



Sveriges lantbruksuniversitet

**Fakulteten för veterinärmedicin och husdjursvetenskap**

Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine and Animal Science**

## **Metabolic effect of whole grain**

**Nualprae Saenbungkhor**

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# Metabolic effect of whole grain

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## Abstract

In Western countries, whole grain products are used as a source of dietary carbohydrates for both humans and animals. Several studies have indicated that consumption of whole grain products generate a lower postprandial insulin response than the refined products. This project aimed to compare the metabolic effects from bread baked on whole grain wheat (WGWB; *Triticum aestivum*), wheat flour (WB) and whole grain rye (WGRB; *Secale cereale*) to get an increased understanding of the mechanism behind the health aspect related to the whole grain cereals. Three multi-catheterized pigs were used in a 3×3 Latin square design, in addition two non-catheterized pigs were used as replicates of two of the pigs in the Latin square. Pigs were fed by WGWB, WGRB and WB for seven days. Blood samples were collected from the catheterized pigs at the last day of each period consecutively at 15, 45, 60, 90, 120, 180, and 240 minutes after feeding. In addition, fecal and urinary samples were collected from all pigs and metabolic profiles from these samples were analyzed using H-NMR. Plasma glucose and insulin was measure with ELISA. The insulin response after consumption of WGRB did not differ from WGWB and WB, however an unexpected effect of period was observed. Principal component analysis of fecal and urine metabolic profiles showed a clear clustering according to diet in urine samples where rye bread was separated from the wheat based diets. A similar tendency was observed for the fecal samples. Furthermore, fecal samples from WB and WGWB showed a significantly higher molar proportion of acetate than fecal samples from WGRB. In conclusion, the fiber content did not influence the insulinaemic response. However, this study shows that wheat and rye generates different metabolic profiles. To get an insight into possible mechanisms behind the different urinary and fecal metabolic profiles further studies with more pigs and longer experimental periods are needed.

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## List of abbreviations

AX	Arabinoxylan
AR	Alkylresorcinol
CCK	Cholecystokinin
CVD	Cardiovascular disease
DF	Dietary fiber
DM	Dry matter
GI	Gastrointestinal
GLP-1	Glucagon-like peptide
LDL	Low-density lipoprotein
NDF	Neutral-detergent fiber
NSP	Non-starch polysaccharide
PAH	Para-aminohippuric acid
PCA	Principal Component Analysis
PYY	Peptide tyrosine tyrosine
SCFA	Short chain fatty acid
SD	Standard Deviation
VFA	Volatile fatty acid
WBC	Water binding capacity
WEAX	Water-extractable arabinoxylan
WB	White wheat flour bread
WGRB	Whole grain rye bread
WGWB	Whole grain wheat bread
T2D	Type 2 Diabetes
TiO <sub>2</sub>	Titanium dioxide
TOAX	Total arabinoxylan
TSP	Trimethylsilyl propanoic acid

## Introduction

In the past decades the nutritionists have become increasingly interested in dietary fiber for their beneficial effect on human health (de Leeuw *et al.*, 2008). Epidemiological evidences have shown that intake of a diet rich in dietary fiber and whole-grain foods are associated with reduction in the risk of developing various life style diseases such as type 2 diabetes mellitus (T2D) and cardiovascular diseases (CVD). Recent studies conclude that body weight is reversely associated with the consumption of high-fiber and whole-grain foods (Koh-Banerjee and Rimm, 2003; Gaesser, 2007). The likely outcome would be through an increased satiety (Slavin and Green, 2007). Possible explanation of this effect is a lower digestibility of macronutrients and hence lower energy density of the diet (Southgate and Durnin, 1970).

