure 1.1**), (1→2) β-units in bacteria and blue green algae, or (1→6) side chains in fungi, yeast, and lichen.

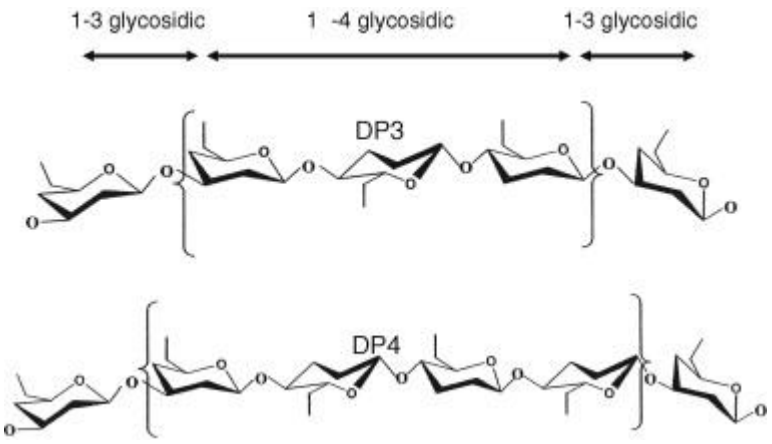

Figure 1.1 Structure of cereal (1 → 3, 1 → 4) β-glucan (Adapted from Vasanthan and Temelli, 2008)

β-Glucan content of cereals ranges from 1% in wheat to 3 to 7% in oats and 5 to 11% in barley (Skendi et al., 2003). Oat and barley β-glucans are similar in structure but differ in the ratio of (1→3) (1→4) linkages, molecular weight, and

thus solubility (Wood and Beer, 1998). The biological activity of β-glucan is related to the macromolecular structure; thus, β-glucan isolated from different sources has different functional characteristics. In human nutrition, cereal β-glucans are used as a functional food ingredient (Malkki, 2004) and the potential health benefits include reduction of bowel transit time (Feldheim and Wisker, 2003), prevention of constipation and thus reduction in risk of colorectal cancer (Hill, 1997), depression of glycemic index (Jenkins et al. 2008), lowering of blood cholesterol (German et al., 1996), promotion of beneficial gut microflora (Tungland, 2003), and increased production of short chain fatty acids (Wisker et al., 2000), biological response modifiers (Bohn and BeMiller, 1995), and immuno-modulators (Zekovic et al., 2005).

Goals pursued in human nutrition and swine production differs considerably. Longevity with excellent health is important for humans while maximum growth rate is the goal for swine producers. Thus, the importance of β-glucan differs between swine and human nutrition. β-Glucans are still under considerable debate, because their effects differ among various stages of swine age and maturity. In weanling pig diets, β-glucan incorporation has been considered beneficial, because systemic immunity was improved (Xiao et al., 2004) and lowered the incidence of sub-clinical or clinical infection and thereby improved growth performance (Dritz et al., 1995). In contrast, inclusion of cereals rich in β-glucan have been considered negative for weaned and grower–finisher pigs due to decreased nutrient digestibility (Bach Knudsen and Hansen, 1991). However, despite its negative effects on digestibility, feeding β-glucan-rich cereals has been

explored, because β-glucan may enhance intestinal health and may thus serve as an alternative for antibiotics as growth promotants.

This review provides an overview of cereal β-glucan, chemical and functional characteristics of dietary fiber, and the role of β-glucan in nutrient flow and intestinal health in swine nutrition. The dietary fiber definition, classification and analysis were also discussed. Finally, the role of cereal β-glucan in human metabolic and intestinal health using the pig model was investigated.

1.2 Definition, classification and analysis of dietary fiber

Definition

The term "dietary fiber" was first used to describe non-digestible plant cell wall constituents (Hipsley, 1953). Later, dietary fiber was related to health and a dietary fiber hypothesis was developed (Trowell, 1972; 1974; Walker, 1974; Painter, 1975). This hypothesis related low incidence of many chronic diseases (chronic bowel diseases, diabetes, coronary heart diseases, and colon cancer) in the human population to a high intake of dietary fiber. The original definition as "the components in plant cell walls that are resistant to all digestive enzymes secreted by any animal" (Trowell,1972) seemed too restrictive. Part of the definition has remained unchanged; however, the components of plant cell walls seemed inappropriate. Some materials that not part of cell walls are also resistant to digestive enzymes and this definition excluded polysaccharides that are used as food additives, e.g., plant gums and modified cellulose. Thus, the definition was

modified to “all polysaccharides and lignin that are resistant to hydrolysis by animal digestive enzymes” (Trowell et al., 1976).

The term dietary fiber was replaced with NSP (Cumming, 1981). Cellulose, hemicellulose, pectin, gum, mucilage, and lignin were included in the definition of dietary fiber (Asp and Johansson, 1984). Two important fiber analyses, neutral detergent fiber (NDF) and acid detergent fiber (ADF), were introduced to describe fiber (Van Soest and Wine, 1967). Two definitions of fiber were used: 1) a physiological, as dietary components resistant to degradation by mammalian enzymes, and 2) a chemical as “sum of NSP and lignin” (Theander et al., 1994).

To define dietary fiber relevant to human nutrition, the American Association of Cereals Chemists (AOCC) reviewed the earlier definitions from 1998 to 2000 and updated it, as “dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in human small intestine with complete or partial fermentation in the large intestine”. Dietary fiber included polysaccharides, oligosaccharides, lignin, and associated plant substances that promotes beneficial physiological effects including laxation and blood cholesterol and / or blood glucose attenuation (DeVries, 2003).

Recently, the CODEX committee on Nutrition and Food on Special Dietary Uses updated the definition of dietary fiber and defined as carbohydrates polymers (a) with 10 or more monomeric units (b), which are not hydrolyzed by the endogenous enzymes in the small intestine of human and belong to the following categories: Edible carbohydrate polymers, naturally occurring in the food as consumed; carbohydrate polymers which have been obtained from raw

food by physical, enzymatic, or chemical means and have been shown to have a physiological effect of benefit to health as demonstrated generally accepted scientific evidence to competent authorities; and synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities. Thus, this new definition was accepted.

In swine nutrition, most studies still define dietary fiber in terms of NDF or ADF, cellulose, hemicelluloses, and lignin. However, detailed composition of soluble and insoluble NSP as part of dietary fiber analysis was reported by few groups (Serena et al., 2008; Le Gall et al., 2009). Swine nutritionists should also define a common method of dietary fiber analysis in nutritional studies to better understand their physiological and metabolic effects.

Classification of dietary fiber

Chemical categorization divided dietary fiber into structural that includes non-cellulose polysaccharides, cellulose, and lignin and non-structural that includes pectin, gums, algal polysaccharides, and modified cellulose (Southgate, 1978). Solubility in water has been an important criterion to classify dietary fiber as soluble and insoluble dietary fiber. Insoluble dietary fiber constitutes cell wall components such as cellulose lignin and hemicellulose and soluble dietary fiber constitutes pectins, gums and mucilages. Their physiological function also differs; insoluble fiber decreases gastric transit time, increases feces bulk, makes feces softer, and thus is important in relieving constipation (Wenk, 2001). In contrast,

soluble fiber increases the viscosity of gastric contents and thus delays gastric emptying, decrease the rate of glucose absorption, enhances immunity and lowers serum cholesterol levels (Brennan, 2005). However, the classification using water-solubility does not fully explain the physiological response. Thus, dietary fiber requires classification that better explains physiological responses and thus metabolic and health benefits. Viscosity and fermentability are two important physio-chemical properties that could be potentially used as classification components and provide a link to physiological responses (Dikeman and Fahey, 2006).

Analysis of dietary fiber

Chemical analyses of feed started with the proximate analysis of feed (Henneberg and Stohmann, 1859). As a component, crude fiber is estimated after sequential extraction with diluted acid and alkali followed by a gravimetric determination of the residue. It is the oldest and most commonly used method to estimate fiber. However, major limitations exist with this analytic method, because the method measures only a small and variable fraction of fiber due to the solubilisation of the structural polysaccharides and lignin. The detergent method was introduced (Van Soest, 1963 a, b; Van Soest and Wine, 1967) resulting in the terms NDF and ADF. These methods measured the fraction of fiber that is insoluble in neutral detergents (NDF) (hemicellulose, cellulose and lignin) and insoluble in acid detergent fiber (ADF) (cellulose and lignin), allowing estimation of hemicellulose by difference. However, the detergent method has a major flaw,

because total fiber is underestimated since pectins and other components are soluble in NDF and are not recovered. Residual starch in high grain diets is another problem encountered in this method that can be overcome by amylase treatment in the NDF method (Schaller, 1976).

The most recent development in the methodology of determination of dietary fiber involved two approaches: the enzymatic-gravimetric method and the enzymatic-chemical method (Asp, 2001).

Enzymatic-gravimetric method. The concept of enzymatic-gravimetric method was first introduced by Williams and Olmstedt (1935). Later, a method was developed by Thomas (1972) and Hellendoorn et al. (1975). Gravimetric methods to measure both soluble and insoluble portions of dietary fiber were developed by Furda (1977, 1981), Schweizer and Wursch (1979, 1981) and Asp and Johansson (1984). Then finally the first version of the enzymatic-gravimetric method was developed (method 985.29; AOAC, 2006). The method involved enzymatic removal of starch and protein, then precipitation of soluble dietary fiber by aqueous ethanol, isolation, and the weighing of residue. The final step is correction of protein and ash in the residue (Asp and Johansson, 1984; Asp et al., 1992; Cho et al., 1997). The method was modified for insoluble and soluble fiber and simplified using 4–morpholine-ethanesulfonic acid-TRIS buffer (method 991.43; AOAC, 2006).

Enzymatic-chemical method. This method involves enzymatic removal of starch, extraction of low molecular weight sugars, and acid hydrolysis of dietary fiber polysaccharides and determination of their monosaccharide residues by gas

liquid chromatography, high performance liquid chromatography or calorimetry. The method was introduced by Widdowson and McCance (1935) and further developed by Southgate (1969 a,b) in an attempt to carry out sequential analysis all carbohydrates, i.e., starch and fiber components. For determination of soluble and insoluble dietary fiber, gas liquid chromatography (GLC) was introduced (Schweizer and Wursch, 1979). In the same year GLC was used in the Uppsala methodology and for soluble and insoluble components along with Klason Lignin (Theander and Aman, 1979). In 25 years, this method was developed further using advanced enzymes and procedures (Englyst and Hudson, 1987).

A review on dietary fiber analysis described importance and application of methodologies and suggested application related to cost and time of particular methodology (McCleary, 2003). Enzymatic-gravimetric method for food labeling and control purposes and enzymatic-chemical for research purposes was recommended (McCleary, 2003).

Since the update of the dietary fiber definition by CODEX Alimentarius, a new method to measure high and low molecular weight dietary fiber was standardized (McCleary et al., 2010). The new method is an enzymatic, gravimetric liquid chromatographic method that is applicable to plant materials, food, and food ingredients, is consistent with the 2008 CODEX definition, including natural occurring, isolated, modified, and synthetic polymers (method 2009.01). This method is for measurement of total dietary fiber (TDF) including resistant starch (RS) and low molecular weight non-digestible oligosaccharides of degree of polymerization ≥ 3. The first step of the method is to solubilize and

hydrolyze non-resistant starch to glucose and maltose using α-amylase and amyloglucosidase for 16 h at 37°C in a water bath. The reaction is terminated using heat and pH and protein is digested using protease. The high molecular weight dietary fiber, insoluble and precipitated soluble, is captured using ethanol or industrial methylated spirit, and then further washed with ethanol and acetone, dried, and weighed. Non-precipitated dietary fiber in the filtrate is quantified using liquid chromatography (LC) after concentrating, desalting through ion exchange resins and further concentrating.

1.3 Characteristics of β-glucan

β-Glucans of oat and barley occur in the wall of endosperm cells along with starch, matrix protein and lipids and occur to some extent in the aleurone (Brennan, 2005). Barley and oat β-glucan consists of linear (1→3) (1→4) β-linked chains. In the linear β-glucan chain, the (1→3) linkages occur singly whereas the (1→4) linkages are found mostly in sequence of 2 or 3 (Skendi et al., 2003). Thus, the molecule is considered as being composed of (1→3)-β-linked cellotriosyl and cellotetraosyl units (Wood, 2001). The oat β-glucan have generally higher molecular weight (MW) (0.065-3×10^6 g/mol) than barley (0.15-2.5×10^6 g/mol) (Beer et al., 1997; Lazaridou et al., 2004). The rheological properties of β-glucan are dependent on the ability of β-glucan to associate with water as determined by proportion of cellotriosyl and cellotetraosyl units, their arrangement, degree of polymerization and thus the MW (Wood, 2001).

1.4 Functional properties of β-glucan

β-Glucan has many functional properties such as water-holding capacity, adsorption of organic materials, cation exchange capacity (Brennan and Cleary, 2005). Solubility was considered very important, earlier; however, recently viscosity and fermentability were identified as two major characteristics responsible for its beneficial physiological functions (Dikeman and Fahey, 2006). Increased digesta viscosity after feeding soluble NSP such as guar gum, rye, and pectin modified digesta passage rate and decreased nutrient digestibility in pigs (Owusu-Asiedu et al., 2006; Serena et al., 2008; Le Gall et al., 2009). The dietary fiber is not digested by porcine enzymes but is instead fermented by the microbial community in distal small and large intestine, thereby producing short-chain fatty acids (Bach Knudsen and Hansen, 1991).

Viscosity

Viscosity was defined first by English scientist Sir Isaac Newton as the relationship between the flow of a fluid and force directed on that fluid and calculated by the equation as shear stress divided by shear rate. Newtonian flow or true viscous flow where shear rate is directly proportional to shear stress and thus resultant viscosity is independent of changes in shear rate and thus single point measurement is adequate to determine viscosity. However, most of fluids are non- Newtonian in nature and the viscosity is dependent on shear rate. The unit of viscosity is millipascal second (mPa•s) or centipoise (cP) whereas 1cP = 1mPa•s[10]. In relation to dietary fiber, viscosity is defined as the ability of the

dietary fiber to thicken when mixed with fluids (Dikeman and Fahey, 2006). This viscous behavior is due to the physical entanglements of the polysaccharides along with trapping water in between (Guillon and Champ, 2003). Viscosity has been described as dynamic viscosity, kinematics viscosity, relative viscosity, and apparent viscosity, etc.; however, apparent viscosity is the most common term used by researchers for dietary fiber effects. Soluble fiber such as β-glucans (oat and barley), arabinoxylans (wheat) and galactomannans (guar gum) increase the viscosity of the digesta when ingested. Along with solubility, MW, particle size, chemical composition and structure, temperature, pH and amount of moisture are associated with viscosity (Dikeman and Fahey, 2006). The MW of β-glucan has most influence on viscosity (Wood, 2007) and had inverse relations with glycemic and insulin responses (Wood et al., 1994). At a constant temperature, polysaccharide concentration directly affects viscosity (Ellis et al., 1995). The presence of β (1→3) linkages leads to kink in straight chain polymer, allows water to get in and form hydrogen bonds with water molecules and hydroxyl group of β-glucan and thus make it water soluble and viscous (Vasanthan and Temelli, 2008). Viscosity affects various physiological effects such as starch digestion, glucose absorption and thus insulin secretion, attenuation of blood lipids, and laxation (Dikeman and Fahey, 2006; Wood, 2007).

Fermentability

Fermentability is defined as the ability of the nutrient to be fermented by the microflora of the intestine, especially large intestine (Johansen et al., 1997). The

extent of fermentation of fiber depends partly on its solubility, because soluble fiber is generally more fermentable whereas insoluble fiber mainly adds bulk to the contents of intestine (Wong et al., 2006). The products of fermentation are SCFA that include acetate, butyrate, lactate, propionate, and valerate (Cummings, 1981). Along with SCFA, several gases such as CH_4, H_2, and CO_2 are formed during fermentation. The SCFA are rapidly absorbed from the large intestine and may provide up to 30% of the maintenance energy requirement of pigs (Yen et al., 1991). Along with the source of energy to whole body, SCFA, especially butyrate, is a source of energy for colonocytes and plays a major role in cell proliferation and differentiation (Wong et al., 2006), and is important in prevention of colon cancer in humans (Bornet et al., 2002).

1.5 β-Glucan and nutrient flow

Nutrient flow is defined as cascade of processes from food entering the mouth to complete nutrient absorption. Cereal β-glucans influences several aspects of the digestive process such as total extent of digestion (Bach Knudsen and Hansen, 1991), site of digestion (Wilfart et al., 2007), enzymatic activity (Hedemann et al., 2006), nutrient absorption (Bach Knudsen et al., 2000), and systemic and gastrointestinal hormonal responses (Ellis et al., 1995; Serena et al., 2009).

Cereal β-glucan decreases nutrient digestibility and absorption in pigs and thus may possibly limit growth (Bach Knudsen et al., 2000). These effects of β-glucan in pigs have therefore been viewed as negative. Cereal β-glucan in human

diets improved insulin and glucose metabolism (Jenkins et al., 2008) and is thus considered positive for metabolic and intestinal health (Wood, 2007). However, β-glucan also provides a nutritional advantage in pregnant sows that have restricted access to feed, because rate of gastric emptying is reduced and thereby prolongs the feeling of satiety following a meal and reduces the period of hunger (Vestergaard, 1997). These digestive and absorptive effects are attributed to the functional property of viscosity, because β-glucan binds with water and increases digesta viscosity (Serena et al., 2009).

Many studies explored the role of dietary fiber in pig diets (Wenk, 2000), including the role of purified β-glucan and cereals rich in β-glucan on metabolic and intestinal health (Wood, 2007). However, information directly related to β-glucan action on digestion and absorption in pigs or using pig as model is scarce. In this review, the impact of β-glucan on growth performance, nutrient digestibility, and site of digestion, effects of enzymes, nutrient absorption, and gastrointestinal and systemic hormones with purified or native β-glucan on pigs of different stages of growth was reviewed.

Growth performance

Cereal β-glucan decreased nutrient digestibility and thus growth performance in weaned and grower pigs (Back Knudsenet al., 1993). However, β-glucan has also been investigated as potential replacement for antibiotics diets for weaning pigs to maintain growth performance. In a study with triticale, wheat and oat-based diets (Hogberg and Lindberg, 2004), the influence of high and low levels of

NSP, β-glucan, and enzyme supplementation on growth performance in weaned pigs was studied. The average daily gain (ADG) was higher in pigs fed diets with a high NSP level that was probably caused by increased gut fill (Jorgensen et al., 1996) and increased weight of viscera (Kass et al., 1980; Jorgensen et al., 1996; McDonald et al., 1999). Other studies with cereal β-glucans indicated similar results. For example, pigs fed diets based on barley containing 4.8% β-glucan had a higher ADG than pigs fed diets based on barley containing 2.6% β-glucan (Jensen et al., 1998). In contrast, oat β-glucan decreased growth rate of grower pigs because it reduced nutrient digestibility (Bach Knudsen et al., 1993). However, oat β-glucan ranging from 1.6 to 4.1% (as-fed) did not affect performance of grower-finisher pigs (Fortin et al., 2003). Similarly, other studies with oat-based diets suggested that β-glucan did not reduce growth performance of pigs (Morris and Burrows, 1986; Brand and Van Der Merwe, 1996). Thus, β-glucan improved growth rate in weaned pigs at lower dietary doses and may decrease growth performance in grower and finisher pigs.

Gastric emptying and digesta transit

Gastric emptying rate determines the degree of distension and the release of nutrient in the duodenum (Lepionka et al., 1997). This rate is a highly controlled mechanism, and also depends on physical and chemical characteristics of gastric content (Mayer et al., 1994). Viscous dietary fiber reduces the gastric emptying rate in pigs (Rainbird and Low, 1986) as viscosity and water-holding capacity are important determinants of the gastric emptying rate (Torsdottir et al., 1991).

Cereal β-glucan increased digesta viscosity and water-holding capacity (Bach Knudsen and Hansen, 1993; Bach Knudsen et al., 2000) and thus could potentially affect gastric emptying rate. In rats, isolated oat β-glucan reduced gastric emptying rate (Begin et al., 1989). In pigs, the effect of oat bran and rolled oats on gastric emptying was measured using solid and liquid markers of digesta (Jorgensen et al., 1996). Results indicated delayed gastric emptying rate of liquid marker in proportion to the amount of β-glucan, similar to results on liquid gastric emptying rate using other soluble dietary fiber (Rainbird and Low, 1986; Miquel et al., 2001). In humans, gastric emptying rate was rapid after oat β-glucan intake (Hlebowicz et al., 2009). In addition to viscosity and water holding capacity, end products of fermentation SCFA also control gastric emptying rate (Ropert et al., 1996). The gastric emptying rate is important physiologically, because it affects nutrient digestion and absorption (Bray, 2002) and reduces aggression in feed restricted sows (Vestergaard, 1997). Another factor that affects nutrient digestibility is mean retention time or digesta passage rate that determines the contact time of substrate with enzymes (Wilfart et al., 2007). Soluble dietary fiber may increase mean retention time or decrease digesta passage rate (Owusu-Asiedu et al., 2006) and insoluble dietary fiber may decrease mean retention time (Le Goff et al., 2002). Studies with purified β-glucan are lacking; however, oat by-products decrease mean retention time (Potkins et al., 1991). The oat byproducts used in the study was insoluble and thus digesta bulk and its effect on colonic motility was the proposed mechanism (Kirwan et al., 1974). In human and rats, soluble oat fiber increased fecal wet weight and reduced total mean retention

time (Anderson et al., 1984; Chen and Raymond, 2008). Although indirect reports of soluble dietary fiber and these important variables exist, an understanding of effects of purified soluble β-glucan on gastric emptying and mean retention time is required.

Nutrient digestibility

Cereal β-glucan increased digesta viscosity after binding with water and decreased nutrient digestibility. Most studies in pigs evaluated nutrient digestibility using oat- or barley-based diets. The 4 different fractions oat β-glucan; 2 oat flours, oat groats and oat bran, were fed to ileal-cannulated grower pigs and results indicated that β-glucan as an integral part of oat fractions decreased nutrient digestibility (Bach Knudsen et al., 1993). The protein, energy and fat digestibility were lower in diets with oat bran. However, ileal digestibility of starch was almost 100% and thus not affected by high β-glucan content. With different barley varieties, complete degradation of starch at the end of ileum was reported (Jensen et al., 1998). Thus, β-glucan or other soluble dietary fiber could slow the rate of starch digestion without affecting digestibility. The digestibility of energy, protein and fat were negatively correlated with the intake of soluble oat β-glucan (Bach Knudsen and Hansen, 1991). Cereal β-glucan as major fraction of oat soluble NSP (Aspinall and Carpenter, 1984) decreased the carbohydrate and lipid digestibility by virtue of its property to increase viscosity (Chen and Anderson et al., 1986). Similarly, low apparent digestibility of organic matter (OM) was observed in the ileum, caecum, colon and rectum after feeding oat β-

glucan (Hogberg and Lindberg, 2004; Hogberg and Lindberg, 2006; Le Gall et al., 2009).

However, effects of β-glucan on nutrient digestibility in weaning pigs are different. The digestibility of DM, GE, CP, ether extract, Ca and P were linearly increased as the dietary concentrations of β-glucan increased in weaning pigs (Hahn et al., 2006). The positive correlation of digestible energy (DE) and total soluble β-glucan in different barley samples were reported; however correlation was negative with ADF and NDF (Fairbairn et al., 1999). Similarly, in sow's soluble oat based diets improved the energy digestibility with no differences for CP digestibility (Renteria-Flores et al., 2008). Two explanations may be related to these results, first, the delayed gastric emptying induced by oat (Schneeman et al., 1998) that improved digestive and absorptive efficiency (Davidson and McDonald, 1998) and/or second, β-glucan were fermented and thus improved digestibility of dietary fiber (Le Goff and Noblet, 2001).

β-Glucan was fermented to some extent in the small intestine by microbial population (**Table 1.1**). The ileal digestibility of β-glucan was 0.45-0.85 (Bach Knudsen et al., 1993), 0.64-0.88 (Bach Knudsen and Hansen 1991), 0.70 to 0.97 (Graham et al., 1986). The marked difference in digestibility of NSP, soluble and insoluble, at the end of the small intestine was reported (Bach Knudsen et al., 1991). The recovery of wheat NSP (insoluble) at the end of small intestine was nearly complete (82-104%) while there was a substantial loss in small intestine of oat NSP.

Table 1.1 Ileal and total tract digestibility coefficient of β-glucan.

	Digestibility coefficient	
Reference	Ileal	Total tract
Millard and Chesson, 1984	0.63	-
Graham et al., 1986	0.70-0.97	1.00
Rakowska et al., 1990	0.64-0.74	0.86-0.96
Bach Knudsen and Hansen, 1991	0.64-0.88	1.00
Bach Knudsen et al., 1993a	0.45-0.85	1.00
Inborr et al., 1993	0.34	-
Li et al., 1996	0.74-0.76	0.99
Pettersson and Lindberg, 1997	0.82-0.90	1.00
Jensen et al., 1998	0.48-0.58	1.00
Hogberg and Lindberg, 2004	0.63-0.73	0.89-0.93
Hogbergand and Lindberg, 2006	-	0.97-0.98
Le Gall et al., 2009	0.41-0.73	-

The depolymerisation of β-glucan begins in the stomach (Johansen et al., 1997) and high solubility of β-glucan makes this an easily degradable substrate by bacterial population colonizing distal part of small intestine (Bach Knudsen et al., 1993; Hogberg and Lindberg 2004).

In conclusion, digestibility of starch may not be affected by β-glucan (Bach Knudsen et al., 1993); however, digestibility of energy, protein and fat were negatively correlated with β-glucan content in grower pig diets (Le Gall et al.,

2009). However, β-glucan improved energy digestibility in the mature gastro-intestinal tract of sows (Le Goff and Noblet, 2001).

Site of digestion

The digestive process starts at duodenum and pancreatic and mucosal enzymatic digestion complete by the end of ileum. β-Glucan and any other undigested nutrient goes into large intestine for microbial digestion known as fermentation (Bach Knudsen et al., 1993). The end products of fermentation are SCFA that contributes to 15-20% of the digestible energy. Soluble β-glucan may affect the site of nutrient digestion and thus shifts the enzymatic digestion of nutrients to microbial fermentation.

Soluble NSP and undigested nutrients are fermneted in the small intestine and large intestine and serve as the main energy substrate for microbes (Bach Knudsen et al., 1991). This process is known as fermentation and the end products are lactic acid (LA) and SCFA such as acetic acid, propionic acid and butyric acid (Latymer et al., 1990). LA is the main product of fermentation in the stomach and small intestine while SCFA are important end products in the large intestine (Clemens et al., 1975; Hogberg and Lindberg, 2004).

Increased production of SCFA are associated with improved intestinal health as SCFA favors production of beneficial microbes such as lactobacilli (Snart et al., 2006) and reduce undesirable bacteria in large intestine (van Der Wielen et al., 2000). The SCFA are absorbed from the intestine and contribute to the

maintenance energy (Bach Knudsen and Hansen, 1991). Apart from supplying energy, SCFA also have other specific effects which will be detailed later.

Digestive enzymes activity

β-Glucan decreases nutrient digestibility and absorption through several mechanisms which may include altering digestive enzyme activity. There are no direct reports of β-glucan on enzymatic activity in pigs; those related to soluble dietary fiber will be discussed. Most of the earlier reports on effects of dietary fiber on enzyme activity used *in vitro* techniques following incubation with purified or non purified fiber sources (Schneeman and Gallaher, 1985; Isaksson et al., 1982). In most cases, enzyme activity was reduced either by non specific binding of the enzymes or presence of specific enzyme inhibitors (Dunaif and Schneeman, 1981). In "*in vivo"* studies using pigs and rat, soluble dietary fiber increased the brush border enzyme activity (Chun et al., 1989; Hedemann et al., 2006).

Glucose absorption

The effects of β-glucan from oat and barley have been studied extensively in humans in relation to glucose metabolism and insulin responses using glucose measurements in peripheral blood (Wood et al., 1994; Panahi et al., 2007; Jenkins et al., 2008). β-Glucan decreased peak glucose flux by increasing viscosity and water-binding capacity of gastrointestinal contents, (Bach Knudsen et al., 2005) and reducing gastric emptying rate (Darwiche et al., 2003). β-Glucan interferes

with enzymatic digestion and mucosal absorption (Isaksson et al., 1982) by formation of the unstirred water layer (Eastwood and Morris, 1992) and thereby decreases transport of glucose to the enterocytes. The glucose responses are related to viscosity of β-glucan and depend on concentration and molecular weight (Wood et al., 1994). The FDA has adopted a recommendation of 3 g/day of β-glucan for nutritional effects (FDA, 1997). Dietary β-glucan may improve glucose metabolism (Jenkins and Jenkins, 1994) and is thus important approach for the prevention and treatment of diabetes. For the quantitative measurements of kinetics of glucose absorption, advanced surgical models are required (Bach Knudsen et al., 2000; 2005). For example, the porto-arterial catheterization model allows collection of blood samples from the portal vein and carotid artery and blood flow measurements and thus following glucose analyses the calculation of net glucose absorption (Rerat et al., 1980). Feeding of oat-based diets did not affect net glucose absorption (Bach Knudsen et al., 2000). Instead, viscous dietary fiber reduced glucose absorption (Nunes and Malmlof, 1992; Ellis et al., 1995). Similarly, feeding soluble dietary fiber lowered glucose absorption rates in sows (Serena et al., 2009). Thus, the model provided further evidence that β-glucan decreases peripheral glucose concentrations and net glucose absorption and rate.

Insulin response

Insulin responses are directly linked to glucose responses and are generally assessed with plasma insulin. Dietary β-glucan decreased insulin responses in healthy humans (Juvonen et al., 2009; Nazare et al., 2009), obese human subjects

(Kim et al., 2009), and type II diabetes patients (Liatis et al., 2009). However, results are not consistent, because insulin responses remained unaffected after consumption of oat or rye β-glucan in human (Tappy et al., 1996; Frank et al., 2004) and animal studies (Bach Knudsen et al., 2000; Bach Knudsen et al., 2005). Peripheral plasma insulin may not represent insulin secretion accurately, because the liver extracts substantial amounts of insulin (Polonsky and Rubenstein, 1984). The C-peptide that is released in equimolar amounts from proinsulin in the pancreas is considered as a more reliable indicator of insulin release into systemic blood, because C-peptide has a lower hepatic extraction and renal excretion than insulin (Polonsky and Rubenstein, 1984). The lower insulin response after feeding soluble dietary fiber was mainly due to reduced glucose absorption that directly reduced pancreatic insulin release (Ellis et al., 1995) or indirectly through effect on incretins, i.e., glucose-stimulated insulinotropic peptides that include glucose dependent insulinotropic peptide (GIP) and Glucagon like peptide (GLP-1) (Rehfeld, 1998).

Incretins and satiety

GLP-1 is secreted from enteroendocrine L-cells, which are located mainly in the distal ileum and colon. In contrast, GIP is released from enteroendocrine K cells (cells) located in the small intestine in response to luminal glucose (Baggio and Drucker, 2007). However, endocrine cells that produce GLP-1 or GIP, and cells that produce both peptides, can be found throughout all regions of the porcine, rat, and human small intestine. Both cells are open-type intestinal

epithelial endocrine cell that come directly in contact with luminal nutrients through its apical surface and are thus stimulated by a variety of nutrient, neural, and endocrine factors. This incretin effect may stimulate 70% of glucose-dependent insulin secretion (Holst, 2009).

Both GLP-1 and GIP enhance glucose-dependent insulin secretion, promote β-cell proliferation and inhibit β-cell apoptosis, and thus increase pancreatic β-cell mass (Baggio and Drucker, 2007) **(Table 1.2).** Recently, incretins have been studied as potential therapy for type-II diabetes (Holst, 2009). The viscous oat bran may decrease the early postprandial GIP flux in humans (Juvonen et al., 2009) and subsequently lower insulin release (Gault et al., 2003). Few swine studies with soluble dietary fiber also reported decrease in GIP (Nunes and Malmlof, 1992; Ellis et al., 1995). In contrast, fermentable dietary fiber increased secretion of GLP-1 in intestinal mucosa and blood (Reimer and McBurney, 1996; Cani et al., 2004) via to two mechanisms: 1) an increase in colonic mass and 2) an increase in gene expression of the proglucagon mRNA precursor of GLP-1. The oat β-glucan has been reported to increase GLP-1 after 14 weeks of treatment (Greenway et al., 2007) and may be related to high fermentability of β-glucan and thus production of butyrate that may stimulate expression of proglucagon mRNA (Drozdowski et al., 2002). However, the significance of incretins and effect of cereal β-glucan has not been established. The role of dietary fiber on satiety through various mechanisms is well known.

Table 1.2 Role of GIP and GLP-1 in various tissues that contribute incretin effect and glucose homeostasis[1]

Incretin	Organ	Effect
GIP	Pancreas	↑ insulin production
		↑ insulin biosynthesis
		↑ β-cell proliferation
		↓ β-cell apoptosis
	Adipose tissue	↑ lipogenesis
GLP-1	Pancreas	↑ insulin production
		↓ glucagon secretion
		↑ insulin biosynthesis
		↑ β-cell proliferation
		↓ β-cell apoptosis
	Liver	↓ glucose production
	Stomach	↓ gastric emptying
	Brain	↓ appetite
	Muscle	↑ glucose uptake and storage

[1]Baggio and Drucker, 2007

Fiber intake slows gastric emptying, lowers plasma insulin, decreases absorption of nutrients, increases products of fermentation SCFA and modifies release of satiety-inducing gastrointestinal peptides (Anderson et al., 2009). The gut hormones involved in satiety are cholecystokinin, GLP-1, ghrelin, and peptide YY (Cummings and Overduin, 2007). This satiety effect is important in sows,

because incorporation of fibrous ingredients reduced activity and stereotyped behavior (Robert et al., 1993; Matte et al., 1994).

1.6 β-Glucan and systemic immunity

The association of β-glucan with immunity has a long history. β-Glucans extracted from various mushrooms and fungi have been used for centuries as medicines to improve immunity in Asia and Eastern Russia (Zekovic et al., 2005). Fungal and yeast β-glucan are biological response modifiers (Bohn and BeMiller, 1995). The (1→3, 1→6) β-glucan increased specific and non-specific immunity in pigs (Xiao et al., 2004). β-Glucan increased the host resistance to various pathogens such as bacteria (Reynolds et al., 1980), virus (Williams and Luzio, 1980), fungal (Browder et al., 1984) and parasitic infections (Goldman and Jaffe, 1991). β-Glucan stimulated non-specific immunomodulation such as activity of macrophages (Konopski et al., 1993), neutrophils (Vetvicka et al., 1996), natural killer cells (Duan et al., 1994), T cells (Sakurai et al., 1992), and B cells (Soltys et al., 1994). Binding of β-glucan to specific receptor sites on the cell surface membrane activates these immune cells (Brown and Gordon, 2001; Vetvicka et al., 1996). Cereal β-glucan was also investigated as immunomodulater *in vitro* and *in vivo* in animal models (Estrada et al., 1997) and may increase secretion of IL-1 (Abel and Czop, 1992; Estrada et al., 1997; Yun et al., 2003). β-Glucan from oats induced secretion of IL-2, IFN-gamma and IL-4 cytokines from spleen cell cultures, important mechanisms of protection against fungal infection.

However beneficial effects are modulated by factors such as hygienic conditions of the farm, age of animals and dose of β-glucan in the diet. This is the reason why others did not observed any effects of β-glucan in pigs (Dritz et al., 1995).

Since 1940, research related to β-glucan and immunity has been abundant and focuses mainly on exploring effects of various types of β–glucan extracted from yeast or fungi against different pathogens in various animal models. However, role of cereal β-glucan in immunity in pigs has not established and needs further investigation.

1.7 β-Glucan and intestinal health

The concept of gut health is not well defined and roughly consists of three major components: 1) diet, 2) mucosa and 3) commensal flora. These components interact to form a dynamic equilibrium to ensure normal functionality of the digestive tract (Montagne et al., 2003) **(Figure 1.2).** Dietary fiber and thus β-glucan are dietary approaches to change the microflora, because β-glucan serves as a substrate for fermentation in distal small and large intestine. Changes in microbial community affect the gut mucosa and thus gut health directly or indirectly via end products of fermentation such as SCFA.

Intestinal morphology

The swine digestive tract consists of mouth, esophagus, stomach, small intestine and large intestine along with salivary glands, liver and pancreas. The

intestinal wall has four major layers: the mucosa, sub mucosa, the muscularis and serosa. The process of small intestine turnover is dynamic process of crypt cell proliferation, migration along the crypt villus axis and then cell death from surface via apoptosis.

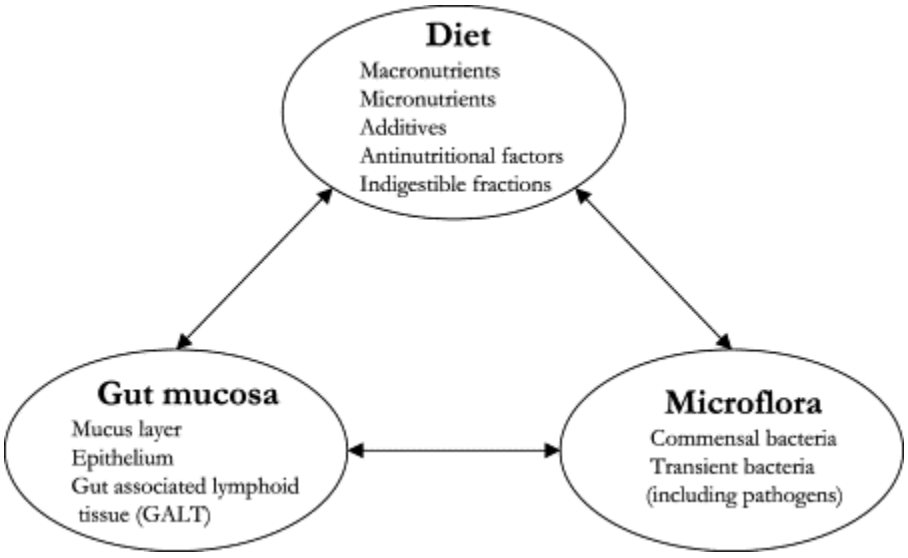

Figure 1.2 Schematic representations of contributions of diet, gut mucosa and microflora for gut health (Adapted from Montagne et al., 2003).

The villus height/ crypt depth ratio is an important parameter of intestinal morphology and is directly related to the digestion and absorption process. The absorptive capacity of small intestinal epithelium is linked with the villus height/ crypt depth ratio. Short villi lead to absolute loss of intestinal surface area and mature absorptive cell and thus decreased sodium and water absorption. This is more important in weaning pigs as they are not capable of fluid absorption to prevent clinical diarrhea and dehydration (Nabuurs, 1998). There are few studies directly studying the effect of β-glucan on intestinal morphology and thus, the present discussion has to rely on studies using soluble viscous fiber.

The effects of soluble dietary fiber in pigs are not consistent and depend on physico-chemical characteristics of fiber, their concentration and most importantly the age of animal (Montagne et al., 2003). In weaning pigs, studies on effects of low and high viscous carboxymethylcellulose (CMC) on intestinal morphology reported shorter intestinal villi and width of small muscle layer with high viscous CMC (McDonald et al., 2001). The crypt depth was higher for both viscous CMC resulting in lower villus height/ crypt depth ratios in high viscous CMC fed group with no difference in low viscous and control fed groups concluding that viscosity is beneficial up to some threshold and then it reduced the ratio and thus absorptive capacity of the intestine. The insoluble fiber (corn cobs) increased the villus height/ crypt depth ratio due to increased villus height in upper jejunum as compared to soluble fiber and control diets (Van Nevel et al., 2006). The fermentable non viscous fiber (wheat straw), increased villus width and crypt depth in jejunum and ileum (Jin et al., 1994). Along with crypt depth, there was increased rate of cell proliferation and cell death supporting the hypothesis that fiber increased the turnover of intestinal mucosa. In contrast, there was no effect of either soluble or insoluble fiber in villus height/ crypt depth in newly weaned pigs as both villi height and crypt depth were reduced in soluble fiber (pectin) fed pigs (Hedemann et al., 2006). However, the number of villi and crypt per millimeter of intestine were increased in pectin fed pigs indicating increased absorptive surface area. The number of native mitosis indicator of epithelial cell proliferation was reduced by high fiber feeding in the same study. However, others did not observe any effect of fiber on villus height/ crypt depth

ratio or intestinal morphology (Bikker et al., 2006). A recent study in sows with high fiber diets with more soluble fiber, there was only an increase in crypt depth with no difference in mitotic count (Serena et al., 2008b).

Both viscosity and fermentability have been linked with the possible mechanisms of dietary fiber to change intestinal morphology in terms of change in villus height/ crypt depth ratio and increased turnover of intestinal mucosa. The presence of high viscous digesta may increase the villus atrophy due to increase rate of villus cell losses which lead to increases crypt cell production generally with increased crypt depth (Montagne et al., 2003). Similarly the products of fermentation, especially butyrate had trophic effects on intestinal cell and thus stimulation of cell proliferation (Blottieres et. al., 1999).

The goblet cells in the intestinal mucosa secrete mucus that contains mucin (glycoprotein) that protects the gut from physical, chemical and infectious agents (Lien et al., 2001). Mucus is continuously removed from lumen by physical, chemical and enzymatic erosion and synthesized and secreted as well from goblet cells. The dietary fiber acts on mucous in both ways as some fibers increase the excretion of mucous, and then compensatory increase in mucous production via increase in goblet cells. The linear increased in mucin in ileal digesta after feeding pea fiber in pigs was reported (Lien et al., 2001). Insoluble fiber like wheat straw, corn cobs and wood cellulose also increased the excretion of mucin (Mariscal-Landin et al., 1995). The abrasive action of insoluble fiber caused this increased excretion which is compensated by increased production as observed in rats (Montagne et al., 2003). Contrary to insoluble fiber, soluble fiber e.g. pectin

decreased the mucin production - mainly acidic and sulfated mucin subtypes (Hedemann et al., 2006). The change in mucin composition is related to its maturity and thus immunity as sulfomucins are more mature and protect against bacterial infection (Deplancke and Gaskins, 2001). The pectins decreased the sulfomucins and thus pectin fed pigs were more susceptible to infection. This increased immature mucin was an outcome of increased epithelial cell turnover (Specian and Oliver, 1991). In sows there was no effect of dietary fiber on mucin characteristics (Serena et al., 2008b).

So concluding the effect of dietary fiber on intestinal morphology, it seems that it depends on individual fiber, its dose and age of the animal but still there is lot to be studied to clearly define its effect and possible mechanism.

Microbial profile

The gastrointestinal tract of pigs contains aerobic and facultative strict anaerobic bacteria species that vary within segment of the tract (Leser et al., 2002), age, physiological state, and diet composition especially type and amount of dietary fiber (Varel, 1987). Many studies using culture or phenotypic analysis showed that most of the bacteria are gram positive strict anaerobic *Eubacterium*, *Fusobacterium*, *Clostridium*, *Peptostreptococcus,* while the *Bacteriodes* cluster constitute the gram negative bacteria (Allisson et al., 1979, Moore et al., 1987; Robinson et al., 1981). The bacterial density ranges from 10^5 - 10^6 in stomach to 10^{10} - 10^{11} per gram of digesta in large intestine. Although the main site of bacterial colonization is caecum and colon, there are significant amounts of

bacterial colonization in the stomach and small intestine of pigs (Jensen et al., 2001). In the gastrointestinal tract, bacteria exist in different microhabitats, mucus layer, mucosal surface and the lumen (Salanitro et al., 1977) and vary between these microhabitats (Pryde et al., 1999). However, mucus- and mucosal-associated microflora is assumed to be subset of the luminal flora because of normal mucus excretion, epithelial turnover and peristaltic movements in the gastrointestinal tract (Leser et al., 2002). The stomach and small intestine contain low number of bacteria that typically adhere to mucus layer or mucosal surface that includes species lactobacilli, streptococci, clostridia and enterobacteria (Jensen, 2001). Recent studies using molecular tools confirmed the presence of mainly gram positive lactobacilli along with clostridia and even absence of bifidobacteria in stomach and small intestine (Leser et al., 2002). In contrast, large intestine, caecum and colon are mainly colonized by gram negative bacteria such as clostridium cluster IV and XIV and *Bacteriodes* (Leser et al., 2002). The physiological significance of commensal microbiota has been well established in animal and human health. The energy and essential vitamins contribution are some of the important role of commensal bacteria (Bach Knudsen, 2001). The commensal bacteria ferments dietary fiber into SCFA and there are significant roles for SCFA in intestinal health and discussed later.

Cereal β-glucan have prebiotic properties because they are not digested by endogenous enzymes, and pass into the distal small intestine and large intestine, serve as a substrate for microbial fermentation, and selectively stimulate the growth and activity of a small number of beneficial bacteria (Gibson et al., 2004).

Oat and barley β-glucan have a prebiotic effect in pigs, rats, and humans. In rats, oat bran and purified β-glucan increase the content of *Lactobacilli* and *Bifidobacteria* in fecal flora (Jaskari et al., 1998; Snart et al., 2006) because β-glucan serves as a substrate for lactobacilli and bifidobacteria (Jonsson and Hemmingsson, 1991). The high ileal lactobacilli count and butyrate producing microorganisms with purified β-glucan was demonstrated by Pieper et al., (2008). Recently, a study investigated the effect of oat and barley with or without enzyme supplementation on lactobacilli and Bifidobacteria and showed that oat based diets, because of more insoluble β-glucan than barley based diet, resulted in greater beneficial microflora (Reilly et al., 2010). Moreover, in the same study it was indicated that endogenous β-glucan supported a higher number of lactobacilli than exogenous purified β-glucan. Cereal β-glucan can thus serve as a prebiotic for changing the microbial community to improve gut health.

In addition to the beneficial effects of dietary fiber on intestinal health, selection of specific fiber fractions may also stimulate overgrowth of pathogenic bacteria. For instance, dietary inclusion of highly viscous CMC has been shown to increase fecal shedding of enterotoxigenic *Escherichia coli* in weaned pigs (Hopwood et al., 2002). The secretion of virulence factors such as heat-labile (LT), heat-stable STa and STb by enterotoxigenic *Escherichia coli* and or heat stable EAST1 by enteroaggregative *Escherichia coli* are responsible for pathogenic effects (Fleckenstein et al., 2010).

Microbial metabolites

β-Glucan is major energy substrate for microbial fermentation (Bach Knudsen et al., 1991) in the small intestine and mainly large intestine. The end-products are lactic acid and SCFA such as acetic acid, propionic acid and butyric acid (Wong et al., 2006). Lactic acid is the main product of fermentation in the stomach and small intestine while SCFA are important end-products in the large intestine (Clemens et al., 1975; Hogberg and Lindberg, 2004). An increased production of SCFA is related to improved gut health. The acidic pH of SCFA favored production of beneficial microbes (Snart et al., 2006) and reduced undesirable bacteria in large intestine (Van der Wielen et al., 2000). The produced SCFA are absorbed by the epithelium and can contribute to the energy requirement (Bach Knudsen and Hansen, 1991). Apart from supplying energy and promoting beneficial microbes such as *Lactobacilli* and B*ifidobacteria*, these SCFA also have specific functions. For example, butyric acid has important role in the metabolism, structure, and function of the colonic epithelium (Wong et al., 2006) and propionate has role in hepatic metabolism (Chen and Anderson, 1986).

The feeding of oat fractions rich in β-glucan increased the concentrations of lactic acid in the stomach and small intestine and of SCFA in the large intestine (Bach Knudsen et al., 1991, 1993). The proportion of butyric acid (Bach Knudsen et al., 1993) and propionic acid were higher after feeding oat- and barley-based diets (Hogberg and Lindberg, 2004, 2006). Increased dietary content of soluble β-glucan favored the growth of lactobacilli (Pluske et al., 2001) that may have increased the production of lactic acid in the stomach and small intestine

(Hogberg and Lindberg, 2004). The feeding of a diet containing oat and other soluble fiber increased the production of SCFA in gastrointestinal tract and thereby increased portal vein SCFA especially butyrate (Bach Knudsen et al., 2000; Bach Knudsen et al., 2005; Serena et al., 2009). Acetate is the principal SCFA. Acetate is absorbed and transported to the liver and energy substrates for muscle and cholesterol synthesis in adipose and mammary tissues (Wong et al., 2006). Propionate is a substrate for hepatic gluconeogenesis and inhibits cholesterol synthesis in the liver (Hamer et al., 2008). The increased butyrate flux is important because butyrate is a major source of energy for colonic mucosa (Roediger, 1982), and an important link to gut health via stimulation of cell proliferation (Comalada et al., 2006), promotion of apoptosis, and prevention of colon cancer (Hamer et al., 2008). In summary, cereal β-glucan increase production of SCFA and thus improves intestinal and metabolic health in humans (Wong et al., 2006); however, the importance in swine still needs to be established.

1.8 Conclusions

The inclusion of β-glucan in human diets as a functional food has been explored extensively. The effects on glucose, insulin reponses have been established in human and animal models. In swine production, β-glucan might be less advantageous due to its negative effect on digestibility of nutrients and thus growth rate of pigs. However, there are potential effects of cereal β-glucan on intestinal morphology, microbiota, and bacterial metabolites and thus intestinal

health and these potential benefits of β-glucan cannot be ignored. Proof for these benefits may require a reconsideration of the role and significance of β-glucan in swine nutrition.

1.9 Overall objectives

The hypothesis of the research project was that viscosity and fermentability are important fiber characteristics that affect nutrient digestibility, fermentability, digesta kinetics, and nutrient absorption and hormone responses in pigs. The approach taken was to use sophisticated surgical models such as ileal-cannulated pigs and porto-arterial catheterized pigs.

The research project was designed with following objectives:

1. To develop a porto-arterial catheterization model and to test the effectiveness of this model for nutrient absorption for long-term studies in swine (Chapter 2).
2. To study the effect of 0, 3, and 6% oat β-glucan on net glucose flux, insulin and incretin secretion, and net SCFA flux (Chapter 3).
3. To clarify the association between glucose and hormones after feeding varying concentrations of β-glucan (Chapter 3).
4. To understand the independent and interactive effects of the 2 functional properties (viscosity and fermentability) of dietary fiber in semi-purified diets on nutrient digestibility, digesta characteristics and passage rate, fermentation, and N retention in ileal-cannulated grower pigs (Chapter 4).

5. To understand the independent and interactive effects of the 2 functional properties (viscosity and fermentability) of dietary fiber in semi-purified diets on the ileal and fecal bacterial community, butyrate production pathway genes, and the occurrence of virulence factor genes of swine-pathogenic *E. coli* in ileal-cannulated grower pigs (Chapter 5).
6. To understand the independent and interactive effects of viscosity and fermentability on net flux of glucose and SCFA and apparent production of insulin and GLP-1(Chapter 6).

1.10 References

Abel, G., and J. K. Czop. 1992. Stimulation of human monocytes β-glucan receptors by glucan particles induces production of TNF-α and IL-1 β. Int. J. Immunopharmacol. 14:1363- 1373.

Allison, M. J., I. M. Robinson, J. A. Bucklin, and G. D. Booth. 1979. Comparison of bacterial populations of the pig cecum and colon based upon enumeration with specific energy sources. Appl. Environ. Microbiol. 37:1142-1151.

Anderson, J. W., L. Story, B. Sieling, W. J. Chen, M. S. Petro, and J. Story. 1984. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. Am. J. Clin. Nutr. 40:1146-1155.

Anderson, J. W., P. Baird, R. H. Davis Jr, S. Ferreri, M. Knudtson, A. Koraym, V. Waters, and C. L. Williams. 2009. Health benefits of dietary fiber. Nutr. Rev. 67:188-205.

AOAC. 2006. Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Arlington, VA.

Asp, G., and C. G. Johansson CG. 1984. Dietary fibre analysis. Nutr. Abst. Rev. 54:735-752.

Asp, N. G., T. F. Schweizer, D. A. T. Southgate, and O. Theander. 1992. Dietary fiber analysis. In Dietary fibre- a component of food. Nutritional Function in health and Diseases. 1992. pp 57-102(TF Schweizer and CA Edwards, editors). London: Springer- Verlag.

Asp, N. G. 2001. Development of dietary fibre methodology. In Advances dietary fiber technology. 77-88. (McCleary, B. V., and L. Prosky, editors) Oxford: Blackwell Science Ltd.

Aspinall, G. O., and R. C. Carpenter. 1984. Structural investigations on the non-starchy polysaccharides of oat bran. Carbohydrates Polymers 4:271-282.

Bach Knudsen, K. E. 2001. Development of antibiotic resistance and options to replace antimicrobials in animal diets. Proc. Nutr. Soc. 60:291-299.

Bach Knudsen, K. E., A. Serena, A. K. Kjaer, H. Jorgensen, and R. Engberg. 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135:1696-1704.

Bach Knudsen, K. E., and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 1. Digestibility and bulking properties of polysaccharides and other major constituents. Br. J. Nutr. 65:217-232.

Bach Knudsen, K. E., B. B. Jensen, and I. Hansen. 1993. Digestion of polysaccharides and other major components in the small and large intestine fed on diets consisting of oat fractions rich in β-D-Glucan. Br. J. Nutr. 70:537-556.

Bach Knudsen, K. E., H. Jorgensen, and N. Canibe. 2000. Quantification of the absorption of nutrients derived from carbohydrate assimilation: Model experiment with catheterised pigs fed on wheat- or oat-based rolls. Br. J. Nutr. 84:449-458.

Baggio, L. L., and D. J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131-2157.

Beer, M. U., P. J. Wood., and J. Weisz. 1997. Molecular weight distribution and (1→3) (1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chem. 74:705-709.

Begin, F., C. Vachon, J. D. Jones, P. J. Wood, and L. Savoie. 1989. Effect of dietary fibers on glycemia and insulinemia and on gastrointestinal function in rats. Can. J. Physiol. Pharmacol. 67:1265-1271.

Bikker, P., A. Dirkzwager, J. Fledderus, P. Trevisi, I. le Huerou-Luron, J. P. Lalles and A. Awati. 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84:3337-3345.

Blottieres, H. M., M. Champ, C. Hoebler, C. Michel and C. Cherbut. 1999. Production and digestive effects of short chain fatty acids. Sci. Aliment. 19:269–290.

Bohn, J. A., and J. N. BeMiller. 1995. (1→3) – β-D-Glucan as biological response modifiers: A review of structure – functional activity relationships. Carbohydrate Polymers 28:3-14.

Bornet, F. R., F. Brouns, Y. Tashiro, and V. Duvillier. 2002. Nutritional aspects of short-chain fructooligosaccharides: Natural occurrence, chemistry, physiology and health implications. Dig. Liver Dis. 34:S111-S120.

Brand, T. S., and J. P. Van der Merwe. 1996. Naked oats as a substitute for maize in diets for weaning and grower- finisher pigs. Anim. Feed Sci. Technol. 57:139-147.

Bray, G. A. 2002. The underlying basis for obesity: Relationship to cancer. J. Nutr. 132:3451S-3455S.

Brennan, C. S., and L. J. Cleary. 2005. The potential role of cereal (1→3, 1→4)-β-D-glucans as functional food ingredients. J. Cereal Sci. 42:1-13.

Brennan, C. S. 2005. Dietary fibre, glycaemic response, and diabetes. Mol. Nutr. Food Res. 49:560-570.

Browder, I. W., D. L. Williams, A. Kitahama, and N. R. Di Luzio. 1984. Modification of post- operative C. albicans sepsis by glucan immunostimulation. Int. J. Immunopharmacol. 6:19-26.

Brown, G. D., and S. Gordon. 2001. Immune recognition. A new receptor for beta glucan. Nature 413:36-37.

Cani, P. D., C. Dewever, and N. M. Delzenne. 2004. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br. J. Nutr. 92:521-526.

Chen W. J. L. and J. W. Anderson. 1986. Hypocholesterolemic effects of soluble fibers. In dietary fiber. Basic and Clinical Aspects, pp 275-286 (G.V. Vahouny and D. Kritchevsky, editors) New York: Plenum Press.

Chen, J., and K. Raymond. 2008. Beta-glucan in the treatment of diabetes and associated cardiovascular risks. Vasc. Health. Risk Manag. 4:1265-1272.

Cho, S., J. DeVries, and L. Prosky. 1997. Dietary fibre Analysis and Applications. Gaithesburg, MD: Association of Official Analytical Chemists International.

Chun, W., T. Bamba, and S. Hosoda. 1989. Effect of pectin, a soluble dietary fiber, on functional and morphological parameters of the small intestine in rats. Digestion 42:22-29.

Clemens, E. T., C. E. Stevens, and M. Southworth. 1975. Sites of organic acid production and pattern of digesta movement in the gastrointestinal tract of swine. J. Nutr. 105:759-768.

Comalada, M., E. Bailon, O. de Haro, F. Lara-Villoslada, J. Xaus, A. Zarzuelo and J. Galvez. 2006. The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. J. Cancer Res. Clin. Oncol. 132:487-497.

Cummings, D. E., and J. Overduin. 2007. Gastrointestinal regulation of food intake. J. Clin. Invest. 117:13-23.

Cummings, J. H. 1981. Dietary fibre. Br. Med. Bull. 37:65-70.

Darwiche, G., O. Bjorgell, and L. O. Almer. 2003. The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy subjects. BMC Gastroenterol. 3:12.

Davidson, M. H., and A. McDonald. 1998. Fiber forms and functions. Nutr. Res. 18:617-624

Deplancke, B., and H. R. Gaskins.2001. Microbial modulation of innate defense: Goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73: 1131S-1141S.

DeVries, J. W. 2003. On defining dietary fibre. Proc. Nutr. Soc. 62:37-43.

Dikeman, C. L., and G. C. Fahey. 2006. Viscosity as related to dietary fiber: A review. Crit. Rev. Food Sci. Nutr. 46:649-663.

Dritz, S. S., J. Shi, T. L. Kielian, R. D. Goodband, J. L. Nelssen, M. D. Tokach, M. M. Chengappa, J. E. Smith, and F. Blecha. 1995. Influence of dietary beta-glucan on growth performance, nonspecific immunity, and resistance to streptococcus suis infection in weanling pigs. J. Anim. Sci. 73:3341-3350.

Drozdowski, L. A., W. T. Dixon, M. I. McBurney, and A. B. Thomson. 2002. Short-chain fatty acids and total parenteral nutrition affect intestinal gene expression. JPEN J. Parenter. Enteral Nutr. 26:145-150.

Duan, X., M. Ackerly, E. Vivier and P. Anderson. 1994. Evidence for the involvement of the β- glucan – binding cell surface lectins in human natural killer cell function. Cell Immunol. 157:393-402.

Dunaif, G., and B. O. Schneeman. 1981. The effect of dietary fiber on human pancreatic enzyme activity in vitro. Am. J. Clin. Nutr. 34:1034-1035.

Eastwood, M. A., and E. R. Morris. 1992. Physical properties of dietary fiber that influence physiological function: A model for polymers along the gastrointestinal tract. Am. J. Clin. Nutr. 55:436-442.

Ellis, P. R., F. G. Roberts, A. G. Low, and L. M. Morgan. 1995. The effect of high-molecular-weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in the growing pig: Relationship to rheological changes in jejunal digesta. Br. J. Nutr. 74:539-556.

Englyst, H. N., and G. Hudson. 1987. Colorimetric method for routine measurement of dietary fibre as non- starch polysaccharides. A comparison with gas- liquid chromatography. Food Chem. 24:63-76.

Estrada, A., C. H. Yun, A. Van Kessel, B. Li, S. Hauta, and B. Laarveld. 1997. Immunomodulatory activities of oat β- glucan in vitro and in vivo. Microbio. Immunol. 41:991-998.

Fadel, J. G., C. W. Newman, R. K. Newman and H. Graham H. 1988. Effects of extrusion cooking of barley on ileal and feacal digestibility of dietary components in pigs. Can. J. Anim. Sci. 68:891-897.

Fadel, J. G., C. W. Newman, R. K. Newman and H. Graham. 1989. Effect of baking hulless barley on the digestibility of dietary components as measured at the ileum and in feces in pigs. J. Nutr. 119:722-726.

Fairbairn, S. L., J. F. Patience, H. L. Classen, and R. T. Zijlstra. 1999. The energy content of barley fed to growing pigs: characterizing the nature of its variability and developing prediction equations for its estimation. J. Anim. Sci. 77: 1502-1512.

Feldheim, W., and E. Wisker. 2003. Dietary fibre and type 2 diabetes mellitus. Forum. Nutr. 56:174-176.

Fleckenstein, J. M, P. R. Hardwidge, G. P. Munson, D. A. Rasko, H. Sommerfelt and H. Steinsland. 2010. Molecular mechanisms of enterotoxigenic Escherichia coli infection. Microbes Infect. 12:89-98.

Food and Drug Adminsitration. 1997. "Food labeling: Health Claims: Oats and coronary heart diseases," Final Rule (21CFR part 101). Fed. Regist. (US) 61: 296-337.

Fortin, A., W. M. Robertson, S. Kibite, and S. J. Landry. 2003. Growth performance, carcass and pork quality of finisher pigs fed oat- based diets containing different levels of β- glucan. J. Anim. Sci.81:449-456.

Frank J., B. Sundberg, A. Kamal-Eldin, B. Vessby and P. Aman. 2004. Yeast-leavened oat breads with high or low molecular weight beta-glucan do not differ in their effects on blood concentrations of lipids, insulin, or glucose in humans. J. Nutr. 134: 1384-1388.

Furda, I. 1977. Fractionation and examination of biopolymers from dietary fibre. Cereal Foods World 22:252-254.

Furda, I. 1981. Simultaneous analysis of soluble and insoluble dietary fibre. In the Analysis of dietary fibre in food, pp. 163-172(WPT James and O Theander, editors.) New York: Marcel Dekker.

Gault, V. A., F. P. O'Harte, and P. R. Flatt. 2003. Glucose-dependent insulinotropic polypeptide (GIP): Anti-diabetic and anti-obesity potential? Neuropeptides. 37:253-263.

German J.B., R. Xu, R. Walzem, J. E. Kinsella, B. Knuckles, M. Nakamura and W. H. Yokoyama. 1996. Effect on dietary fats and barley fiber on total cholesterol and lipoprotein cholesterol distribution in plasma of hamsters. Nutr. Res.16:1239-1249.

Gibson, G. R., H. M. Probert, J. V. Loo, R. A. Rastall, and M. B. Roberfroid. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr. Res. Rev. 17:259-275.

Goldman, R., and C. L. Jaffe. 1991. Administration of β- glucan following Leishmania major infection suppress disease progression in mice. Parasite Immunol. 13:137-147.

Graham, H., K. Hesselman, and P. Aman. 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. J. Nutr. 116:242-251.

Greenway, F., C. E. O'Neil, L. Stewart, J. Rood, M. Keenan, and R. Martin. 2007. Fourteen weeks of treatment with viscofiber increased fasting levels of glucagon-like peptide-1 and peptide-YY. J. Med. Food. 10:720-724.

Guillon, F., and M. Champ. 2003. Structural and physical properties of dietary fibers, and consequences of processing on human physiology. Food Res. Int. 33:233-245.

Hahn, T. W., J. D. Lohakare, S. L. Lee, W. K. Moon, and B. J. Chae. 2006. Effects of supplementation of β-Glucan on growth performance, nutrient digestibility and immunity in weaning pigs. J. Anim. Sci. 84:1422-1428.

Hamer, H. M., D. Jonkers, K. Venema, S. Vanhoutvin, F. J. Troost, and R. J. Brummer. 2008. Review article: The role of butyrate on colonic function. Aliment. Pharmacol. Ther. 27:104-119.

Hedemann, M. S., M. Eskildsen, H. N. Laerke, C. Pedersen, J. E. Lindberg, P. Laurinen, K. E. Knudsen. 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. J. Anim. Sci. 84:1375-1386.

Hellendoorn, E. W., M. G. Noordhoff and J. Slagman. 1975. Enzymatic determination of the indigestible residue (dietary fibre) content of human food. J. Sci. Food Agri. 26:1461-1468.

Hennerberg, W., and F. Stohmann. 1860. Beitrage zur Begrundung einer rationellen Futterung der Wiederkauer volume 1. Braunschweig. Germany

Hill, M. J. 1997. Cereals, cereal fibre and colorectal cancer risk: A review of the epidemiological literature. Eur. J. Cancer Prev. 6:219-225.

Hipsley E. H. 1953. Dietary “fibre” and pregnancy toxiaemia. Bri. Med. J. 2:420-422.

Hlebowicz, J., J. M. Jonsson, S. Lindstedt, O. Bjorgell, G. Darwich, and L. O. Almer. 2009. Effect of commercial rye whole-meal bread on postprandial blood glucose and gastric emptying in healthy subjects. Nutr. J. 8:26.

Hogberg, A., and J. E. Lindberg JE. 2004. Influence of cereal non- starch polysaccharides and enzyme supplementation on digestion site and gut environment in weaned piglets. Anim. Feed Sci. Technol. 116:113-128.

Hogberg, A., and J. E. Lindberg. 2006. The effect of level and type of cereal non-starch polysaccharides on the performance, nutrient utilization and gut environment of pigs around weaning. Anim. Feed Sci. Technol. 127:200-219.

Holst, J. J., T. Vilsboll, and C. F. Deacon. 2009. The incretin system and its role in type 2 diabetes mellitus. Mol Cell Endocrinol. 297:127-36.

Hopwood, D. E., D. W. Pethick, and D. J. Hampson. 2002. Increasing the viscosity of the intestinal contents stimulates proliferation of enterotoxigenic *Escherichia coli* and *Brachyspira pilosicoli* in weaner pigs. Br. J. Nutr. 88:523-532.

Inborr, J., M. Schmitz, and F. Ahrens. 1993. Effect of adding fibre and starch degrading enzymes to a barley/wheat based diet on performance and nutrient digestibility in different segments of the small intestine of early weaned pigs. Anim. Feed Sci. Technol. 44:113-127.

Isaksson, G., I. Lundquist, and I. Ihse. 1982. Effect of dietary fiber on pancreatic enzyme activity in vitro. Gastroenterology. 82:918-924.

Jaskari, J., P. Kontula, A. Siitonen, H. Jousimies-Somer, T. Mattila-Sandholm, and K. Poutanen. 1998. Oat beta-glucan and xylan hydrolysates as

selective substrates for bifidobacterium and lactobacillus strains. Appl. Microbiol. Biotechnol. 49:175-181.

Jenkins, A. L., and D. J. Jenkins. 1994. Dietary fibre, glycaemic index and diabetes. S. Afr. Med. J. Suppl:36-37.

Jenkins, A. L., D. J. Jenkins, T. M. Wolever, A. L. Rogovik, E. Jovanovski, V. Bozikov, D. Rahelic, and V. Vuksan. 2008. Comparable postprandial glucose reductions with viscous fiber blend enriched biscuits in healthy subjects and patients with diabetes mellitus: Acute randomized controlled clinical trial. Croat. Med. J. 49:772-782.

Jensen M. S., K. E. Bach Knudsen, J. Inborr and K. Jakobsen. 1998. Effect of β-glucanase supplementation on pancreatic enzyme activity and nutrient digestibility in piglets fed diets based on hulled and hulless barley varieties. Anim. feed Sci. Technol. 72:329-345.

Jensen, B. B. 2001. Possible ways for modifying type and amount of products from microbial fermentation in the gut. In: Gut environment of pigs (Editors, A. Piva, K. E. Bach Knudsen and J. E. Lindberg) Nottingham Univ. Press, Nottingham, UK. pp 181-191.

Jin, L., L. P. Reynolds, D. A. Redmer, J. Caton and J. D. Screnshaw. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, morphology in growing pigs. J. Anim. Sci. 72:2270–2278

Johansen, H. N., K. E. Bach Knudsen, K. E. Wood, and R. G. Fulcher. 1997. Physico-chemical properties and the degradation of oat bran polysaccharides in the gut of pigs. J. Sci. Food Agri. 73:81-92.

Jonsson, E., and S. Hemmingsson. 1991. Establishment in the piglet gut of lactobacilli capable of degrading mixed-linked β-glucan. J. Appl. Bacteriol. 70:512–516.

Jorgensen, H., X. Q. Zhao, and B. O. Eggum. 1996. The influence of dietary fibre and environmental temperature on the development of the

gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. Br. J. Nutr. 75:365-378.

Juvonen, K. R., A. K. Purhonen, M. Salmenkallio-Marttila, L. Lahteenmaki, D. E. Laaksonen, K. H. Herzig et al. 2009. Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. J. Nutr. 139:461-466.

Kass, M. L., P. J. Van Soest, W. G. Pond, B. Lewis and R. E. McDowell. 1980. Utilization of dietary fiber from alfalfa by growing swine.1. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. J. Anim. Sci. 50:175-191.

Kim, H., K. S. Stote, K. M. Behall, K. Spears, B. Vinyard, and J. M. Conway. 2009. Glucose and insulin responses to whole grain breakfasts varying in soluble fiber, beta-glucan: A dose response study in obese women with increased risk for insulin resistance. Eur. J. Nutr. 48:170-175.

Kirwan, W. O., A. N. Smith, A. A. McConnell, W. D. Mitchell, and M. A. Eastwood. 1974. Proceedings: Actions of bran on colonic motility related to its physical properties. Gut. 15:828.

Konopski, Z., R. Seljelid, and T. Eskeland. 1993. Cytokines and PGE2 modulate the phagocytic, function of the β- glucan receptor in macrophages. Scandinavian J. Immunol. 37:587- 592.

Latymer, E. A., A. G. Low, K. Fadden, I. E. Sambrook, S. C. Woodley, and H. D. Keal. 1990. Measurement of transit time of digesta through sections of gastrointestinal tract of pigs fed with diets containing various sources of dietary fibre (non-starch polysaccharides). Arch. Tierernahr. 40:667-680.

Lazaridou, A., C. G. Biliaderis, M. Micha-Screttas, and B. R. Steele. 2004. A comparative study on structure-function relations of mixed-linkage (1→3), (1→4) linear beta D-glucan. Food Hydrocolloids 18:837-855.

Le Gall, M., A. Serena, H. Jorgensen, P. K. Theil, and K. E. Bach Knudsen. 2009. The role of whole-wheat grain and wheat and rye ingredients on the

digestion and fermentation processes in the gut - a model experiment with pigs. Br. J. Nutr. 102:1590-1600.

Le Goff, G., and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci. 79:2418-2427.

Le Goff, G., J. van Milgen, and J. Noblet. 2002. Influence of dietary fibre on digestive utilization and rate of passage in growing pigs, finishing pigs and adult sows. Anim. Sci. 74:503-515.

Lepionka, L., C. H. Malbert, and J. P. Laplace. 1997. Proximal gastric distension modifies ingestion rate in pigs. Reprod. Nutr. Dev. 37:449-457.

Leser, T. D., J. Z. Amenuvor, T. K. Jensen, R. H. Lindecrona, M. Boye, and K. Møller. 2002. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl. Environ. Microbiol. 68:673-690.

Li, J., J. Xing, X. Li, X. Wang, L. Zhao, S. Lv, and D. Huang D. 2005. Effects of β- glucan extracted from Saccharomyces cerevisiae on humoral and cellular immunity in weaned pigs. Archives Anim. Nutr. 59:303-312.

Liatis, S., P. Tsapogas, E. Chala, C. Dimosthenopoulos, K. Kyriakopoulos, E. Kapantais et al. 2009. The consumption of bread enriched with betaglucan reduces LDL-cholesterol and improves insulin resistance in patients with type 2 diabetes. Diabetes Metab. 35:115-120.

Lien, K. A., W.C. Sauer and J.M. He. 2001. Dietary influences on the secretion into and degradation of mucin in the digestive tract of monogastric animals and humans. J. Anim. Feed Sci. 10:223-245.

Lindberg, J. E., A. Arvidsson, and J. Wang. 2003. Influence of naked barley cultivar with normal, amylase- rich or amylopectin – rich starch and enzyme supplementation on digestibility and piglet performance. Anim. Feed Sci. Technol. 104:121-131.

Malkki, Y. 2004. Trends in dietary fiber research and development. Acta Alimentaria. 33:39-62

Mariscal-Landin, G. B. Sève, Y. Colléaux and Y. Lebreton. 1995. Endogenous amino nitrogen collected from pigs with end-to-end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. J. Nutr. 125:136–146

Matte, J. J., S. Robert, C. L. Girard, C. Farmer, and G. P. Martineau. 1994. Effect of bulky diets based on wheat bran or oat hulls on reproductive performance of sows during their first two parities. J. Anim. Sci. 72:1754-1760.

Mayer, E. A. 1994. The physiology of gastric storage and emptying. In physiology of the gastrointestinal tract, 929-976 (L. R. Johnson, editor). New York: Raven press.

McCleary, B. V. 2003. Dietary fiber analysis. Proc. Nutr. Soc. 62:3-9.

McCleary, B. V., J. W. DeVries, J. I. Rader, G. Cohen, L. Prosky, D. C. Mugford, M. Champ, and K. Okuma. 2010. Determination of total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: Collaborative study. J. AOAC Int. 93:221-233.

McDonald, D. E., D. W. Pethick, J. R. Pluske, and D. J. Hampson. 1999. Adverse effects of soluble non- starch polysaccharides (guar gum) on piglet growth and experimental colibacillosis immediately after weaning. Res. In Vet. Sci. 67:245-250.

McDonald, D. E., D. W. Pethick, B. P. Mullan, and D. J. Hampson. 2001. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic escherichia coli in newly-weaned pigs. Br. J. Nutr. 86:487-498.

Millard, P., and A. Chesson. 1984. Modification to Swede (Brassica napus L.) anterior to the terminal ileum of pigs: some implications for the analysis of dietary fibre. Brit. J. Anim. Nutr. 52:583-594.

Miquel, N., K. E. Knudsen, and H. Jorgensen. 2001. Impact of diets varying in dietary fibre characteristics on gastric emptying in pregnant sows. Arch. Tierernahr. 55:121-145.

Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between DF and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95-117.

Moore, W. E. C., L. V. H. Moore, E. P. Cato, T. D. Wilkins and E. T. Kornega. 1987. Effect of high-fiber and high-oil diets on the faecal flora of swine. Appl. Environ. Microbiol. 53:638-644.

Morris, J. R., and V. D. Burrows. 1986. Naked oats in grower- finisher pig diets. Canadian J. Anim. Sci. 66:833-836.

Nabuurus, M. J. 1998. Weaning piglets as a model for studying pathophysiology of diarrhoea. Vet. Quart**.** 20:42-45.

Nazare, J. A., S. Normand, A. Oste Triantafyllou, A. Brac de la Perriere, M. Desage, and M. Laville. 2009. Modulation of the postprandial phase by beta-glucan in overweight subjects: Effects on glucose and insulin kinetics. Mol. Nutr. Food Res. 53:361-369.

Nunes, C. S., and K. Malmlof. 1992. Effects of guar gum and cellulose on glucose absorption, hormonal release and hepatic metabolism in the pig. Br. J. Nutr. 68:693-700.

Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. Van Kessel, P. H. Simmins, and R. T. Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. J. Anim. Sci. 84:843-852.

Painter, N. S. 1975. Diverticular diseases of colon. London. Heinemann.

Panahi, S., A. Ezatagha, F. Temelli, T. Vasanthan, and V. Vuksan. 2007. Beta-glucan from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. J. Am. Coll. Nutr. 26:639-644.

Pettersson, A., and J. E. Lindberg. 1997. Ileal and total tract digestibility in pigs of naked and hulled barley with different starch composition Anim. Feed Sci. Technol. 66: 97-109.

Pieper, R., R. Jha, B. Rossnagel, A. G. Van Kessel, W. B. Souffrant, and P. Leterme. 2008. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiol. Ecol. 66:556-566.

Pluske, J. R. 2001. Morphological and functional changes in the small inetstine of the newly-waened pig. In Gut environnent of pigs (Editors A. Piva, K. E. Bach Knudsen, and J. E. Lindberg) Nottingham Uni. Press, Nottingham, UK. pp- 1-27.

Polonsky, K. S., and A. H. Rubenstein. 1984. C-peptide as a measure of the secretion and hepatic extraction of insulin. pitfalls and limitations. Diabetes. 33:486-494.

Potkins, Z. V., T. L. Lawrence, and J. R. Thomlinson. 1991. Effects of structural and non-structural polysaccharides in the diet of the growing pig on gastric emptying rate and rate of passage of digesta to the terminal ileum and through the total gastrointestinal tract. Br. J. Nutr. 65:391-413.

Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C. S. and H. J. Flint. 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiol. Lett. 217:133-139.

Rainbird, A. L., and A. G. Low. 1986. Effect of various types of dietary fibre on gastric emptying in growing pigs. Br. J. Nutr. 55:111-121.

Rakowska, M., T. Zebrowska, M. Neumann, K. Medynska, and K. Raczynska-Bojanowska. 1990. The apparent ileal and faecal digestibilities of amino

acids and carbohydrates of rye, barley and triticum grains in pigs. Arch Tierernahr. 40:695-701.

Rehfeld, J. F. 1998. The new biology of gastrointestinal hormones. Physiol. Rev. 78:1087-1108.

Reilly, P., T. Sweeney, C. O'Shea, K. M. Pierce, S. Figat, A. G. Smith, D. A. Gahan, and J. V. O'Doherty. 2010. The effect of cereal-derived beta-glucans and exogenous enzyme supplementation on intestinal microflora, nutrient digestibility, mineral metabolism and volatile fatty acid concentrations in finisher pigs. Anim. Feed Sci. Technol. 158:165-176.

Reimer, R. A., and M. I. McBurney. 1996. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology. 137:3948-3956.

Renteria-Flores, J. A., L. J. Johnston, G. C. Shurson, and D. D. Gallaher. 2008. Effect of soluble and insoluble fiber on energy digestibility, nitrogen retention, and fiber digestibility of diets fed to gestating sows. J. Anim. Sci. 86:2568-2575.

Rerat, A., P. Vaugelade, and P. Villiers. 1980. A New Method for Measuring the Absorption of Nutrients in the Pig: Critical Examination. Pages 177-178-216 Low, A. G., and I. G. Partridge, eds. National Institute for Research in Dairying, Reading, England, and Hannah Research Institute, Ayr, Scotland.

Reynolds, J. A., M. D. Kastello, D. G. Harrington, C. L. Crabbs, C. J. Peters, C. J. Jemski , G. H. Scott, and N. R. Di Luzio. 1980. Glucan –induced enhancement of host resistance to selected infectious diseases. Infect. Immunol. 30:51-57.

Robert, S., J. J. Matte, C. Farmer, C. L. Girard and G. -P. Martineau. 1993. High-fibre diets for sows: effects on stereotypies and adjunctive drinking. Appl. Anim. Behav. Sci. 37:297-309.

Robinson, I. M., M. J. Allison, and J. A. Bucklin. 1981. Characterization of the cecal bacteria of normal pigs. Appl. Environ. Microbiol. 41:950-955.

Roediger, W. E. 1982. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology. 83:424-429.

Ropert, A., C. Cherbut, C. Roze, A. Le Quellec, J. J. Holst, X. Fu-Cheng, S. Bruley des Varannes and J. P. Galmiche. 1996. Colonic fermentation and proximal gastric tone in humans. Gastroenterology. 111:286-296.

Salanitro, J. P., I. G. Blake, and P. A. Muirhead. 1977. Types and distribution of anaerobic bacteria in the large intestine of pigs. Appl. Environ. Microbiol. 37:187-193.

Sakurai, T., N. Ohno, and T. Yadomae. 1996. Effects of fungal β- glucan and IFN-γ on the secretory functions of murine alveolar macrophages. J. Leukocyte Bio. 60:118-124.

Schaller, D. A. 1976. Analysis of dietary fiber. Food Prod. Dev.11:70-72.

Schneeman, B. O., and D. Gallaher. 1985. Effects of dietary fiber on digestive enzyme activity and bile acids in the small intestine. Proc. Soc. Exp. Biol. Med. 180:409-414.

Schneeman, B. O. 1998. Dietary fiber and gastrointestinal function. Nutr. Res. 18:625-632

Schweizer, T. F., and P. Wursch. 1979. Analysis of dietary fibre. J. Sci. Food Agri. 30: 613-619.

Schweizer, T. F., and P. Wursch. 1981. Analysis of dietary fibre In the Analysis of Dietary Fibre in Food pp. 203-216 (WPT James and O Theander, editors.) New York: Marcel Dekker.

Serena, A., H. Jorgensen, and K. E. Bach Knudsen. 2008a. Digestion of carbohydrates and utilization of energy in sows fed diets with contrasting levels and physicochemical properties of dietary fiber. J. Anim. Sci. 86:2208-2216.

Serena, A., M. S. Hedemann, and K. E. Bach Knudsen. 2008b. Influence of dietary fiber on luminal environment and morphology in the small and large intestine of sows. J. Anim. Sci. 86:2217-2227.

Serena, A., H. Jorgensen, and K. E. Bach Knudsen. 2009. Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber. J. Anim. Sci. 87: 136-147.

Simoes Nunes, C., and K. Malmlof. 1992. Glucose absorption, hormonal release and hepatic metabolism after guar gum ingestion. Reprod. Nutr. Dev. 32:11-20.

Skendi, A., C. G.Biliaderis, and A. Lazaridou, and M. S. Izydorczyk, MS. 2003. Structure and rheological properties of water soluble beta-glucan from oat cultivars of Avena sativa and Avena bysantina**.** J. Cereal Sci. 38:15-31.

Snart, J., R. Bibiloni, T. Grayson, C. Lay, H. Zhang, G. E. Allison et al. 2006. Supplementation of the diet with high-viscosity beta-glucan results in enrichment for lactobacilli in the rat cecum. Appl. Environ. Microbiol. 72:1925-1931.

Soltys, J., M. Benkova, and Z. Boroskova. 1994. Immunorestorative effect of glucan immunomoduator on guinea pigs with experimental ascariosis. Vet. Immun. Immunopath. 42:379-388.

Southgate D. A. T. 1969. Determination of carbohydrates in food. II Available carbohydrates. J. Sci. Food Agric. 20:326- 330.

Southgate, D. A. T., G. J. Hudson, and H. N. Englyst. 1978. The analysis of dietary fiber: the choices for the analyst. J Sci. Food Agric. 29:979-988.

Specian, R. D., and M.G. Oliver. 1991. Functional biology of intestinal goblet cells. Am. J. Physiol**.** 260:183-193.

Tappy L., E. Gugolz and P. Wursch. 1996. Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects. Diabetes care. 19: 831-834.

Thacker, P. A., G. L. Campbell, and J. GrootWassink. 1992. The effects of organic acids and enzyme supplementation on the performance of pigs fed barley based diets. Canadian J. Anim. Sci. 72:395-402.

Theander, O., and P. Aman. 1979. Studies on dietary fiber. I. Analysis and chemical characterization of water soluble and water-insoluble dietary fibers. Swedish J. Agric. Res. 9:97-106.

Theander, O., P. Aman, E. Westerlund, and H. Graham. 1994. Enzymatic/chemical analysis of dietary fiber. J. AOAC Int. 77:703-709.

Thomas, B. 1972. Beitrage zur Nomenklatur und Analytik pflanzlicher Zellwandsubstanzen. Getreide, Mehl und Brot. 26:158-165.

Torsdottir, I., M. Alpsten, G. Holm, A. S. Sandberg, and J. Tolli. 1991. A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. J. Nutr. 121:795-799.

Trowell H. 1972. Dietary fibre and coronary heart diseases. Europ. J. Bio. Res. 17:345- 349

Trowell, H. 1974. Definition of fibre. Lancet. 503.

Trowell, H., D. A. T. Southgate, T. M. S. Wolever, A. R. Leeds, M. A. Gassull, D. J. A. Jenkins. 1976. Dietary fiber redefined. Lancet. 967.

Tungland B. C. 2003. Fructooligosaccharides and other fructans: structures and occurrence, production, regulatory aspects, food applications and nutritional health significance. ACS Symp. Series 849:135-152.

Van Der Wielen, P. W., S. Biesterveld, S. Notermans, H. Hofstra, B. A. Urlings, and F. van Knapen. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl. Environ. Microbiol. 66:2536-2540.

Van Nevel, C. J., N. A. Dierick, J. A. Decuypere, and S. M. De Smet. 2006. In vitro fermentability and physicochemical properties of fibre substrates and their effect on bacteriological and morphological characteristics of the gastrointestinal tract of newly weaned piglets. Arch. Anim. Nutr. 60:477-500.

Van Soest, P. 1963a. Use of detergents in the analysis of fibrous feeds. I preparation of fiber residues of low nitrogen content. J. offic. Agri. Chemists. 46:825-829.

Van Soest, P. 1963b. Use of detergents in the analysis of fibrous feeds. II.A rapid method for the determination of fiber and lignin. J. offic. Agri. Chemists 46:829-835.

Van Soest, P., and R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds .IV Determination of plant cell wall constituents. J. offic. Agri. Chemists. 50:50-55.

Varel, V. H. 1987. Activity of fiber-degrading microorganisms in the pig large intestine. J. Anim. Sci. 65:488-496.

Vasanthan, T., and F. Temelli. 2008. Grain fractionation technologies for cereal β-glucan concentration. Food Res. Int. 41:876-881.

Vestergarrd, E. M. 1997. The effect of dietary fiber on welfare and productivity of sows. PhD thesis. The Royal Veterinary and Agricultural University, Copenhagen.

Vetvicka, V., B. P. Thornton, and G. D. Ross. 1996. Soluble β- glucan polysaccharide binding to the lectin site of neutrophils or natural killer cell complement receptor type 3(CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J. Clin. Investigation 98:50-61.

Walker, A. R. P. 1974. Dietary fiber and the patterns of diseases. Ann. Intern. Med. 80:663-664.

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Technol. 90:21-33.

Widdowson, E. M., and R. A. McCance. 1935. The available carbohydrates of fruits. Determination of glucose, fructose, sucrose and starch. Biochem. J. 29:151-156.

Wilfart, A., L. Montagne, P. H. Simmins, J. van Milgen, and J. Noblet. 2007. Sites of nutrient digestion in growing pigs: Effect of dietary fiber. J. Anim. Sci. 85:976-983.

Williams, D. L., and N. R. Di Luzio. 1980. Glucan – induced modification of murine viral hepatitis. Sci. 208:67-69.

Williams, R. D., and W. Olmstedt. 1935. A biochemical method for determining indigestible residue (crude fibre) in faeces: lignin, cellulose, and non-water-soluble hemicellulose. J. Biol. Chem. 108:653-666.

Wisker, E., M. Daniel, G. Rave, and W. Feldheim. 2000. Short-chain fatty acids produced in vitro from fibre residues obtained from mixed diets containing different breads and in human faeces during the ingestion of the diets. Br. J. Nutr. 84:31-37.

Wong, J. M., R. de Souza, C. W. Kendall, A. Emam, and D. J. Jenkins. 2006. Colonic health: Fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235-243.

Wood, P. J., J. T. Braaten, F. W. Scott, K. D. Riedel, M. S. Wolynetz, and M. W. Collins. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Br. J. Nutr. 72:731-743.

Wood, P. J., and M. U. Beer. 1998. ‘Functional oat products’, in G Mazza, Functional Foods. Biochemical and processing aspects, Lancaster, Pennsylvania, technomic publishing, 1-37.

Wood, P. J. Cereal β-glucan in diet and health. 2001. J. Cereal Sci. 46:230-238.Wood, P. J., 2001. Cereal β-glucan: structure, properties and health claims. In: McCleary, B. V., and L. Prosky (Eds), Advanced dietary fiber technology. Blackwell science, London, pp.315-327.

Xiao, Z., C. A. Trincado, and M. P. Murtaugh. 2004. Beta-glucan enhancement of T cell IFNgamma response in swine. Vet. Immunol. Immunopathol. 102:315-320.

Yen, J. T., J. A. Nienaber, D. A. Hill, and W. G. Pond. 1991. Potential contribution of absorbed volatile fatty acids to whole-animal energy requirement in conscious swine. J. Anim. Sci. 69:2001-2012.

Yun, C. H., A. Estrada, A. V. Kessel, B. C. Park, and B. Laarveld. 2003. β-glucan, extracted from oat, enhances diseases resistance against bacterial and parasitic infections. Immun. Med. Microbio. 35:67-75.

Zekovic, D. B., S. Kwiatkowski, M. M. Vrvic, D. Jakovljevic, and C. A. Moran. 2005. Natural and modified (1-->3)-beta-D-glucan in health promotion and disease alleviation. Crit. Rev. Biotechnol. 25:205-230.

Chapter 2: Technical Note: An improved surgical model for the long-term studies of kinetics and quantification of nutrient absorption in swine[1]

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2.1 Abstract

An improved technique to study kinetics and quantitative absorption of nutrients in pigs was described. Three female pigs (35 kg BW) were surgically modified with catheters in the hepatic portal vein and carotid artery and an ultrasonic flow probe around the portal vein. Catheter placement and patency was secured using distal modifications (rings and holes) and non-absorbable suture. Catheters and flow probe cable were tunneled subcutaneously following exteriorization for further protection. Fibrosis and adhesions in the body cavity were minimized by avoiding excessive manipulation and drying of viscera. Pigs were supported during recovery by i.v. fluid therapy of AA and electrolytes until regular feeding resumed. Catheters were flushed daily with heparinized saline (200 IU/L). After 10 d, pigs were fed a diet based on wheat and soybean meal for 6 consecutive 7-d periods. On d 7, blood was collected postprandially every 15 min from -15 to 60 min, 30 min to 240 min, 60 min to 480 min, and 120 min to 720 min. Blood flow was measured simultaneously. Plasma was analyzed for glucose, and net glucose absorption was calculated from plasma portal-arterial differences × plasma flow [blood flow × (1 – hematocrit)]. The specific improvements for long-term use of this model are distal modifications of the catheters, post-operative treatment using parental nutrition and gut motility drug, prevention of infection of body cavity by further tunneling of catheters and blood flow probe cable, and use of ultrasonic blood flow probes and meter. Blood flow measurements using an ultrasonic blood flow probe was not changed after 52 d as compared to 10 d post surgery, indicating the reliability of this model. This

catheterized pig model will, thus, allow the long-term study of the kinetics of nutrient absorption.

2.2 Introduction

Dietary nutrient characteristics affect the kinetics of nutrient absorption, gastrointestinal and pancreatic hormone responses, and nutrient metabolism. Catheterization of the portal vein and carotid artery and simultaneous installation of a blood flow probe in the pig (Rerat et al., 1980) is an excellent model to study the kinetics of absorption. The difference in nutrient concentration between blood in the portal vein and carotid artery is multiplied with simultaneously-measured blood flow through the portal vein. The net nutrient flux into the portal vein and absorption kinetics has been measured for carbohydrates (Rerat et al., 1984), AA (Caine et al., 1999), urea, and ammonia (Van Leeuwen et al., 1995).

Major constraints for successful application of the model are complicated surgical procedures, post-operative management, catheter maintenance, and accurate blood flow measurements. Surgical procedures have been modified in individual laboratories to accommodate surgical capabilities, material availability, and model application (e.g., Bajjalieh et al., 1981; Yen and Killefer, 1987; Ten Have et al., 1995; Van Leeuwen et al., 1995). Emergence of ultrasonic, instead of electromagnetic flow probes, improved blood flow measurements. However, in last decade, the model was used sparsely (Bach Knudsen et al., 2000, 2005; Reverter et al., 2000; Lambert et al., 2002; Yen et al., 2004; Le Floc'h and Sève,

2005), in part due to the constraints. Therefore, a need exists to describe the current advances in the model and thereby maximize use in nutrition studies.

The objectives of the present study were to improve the method for catheterization of the portal vein and carotid artery, post-operative management, and establishment of blood flow measurements using an ultrasonic flow meter and to test the effectiveness of this model for nutrient absorption for long-term studies in swine.

2.3 Materials and methods

The animal use protocol was approved by the Animal Care Committee of the University of Alberta, in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Animals and Diets

The animal experiment was conducted at the Swine Research and Technology Centre at the University of Alberta (Edmonton, Alberta, Canada). Three female pigs (35 to 40 kg BW) were moved to pens 1 wk prior to surgery and handled daily to habituate to close human contact for better post-operative management and blood sampling. Pigs were fed at 0800 and 2000 a diet based on wheat and soybean meal along with water containing electrolytes (Gatorade; Quaker Oats, Chicago, IL). Pigs were fasted for 12 h before surgery but had free access to water.

Catheter Preparation and Ultrasonic Blood Flow Meter

The portal and arterial catheters were prepared using 1-m-long polyvinyl tubing (inner diameter, 1.02 mm; outer diameter, 1.78 mm; Saint-Gobain Performance Plastics, Cleveland, OH) with an 18-gauge blunt needle as an adaptor. For the portal vein catheter, two 2-mm rings of tubing (inner diameter, 1.27 mm; outer diameter, 2.29 mm) were installed 1 cm apart by stretching the rings using forceps, with the first ring 2.5 cm from the catheter tip (**Figure 2.1**). Two small holes were cut in the sides of the catheter between the tip and first ring with small scissors to have an alternate route of blood collection. In preliminary work (data not shown), postmortem examination of pigs of non-patent catheters occasionally revealed a catheter tip placed against the wall of the portal vein. For the carotid artery catheter, one 2-mm ring was installed 10 cm from the tip of the catheter. Rings were glued with Loctite 406 (Loctite Corp., Rocky Hill, CT) and edges were made smooth with a file after complete drying. Both catheters were flushed and dried for 48 h with Tridodecylmethylammonium complex heparinate (TDMAC-heparin; Polysciences Inc., Warrington, PA) as an anticoagulant. Pouches to secure the exteriorized catheters were constructed from 10-cm wide strips of Elastoplasts and Velcro. The dimensions of arterial and portal pouches were 10 × 22 cm and 12 × 30 cm, respectively.

To measure blood flow, an ultrasonic flow meter (model TS 206; Transonic Systems, Ithaca, NY) with 14-mm flow probes (S series, back cable exit, U reflectors) was used.

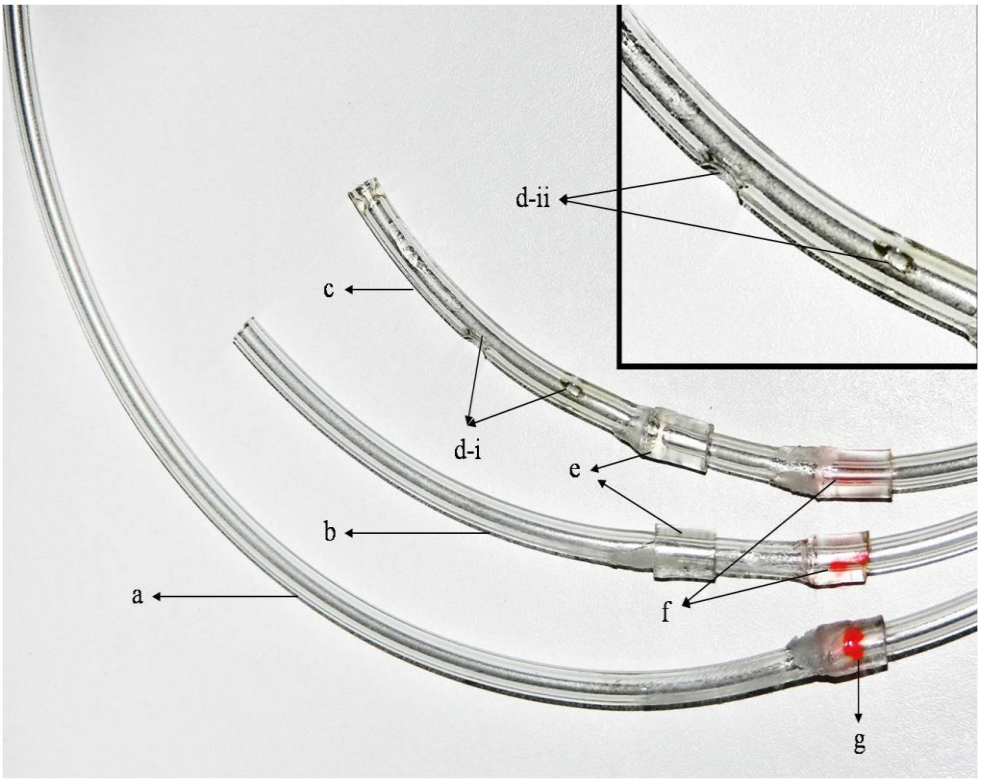

Figure 2.1 Carotid artery unmodified and modified (with holes) portal vein catheters. (a) Carotid artery catheter; (b) Unmodified portal vein catheter; (c) Modified portal vein catheter; (d-i) Holes of the portal vein catheter; (d-ii) Enlarged view of holes of portal vein catheter; (e) Inner rings of portal vein catheter; (f) Outer rings of portal vein catheter; and (g) Ring of carotid artery catheter.

Windaq data acquisition software (Dataq Instruments, Akron, OH) was used to measure pulsatile blood flow continuously by computer. Data were transferred from the flow meter to the computer with a Bayonet Neill-Concelman cable connector. The 10 min average flow rate at each collection time point was used for calculations of quantitative absorption.

Surgery

Preparation. Pigs were sedated first with an i.m. injection of 12 mg/kg BW Ketalean (Ketamine HCl; Biomeda MTC, Cambridge, Ontario, Canada), 2 mg/kg BW Rompun (Xylazine; Bayer Cross, Toronto, Ontario, Canada), and 0.05 mg/kg BW Atropine (Atropine sulphate; Rafter, Calgary, Alberta, Canada). After 30 min, general anesthesia was induced using 4% isoflurane (Isoflo, Abbott Laboratories Ltd., Saint-Laurent, Québec, Canada) provided using a face mask. Then, an endotracheal tube was inserted and connected to a closed-circuit anesthetic apparatus to maintain general anesthesia with isoflurane during surgery. A catheter was inserted in the ear vein for continuous i.v. infusion of 1,500 mL Ringer lactate (solution of NaCl, Na lactate, KCl, and $CaCl_2$; Hospira, Montreal, Quebec, Canada) during surgery. Prior to surgery, 0.2 mg/kg BW Metacam (Boehringer Ingelheim, Burlington, Ontario, Canada) was administered s.c. The pig was placed in dorsal recumbency position. The skin at the mid line and right side was shaved and prepared aseptically with Betadine (7.5% Povidone-Iodine; Purdue Pharma, Pickering, Ontario, Canada).