In Western countries, whole grain products are used as a source of dietary carbohydrate for both humans and animals. Products from whole grain include the endosperm, germ, bran and are rich in dietary fiber (Slavin, 2003). Several studies in humans have indicated that consumption of whole grain products generate a lower postprandial insulin response than refined products (Leinonen *et al.*, 1999; Bjorck *et al.*, 2007). Dietary fiber not only affect satiety in humans but also in mono-gastric animals, including the pig (de Leeuw *et al.*, 2008; Bolhuis *et al.*, 2010). In restrictedly fed animals such as gestating sows, dietary fiber has been observed to decrease behavioral problems induced from hunger, for example rooting and bar-chewing (Meunier-Salaün *et al.*, 2001). Even though the diet for gestation sows are formulated to meet their nutritional requirement. In general, the amount of the feed that is given cannot satisfy the sows behavior and welfare needs. Feeding motivation is observed in gestation sows and is noticeable if the diet is unable to provide sufficient satiety (de Leeuw *et al.*, 2008). Dietary fiber has gained much attention for its functional properties which is able to reduce behavioral problem related to insufficient satiety (Meunier-Salaün, *et al.*, 2001). However, not all types of fiber have the same ability to induce satiety (Slavin, 2010).

In the gastrointestinal tract, dietary fiber may have beneficial effects on the digestion and absorption. In the small intestine, soluble DF increases the luminal viscosity as well as increases the water binding capacity (WBC) of the digesta, leading to slower movement rate of digesta and glucose transportation rate to enterocyte (Ellis *et al.*, 1995). In the large intestine, and in the colon particular, the residue from undigested nutrients mostly from non-digestible carbohydrate will activate the bacterial fermentation and generate short chain fatty acids (SCFA) (Gråsten *et al.*, 2000).

The interest in research regarding dietary fiber in monogastric animals has been high during the last decades. At the beginning by-products from human food with high dietary fiber content was used as an alternative feed ingredient because they were cheap. Due to increased cost of traditional feed ingredients, there have been many attempts to seek for non-conventional feed sources that will meet the requirement of the animal at an acceptable cost. Many of these non-conventional feed sources also have high dietary fiber content, which creates a need for a good knowledge about effects associated with dietary fiber (de Leeuw *et al.*, 2008). Moreover, the

functional properties associated with dietary fiber such as increased satiety and beneficial effects on gut health have given dietary fiber an important role in mono-gastric nutrition.

Moreover, the pig is used as a biomedical model for energy metabolism and obesity in humans because of the similarity of metabolic functions and cardiovascular system as well as similar proportion and size of internal organs. Furthermore, postnatally both pig and human lack brown fat tissue. Brown fat is considered to regulate energy balance and energy homeostasis (Spurlock and Gabler, 2008).

This Master thesis is aimed to study the metabolic effect of whole grain wheat and rye to get an increased understanding of the mechanism behind the health aspect related to the whole grain.

## Literature review

### Dietary fiber – definition and analytical method

The term “Dietary fiber” was defined by Trowell (1972) as “the proportion of food which is derived from the cellular walls of plants which is digested very poorly in human being”. More recently dietary fiber has been described as non-digestible carbohydrates and lignins which are not degraded in the upper gastrointestinal tract (Topping and Clifton, 2001). In 2009, the definition of dietary fiber was updated by CODEX and states that:

“Dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans”

Dietary fiber are commonly classified as soluble or insoluble in water, as an attempt to chemically group fibers according to their physiological effects (Weickert and Pfeiffer, 2008). However, it appears that there is no single analytical method able to meet the requirements to describe both chemical and nutritional component of dietary fiber in the diet. Analytical methods and advanced techniques are continuously improved. The neutral detergent fiber (NDF) analysis, which was developed by Van Soest (1967), is widely used for analysis of insoluble fiber in animal nutrition. However, soluble fiber, i.e. pectin, gums,  $\beta$ -glucan and mucilage are lost in the NDF-analysis and instead dialysis or organic solvent precipitation methods are applicable (Kay, 1982). Non-starch polysaccharides (NSP) are the total of non-degradable polysaccharides which are not degraded by endogenous enzymes. The enzymatic-chemical analysis estimate the dietary fiber (DF) content based on the monomeric composition of the NSP and can be is further divided into two fractions, the soluble and the insoluble (Bach Knudsen, 2001).

### Physiological properties in relation to fiber source

Soluble fiber includes pectins, gums and  $\beta$ -glucans, and are characterized by their ability to dissolve in water and by its viscous and gel-forming properties. The latter will slow down the rate of digestion in the small intestine which results in decreased absorption of macronutrients. (Khoury *et al.*, 2012).