Portal Vein Catheter and Flow Probe. The pig was placed on a surgery table equipped with a recirculating warm water pad and covered with disposable surgical drape (Jorgensen Laboratory Inc., Loveland, CO). An incision was cut in the skin from the base of the sternum down the midline of the abdomen to the umbilicus. After opening the muscle layers and peritoneum, 4 towel clamps (2 on each side) were installed in the muscle layer and attached to the side of the surgery table using stainless-steel chains to open the surgery site. Sterile surgical

towels (45.7 × 45.7 cm; Dukal Corp., Hauppauge, NY) soaked in warm normal saline (0.9% NaCl solution) were used to retract the stomach, spleen, and intestines away from the surgical site. A V-shaped purse-string suture with 22-mm curved needle (Vicryl, Polyglactin 910; Ethicon Inc., Somerville, NJ) was installed in the wall of the portal vein 2.5 cm posterior to the bifurcation of the portal vein into the liver (**Figure 2.2**). A 14-gauge needle with stopper was used to create an opening into the portal vein in the middle of the purse-string suture. The needle was removed and the bleeding was stopped by applying pressure with gauze pads. The catheter was inserted into the portal vein using a wire guide (Cook Canada Inc., Stouffville, Ontario, Canada) until the first ring of the catheter was inserted into the sheath covering the portal vein. Then the suture was closed using 4 square knots. The catheter was secured to the sheath using a non-absorbable suture (Prolene 4-0, polypropylene; Ethicon Inc., Somerville, NJ) with 6 square knots around the second ring. Catheter patency and position were checked after withdrawing the wire guide and flushing with 200 IU/mL heparin saline solution. The site of catheterization was lavaged repeatedly with warm normal saline. Subsequently, upstream from the catheter near the lymph nodes the connective tissue around the vein was dissected free to create clear space around the vein. The flow probe was installed and immersed into normal saline to check the probe function. The flow probe was exteriorized by cutting a hole in the right flank at the base of the vertebral ribs. The catheter was exteriorized using a tunneling rod 3 cm caudal from the probe cable. Both flow probe cable and

catheter were wrapped in sterile surgical towel to maintain sterility until further tunneling.

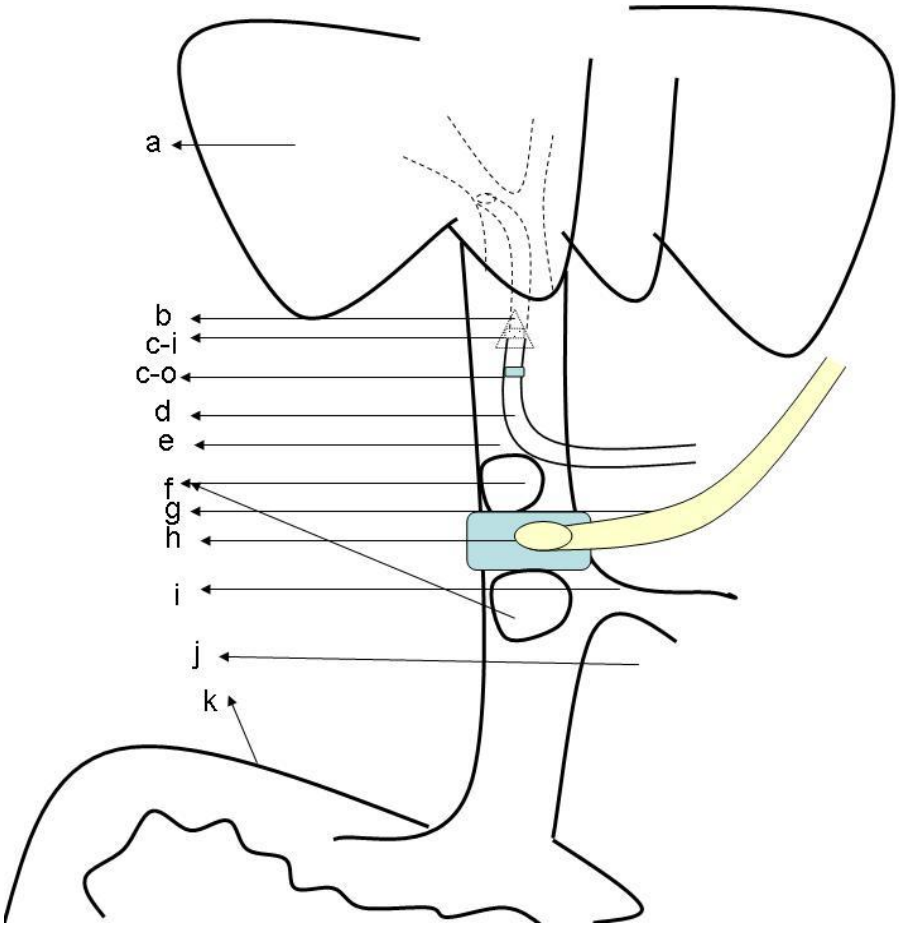

Figure 2.2 Schematic of portal vein catheter and probe placement (Adapted from Ten Have et. al., 1996): (a) Liver; (b) V-shaped purse-string suture; (c-i) Ring of the portal vein catheter inside the portal vein; (c-o) Ring of the portal vein catheter outside the portal vein; (d) Portal vein catheter; (e) Portal vein; (f) Lymph nodes; (g) Probe cable; (h) Probe body; (i) Gastro-splenic vein; (j) Pancreas; and (k) Intestine.

The abdominal cavity was filled with warm normal saline and then closed by suturing separately the peritoneum, muscle layer, and subcutaneous tissue using a continuous suture (PDS II, Polydioxanone; Ethicon Inc., Somerville, NJ) and the skin using a single interrupted suture (Novafil; Covidien, Mansfield, MA).

Carotid Artery Catheter. For the arterial catheter, the skin was incised on the right side of the neck, close to the trachea. The carotid artery was dissected free and damage to the vagus nerve was avoided. A square knot was tied around the artery with vicryl suture towards cranial side to stop blood flow to head. A loose loop of suture was inserted beneath the artery towards the heart and lifted to stop blood flow to the site of catheterization. The artery wall was dissected and cut with small iris scissors and the catheter was inserted gently 10 cm (to reach the vessel junction between carotid and sub-clavian arteries) until the ring passed the suture. The catheter was secured by tying 4 square knots with vicryl. An additional suture was made with the non-absorbable suture (Prolene, polypropylene; Ethicon Inc., Somerville, NJ) around the catheter using 6 square knots to ensure secure placement. The catheter was tunneled dorso-caudally beneath the skin and exteriorized from the right side to the nape using a tunneling rod and the incision was closed by suturing the skin using a simple interrupted suture.

External Protection. The blood flow probe cable was tunneled subcutaneously 10 cm dorsally. The portal vein catheter was tunneled subcutaneously dorsally to exit near the midline in the mid lumbar region. The probe cable and portal catheter were secured together in a pouch. The arterial

catheter was also tunneled dorsally and caudally to exit just behind the shoulders and was secured in a pouch.

Post-operative Management

After surgery, the antibiotic Cefazolin-Na (25 mg/kg BW, every 8 h; Cefazolin-Na, Novopharm Ltd., Toronto, Ontario, Canada), the analgesic Torbugesic (0.4 mg/kg BW, every 6 h i.v.; Butorphanol tartrate, Wyeth Animal Heath, Guelph, Ontario, Canada) and the analgesic/anti-inflammatory drug Meloxicam (0.1 mg/kg BW, every 24 h orally; Metacam Oral Suspension, Boehringer Ingelheim, Burlington, Ontario, Canada) were given for 3 d. On the evening of the surgery day and following morning, 2 mL/kg BW of a cocktail containing dextrose, electrolytes, AA, and B-complex vitamins (Aminolean, Vetoquinol N.-A. Inc., Lavaltrie, Quebec, Canada) was given i.v. via the portal vein catheter as a slow drip to provide parental nutrition. To induce gut motility and reduce post-operative nausea, Metaclopromide HCl (10 mg i.m., every 8 h; Sandoz Canada Inc., Boucherville, QC) was given for 2 d. Twice daily, the pig's body temperature, pain, and general health was monitored.

The day following surgery, 100 g of feed was offered in the morning and evening. Feed allowance was gradually increased to 1 kg/d on d 5 post surgery. The catheters were flushed aseptically daily with 200 IU/L of heparinized normal saline to maintain their patency. Sutures were removed using a half dose of Rompun and Ketalean 10 d after surgery. Pigs were wearing stretchable shirts to cover arterial and venous pouch to further secure catheters and probes.

Experimental Sampling, Measurement of Blood Flow and Sample Analysis

Blood samples were collected from 3 pigs 1 d every week in heparinized tubes (BD Canada, Oakville, Ontario, Canada) from the carotid artery and portal vein. Blood was collected every 15 min from -15 to 60 min, then every 30 min to 240 min, then every 60 min to 480 min, and 600 min and 720 min postprandially; blood flow was measured simultaneously. During measurements, the probe was attached to the flowmeter with a cable. Flow was recorded continuously at each collection for 10 min using windaq software. After the collection, the catheter was flushed with 10 mL of 10 IU/mL heparinized saline to prevent clotting and replace the fluid loss. Plasma was analyzed for glucose (glucose oxidase kit; Diagnostics Chemicals Ltd., Charlottetown, Prince Edward Island, Canada). Net glucose absorption was calculated from plasma portal-arterial differences × plasma flow (Rerat et al., 1980). Plasma flow rate was calculated from blood flow rate using the following equation: plasma flow = blood flow × (1 – hematocrit). Blood collection and blood flow measurements were done weekly for consecutive 6 wks.

Calculations and Statistical Analysis

Net glucose absorption was calculated from plasma portal-arterial differences and plasma flow measurements using the formula $q = (Cp - Ca)F(dt)$ (Rerat et al., 1980). Cumulative net glucose absorption can be calculated subsequently using the formula

$$\mathbf{Q} = \sum_{t0}^{t1} q$$

In the formulas, q is the amount of nutrients absorbed within time period dt, Cp and Ca are the concentration of nutrient in portal and arterial plasma respectively, F is the plasma flow in the portal vein, and Q is the amount of nutrient absorbed production from time t_0 to t_1. Blood flow rates of period 1 and 6 were compared using t-test. Plasma carotid and portal glucose and net glucose absorption were analyzed as repeated measures using the MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC).

2.4 Results and Discussion

Surgery

The surgeries averaged 5 h, despite anatomical variations inside the body cavity such as location and size of the portal vein and lymph nodes on the portal vein (Hecker, 1974). The dorsal recumbency position combined with a mid line incision made the area of the portal vein accessible for catheterization and blood flow probe implantation. Use of wet surgical towels to keep the viscera away from the surgical site further facilitated access.

The surgical approach using a mid-line incision was developed by Rerat et al. (1980). Another approach, the left lateral recumbency position, has been used (Bajjalieh et al., 1981; Yen and Killefer, 1987); however, the working field is smaller than with the present approach. The portal vein catheterization procedure using a purse string suture was developed by Rerat et al. (1980); however, in the present study a wire guide, instead of a modified needle, was used to guide the catheter into the vein. In swine and sheep, rings have been used previously to

support suturing catheters outside the blood vessel to tissue (Mineo et al., 1991; Trottier et al., 1995); however, installation of the first of 2 rings inside the vein to secure catheter placement was a novel approach first described in the present study. Previously, portal catheters have been secured with a nylon mesh, glued to the catheter, and sutured to connective tissue (Yen and Killefer, 1987). Catheters with silicon rosettes may prevent post surgical changes in catheter length (Van Leeuwen et al., 1994). The technique to install the flow probe was similar to that described previously for the external pudic artery in swine (Renaudeau et al., 2002) and ruminal arteries in sheep (Remond et al., 1993). The carotid artery catheterization procedure is a standard methodology (e.g., Rerat et al., 1980).

Recovery and Post-operative Outcome

Pigs did not have complications on the d of surgery, and recovered from surgery without clinical signs of infection. After surgery, parental nutrition served as supply of essential AA, electrolytes, and energy to the intestine and pig until oral feeding resumed. Preoperative habituation of pigs allowed for post-operative management. Therapy with antibiotics for 3 d and sterile surgical techniques prevented post-operative infections. On d 2 after surgery, 1 pig started vomiting, refused to eat, and was lethargic; both catheters were functional. Following gut motility medication treatment for 2 ds, pig recovered fully. The successful recovery post surgery is likely due to factors during and after surgery, e.g., the use of sterile saline towels to hold viscera to prevent adhesions and infections inside the body cavity, subcutaneous tunneling of the probe cable to reduce tracking of

skin contamination along the cable to prevent infection, parental nutrition, broad spectrum antibiotics, and the gut motility drug. The 2 rings on tip of the catheter to secure placement, use of anticoagulant coating (TDMAC-heparin), and regular flushing of catheters with heparinized saline maintained patency of the portal vein catheter for 52 d post surgery, indicating that blood can be sampled long-term in catheterized swine (Trottier et al., 1995). The necessity of the extra holes in catheters cannot be demonstrated experimentally, and we have since completed studies successfully in our combined laboratories using portal catheters with and without the extra holes.

Blood Flow Rate Measurements

Blood flow is the critical factor for quantitative nutrient absorption measurements and a major constraint for chronic studies. Rate of blood flow has been measured using electromagnetic flow probes (Bajjalieh et al., 1981) or the indicator dilution method (Yen and Killefer, 1987). Some have simply used reported blood flow values (Van Leeuwen et al., 1994). In the present study, ultrasonic flow probes replaced old electromagnetic technology with the advantage that the probe is placed in alignment with the vessel and data can be collected immediately following implantation. The electromagnetic flow probe has major limitations for chronic studies as proliferation of fibrous tissues between the sensor of flow probe and vessel wall reduces its flow rate abnormally low after 13 to 28 d (Rerat et al., 1980).

The flow rate of portal vein at the time of feeding was 1.11 L/min at 10 d after surgery and 1.16 L/min at 52 d after surgery (**Table 2.1**). The average flow rate was 28.3 mL/(kg·min), similar to previous reports using ultrasonic blood flow probes (Bach Knudsen et al., 2000, 2005).

Table 2.1 Comparison of portal vein blood flow 10 and 52 d after surgery

Blood flow rate (L/min)	Day after surgery		Pooled	
	10	52	SEM	*P*-value
Pre-prandial[1]	1.11	1.16	0.16	0.79
Post-prandial[2]	1.23	1.30	0.16	0.67

[1]Means of 3 observations of blood flow between 5 min before and 5 min after morning feeding.

[2]Means of 3 observations of flow between 5 min before and 5 min after 120 min of morning feeding.

Blood flow at the time of feeding and post prandial did not differ between 10 and 52 d after surgery (periods 1 and 6, respectively), indicating that blood flow rate might remain constant within studies. Physiologically, blood flow normally plateaus after pigs reach 30 to 50 kg BW and differences are, thus, abnormal and likely due to the measurement technique.

A higher blood flow was measured with electromagnetic flow probes ranging from 38 to 55 mL/(kg·min) (Rerat et al., 1980, 1984; Simoes and Malmlof, 1992), a technology that is only reliable for 2 to 4 weeks after surgery. The new

ultrasonic probe has an advantage of taking measurements for longer periods post surgery (Ellis et al., 1995).

Quantitative Absorption Measurements

Baseline glucose in the portal vein and carotid artery was 5.5 mmol/L, and, then, increased to 10.1 mmol/L in the portal vein and 6.4 mmol/L in the carotid artery (**Figure 2.3**).

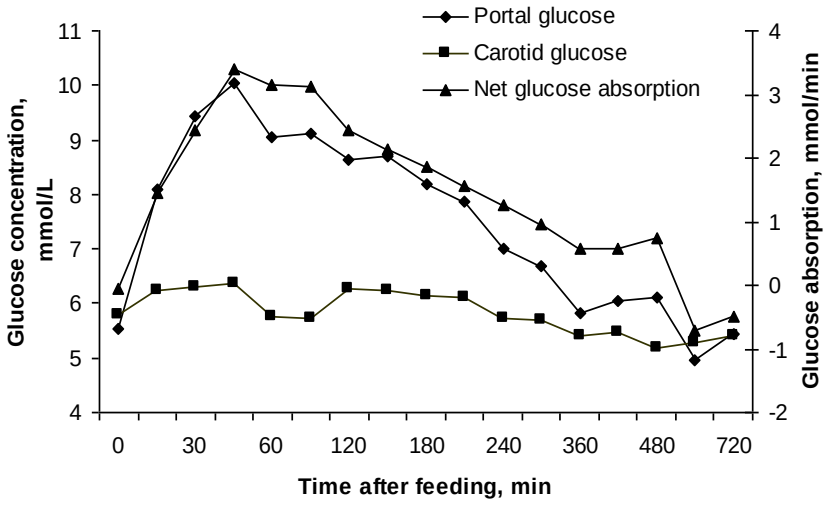

Figure 2.3 Portal and arterial plasma concentrations of glucose (mmol/L) and net glucose absorption rate (mmol/min) after feeding a diet based on wheat and soybean meal (based on 6 observations). For the 3 variables, a time effect existed ($P < 0.001$). The SEM was 0.36 for carotid glucose, 0.37 for portal glucose, and 0.43 for net glucose absorption.

The net glucose absorption from the intestine into the systemic blood circulation was -0.05 mmol/min prior to the meal and peaked at 3.39 mmol/min at 45 min postprandially. Although arterial and portal glucose concentrations and

absorption kinetics cannot be compared with other studies because of differences in diets, the suitability of the present model has been demonstrated. The kinetics of glucose absorption affects glucose metabolism (Jenkins et al., 1995). In humans, effects of starch and fiber sources on glucose metabolism are normally studied using glucose measurements in peripheral blood. However, glucose homeostasis is tightly controlled by insulin and glucagon in the systemic circulation in normal human subjects.

Furthermore, ethical and practical reasons limit experimental surgeries in human subjects. The present model in swine might, thus, be used as a model for nutrient absorption studies in humans. The techniques to catheterize the portal vein and carotid artery and install a blood flow probe in the pig were described. Major improvements for long-term use of the model may be distal modifications of the catheters, specific post-operative treatment of parental nutrition and gut motility drug, prevention of infection of body cavity by subcutaneous tunneling of catheter and blood flow probe cable, and use of ultrasonic blood flow probe and meter. The model can be used for several weeks following surgery to study effects of dietary ingredients on kinetics and total absorption of nutrients and hormones production and chronic metabolic studies.

2.5 References

Bach Knudsen, K. E., H. Jørgensen, and N. Canibe. 2000. Quantification of the absorption of nutrients derived from carbohydrate assimilation: model experiment with catheterised pigs fed on wheat- or oat-based rolls. Br. J. Nutr. 84:449-458.

Bach Knudsen, K. E., A. Serena, A. K. B. Kjaer, H. Jørgensen, and R. Engberg. 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135:1696-1704.

Bajjalieh, N. L., A. H. Jensen, G. R. Frank, and D. E. Brown. 1981. A technique for monitoring nutrient absorption in the conscious, unrestrained pig. J. Anim. Sci. 52:101-107.

Caine W. R., M. W. A. Verstegen, W. C. Sauer, S. Tamminga, and P. van Leeuwen. 1999. Flux of amino acids and peptides across the portal vein-drained tissue of pigs. J. Anim. Feed Sci. 8:27-43.

CCAC. 1993. Guide to the care and use of experimental animals. Vol. 1. 2nd ed. Canadian Council on Animal Care, Ottawa, Ontario, Canada.

Ellis, P. R., F. G. Roberts, A. G. Low, and L. M. Morgan. 1995. The effect of high molecular-weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in growing pig: relationship to rheological changes in jejunal digesta. Br. J. Nutr. 74:539-556.

Hecker, J. F. 1974. Experimental surgery on small ruminants. Butterworths, Sydney, Australia.

Jenkins, D. J. A., R. G. Josse, A. L. Jenkins, T. M. S. Wolever and V. Vuksan. 1995. Implications of altering the rate of carbohydrate absorption from the gastrointestinal tract. Clin. Invest. Med. 18:296-302.

Lambert, B. D., B. Stoll, H. Niinikoski, S. Pierzynowski, and D. G. Burrin. 2002. Net portal absorption of enterally fed alpha-ketoglutarate is limited in young pigs. J. Nutr. 132:3383-3386.

Le Floc’h, N., and B. Sève. 2005. Catabolism through the threonine dehydrogenase pathways does not account for the high first-pass extraction rate of dietary threonine by the portal drained viscera in pigs. Br. J. Nutr. 93:447-456.

Mineo, H., T. Oyamada, M. Akiyama, T. Yasuda and S. Kato. 1991. A new method of catheterization of the hepatic and portal veins in sheep for long-term blood sampling. Small Rumin. Res. 5:293-300.

Remond, D., C. Poncet, and J. Lefaivre. 1993. Technical note: ruminal vein catheterization and continuous blood flow measurement in ruminal arteries of sheep. J. Anim. Sci. 71:1276-1280.

Renaudeau, D., Y. Lebreton, J. Noblet, and J. Y. Dourmad. 2002. Measurement of blood flow through the mammary gland in lactating sows: methodological aspects. J. Anim. Sci. 80:196-201.

Rerat, A., P. Vaugelade, and P. Villiers. 1980. A new method for measuring the absorption of nutrients in the pig: Critical examination. Pages 177-216 in Current Concepts of Digestion and Absorption in Pigs. Tech. Bull. 3. A. G. Low and I. G. Partridge, ed. National Institute for Research in Dairying, Reading, England, and Hannah Research Institute, Ayr, Scotland.

Rerat, A. A., P. Vaissade, and P. Vaugelade. 1984. Absorption kinetics of some carbohydrates in conscious pigs. Br. J. Nutr. 51:517-529.

Reverter, M., T. Lundh, H. L. Gonda, and J. E. Lindberg. 2000. Portal net appearance of amino acids in growing pigs fed a barley-based diet with inclusion of three different forage meals. Br. J. Nutr. 84:483-494.

Simoes Nunes, C., and K. Malmlof. 1992. Effects of guar gum and cellulose on glucose absorption, hormonal release and hepatic metabolism in the pig. Br. J. Nutr. 68:693-700.

Ten Have, G. A. M., M. C. F. Bost, J. C. A. W. Suyk-Wierts, A. E. J. M. van den Bogaard, and N. E. P. Deitz. 1996. Simultaneous measurement of metabolic flux in portally-drained viscera, liver, spleen, kidney and hindquarter in the conscious pig. Lab Anim. 30:347-358.

Trottier, N. L., C. F. Shipley, and R. A. Easter. 1995. A technique for the venous cannulation of the mammary gland in the lactating sow. J. Anim. Sci. 73:1390-1395.

Van Leeuwen, P., H. G. D. Leuvenink, W. M. Haasbroek, G. Priem, M. Bosch, and D. J. van Kleef. 1995. A portal vein catheterization technique in pigs and sheep, and postprandial changes of pO2, pCO2, pH, urea, ammonia, and creatinine and proteins in portal and arterial blood measured in pigs. J. Anim. Physiol. Anim. Nutr. 73:38-46.

Yen, J. T., and J. Killefer. 1987. A method for chronically quantifying net absorption of nutrients and gut metabolites into hepatic portal vein in conscious swine. J. Anim. Sci. 64:923-934.

Yen, J. T., V. H. Varel, and J. A. Neienaber. 2004. Metabolic and microbial responses in western crossbred and in Meishan growing pigs fed a high-fiber diet. J. Anim. Sci. 82:1740-1755.

Chapter 3: Dietary oat β-glucan reduces peak net glucose flux and insulin production, and modulates plasma incretin in portal-vein catheterized grower pigs[2]

[2]The version of this chapter has been accepted in *Journal of Nutrition*

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3.1 Abstract

Net glucose and short chain fatty acid (SCFA) flux and insulin secretion into the portal vein might be associated with the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The objectives were to clarify this association and to study the impact of 2 doses of dietary oat β-glucan on the variables. Three 35-kg portal vein-catheterized pigs fed 3 diets containing 0, 3, or 6% oat β-glucan concentrate (BG0, BG3, and BG6) for 7-d in a repeated 3 × 3 Latin square. On d 7, blood was sampled for 12 h postprandially. Net glucose flux and apparent hormone production were calculated from plasma portal-arterial differences × flow. Postprandially, pigs fed BG6 had lower ($P < 0.05$) portal glucose at 15, 30, and 45 min, and a lower ($P < 0.05$) net glucose flux during the first h. Pigs fed BG6 tended to have lower ($P < 0.10$) portal C-peptide without lowering insulin, indicating that pigs fed BG6 had lower actual insulin release combined with a higher prehepatic retention of insulin. Pigs fed BG6 had lower ($P < 0.05$) portal GIP and GLP-1, which in turn were correlated ($R^2 = 0.81$ and 0.88, respectively; $P < 0.01$) with portal glucose. Pigs fed BG3 and BG6 had a higher ($P < 0.05$) net SCFA flux than pigs fed BG0, indicating increased fermentation. In conclusion, dietary supplementation of 6% oat β-glucan concentrate decreased net glucose flux, increased net SCFA flux, and decreased peak apparent insulin production, changes that were associated with GIP and GLP-1 mediation.

3.2 Introduction

The kinetics of glucose absorption affects glucose metabolism and insulin responses, two major factors for control and prevention of type-II diabetes in humans (Jenkins and Jenkins, 1994). Among soluble non starch polysaccharide (NSP), effects of β-glucan concentrate derived from oat and barley have been studied extensively in humans in relation to glucose metabolism and insulin responses using glucose measurements in peripheral blood (Jenkins et al., 2008; Kim et al., 2009; Panahi et al., 2007). Soluble NSP may decrease peak glucose flux by increasing viscosity and water-binding capacity of gastrointestinal contents (Bach Knudsen et al., 2005) and interfering with enzymatic digestion and mucosal absorption (Isaksson et al., 1982). To study the kinetics of nutrient uptake and hormone production and the control of glucose homeostasis by insulin, advanced surgical models or isotope techniques are ideal but their use is obviously limited in normal human subjects. Instead, the porcine porto-arterial catheterization model allows studying the effects of changes in dietary composition on net nutrient flux and apparent production of gastrointestinal and pancreatic hormones (Rerat et al., 1984).

The incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released from L and K cells (enteroendocrine cells) of the large and small intestine, respectively, in response to luminal glucose (Baggio and Drucker, 2007) and might thus be reduced by viscous NSP (Juntunen et al., 2002). Both GLP-1 and GIP enhance glucose-dependent insulin secretion, promote β-cell proliferation and inhibit β-cell apoptosis, and thus increase

pancreatic β-cell mass (Baggio and Drucker, 2007). Recently, incretins have been studied as potential therapy for type-II diabetes (Holst et al., 2009). Undigested nutrients and NSP are fermented in the intestine producing SCFA that serve as nutrients systemically and for the colonic epithelium, regulate proliferation, differentiation and gene expression, and promote growth of *Bifidobacterium* and *Lactobacillus* species (Wong et al., 2006). The SCFA increase the expression of the proglucagon gene in L cells of intestine and thus may increase GLP-1 secretion (Reimer and McBurney, 1996). However, the knowledge about incretin modulation by dietary viscous and fermentable NSP such as oat β-glucan is limited (Juntunen et al., 2002). Moreover, the complex network of glucose kinetics, insulin and incretin responses, and SCFA flux in response to feeding oat β-glucan is poorly understood.

Thus, a porcine porto-arterial catheterization model was used to test the hypotheses that β-glucan as a soluble NSP decrease the peak net glucose flux and insulin secretion and increase net SCFA flux and that these changes are associated with incretins. The objectives were to clarify this association and to study the impact of 2 doses of dietary oat β-glucans on the variables.

3.3 Materials and Methods

Experimental designs and procedures

The animal protocol was approved by the Animal Care and Use Committee for Livestock at the University of Alberta and was conducted at the Swine Research and Technology Centre. Three female pigs (35 to 40 kg BW) were

catheterized in the portal vein and carotid artery using modified polyvinyl tube catheters; a 14-mm blood flow probe (Transonic Systems, Ithaca, NY) was implanted around portal vein (Hooda et al., 2009). Post-operative management included antibiotics, analgesics, gut motility drugs, and intravenous fluids for 3 d. Catheters were flushed aseptically daily with 200 IU/L of heparinized normal saline to maintain their patency and were secured using pouches. Only three pigs were used due to the complexity of surgery and maintenance requirements of the animals.

Diets. The diets (**Table 3.1**) were based on wheat, soybean meal, canola oil, and vitamin and mineral premix. The control diet (BG0) did not contain supplemental oat β-glucan. The 50% oat β-glucan concentrate (Viscofiber, Cevena Bioproducts, Edmonton, Alberta, Canada) was included in two diets to contain 3 and 6% of supplemented β-glucan (BG3 and BG6, respectively). Diets were formulated to be isocaloric and had an equal content of total carbohydrates and vitamin and mineral premix. Pigs were fed one of three diets for 7 d in a double 3 × 3 Latin square repeated over time, to have 6 observations per diet. Pigs were fed 1.00 kg/d until 40 kg BW, 1.10 kg/d until 45 kg BW, 1.20 kg/d until 50 kg BW, and 1.50 kg/d until 65 kg BW. Feed was divided into two equal meals and fed at 0800 and 2000 h with free access to water.

Sampling protocol. Blood was collected on d 7 of each period in heparinized and EDTA tubes from the carotid artery and portal vein. Blood was collected every 15 min from -15 to 60 min, then every 30 min up to 240 min, then every 60 min up to 480 min, and 600 min and 720 min postprandially.

Table 3.1 Ingredient and chemical composition of diets varying in β-glucan concentration

	Diets		
Item	BG0	BG3	BG6
Ingredient	*%, as fed basis*		
Wheat	72.20	66.48	60.75
Soybean meal	22.00	20.35	18.70
Canola oil	1.80	1.67	1.55
Premix[1]	4.00	4.00	4.00
Oat BG[2]	-	7.50	15.00
Chemical composition	*%(DM basis)*		
β-glucan	0.62	4.29	7.05
Gross energy, MJ/kg	18.51	18.53	18.65
Starch	44.24	40.78	37.62
Soluble dietary fiber	4.20	6.41	10.69
Insoluble dietary fiber	17.69	23.97	21.84
Total dietary fiber	21.89	30.38	32.53
Carbohydrates (starch + total dietary fiber)	66.13	71.16	70.15

[1]Provided the following per kg of diet: Na, 3.18 g as salt; Cl, 4.90 g as salt; Fe, 214 mg as ferrous sulfate; Zn, 107 mg as $ZnCO_3$; Mn, 48.6 mg as $MnSO_4$; Cu, 124 mg as $CuSO_4$; I, 0.36 mg as $K(IO_3)_2$; Co, 0.06 mg as $CoSO_4$; Se, 0.054 mg as Na_2SeO_3; retinol, 2.7 mg; cholecalciferol, 24.8 μg; α-tocopherol, 4.8 μg; menadione, 0.62 mg; vitamin B12, 0.02 mg; riboflavin, 5 mg; niacin, 22 mg; D-pantothenic acid, 15 mg; biotin, 0.09 mg; and choline, 260 mg.

[2]50% oat β-glucan concentrate containing (%): moisture, 6.58; total carbohydrate, 80.70; total dietary fiber, 70.64; soluble dietary fiber, 57.27; insoluble dietary fiber, 13.37; β-glucan, 50.45; starch, 7.17; protein, 4.69; lipids - 3.98; and ash, 4.05.

Blood flow was measured simultaneously (model TS 206; Transonic Systems) and was recorded continuously at each collection for 10 min using Windaq software (Dataq Instruments, Akron, OH). After collection, catheters were flushed with 10 mL of 10 IU/L heparinized saline to prevent clotting and replace the fluid loss. Hematocrit values were measured immediately using the standard method. Blood was centrifuged at 1500 × g for 10 min and plasma was frozen at -20ºC in heparinized tube for glucose, insulin, SCFA, and C-peptide analyses and in EDTA tubes at -80ºC for GLP-1 and GIP analyses.

Analytical procedures. Diets were analyzed in duplicate for dry matter by drying at 135°C in an airflow-type oven for 2 h (method 930.15; (AOAC, 2006)), and gross energy by adiabatic bomb calorimetry (Model 5003, Ika-Werke GMBH & Co. KG, Staufen, Germany). The NSP were analyzed by the modified procedure of Englyst et al. (1994). Total starch, mixed linked β-glucan and total dietary fiber were analyzed using a kit (Megazyme Int. Ireland Ltd., Bray, Ireland) based on enzymatic analysis (methods 996.11, 995.16, and 985.29 respectively; (AOAC, 2006).

Glucose and SCFA were analyzed in all plasma samples; however, insulin, C-peptide, GLP-1, and GIP were analyzed only in plasma collected until 480 min postprandially. Plasma was analyzed for glucose using a glucose oxidase kit (Trinder, 1969) (Diagnostics Chemicals Ltd., Charlottetown, Prince Edward Island, Canada) and for SCFA (acetate, propionate, butyrate, valerate, caprionate, isovalerate, and isobutyrate) using the procedure described by Brighenti (1998) using isocaprionate as internal standard. Briefly, 400 µL of plasma was mixed

with 50 µL internal standard and then deproteinized by addition of 32 µL of 25% phosphoric acid, followed by incubating at 60ºC for 30 min. The solution was centrifuged at 8,000 g for 30 min to remove the proteins and the supernatant was analyzed for SCFA using GC.

Insulin was analyzed by RIA using porcine insulin kit (Linco Research, St. Charles, MO; intra-assay CV = 6.4 and interassay CV = 9.9). The C-peptide was analyzed by RIA using porcine C-peptide kit (Linco Research; intra-assay CV = 9.7 and interassay CV = 5.8). The GLP-1 was quantified using a double antibody RIA after extraction with alcohol (Linco Research; intra-assay CV = 11.9 and interassay CV = 4.4) and combined extraction and assay recoveries of cold spiked kit quality control was 70.2 ± 5.9%. The GIP was analyzed by RIA (Bachem Americas, Inc., Torrance, CA; intra-assay CV = 7.7 and interassay CV = 15.2). For GIP analysis, plasma was thawed at 4 °C and 85 µg of aprotinin (Roche Diagnostics, Laval, Quebec, Canada) in 25 µl deionized water was added to each mL of plasma during thawing.

Calculations and Statistical Analyses

Calculations. Net nutrient flux and apparent hormone production were calculated from plasma portal-arterial differences and plasma flow measurements using the formula

$$q = (Cp - Ca)F(dt)$$ (Rerat et al., 1984),

where q is the amount of nutrient absorbed or hormone produced within time period dt, Cp and Ca are the concentration of nutrient or hormone in portal and

arterial plasma, respectively, and F is the plasma flow in the portal vein. Plasma flow rate was calculated from blood flow rate using the following equation: plasma flow = blood flow × (1 − (hematocrit/100)). Cumulative glucose flux can be calculated subsequently using the formula

$$\mathbf{Q} = \sum_{t0}^{t1} q$$

where Q is the amount of nutrient absorbed or hormone produced from time t_0 to t_1. The term net glucose and SCFA flux is used for net portal appearance of these nutrients after utilization by intestine. For insulin and incretins, the term apparent production was used due to the pulsatile secretion, hepatic extraction of insulin, and variable half life (Ellis et al., 1995). The adjusted total glucose flux is calculated as total glucose flux divided by the percentage of starch in the diet.

***Statistical analyses*.** Data were analyzed using the MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC) using pig as the experimental unit. Results were reported as least-squares means with $P < 0.05$ defined as significant and $0.05 \leq P < 0.10$ as trends. Arterial and portal nutrient and hormone data and net nutrient flux and apparent hormone production were analyzed as repeated measures. The statistical model included period within square and pig as random effects and diet, time, and diet × time as fixed effects; therefore, $n = 3$ per diet for mean separation (Littell et al., 1991). Means were separated for diet using the PDIFF statement in the Mixed model, for individual time points after detecting a significant diet effect using SLICE/time. Principal component (PC) analysis was performed using JMP software of SAS. The loading plot was used to observe

correlations among all portal variables of the first two eigenvalues, i.e., PC 1 and PC 2. Subsequently, specific relationships between portal glucose and portal concentrations of insulin and incretins were analyzed at 12 time points using the weighted linear regression analysis using the REG procedure of SAS with predicted values of the dependent variable adjusted for period, pig, and diet effects (St-Pierre, 2001).

3.4 Results

Diets

Gross energy was similar for the three diets (**Table 3.1**). The dietary fiber replaced starch in the BG-supplemented diets, similar to the approach reported by other groups (Bach Knudsen et al., 2000; Serena et al., 2009). Total dietary fiber and NSP were higher in BG6 and BG3 than BG0, with mainly an increase in soluble dietary fiber and NSP (**Table 3.1** and **Appendix 1**, respectively), whereas starch was higher in BG0 than BG3 and BG6. The dietary β-glucan content was slightly higher than expected, because the rest of the diet contained some β-glucan, likely from wheat.

Blood flow

Portal blood flow was 0.86 L/min before feeding and increased ($P < 0.001$) to a maximum of 1.33 L/min at 90 min postprandial; portal flow returned to basal pre-feeding value at 300 min (data not shown). Mean blood flow was 1.17 L/min, which converts to 28.3 mL/ (kg BW • min), and was not influenced by diet.

Glucose kinetics

Portal glucose at the time of feeding was tended to be higher ($P < 0.10$) for pigs fed BG0 compared to pigs fed BG3 and BG6 and increased ($P < 0.05$) to a maximum at 45 min postprandial (Appendix 3). Portal glucose was lower ($P < 0.05$) postprandially in pigs fed BG3 and BG6 than BG0 by 19 and 28%, 7 and 17%, and 8 and 14% at 15, 30 and 45 min, respectively. Net glucose flux increased ($P < 0.05$) immediately postprandially and peaked at 45 min at a concentration that was less ($P < 0.05$) in pigs fed BG6 than pigs fed BG0 and BG3 (**Figure 3.1**). Total glucose flux during the first h was 22 and 51% lower ($P < 0.05$), respectively, for pigs fed BG3 and BG6 than pigs fed BG0 (**Figure 3.2A**). After adjustments for the different starch content among diets (**Figure 3.2B**), treatment differences in total glucose flux in the first h after feeding β-glucan diets were maintained, indicating that differences in dietary starch content of diets were not responsible for the observed glucose flux effects.

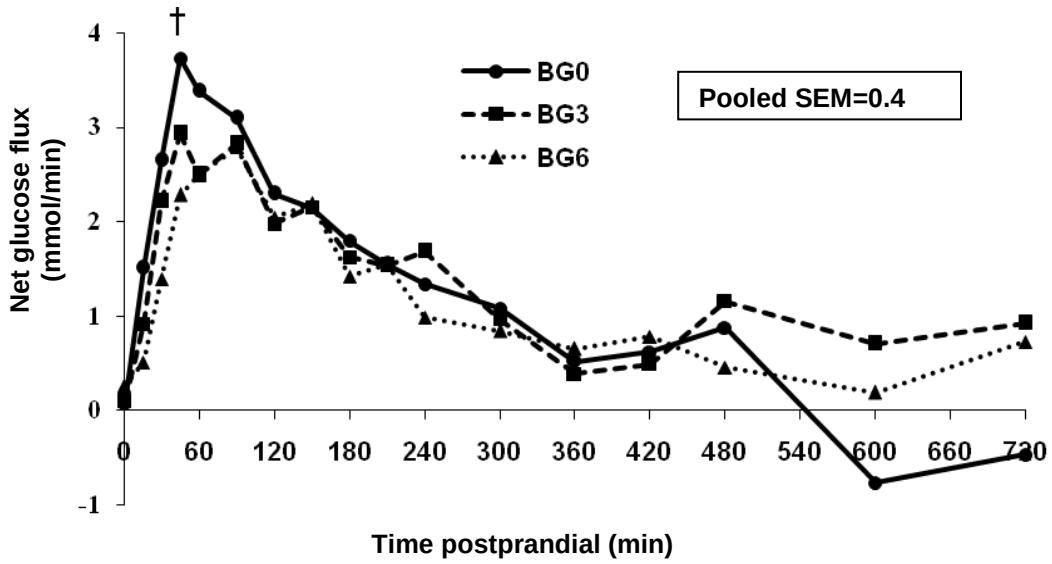

Figure 3.1 Net portal flux of glucose of pigs fed diets varying in β-glucan concentration (n = 3). Symbol indicates that means differ ($P < 0.05$). † BG0 > BG6

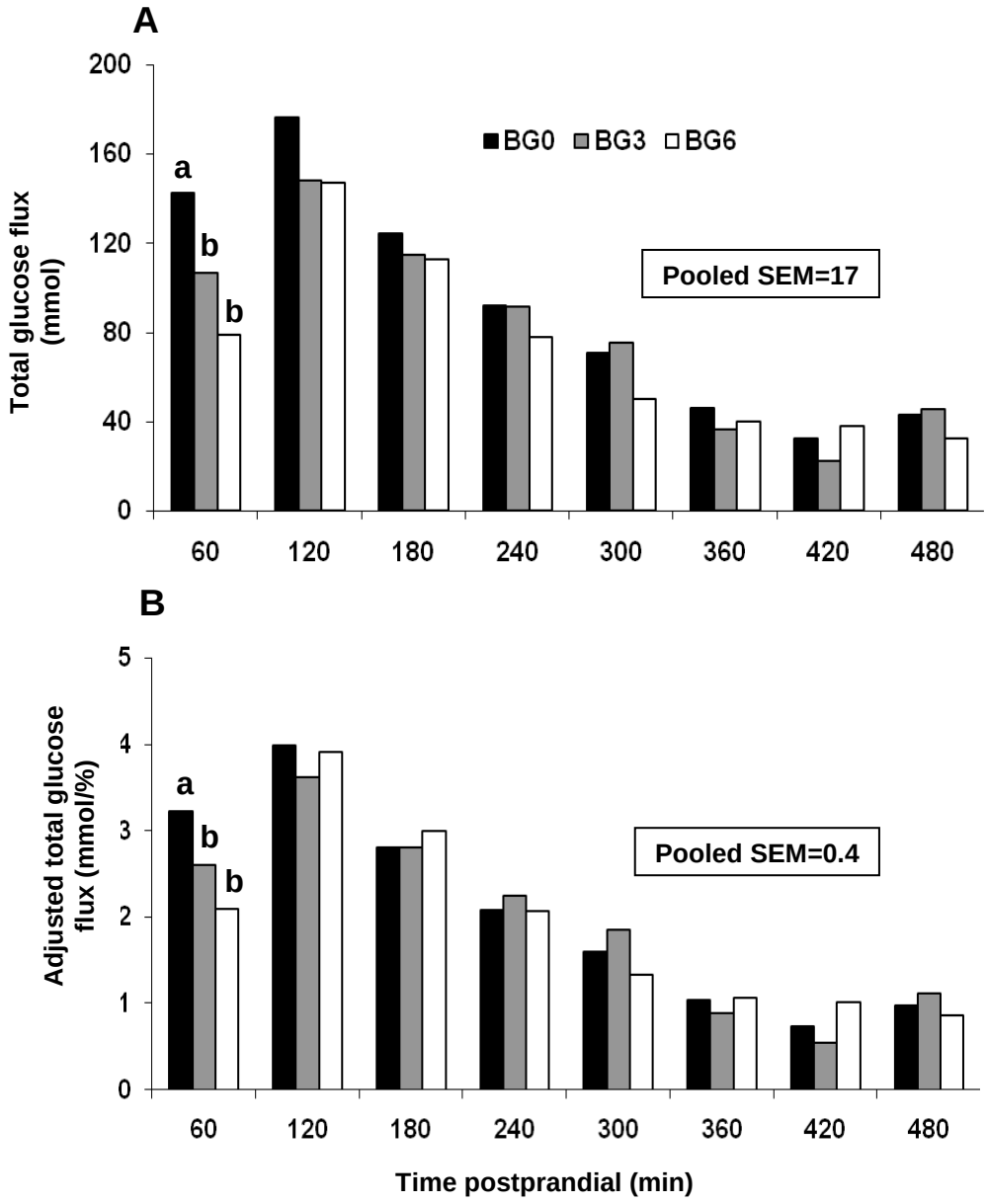

Figure 3.2 Total glucose flux (A) and adjusted (for starch content of diet) total glucose flux (B) of pigs fed diets varying in β-glucan concentration (n = 3). Means without a common letter differ ($P < 0.05$).

Insulin responses

At 30 min postprandial, portal insulin peaked for all pigs and did not differ among diets. At 90 min postprandial, portal insulin was 60% lower ($P < 0.05$) for pigs fed BG6 than pigs fed BG0 (**Appendix 4**). Portal C-peptide followed the same trend as insulin and was lowest ($P < 0.05$) for pigs fed BG6 at 90 min postprandial (**Appendix 4**). Similar to portal concentrations, the apparent production of insulin was less ($P < 0.05$) at 90 and 120 min postprandial in pigs fed BG6 than pigs fed BG0 (Figure 3.3A). Similarly, apparent production of C-peptide was less ($P < 0.05$) in pigs fed BG6 and BG3 than pigs fed BG0 at 90 min and was less ($P < 0.05$) only in pigs fed BG6 at 120 min postprandial (**Figure 3.3B**). Mean arterial insulin and C-peptide from 0 to 480 min was not affected by diet. Pigs fed BG6 tended to have lower ($P < 0.10$) mean portal C-peptide than pigs fed BG0 and BG3. Mean apparent production of C-peptide tended to be lower ($P < 0.10$) in pigs fed BG6 than BG0 without affecting the apparent production of insulin (**Appendix 2**).

Incretins

Both arterial and portal GIP were affected by time ($P < 0.001$), increased postprandially and reached a maximum that was lower ($P < 0.05$) for pigs fed BG3 and BG6 than for pigs fed BG0 during the early postprandial phase (**Figure 3.4A**). Portal GLP-1 was affected by time ($P < 0.05$); pigs fed BG6 had a lower ($P < 0.05$) portal GLP-1 at 90, 120, and 180 min postprandial than pigs fed BG0 and BG3 (**Figure 3.4B**).

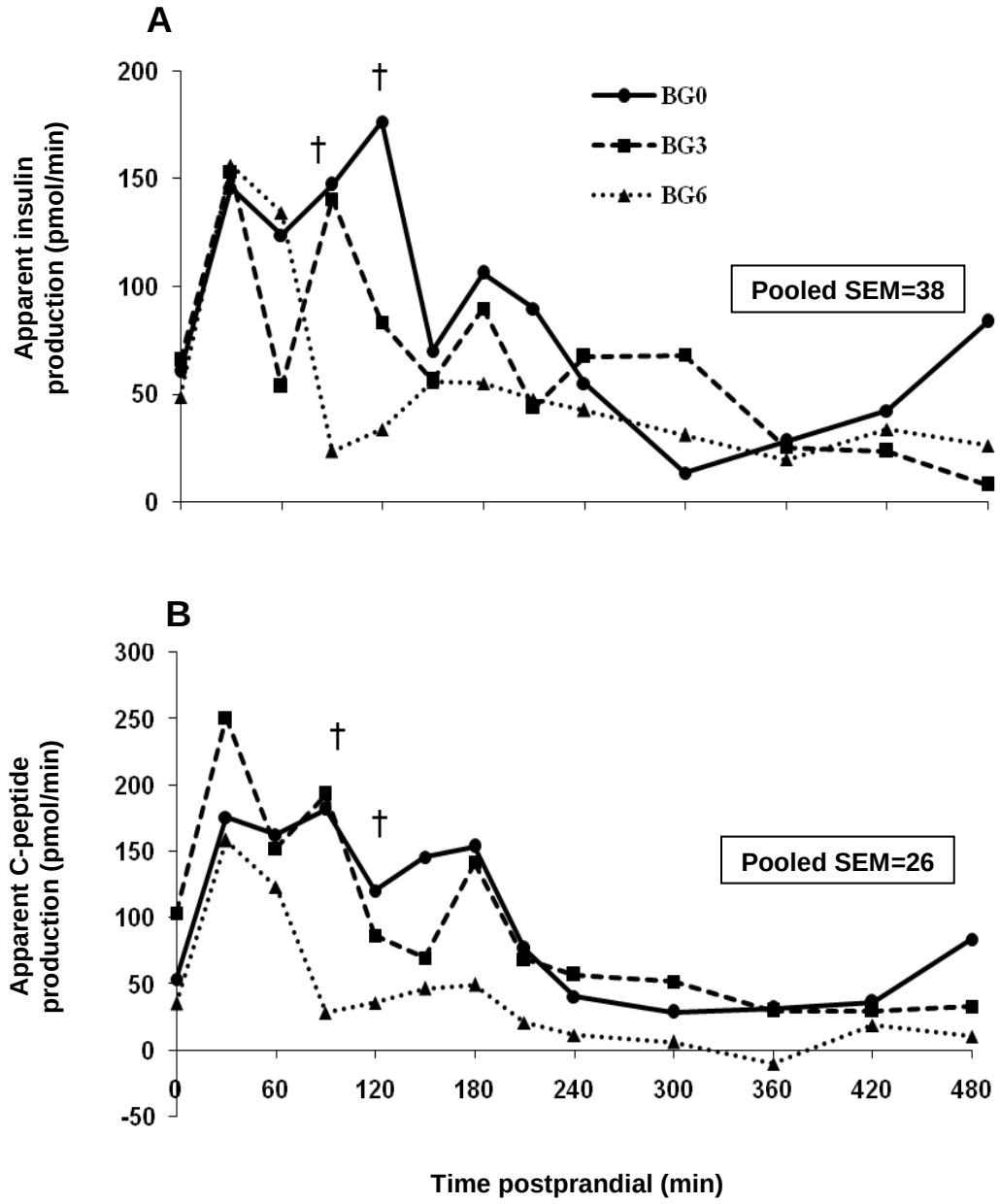

Figure 3.3 Apparent production of insulin (A), and C-peptide (B) of pigs fed diets varying in β-glucan concentration (n = 3). Symbol indicates that means differ (P < 0.05). † BG0 > BG6.

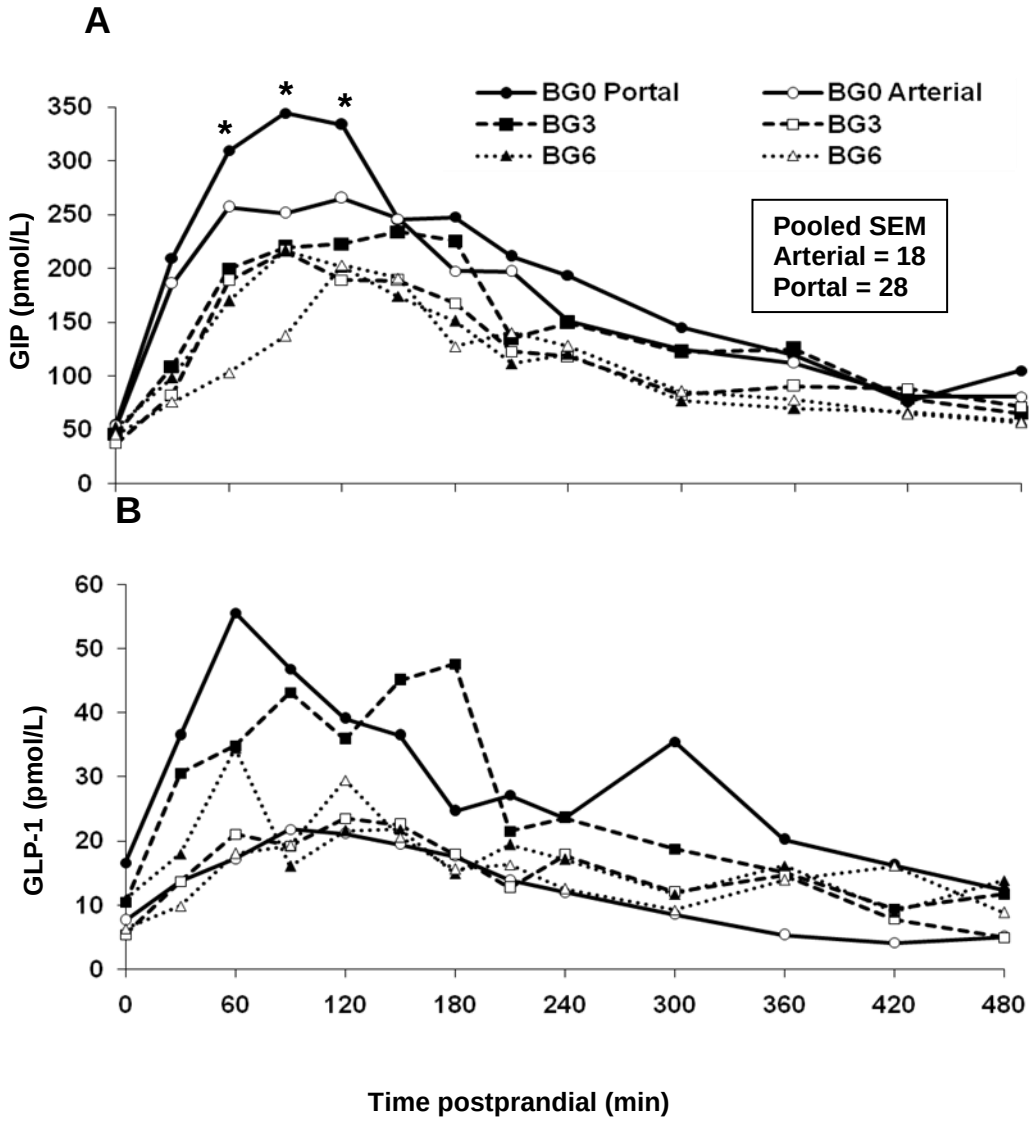

Figure 3.4 Portal and arterial plasma GIP (A) and GLP-1 (B) of pigs fed diets varying in β-glucan concentration (n = 3). Symbol indicates that means differ (P < 0.05). * BG0 > BG3 and BG6, † BG0 > BG6.

Pigs fed BG3 and BG6 had a lower ($P < 0.05$) mean arterial GIP, while pigs fed BG6 tended to have lower ($P < 0.10$) mean portal GIP and apparent production of GIP (**Appendix 2**). Similarly, mean portal GLP-1 was lower in pigs fed BG6 than pigs fed BG0 and BG3 without an effect on arterial GLP-1 and apparent production of GLP-1 (**Appendix 2).**

SCFA

Pigs fed BG6 had higher ($P < 0.05$) portal propionate and butyrate and tended to have higher ($P < 0.10$) total SCFA and acetate than pigs fed BG0 (**Appendix 2** and **Appendix 3**). Pigs fed BG6 had a higher ($P < 0.05$) net flux of propionate and butyrate than pigs fed BG0 and BG3 (**Appendix 2)**.

Principle component and regression analyses

The loading plot (**Figure 3.5**) showed portal glucose, insulin, C-peptide, GLP-1, GIP, and SCFA as affected by PC 1 and PC 2 and correlations among these variables. This loading plot indicated three clusters: insulin positively affected by PC 1 and negatively by PC 2, glucose and incretin positively affected by both PC 1 and PC 2, and SCFA positively affected by PC 2 and negatively by PC 1. The latter was negatively correlated to the other two clusters (angles between arrows was > 90º). The relations of portal glucose with portal insulin ($R^2 = 0.70$; $P < 0.001$), C-peptide ($R^2 = 0.78$; $P < 0.001$), GIP ($R^2 = 0.81$; $P < 0.001$), and GLP-1 ($R^2 = 0.88$; $P < 0.001$) indicated positive and significant relations (**Appendix 5**).

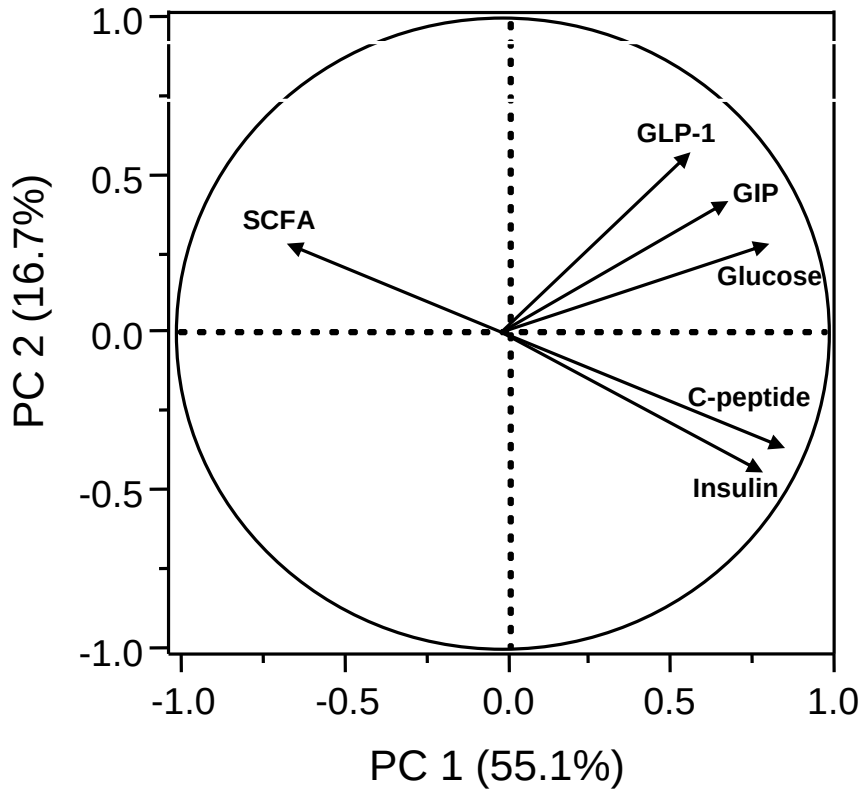

Figure 3.5 Loading plot showing correlations among portal plasma glucose, insulin, C-peptide, GLP-1, GIP, and SCFA of the first two eigenvalues (PC 1 and PC 2) of pigs fed diets varying in β-glucan concentration.

However, portal SCFA was strongly and negatively related to portal glucose ($R^2 = 0.52$; $P < 0.01$) and portal GLP-1 ($R^2 = 0.54$; $P < 0.001$) (**Appendix 5**).

3.5 Discussion

Dietary oat β-glucan lowers glucose and insulin responses and thus affects the entero-insular axis, and also stimulates fermentation. The association of incretins to these responses is poorly understood. To our knowledge, the present study provides novel evidence that the incretins are associated with the reduced net

glucose flux and insulin secretion caused by oat β-glucan in a dose-dependent manner.

Porto-arterial catheterization model

Oat is considered a functional food, since oat was linked to lower blood cholesterol (Ellegard and Andersson, 2007) and glucose (Brennan, 2005). However, effects on lowering glucose and insulin were not consistent among short-term studies (Biorklund et al., 2008; Kim et al., 2009; Maki et al., 2007). Studies in humans with collected peripheral blood (Panahi et al., 2007; Biorklund et al., 2008) did not allow the quantification of the association between kinetics of nutrient absorption and insulin and incretin secretion. Hence, the porcine porto-arterial catheterization model was used following dietary supplementation of oat β-glucan. Portal blood allows quantitative measurements of absorbed nutrients albeit following epithelial utilization while arterial blood represents the basal concentration of nutrients reaching the intestine; their difference is the qualitative nutrient flux (Rerat et al., 1980). Simultaneous blood flow measurements allowed quantification of the net nutrient flux in long-term studies (Hooda et al., 2009). Interestingly, the same biological mechanism applies for insulin and incretins, because both are drained into the portal vein (Burcelin et al., 2007).

Kinetics of glucose absorption

In the present study, oat β-glucan altered the kinetics of glucose absorption. 6% dietary oat β-glucan reduced the peak and total net glucose flux during first h

after feeding, similar to soluble NSP in pigs (Ellis et al., 1995) and in normal (Panahi et al., 2007; Juvonen et al., 2009), obese (Kim et al., 2009) and diabetic humans (Jenkins et al., 2008). The high viscosity of β-glucan may explain the lower glucose flux (Tappy et al., 1996), because increased digesta viscosity reduces gastric emptying rate (Darwiche et al., 2003), and also slows digestion and absorption (Edwards et al., 1988). Specifically, high digesta viscosity decreases enzyme diffusion (Schneeman and Gallaher, 1985) and stimulates the formation of the unstirred water layer (Eastwood and Morris, 1992) and thereby decreases transport of glucose to the enterocytes. Thus, 6% dietary oat β-glucan lowers the glucose response in pigs fed a wheat-based diet confirming that oat β-glucan is a functional food for diabetes management.

Insulin responses

Insulin responses are generally assessed with plasma insulin concentrations. However, C-peptide that is released in equimolar amounts from proinsulin in the pancreas is considered as a more reliable indicator of insulin release into systemic blood, because C-peptide has a lower hepatic extraction than insulin (Polonsky and Rubenstein, 1984). The lower apparent production of C-peptide in pigs fed BG6 indicated clearly that insulin release was reduced even though portal insulin did not differ between pigs fed BG6 and BG0. This contrast emphasizes the importance of measuring C-peptide simultaneously with insulin (Polonsky and Rubenstein, 1984). The lower pancreatic insulin response in pigs fed BG6 was probably due to reduced glucose absorption that directly reduced pancreatic

insulin release (Ellis et al., 1995), similar to observations in humans after eating β-glucan-enriched breads (Kim et al., 2009) or beverages (Juvonen et al., 2009). The glucose and insulin responses in pigs fed BG6 but not to BG3 highlighted the importance of dose-dependent responses, similar to observed in humans (Jenkins et al., 2008; Tappy et al., 1996).

Incretins

The incretins GIP and GLP-1 are released from enteroendocrine K and L cells, respectively, stimulate glucose-induced insulin secretion (Rehfeld, 1998), and are thus important in glucose homeostasis (Burcelin et al., 2007). Carbohydrate intake and intraluminal glucose stimulate incretin release (Schirra et al., 1996). The lower apparent production of GIP and GLP-1 in pigs fed BG6 is thus in agreement with other viscous NSP decreasing the early postprandial GIP flux (Juvonen et a., 2009; Morgan et al., 1990; Simoes and Malmlof, 1992) and subsequent lower insulin release (Gault et al., 2003) In contrast, fermentable NSP increased secretion of GLP-1 in intestinal mucosa and blood (Reimer and McBurney, 1996; Cani et al., 2004) that was linked to two mechanisms: 1) an increase in colonic mass (Cani et al., 2004) and 2) an increase in gene expression of the proglucagon mRNA precursor of GLP-1 (Reimer and McBurney, 1996). Butyrate may also stimulate expression of proglucagon mRNA (Drozdowski et al., 2002). The decreased portal GLP-1 in the present study despite an increased net butyrate flux could be explained as follows: 1) the duration of oat β-glucan intake was not sufficient to increase colonic mass, 2) the fermentation did not

occur near the L-cells, and 3) the formation of an unstirred layer at mucosal surface that prevented the interaction of nutrients with the apical surface of enteroendocrine cells (Juvonen et al., 2009) that is required for stimulation for GLP-1 incretin secretion (Baggio and Drucker, 2007). The inverse association between portal SCFA and GLP-1 thus challenge the paradigm that increased SCFA stimulates GLP-1 secretion.

SCFA

The most interesting change in net SCFA flux was observed in the late postprandial phase that was achieved via the 12 h collection. The feeding of a diet containing soluble fiber increased production of SCFA in gastrointestinal tract and thereby increased portal propionate and butyrate absorption and thereby increased net SCFA flux (Serena et al., 2009). The disappearance of β-glucan by the end of large intestine (data not shown) indicated that β-glucan is fermented completely in the intestine. The increased butyrate flux is important because butyrate is a major source of energy to colonic mucosa (Roediger, 1982), and an important link to gut health via stimulation of cell proliferation (Comalada et al., 2006), promotion of apoptosis, and prevention of colon cancer (Wong et al., 2006; Hamer et al., 2008). Propionate acts as substrate for hepatic gluconeogenesis and inhibits cholesterol synthesis in liver (Hamer et al., 2005). Dietary oat β-glucan may thus improve intestinal and metabolic health.

Glucose link to hormones

The principle component analysis demonstrated strong relations among portal glucose, insulin, incretins, and SCFA, with SCFA inversely related to all others. Further specific regression analyses between portal glucose and portal insulin, C-peptide, GIP, and GLP-1 indicated that glucose in portal blood acts as stimulant for secretion of incretins and insulin. Glucose sensors in the arterial blood supplying the β-cells of Islets of Langerhans and in the portal vein may stimulate the insulin secretion (Jenkins et al., 1995).

The present study demonstrated that 6% oat β-glucan lowered peak net glucose flux in the pig concurrently with an attenuated incretin and insulin response, thereby explaining some of the underlying physiology and metabolism of dietary NSP. The principle component analysis of portal variables indicated strong associations among glucose and incretins providing further evidence that portal glucose acts as strong stimulus for incretin release. Incretins in turn affect insulin release and thereby enforce the existence of the entero-insular axis or cross- talk between intestine and pancreas to control blood glucose. Finally, increased fermentation and net flux of propionate and butyrate provided solid evidence that oat β-glucan are fermented and are beneficial to manage human metabolic diseases and gut health.

3.6 References

AOAC, 2006 Official methods of analysis.18th ed. Assoc. Off. Anal. Chem. Arlington, VA

Bach Knudsen, K. E., H. Jorgensen, and N. Canibe. 2000. Quantification of the absorption of nutrients derived from carbohydrate assimilation: Model experiment with catheterised pigs fed on wheat- or oat-based rolls. Br. J. Nutr. 84:449-458.

Bach Knudsen, K. E., A. Serena, A. K. Kjaer, H. Jorgensen, and R. Engberg. 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135:1696-1704.

Baggio, L. L., and D. J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131-2157.

Biorklund, M., J. Holm, and G. Onning. 2008. Serum lipids and postprandial glucose and insulin levels in hyperlipidemic subjects after consumption of an oat beta-glucan-containing ready meal. Ann. Nutr. Metab. 52:83-90.

Brennan, C. S. 2005. Dietary fibre, glycaemic response, and diabetes. Mol. Nutr. Food Res. 49:560-70.

Brighenti, F. 1998. Summary of the conclusion of the working group on profibre interloboratory study on determination of short chain fatty acids in blood. In: Gullion R, Amado F, Amaral-Collaco JA, Anderson H, Asp NG, Bach Knudsen KE, Champ M, Mathers J, Robertson JA, Rowland I, Van Loo J, editors. Functional Properties of non-digestible carbohydrates. Science, Research and Development, Brussels: European Commission, DG XII. p. 150-3.

Burcelin, R., P. D. Cani, and C. Knauf. 2007. Glucagon-like peptide-1 and energy homeostasis. J. Nutr. 137:2534S-2538S.

Cani, P. D., C. Dewever, N. M. Delzenne. 2004. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br. J. Nutr. 92:521-526.

Comalada, M., E. Bailon, O. de Haro, F. Lara-Villoslada, J. Xaus, A. Zarzuelo, and J. Galvez. 2006. The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. J. Cancer Res. Clin. Oncol. 132:487-497.

Darwiche, G., O. Bjorgell, and L. O. Almer. 2003. The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy subjects. BMC Gastroenterology. 3:12.

Drozdowski, L. A., W. T. Dixon, M. I. McBurney, and A. B. Thomson. 2002. Short-chain fatty acids and total parenteral nutrition affect intestinal gene expression. J. Parenter. Enteral Nutr. 26:145-150.

Eastwood, M. A., and E. R. Morris. 1992. Physical properties of dietary fiber that influence physiological function: A model for polymers along the gastrointestinal tract. Am. J. Clin. Nutr. 55:436-442.

Edwards, C. A., I. T. Johnson, and N. W. Read. 1988. Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? Eur. J. Clin. Nutr. 42:307-12.

Ellegard, L., and H. Andersson. 2007. Oat bran rapidly increases bile acid excretion and bile acid synthesis: An ileostomy study. Eur. J. Clin. Nutr. 61:938-945.

Ellis, P. R., F. G. Roberts, A. G. Low, and L. M. Morgan. 1995. The effect of high-molecular-weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in the growing pig: Relationship to rheological changes in jejunal digesta. Br. J. Nutr. 74:539-56.

Englyst, H. N., M. E. Quigley, and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119:1497-1509.

Gault, V. A., F. P. O'Harte, and P. R. Flatt PR. 2003. Glucose-dependent insulinotropic polypeptide (GIP): Anti-diabetic and anti-obesity potential? Neuropeptides 37:253-263.

Hamer, H. M., D. Jonkers, K. Venema, S. Vanhoutvin, F. J. Troost, and R. J. Brummer. Review article: The role of butyrate on colonic function. Aliment. Pharmacol. Ther. 27:104-119

Holst, J. J., T. Vilsboll, and C. F. Deacon. 2009. The incretin system and its role in type 2 diabetes mellitus. Mol. Cell Endocrinol. 297:127-136.

Hooda, S., J. J. Matte, C. W. Wilkinson, and R. T. Zijlstra. 2009. Technical note: An improved surgical model for the long-term studies of kinetics and quantification of nutrient absorption in swine. J. Anim. Sci. 87:2013-2019.

Isaksson, G., I. Lundquist, and I. Ihse. 1982. Effect of dietary fiber on pancreatic enzyme activity in vitro. Gastroenterology 82:918-924.

Jenkins, A. L., D. J. Jenkins, T. M. Wolever, A. L. Rogovik, E. Jovanovski, V. Bozikov, D. Rahelic, and V. Vuksan. 2008. Comparable postprandial glucose reductions with viscous fiber blend enriched biscuits in healthy subjects and patients with diabetes mellitus: Acute randomized controlled clinical trial. Croat Med. J. 49:772-782.

Jenkins, A. L., and D. J. Jenkins.1994. Dietary fibre, glycaemic index and diabetes. S Afr Med J. 1994:36-37.

Jenkins, D. J., R. G. Josse, A. L. Jenkins, T. M. Wolever, and V. Vuksan. 1995. Implications of altering the rate of carbohydrate absorption from the gastrointestinal tract. Clin. Invest. Med. 18:296-302.

Juntunen, K. S., L. K. Niskanen, K. H. Liukkonen, K. S. Poutanen, J. J. Holst, and H. M. Mykkanen. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. Am. J. Clin. Nutr. 75:254-262.

Juvonen, K. R., A. K. Purhonen, M. Salmenkallio-Marttila, L. Lahteenmaki, D. E. Laaksonen, K. H. Herzig, M. I. Uusitupa, K. S. Poutanen and L. J.

Karhunen. 2009. Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. J. Nutr. 139:461-466.

Kim, H., K. S. Stote, K. M. Behall, K. Spears, B. Vinyard, and J. M. Conway. 2009. Glucose and insulin responses to whole grain breakfasts varying in soluble fiber, beta-glucan: A dose response study in obese women with increased risk for insulin resistance. Eur. J. Nutr. 48:170-175.

Littell, R. C., R. F. Freund, and P.C. Spector. 1991. SAS system for linear models. 3rd ed. SAS Inst. Inc., Cary, NC.

Maki, K. C., M. H. Davidson, M. S. Witchger, M. R. Dicklin, and P. V. Subbaiah. 2007. Effects of high-fiber oat and wheat cereals on postprandial glucose and lipid responses in healthy men. Int. J. Vitam. Nutr. Res. 77:347-356.

Morgan, L. M., J. A. Tredger, J. Wright, and V. Marks. 1990. The effect of soluble- and insoluble-fibre supplementation on post-prandial glucose tolerance, insulin and gastric inhibitory polypeptide secretion in healthy subjects. Br. J. Nutr. 64:103-110.

Panahi, S., A. Ezatagha, F. Temelli, T. Vasanthan, and V. Vuksan. 2007. Beta-glucan from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. J. Am. Coll. Nutr. 26:639-644.

Polonsky, K. S., and A. H. Rubenstein. 1984. C-peptide as a measure of the secretion and hepatic extraction of insulin. pitfalls and limitations. Diabetes 33:486-494.

Rehfeld, J. F. 1998. The new biology of gastrointestinal hormones. Physiol Rev. 78:1087-1108.

Reimer, R. A., and M. I. McBurney. 1996. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology 177:3948-3956.

Rerat, A., P. Vaugelade, and P. Villiers. 1980. A new method for measuring the absorption of nutrients in the pig: Critical examination. Pages 177–216 in Current Concepts of Digestion and Absorption in Pigs. Tech. Bull. 3. A. G. Low and I. G. Partridge, ed. National Institute for Research in Dairying, Reading, England, and Hannah Research Institute, Ayr, Scotland.

Rerat, A. A., P. Vaissade, and P. Vaugelade. 1984. Absorption kinetics of some carbohydrates in conscious pigs. 2. Quantitative aspects. Br. J. Nutr. 51:517-529.

Roediger, W. E. 1982. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 83:424-429.

Schirra, J., M. Katschinski, C. Weidmann, T. Schafer, U. Wank, R. Arnold, and B. Goke. 1996. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J. Clin. Invest. 97:92-103.

Schneeman, B. O., and D. Gallaher. 1985. Effects of dietary fiber on digestive enzyme activity and bile acids in the small intestine. Proc. Soc. Exp. Biol. Med. 180:409-414.

Serena, A., H. Jorgensen, and K. E. Bach Knudsen. 2009. Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber. J. Anim. Sci. 87:136-147.

Simoes Nunes, C., and K. Malmlof. 1992. Glucose absorption, hormonal release and hepatic metabolism after guar gum ingestion. Reprod. Nutr. Dev. 32:11-20.

St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741-755.

Tappy, L., E. Gugolz, and P. Wursch. 1996. Effects of breakfast cereals containing various amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM subjects. Diabetes Care 19:831-834.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6:24-27.

Wong, J. M., R. de Souza, C. W. Kendall, A. Emam, and D. J. Jenkins. 2006. Colonic health: Fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235-243.

Chapter 4: Effects of viscosity and fermentability of dietary fiber on nutrient digestibility and digesta characteristics in ileal-cannulated grower pigs[3]

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4.1 Abstract

Relative contributions of 2 functional properties, viscosity and fermentability of dietary fiber on apparaent ileal digestibility (AID) and apparent total tract digestibility (ATTD), digesta passage rate, N retention, and SCFA concentration have not been established. Thus, 8 ileal-cannulated pigs randomized in a double 4 x 4 Latin square were fed 4 diets based on cornstarch and casein supplemented with 5% of actual fiber, either low fermentable, low viscous cellulose (CEL), low fermentable, high viscous carboxymethylcellulose (CMC), high fermentable, low viscous oat β-glucan (LBG), or high fermentable, high viscous oat β-glucan (HBG). Viscosity and fermentability interacted to affect ($P < 0.001$) average daily gain (ADG), digesta viscosity, AID and ATTD of nutrients and tended to affect ($P < 0.10$) digesta passage rate and digesta butyrate. Pigs fed CMC had the lowest ($P < 0.05$) digesta passage rate and the highest ($P < 0.001$) AID of energy, crude protein, and DM and ATTD of energy and DM. The post-ileal DM digestibility was highest ($P < 0.001$) for pigs fed CEL and HBG. Post-ileal DM digestibility had a negative, curvilinear relation with the AID of energy, crude protein, and ash ($R^2 = 0.85$, 0.72, and 0.73, respectively; $P < 0.001$). Digesta viscosity had a less strong relation with AID of energy, crude protein, and ash ($R^2 = 0.45$, 0.36 and 0.36, respectively; $P < 0.001$). In conclusion, negative effects of dietary fiber on digesta characteristics and nutrient digestibility are affected by interactions of viscosity and fermentability via digesta viscosity and digesta passage rate.

4.2 Introduction

Dietary fiber is an important component of feedstuffs, but is not digested by porcine digestive enzymes. Dietary fiber may be fermented, mostly in the large intestine, and may reduce digestibility of other macronutrients (Owusu-Asiedu et al., 2006). The physiological effects of dietary fiber are attributable to 2 functional properties, viscosity and fermentability (Dikeman and Fahey, 2006). Soluble fiber binds water, increases digesta viscosity, modifies digesta passage rate, and thereby reduces nutrient digestibility in the small intestine (Graham et al., 1986; Renteria-Flores et al., 2008). Non-digested nutrients along with fermentable fiber pass into the large intestine and are fermented by microbial populations, thereby producing SCFA (Bach Knudsen and Hansen, 1991).

Effects of types of dietary fiber on nutrient digestibility have been linked to structural characteristics and solubility of fiber (Kirchgessner et al., 1991; Renteria-Flores et al., 2008; Serena et al., 2008). However, the role and specific contributions of viscosity and fermentability are largely unknown. Moreover, most studies investigating the role of fiber are based on feeding fiber-rich feeds with fiber as part of intact plant cell wall; therefore, specific effects of functional properties or structural effects of fiber cannot be differentiated (Bach Knudsen et al., 1993). Thus, the concept of feeding purified fiber fractions with semi-purified diets was adopted to study specific contributions of viscosity and fermentability and their interactions.

The hypothesis of the study was that interactive effects of viscosity and fermentability of fiber independently affect digesta characteristics and nutrient digestibility in the pig. The objectives were to understand the independent and

interactive effects of the 2 functional properties of fiber in semi-purified diets on nutrient digestibility, digesta characteristics and passage rate, fermentation, and N retention in ileal-cannulated grower pigs.

4.3 Materials and Methods

Animals and Diets

The animal protocol was approved by the Animal Care Committee of the University of Alberta, and followed guidelines established by the Canadian Council on Animal Care (CCAC, 1993). The animal experiment was conducted at the Swine Research and Technology Centre at the University of Alberta (Edmonton, Alberta, Canada).

Eight crossbred barrows (initial BW, 20 to 25 kg; Duroc × Large white/Landrace; Genex Hybrid; Hypor, Regina, Saskatchewan, Canada) were moved 1 wk prior to surgery into individual metabolism pens (1.2 × 1.2 m). Each pen was equipped with a single-space feeder and a low-pressure bowl drinker. Pigs were surgically modified with a T-cannula at the distal ileum. At 10 d post surgery, 8 pigs were randomly assigned to 1 of 4 experimental diets according to a double 4 × 4 Latin square design. The daily feed allowance was adjusted to 3 × maintenance of energy (3 × 110 kcal DE/kg $BW^{0.75}$; NRC 1998) and was fed as mash in 2 equal meals at 0800 and 1600 with free access to water.

The 4 semi-purified experimental diets were based on corn starch, casein, and canola oil (**Table 4.1**). Diets contained 5% of actual dietary fiber, corrected for impurities, from 4 sources differing in viscosity and fermentability: low fermentable, low viscous cellulose (**CEL**; TIC Pretested ®

TICACEL 100 cellulose powder; TIC Gums Inc., White Marsh, MD), low fermentable, high viscous carboxymethylcellulose (**CMC**; TIC Pretested ® CMC 6000 fine powder; TIC Gums Inc., White Marsh, MD), high fermentable, low viscous oat β-glucan (**LBG**; Oat Vantage®; GTC Nutrition, Golden, CO), or high fermentable, high viscous oat β-glucan (**HBG**; Viscofiber®; Cevena Bioproducts, Edmonton, Alberta, Canada).

High and low viscous fiber were selected based on in vitro viscosity determined in a 0.5% solution using a rheometer (UDS 200; Paar Physica, Glenn, VA) at shear rate of 12.9/s and temperature 20ºC (Ghotra et al., 2009). Gas production, as a function of fermentation of fiber sources was determined for 12 h using an in vitro gas production technique (Jha et al., 2009). Diets were fortified with vitamin and mineral to meet or exceed requirements (NRC, 1998). Titanium dioxide (**TiO_2**) was included in the diets as an indigestible marker.

Experimental procedure

Each 17-d experimental period consisted of a 10-d of acclimation to diets, followed by a 3-d collection of feces and urine, and a 4-d collection of ileal digesta. Feces were collected from 0800 to 1600 after attaching bags to rings glued around anus (Van Kleef et al., 1994). Urine was collected in buckets containing 25 mL of concentrated sulphuric acid for 24 h and weighed and pooled for 3 d of collection. Ileal digesta for digestibility measurements were collected from 0800 to 1600 h into plastic bags (length, 10 cm; diameter, 4 cm) containing 8 mL of 10% (vol/vol) formic acid to minimize bacterial fermentation. Every third bag of digesta was collected without formic acid for SCFA analyses.

Table 4.1 Ingredient composition of the experimental diets containing 4 fiber sources (as-fed basis)[1]

	Low fermentable		High fermentable	
	Low viscous	High viscous	Low viscous	High viscous
Ingredient, %	CEL	CMC	LBG	HBG
Corn starch[2]	72.90	71.85	68.85	67.46
Ca caseinate[3]	16.00	16.00	16.00	16.00
Cellulose[4]	5.20	-	-	-
Carboxymethylcellulose[5]	-	6.25	-	-
Low viscous oat β-glucan[6]	-	-	8.95	-
High viscous oat β-glucan[7]	-	-	-	9.25
Dicalcium phosphate	1.20	1.20	1.20	1.20
Celite[8]	1.00	1.00	1.00	1.00
Canola oil	1.00	1.00	1.00	1.00
Limestone	0.90	0.90	0.90	0.90
Mineral premix[9]	0.50	0.50	0.50	0.50
Vitamin premix[10]	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Titanium dioxide	0.30	0.30	0.30	0.30

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan.

[2]Melojel (National Starch and Chemical Co., Bridgewater, NJ).

[3]Spray-dried Ca caseinate (American Casein Co., Burlington, NJ).

[4]TIC Pretested® TICACEL 100 cellulose powder (TIC Gums Inc., White Marsh, MD); 100 g contains 99 g of insoluble dietary fiber, 28 mg of Na, and 3 mg of Ca.

[5]TIC Pretested® CMC 6000 fine powder (TIC Gums Inc., White Marsh, MD); 100 g contains 80 g of soluble dietary fiber, 7,943 mg of Na, 9 mg of Ca, and 19 mg K.

[6]OatVantage™ oat bran concentrate (GTC Nutrition, Golden, CO); 100 g contains 54 g of β-glucan.

[7]Viscofibre® oat β-glucan concentrate (Cevena Bioproducts, Edmonton, Alberta, Canada); 100 g contains 45 g of β-glucan.

[8]Acid-insoluble ash (Celite Corp., Lompoc, CA).

[9]Provided per kilogram of diet: Zn, 100 mg as zinc sulfate; Fe, 80 mg as ferrous sulfate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulfate; I, 0.5 mg as calcium iodate; and Se, 0.1 mg as sodium selenate.

[10]Provided per kilogram of diet: vitamin A, 8250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine,1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 0.025 mg.

For digesta passage rate, on d 7 of collection, 40 mL of liquid marker (**Cr-EDTA**) and 1 g of solid marker (Ytterbium oxide; $\mathbf{Yb_2O_3}$) were mixed into the morning single meal. The meal was fed and digesta was collected at 90, 180, 270, 360, 540, and 720 min after feed consumption (Wilfart et al., 2007). The collected feces, urine, and digesta were pooled by pig observation and immediately frozen at –20ºC. Feces and digesta were homogenized, subsampled, freeze-dried, and ground finely over a 0.5-mm screen in a centrifugal mill (model ZM1, Retsch; Brinkman Instruments, Rexdale, Ontario, Canada).

Chemical Analyses

Diets, digesta, and feces were analyzed in duplicate for DM, GE, starch, CP, ether extract, and dietary fiber (soluble, insoluble, and total). The DM was analyzed by drying at 135°C in an airflow-type oven for 2 h (method 930.15; AOAC, 2006), GE by an adiabatic bomb calorimeter (model 5003, Ika-Werke GMBH & Co. KG, Staufen, Germany), CP using oxidation (N × 6.25; FP-428 N determinator, Leco Corp., St. Joseph, MI), ash (method 9420.5; AOAC, 2006), and ether extract using Goldfish Extraction apparatus using diethyl ether solvent (method 920.39; AOAC, 2006). The AA in digesta and feed were analyzed by ion-exchange chromatography (Htoo et al., 2007). Total starch, dietary fiber, and mixed linked β-glucan were analyzed using kits (Megazyme International Ireland Ltd., Bray, Ireland) based on enzymatic analysis (methods 996.11, 2002.02, 985.29, and 995.16, respectively; AOAC, 2006). The TiO_2 was analyzed using spectrophotometer (method 540.91; AOAC, 2006). Fresh fecal and digesta samples were analyzed for DM (method 930.15;

AOAC, 2006) and SCFA using gas chromatography (Htoo et al., 2007). Digesta samples were analyzed for pH (Accumet Basic AB15; Fischer Scientific, Fairlawn, NJ) and viscosity (DV-I Viscometer; Brookfield, Middleboro, MA). Viscosity values were converted to a log scale to reach a normal distribution. The Cr-EDTA and Yb_2O_3 were analyzed using an atomic absorption spectrometer (Varian SpectAA 240 FS, Mississauga, Ontario, Canada) using a standard procedure (Siddons et al., 1985). Urinary N was analyzed using Kjeldahl (method 968.06; AOAC, 2006).

Calculations

The apparent ileal (**AID**) and apparent total tract digestibility (**ATTD**) of DM, CP, ash, starch, and energy were calculated using the TiO_2 concentration of feed, digesta, and feces. The post-ileal DM digestibility was calculated as difference between ATTD and AID of DM. The equation described by Wilfart et al. (2007) was used for calculation of mean retention time.

$$MRT_{digesta} = \left(\sum_{i=1}^{n} Citi \right) / \left(\sum_{i=1}^{n} Ci \right)$$

where Ci is the marker concentration at time t_i after ingestion of the marked meal. The digesta passage rate were calculated from the linear relationship following first-order kinetics as described by the equation log Y = a + bX, where Y is log of Yb_2O_3 concentration (mg of Yb_2O_3/kg of DM) and X is time (h) and thus the slope (b) of the line is the digesta passage rate (Potkins et al., 1991).

Statistical Analyses

To compare differences among diets, data were subjected to ANOVA using the mixed procedure (SAS Inst. Inc., Cary, NC). The fixed effect of diet (n = 4) and the random effect of experimental period (n = 4) and pigs (n = 8) were included in the main model. The pig was used as the experimental unit and means were reported as least-squares means. Specific effects of viscosity and fermentability and their interaction were analyzed using contrast statements. In case viscosity and fermentability interacted ($P < 0.10$), means were separated using the probability of difference. Differences were considered significant if $P < 0.05$ and were described as tendencies if $0.05 \leq P < 0.10$. Principal component (**PC**) analysis was performed using multivariate analysis of JMP software (version 8.0.1; SAS) and the loading plot was used to determine correlations among individual variables of the first 2 eigenvalues (i.e., PC 1 and 2). The relationship between the AID of energy, CP, and ash with post-ileal DM digestibility (fermentability) and viscosity were analyzed using the weighted linear and non-linear regression analysis (PROC REG, SAS) with predicted values of the dependent variable adjusted for period and pig effects (St-Pierre, 2001).

4.4 Results

Pigs and Diets

All pigs remained healthy throughout the experiment. The starch content differed slightly among diets (**Table 4.2**). Dietary fiber content was mostly insoluble for CEL whereas CMC and LBG contained mostly soluble dietary fiber. In contrast, the HBG diet contained an equal amount of insoluble and

soluble dietary fiber. The CP and AA content was higher in LBG and HBG, because the 2 oat β-glucan concentrates contained some CP. Similarly, the GE content was higher in LBG and HBG and could be related to high energy content in oat β-glucan concentrates. Diets met the minimum requirements of nutrients for pig of that BW group.

ADG, digesta characteristics, and N balance

Viscosity and fermentability interacted ($P < 0.001$; **Table 4.3**) to change ADG of pigs; specifically, pigs fed CMC had a 38% lower ($P < 0.05$) ADG than pigs fed the other 3 fiber sources. Viscosity and fermentability did not affect the pH of ileal digesta. Viscosity and fermentability interacted ($P < 0.001$; **Table 4.3**) to change digesta viscosity and DM content of digesta and feces. Pigs fed CMC had the highest ($P < 0.05$) digesta viscosity followed by HBG and LBG, whereas pigs fed CEL had the lowest ($P < 0.05$) digesta viscosity. The DM content of fresh digesta was highest ($P < 0.05$) for pigs fed CEL, followed by LBG and HBG, and was lowest ($P < 0.05$) for pigs fed CMC. The DM content of fresh feces was half ($P < 0.05$) for pigs fed CMC than for pigs fed the other 3 fiber sources.

Total N intake tended to be higher ($P < 0.10$; **Table 4.3**) after feeding high than low viscosity fiber sources. Viscosity and fermentability interacted ($P < 0.05$) to affect fecal N loss; specifically, pigs fed CMC had a lower ($P < 0.05$) fecal N than CEL and HBG. Viscosity and fermentability did not affect urinary N loss and N retention. Viscosity and fermentability tended to interact ($P < 0.10$) to affect fecal/urinary N ratio. Pigs fed CMC had lower fecal/urinary N ($P < 0.05$) than pigs fed HBG.

Table 4.2 Analyzed chemical composition and in vitro viscosity of experimental diets containing 4 fiber sources (as-fed basis)[1]

	Low fermentable		High fermentable	
	Low viscous	High viscous	Low viscous	High viscous
Item	CEL	CMC	LBG	HBG
Starch, %	62.95	60.22	60.23	57.53
Dietary fiber, %				
Insoluble	5.38	1.94	1.96	4.53
Soluble	0.00	4.71	6.10	4.34
Total	5.38	6.65	8.06	8.87
β-glucan, %	0.00	0.00	5.09	4.59
CP, %	14.83	14.71	16.27	15.69
GE, Mcal/kg	3.80	3.82	3.91	3.93
Ether extract, %	0.40	0.26	0.68	0.64
Ash, %	4.47	5.26	4.64	4.97
Indispensable AA, %				
Arg	0.35	0.35	0.41	0.38
His	0.41	0.38	0.42	0.43
Ile	0.86	0.85	0.90	0.89
Leu	1.40	1.40	1.48	1.45

Lys	1.15	1.15	1.19	1.17
Met	0.25	0.24	0.27	0.25
Phe	0.75	0.74	0.80	0.78
Thr	0.55	0.50	0.60	0.57
Val	1.00	0.99	1.06	1.04
Dispensable AA, %				
Ala	0.46	0.46	0.52	0.51
Asp	1.04	1.02	1.13	1.20
Glu	3.29	3.25	3.52	3.37
Gly	0.31	0.31	0.43	0.37
Tyr	0.50	0.48	0.53	0.53
In-vitro viscosity, mPa•s, log[2]	0.40	3.29	1.69	3.54
Cumulative gas production, ml/g	36	19	187	167

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan.

[2]In vitro viscosity of the 4 test ingredients was (mPa•s, log[2]): CEL, 0.49, CMC, 2.46, LBG, 1.38, and HBG, 2.32.

Table 4.3 The ADG, physiochemical characteristics of digesta, and N balance of pigs fed experimental diets containing 4 fiber sources[1,2]

	Low fermentable		High fermentable					
	Low viscous	High viscous	Low viscous	High viscous		*P*-value		
Item	CEL	CMC	LBG	HBG	SEM	V	F	V × F
ADG, g/d	540[a]	330[b]	530[a]	570[a]	50	0.006	<0.001	<0.001
pH	4.7	5.0	5.0	5.0	0.2	0.335	0.456	0.449
Digesta viscosity, mPa•s, log	2.5[c]	4.1[a]	3.2[b]	3.3[b]	0.2	<0.001	<0.001	<0.001
Fresh digesta DM, %	17.2[a]	5.5[c]	8.6[b]	9.0[b]	0.9	<0.001	0.019	<0.001
Fresh feces DM, %	51.3[a]	25.9[b]	49.9[a]	55.4[a]	4.7	<0.001	<0.001	<0.001
Total N intake, g/d	46.8	47.7	46.1	47.5	8.1	0.089	0.501	0.677
Fecal N loss, g/d	7.6[a]	5.2[b]	6.9[ab]	8.0[a]	1.7	0.391	0.159	0.029
Urinary N loss, g/d	9.5	12.0	10.5	10.5	1.7	0.218	0.792	0.240
N retention, g/d	30.4	31.1	28.8	29.3	5.6	0.633	0.223	0.943
Fecal/urinary N ratio	0.7[ab]	0.5[b]	0.6[ab]	0.8[a]	0.1	0.510	0.769	0.094

[abc]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan; V = viscosity; F = fermentability.

[2]Means are least-squares means based on 8 observations per diet.

Digesta kinetics

Viscosity and fermentability did not affect mean retention time for liquid digesta (**Table 4.4**). However, high viscous fiber sources increased ($P < 0.05$) the mean retention time of solid digesta by 18% compared to low viscous fiber sources. Finally, viscosity and fermentability tended to interact ($P < 0.10$) to affect digesta passage rate. The feeding of CMC reduced ($P < 0.05$) digesta passage rate by 66% compared to the other 3 fiber sources.

Apparent ileal, total tract and post-ileal nutrient digestibility

Viscosity and fermentability of fiber interacted to affect AID of nutrients ($P < 0.001$; **Table 4.5**). The AID of CP, DM, and ash were highest ($P < 0.05$) in CMC fed pigs, intermediate for LBG and were lowest ($P < 0.05$) for CEL and HBG fed groups. The AID of energy was 10 to 12 percentage units higher ($P < 0.05$) in pigs fed CMC than other 3 fiber sources, and DE content followed a similar pattern. Viscosity and fermentability of fiber interacted to affect ($P < 0.001$; Table 5) the ATTD of nutrient. The ATTD of ash was higher ($P < 0.05$) in pigs fed CMC than the other 3 fiber sources. The ATTD of CP was highest ($P < 0.05$) for CMC fed pigs and was lowest ($P < 0.05$) for HBG. The ATTD of DM and energy were highest ($P < 0.05$) in pigs fed LBG and lowest ($P < 0.05$) for CEL fed groups.

The post-ileal ash digestibility as percentage of intake was lower ($P < 0.05$) for pigs fed viscous fiber. Viscosity and fermentability tended to interact ($P < 0.1$) to affect post-ileal digestibility of CP and was highest ($P < 0.05$) in pigs fed CEL and lowest ($P < 0.05$) in CMC fed groups.

Table 4.4 Mean retention time of solid and liquid digesta and the digesta passage rate of pigs fed experimental diets containing 4 fiber sources[1,2]

	Low fermentable		High fermentable					
	Low viscous	High viscous	Low viscous	High viscous		*P*-value		
Item	CEL	CMC	LBG	HBG	SEM	V	F	V × F
Mean retention time, h								
Liquid digesta	5.4	6.1	6.1	5.8	0.5	0.528	0.528	0.981
Solid digesta	5.1	6.8	6.0	6.3	0.5	0.015	0.333	0.913
Digesta passage rate[3]	0.30[a]	0.10[b]	0.29[a]	0.29[a]	0.07	0.124	0.010	0.095

[ab]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan; V = viscosity; F = fermentability.

[2]Means are least-squares means based on 8 observations per diet.

[3]Calculated from linear relationship following first-order kinetics as described by the equation log Y = a + bX, where Y is log of Yb_2O_3 concentration (mg of Yb_2O_3/kg of DM) and X is time (h) and thus the slope (b) of the line is rate constant.

Similarly, viscosity and fermentability interacted ($P < 0.05$) to affect post-ileal digestibility of DM and energy and lowest ($P < 0.05$) after feeding CMC as compared to other 3 fiber fed groups.

Post-ileal digestibility of ash as % of entering in cecum was affected by both viscosity ($P < 0.05$) and fermentability ($P < 0.05$). Viscosity and fermentability interacted ($P < 0.05$) to affect post-ileal digestibility of DM and energy and were lowest ($P < 0.05$) for CMC fed pigs than other 3 fiber (**Table 4.5**).

Apparent ileal AA digestibility

Viscosity and fermentability interacted ($P < 0.01$; **Table 4.6**) to change the AID of all indispensable and dispensable AA specifically, pigs fed CMC had higher ($P < 0.05$) AID than pigs fed the other 3 fiber sources.

Among the indispensable AA, pigs fed CMC had the highest ($P < 0.05$) AID of Ile, Lys, Met, Thr, and Val or pigs fed CMC and LBG highest ($P < 0.05$) AID for Arg, Leu and Phe. Pigs fed HBG had the lowest ($P < 0.05$) AID of Arg, His, Lys, and Met or pig fed CEL and HBG had lowest ($P < 0.05$) AID for Ile, Leu, Phe, Thr, and Val.

Digesta SCFA

High fermentable fiber sources reduced ($P < 0.05$; **Table 4.7**) digesta acetate by 33%, increased $P < 0.01$) caproate by 171%, and tended to reduce ($P < 0.10$) digesta total SCFA by 32%. High viscous fiber sources tended to reduce ($P < 0.10$) propionate by 50%. Viscosity and fermentability tended to interact ($P < 0.10$) for butyrate, isobutyrate, and isovalerate. Pigs fed CEL had

a lower ($P < 0.05$) butyrate and higher ($P < 0.05$) isobutyrate and isovalerate than pigs fed the other 3 fiber sources. High viscous fiber sources increased ($P < 0.05$; **Table 4.7**) the digesta concentrations of L- Lactate and total lactate.

High viscous fiber sources increased ($P < 0.001$; **Table 4.7**) the molar ratio of digesta SCFA for acetate and reduced ($P < 0.01$) the molar ratio of propionate and butyrate compared to low viscous fiber sources.