Soluble fiber is considered to slow down glucose and lipid absorption from the small intestine and may also delay stomach emptying. This leads to a reduction of the postprandial glucose response and has a beneficial effect on insulin sensitivity, which may help control diabetes. Moreover, it also lowers blood cholesterol levels (LDL) by interfering with the absorption of dietary cholesterol (Jenkins *et al.*, 2000). These soluble fiber polysaccharides will be fermented by the microflora in large intestine yielding SCFA that are absorbed and generate energy (Topping, 1991).

Common sources of soluble fiber are: oatmeal, oat cereal, lentils, apples, oranges, pears, oat bran, strawberries, nuts, flaxseeds, beans, dried peas, blueberries, psyllium, cucumbers, celery and carrots (Slavin, 2008).

Insoluble fiber includes cellulose, lignin and hemicellulose and is considered as gut-healthy fiber because of the laxative effect by adding bulk to the stool and appears to decrease intestinal transit time which is linked with laxation benefits and bowel habit (Cummings and Stephen, 2007; Slavin and Lloyd, 2012). As insoluble fiber induces an increased passage rate through the gut, this would result in a decreased digestion and nutrient absorption (Lattimer and Haub, 2010).

Common sources of insoluble fiber are: whole wheat, whole grains, wheat bran, corn bran, seeds, nuts, barley, couscous, brown rice, bulgur, zucchini, celery, broccoli, cabbage, onions, tomatoes, carrots, cucumbers, green beans, dark leafy vegetable, raisins, grapes, fruit and root vegetable skins (Slavin, 2008).

Food processing can either enhance or reduce the fiber content of fruits and vegetables. For example, peeling will decrease the fiber content (Marlett and Cheung, 1997). Heat treatment (e.g., extruding) or baking will increase the fiber content of the product, by concentrating the fiber by removing water or by generating Maillard products (Slavin and Lloyd, 2012).

In the aspect of physiological properties of dietary fiber bulkiness, fermentability and viscosity are important to consider. These factors can contribute to the satiating potential of high dietary fiber diets both in humans and pigs (Wanders *et al.*, 2011; Souza da Silva *et al.*, 2012). The underlying mechanisms associated with the satiating effect of dietary fiber can be related to several different mechanisms. One factor is dilution of the energy density of the diet (Burton-Freeman, 2000). Moreover, the bulkiness increases saliva production, gastric enzyme and pancreatic secretion from increased chewing activity which promote satiation (Slavin and Green, 2007; Benelam, 2009), delays gastric emptying and reduces intestinal transit time (Brownlee, 2011). Furthermore, the production from microbial fermentation in colon which yield SCFA, mainly acetate, propionate, and butyrate which may also influences the feeling of satiety (Darzi *et al.*, 2011). SCFA are able to induce the secretion of satiety-related peptide, for example peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) from entero-endocrine cells (Delzenne and Cani, 2005). Delayed gastric emptying and lower transit time result in increasing nutrient digestion and absorption while appetite is reduced (Sleeth *et al.*, 2010). Additionally, previous studies have found that even if the energy from SCFA is utilized less efficiently than energy from glucose, SCFA can greatly contribute to the energy supply of pigs (Jørgensen *et al.*, 1997; Gerrits *et al.*, 2012).

Novel studies have provided new insights, suggesting that the improvement of insulin sensitivity and, modulation of gut hormones secretion are more related to the consumption of insoluble cereal dietary fiber and whole grains than to consumption of soluble fiber (de Munter *et al.*, 2007; Schulze *et al.*, 2007; Weickert and Pfeiffer, 2008). A cohort study from the US published in 2000, indicated that bran products and whole grain from corn and wheat are the main source of cereal dietary fiber in human diet (McKee and Latner, 2000). These studies reiterated that high cereal dietary fiber consumption was associated with a reduced risk of developing diabetes (de Munter, *et al.*, 2007; Schulze *et al.*, 2007).

### Properties of Whole grain

Foods are considered to be whole grains if all of the kernel components (i.e., bran, germ, and endosperm) are present. Generally, whole grains are characterized by a higher content of DF, bioactive components and other nutritional beneficial compounds when compared with refined products. Basically, whole grains contains approximately 12% of total DF (largely insoluble), and there is a significant correlation between the consumption of cereal DF and whole grain (Schulze *et al.*, 2007). Also, there is evidence showing that whole grain generates an effect on the metabolism by increasing the viscosity, delaying gastric emptying and effectively slowing glucose absorption, which influences the insulin metabolism (Andersson *et al.*, 2009; Hartvigsen *et al.*, 2014).

Table 1. Macronutrient compositions of rye and wheat grains (% of dry matter)\*#

	<b>Whole grain rye flour (100)</b>	<b>Whole grain wheat flour (100)</b>	<b>White wheat flour (66)</b>
<b>Fat</b>	8-13	12-14	13
<b>Protein</b>	2-3	3	1
<b>Starch</b>	56-70	67-70	84
<b>Ash</b>	2	20.5	
<b>Total fiber</b>	15-17	10-13	3
<b>Of which soluble fiber</b>	3-4	1-2	1-2

\*Data in the table was derived from the following references: Andersson *et al.*, 1993; Clydesdale 1994; Hansen *et al.* 2004; Härkönen *et al.* 1997; Lasztity 1998; Nilson *et al.*; 1997; Welch 1995; Vollendoff and Marlet 1991; Åman *et al.* 1997.

#The number in the bracket indicates the extraction rate.

Extraction rate is the proportion of the flour derived from a known quantity of grain.

Extraction rate of 100 is comparable to whole grain flour.

Extraction rate of 66 is equal to 66% of grain is milled in this flour which corresponds to white wheat flour.

The dominant dietary fiber in wheat and rye is arabinoxylan. In whole grains, arabinoxylan and  $\beta$ -glucan are present in both soluble and insoluble form. Several studies have shown beneficial effect of arabinoxylan and  $\beta$ -glucan on reduction of glucose and insulin response compared to

products with low dietary fiber content (Garcia *et al.*, 2007; Wood, 2010). Moreover, dietary fiber products in which the intact kernels are included also have an impact on glycaemia level owing to their physical form, and which slows down the rate of starch hydrolysis in the gut (Hartvigsen *et al.*, 2014).

### Fiber and Satiety

Satiety is the state generated as a result of consumption (Blundell *et al.*, 1996) or the meal initiation (Graaf *et al.*, 2004). The mechanism of postprandial satiety might be illustrated by the function of a gut sensor system which signals from the GI tract to the appetite center in the brain where both neural and endocrine signals are involved. As the food appears in the stomach it stimulates the mechanical and chemical receptors to transmit the signals via the vagus nerve to brain stem (Fraser *et al.*, 1995; Willing and Berthoud, 1997; Mathis *et al.*, 1998).

Satiation is the mechanism which operates while foods are being digested (Blundell *et al.*, 1996) or at the meal termination (Graaf *et al.*, 2004). The termination of meal relies on short-term signals from the distension of stomach and/or on gut hormones (for example, cholecystokinin (CCK) and GLP-1). The sensitivity of these short-term signals is also affected by the long-term signals hormones such as leptin, insulin and ghrelin (McMinn *et al.*, 2000; Wang *et al.*, 2000; Havel, 2001)

The consumption of dietary fiber has been widely accepted to suppress energy intake by promoting satiation and satiety (Blundell and Burley, 1987). Satiety and satiation are part of the appetite control system and are involved in restriction of energy control. Satiety is defined as the feeling of fullness from the previous meal consumption and inhibits eating between meals (Schroeder *et al.*, 2009).

The viscous fiber (i.e., pectin, psyllium and guar gum) effectively increase satiety and influence the satiety even in a small amount. The effect is linked to the effect of viscosity on both food digestion and nutrient absorption, by prolonging the available time to spur pre- and post-absorptive satiety mechanism (Slavin and Green, 2007).

### Glycemic index VS appetite, hunger and satiety

Glycemic index (GI) is a ranking system for carbohydrate based diets which is defined as an indicator of the potential of glycemic carbohydrates in different kinds of food to elevate the level of blood glucose within two hours after consumption. This value is obtained from ingestion of a 50 g carbohydrate portion of a test food, the glucose response of that diet is measured according to the increased areas under the blood glucose response curve (IAUC) (Jenkins *et al.*, 1981).