Table 4.5 Apparent ileal digestibility (AID) and total tract digestibility (ATTD) of nutrients in pigs fed experimental diets containing 4 fiber sources[1,2]

	Low fermentable		High fermentable					
	Low viscous	High viscous	Low viscous	High viscous		*P*-value		
Item	CEL	CMC	LBG	HBG	SEM	V	F	V × F
AID, %								
Ash	-41.7[c]	34.0[a]	0.9[b]	-26.0[c]	8.0	0.003	0.236	<0.001
CP	72.8[c]	85.6[a]	77.7[b]	72.8[c]	1.8	0.012	0.012	<0.001
DM	69.8[c]	86.5[a]	75.9[b]	72.6[c]	1.3	<0.001	<0.001	<0.001
Energy	76.1[b]	89.6[a]	78.0[b]	77.5[b]	1.1	<0.001	0.002	<0.001
Starch	98.5	97.7	96.1	98.6	1.3	0.694	0.350	0.189
DE, Mcal/kg	2.8[c]	3.4[a]	3.1[b]	3.0[b]	0.1	<0.001	0.749	<0.001
ATTD, %								
Ash	-49.3[b]	40.2[a]	-26.3[b]	-35.6[b]	15.9	<0.001	0.016	<0.001
CP	85.0[ab]	88.8[a]	86.3[ab]	83.2[b]	1.5	0.814	0.142	0.025
DM	83.6[b]	87.9[a]	88.2[a]	85.4[ab]	1.3	0.432	0.319	0.003
Energy	89.6[b]	90.5[ab]	92.5[a]	89.8[b]	0.8	0.257	0.141	0.027
Post-ileal digestibility,[3] % of intake								
Ash	22.3	14.9	56.8	-9.6	8.8	0.038	0.233	0.128

CP	13.0[a]	4.0[b]	8.9[ab]	10.4[ab]	2.6	0.171	0.680	0.065
DM	13.8[a]	1.4[b]	11.4[a]	12.8[a]	1.9	0.006	0.019	<0.001
Energy	13.5[a]	0.8[b]	11.8[a]	12.3[a]	1.5	<0.001	0.005	0.006
Post-ileal digestibility, % of entering cecum								
Ash	-2.9	28.0	-32.0	-8.8	14.4	0.032	0.011	0.732
CP	45.7	41.0	27.9	35.4	8.9	0.877	0.222	0.514
DM	45.4[a]	25.5[b]	42.6[a]	46.0[a]	6.1	0.108	0.135	0.042
Energy	56.2[a]	22.8[b]	55.3[a]	53.6[a]	5.2	0.005	0.012	0.009

[abc]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan; V = viscosity; F = fermentability.

[2]Means are least-squares means based on 8 observations per diet.

[3]Calculated as difference of ATTD minus AID of nutrients.

Table 4.6 Apparent ileal digestibility of AA in pigs fed experimental diets containing 4 fiber sources[1,2]

	Low fermentable		High fermentable					
	Low viscous	High viscous	Low viscous	High viscous		*P*-value		
Item	CEL	CMC	LBG	HBG	SEM	V	F	V × F
Indispensable AA, %								
Arg	78.6[b]	85.2[a]	80.8[ab]	67.0[c]	2.3	0.119	0.002	<0.001
His	90.7[ab]	93.4[a]	90.1[b]	87.0[c]	1.0	0.871	0.002	0.008
Ile	80.2[c]	90.5[a]	85.5[b]	81.0[c]	1.7	0.059	0.157	<0.001
Leu	88.0[bc]	92.7[a]	89.4[ab]	84.9[c]	1.1	0.959	0.011	<0.001
Lys	87.2[b]	92.0[a]	87.6[b]	83.6[c]	1.1	0.758	0.004	0.002
Met	95.0[b]	98.0[a]	94.9[b]	91.8[c]	0.6	0.927	<0.001	<0.001
Phe	88.7[ab]	92.5[a]	89.2[a]	83.3[b]	1.3	0.440	0.004	0.002
Thr	74.3[bc]	85.6[a]	78.6[b]	71.3[c]	2.5	0.248	0.007	<0.001
Val	79.6[bc]	88.8[a]	83.1[b]	77.1[c]	1.6	0.259	0.008	<0.001
Dispensable AA, %								

Ala	71.5[b]	82.0[a]	75.3[ab]	63.0[c]	2.9	0.746	0.009	<0.001
Asp	77.3[b]	85.5[a]	77.0[b]	69.8[c]	2.3	0.813	<0.001	<0.001
Glu	85.4[b]	92.4[a]	87.6[b]	86.1[b]	1.4	0.014	0.055	<0.001
Gly	50.4[a]	60.5[a]	52.5[a]	37.5[b]	4.9	0.488	0.008	0.002
Ser	67.7[b]	86.6[a]	75.6[c]	71.9[bc]	3.2	0.002	0.125	<0.001
Tyr	92.2[a]	93.9[a]	91.3[a]	85.4[b]	1.0	0.065	<0.001	0.002

[abc]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan; V = viscosity; F = fermentability.

[2]Means are least-squares means based on 8 observations per diet.

Table 4.7 Digesta SCFA concentrations and molar ratio in pigs fed experimental diets containing 4 fiber sources[1,2]

	Low fermentable		High fermentable					
	Low viscous	High viscous	Low viscous	High viscous		*P*-value		
Item	CEL	CMC	LBG	HBG	SEM	V	F	V × F
Concentration, µmol/g fresh matter								
Acetate	18.8	15.5	10.1	13.0	3.3	0.923	0.027	0.209
Propionate	2.8	1.3	2.4	1.4	0.5	0.061	0.352	0.299
Butyrate	1.8[a]	0.5[b]	0.7[b]	0.5[b]	0.3	0.024	0.096	0.080
Isobutyrate	0.22[a]	0.07[b]	0.01[b]	0.01[b]	0.05	0.064	0.002	0.050
Valerate	0.12	0.08	0.06	0.04	0.02	0.120	0.033	0.722
Isovalerate	0.41[a]	0.14[b]	0.04[b]	0.04[b]	0.07	0.029	<0.001	0.029
Caproate	0.17	0.10	0.38	0.39	0.08	0.585	0.002	0.563
Total SCFA	26.1	17.8	13.8	16.1	4.7	0.416	0.068	0.154
D-lactate	0.19	2.47	1.06	3.01	1.08	0.057	0.506	0.875
L-Lactate	7.9	19.2	11.6	17.5	3.6	0.019	0.771	0.440
D+L-lactate	8.1	21.6	12.7	20.5	4.3	0.021	0.697	0.514

Molar ratio, %								
Acetate	75.9	87.8	75.1	82.5	2.5	<0.001	0.404	0.226
Propionate	13.9	7.6	16.6	9.3	1.9	<0.001	0.492	0.861
Butyrate	5.6	2.3	4.4	3.2	0.6	0.006	0.488	0.218

[abc]Within a row, means without a common superscript differ ($P < 0.05$).

[1]CEL = cellulose; CMC = carboxymethylcellulose; LBG = low viscous oat β-glucan; HBG = high viscous oat β-glucan; V = viscosity; F = fermentability; SCFA, short-chain fatty acid.

[2]Means are least-squares means based on 8 observations per diet.

Principal component analysis

The PC analysis of AID of DM, digesta total SCFA, digesta viscosity and passage rate, and ADG is shown as a loading plot (**Figure 4.1**). The plot revealed 2 clusters: a first cluster including digesta total SCFA, digesta passage rate, and ADG that was affected by PC 1 and second cluster including AID of DM and digesta viscosity that was affected by PC 2. Variables within a cluster were positively correlated. The 2 clusters were correlated negatively, because the angle between the 2 clusters exceeded 90°. Thus, the AID of DM was related positively to ileal digesta viscosity, whereas digesta total SCFA were related positive to digesta passage rate.

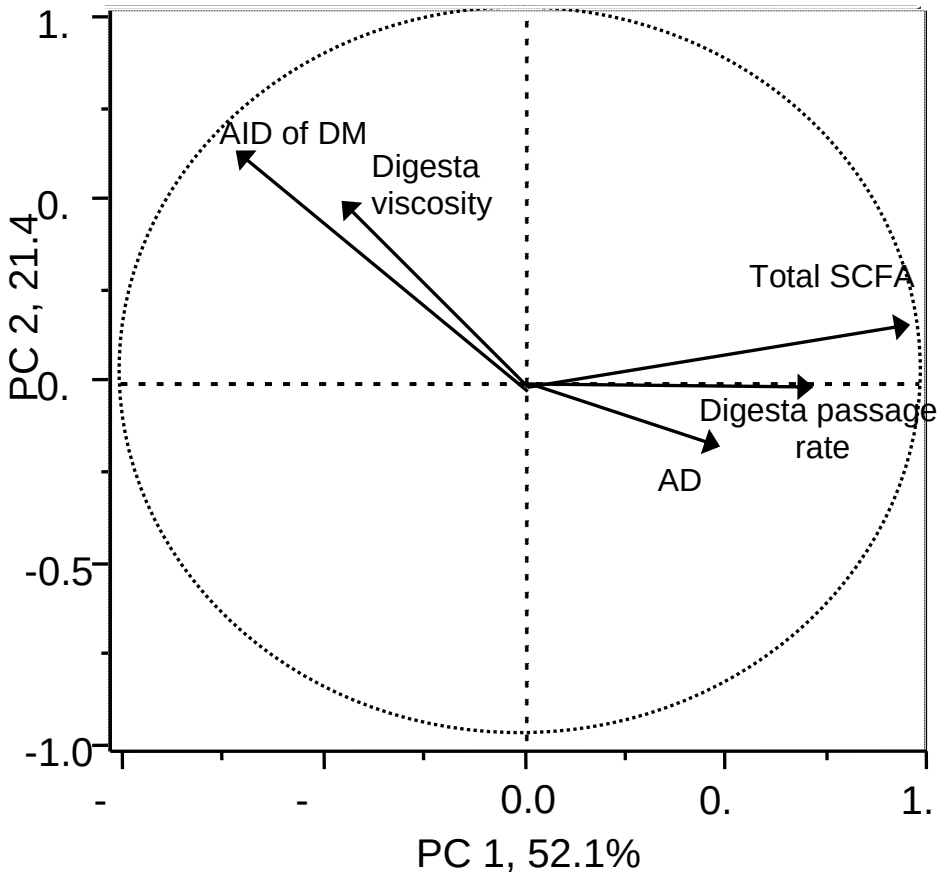

Figure 4.1. Loading plot of PC analysis showing correlations among AID of DM, total SCFA, digesta viscosity, digesta passage rate, and ADG of the first two eigenvalues (PC 1 and PC 2).

Relations between fermentability and viscosity with nutrient digestibility

Post-ileal DM digestibility was related strongly, inversely, and curvilinearly (R^2 = 0.85, 0.72, 0.73, respectively; $P < 0.001$; **Figure 4.2**) to the AID of energy, CP, and ash. Instead, digesta viscosity was related not as strong (R^2 = 0.45, 0.36, respectively; $P < 0.001$; **Figure 4.3**) to the AID of energy, CP, and ash.

4.5 Discussion

The effects of dietary fiber on nutrient digestibility are known well; however, a dearth of studies explains the role of the 2 functional properties of fiber, i.e., viscosity and fermentability and their interactions on nutrient digestibility. Viscosity is defined as the ability to thicken when mixed with fluid and most of the soluble fiber exhibit this functional property (Dikeman and Fahey, 2006). Increased digesta viscosity after feeding soluble fiber such as guar gum, rye, and pectin decreased nutrient digestibility in pigs (Owusu-Asiedu et al., 2006; Serena et al., 2008). In contrast, very high viscous carboxymethylcellulose improved CP digestibility in weaned pigs (Fledderus et al., 2007). The fiber is not digested by porcine enzymes but is instead fermented by the microbial community in distal small and large intestine. The amount of viscosity may impact the amount of fermentation, because non-viscous fiber may increase digesta passage rate and reduce small intestine digestibility, and thus partly shifts enzymatic digestion to microbial fermentation (Owusu-Asiedu et al., 2006). Similarly, an interaction of viscosity and fermentability affected most of the variables related to digestion, digesta characteristics, and fermentation.

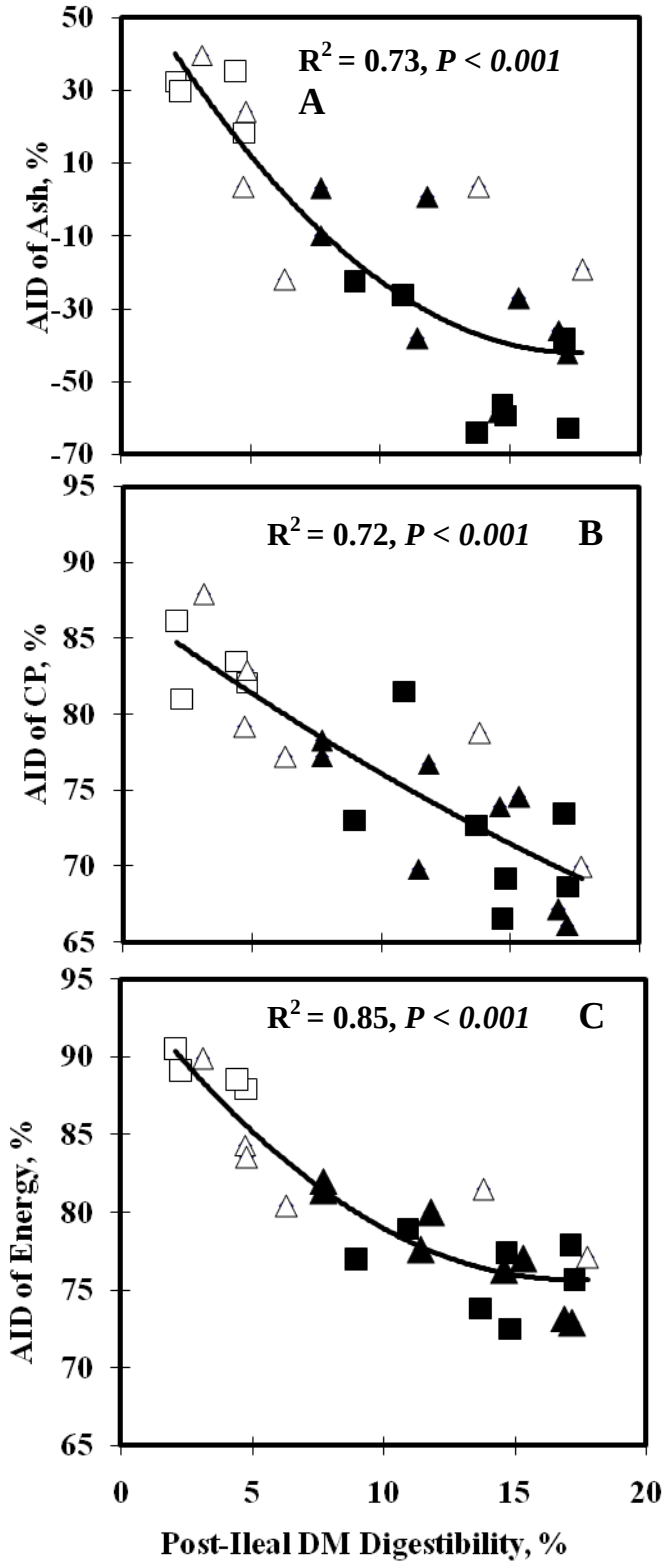

Figure 4.2 Relations between post-ileal DM digestibility and AID of ash (panel A), CP (panel B), and energy (panel C) of pigs fed experimental diets containing either 5% cellulose (CEL; ■), carboxymethylcellulose (CMC; □), low viscous oat β-glucan (LBG; △), or high viscous oat β-glucan (HBG; ▲).

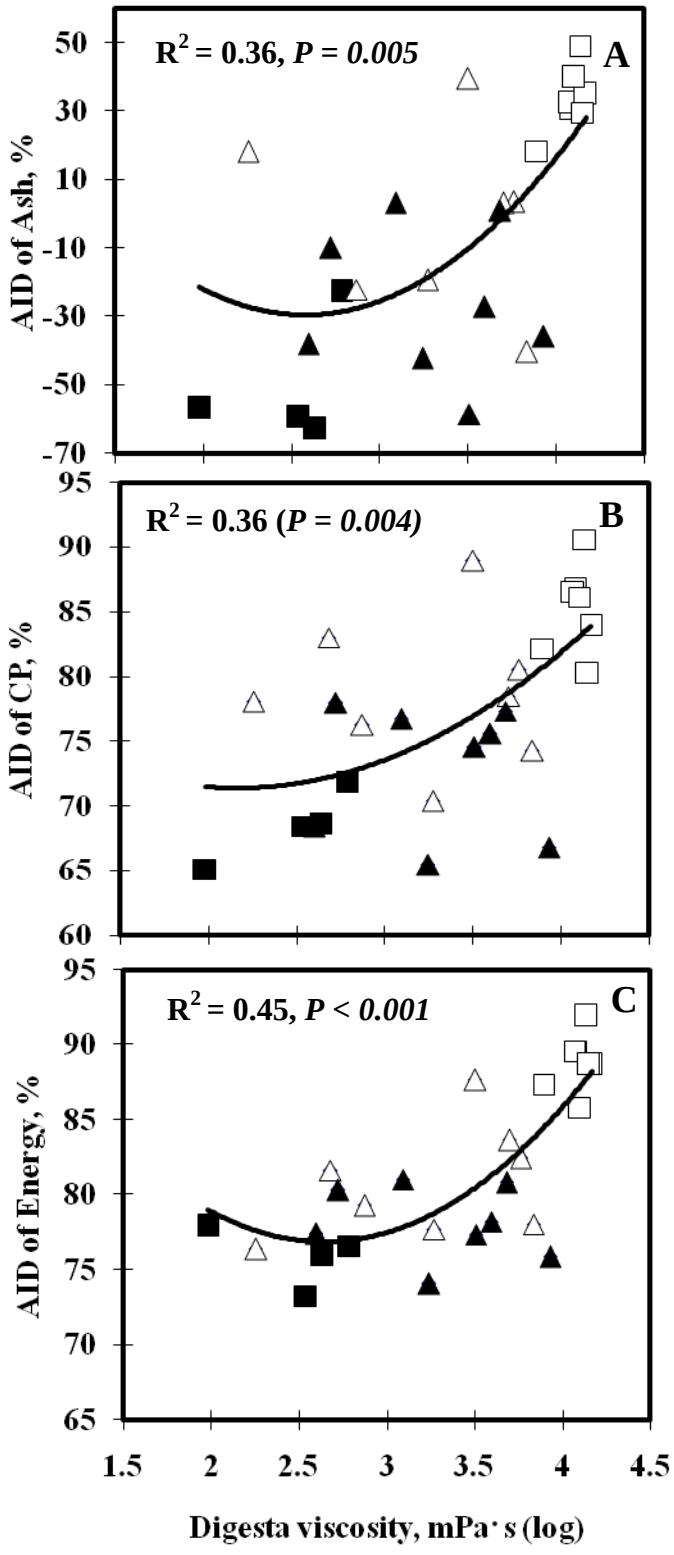

Figure 4.3 Relations between digesta viscosity of pigs with AID of ash (panel A), CP (panel B, and energy (panel C) of pigs fed experimental diets containing either 5% cellulose (CEL; ■), carboxymethylcellulose (CMC; □), low viscous oat β-glucan (LBG; △), or high viscous oat β-glucan (HBG; ▲).

In the present study, the interaction of viscosity and fermentability affected ADG and digesta characteristics. The ADG was lowest for pigs fed the high viscosu CMC diet compared to pigs fed the other 3 fiber diets. The watery digesta and feces for pigs fed CMC diets indicated less water retention and the onset of diarrhea, similar to observation with weaned pigs fed CMC or pearled barley (McDonald et al., 2001; Montagne et al., 2003; Hopwood et al., 2004), and the watery digesta was related to high digesta viscosity. The high digesta viscosity by inclusion of CMC in diets slows digesta passage rate and reduces nutrient absorption, and thereby creates a favorable environment for microbial pathogens to proliferate (van der Klis et al., 1993). Indeed, pigs fed the CMC diet in the present study had increased prevalences of *Escherichia coli* virulence factors in feces (Metzler-Zebeli et al., 2010). In this context, CMC may bind with the intestinal mucus layer and change its composition to facilitate the binding of *Escherichia coli* to intestinal mucus (Rossi et al., 1996). Inclusion of CMC in broiler chick diets reduced Na and water retention (Johnson and Gee, 1986) that could further explain the diarrhea caused by CMC. Thus, the present study indicated a strong association between digesta viscosity and reduced BW gain.

Interactions of viscosity and fermentability of fiber modified digesta viscosity. Dietary CMC caused high digesta viscosity in swine (Piel et al., 2005) and poultry (Waldenstedt et al., 2000) that was related to the high solubility of CMC (Johnson and Gee, 1986). The high digesta viscosity matched perfectly with the high diet viscosity and thus appeared to confirm the paradigm that a high diet viscosity results in a high digesta viscosity (Dikeman and Fahey, 2006). Indeed, pigs fed the HBG diet with high diet

viscosity also had high digesta viscosity, similar to studies in rats (Gallaher et al., 1999). However, in spite of low diet viscosity, pigs fed the LBG diet had the same digesta viscosity as pigs fed the HBG diet. The uplift in digesta viscosity for LBG might be due to linkages of starch fragments to β-glucan increasing viscosity (Faraj et al., 2006). The exact location and mechanism in the gastro-intestinal tract for the equalization of digesta viscosity between LBG and HBG requires further analysis, but points to diet viscosity not being entirely dependable to predict digesta viscosity.

Digesta viscosity affected digesta passage rate and thereby nutrient digestibility (Owusu-Asiedu et al., 2006). Specifically, high viscous CMC reduced digesta passage rate similar to pigs fed soluble fiber that had an increased digesta viscosity and decreased digesta passage rate (Bach Knudsen and Hansen, 1991; Owusu-Asiedu et al., 2006). In pigs fed diets containing CMC, increased digesta viscosity can partly reduce passage rate by reducing the gastric emptying rate (Montagne et al., 2003). Effects of soluble or insoluble fiber on digesta passage rate are not consistent among studies. For example, soluble fiber did not alter digesta passage rate in pigs (Latymer et al., 1990; Van Leeuwen et al., 2006) similar to the present study where digesta viscosity of pigs fed diets containing HBG and LBG was not high enough to reduce the digesta passage rate that thus remained comparable to pigs fed the low viscous CEL diet. The fiber may increase the peristaltic action of intestine (Wenk, 2001) or increase digesta bulk (Stanogias and Pearce, 1985) that might compensate effects of digesta viscosity on digesta passage rate. In the present study, PC analysis indicated that reduced digesta passage rate was positively correlated to nutrient digestibility, thereby confirming the paradigm that slow

digesta passage rate leads to more time for enzymatic digestion thereby improving digestibility (Fledderus et al., 2007). This proposed mechanism might have resulted in highest AID of CP and AA in pigs fed high viscous CMC. In contrast to pigs fed CMC, pigs fed CEL as an insoluble fiber had a higher digesta passage rate and lower mean retention time in the ileum (Wilfart et al., 2007). Thus, CEL decreased contact time between digestive enzymes and substrates thereby explaining the lower DM digestibility in pigs fed CEL, similar to reduced CP digestibility in pigs fed cellulose (Owusu-Asiedu et al., 2006). In pigs fed HBG and LBG, the higher digesta passage rate compared to pigs fed CMC likely caused lower AID of CP and energy. Moreover, digesta viscosity might have caused changes in gut motility, and mixing of digesta and thus lower nutrient digestibility (Montagne et al., 2003).

The results for ash digestibility were unique in the present study. The ATTD of ash was negative after feeding the fiber sources except for CMC, similar to recent findings in sows (Serena et al., 2008). The negative digestibility could be due to endogenous secretions of minerals (Dierick et al., 1989) or due to increased mineral requirements for microbial activity (Metzler et al., 2009). The positive ATTD of ash values with high viscous CMC could be due to the decreased digesta passage rate, which in turn increased the digestion and absorption in the small intestine (Powell et al., 1994). In contrast, with HBG and LBG diets, increased fermentation and thus more microbial mass and thus more secretion of minerals for microbial requirements (Demigné et al., 1989). The CEL as an insoluble dietary fiber might have damaged the mucosa and thus inhibit transcellular carrier-mediated mineral absorption (Oku et al., 1982).

Feeding pigs fiber has been studied as a strategy to shift the excretion of N from urine to feces and thereby minimize ammonia emission from pig barns (Kreuzer et al., 1998). Dietary fermentable fiber increase intestinal microbial populations that require N for their protein synthesis and thereby reduce N absorption by the pig or stimulate an N flux from the pig into its intestine effectively reducing excretion of excess N in urine (Canh et al., 1998). In the present study, fermentable fiber caused a trend to increase the ratio of fecal to urinary N indicating a shift of N from urine to feces, similar to previous studies (e.g., Zervas and Zijlstra, 2002). However, fermentable fiber did not cause a shift as strong in the present study because highly digestible protein feedstuffs were fed and the supply of AA matched their requirements well so that less excess N was available to be shifted to N excretion in feces. Regardless, the observed shift provides further evidence that fermentable fiber have a major role to play to reduce environmental concerns related to pig production.

The presence of SCFA in digesta indicated that the intestinal microflora did start fermentation of purified fiber sources by the end of ileum, similar to other studies in grower pigs (Bach Knudsen et al., 1991) and sows (Serena et al., 2008). The fermentation of these sources continued in the large intestine (Metzler-Zebeli et al., 2010). The post-ileal DM digestibility, an indicator of fermentation (Baumgärtel et al., 2008), did not differ among pigs fed CEL, LBG, and HBG diets. Post-ileal DM digestibility was inversely related (r^2 = 0.85) to the AID of energy, indicating that DM not digested in the small intestine is fermented in the large intestine. The higher digesta passage rate in pigs fed CEL, LBG, and HBG diets increase the flow of DM into the large

intestine for fermentation. This proposed mechanism was confirmed by the PC analysis, because digesta SCFA was positively correlated to digesta passage rate and negatively to nutrient digestibility. Interestingly, the interaction of viscosity and fermentability affected digesta butyrate, valerate, caproate and isovalerate and these SCFA were higher in digesta of pigs fed diets containing CEL. Although cellulose itself is likely low fermentable, feeding CEL resulted in higher ileal butyrate concentrations than high fermentable LBG and HBG that could be attributed to the increased availability of easily fermentable substrate. The increased digesta butyrate in pigs fed CEL compared to pigs fed other diets was further investigated by our group using samples collected from the same pigs. Increased butyrate concentrations in feces came along with higher fecal gene copies of butyryl-CoA CoA transferase, which can be used to assess the major butyrate-producing bacterial species (Louis et al., 2010), in pigs fed CEL compared to pigs fed CMC (Metzler-Zebeli et al., 2010). Higher digesta butyrate concentrations may be beneficial for intestinal health, because butyrate is the preferred source of energy for colonocytes and prevents colon cancer (Wong et al., 2006). Furthermore, increased digesta lactate concentrations in pigs fed CMC and HBG supported that intestinal absorption and bacterial utilization of lactate (Louis et al., 2007) can be markedly impaired by higher digesta viscosity in the upper gastro-intestinal tract (Wenk, 2001).

In conclusion, pigs fed high viscous CMC had the highest AID of nutrients, by slowing down the rate of digesta passage. In contrast, faster digesta passage reduced the AID of nutrients in pigs fed CEL group compared to pigs fed CMC, thereby increased nutrient flow into the large intestine so

that part of the nutrients that would normally be digested were instead fermented. Thus, negative effects of fiber on digesta characteristics and nutrient digestibility are affected by interactions of viscosity and fermentability via digesta viscosity and digesta passage rate.

4.6 References

AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Bach Knudsen, K. E., and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 1. Digestibility and bulking properties of polysaccharides and other major constituents. Br. J. Nutr. 65:217-232.

Bach Knudsen, K. E., B. B. Jensen, J. O. Andersen, and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. Br. J. Nutr. 65:233-248.

Bach Knudsen, K. E., B. B. Jensen, and I. Hansen. 1993. Digestion of polysaccharides and other major components in the small and large intestine of pigs fed on diets consisting of oat fractions rich in β-D-glucan.Br. J. Nutr. 70:537-556.

Baumgartel, T., B. U. Metzler, R. Mosenthin, R. Greiner, and M. Rodehutscord. 2008. Precaecal and postileal metabolism of P, Ca and N in pigs as affected by different carbohydrate sources fed at low level of P intake. Arch. Anim. Nutr. 62:169-81.

Canh, T. T., A. L. Sutton, A. J. A. Aarnink, M. W. A. Verstegen, J. W. Schrama, and G. C. M. Bakker. 1998. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. J. Anim. Sci. 76:1887-1895.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. 2nd ed., vol. 1. Ottawa, Ontario, Canada.

Demigné, C., M. A. Levrat, and C. Rémésy. 1989. Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. J. Nutr. 119:1625-1630.

Dierick, N. A., I. J. Vervaeke, D. I. Demeyer, and J. A. Decuypère. 1989. Approach to the energetic importance of fibre digestion in pigs. I. Importance of fermentation in the overall energy supply. Anim. Feed Sci. Technol. 23:141-167.

Dikeman, C. L., and G. C. Fahey. 2006. Viscosity as related to dietary fiber: A review. Crit. Rev. Food Sci. Nutr. 46:649-663.

Faraj A, T. Vasanthan, and R. Hoover. 2006. The influence of alpha-amylase-hydrolysed barley starch fractions on the viscosity of low and high purity barley beta-glucan concentrates. Food Chem. 96:56-65.

Fledderus, J., P. Bikker, and J. W. Kluess. 2007. Increasing diet viscosity using carboxymethylcellulose in weaned piglets stimulates protein digestibility. Livest. Sci. 109:89-92.

Gallaher, D. D., K. J. Wood, C. M. Gallaher, L. F. Marquart, and A. M. Engstrom. 1999. Intestinal contents supernatant viscosity of rats fed oat based muffins and cereal products. Cereal Chem. 76:21-24.

Ghotra, B. S., T. Vasanthan, and F. Temelli. 2009. Rheological properties of aqueous blends of high purity barley beta-glucan with high purity commercial food gums. Food Chem. 117:417-425.

Graham, H., K. Hesselman, and P. Aman. 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. J. Nutr. 116:242-251.

Hopwood, D. E., D. W. Pethick, J. R. Pluske, and D. J. Hampson. 2004. Addition of pearl barley to a rice-based diet for newly weaned piglets increases the viscosity of the intestinal contents, reduces starch digestibility and exacerbates post-weaning colibacillosis. Br. J. Nutr. 92:419-427.

Htoo, J. K., B. A. Araiza, W. C. Sauer, M. Rademacher, Y. Zhang, M. Cervantes, and R. T. Zijlstra. 2007. Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. J. Anim. Sci. 85:3303-3312.

Jha R, J. Bindelle and B.Rossnagel, A. Van Kessel and P. Leterme. 2009. *In vitro* evaluation of the fermentation characteristics of the carbohydrate fractions of hulless barley and other cereals in the gastrointestinal tract of pigs. Anim. Feed Sci. Technol. (*Submitted*)

Johnson, I. T., and J. M. Gee. 1986. Gastrointestinal adaptation in response to soluble non-available polysaccharides in the rat. Br. J. Nutr. 55:497-505.

Kirchgessner, M., H. L. Muller, and F. X. Roth. 1991. Digestibility, intestinal passage time and energy value of wheat bran in model studies in sows. Z. Ernahrungswiss. 30:118-130.

Kreuzer, M., A. Machmuller, M. M. Gerdemann, H. Hanneken, and M. Wittmann. 1998. Reduction of gaseous nitrogen loss from pig manure using feeds rich in easily-fermentable non-starch polysaccharides. Anim. Feed Sci. Tech. 73:1-19.

Latymer, E. A., A. G. Low, K. Fadden, I. E. Sambrook, S. C. Woodley, and H. D. Keal. 1990. Measurement of transit time of digesta through sections of gastrointestinal tract of pigs fed with diets containing various sources of dietary fibre (non-starch polysaccharides). Arch. Tierernahr. 40:667-680.

Louis, P., K. P. Scott, S. H. Duncan, and H. J. Flint. 2007.Understanding the effects of diet on bacterial metabolism in the large intestine. J. Appl. Microbiol. 102:1197-1208.

Metzler, B. U., W. Vahjen, T. Baumgärtel, M. Rodehutscord, and R. Mosenthin. 2009. Changes in bacterial populations in the ileum of pigs fed low-phosphorus diets supplemented with different sources of fermentable carbohydrates. Anim. Feed Sci. Technol. 148:70-91.

Metzler-Zebeli B. U., S. Hooda, R. Pieper, R. T. Zijlstra, A. G. van Kessel, R. Mosenthin, and M. G. Gänzle. 2010. Non-starch polysaccharides modulate bacterial microbiota, pathways for butyrate production, and abundance of pathogenic Escherichia coli in the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 76:3692-3701.

McDonald, D. E., D. W. Pethick, B. P. Mullan, and D. J. Hampson. 2001. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic escherichia coli in newly-weaned pigs. Br. J. Nutr. 86:487-498.

Montagne, L., and J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95-117.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Oku, T., F. Konishi, and N. Hosoya. 1982. Mechanism of inhibitory effect of unavailable carbohydrate on intestinal calcium absorption. J. Nutr. 112:410-415.

Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. Van Kessel, P. H. Simmins, and R. T. Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. J. Anim. Sci. 84:843-852.

Piel, C., L. Montagne, B. Seve, and J. P. Lalles. 2005. Increasing digesta viscosity using carboxymethylcellulose in weaned piglets stimulates ileal goblet cell numbers and maturation. J. Nutr. 135:86-91.

Potkins, Z. V., T. L. Lawrence, and J. R. Thomlinson. 1991. Effects of structural and non-structural polysaccharides in the diet of the growing pig on gastric emptying rate and rate of passage of digesta to the terminal ileum and through the total gastrointestinal tract. Br. J. Nutr. 65:391-413.

Powell, J. J., M. W. Whitehead, S. Lee, and R. P. H. Thompson. 1994. Mechanisms of gastrointestinal absorption: dietary minerals and the influence of beverage ingestion. Food Chem. 51:381-388.

Renteria-Flores, J. A., L. J. Johnston, G. C. Shurson, and D. D. Gallaher. 2008. Effect of soluble and insoluble fiber on energy digestibility, nitrogen retention, and fiber digestibility of diets fed to gestating sows. J. Anim. Sci. 86:2568-2575.

Rossi, S., M. C. Bonferoni, F. Ferrari, M. Bertoni, and C. Caramell. 1996. Characterization of mucin interaction with three viscosity grades of sodium carboxymethylcellulose. Comparison between rheological and tensile testing. Eur. J. Pharma. Sci. 4:189-196.

Serena, A., H. Jorgensen, and K. E. Bach Knudsen. 2008. Digestion of carbohydrates and utilization of energy in sows fed diets with contrasting levels and physicochemical properties of dietary fiber. J. Anim. Sci. 86:2208-2216.

Siddons, R. C., J. Paradine, D. E. Beever, and P. R. Cornell. 1985. Ytterbium acetate as a particulate-phase digesta-flow marker. Br. J. Nutr. 54:509-519.

Stanogias, G., and G. R. Pearce. 1985. The digestion of fibre by pigs. 1. The effects of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage. Br. J. Nutr. 53:513-530.

St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741-755.

van der Klis, J. D., A. van Voorst, and C. van Cruyningen. 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the physico-chemical conditions in the gastrointestinal tract of broilers. Br. Poult. Sci. 34:971-983.

Van Kleef, D. J., K. Deuring, and P. Van Leeuwen. 1994. A new method of feces collection in the pig. Lab. Anim. 28:78-79.

Van Leeuwen, P., A. H., van Gelder, J. A. de Leeuw, and J. D. van der Klis. 2006. An animal model to study digesta passage rate in different compartments of the gastrointestinal tract (GIT) as affected by dietary composition. Curr. Nutr. Food Sci 2:97-105.

Waldenstedt, L., K. Elwinger, A. Lunden, P. Thebo, M. R. Bedford, and A. Uggla. 2000. Intestinal digesta viscosity decreases during coccidial infection in broilers. Br. Poult. Sci. 41:459-464.

Wenk, C., 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Tech. 90:21-33.

Wilfart, A., L. Montagne, H. Simmins, J. Noblet, and J. Milgen. 2007. Digesta transit in different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by wheat bran. Br. J. Nutr. 98:54-62.

Wong, J. M., R. de Souza, C. W. Kendall, A. Emam, and D. J. Jenkins. 2006. Colonic health: fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235-243.

Zervas, S., and R. T. Zijlstra. 2002. Effects of dietary protein and oathull fiber on nitrogen excretion patterns and postprandial plasma urea profiles in grower pigs. J. Anim. Sci. 80:3238-3246.

Chapter 5: Non-starch polysaccharides modulate bacterial microbiota, pathways for butyrate production, and abundance of pathogenic *Escherichia coli* in the gastrointestinal tract of pigs[4]

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5.1 Abstract

The impact of non-starch polysaccharides (NSP) differing in their functional properties on the intestinal bacterial community composition, prevalence of butyrate-production pathway genes, and the occurrence of *Escherichia coli* virulence factors was studied in eight ileal-cannulated growing pigs using terminal-restriction-fragment-length-polymorphism (TRFLP) and quantitative PCR. A cornstarch-casein based diet was supplemented with low viscous, low fermentable cellulose (CEL), high viscous, low fermentable carboxymethylcellulose (CMC), low viscous, high fermentable oat β-glucan (LBG) and high viscous, high fermentable oat β-glucan (HBG). Only minor effects of NSP fractions on the ileal bacterial community were observed but NSP clearly changed the digestion in the small intestine. When compared to CMC, more fermentable substrate was transferred into the large intestine with CEL, LBG and HBG, resulting in higher postileal dry matter disappearance. Linear discriminant analysis of NSP and TRFLP profiles and 16S rRNA gene copy numbers of major bacterial groups revealed that CMC resulted in a distinctive bacterial community compared to the other NSP that was characterized by higher numbers of total bacteria, *Bacteroides-Prevotella-Porphyromonas*, *Clostridium* cluster XIVa, *Enterobacteriaceae*, and increased prevalence of *E. coli* virulence factors in feces. Fecal gene copies of butyryl-coenzyme A (CoA) CoA tranferase were higher than for butyrate kinase and were affected by NSP. The present results suggest that the NSP fractions clearly and distinctly affected the taxonomic composition and metabolic features of the fecal microbiota. However, the

effects were more linked to the individual NSP and to their effect on nutrient flow into the large intestine than to their shared functional properties.

5.2 Introduction

The porcine intestinal microbiota changes in response to dietary carbohydrate composition due to specific substrate preferences of bacteria (Castillo et al., 2007). Therefore, inclusion of specific non-starch polysaccharides (NSP) in the diet of pigs allows manipulation of the composition of the intestinal microbiota. The NSP can also reduce digestibility of nutrients in the small intestine (Dikeman and Fahey, 2006). The resulting changes in nutrient flow alter the availability of fermentable substrate in the different sections of the gut and thus may modify the bacterial community structure. Differences in the fermentability of individual NSP may not only affect the kinetics of their degradation by intestinal bacteria, but also may change the composition of the fermentation end-products (Williams et al., 2005). Particularly, butyrate is an important metabolite because of its potential to affect gene expression and to improve cellular development in enterocytes (Pryde et al., 2002). The ability of gut microbiota to produce butyrate can vary considerably in response to environmental factors, such as diet composition (Bach Knudsen et al., 2003). However, the number of butyrate-producing bacteria has been difficult to estimate in complex fecal samples by targeting the 16S rRNA gene, because these bacteria do not form a homogeneous phylogenetic group, and both butyrate producers and non-butyrate producers are found within the same phylogenetic clusters belonging to *Clostridium* clusters I, III, IV, XI, XIVa, XV and XVI (Louis et al., 2007). Two alternative

pathways for butyrate formation have been described in bacteria harboring the rumen and human colon (Diez-Gonzalez et al., 1999; Louis et al., 2006). The majority of human colonic butyrate producers use butyryl-coenzyme A (CoA) CoA transferase, whereas soil bacteria mostly utilize the butyrate kinase for the last step of butyrate formation (Louis et al., 2004; Louis et al., 2007). However, information about the butyrate pathways used by intestinal bacteria in pigs is not available.

In addition to the effects of functional properties of NSP on intestinal physiology and fermentation processes, selection of specific NSP fractions may also prevent or stimulate overgrowth of pathogenic bacteria. For instance, dietary inclusion of highly viscous CMC has been shown to increase fecal shedding of enterotoxigenic *Escherichia coli* in weaned pigs (Hopwood et al., 2002). There is a need to identify those dietary NSP fractions that may either rise or reduce the numbers of potential pathogenic bacteria to formulate diets exerting beneficial effects on gut health, which is particularly important in antibiotic-free feeding regimens.

Most studies pertaining on the effect of diet composition on the bacterial community in pigs have employed natural NSP sources and cereal based diets, thereby resulting in a mixture of different soluble and insoluble NSP showing considerable interactions and modification on intestinal bacterial ecophysiology (Castillo et al., 2007; Pieper et al., 2008; Pieper et al., 2009). Purified NSP fractions are increasingly available from the bio-processing industry for use in food preparation and potentially animal feeds, where economics and possible health benefits warrant. However, less is known about the fermentative properties of purified NSP fractions compared to NSP in the

grain matrix (Pieper et al., 2009) which may also differ according to their origin.

The aim of the present study was to examine the effects of four purified NSP fractions differing in their functional properties, i.e. viscosity and fermentability, on the ileal and fecal bacterial community, butyrate production pathway genes, and the occurrence of virulence factor genes of swine-pathogenic *E. coli*, including enterotoxigenic and enteroaggregative *E. coli* (Frydendahl, 2002; Han et al., 2007).

5.3 Materials and Methods

Animals and Diets

A total of 8 crossbred Duroc × Landrace pigs (average weight, 22 ± 1.4 kg) from the herd of the Swine Research and Technology Centre, Edmonton, AB, Canada were surgically fitted with a simple T-cannula at the distal ileum (Li et al., 1993). The animal protocol was approved by the University of Alberta Animal Care and Use Committee for Livestock, and followed principles established by the Canadian Council on Animal Care (CCAC, 1993). The pigs were assigned to one of four diets in a double 4 × 4 Latin square resulting in 8 observations per diet. A semi-purified diet based on cornstarch and casein was formulated to meet or to exceed the nutrient requirements for growing pigs (**Table 4.1**; NRC, 1998). The basal diet was supplemented with 5% active NSP ingredient of four purified NSP fractions: 1) low fermentable, low viscous cellulose (CEL; TIC Pretested Ticacel MCC FG-100; TIC GUMS, White Marsh, MD), 2) low fermentable, high viscous carboxymethylcellulose (CMC; TIC Pretested Ticalose CMC 6000 F, TIC

GUMS), 3) high fermentable, low viscous oat β-glucan (LBG; OatVantage, GTC Nutrition, Missoula, MT), and 4) high fermentable, high viscous oat β-glucan (HBG; Viscofiber, Cevena Bioproducts, Edmonton, AB, Canada). To reach 5% of the active NSP in the diet, the inclusion levels of the NSP fractions were 5.20, 6.25, 8.95, and 9.25% for CEL, CMC, LBG and HBG. The NSP fractions were selected based on their in vitro viscosity that was 0.3, 285, 20, and 210 mPas for CEL, CMC, LBG and HBG, respectively, as determined in 0.5% NSP solution using a rheometer (UDS 200, Paar Physica, Glenn, VA) at a shear rate of 12.9/s and 20ºC. The viscosity of β-glucan is linked to their molecular weight and has been reported to increase about tenfold with a doubling of the molecular weight (Eastwood and Morris, 1992). Titanium dioxide was added to the diet as digestibility marker. The pigs were allowed to consume the experimental diets at a rate of approximately 3% of their maintenance requirement for energy (3 x 110 kcal DE/kg $BW^{0.75}$; NRC, 1998). They were fed twice daily two equal meals in mash form at 8 a.m. and 4 p.m.

Collection of intestinal samples

Each experimental period comprised 17 d; an adaptation period of 10 d was followed by 3-d collection of feces and 8-h collection of ileal effluent on 4 d beginning at 8 a.m. to 4 p.m. Feces were collected using plastic bags attached to the skin around the anus (Van Kleef et al., 1994). The bags were changed each time feces were voided and, after taking subsamples of fresh feces for the analysis of short-chain fatty acids (SCFA), the feces were stored at -20°C until being freeze-dried. For bacterial DNA extraction, subsamples of

freshly voided feces were taken and immediately stored at -20°C. The ileal collection procedure was adapted from Li et al. (1993) using plastic tubings attached to the barrel of the cannula by elastic bands. Twice during collection of ileal effluent (11 a.m. and 2 p.m.), subsamples of ileal effluent (approximately 50 mL) were collected for bacterial DNA analysis and immediately stored at -20°C. The ileal effluent was pooled within each animal and stored at -20°C. A subsample of fresh ileal effluent was stored separately for SCFA analysis. The remaining ileal and fecal samples were freeze-dried prior to analyses of dry matter and protein.

T-RFLP analysis

Total genomic DNA was extracted from ileal effluent and feces using a FastDNA kit (Qiagen, Mississauga, Ontario, Canada) according to the manufacturer's instructions. Partial fragments of bacterial 16S rRNA genes were amplified by PCR using universal forward primer S-D-Bact-0008-a-S-20 (AGAGTTTGATCMTGGCTCAG), labelled with 6-carboxyflourescein (6-FAM) and reverse primer S-D-Bact-0926-a-S-20 (CCGTCAATTCATTTGAGTTT; 37). The purified PCR product (200 ng) was digested at 37°C overnight using 15U of *Msp*I (Fermentas, Burlington, CA) in 2 µL reaction buffer and UV-sterilized Millipore water, made up to 20 µL. Two µL of the digestion solution were subsequently mixed with 9 µL of formamide and 0.5 µL of an internal size standard (ABI GeneScan 600 LIZ size standard) and denatured at 95°C for 5 min followed by cooling on ice for 2 min. Fragment sizes were analyzed using an ABI 3130xl Genetic Analyzer in gene scan mode and GeneMapper v3.7 software (Applied Biosystems,

Ontario, Canada). Fragments that were different in less than + /- 3bp were considered to be identical as binning criteria.

Genomic DNA extraction for quantitative PCR (qPCR)

Nucleic acids were extracted from ileal effluent and feces of pigs using phenol-chloroform essentially as described by Knarreborg et al. (2002). For DNA extraction, 200 mg of sample was weighed into a sterile tube containing 300-400 mg of sterile zirconium beads (diameter, 0.1 mm) and suspended in 1 mL of TN150 buffer (10 mM Tris-HCl, 150 mM NaCl [pH 8.0]). The suspension was vortexed and centrifuged at 14,600 x *g* for 5 min. The pellet was washed twice with 1 mL of TN150 buffer and was resuspended in 1 mL of TN150 buffer. The cells were lysed by physical disruption in a mini-bead beater (Biospec Products, Barlesville, Okla.) at 5,000 rpm for 3 min and placed on ice to cool for 5 min. Subsequently, the sample was centrifuged at 14,600 x *g* for 5 min. A total of 900 μL of the supernatant was extracted twice with 1 mL TE (10 mM Tris, 1 mM EDTA [pH 8.5])-saturated phenol, followed by extraction with an equal volume of chloroform-isoamyl alcohol (24:1). The nucleic acids were precipitated with 2 volumes of cold ethanol (-20°C) and 0.1 volume of 5 M potassium acetate and stored overnight at -20°C. The DNA was collected by centrifugation at 14,600 x *g* for 20 min at 4°C, dried at room temperature for 1 h and dissolved in 50 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]). Prior to qPCR, DNA was diluted 5 times with sterilized Millipore water.

Quantitative PCR

Quantitative PCR was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using the detection software (Version 2.01, Applied Biosystems). Each reaction was run in duplicate in a volume of 25 µl in optical reaction plates sealed with optical adhesive film (Applied Biosystems). The reaction mixture consisted of 12.5 µl Fast SYBR Green Mastermix (Applied Biosystem), 1 µl (10 µM) of primers (Table 1), and 1 µl of template DNA of ileal or fecal samples. To account for the degeneracy of the butyryl-CoA CoA transferase and butyrate kinase primers higher primer concentrations were used in the reaction mixture which was 20 µM each. For amplification of the butyrate kinase gene and virulence factor genes 12.5 µL QuantiFast SYBR Green Mastermix (QIAGEN, Mississauga, ON, Canada) was used. Amplification involved one cycle at 95°C for 5 min for initial denaturation followed by 40 cycles of denaturation at 95°C for 15 s, primer annealing at the optimal temperatures (**Table 5.1**) for 30 s and extension at 72°C for 30 s, one cycle of 95°C for 1 min, one cycle of 55°C for 1 min, and a stepwise increase of the temperature from 55 to 95°C (at 10 s per 0.5°C) to obtain melt curve data. Data were collected at the extension step. Melting curves were checked after amplification in order to assure correct amplification results. Standard curves were generated using serial dilutions of the purified and quantified PCR products generated by standard PCR using the primers shown in Table 1 and genomic DNA from pig intestinal contents (Lee et al., 2006). The detection limit was 10^2, 10^4 and 10^3 copy numbers/g wet digesta for the group specific primers, butyrate enzyme genes primers and *E. coli* virulence factors primers, respectively.