Table 2. Classification of food according to their glycemic index (GI; adapted from: Brand-Miller *et al.*, 2003a)

<b>Classification</b>	<b>GI range</b>	<b>Examples</b>
<b>Low GI</b>	< 55	Whole grains, pasta, lentils, beans, most fruit and vegetables
<b>Medium GI</b>	56-69	Sucrose, brown rice, basmati rice
<b>High GI</b>	>70	Baked potato, white bread, white rice (e.g. jasmine), corn

The value is then expressed as a percentage of the response to the same amount of carbohydrate from standard carbohydrate, for example white bread. Carbohydrates which are slowly broken down during the digestion have a lower than carbohydrates that are rapidly broken down and constantly release glucose into blood circulation. The GI rating system classifies foods into three categories, low, medium and high GI (Table1; Brand-Miller *et al.*, 2003a).

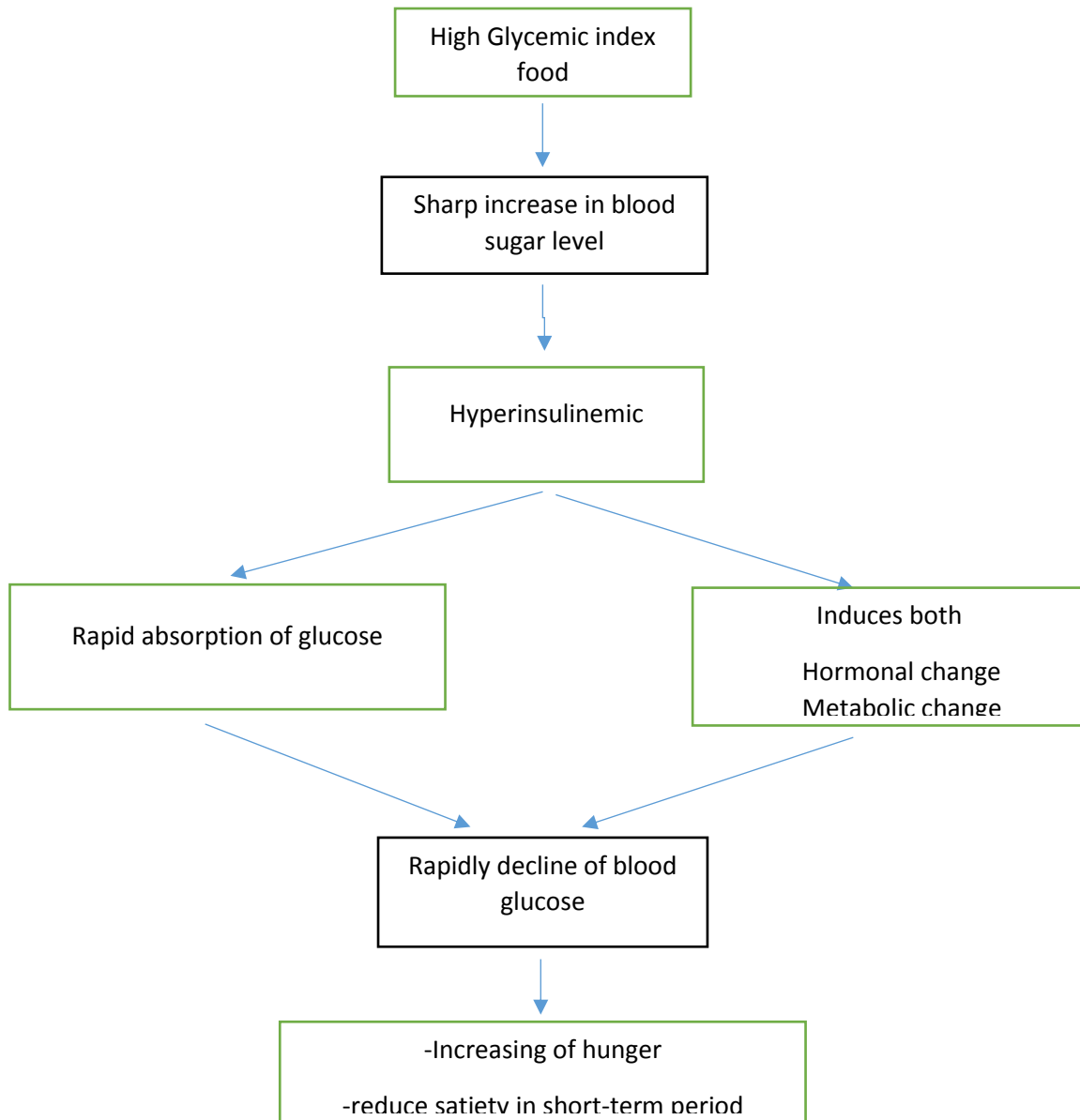


Figure 1. Interaction between high glycaemic index food and feeling of satiety. Modified from Yoshimi *et al.* (2009).

In a short-term human study (1-3 days), objects which received the test meals with high GI raised their food intake during the following meal. On the other hand, the objects that received the low GI had a tendency to prolonged satiety (Ludwig *et al.*, 1999; Ball *et al.*, 2003; Warren *et al.*, 2003). With the consumption of high GI food, glucose will be rapidly absorbed resulting in metabolic and hormonal changes which enhance the food intake (see figure 2). The low GI meal showed a lower insulin response than moderate and high GI meal (Ball *et al.*, 2003)

## Nutrients of Wheat

Wheat (*Triticum aestivum*) is composed of an outer layer of bran and inner layer of endosperm and the germ. The fiber composition in wheat grains are approximately 6.0 % arabinoxylan, 0.6 %  $\beta$ -glucan, 1.8 % cellulose and 1.0 % fructan (Karppinen *et al.*, 2003; Vitaglione *et al.*, 2008; Stone and Morell, 2009).