Table 5.1 Oligonucleotide primers used to profile intestinal samples

Bacterial group	Primer sequences (5`-3`)	Annealing temp (°C)	Reference
Domain bacteria	F: CGGYCCAGACTCCTACGGG	60	19
(200 bp)	R: TTACCGCGGCTGCTGGCAC		
Lactobacillus spp.	F: AGCAGTAGGGAATCTTCCA	62	46
(341 bp)	R: CACCGCTACACATGGAG		14
Enterococcus spp.	F: CCCTTATTGTTAGTTGCCATCATT	60	39
(144 bp)	R: ACTCGTTGTACTTCCCATTGT		
Bifidobacterium spp.	F: TCGCGTCYGGTGTGAAAG	60	39
(243 bp)	R: CCACATCCAGCRTCCAC		
Streptococcus spp.	F: AGAGTTTGATCCTGGCTCAG	60	33
(485 bp)	R: GTTAGCCGTCCCTTTCTGG		10
Clostridium cluster XIVa	F: AAATGACGGTACCTGACTAA	60	28
(438-441 bp)	R: CTTTGAGTTTCATTCTTGCGAA		
Clostridium cluster IV	F: GCACAAGCAGTGGAGT	60	29
(130 bp)	R: CTTCCTCCGTTTTGTCAA		
Clostridium cluster I	F: ATGCAAGTCGAGCGAKG	60	39
(120 bp)	R: TATGCGGTATTAATCTYCCTTT		
Bacteroides-Prevotella-	F: GGTGTCGGCTTAAGTGCCAT	60	39
Porphyromonas (140 bp)	R: CGGAYGTAAGGGCCGTGC		
Enterobacteriaceae family	F: CATTGACGTTACCCGCAGAAGAAGC	63	4
(195 bp)	R: CTCTACGAGACTCAAGCTTGC		
Butyryl-CoA CoA transferase	F: GCIGAICATTTCACITGGAAYWSITGGCAYATG	53	24
(530 bp)	R: CCTGCCTTTGCAATRTCIACRAANGC		
Butyrate kinase	F: GTATAGATTACTIRYIATHAAYCCNGG	53	26
(301 bp)	R: CAAGCTCRTCIACIACIACNGGRTCNAC		
STa	F: ATGAAAAAGCTAATGTTGGC	56	13
(193 bp)	R: TACAACAAAGTTCACAGCAG		
STb	F: AATATCGCATTTCTTCTTGC	56	13

(204 bp)	R: GCATCCTTTTGCTGCAAC		
LT	F: CTATTACAGAACTATGTTCGG	56	13
(291 bp)	R: TACTGATTGCCGCAATTG		
EAST1	F: TGCCATCAACACAGTATATCC	56	13
(109 bp)	R: GCGAGTGACGGCTTTGT		

Analytical methods

Samples of diets, freeze-dried ileal effluent and feces were finely ground to pass through a 0.5-mm mesh screen (Lab Retsch mill, Haan, Germany). Dry matter, crude protein and titanium dioxide were analyzed according to AOAC (2006). Feces were analyzed for SCFA by gas chromatography as described by Htoo et al. (2007).

Data presentation

Results for TRFLP analysis, gene copy numbers of bacterial groups, butyrate enzymes, and *E. coli* virulence factors, and fecal SCFA are expressed on the basis of wet weight of ileal effluent and feces rather than dry weight to illustrate the actual *in situ* situation in the gastrointestinal tract. Ileal protein flow was expressed on a dry matter basis.

Calculations

Ileal flow of dry matter and crude protein represents the amount of dry matter and protein present in ileal effluent, and was calculated according to the following equation:

$$D_O = A_I \times (I_D / I_I)$$

where D_O is the total output of a nutrient in ileal effluent (g/kg of dry matter intake), A_I is the concentration of a nutrient in ileal effluent (g/kg of dry matter intake), I_D is the marker concentration in the assay diet (g/kg of dry matter), and I_I is the marker concentration in ileal effluent (g/kg of dry matter). Dry matter disappearance in the large intestine was calculated as difference between fecal and ileal dry matter content.

Statistical Analysis

Data were analyzed according to a double 4 × 4 Latin square design using the mixed procedure (PROC MIXED) of the Statistical Analysis System (SAS Inst. Inc., Cary, NC). Fixed effects included animal and treatment effect. Period and animal within a square were considered as random effects, assuming a compound symmetry variance-covariance structure (type = cs). To detect any influential observation on the model, a test was performed using the Cook's distance (Cook's D) as criteria. Any observation with a Cook's D greater than 0.5 was considered as influential and hence deleted from further analysis. Degrees of freedom were approximated using Kenward-Rogers method (ddfm = kr). A probability level of $P \leq 0.05$ was defined as significant, whereas a trend was considered at $0.05 < P \leq 0.10$.

The TRFLP results were analyzed using Statistica software (version 6.0, Statsoft, Tulsa, OK). The profiles were normalized and only fragments with a relative peak area ratio of $Pi \geq 1\%$ were considered for further analyses. The total number of distinct fragments (Richness, *S*) was counted, and Shannon index $[H' = -\sum_{i=1}^{S} (pi)(\log\ pi)]$ and Simpson Index $[1 - D = \sum_{i=1}^{S} (pi^2)]$ were calculated as ecological measures of the relative distribution of bacterial groups in the community. The Shannon and Simpson's index take into account the number of species and the evenness (Shannon) or relative distribution (Simpson's) of the species as represented by TRFs. The indices are increased either by having additional unique TRFs, or by having a greater evenness of TRFs. Mean values of these parameters were compared by ANOVA followed by Tukey HSD test. Individual TRF's were assigned to microbial species using the MICA II online analysis tool (http://mica.ibest.uidaho.edu/trflp.php)

by using the above mentioned primer set and *Msp*I for an *in silico* virtual digest against the RDP database.

Discrimination model was developed using linear discriminant analysis of JMP software (version 8.0.1, SAS Institute Inc., Cary, NC) to examine potential relationships between NSP fractions and TRFs and NSP fractions and gene copies of bacterial groups. Principal component (PC) analysis was performed to examine any potential grouping of gene copies of bacterial groups, butyrate-production pathway genes, *E. coli* virulence factors, ileal flow of dry matter and postileal dry matter disappearance according to the different diets used in this study by means of JMP software.

The loading plot shows the variables responsible for the variation within the dataset, and the correlations among individual variables of the first two eigenvalues (i.e., PC 1 and PC 2). This gives a graphical representation of the extent to which each factor accounts for the variance in the data and shows the relationship between the different variables.

5.4 Results

Ileal flow of dry matter and protein, dry matter disappearances in the large intestine and concentrations of SCFA in feces

Dry matter content of ileal effluent and feces was lower ($P < 0.01$) for CMC than for LBG and HBG (**Table 5.2**). Ileal flow of dry matter and protein was higher ($P < 0.01$) for CEL, LG and HG compared to CMC. As a result, the postileal dry matter disappearance was greater ($P = 0.01$) for CEL, LG and HG compared to CMC. Fecal concentrations of total SCFA, including acetate, propionate and butyrate were lower ($P < 0.05$) in pigs fed the CMC rather than

CEL, LBG and HBG diets. Inclusion of LG and HG additionally increased concentrations of iso-butyrate and iso-valerate in feces ($P < 0.01$).

TRFLP analysis

The TRFLP analysis of ileal samples showed patterns dominated by relatively few major TRFs, whereas a total of 75 different TRFs were obtained in feces (**Appendix 6 and 7**). Measures of species richness (4.1±1.8 vs. 14.5±4.3), and Shannon (0.34±0.21 vs. 0.86±0.22) and Simpson's (0.40±0.24 vs. 0.76±0.13) diversity were higher in feces than ileum (**Table 5.3**). Among diets, diversity indices were not significantly different. However, a trend ($P < 0.10$) towards higher diversity with CMC as compared to the other treatments was observed, whereas CEL showed relatively lower diversity indices (**Table 5.3**) and similar trends for ecological measures were obtained in ileal effluents and fecal samples.

Terminal restriction fragments identified in ileal effluent could be assigned to *Streptococcus/Lactococcus* spp., *Lactobacillus* spp., *Clostridium* cluster I, XIVa and XVIII, and *Fibrobacter* spp. Ileal communities were dominated by TRFs representing *Streptococcus agalactiae*-like species in almost all animals. In feces, the following bacterial groups and clusters could be identified: *Streptococcus* spp., *Lactobacillus/Enterococcus/Oenococcus* spp., *Clostridium* cluster I, IV, XI, XIVa and XVIII. Furthermore, TRFs that contributed only about 1 to 2% to all TRFs were *Corynebacterium, Collinsella*, *Fibrobacter*, *Selenomonas* and *Desulfovibrio* species-like phylotypes. Two TRFs in ileal effluent and 21 TRFs in feces could not be assigned to known species.

Bacterial populations

A set of 10 group-specific primers was employed to quantify bacterial populations in ileal effluent and feces. Bacterial populations in ileal effluent were only slightly affected by the NSP fractions (**Table 5.4**). Gene copy number of total bacteria in the distal ileum was lower ($P < 0.05$) for LBG compared to CEL, CMC and HBG. The *Clostridium* cluster I was only detectable in ileal effluent of pigs fed the CEL diet. Supplementation of CMC resulted in the highest ileal gene copy number of the *Enterobacteriaceae* family ($P < 0.05$). In feces, gene copy number of total bacteria was highest for CMC and lowest for LBG ($P < 0.05$). Fecal gene copy number of the *Clostridium* cluster XIVa was increased by CMC and reduced by LBG compared to CEL, whereas the fecal number of the *Clostridium* cluster IV was lower for both CMC and LBG compared to CEL ($P < 0.05$). Additionally, CMC and LBG caused lower ($P < 0.05$) gene copy numbers of the *Clostridium* cluster I in feces compared to CEL and HBG.

The CMC increased the fecal gene copy number of *Bacteroides-Prevotella-Porphyromonas* group compared to the other NSP fractions, whereas HBG reduced it compared to CEL and CMC ($P < 0.05$). Finally, the fecal gene copy number of the *Enterobacteriaceae* family was distinctly higher ($P < 0.05$) for CMC compared to LBG and HBG.

Table 5.2 Characteristics of ileal effluent and feces and SCFA in feces of pigs fed diets supplemented with viscous and fermentable non-starch polysaccharide fractions[a]

	Low fermentable		High fermentable			
Item	Low viscous CEL	High viscous CMC	Low viscous LBG	High viscous HBG	SEM	P-value
Dry matter content (g/kg wet wt)						
Ileal effluent	145[a]	55[c]	86[b]	88[b]	5.5	0.001
Feces	573[a]	268[b]	571[a]	554[a]	30.0	<0.001
Ileal flow (g/kg dry matter intake)						
Dry matter	308[a]	165[c]	242[b]	277[a,b]	17.4	<0.001
Crude protein	42[a,b]	26[c]	39[b]	47[a]	2.0	<0.001
Postileal dry matter disappearance (g/kg dry matter intake)	129[a]	23[b]	102[a]	126[a]	20.3	0.010
SCFA in feces (μmol/g wet wt)						
Total	61[a]	28[b]	69[a]	64[a]	6.9	0.005
Acetate	43[a]	19[b]	42[a]	41[a]	4.9	0.015
Propionate	9[a]	5[b]	10[a]	9[a]	1.2	0.038
Butyrate	5[a]	1[b]	7[a]	5[a]	0.9	0.004
Isobutyrate	1.4[b]	0.7[c]	2.8[a]	2.3[a]	0.22	<0.001
Valerate	1.1[b]	0.5[c]	2.1[a]	1.5[b,c]	0.19	<0.001
Isovalerate	2.3[b]	1.1[b]	4.6[a]	3.6[a]	0.47	<0.001
Caproate	0.2[a,b]	0.2[b]	0.4[a]	0.3[a]	0.06	0.159

[a]Data are presented as least-square means (n = 8). Values within a row not having the same superscript are significantly different (P < 0.05). CEL, cellulose; CMC, carboxymethylcellulose; LBG, low viscous oat β-glucan; and HBG, high viscous oat β-glucan.

Table 5.3 Species richness, and Shannon and Simpson's indices of diversity as calculated from normalized TRFLP profiles in ileal effluents and feces of pigs fed diets supplemented with viscous and fermentable non-starch polysaccharide fractions[a]

	Low fermentable		High fermentable			
Item	Low viscous CEL	High viscous CMC	Low viscous LBG	High viscous HBG	Pooled SEM	*P*-Value
Ileal effluent						
Species richness	3.50	4.25	3.50	5.00	1.78	0.288
Shannon index	0.23	0.42	0.29	0.43	0.20	0.160
Simpson index	0.26	0.50	0.35	0.48	0.23	0.156
Feces						
Species richness	11.60	16.25	13.67	15.14	4.19	0.277
Shannon index	0.68	0.98	0.80	0.90	0.21	0.086
Simpson index	0.65	0.83	0.72	0.79	0.12	0.076

[a]Data are presented as least-square means (n = 8). CEL, cellulose; CMC, carboxymethylcellulose; LBG, low viscous oat β-glucan; and HBG, high viscous oat β-glucan.

Table 5.4 Bacterial groups in ileal effluent and feces (log_{10} 16S rRNA gene copies/g wet wt) of pigs fed diets supplemented with viscous and fermentable non-starch polysaccharide fractions [a]

Bacterial groups (log_{10} 16S rRNA gene copies/g wet wt)	Low fermentable		High fermentable		Pooled SEM	P-Value
	Low viscous CEL	High viscous CMC	Low viscous LBG	High viscous HBG		
Ileal effluent						
Total bacteria	9.5[a]	9.8[a]	8.5[b]	9.6[a]	0.18	0.001
Lactobacillus spp	7.9	8.3	7.7	8.3	0.24	0.184
Enterococcus spp	8.1[a,b]	8.0[a,b]	7.6[b]	8.5[a]	0.28	0.227
Streptococcus spp.	8.1[a]	8.2[a]	7.8[b]	8.2[a]	0.08	0.006
Bifidobacterium spp	6.9	7.1	6.9	7.5	0.37	0.655
Clostridium cluster XIVa	6.8	7.3	6.4	6.9	0.36	0.387
Clostridium cluster IV	5.2	3.0	4.5	5.1	0.94	0.363
Clostridium cluster I[b]	6.3±0.34	< 2	< 2	< 2	-	-
Bacteroides-Prevotella-Porphyromonas	5.6	6.3	5.5	6.4	0.35	0.197
Enterobacteriaceae family	8.2[b]	9.1[a]	7.6[c]	8.8[a,b]	0.27	0.006
Feces						
Total bacteria	10.1[b]	11.3[a]	9.2[c]	9.7[b,c]	0.25	<0.001
Lactobacillus spp	6.9[a]	5.6[b]	6.2[a,b]	6.1[a,b]	0.41	0.202
Enterococcus spp	8.1[a]	6.9[b,c]	6.6[c]	7.4[a,b]	0.29	0.010
Streptococcus spp.	7.8[b]	8.5[a]	7.4[b]	7.7[b]	0.18	0.004
Bifidobacterium spp	7.5	7.0	7.2	7.8	0.39	0.525
Clostridium cluster XIVa	7.7[b]	8.7[a]	6.4[c]	7.1[b,c]	0.40	0.007
Clostridium cluster IV	7.8[a]	6.5[b]	6.6[b]	7.1[a,b]	0.33	0.059

Clostridium cluster I	7.2[a]	2.5[b]	3.3[b]	6.5[a]	0.93	0.005
Bacteroides-Prevotella-Porphyromonas	8.9[b]	10.5[a]	8.0[c]	8.4[b,c]	0.20	<0.001
Enterobacteriaceae family	9.0[a,b]	10.3[a]	7.0[c]	7.6[b,c]	0.58	0.004

[a]Data are presented as least-square means (n = 8). Values within a row not having the same superscript are significantly different ($P < 0.05$). CEL, cellulose; CMC, carboxymethylcellulose; LBG, low viscous oat β-glucan; and HBG, high viscous oat β-glucan.

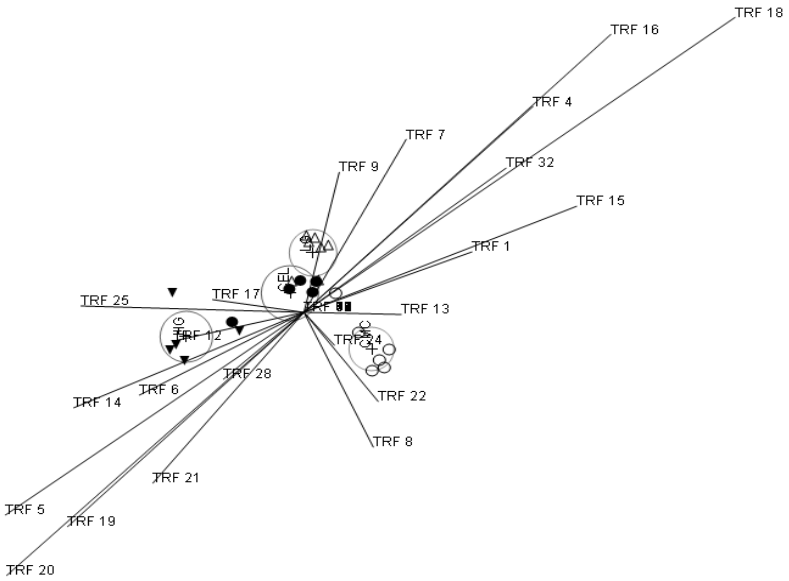
[b] Mean ± SE. Detection limit: 2 $\log_{10}$ 16S rRNA gene copies/g wet wt.

Multivariate analysis

Linear discriminant analysis of NSP fractions and TRFs (**Figure 5.1a**) divided the effects of NSP fractions in three clusters: CEL and LBG diets overlapping in their 95% confidence intervals, CMC diet, and HBG diet. The CEL and LBG diet were more related to TRF9 (*Bacteroides* species-like phylotype), whereas the CMC diet was linked to TRF22 (*Clostridium polysaccharolyticum*-like phylotype). The HBG diet was more related to TRF14 (*Clostridium innocuum*-like phylotypes). According to the linear discriminant analysis of NSP fractions and gene copy numbers of bacterial groups (**Figure 5.1b**), samples were also divided into three clusters; however, CEL and HBG formed a single cluster as indicated by the intersecting 95% confidence intervals. The LBG diet was different from CEL, HBG and CMC, whereby CMC was drastically different from the other NSP fractions. Here, gene copy numbers of *Bifidobacterium* spp. and *Clostridium* cluster I discriminated best for CEL and HBG, whereas *Clostridium* cluster IV and *Streptococcus* spp. discriminated best for CMC, and *Clostridium* cluster XIVa for LBG.

Figure 5.1c depicts the loading plot showing the individual qPCR data, fecal SCFA, ileal flow and disappearance of dry matter in the large intestine responsible for the variation of the first two eigenvalues (PC 1 and PC 2) and the correlations among individual variables. The first PC component accounted for 38.8% of the variation, PC 2 explained 15.2%.

A


TRF 18
TRF 16
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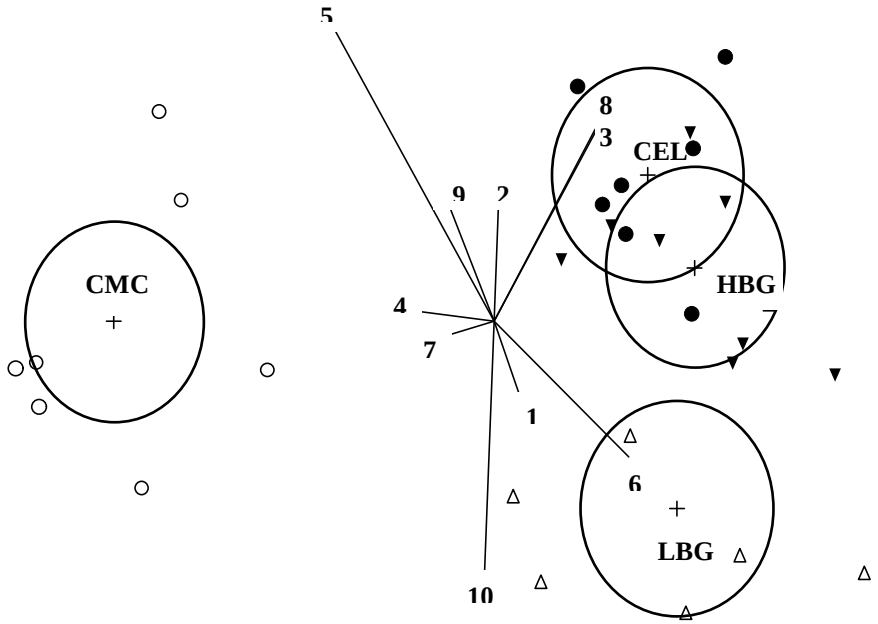
B


5
8
3
CEL
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CMC
HBG
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LBG
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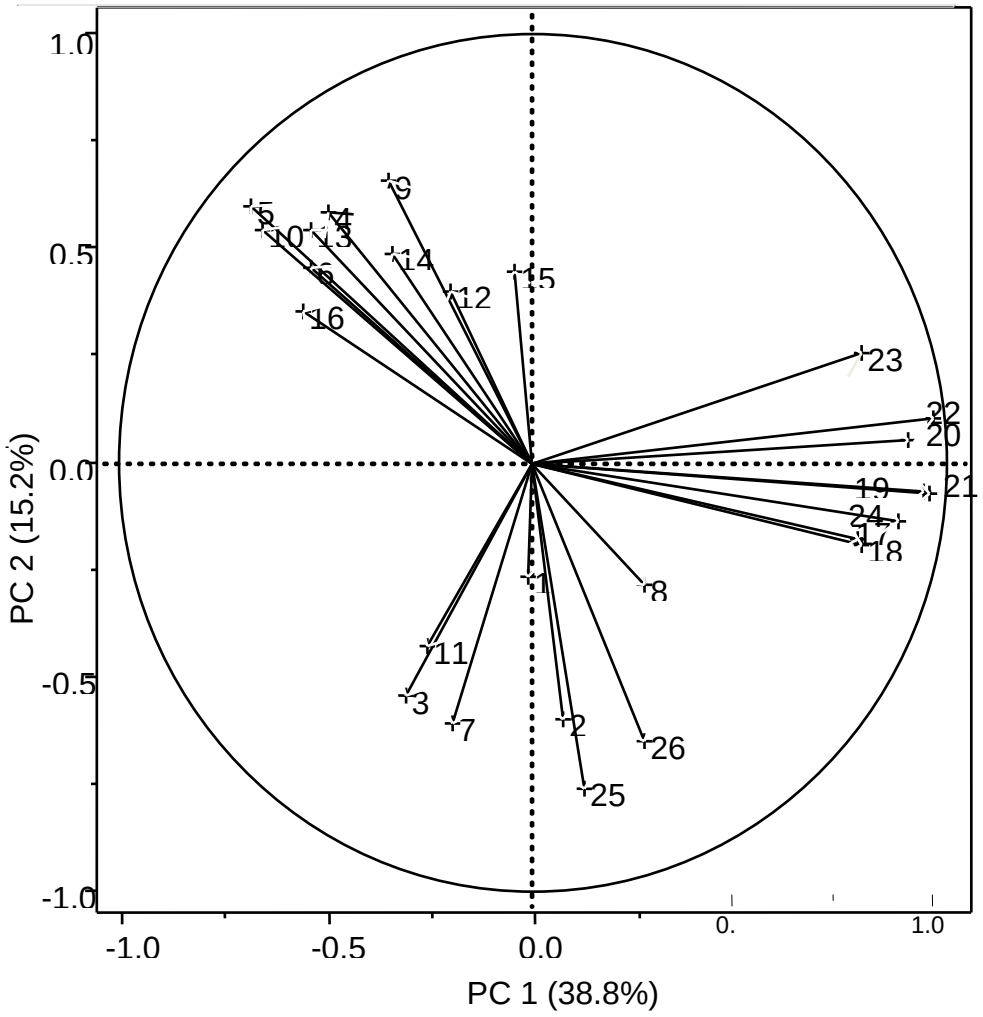

Figure 5.1 Linear discriminant analysis of the NSP fractions and TRFs (a) and gene copies of bacterial groups (b): cellulose (CEL; ●), carboxymethylcellulose (CMC; O), low viscous oat β-glucan (LBG; △) and high viscous oat β-glucan (HBG; ▼). (c) Loading plot showing the correlations among gene copies of bacterial groups in feces, *E. coli* virulence factors in feces, butyryl-Coenzyme A (CoA) CoA transferase and butyrate kinase in feces, fecal SCFA and ileal flow and disappearance of fermentable substrate in the large intestine of the first two eigenvalues (PC 1 and PC 2). 1, *Lactobacillus* spp.; 2, *Enterococcus* spp.; 3, *Bifidobacterium* spp.; 4, *Streptococcus* spp.; 5, *Bacteroides-Prevotella-Porphyromonas*; 6, *Clostridium* cluster XIVa; 7, *Clostridium* cluster IV; 8,

Clostridium cluster I; 9, *Enterobacteriaceae*; 10, total bacteria; 11, butyryl-CoA CoA transferase; 12, butyrate kinase; 13, EAST1; 14, STa; 15, STb; 16, LT; 17, acetate; 18, propionate; 19, iso-butyrate; 20, butyrate; 21, iso-valerate; 22, valerate; 23, caproate; 24, total SCFA; 25, ileal flow of dry matter; and 26, postileal dry matter disappearance.

The SCFA form a cluster located on the right part of the graph and were highly influenced by PC 1. *Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Clostridium* cluster I and IV and butyryl-CoA CoA transferase formed a second cluster at the bottom of the loading plot and were negatively correlated to PC 2. A third cluster was formed by total bacteria, *Enterobacteriaceae*, *Bacteroides-Prevotella-Porphyromonas* group, *Streptococcus* spp., *Clostridium* cluster XIVa, *E. coli* virulence factors and butyrate kinase that were influenced by both PC 1 and PC 2 and were negatively correlated to the other two clusters (angles between arrows for these clusters >90°). Variables within these three cluster were positively related among each other as indicated by the small angles between the arrows for these variables (<90°).

Butyrate-production pathway genes

Butyrate-producing bacteria in feces were determined by targeting genes for the enzymes butyryl-CoA CoA transferase and butyrate kinase (**Figure 5.2**). The butyryl-CoA CoA transferase occurred in higher gene copy numbers (7.9 to 8.8 $\log_{10}$ DNA gene copies/g wet wt) compared to the butyrate kinase (5.7 to 6.6 $\log_{10}$ DNA gene copies/g wet wt). Dietary supplementation of CMC resulted in a lower

($P < 0.05$) gene copy number of the butyryl-CoA CoA transferase compared to the CEL diet. In contrast, CMC increased ($P < 0.05$) the gene copy number of the butyrate kinase compared to LBG and HBG.

Escherichia coli virulence factors

The heat-stable enterotoxin of enteroaggregative *E. coli* was the dominating virulence factor detected in both ileal and fecal samples ranging from 6.0 to 8.9 $\log_{10}$ DNA gene copies/g wet wt.

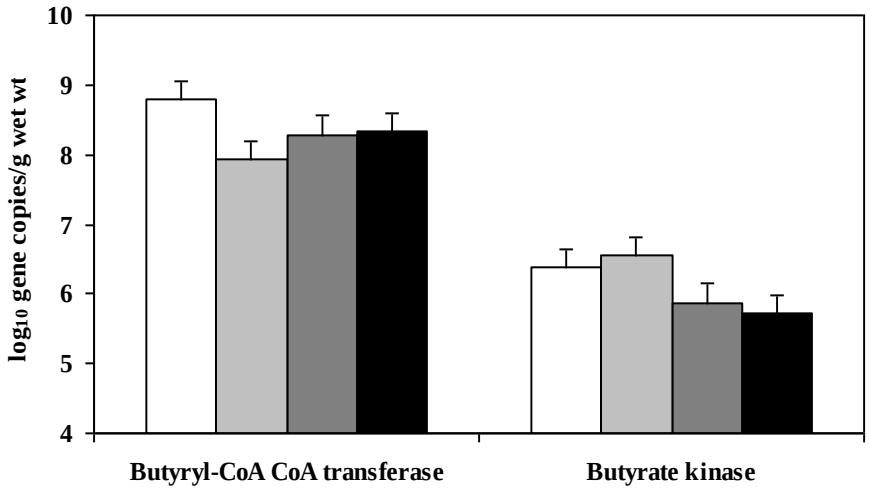

Figure 5.2 Gene copy numbers of butyryl-coenzyme A (CoA) CoA-transferase and butyrate kinase in feces of pigs fed diets supplemented with cellulose (□), carboxymethylcellulose (■), low-viscous oat β-glucan (■) or high-viscous oat β-glucan (■). The detection limit was 4 $\log_{10}$ gene copies/g wet wt.

In ileal effluent, EAST1 was affected by NSP (P = 0.026) and was significantly higher for CMC and HBG compared to LBG (6.8, 7.5, 6.0 and 7.3 ± 0.35 $\log_{10}$ DNA gene copies/g wet wt for CEL, CMC, LBG and HBG).

Virulence factors of enterotoxigenic *E. coli* STa, STb and LT were below detection limit in the ileal effluent. In feces, EAST1 was higher by 1.4 to 2.5 log units for CMC compared to the other NSP fractions (P < 0.05; **Figure 5.3**).

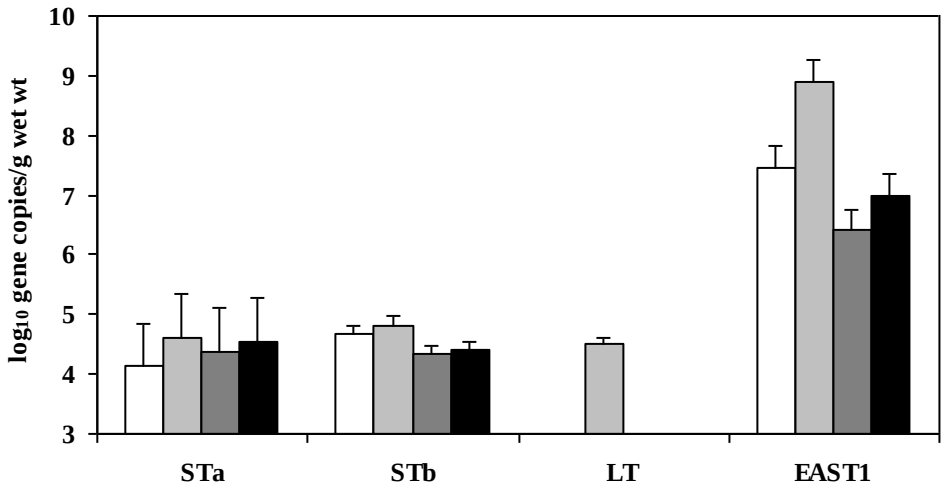

Figure 5.3 Gene copy numbers of virulence factors (heat-stable enterotoxins (STa and STb), heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* and heat-stable enterotoxin (EAST1) of enteroaggregative *E. coli*) in feces of pigs fed diets supplemented cellulose (□), carboxymethylcellulose (■), low-viscous oat β-glucan (■) or high-viscous oat β-glucan (■). The detection limit was 3 $\log_{10}$ gene copies/g wet wt.

The gene copy number of STa did not differ in feces, whereas the number of STb was higher ($P < 0.05$) for CMC compared to LG. The LT was only detectable in feces of pigs fed the CMC diet.

5.5 Discussion

In the present study, we used a polyphasic approach to study the effects of four purified NSP fractions of low and high viscosity and fermentability on the taxonomic composition of the ileal and fecal microbiota and at metabolic level on butyrate-producing bacteria and *E. coli* virulence factors using TRFLP and qPCR. Because purified NSP fractions may affect the bacterial community structure in a different way when added to a cereal based diet due to the NSP in the grain matrix (Pieper et al., 2008), a semi-purified diet was employed in the present experiment.

The NSP fractions differently affected the small intestinal digestion and markedly changed the availability of fermentable substrate in the large intestine. However, evidence did not exist that the shared functional properties affected digestive processes and endogenous nitrogen losses (Souffrant, 2001) consistently among the NSP fractions, suggesting that the specific chemical structures of the NSP are as relevant as shared rheological properties (Dikeman and Fahey, 2006; Wenk, 2001). Correspondingly, consistent effects of viscosity and fermentability were not observed for gene copies of bacterial groups in ileal effluent. Cellulose resulted in a faster transit compared to CMC and HBG (data not shown); thus, beside the retention time in the small intestine the accessibility of dietary nutrients appeared to be a critical factor for bacterial growth. Increased digesta viscosity

impairs intestinal contractions (Lentle and Jansson, 2008) thereby preventing mixing of digesta and bacteria, and thus access of bacteria to new substrate. Low viscous CEL, in turn, did likely not impair intestinal contractions and hence digesta mixing. According to the TRFLP profiles, *Streptococcus agalactiae*-like phylotypes dominated the ileal microbiota, followed by phylotypes belonging to *Clostridium* cluster XIVa. Surprisingly, TRFs representing *Enterobacteriaceae* species were not detected in ileal effluent or feces which can be likely associated with the utilization of only one restriction enzyme (Matsumoto et al., 2005), whereas the high rRNA gene copy numbers of *Enterobacteriaceae* produced with qPCR confirmed the prevalence of this bacterial group in the gut of pigs (Leser et al., 2002; Scharek et al., 2005).

The effect of the different purified NSP fractions on the formation of fermentation end products and bacterial numbers in feces mostly depended on changes in the ileal flow of dry matter (i.e. NSP fractions and other non-digested dietary ingredients) into the large intestine. In this context, low fermentable CEL resulted in similar postileal dry matter disappearance and SCFA concentrations in feces compared to high fermentable LBG and HBG and significantly higher compared to low fermentable CMC.

Diversity indexes indicated that CMC supported a higher fecal bacterial diversity when compared to the other NSP. Linear discriminant analysis of qPCR and TRFLP data confirmed that the fecal bacterial community structure in pigs fed the CMC diet differed from pigs fed the other NSP fractions. For instance, cellulolytic *C. polysaccharolyticum*-like phylotypes (TRF22) (Warnick et al.,

2002) discriminated best for the CMC diet, whereas fibrolytic and amylolytic *Bacteroides* species-like phylotypes (TRF9) (Smith et al., 2006) were the best discrimination variable for CEL and LBG. This may indicate that not only the NSP fractions, but also the starch content in digesta may have modulated the bacterial community in pigs fed these diets.

Low fermentable CMC resulted in highest gene copy numbers of total bacteria in feces compared to the other NSP fractions. Generally, *Clostridium* cluster IV and XIVa and the *Bacteroides-Prevotella-Porphyromonas* group are the dominating strictly anaerobic bacterial groups in the large intestine of pigs (Leser et al., 2002). Carboxymethylcellulose clearly promoted the growth of the *Clostridium* cluster XIVa and particularly of the *Bacteroides-Prevotella-Porphyromonas* group and *Enterobacteriaceae* compared to the other NSP. In contrast, the CEL diet favored the growth of *Clostridium* cluster IV. In addition to cellulose, the availability of other easily fermentable substrates in the ileal effluent, such as starch and protein, may have supported the higher numbers of *Clostridium* cluster IV as it contains both fibrolytic and non-fibrolytic species (Louis and Flint, 2009; Louis et al., 2007) including some butyrate-producing bacteria, such as *Butyrivibrio fibrisolvens* (Asanuma et al., 2005).

Cellulose, LBG and HBG caused higher butyrate concentrations in feces compared to CMC. However, the measurement of butyrate in colonic digesta and portal blood is insufficient as butyrate is mainly catabolized by colonocytes (Bach Knudsen et al., 2003) and the various *Clostridium* clusters contain both butyrate and non-butyrate producers (Louis et al., 2007). In humans, butyryl-CoA CoA

transferase and butyrate kinase genes are used as marker genes to detect butyrate-producing bacteria in the colon (Louis and Flint, 2007; Louis et al., 2004). Similar to human butyrate-producers (Louis et al., 2004), the main route of butyrate formation in the hindgut of pigs is the butyryl-CoA CoA transferase pathway. Moreover, the loading plot of PCA indicated that the ileal flow of dry matter into the large intestine was positively correlated to the gene copies of butyryl-CoA CoA transferase, suggesting that the availability of fermentable substrate and not only the NSP fractions were important for butyrate-producers using the butyryl-CoA CoA transferase pathway. Similarly, butyryl-CoA CoA transferase correlated to lactic acid producing groups, such as lactobacilli, bifidobacteria and enterococci. This may be related to cross-feeding of butyrate-producing bacteria with lactate (Louis et al., 2007). The butyrate kinase was negatively correlated with the ileal flow of fermentable substrate and its gene copy numbers were increased by CMC.

The CEL and HBG diets markedly raised the gene copy numbers of *Clostridium* cluster I. Although this cluster contains fibrolytic and butyrate-producing bacteria (e.g. *C. cellulovorans*), other members such as *C. perfringens* may be harmful for the host (Mclane et al., 2006). A TRF representing a *C. perfringens*-like phylotype was identified in feces and a TRF was recognized as *Clostridium bifermentans*-like phylotype that represents a potential pathogenic bacterium belonging to *Clostridium* cluster XI (Scanlan et al., 1994). However, adverse effects of high viscous NSP on gut health are mainly attributed to pathogenic *E. coli* (Langhout et al., 1999; Smits et al., 1998). High viscous CMC

favored growth of pathogenic *E. coli* in weaning pigs (Hopwood et al., 2002; McDonald et al., 2001); however, these effects were generally confined to the immediate period after weaning (Hampson, 1994; Hopwood et al., 2002). The loose feces in combination with the high ileal and fecal rRNA gene copy numbers of *Enterobacteriaceae* in growing pigs used in the present study indicate that older pigs are also susceptible to overgrowth of enteropathogenic bacteria when the diet contains CMC. Quantitative PCR of virulence factors revealed that particularly enteroaggregative *E. coli* were present in higher gene copy numbers in the distal ileum and in feces. Moreover, the heat-labile enterotoxin LT was exclusively detectable in feces of pigs fed the CMC diet as compared to the other NSP. Similar gene copy numbers of EAST1, STa and STb in feces of pigs fed CEL, LBG and HBG did not cause any signs of diarrhea. The lower numbers of *Enterobacteriaceae* and *E. coli* virulence factors with high viscous HBG suggest that factors other than viscosity are involved in the stimulation of pathogenic *E. coli*. The CMC diet may have influenced the proliferation of pathogenic *E. coli* through changes in the mucus composition and the amount of mucus produced (Piel et al., 2005).

In conclusion, this study disclosed that the intestinal bacterial community, genes of alternative pathways of butyrate production, and *E. coli* virulence factors are specifically modulated by supplementing a semi-purified diet with CEL, CMC, LBG or HBG. Changes may be attributable to bacterial fermentation of NSP; additionally, NSP altered the ileal flow of nutrients into the large intestine. Effects of the NSP fractions were linked to the individual NSP fractions rather

than to their shared functional properties, i.e. viscosity and fermentability. Comparable to human colonic microbiota, gene copy numbers of butyryl-CoA CoA transferase were higher than numbers of butyrate kinase genes, indicating that this pathway is the dominant butyrate-production pathway in the large intestine of pigs. Although increasing intestinal viscosity was generally associated with impaired gut health (Langhout et al., 1999; Smits et al., 1998), only CMC increased the susceptibility of pigs to overgrowth of pathogenic *E. coli*, suggesting that the use of CMC in diets for growing pigs is detrimental as compared to the other NSP fractions investigated.

5.6 References

AOAC. 2006. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th ed. Arlington, VA.

Asanuma, N., M. Ishiwata, T. Yoshii, M. Kikuchi, Y. Nishina, and T. Hino. 2005. Characterization and transcription of the genes involved in butyrate production in *Butyrivibrio fibrisolvens* type I and II strains. Curr. Microbiol. 51:91-94.

Bach Knudsen, E. K., A. Serena, N. Canibe, and K. S. Juntunen. 2003. New insight into butyrate metabolism. Proc. Nutr. Soc. 62:81-86.

Bartosch, S., A. Fite, G. T. Macfarlane, and M. E. T. McMurdo. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl. Environ. Microbiol. 70:3575-3581.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. 2nd ed., vol. 1. Ottawa, ON, Canada.

Castillo, M., G. Skene, M. Roca, M. Anguita, I. Badiola, S. H. Duncan, H. J. Flint, and S. M. Martín-Orúe. 2007. Application of 16S rRNA gene-targetted fluoresensce in situ hybridization and restriction fragment length polymorphism to study porcine microbiota along the gastrointestinal tract in response to different sources of dietary fibre. FEMS Microbiol. Ecol. 59:138-146.

Diez-Gonzalez, F., D. R. Bond, E. Jennings, and J. B. Russell. 1999. Alternative schemes of butyrate production in *Butyrivibrio fibrisolvens* and their relationship to acetate utilization, lactate production, and phylogeny. Arch. Microbiol. 171:324-330.

Dikeman, C. L., and G. C. Fahey. 2006. Viscosity as related to dietary fiber: a review. Crit. Rev. Food Sci. Nutr. 46:649-663.

Eastwood, M. A., and E. R. Morris. 1992. Physical properties of dietary fiber that influence physiological function: A model for polymers along the gastrointestinal tract. Am. J. Clin. Nutr. 55:436-442.

Franks, A. H., H. J. Harmsen, G. C. Raangs, G. J. Jansen, F. Schut, and G. W. Welling. 1998. Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 64:3336-3345.

Frydendahl, K. 2002. Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Vet. Microbiol. 85:169-182.

Hampson, D. J. 1994. Postweaning *Escherichia coli* in pigs, p. 171-191. *In* C. L. Gyles (ed.), *Escherichia coli* in Domestic Animals and Humans. CAB International, Wallingford, UK.

Han, W., B. Liu, B. Cao, L. Beutin, U. Krüger, H. Liu, Y. Li, Y. Liu, L. Feng, and L. Wang. 2007. DNA microarray-based identification of serogroups and

virulence gene patterns of *Escherichia coli* isolates associated with porcine postweaning diarrhea and edema disease. Appl. Environ. Microbiol. 73:4082-4088.

Heilig, H. G. H. J., E. G., Zoetendal, E. E. Vaughan, P. Marteau, A. D. L. Akkermans, and W. M. de Vos. 2002. Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. Appl. Environ. Microbiol. 68:114-123.

Hopwood, D. E., D. W. Pethick, and D. J. Hampson. 2002. Increasing the viscosity of the intestinal contents stimulates proliferation of enterotoxigenic *Escherichia coli* and *Brachyspira pilosicoli* in weaner pigs. Br. J. Nutr. 88:523-532.

Htoo, J. K., B. A. Araiza, W. C. Sauer, M. Rademacher, Y. Zhang, M. Cervantes, R. T. Zijlstra. 2007. Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. J. Anim. Sci. 85:3303-3312.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol. 68:5918-5924.

Langhout, D. J., J. B. Schutte, P. Van Leeuwen, J. Wiebenga, and S. Tamminga. 1999. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br. Poult. Sci. 40:340-347.

Lee, D.-H., Y.-G. Zo, and S.-J. Kim. 1996. Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism. Appl. Environ. Microbiol. 62:3112-3120.

Lee, C., J. Kim, S. G. Shin, and S. Hwang. 2006. Absolute and relative QPCR quantification of plasmid copy number in *Escherichia coli.* J. Biotechnol. 123:273-280.

Lentle, R. G., and P. W. M. Jansson. 2008. Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: a review. J. Comp. Physiol. 178:673-690.

Leser, T. D., J. Z. Amenuvor, T. K. Jensen, R. H. Lindecrona, M. Boye, and K. Møller. 2002. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl. Environ. Microbiol. 68:673-690.

Li, S., W. C. Sauer, and M. Z. Fan. 1993. The effect of dietary crude protein level on amino acid digestibility in early-weaned pigs. J. Anim. Physiol. Anim. Nutr. 70:26-37.

Louis, P., and H. J. Flint. 2007. Development of a semiquantiative degenerate real-time PCR-based assay for estimation of numbers of butyryl-Coenzyme A (CoA) CoA transferase genes in complex bacterial samples. Appl. Environ. Microbiol. 73:2009-2012.

Louis, P., and H. J. Flint. 2009. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol. Lett. 254:1-8.

Louis, P., S. H. Duncan, S. I. McCrae, J. Millar, M. S. Jackson, and H. J. Flint. 2004. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. Appl. Environ. Microbiol. 186:2099-2106.

Louis, P., K. P. Scott, S. H. Duncan, and H. J. Flint. 2007. Understanding the effects of diet on bacterial metabolism in the large intestine. J. Appl. Microbiol. 102:1197-1208.

Matsuki, T., K. Watanabe, J. Fujimoto, Y. Miyamoto, T. Takada, K. Matsumoto, H. Oyaizu, and R. Tanaka. 2002. Development of 16S rRNA-gene

targeted group-specific primers for the detection and identification of predominant bacteria in human feces. Appl. Environ. Microbiol. 68:5445-5451.

Matsuki, T., K. Watanabe, J. Fujimoto, T. Takada, and R. Tanaka. 2004. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. Appl. Environ. Microbiol. 70:7220-7228.

Matsumoto, M., M. Sakamoto, H. Hayashi, and Y. Benno. 2005. Novel phylogenetic assignment database for terminal-restriction fragment length polymorphism analysis of human colonic microbiota. J. Microbiol. Meth. 61:305-319.

McDonald, D. E., D. W. Pethick, B. P. Mullan, and D. J. Hampson. 2001. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic *Escherichia coli* in newly-weaned pigs. Br. J. Nutr. 86:487-498.

McLane, B. A., F. A. Uzal, M. E. F. Miyakawa, D. Lyberly, and T. Wilkins. 2006. The enterotoxic clostridia. Prokaryotes 4:698-752.

Nübel, U., B. Engelen, A. Felske, J. Snaidr, A. Wieshuber, R. I. Amann, W. Ludwig, and H. H. Backhaus. 1996. Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. J. Bacteriol. 178:5636-5643.

Nutrient Requirement Council. 1998. Nutrient Requirements of Swine. 10th ed. National Academic Press, Washington, DC.

Piel, C., L. Montagne, B. Sève, and J. P. Lallès. 2005. Increasing digesta viscosity using carboxymethylcellulose in weaned piglets stimulates ileal goblet cell numbers and maturation. J. Nutr. 135:86-91.

Pieper, R., R. Jha, B. Rossnagel, A. G. Van Kessel, W. B. Souffrant, and P. Leterme. 2008. Effect of barley and oat cultivars with different

carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiol. Ecol. 66:556-566.

Pieper, R., J. Bindelle, B. Rossnagel, A. G. Van Kessel, and P. Leterme. 2009. Effect of carbohydrate composition in barley and oat cultivars on microbial ecophysiology and the proliferation of *Salmonella enterica* in an in vitro model of the porcine gastrointestinal tract. Appl. Environ. Microbiol. 75:7706-7716.

Pryde, S. E., S. H. Duncan, G. L. Hold, C. S. Stewart, and H. J. Flint. 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiol. Lett. 217:133-139.

Rinttilä, T., A. Kassinen, E. Malinen, L. Krogius, and A. Palva. 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J. Appl. Microbiol. 97:1166-1177.

Scanlan, D. R., M. A. Smith, and H. D. Isenberg, S, Engrassia, and E. Hilton. 1994. *Clostridium bifermentans* bacteremia with metastatic osteomyelitis. J. Clin. Microbiol. 32:2867-2868.

Scharek, L., J. Guth, K. Reiter, K. D. Weyrauch, D. Taras, P. Schwerk, P. Schierack, M. F. Schmidt, L. H. Wieler, and K. Tedin. 2005. Influence of a probiotic *Enterococcus faecium* strain on development of the immune system of sows and piglets. Vet. Immunol. Immunopathol. 105:151-161.

Smith, C. J., E. R. Rocha, and B. J. Paster. 2006. The medically important *Bacteroides* spp. in health and disease. Prokaryotes 7:381-427.

Smits, C. H., A. Veldman, H. J. Verkade, and A. C. Beynen. 1998. The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. Poultry Sci. 77:1534-1539.

Souffrant, W. B. 2001. Effect of dietary fibre on ileal digestibility and endogenous nitrogen losses in the pig. Anim. Feed. Sci. Technol. 90:93-102.

Van Kleef, D. J., K. Deuring, and P. van Leeuwen. 1994. A new method of faeces collection in the pig. Lab Anim. 28:78-79.

Walter, J., C. Hertel, G. W. Tannock, C. M. Lis, K. Munro, and W. P. Hammes. 2001. Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 67:2578-2585.

Warnick, T. A., B. A. Methé, and S. B. Leschine. 2002. *Clostridium phytofermentans* sp. nov., a cellulolytic mesophile from forest soil. Int. J. Syst. Evol. Microbiol. 52:1155-1160.

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. Anim. Feed Sci. Technol. 90:21-33.

Williams, B. A., M. W. Bosch, A. Awati, S. R. Konstantinov, H. Smidt, A. D. L. Akkermans, M. W. A. Verstegen, and S. Tamminga. 2005. In vitro assessment of grastointestinal tract (GIT) fermentation in pigs: Fermentable substrates and microbial activity. Anim. Res.54:191-202.

Chapter 6: High viscous and high fermentable dietary fiber increases net butyrate flux, and insulin and GLP-1 production but does not affect net glucose flux in portal-vein catheterized grower pigs[5]

[5]This chapter will be submitted to the Journal of Nutrition.

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6.1 Abstract

Dietary fiber may lower glycemic and insulin responses and increase short chain fatty acid (SCFA); however, specific contributions of viscosity and fermentability of fiber are unknown. Thus, the effects of these functional properties on the net nutrient flux and insulin responses were studied in 4 pigs with 2 catheters [portal vein and carotid artery] and 1 blood flow probe [portal vein]. Semi-purified [cornstarch, casein] diets containing 5% actual dietary fiber sources were fed in a 2 (low and high viscous) × 2 (low and high fermentable) factorial arrangement. Diets contained low viscous, low fermentable cellulose (CEL-LF), high viscous, low fermentable carboxymethylcellulose (CMC), low viscous, high fermentable cellulose (CEL-HF), and high viscous, high fermentable oat β-glucan (HBG). Blood was sampled for 12 h postprandial and net nutrient flux and apparent hormone production were calculated from plasma portal minus arterial differences × flow. Fermentability tended ($P < 0.10$) to increase arterial glucose without any effects on portal glucose and net glucose flux. Viscosity reduced ($P < 0.05$) apparent insulin production in pigs. Viscosity and fermentability interacted to increase ($P < 0.05$) apparent C-peptide and glucagon like peptide (GLP-1) production that was highest ($P < 0.05$) in pigs fed HBG. Viscosity increased ($P < 0.05$) net acetate flux. Viscosity and fermentability interacted to increase ($P < 0.05$) net propionate and butyrate flux that were highest ($P < 0.05$) in pigs fed HBG in the late postprandial phase confirming fermentation of β-glucan. In conclusion, high viscous and high fermentable dietary fiber

increased net butyrate flux and insulin and GLP-1 production but did not affect net glucose flux.

6.2 Introduction

Intake of dietary fiber has been linked to beneficial metabolic and intestinal health (Anderson et al., 2009), because dietary fiber lowers glucose and insulin responses (Jenkins et al., 2008), modifies gastrointestinal peptides (Karhunen et al., 2010; Hooda et al., 2010a), increases production of short chain fatty acid (SCFA; Wisker et al., 2000), and promotes a beneficial gut microflora (Wong et al., 2006). The physiological effects of dietary fiber have been attributed to 2 main functional properties: viscosity and fermentability (Dikeman and Fahey, 2006). Viscosity was associated with reduced glucose and insulin responses (Jenkins et al., 2008; Kim et al., 2009; Hooda et al., 2010a) because soluble dietary fiber increases digesta viscosity (Hooda et al., 2010b), reduces gastric emptying (Darwiche et al., 2003), and slows digestion and absorption (Edwards et al., 1988).

Fermentable dietary fiber is not digested by gastrointestinal enzymes, but is fermented by the microflora in the distal small and large intestine. The fermentation produces SCFA that serve as nutrients systemically for the colonic epithelium and regulate proliferation, differentiation and gene expression, especially butyrate (Wong et al., 2006). The SCFA increased the expression of the proglucagon gene in enteroendocrine L cells and may increase glucagon like peptide (GLP-1) secretion (Reimer and McBurney, 1996). The GLP-1 as an

anorexigenic hormone is strongly linked to enhanced satiety (Nilsson et al., 2008). The GLP-1 has also been studied as potential therapy for type-II diabetes, because it enhances glucose-dependent insulin secretion, promotes β-cell proliferation and inhibits β-cell apoptosis, and thus increases pancreatic β-cell mass and insulin sensitivity (Baggio and Drucker, 2007). Thus, fermentable dietary fiber has been associated with increased GLP-1 (Cani and Delzenne, 2009; Tarini and Wolever, 2010), whereas soluble and viscous fiber attenuates GLP-1 responses (Juvonen et al., 2009; Karhunen et al., 2010). However, the interactive effects of viscosity and fermentability on SCFA and GLP-1 and subsequent effects on glucose and insulin responses are poorly understood.

Previously with ileal-cannulated pigs, viscosity and fermentability interacted to affect digesta characteristics and digestion and fermentation variables (Hooda et al., 2010b). In the previous study it has also reported that even cellulose was not likely fermented, but increased the fermentability of diet. Thus, instead of fermentability of fiber source, the fermentability of diet was considered in present design. Using this treatment arrangement, the porcine porto-arterial catheterization model tested the hypothesis that viscosity and fermentability will affect net flux of glucose and SCFA and apparent production of insulin, C-peptide, and GLP-1. Thus, the objectives were to clarify the independent and interactive effects of viscosity and fermentability of dietary fiber on kinetics of nutrient flux and hormones production during the 12-h postprandial period.

6.3 Materials and Methods

Experimental Design and Procedures

The animal protocol was approved by the Animal Care and Use Committee for Livestock at the University of Alberta and was conducted at the Swine Research and Technology Centre.

Surgical model. Four female pigs (35 to 40 kg BW) were catheterized in the portal vein and carotid artery using modified polyvinyl tube catheters. Furthermore, a 14-mm blood flow probe (Transonic Systems, Ithaca, NY) was implanted around the portal vein (Hooda et al., 2009). Post-operative management included antibiotics, analgesics, gut motility drugs, and intravenous fluids for 3 d. Catheters were flushed aseptically daily with 200 IU of heparinized saline to maintain their patency and were secured using pouches.

Experimental diets. The 4 semi-purified experimental diets were based on corn starch, casein, and canola oil (**Table 6.1**). Diets contained 5% of actual NSP, following correcting for impurities, from 4 sources differing in viscosity and fermentability in a 2 × 2 factorial arrangement: low fermentable, low viscous cellulose (CEL-LF; Solka-Floc®; International Fiber Corp., North Tonawanda, NY), low fermentable, high viscous carboxymethylcellulose (CMC; TIC Pretested ® CMC 6000 fine powder; TIC Gums Inc., White Marsh, MD), high fermentable, low viscous cellulose (CEL-HF; TIC Pretested ® TICACEL 100 cellulose powder; TIC Gums Inc.) or high fermentable, high viscous oat β-glucan (Oat Vantage®; GTC Nutrition, Golden, CO). Four NSP sources were selected for viscosity and fermentability characteristics based on digesta viscosity and

fermentation results from a previous trial with ileal-cannulated pigs (Hooda et al., 2010b). Content of vitamin and mineral premix was equal among diets. Pigs were fed one of four diets for 7 d in a 4 × 4 Latin square to obtain 4 observations per diet. Pigs were fed 1.00 kg/d until 40 kg BW, 1.10 kg/d until 45 kg BW, 1.20 kg/d until 50 kg BW and 1.50 kg/d until 65 kg BW. The daily feed allowance was divided into two equal meals and fed at 0800 and 2000 h with free access to water.

Sampling protocol. Blood was collected on d 7 of each period in heparinized and EDTA tubes from the carotid artery and portal vein. Blood was collected every 15 min from -15 to 60 min, then every 30 min up to 240 min, then every 60 min up to 480 min, and 600 min and 720 min postprandially. Blood flow was measured simultaneously (model T402; Transonic Systems) and was recorded continuously at each collection for 10 min using Windaq software (Dataq Instruments, Akron, OH). After collection, the catheters were flushed with 10 mL of 10 IU/mL heparinized saline to prevent clotting and replace the fluid loss. Hematocrit values were measured immediately using standard methods. Blood was centrifuged at 1500 × g for 10 min and plasma was frozen at -20ºC in heparinized tubes for glucose, insulin, SCFA, and C-peptide analyses and in EDTA tubes at -80ºC for GLP-1analysis.

Table 6.1 Ingredient composition of the experimental diets containing 4 dietary fiber sources (as-fed basis)[1]

	Low fermentable		High fermentable	
Ingredient, %	Low viscous CEL-LF	High viscous CMC	Low viscous CEL-HF	High viscous HBG
Corn starch[2]	71.00	71.00	71.00	71.00
Calcium caseinate[3]	16.00	16.00	16.00	16.00
Solkafloc[4]	5.00	-	-	-
Carboxymethylcellulose[5]	-	6.25	-	-
Cellulose[6]	-	-	5.20	-
High viscous oat β-glucan[7]	-	-	-	9.10
Dicalcium phosphate	1.20	1.20	1.20	1.20
Celite[8]	4.10	2.85	3.90	-
Canola oil	1.00	1.00	1.00	1.00
Limestone	0.90	0.90	0.90	0.90
Mineral premix[9]	0.50	0.50	0.50	0.50
Vitamin premix[10]	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Calculated nutrient composition				
DE, MJ/Kg	15.07	15.07	15.07	15.08
Lysine, %	1.17	1.17	1.17	1.18

[1]CEL-LF = cellulose, low fermentable; CMC = carboxymethylcellulose; CEL-HF = cellulose, high fermentable; HBG = high viscous oat β–glucan.

[2]Melojel (National Starch and Chemical Co., Bridgewater, NJ).

[3]Spray-dried calcium caseinate (American Casein Co., Burlington, NJ).

[4]International Fiber Corp. (North Tonawanda, NY).

[5]TIC Pretested ® CMC 6000 fine powder (TIC Gums Inc., White Marsh, MD); 100 g contains 80 g of soluble dietary fiber, 7943 mg of Na, 9 mg of Ca, and 19 mg K.

[6]TIC Pretested ® TICACEL 100 cellulose powder (TIC Gums Inc., White Marsh, MD); 100 g contains 99 g of insoluble dietary fiber, 28 mg of Na, and 3 mg of Ca.

[7]OatVantage® oat bran concentrate (GTC Nutrition, Golden, CO); 100 g contains 54 g of β-glucan.

[8]Acid-insoluble ash (Celite Corp., Lompoc, CA).

[9]Provided per kilogram of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO_3.

[10]Provided per kilogram of diet: retinol, 2.5 mg; cholecalciferol, 20.6μg; α-tocopherol, 2.7μg; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine,1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 0.025 mg.

Analytical procedures. Diets were analyzed in duplicate for dry matter by drying at 135°C in an airflow-type oven for 2 h (method 930.15 ;(AOAC, 2006), and gross energy by adiabatic bomb calorimetry (Model 5003, Ika-Werke GMBH & Co. KG, Staufen, Germany). The NSP were analyzed by the modified procedure (Englyst et al., 1994).

Total starch and mixed linked β-glucan were analyzed using a kit (Megazyme International Ireland Ltd., Bray, Ireland) based on enzymatic analysis (method AOAC 996.11, and AOAC 995.16;(AOAC, 2006).

Glucose, insulin, C-peptide and SCFA were analyzed in all plasma samples; however, GLP-1 was analyzed only in plasma samples collected until 480 min after feeding. Plasma glucose was analyzed using a glucose oxidase kit (Trinder, 1969) (Diagnostics Chemicals Ltd., Charlottetown, Prince Edward Island, Canada). Plasma SCFA (acetate, propionate, butyrate, valerate, caprionate, isovalerate, and isobutryate) was analyzed by GC using the procedure described by Brighenti, (1994) with isocaprionate as internal standard.

Insulin was analyzed by RIA using a porcine insulin kit (Linco Research, St. Charles, MO; intra-assay CV = 13.5 and interassay CV = 15.4). The C-peptide was analyzed using porcine C-peptide RIA (Linco Research; intra-assay CV = 10.5 and interassay CV = 13.8). The GLP-1 were quantified using a double antibody RIA after extraction with alcohol (Linco Research; intra-assay CV = 10.6 and interassay CV = 5.78) and combined extraction and assay recovery of cold spiked kit quality control was 64.1 ± 2.5 %.

Calculations and Statistical Analyses

Calculations. Net nutrient flux and hormone production were calculated from plasma portal-arterial differences and plasma flow measurements using the formula

$$q = (Cp - Ca)F(dt)$$ (Rerat et al., 1980)

In the formula, q is the amount of nutrient absorbed or hormone produced within time period dt, Cp and Ca are the concentration of nutrient or hormone in portal and arterial plasma respectively, F is the plasma flow in the portal vein.

Plasma flow was calculated from blood flow rate using the following equation: plasma flow = blood flow × (1 – (hematocrit/100)). The term net glucose and SCFA flux was used for the net portal appearance of these nutrients after utilization by the intestine following absorption (Rerat, 1971). For insulin, C-peptide and GLP-1, the term apparent production was used due to the pulsatile secretion, hepatic extraction of insulin, and variable half life (Ellis et al., 1995).

Statistical analyses. Data were analyzed using the MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC) using pig as the experimental unit. Results were reported as least-squares means with $P < 0.05$ defined as significant and $0.05 \leq P < 0.10$ as trends. Arterial and portal nutrient and hormone data and net nutrient flux and apparent hormone production were analyzed as repeated measures. The statistical model included collection period and pig as random effects and viscosity, fermentability, time, and three way interactions as fixed effects. Means were separated for interactions of viscosity and fermentability using a contrast statement in the Mixed model, for individual time points after detecting a significant diet effect using SLICE/time.

6.4 Results

Diets and Pigs

The starch, total NSP, protein, and GE content were similar among the four diets (**Table 6.2**). The dietary fiber content was mostly insoluble for CEL-LF and CEL-HF whereas CMC and HBG contained mostly soluble dietary fiber. Diets

met the minimum requirements of nutrients for pig of that BW group. All pigs remained healthy throughout the experiment.

Glucose and SCFA Kinetics

Arterial glucose tended to be higher ($P < 0.10$) for high than low fermentable dietary fiber. Viscosity, fermentability or their interactions did not affect portal glucose (**Table 6.3**). Net glucose flux increased ($P < 0.05$) immediately postprandially and peaked at 60 min. The functional properties of dietary fiber did not affect the net glucose flux (**Figure 6.1**).

Portal total SCFA tended to be higher ($P < 0.10$) for high than low viscous dietary fiber; however, functional properties of dietary fiber did not affect arterial total SCFA (**Table 6.3).** The net acetate flux was higher ($P < 0.05$) for high than low viscous dietary fiber at 45, 90, 120, 180, 420, 480, and 720 min postprandial (**Figure 6.2A**).

The interactions of viscosity and fermentability ($P < 0.05$) increased net propionate and butyrate flux during the late postprandial phase (**Figure 6.2B, C**). Pigs fed HBG had a higher ($P < 0.05$) net propionate and butyrate flux than pigs fed the other three fiber sources.

Insulin Responses

Viscosity and fermentability interacted ($P < 0.05$) to increase arterial insulin at 15, 30, 45, 60, and 90 min postprandial (**Appendix 8**).

Table 6.2 Analyzed chemical composition of experimental diets containing 4 dietary fiber sources (as-fed basis)[1]

	Low fermentable		High fermentable	
Item	Low viscous CEL-LF	High viscous CMC	Low viscous CEL-HF	High viscous HBG
Starch, %	64.35	64.49	63.93	65.42
Total NSP, %	8.16	7.23	8.32	7.45
Soluble	1.67	5.94	0.90	5.57
Insoluble	6.49	1.29	8.23	1.88
Carbohydrates (starch + NSP), %	72.51	71.72	72.25	72.87
Protein, %	15.46	15.21	14.89	16.33
Gross energy, MJ/kg	16.04	16.14	16.15	16.73

[1]CEL-LF = cellulose, low fermentable; CMC = carboxymethylcellulose; CEL-HF = cellulose, high fermentable; HBG = high viscous oat β–glucan.

Table 6.3 Mean arterial and portal plasma concentration of nutrients and hormones (0 to 720 min after feeding) of pigs fed experimental diets containing 4 dietary fiber sources[1].

	Low fermentable		High fermentable			*P*-value		
Item	Low viscous CEL-LF	High viscous CMC	Low viscous CEL-HF	High viscous HBG	SEM	V	F	V × F
Arterial								
Glucose, mmol/L	5.29	5.46	5.91	5.95	0.25	0.713	0.052	0.101
Total SCFA, µmol/L	348	359	381	353	42	0.835	0.757	0.641
Acetate	290	292	317	291	33	0.729	0.699	0.669
Propionate	26.9	41.8	39.7	37.6	7.54	0.370	0.514	0.247
Butyrate	1.67	2.89	3.05	5.72	0.65	0.011	0.004	0.304
Insulin, pmol/L	101	98	162	184	28	0.734	0.015	0.678
C-peptide, pmol/L	249	281	375	423	62	0.488	0.025	0.894
GLP-1, pmol/L	4.18	5.75	3.89	5.52	1.25	0.261	0.823	0.976
Portal								
Glucose, mmol/L	8.18	7.72	8.54	8.14	0.52	0.319	0.161	0.602
Total SCFA, µmol/L	752	814	777	1022	85	0.065	0.135	0.276
Acetate	618	667	641	736	71	0.327	0.506	0.760

Propionate	84	101	102	177	20	0.025	0.041	0.261
Butyrate	10.0[b]	11.7[b]	12.8[b]	34.5[a]	2.8	<0.001	<0.001	<0.001
Insulin, pmol/L	203	149	282	252	45	0.299	0.030	0.766
C-peptide, pmol/L	435	406	555	733	86	0.398	0.015	0.261
Net Flux/Production								
Glucose, mmol/min	1.57	1.61	1.57	1.67	0.22	0.751	0.872	0.734
Total SCFA, µmol/min	247	324	239	396	46	0.018	0.457	0.403
Acetate	200	268	187	260	34	0.050	0.763	0.938
Propionate	35.5[b]	45.1[b]	36.7[b]	86.4[a]	11.5	0.018	0.064	0.050
Butyrate	4.7[b]	5.9[b]	6.8[b]	17.3[a]	1.42	0.001	0.001	0.004
Insulin, pmol/min	77.1	31.0	95.5	47.7	31.0	0.049	0.445	0.974
C-peptide, pmol/min	126[ab]	85[b]	147[ab]	222[a]	33	0.365	0.012	0.023
GLP-1, pmol/min	6.58	6.78	8.37	8.23	2.65	0.991	0.553	0.948

[1]CEL-LF = cellulose, low fermentable; CMC = carboxymethylcellulose; CEL-HF = cellulose, high fermentable; HBG = high viscous oat β-glucan, V= viscosity, F= fremnetability.

[2]Values are means, $n = 4$. Within a row, means without a common superscript differ, $P < 0.05$.

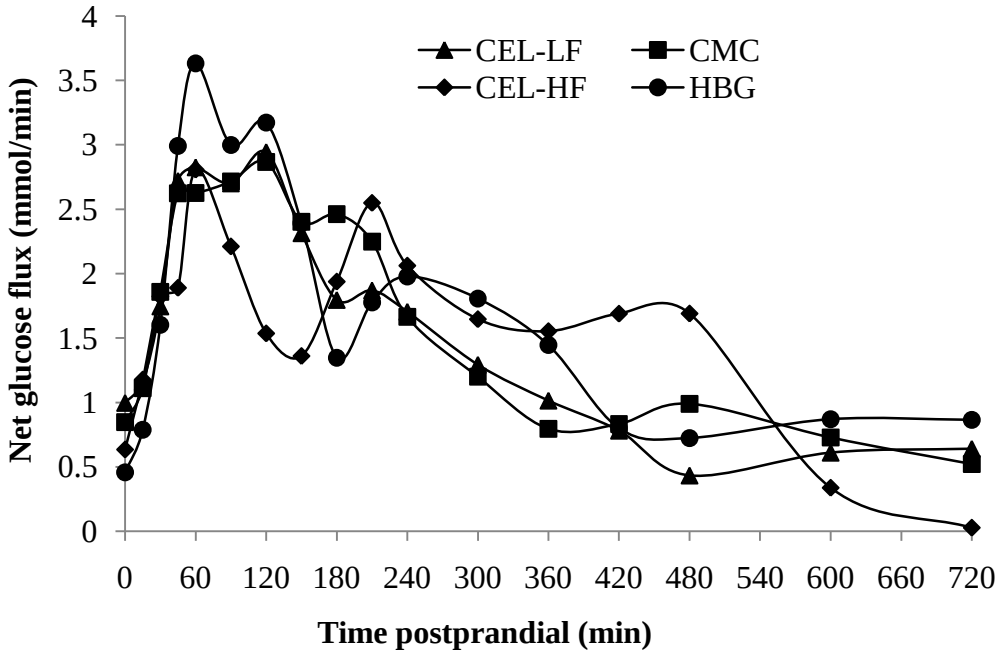


Figure 6.1 Net portal flux of glucose of pigs fed experimental diets containing 4 dietary fiber sources (n = 4). For viscosity, $P > 0.05$; fermentability, $P > 0.05$; viscosity × fermentability, $P > 0.05$; viscosity × fermentability × time, $P > 0.05$.

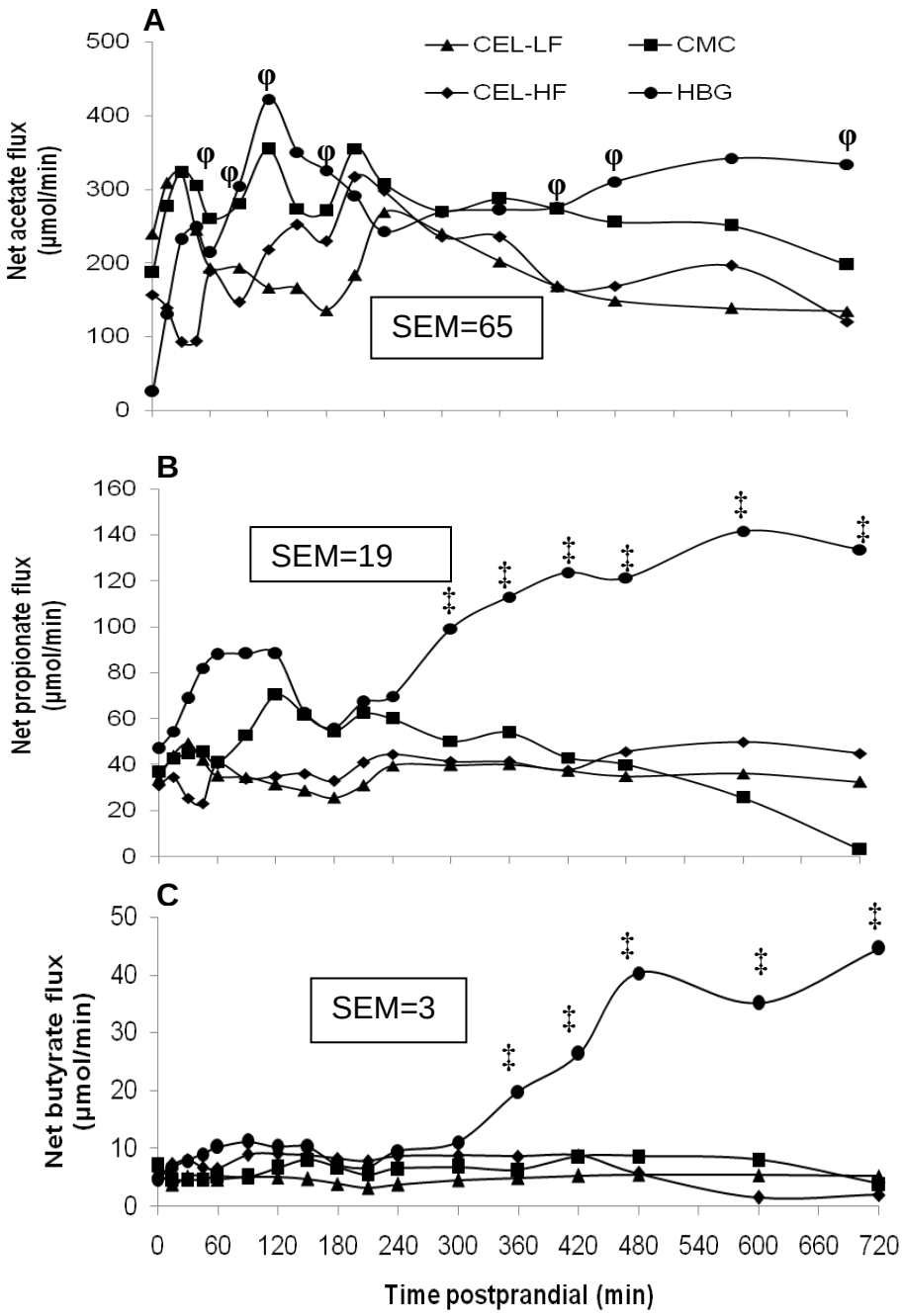


Figure 6.2 Net flux of acetate (A) propionate (B) and butyrate (C) in growing pigs fed experimental diets containing 4 dietary fiber sources (n = 4). For net flux of acetate, viscosity × time, $P < 0.05$, φ viscosity $P < 0.05$ For net flux of propionate and butyrate, viscosity × fermentability × time, $P < 0.05$ and symbol indicates that means differ for a specific time point ($P < 0.05$). ‡ HBG > CEL-HF = CEL-LF = CMC.

At 15 min, arterial insulin was highest ($P < 0.05$) for pigs fed HBG, followed by pigs fed CEL-HF and CEL-LF, and was lowest ($P < 0.05$) for CMC fed pigs, whereas at 30 and 45 min postprandial, arterial insulin was higher ($P < 0.05$) for pigs fed HBG and CEL-HF than for pigs fed CEL-LF and CMC (**Appendix 8**). At 60 and 90 min postprandial arterial insulin was higher ($P < 0.05$) for HBG fed groups and similar among pigs fed the other three dietary fiber sources (**Appendix 8**). High viscosity reduced ($P < 0.05$) portal insulin at 15, 30, 45, and 90 min, whereas high fermentability increased ($P < 0.05$) portal insulin at 0, 30, and 45 min postprandial (**Appendix 8**).

Arterial and portal C-peptide followed the same trend as arterial insulin. Viscosity and fermentability interacted ($P < 0.05$) to increase arterial and portal C-peptide at 15, 30, 45, 60, 90, and 120 min postprandial. At 15, 30, and 45 min postprandial, arterial C-peptide was higher ($P < 0.05$) for pigs fed HBG and CEL-HF than for pigs fed CEL-LF and CMC (**Appendix 9**). At 60 and 90 min postprandial, arterial C-peptide was higher ($P < 0.05$) for pigs fed HBG and similar among the pigs fed other three dietary fiber sources (**Appendix 9**). Portal C-peptide was highest ($P < 0.05$) in pigs fed HBG at all postprandial time points (**Appendix 9**). Similar to portal insulin, high viscosity ($P < 0.05$) reduced the apparent production of insulin at 0, 15, 30, 45, and 60 min postprandial (**Figure 6.3A**). However, viscosity and fermentability interacted ($P < 0.05$) to affect apparent production of C-peptide from 15 to 90 min postprandial and was highest ($P < 0.05$) in pigs fed HBG and similar among pigs fed the other three dietary fiber sources (**Figure 6.3B**).

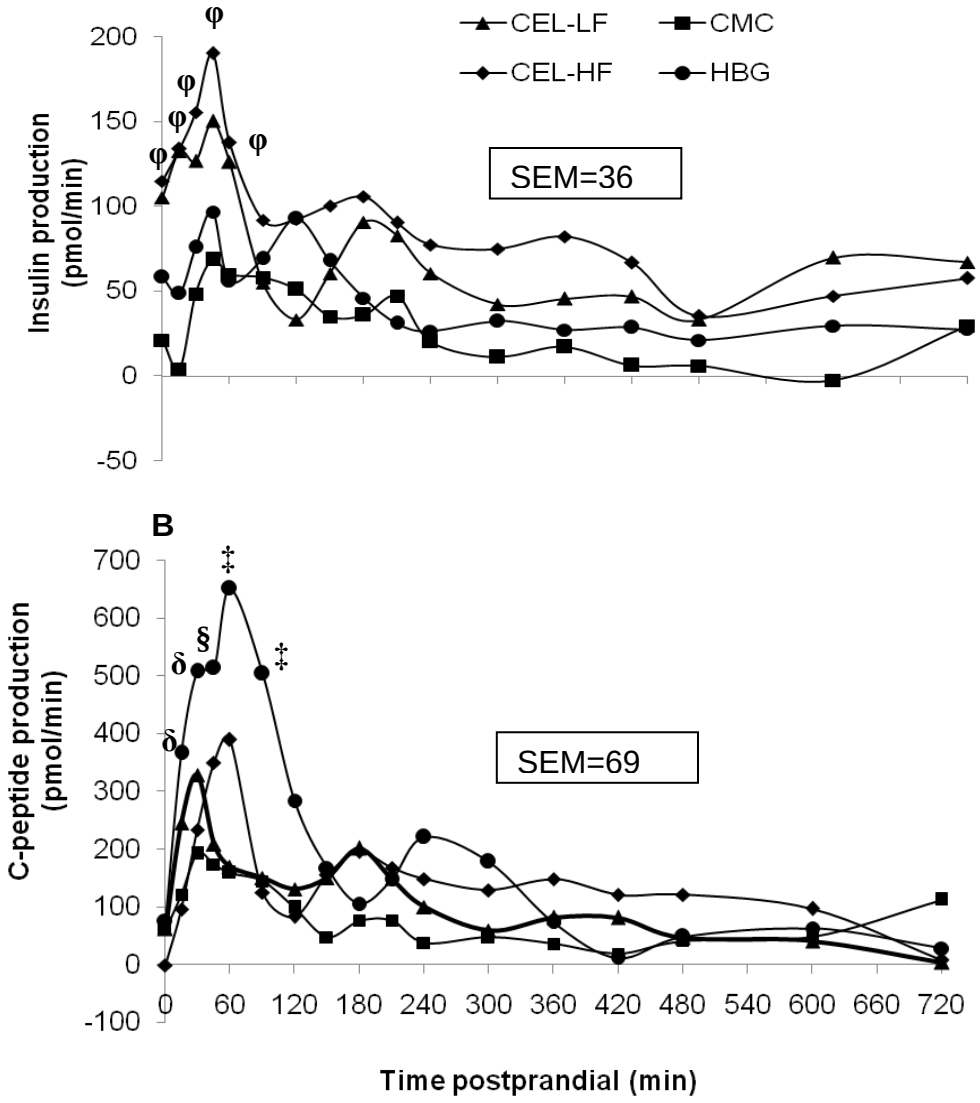


Figure 6.3 Apparent production of insulin (A) and C-peptide (B) in growing pigs fed experimental diets containing 4 dietary fiber sources (n = 4). For apparent production of insulin, viscosity × time, $P < 0.05$, φ viscosity $P > 0.05$. For apparent production of C-peptide, viscosity × fermentability × time, $P < 0.05$ and symbol indicates that means differ for a specific time point ($P < 0.05$). δ HBG ≥ CEL-LF ≥ CMC = CEL-HF; § HBG = CEL-HF > CEL-LF = CMC; ‡ HBG > CEL-HF = CEL-LF = CMC.

GLP-1

Viscosity, fermentability and their interactions did not affect arterial and portal GLP-1 (**Appendix 8**). However, interactions of viscosity and fermentability changed the apparent production of GLP-1 at 30, 60, 90, and 210 min postprandial (**Figure 6.4**). At 30 and 60 min postprandial, the apparent production of GLP-1 was higher ($P < 0.05$) for pigs fed HBG and CEL-HF than for pigs fed CEL-LF and CMC (**Figure 6.4**). At 90 min postprandial, the apparent production of GLP-1 was higher ($P < 0.05$) for pigs fed HBG and did not differ among pigs fed the other three dietary fiber sources (**Figure 6.4**). At 210 min postprandial, apparent production of GLP-1 was highest for pigs fed CEL-HF, followed by pigs fed CEL-LF, and was lowest for pigs fed CMC and HBG (**Figure 6.4**).

6.5 Discussion

Current paradigms are that viscous dietary fiber lowers glucose and insulin responses (Jenkins et al., 2008) and fermentable dietary fiber stimulates fermentation and links to increased GLP-1 secretion (Reimer and McBurney, 1996; Cani et al., 2004). However, the interactive effects of viscous and fermentable dietary fiber on these nutrient and hormone responses are poorly understood.

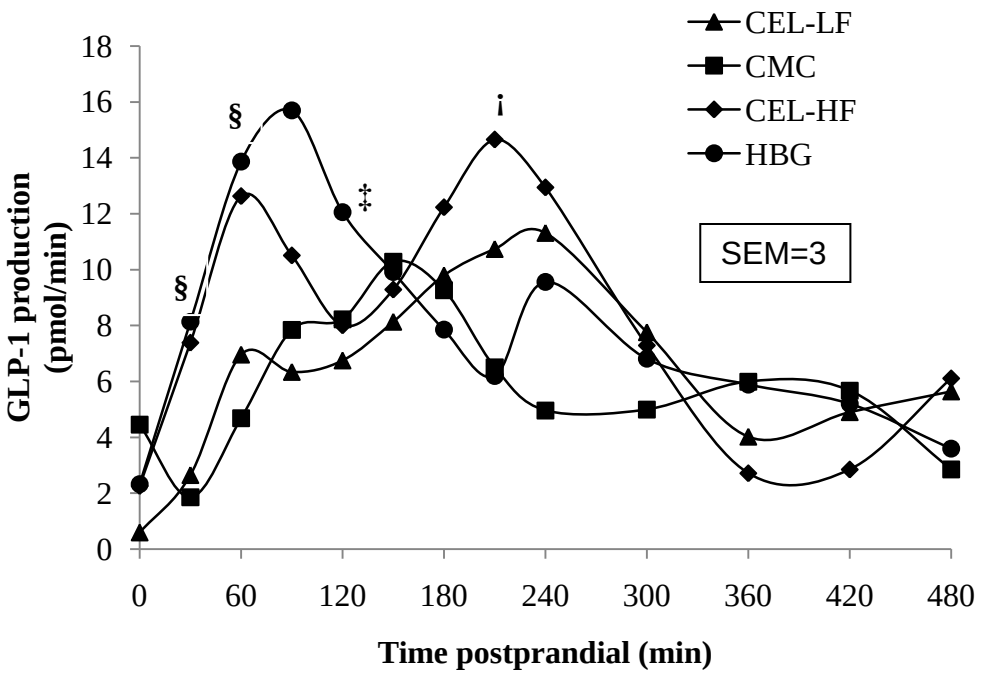


Figure 6.4 Apparent production of GLP-1 in growing pigs fed experimental diets containing 4 dietary fiber sources (n = 4). Viscosity × fermentability × time, P < 0.05 and symbol indicates that means differ for a specific time point (P < 0.05). § HBG = CEL-HF > CEL-LF = CMC; ‡ HBG > CEL-HF = CEL-LF = CMC; ¡ CEL-HF > CEL-LF > CMC = HBG

Moreover, most human studies use peripheral blood concentration as an indicator of nutrient absorption and hormones secretion (Panahi et al., 2007; Juvonen et al., 2009), which does not provide quantitative measurements. Instead, the porcine porto-arterial catheterization model allows studying effects of changes in dietary composition on net nutrient flux and production of gastrointestinal and pancreatic hormones (Rerat et al., 1980) and was used to study the effects of viscous and fermentable dietary fiber.

Kinetics of glucose absorption

In the present study, fermentable dietary fiber tended to increase arterial glucose. However, fiber properties did not change portal glucose and net glucose flux, similar to reports in humans (Hlebowicz, 2009; Freeland et al., 2010) and pigs (Bach Knudsen et al., 2000; Bach Knudsen et al., 2005). Using corn starch, data in the present study indicated that the digestion kinetics of rapidly digestible starch could not be reduced using concentrated viscous or fermentable fiber, in contrast to β-glucan concentrate reducing digestion kinetics of native starch contained in ground wheat in a previous study (Hooda et al., 2010a). Cereal-based dietary fiber has been associated with reduced glucose absorption, because dietary fiber as a part of endosperm wall structure entraps starch and protein (Serena et al., 2009; Karhunen et al., 2010). The fermentable dietary fiber was associated with increased jejunum SGLT-1 mRNA abundance and increased GLUT-2 glucose transporters concentrations in the small intestine (Massimino et al., 1998). The GLUT-2 and SGLT-1 are high capacity facilitative transporters for glucose and ensure the movement of glucose across enterocytes (Foley and Bell, 1990). The end product of fermentable fiber fermentation SCFA, specifically butyrate, may increase the abundance of glucose transporters (Tappenden et al., 1997; Mangian and Tappenden, 2009) and that could lead to increase arterial glucose by fermentable dietary fiber.

SCFA

High viscosity and high fermentability increased portal propionate and arterial butyrate but not arterial acetate and propionate. Pigs fed HBG had the highest portal butyrate. Arterial SCFA represent concentrations after hepatic metabolism of acetate and propionate (Remesy et al., 1980) and thus may not reflect net flux. High viscosity decreased nutrient digestibility and may shift the enzymatic digestion to microbial fermentation (Renteria-Flores et al., 2008) and thus increase production of SCFA. The most interesting change in net propionate and butyrate flux occurred in the late postprandial phase, observations achieved via the 12 h collection. The increased flux of propionate and butyrate indicated fermentation of oat β-glucan (Hooda et al., 2010a). The disappearance of β-glucan by the end of large intestine (data not shown) confirmed that β-glucan is fermented completely in the intestine. The increased butyrate flux is important because butyrate is a major source of energy to colonic mucosa (Roediger, 1982) and an important link to gut health via stimulation of cell proliferation (Comalada et al., 2006), promotion of apoptosis, and prevention of colon cancer (Hamer et al., 2008). Propionate acts as substrate for hepatic gluconeogenesis and inhibits cholesterol synthesis in liver (Hamer et al., 2008). Increase in late postprandial butyrate flux might have caused increased GLP-1 during hyperglycemic phase as butyrate has been reported to increase mRNA proglucagon gene expression (Drucker, 1998). Contrary to results in previous study cellulose diet did not increase the net SCFA flux specifically butyrate despite higher fecal concentration in ileal cannulated pigs (Metzler-Zebeli et al., 2010) and could be due to

insufficient adaption period for fermentation in the present study vs. the study with ileal-cannulated pigs (6 vs. 10 d). The adaptation is significant for to adaption of microbial community to new substrates. Thus, these results further confirmed the results of previous study that dietary oat β-glucan increased propionate and butyrate production and flux and thus improve intestinal and metabolic health.

Insulin responses

Insulin responses are generally assessed with plasma insulin concentrations. However, C-peptide that is released in equimolar amounts from proinsulin in the pancreas is considered as a more reliable indicator of insulin release into systemic blood, because C-peptide has a lower hepatic extraction than insulin (Polonsky and Rubenstein, 1984). Differences between the apparent production of insulin and C-peptide in pigs fed HBG may indicate more hepatic extraction of insulin than in pigs fed the other three dietary fiber sources. This difference emphasizes the importance of measuring C-peptide simultaneously with insulin (Polonsky and Rubenstein, 1984) and C-peptide was used as indicator of insulin secretion rather than insulin itself. The interactions of viscosity and fermentability increased the apparent production of insulin (indicated by C-peptide production) that contradicts various studies reporting that oat or barley β-glucan decreased insulin responses (Ellis et al., 1995; Juvonen et al., 2009; Kim et al., 2009). Oat β-glucan occurs in the wall of the endosperm and requires extraction that may affect physiochemical properties and thereby physiological responses (Burkus and

Temelli, 1998; Burcelin et al., 2007). In addition, physiochemical properties of β-glucan may alter during passage through the gastro-intestinal tract. Specifically, ileal-cannulated pigs fed high and low viscous β-glucan had the same viscosity of ileal digesta (Hooda et al., 2010b). However, the origin of viscosity alteration in the gastro-intestinal tract was not clarified.

Oat β-glucan is fermented and increased the production (Hooda et al., 2010b) and absorption (in present study) of SCFA, specifically butyrate, that may increase GLP-1 (Bosch et al., 2009; Freeland et al., 2010). The incretin GLP-1 increases glucose-dependent insulin secretion (Baggio and Drucker, 2007) and thus explains the higher insulin production in pigs fed HBG.

GLP-1

The GLP-1 is released from enteroendocrine L cells, stimulates glucose-induced insulin secretion (Rehfeld, 1998), and is thus important in glucose homeostasis (Burcelin et al., 2007). Pigs fed HBG and CEL-HF had a higher GLP-1 production during the early postprandial period and thus in agreement with fermentable dietary fiber increasing the early postprandial GLP-1 flux in other studies (Reimer and McBurney, 1996; Cani et al., 2004; Greenway et al., 2007; Tarini and Wolever, 2010). This increase was linked to two mechanisms: 1) an increase in colonic mass (Cani et al., 2007) and thus increase in number of L cells (Tappenden et al., 1998), and 2) an increase in gene expression of the proglucagon mRNA, precursor of GLP-1 (Reimer and McBurney, 1996). The end products of fermentation SCFA, specifically butyrate, may also stimulate

expression of proglucagon mRNA (Drozdowski et al., 2002). In contrast, viscous dietary fiber may decrease the early postprandial GLP-1 flux (Juvonen et al., 2009; Karhunen et al., 2010; Hooda et al., 2010a). Viscous dietary fiber forms an unstirred layer at mucosal surface that might prevents the interaction of nutrients with the apical surface of enteroendocrine cells (Juvonen et al., 2009) that is required for stimulation of GLP-1 secretion (Baggio and Drucker, 2007). The GLP-1 reaches to pancreatic β-cell, bind to receptors and initiate a cascade of events for insulin secretion (Burcelin et al., 2007). The higher GLP-1 production after feeding HBG diet could explain the higher insulin secretion and thus could be potential nutritional approach for increased endogenous production of insulin in type-II diabetes patients.

The present study demonstrated that interactions of viscosity and fermentability in oat β-glucan did not affect net glucose flux in the pig but increased insulin responses. The increase GLP-1 by fermentable dietary fiber indicated that oat β-glucan and fermentable cellulose could be potential nutritional approach to increase GLP-1 that has been considered important for satiety and glucose homeostasis and thus prevention of diabetes. Finally, increased fermentation and net flux of propionate and butyrate provided solid evidence that oat β-glucan are fermented and are beneficial to manage human metabolic diseases and gut health.

6.6 References

Anderson, J. W., P. Baird, R. H. Davis Jr, S. Ferreri, M. Knudtson, A. Koraym, V. Waters, and C. L. Williams. 2009. Health benefits of dietary fiber. Nutr. Rev. 67:188-205.

AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Bach Knudsen, K. E., H. Jorgensen, and N. Canibe. 2000. Quantification of the absorption of nutrients derived from carbohydrate assimilation: Model experiment with catheterised pigs fed on wheat- or oat-based rolls. Br. J. Nutr. 84:449-458.

Bach Knudsen, K. E., A. Serena, A. K. Kjaer, H. Jorgensen, and R. Engberg. 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135:1696-1704.

Baggio, L. L., and D. J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131-2157.

Bosch, G., A. Verbrugghe, M. Hesta, J. J. Holst, A. F. van der Poel, G. P. Janssens and W. H. Hendriks. 2009. The effects of dietary fibre type on satiety-related hormones and voluntary food intake in dogs. Br. J. Nutr. 102:318-325.

Brighenti F. 1998. Summary of the conclusion of the working group on profibre interloboratory study on determination of short chain fatty acids in blood. In: Gullion R, Amado F, Amaral-Collaco JA, Anderson H, Asp NG, Bach Knudsen KE, Champ M, Mathers J, Robertson JA, Rowland I, Van Loo J, editors. Functional Properties of non-digestible carbohydrates. Science, Research and Development, Brussels: European Commission, DG XII. p. 150-3.

Burcelin, R., P. D. Cani, and C. Knauf. 2007. Glucagon-like peptide-1 and energy homeostasis. J. Nutr. 137:2534S-2538S.

Burkus, Z., and F. Temelli. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Cani, P. D., C. Dewever, and N. M. Delzenne. 2004. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br. J. Nutr. 92:521-526.

Cani, P. D., S. Hoste, Y. Guiot, and N. M. Delzenne. 2007. Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. Br. J. Nutr. 98:32-37.

Cani, P. D., and N. M. Delzenne. 2009. The role of the gut microbiota in energy metabolism and metabolic disease. Curr. Pharm. Des. 15:1546-1558.

Comalada, M., E. Bailon, O. de Haro, F. Lara-Villoslada, J. Xaus, A. Zarzuelo et al. 2006. The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. J. Cancer Res. Clin. Oncol. 132:487-497.

Darwiche, G., O. Bjorgell, and L. O. Almer. 2003. The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy subjects. BMC Gastroenterolo. 3:12.

Dikeman, C. L., and G. C. Fahey. 2006. Viscosity as related to dietary fiber: A review. Crit. Rev. Food Sci. Nutr. 46:649-663.

Drozdowski, L. A., W. T. Dixon, M. I. McBurney, and A. B. Thomson. 2002. Short-chain fatty acids and total parenteral nutrition affect intestinal gene expression. JPEN J. Parenter. Enteral Nutr. 26:145-150.

Drucker, D. J. 1998. Glucagon-like peptides. Diabetes. 47:159-169.

Edwards, C. A., I. T. Johnson, and N. W. Read. 1988. Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? Eur. J. Clin. Nutr. 42:307-312.

Ellis, P. R., F. G. Roberts, A. G. Low, and L. M. Morgan. 1995. The effect of high-molecular-weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in the growing pig: Relationship to rheological changes in jejunal digesta. Br. J. Nutr. 74:539-556.

Englyst, H. N., M. E. Quigley, and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119:1497-1509.

Foley, J. E., and P. A. Bell. 1990. Molecular biology as a tool in the study of glucose transport and metabolism. Ann. Med. 22:177-179.

Freeland, K. R., C. Wilson, and T. M. Wolever. 2010. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. Br. J. Nutr. 103:82-90.

Greenway, F., C. E. O'Neil, L. Stewart, J. Rood, M. Keenan, and R. Martin. 2007. Fourteen weeks of treatment with viscofiber increased fasting levels of glucagon-like peptide-1 and peptide-YY. J. Med. Food. 10:720-724.

Hamer, H. M., D. Jonkers, K. Venema, S. Vanhoutvin, F. J. Troost, and R. J. Brummer. 2008. Review article: The role of butyrate on colonic function. Aliment. Pharmacol. Ther. 27:104-119.

Hlebowicz, J. 2009. Postprandial blood glucose response in relation to gastric emptying and satiety in healthy subjects. Appetite 53:249-252.

Hooda, S., J. J. Matte, C. W. Wilkinson, and R. T. Zijlstra. 2009. Technical note: An improved surgical model for the long-term studies of kinetics and

quantification of nutrient absorption in swine. J. Anim. Sci. 87:2013-2019.

Hooda, S., J. J. Matte, T. Vasanthan, and R. T. Zijlstra. 2010a. Dietary oat β-glucan reduces peak net glucose flux and insulin production, and modulates plasma incretin in portal-vein catheterized grower pigs. Accepted in J. Nutr doi;10.3945/jn.110.122721

Hooda, S., Metzler-Zebeli, B. U., T. Vasanthan, and R. T. Zijlstra. 2010b. Effects of viscosity and fermentability of non-starch polysaccharides on nutrient digestibility and digesta characteristics in ileal-cannulated grower pigs. Submitted in Br. J. Nutr.

Jenkins, D. J., C. W. Kendall, G. McKeown-Eyssen, R. G. Josse, J. Silverberg, G. L. Booth, E. Vidgen, A. R. Josse, T. H. Nguyen, S. Corrigan, M. S. Banach, S. Ares, S. Mitchell, A. Emam, L. S. Augustin, T. L. Parker, and L. A. Leiter. 2008. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: A randomized trial. JAMA. 300:2742-2753.

Juvonen, K. R., A. K. Purhonen, M. Salmenkallio-Marttila, L. Lahteenmaki, D. E. Laaksonen, K. H. Herzig, M. I. Uusitupa, K. S. Poutanen, and L. J. Karhunen. 2009. Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. J. Nutr. 139:461-466.

Karhunen, L. J., K. R. Juvonen, S. M. Flander, K. H. Liukkonen, L. Lahteenmaki, M. Siloaho D. E. Laaksonen, K. H. Herzig, M. I. Uusitupa, and K. S. Poutanen. 2010. A psyllium fiber-enriched meal strongly attenuates postprandial gastrointestinal peptide release in healthy young adults. J. Nutr. 140:737-744.

Kim, H., K. S. Stote, K. M. Behall, K. Spears, B. Vinyard, and J. M. Conway. 2009. Glucose and insulin responses to whole grain breakfasts varying in soluble fiber, beta-glucan: A dose response study in obese women with increased risk for insulin resistance. Eur. J. Nutr. 48:170-175.

Mangian, H. F., and K. A. Tappenden. 2009. Butyrate increases GLUT2 mRNA abundance by initiating transcription in Caco2-BBe cells. JPEN J. Parenter. Enteral Nutr. 33:607-617.

Massimino, S. P., M. I. McBurney, C. J. Field, A. B. Thomson, M. Keelan, M. G. Hayek et al. 1998. Fermentable dietary fiber increases GLP-1 secretion and improves glucose homeostasis despite increased intestinal glucose transport capacity in healthy dogs. J. Nutr. 128:1786-1793.

Metzler-Zebeli, B. U., S. Hooda, R. Pieper, R. T. Zijlstra, A. G. van Kessel, R. Mosenthin and M. G. Gaenzle.2010. Non-starch polysaccharides modulate bacterial microbiota, pathways for butyrate production, and abundance of pathogenic escherichia coli in the pig gastrointestinal tract. Appl. Environ. Microbiol. 76:3692-3701.

Nilsson, A. C., E. M. Ostman, J. J. Holst, and I. M. Bjorck. 2008. Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. J. Nutr. 138:732-739.

Panahi, S., A. Ezatagha, F. Temelli, T. Vasanthan, and V. Vuksan. 2007. Beta-glucan from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. J. Am. Coll. Nutr. 26:639-644.

Polonsky, K. S., and A. H. Rubenstein. 1984. C-peptide as a measure of the secretion and hepatic extraction of insulin. pitfalls and limitations. Diabetes. 33:486-494.

Rehfeld, J. F. 1998. The new biology of gastrointestinal hormones. Physiol. Rev. 78:1087-1108.

Reimer, R. A., and M. I. McBurney. 1996. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology 137:3948-3956.

Remesy, C., C. Demigne, and F. Chartier. 1980. Origin and utilization of volatile fatty acids in the rat. Reprod. Nutr. Dev. 20:1339-1349.

Renteria-Flores, J. A., L. J. Johnston, G. C. Shurson, and D. D. Gallaher. 2008. Effect of soluble and insoluble fiber on energy digestibility, nitrogen retention, and fiber digestibility of diets fed to gestating sows. J. Anim. Sci. 86:2568-2575.

Rerat, A. 1971. Demonstration of a quantitative method for studying absorption in the pig. Annales de Biologie Animal, Bichimie, Biophysique. 11:277.

Rerat, A., P. Vaugelade, and P. Villiers. 1980. A New Method for Measuring the Absorption of Nutrients in the Pig: Critical Examination. Pages 177-178-216 Low, A. G., and I. G. Partridge, eds. National Institute for Research in Dairying, Reading, England, and Hannah Research Institute, Ayr, Scotland.

Roediger, W. E. 1982. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 83:424-429.

Serena, A., H. Jorgensen, and K. E. Bach Knudsen. 2009. Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber. J. Anim. Sci. 87:136-147.

Tappenden, K. A., A. B. Thomson, G. E. Wild, and M. I. McBurney. 1997. Short-chain fatty acid-supplemented total parenteral nutrition enhances functional adaptation to intestinal resection in rats. Gastroenterology 112:792-802.

Tappenden, K. A., L. A. Drozdowski, A. B. Thomson, and M. I. McBurney. 1998. Short-chain fatty acid-supplemented total parenteral nutrition alters intestinal structure, glucose transporter 2 (GLUT2) mRNA and protein, and proglucagon mRNA abundance in normal rats. Am. J. Clin. Nutr. 68:118-125.

Tarini, J., and T. M. Wolever. 2010. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Appl. Physiol. Nutr. Metab. 35:9-16.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6:24-27.

Wisker, E., M. Daniel, G. Rave, and W. Feldheim. 2000. Short-chain fatty acids produced in vitro from fibre residues obtained from mixed diets containing different breads and in human faeces during the ingestion of the diets. Br. J. Nutr. 84:31-37.

Wong, J. M., R. de Souza, C. W. Kendall, A. Emam, and D. J. Jenkins. 2006. Colonic health: Fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235-243.

Chapter 7: Summary and Conclusions

7.1 Conclusions

Intake of dietary fiber has been associated with beneficial effects for metabolic and intestinal health (Anderson et al., 2009). The physiological effects of dietary fiber have been attributed to 2 main functional properties: viscosity and fermentability (Dikeman and Fahey, 2006). Viscosity is defined as the ability of dietary fiber to thicken after mixing with water and to form gels (Dikeman and Fahey, 2006). This viscous property is due to physical entanglements of the polysaccharides along with trapping of water in between polysaccharides (Guillon and Champ, 2003). Viscosity has been associated with reduced glucose, insulin, and incretin responses (Jenkins et al., 2008; Kim et al., 2009) because increased digesta viscosity reduces gastric emptying (Darwiche et al., 2003) and nutrient digestibility in the small intestine (Graham et al., 1986; Renteria-Flores et al., 2008). Fermentability is defined as the ability of the nutrient to be fermented by the microflora especially in the large intestine. Non-digested nutrients along with fermentable dietary fiber pass into the large intestine to be fermented by microbial populations. Fermentable fiber promotes growth of beneficial gut microflora (Wong et al., 2006) that produces short-chain fatty acids as an end-product (Bach Knudsen and Hansen, 1991). The SCFA especially butyrate, serve as nutrients; regulate proliferation, differentiation, and gene expression in the colonic epithelium (Wong et al., 2006).

Recently, incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) have gained importance as therapy for type II

diabetes (Holst et al., 2009). The GLP-1 and GIP are released from L and K cells (enteroendocrine cells) of the large and small intestine, respectively, in response to luminal glucose (Baggio and Drucker, 2007). Viscous dietary fiber may reduce incretin production (Juntunen et al., 2003). In contrast, fermentable fiber may increase SCFA production that may increase the expression of the proglucagon gene in L cells of intestine and thus may increase GLP-1 secretion (Reimer and McBurney, 1996). Both GLP-1 and GIP enhance glucose-dependent insulin secretion, promote β-cell proliferation and inhibit β-cell apoptosis, and thus increase pancreatic β-cell mass (Baggio and Drucker, 2007).

The effects on lowering glucose and insulin were not consistent among short-term studies (Maki et al., 2007; Biorklund et al., 2008; Kim et al., 2009). Studies in humans with the collection of peripheral blood (Panahi et al., 2007; Biorklund et al., 2008) did not allow the quantification of the kinetics of nutrient absorption and insulin and incretin secretion. Hence, the porcine porto-arterial catheterization model was used. Portal blood allows quantitative measurements of absorbed nutrients albeit following epithelial utilization while arterial blood represents the basal concentration of nutrients reaching the intestine. The difference in concentration is the qualitative nutrient flux. Simultaneous blood flow measurements allowed quantification of the net nutrient flux in long-term studies (Rerat et al., 1980). Thus, the first objective of the thesis was to develop an improved surgical model for long-term studies of kinetics and quantification of nutrient absorption in swine (Chapter 2). The model was used for further studies for this thesis and other research projects. The development of model was major

challenge as the model is invasive, requires detailed maintenance and care; thus, few experimental units can be used per experiment.

The second study (Chapter 3) used the port-arterial surgical model to study the effects of 2 doses of oat β-glucan on peak net glucose flux, net SCFA flux, insulin secretion and association with incretin. The study demonstrated that 6% oat β-glucan lowered peak net glucose flux in the pig concurrently with attenuated incretin and insulin responses, thereby explaining some of the underlying physiology and metabolism of dietary fiber. The principle component analysis of portal vein variables indicated strong associations among glucose and incretins providing further evidence that portal glucose acts as a strong stimulus for incretin release. Incretins in turn affect insulin release and thereby enforce the existence of the entero-insular axis or cross-talk between intestine and pancreas to control blood glucose. Finally, increased fermentation and net flux of propionate and butyrate provided solid evidence that oat β-glucan are fermented and may be beneficial to manage human metabolic diseases and gut health.

The results on effects of oat β-glucan on glucose, insulin, incretin and SCFA responses were interesting but the specific contributions of viscosity and fermentability were not explained. In animal nutrition, most studies investigating the role of dietary fiber are based on including feedstuffs rich in dietary fiber that contain fiber as part of intact plant cell wall. Therefore, specific effects of functional properties or structural effects of dietary fiber cannot be differentiated (Bach Knudsen et al., 1993). Thus, the concept of feeding purified dietary fiber fractions with semi-purified diets was adopted in the second and third study to

separate specific contributions of viscosity and fermentability and their interactions on nutrient digestibility, digesta kinetics, fermentation, SCFA production in ileal-cannulated growing pigs. The conclusion was that pigs fed high viscous carboxymethylcellulose (CMC) had the highest apparent ileal digestibility (AID) of nutrients, by slowing down the rate of digesta passage (Chapter 4). In contrast, a faster digesta passage reduced the AID of nutrients in pigs fed cellulose (CEL) compared to pigs fed CMC, thereby increased nutrient flow into the large intestine so that part of the nutrients that would normally be digested were instead fermented. Thus, negative effects of dietary fiber on digesta characteristics and nutrient digestibility are affected by interactions of viscosity and fermentability via digesta viscosity and digesta passage rate.

In the fourth study, the impact of non-starch polysaccharides (NSP) differing in their functional properties on intestinal bacterial community composition, prevalence of butyrate-production pathway genes, and the occurrence of *Escherichia coli* virulence factors was studied in 8 ileal-cannulated grower pigs using terminal-restriction-fragment-length-polymorphism (TRFLP) and quantitative PCR (Chapter 5). In conclusion, CMC promoted growth of the *Clostridium* cluster XIVa and particularly of the *Bacteroides-Prevotella-Porphyromonas* group and *Enterobacteriaceae* compared to the other NSP. In contrast, CEL favored growth of *Clostridium* cluster IV that was attributable to bacterial fermentation of NSP. Additionally, the NSP altered the ileal flow of nutrients into the large intestine. Comparable to human colonic microbiota, gene copy numbers of butyryl-CoA CoA transferase were higher than numbers of

butyrate kinase genes, indicating that this pathway is the dominant butyrate-production pathway in the large intestine of pigs. Although increasing intestinal viscosity was generally associated with impaired gut health (Langhout et al., 1999; Smits et al., 1998), only CMC increased the susceptibility of pigs to overgrowth of pathogenic *E. coli*, suggesting that the use of CMC in diets for growing pigs is detrimental as compared to the other NSP fractions.

In the last study, the viscosity and fermentability of diet was considered instead of only dietary fiber. The objectives of the last study were to clarify the independent and interactive effects of viscosity and fermentability of dietary fiber on kinetics of nutrient flux and hormones production during the 12-h postprandial period (Chapter 6). The results demonstrated that interactions of viscosity and fermentability in oat β-glucan did not affect net glucose flux in the pig but increased insulin responses. The increased GLP-1 by fermentable dietary fiber indicated that oat β-glucan and fermentable cellulose could be potential nutritional approach to increase GLP-1 that has been considered important for satiety and glucose homeostasis and thus prevention of diabetes (Holst et al., 2009). Finally, increased fermentation and net flux of propionate and butyrate provided solid evidence that oat β-glucan are fermented and may be beneficial to manage human metabolic diseases and gut health.

7.2 Challenges and Limitations

Overall, the research project clarified the individual and interactive effects of viscosity and fermentability using concentrated fiber sources on nutrient flow and

intestinal health and provided a proposed mechanism of their effects. However, challenges existed throughout the project. The porto-arterial catheterization model is limited in experimental units and also has variablility than other surgical models (Bach Knudsen et al., 1992).

Oat and barley β-glucan are generally similar in structure but differ in the ratio of (1→3) (1→4) linkages, molecular weight (MW) and thus solubility (Wood and Beer, 1998). The MW of oat β-glucan is around 3×10^6 and more concentrated in the outer layers of oat grain endosperm, while the barley has lower MW ($2\text{-}2.5\times10^6$) and distributed more uniformly throughout the endosperm of barley grain. It is possible to isolate highly purified concentrations of β-glucan but that compromise the molecular weight and high solubility that is critical for viscosity development (Vasanthan and Temelli, 2008). The wet water processing technologies have yielded very high purity products but lower viscous solution as endogenous enzymes in aqueous solution led to degradation of β-glucan. Thus for the study low purified β-glucan (50%) with maintained viscosity were used. The choice of other viscous and fermentable fiber sources was a major challenge because in vitro lab analysis results did not coincide with in vivo measurements in swine digesta. For example, LBG had a lower in vitro viscosity than HBG, but ileal digesta viscosity was not different between pigs fed LBG and HBG. Furthermore, the magnitude of viscosity was not similar. Pigs fed CMC had a very high digesta viscosity compared to pigs fed HBG. The two sources of oat β-glucan extracted using different technologies caused different glucose, insulin and incretin responses. The CEL was not itself fermented but increased digesta

passage rate and thereby decreased nutrient digestibility and increased fermentation and production of SCFA in the large intestine. Cellulose from different sources behaved differently in terms of effect on insulin and GLP-1 production. Thus, a more detailed description of effects of different concentrated fiber sources in terms of physiochemical and functional characteristics is required both using in vitro and in vivo to clearly define their physiological effects.

Differences in length of the adaption period among studies may have caused differences in effects on fermentation. The same cellulose source caused increased concentration of SCFA in ileal-cannulated pigs but did not increase SCFA absorption in porto-arterial catheterized pigs.

The ileal-cannulated and porto-arterial catheterization models allowed collection of samples for measurement of nutrient digestibility and absorption. However, both models provide only the end-point of processes, but not details about the dynamic process of complete digestion and absorption. The feeding of semi-purified diets especially the high viscous diets was the challenge, because more time was required to adapt the pigs to these diets, because swine are used to coarse cereal-based diets.

7.3 Future Research

Dietary fiber is an important nutrient in animal and human nutrition; thus, a need exists to understand its effect on gastrointestinal physiology. Numerous publications studied the effects of dietary fiber on nutrient flow and intestinal health. However, evidence explaining results of functional properties on changes

in gastrointestinal tract characteristics, digesta transit and thus the mechanisms are rare. We studied few dietary fiber sources in relation to viscosity and fermentability, however, numerous fiber sources exist that could be potentially used as functional fiber in animal as well as in human nutrition. Studies using animal models specifically surgical and slaughter models are very good for linking the effects to the digesta characteristics and explain the mechanism.

Inulin and fructo-oligosaccharides have been studied extensively as prebiotic; however there are many others dietary fibers that could be prebiotic but their potential have been not explored. The advancement of new genomic techniques replaced the traditional plating methods for qualitative and quantitative measurements of gut microbiota. Thus using these techniques the potential of different kinds of dietary fiber can be explored to manipulate the gut microbiota to improve gut health. There is a need of not only exploring the gut microbiology but also detailed the metabolic pathways used by gut microflora and their effect on host physiology.

The concept of gut health involves the dynamic equilibrium of diet, gut mucosa and gut microbiota (Montagne et al., 2003). Thus apart from gut microbiota, there is a need to study the potential of other dietary fiber specifically with regards to viscosity and fermentability on intestine morphology, quality and quantity of intestinal secretions. The complex interaction of gut microbiota and host mucosa as influenced by various dietary fiber sources needs to be explored in more detail.

The incretins and specifically GLP-1 has gained attention in past few years as a potential therapy for type II diabetes (Holst, 2009). Few studies indicated that increased GLP-1 and thus insulin secretion could be manipulated by nutritional interventions specifically fermentable dietary fiber (Reimer and McBurney, 1996). Our results indicated that oat β-glucan could be potential dietary fiber but there is a need for further studies to explore the optimum viscosity and fermentability to achieve the desired effect. Studies using the same treatment arrangement using slaughter model may explain the mechanism behind effects on GLP-1 and insulin release. Gene expression studies using enteroendocrine cells will explain the exact site and effects of viscosity and fermentability.

This thesis studied the effects on healthy pigs and that can be applied to healthy humans. However, metabolism is different in insulin resistant, type II diabetes and humans with metabolic syndrome and thus studies are warranted using a diseased animal model. And last but not least, the finding using animal models should be validated in humans.

7.4 References

Anderson, J. W., P. Baird, R. H. Davis Jr., S. Ferreri, M. Knudtson, A. Koraym, V. Waters, and C. L. Williams. 2009. Health benefits of dietary fiber. Nutr. Rev. 67:188-205.

Bach Knudsen, K. E., and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 1. Digestibility and bulking properties of polysaccharides and other major constituents. Br. J. Nutr. 65:217-232.

Bach Knudsen, K. E., B. B. Jensen, and I. Hansen. 1993. Digestion of polysaccharides and other major components in the small and large

intestine fed on diets consisting of oat fractions rich in β-D-Glucan. Br. J. Nutr. 70:537-556.

Bach Knudsen K. E. 1992. Methodological aspects of in vivo methods for measuring of starch digestibility: animal models, in Methodological Aspects of In Vivo Methods for Measurement of Starch Digestibility. Ed by Gudmand-Høyer E , EURESTA, Vedbæk, pp 40-56.

Baggio, L. L., and D. J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131-2157.

Biorklund M, J. Holm, and G. Onning G. 2008. Serum lipids and postprandial glucose and insulin levels in hyperlipidemic subjects after consumption of an oat beta-glucan-containing ready meal. Ann. Nutr. Metab. 52:83-90.

Darwiche, G., O. Bjorgell, and L. O. Almer. 2003. The addition of locust bean gum but not water delayed the gastric emptying rate of a nutrient semisolid meal in healthy subjects. BMC Gastroenterol. 3:12-17.

Dikeman, C. L., and G. C. Fahey. 2006. Viscosity as related to dietary fiber: A review. Crit. Rev. Food Sci. Nutr. 46:649-663.

Graham, H., K. Hesselman, and P. Aman. 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. J. Nutr. 116:242-251.

Guillon, F., and M. Champ. 2003. Structural and physical properties of dietary fibers, and consequences of processing on human physiology. Food Res. Int. 33:233-245.

Holst, J. J., T. Vilsboll, and C. F. Deacon. 2009. The incretin system and its role in type 2 diabetes mellitus. Mol. Cell Endocrinol. 297:127-136.

Jenkins, A. L., D. J. Jenkins, T. M. Wolever, A. L. Rogovik, E. Jovanovski, V. Bozikov, D. Rahelic, and V. Vuksan. 2008. Comparable postprandial glucose reductions with viscous fiber blend enriched biscuits in healthy subjects and patients with diabetes mellitus: Acute randomized controlled clinical trial. Croat. Med. J. 49:772-782.

Juntunen, K. S., L. K. Niskanen, K. H. Liukkonen, K. S. Poutanen, J. J. Holst, and H. M. Mykkanen. Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. Am J Clin Nutr. 75:254-262.

Juvonen, K. R., A. K. Purhonen, M. Salmenkallio-Marttila, L. Lahteenmaki, D. E. Laaksonen, K. H. Herzig et al. 2009. Viscosity of oat bran-enriched beverages influences gastrointestinal hormonal responses in healthy humans. J. Nutr. 139:461-466.

Kim, H., K. S. Stote, K. M. Behall, K. Spears, B. Vinyard, and J. M. Conway. 2009. Glucose and insulin responses to whole grain breakfasts varying in soluble fiber, beta-glucan: A dose response study in obese women with increased risk for insulin resistance. Eur. J. Nutr. 48:170-175.

Langhout, D. J., J. B. Schutte, P. Van Leeuwen, J. Wiebenga, and S. Tamminga. 1999. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br. Poult. Sci. 40:340-347.

Maki, K. C., M. H. Davidson, M. S. Witchger, M. R. Dicklin, and P. V. Subbaiah. 2007. Effects of high-fiber oat and wheat cereals on postprandial glucose and lipid responses in healthy men. Int. J. Vitam. Nutr. Res. 77:347-356.

Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between DF and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108:95-117.

Panahi, S., A. Ezatagha, F. Temelli, T. Vasanthan, and V. Vuksan. 2007. Beta-glucans from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. J Am Coll Nutr. 26:639-644.

Reimer, R. A., and M. I. McBurney. 1996. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology 137:3948-3956.

Renteria-Flores, J. A., L. J. Johnston, G. C. Shurson, and D. D. Gallaher. 2008. Effect of soluble and insoluble fiber on energy digestibility, nitrogen retention, and fiber digestibility of diets fed to gestating sows. J. Anim. Sci. 86:2568-2575.

Rerat, A., P. Vaugelade, and P. Villiers. 1980. A New Method for Measuring the Absorption of Nutrients in the Pig: Critical Examination. Pages 177-178-216 Low, A. G., and I. G. Partridge, eds. National Institute for Research in Dairying, Reading, England, and Hannah Research Institute, Ayr, Scotland.

Smits, C. H., A. Veldman, H. J. Verkade, and A. C. Beynen. 1998. The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. Poultry Sci. 77:1534-1539.

Vasanthan, T., and F. Temelli. 2008. Grain fractionation technologies for cereal β-glucan concentration. Food Res. Int. 41:876-881.

Wong, J. M., R. de Souza, C. W. Kendall, A. Emam, and D. J. Jenkins. 2006. Colonic health: Fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235-243.

Appendix 1 Non-starch polysaccharide (NSP) composition of diets varying in β-glucan concentration (Chapter 3)

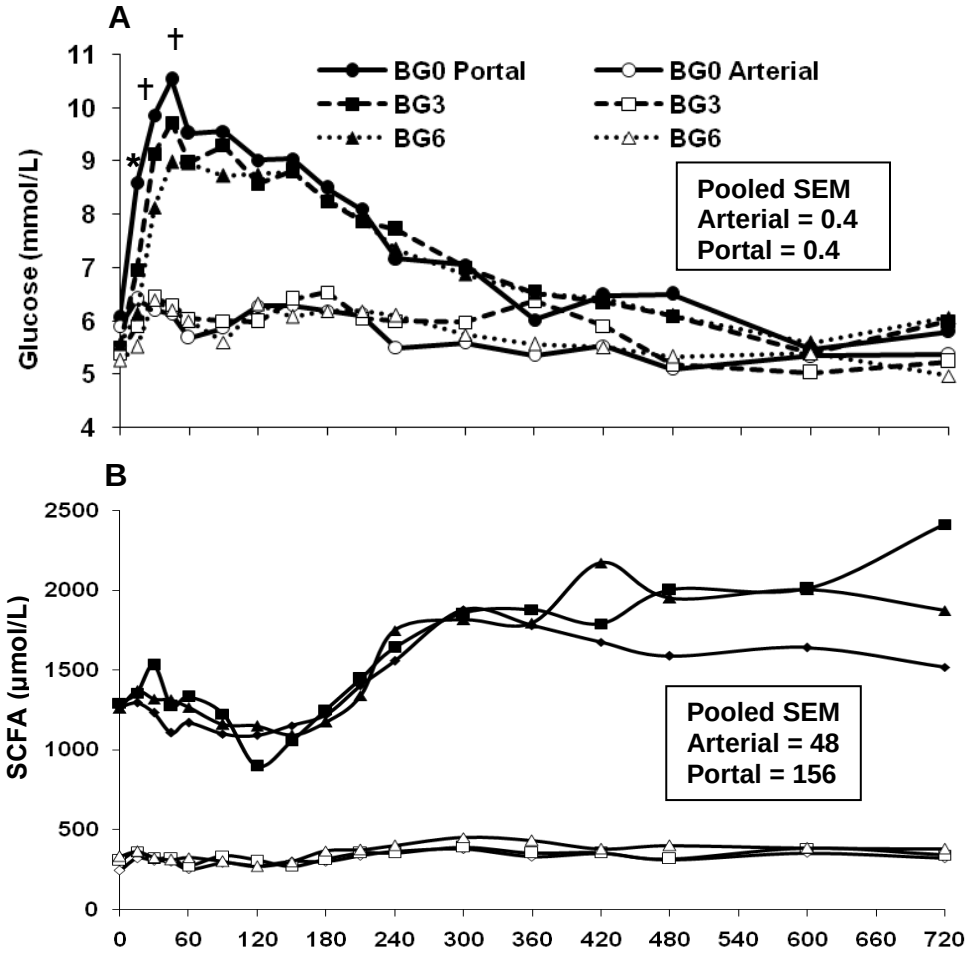
Item	Diets		
	BG0	BG3	BG6
Chemical composition	*% (DM basis)*		
Total NSP	10.31	17.82	20.68
Soluble	2.72	7.14	11.21
Insoluble	7.59	10.69	9.47
Arabinose	2.00	2.15	2.75
Galactose	1.24	1.11	1.28
Glucose	4.23	11.27	12.19
Mannose	0.24	0.27	0.35
Rhamnose	0.06	0.06	0.08
Ribose	0.06	0.07	0.08
Xylose	2.53	2.95	4.04
Fucose	0.06	0.06	0.07

Appendix 2 Mean portal and arterial plasma concentrations, net flux of nutrients and apparent production of hormones (0 to 720 min after feeding) of pigs fed diets varying in β-glucan concentration[1](Chapter 3).

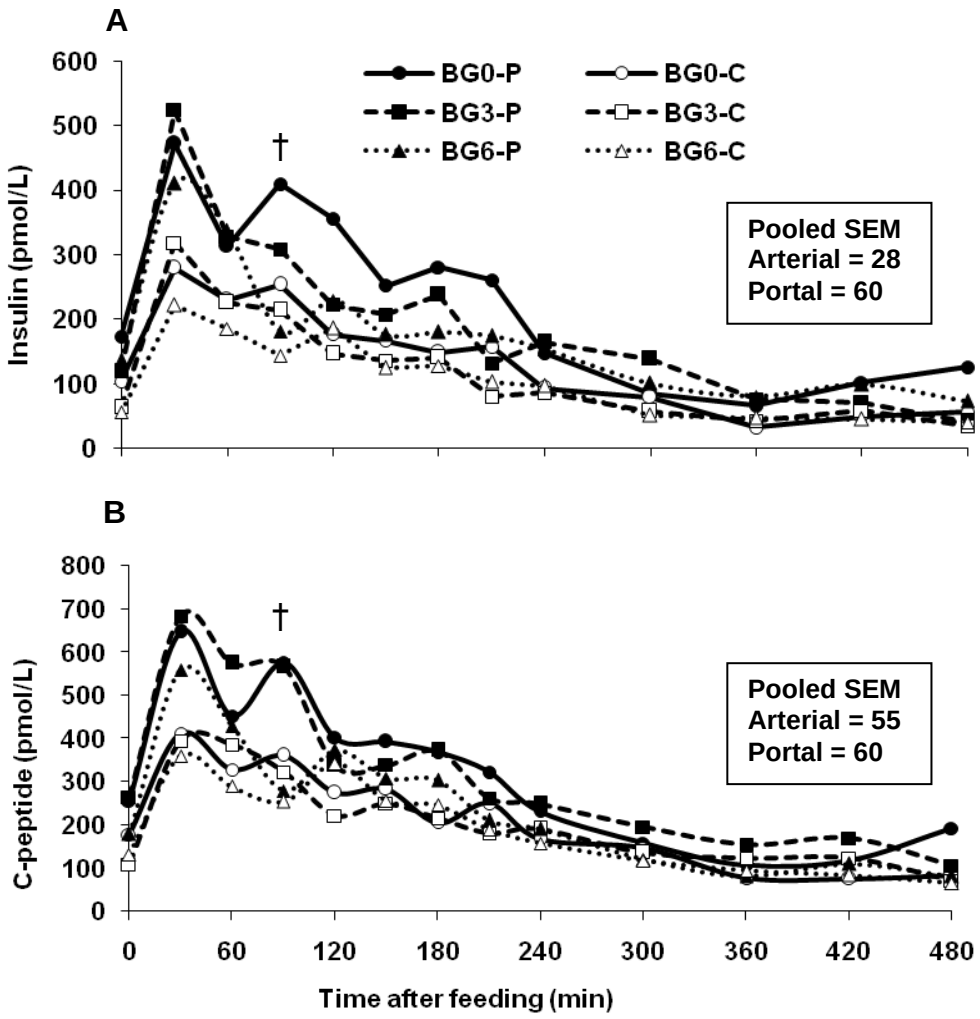
	Carotid artery					Portal vein				
Item	BG0	BG3	BG6	SEM	*P*-value	BG0	BG3	BG6	SEM	*P*-value
Blood flow, *L/min*	-	-	-	-	-	1.15	1.17	1.18	0.12	0.954
Glucose, *mmol/L*	5.81	5.92	5.78	0.31	0.923	7.84	7.54	7.36	0.19	0.257
Glucose flux, *mmol/min*	-	-	-	-	-	1.49	1.47	1.27	0.21	0.665
Insulin, *pmol/L*	140	123	110	11	0.124	234	197	179	29	0.281
Insulin production, *pmol/min*	-	-	-	-	-	87.8	67.5	57.4	18.7	0.461
C-peptide, *pmol/L*	219	209	199	36	0.988	325	329	248	25	0.083
C-peptide production, *pmol/min*	-	-	-	-	-	99.2	97.5	41.1	18.1	0.084
GIP, *pmol/L*	169[a]	126[b]	111[b]	9	0.008	200	149	121	19	0.059
GIP production, *pmol/min*						23.6	14.2	3.4	5.3	0.071
GLP-1, *pmol/L*	13.0	14.9	15.2	1.5	0.572	30.1[a]	26.8[a]	17.4[b]	2.7	0.031
GLP-1 production, *pmol/min*	-	-	-	-	-	11.0	10.8	2.6	3.9	0.290
Total SCFA, *µmol/L*	315	335	361	44	0.748	1,356	1,494	1,603	61	0.071
Total SCFA flux, *µmol/min*	-	-	-	-	-	760	862	927	63	0.148

Acetate, *µmol/L*	263	283	304	34	0.681	902	990	992	40	0.054
Acetate flux, *µmol/min*	-	-	-	-	-	463	533	513	48	0.501
Propionate, *µmol/L*	25.5	26.5	29.0	6.9	0.928	236[a]	298[b]	374[c]	20	0.020
Propionate flux, *µmol/min*	-	-	-	-	-	151[a]	198[b]	255[c]	20	0.031
Butyrate, *µmol/L*	11.1	14.6	18.3	1.6	0.06	103[a]	136[b]	157[c]	9	0.007
Butyrate flux, *µmol/min*	-	-	-	-	-	65.9[a]	87.4[a]	103[b]	8.9	0.017
Valerate, *µmol/L*	0.57	0.47	0.47	0.12	0.341	20.1	20.6	24.6	1.7	0.192
Caprionate, *µmol/L*	1.82	1.44	1.67	0.25	0.408	5.12	6.54	6.49	1.46	0.327
Branched chain fatty acid µmol/L										
Isobutryate, *µmol/L*	4.46	3.66	3.44	1.00	0.688	14.0	14.5	13.8	0.8	0.672
Isovalerate, *µmol/L*	7.29	4.94	4.14	2.03	0.454	22.7	21.9	19.8	1.3	0.190

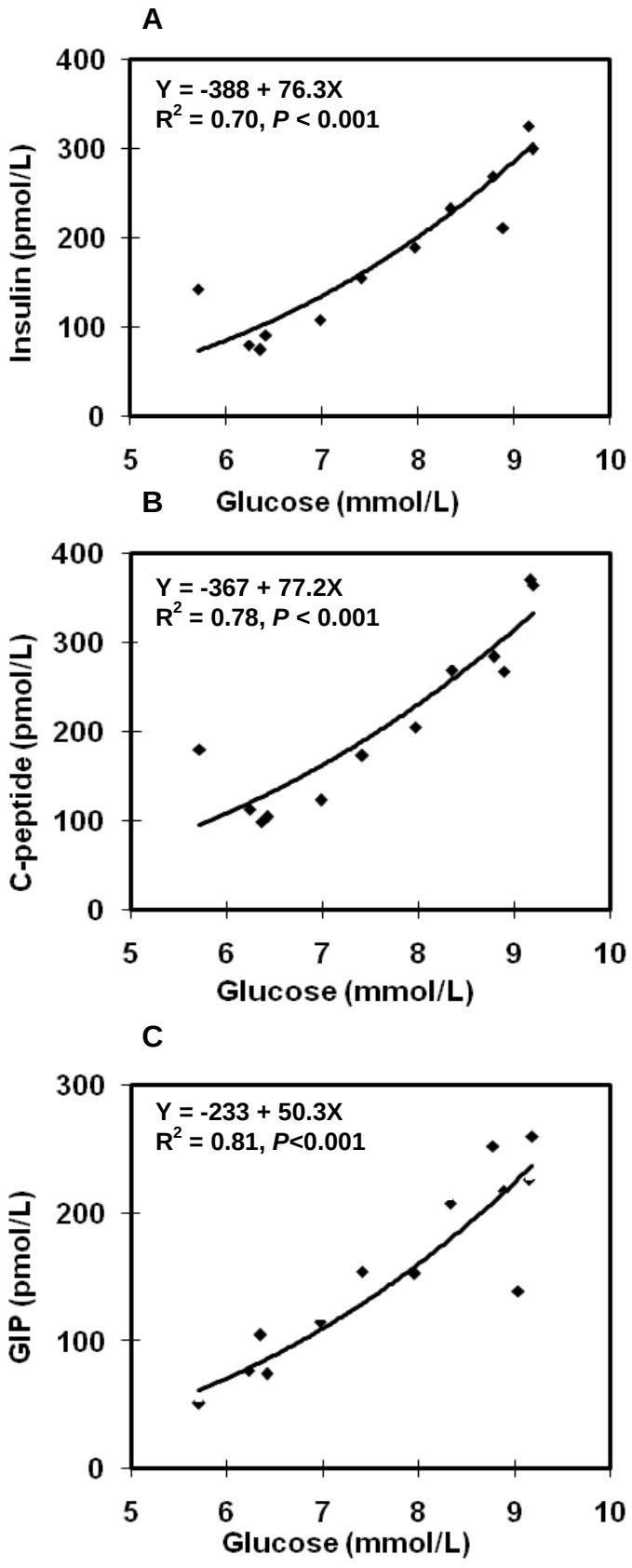
[1]Values are means, $n = 3$. Means in a row within variable with superscripts without a common letter differ, $P < 0.05$

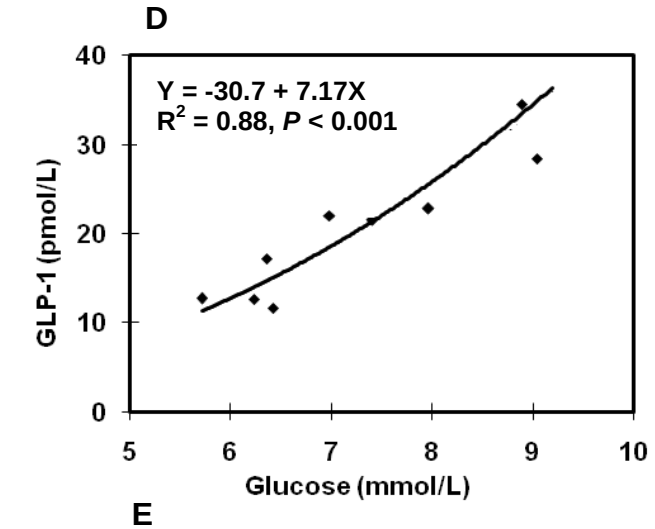


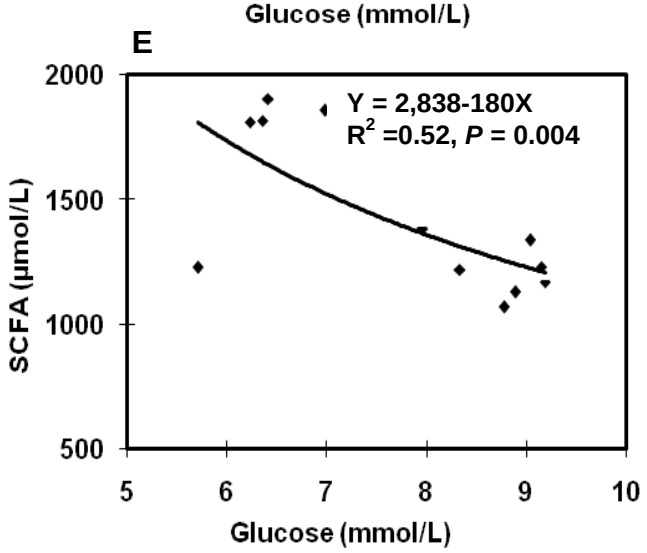
Appendix 3 Portal and arterial plasma concentrations of glucose (A) and SCFA (B) of pigs fed diets varying in β-glucan concentration (n = 3). Symbols indicate that means differ ($P < 0.05$). * BG0 > BG3 and BG6; † BG0 > BG6 (Chapter 3).

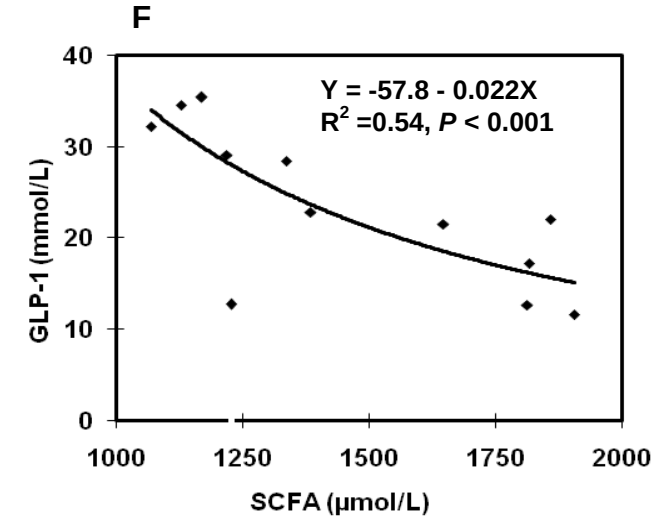


Appendix 4 Portal and arterial plasma concentrations of insulin (A), and C-peptide (B) of pigs fed diets varying in β-glucan concentration (n = 3). Symbol indicates that means differ ($P < 0.05$). † BG0 > BG6 ($P < 0.05$) (Chapter 3).


A
Y = -388 + 76.3X
R2 = 0.70, P < 0.001
Insulin (pmol/L)
400
300
200
100
0
5
6
7
8
9
10
B
Glucose (mmol/L)
Y = -367 + 77.2X
R2 = 0.78, P < 0.001
C-peptide (pmol/L)
400
300
200
100
0
5
6
7
8
9
10
Glucose (mmol/L)
C
Y = -233 + 50.3X
R2 = 0.81, P<0.001
GIP (pmol/L)
300
200
100
0
5
6
7
8
9
10
Glucose (mmol/L)


D
Y = -30.7 + 7.17X
R2 = 0.88, P < 0.001
GLP-1 (pmol/L)
Glucose (mmol/L)
0
10
20
30
40
5
6
7
8
9
10


E
Y = 2,838-180X
R2 =0.52, P = 0.004
SCFA (µmol/L)
Glucose (mmol/L)
500
1000
1500
2000
5
6
7
8
9
10


F
Y = -57.8 - 0.022X
R2 =0.54, P < 0.001
GLP-1 (mmol/L)
SCFA (µmol/L)
0
10
20
30
40
1000
1250
1500
1750
2000

Appendix 5 Relation between portal glucose and portal insulin (A), C-peptide (B), GIP (C), GLP-1 (D) and SCFA (E) in pigs fed varying concentrations of β-glucan (Chapter 3).

Appendix 6 Number, size, mean contribution of individual TRF's to the total bacterial community, and closest cultured relative of TRFs found in the distal ileum of pigs fed diets supplemented with viscous and fermentable non-starch polysaccharide fractions (Chapter 5).

TRF #	Size Bp	Contribution of individual TRF to the total bacterial community composition[1]				Closest cultured relative
		CEL	CMC	LG	HG	
TRF 1	67	0.01	0.03	0.02	0.04	uncultured pig clone
TRF 2	73	-	0.10	-	0.08	*Streptococcus sobrinus*
TRF 3	131	-	-	-	0.03	*Fibrobacter intestinalis*
TRF 4	147	0.03	0.13	0.22	0.08	*Clostridium clostridioforme*
TRF 5	177	0.05	0.01	0.01	0.01	*Lactobacillus delbrueckii*
TRF 6	190	0.01	0.03	0.01	0.01	*L. gasseri/L.johnsonii*
TRF 7	292	-	-	-	0.01	*Clostridium ramosum*
TRF 8	451	-	0.11	0.03	0.02	uncultured pig clone
TRF 9	517	0.01	0.02	0.02	0.01	*Sarcina ventriculi*
TRF 10	551	0.83	0.54	0.69	0.65	*Streptococcus agalactiae*
TRF 11	559	0.06	0.03	0.02	0.07	*Lactococcus garvieae*

[1]Only TRFs with a contribution to the total bacterial community of ≥1% were considered.

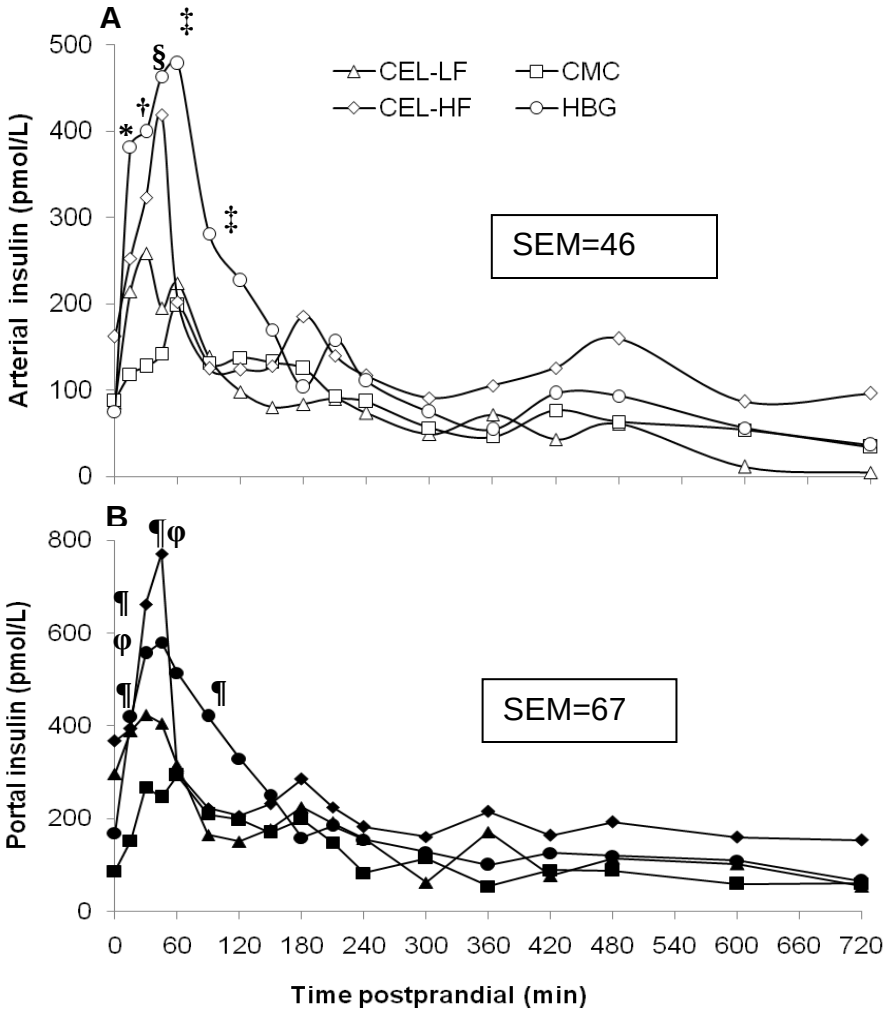
Appendix 7 Number, size, mean contribution of individual TRF's to the total bacterial community, and closest cultured relative of TRFs found in feces of pigs fed diets supplemented with viscous and fermentable non-starch polysaccharide fractions (Chapter 5).

TRF #	Size Bp	Contribution of individual TRF to the total bacterial community composition[1]				Closest cultured relative
		CEL	CMC	LG	HG	
1	32	-	0.01	-	0.01	*Lactobacillus fermentum*
2	43	-	-	-	0.01	Pseudo-TRF
6	72	-	0.01	-	-	*Clostridium glycyrrhizinilyticum*
7	78	-	0.01	-	-	*Sorangium cellulosum*
8	87	-	0.02	0.01	0.02	*Bacteroides*
9	92	0.03	0.04	0.07	0.04	*Bacteroides*
12	119	-	-	-	0.01	*Sulfobacillus acidophilus*
13	129	-	0.01	-	0.04	*Collinsella stercoris*
16	147	0.06	0.04	0.04	0.01	*Clostridium clostridioforme*
17	160	0.01	0.01	-	0.01	uncultured bacterium
18	165	0.01	0.01	0.01	0.04	*Corynebacterium tuscaniense*
19	174	-	0.01	0.01	-	uncultured bacterium
20	189	0.02	0.01	0.01	0.01	*Lactobacillus gasseri*
21	192	-	0.02	-	-	*Clostridium irregulare/ Cl.*

22	202	0.03	0.06	0.01	0.03	*Clostridium polysaccharolyticum*
24	210	0.01	0.02	0.02	0.03	uncultured bacterium
25	217	-	-	-	0.02	*Ruminococcus obeum*
32	266	0.01	0.01	-	0.01	*Fibrobacter succinogenes*
33	273	-	0.01	-	-	uncultured bacterium
34	277	0.01	0.01	-	0.01	uncultured bacterium
35	283	0.01	0.02	0.01	0.03	*Ruminococcus flavefaciens*
36	292	0.01	0.02	0.02	0.04	*Clostridium ramosum*
37	294	0.02	0.01	0.01	0.04	*Clostrium phytofermentans*
38	309	-	-	0.01	-	uncultured bacterium
39	310	-	0.04	0.02	0.03	*Butyrivibrio fibrisolvens*
41	329	-	0.01	-	0.03	*Oenococcus*
48	391	-	0.01	0.02	0.01	*Selenomonas ruminantium*
52	427	-	0.01	0.05	0.03	uncultured bacterium
55	450	0.03	0.05	0.01	0.01	uncultured bacterium
57	466	-	0.01	-	-	*Clostridium paradoxum*
58	470	-	0.03	0.05	0.02	*Peptostreptococcus*
59	478	-	-	-	0.01	uncultured bacterium
60	480	0.03	0.01	0.01	0.03	uncultured bacterium
61	489	0.45	0.18	0.11	0.09	uncultured bacterium
62	496	0.01	-	-	-	*Desulfovibrio defluvii*
63	498	0.01	0.02	0.01	0.01	*Desulfovibrio*
64	515	0.01	0.02	-	-	*Clostridium cellulovorans*

65	517	0.04	0.03	0.03	0.01	*Clostridium proteolyticum*
66	523	-	0.02	0.06	0.02	*Clostridium perfringens-like*
67	531	0.01	0.02	0.01	0.01	uncultured bacterium
68	541	0.02	0.03	0.03	0.02	uncultured bacterium
69	551	0.16	0.16	0.29	0.26	*Streptococcus agalactiae*
70	560	0.01	0.01	0.02	0.01	*Enterococcus*
72	575	-	0.01	0.01	-	*Lactobacillus*
75	598	0.01	-	-	-	uncultured bacterium

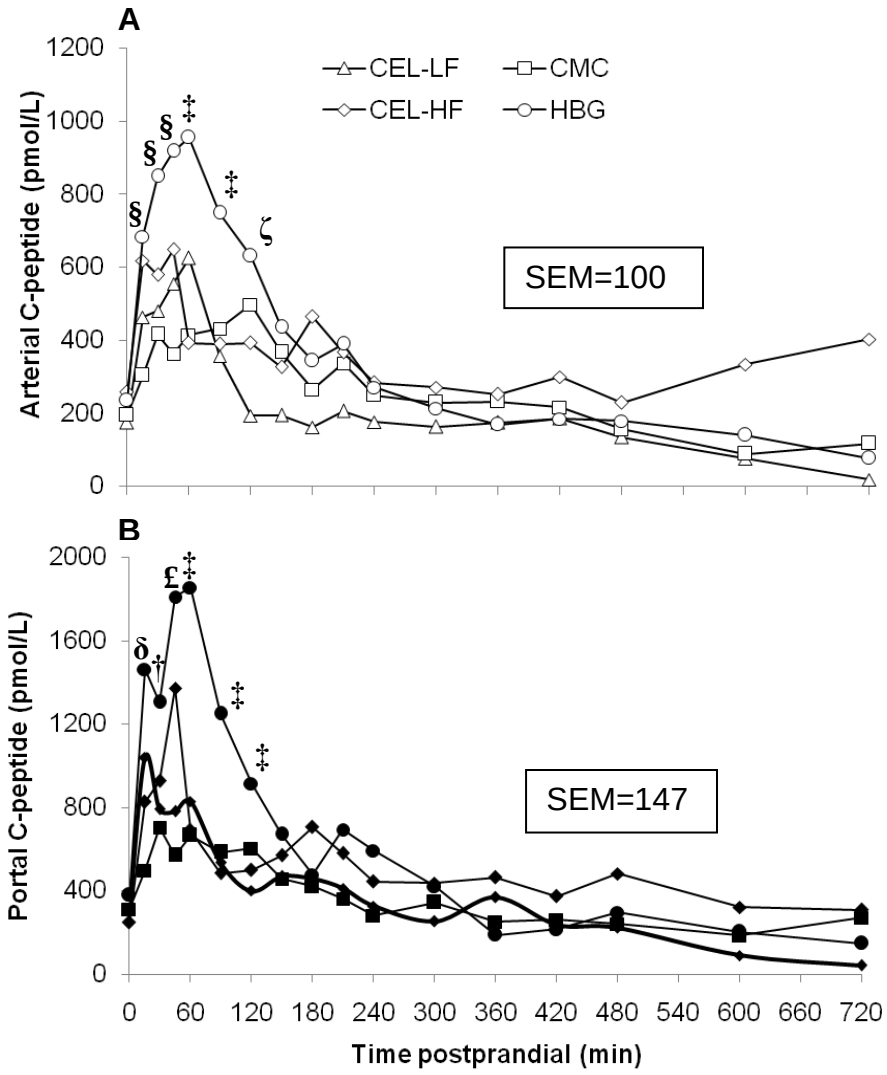
[1]Only TRFs with a contribution to the total bacterial community of ≥1% were considered



Appendix 8 Arterial (A) and portal (B) plasma concentrations of insulin in growing pigs fed experimental diets containing 4 dietary fiber sources (n = 4). For arterial insulin, viscosity × fermentability × time, $P < 0.05$ and symbol indicates that means differ for a specific time point ($P < 0.05$). * HBG > CEL-LF, CEL-HF > CMC; † HBG = CEL-HF ≥ CEL-LF > CMC; § HBG = CEL-HF > CEL-LF = CMC; ‡ HBG > CEL-HF = CEL-LF = CMC.

For portal insulin, viscosity × time, $P < 0.05$, fermentability × time, $P < 0.05$

Φ viscosity $P > 0.05$, ¶ fermentability $P > 0.05$. (Chapter 6).



Appendix 9 Arterial (A) and portal (B) plasma concentrations of insulin in growing pigs fed experimental diets containing 4 dietary fiber sources (n = 4). For arterial and portal C-peptide, viscosity × fermentability × time, $P < 0.05$ and symbol indicates that means differ for a specific time point ($P < 0.05$). § HBG = CEL-HF > CEL-LF = CMC; ‡ HBG > CEL-HF = CEL-LF = CMC; † HBG = CEL-HF ≥ CEL-LF > CMC; δ HBG ≥ CEL-LF ≥ CMC = CEL-HF; £ HBG > CEL-HF > CEL-LF = CMC. ζ HBG = CMC > CEL-HF = CEL-LF (Chapter 6).