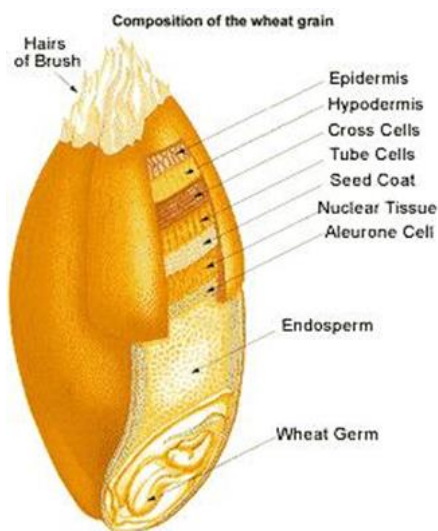


Figure 2. Composition of wheat kernel (source: Wheat and Flour, 2014).

## Nutrients of Rye

When comparing the carbohydrate composition between whole grain rye and whole grain wheat, rye has higher content of fructans, arabinoxylans (AX) and mixed linked  $\beta$  (1-3;1-4)-D-glucans ( $\beta$ -glucans) (Knudsen *et al.*, 2005). The fiber composition in rye grains are approximately 8-10 % arabinoxylan, 1.7-2 %  $\beta$ -glucan, 1.0-1.7 % cellulose and 4.6-6% fructan (Nilsson *et al.*, 1997; Karppinen *et al.*, 2003; Vitaglione *et al.*, 2008; Stone and Morell, 2009). Besides dietary fiber, rye also contains bioactive components such as cinnamic acids, alkylresorcinols, lignans, sterols, vitamins and minerals (Rakha *et al.*, 2009).

## Bioactive compound in cereal grains

Bioactive compounds are extra nutritional substances which generally occur in minute amounts in foods. These substances have beneficial effect to both human and animal health (Kris *et al.*, 2002). The majority of bioactive compounds of whole-grains are mostly located in the bran or

germ fraction while unsaturated fatty acids are mostly found in the germ (Kulawinek *et al.*, 2007).

### Arabinoxylan

Arabinoxylan (AX) is a constituent of hemicellulose which comprise of a xylose backbone with arabinose side chains. In whole grain, AX is considered as a major component of DF which can be found in both endosperm and bran (Lattimer and Haub, 2010). AX generates various effects on the digestive system, for example the undigested AX affect the activities of the enzymes which are produced by the fermenting bacteria and stimulate the probiotic bacteria in the large intestine (Crittenden *et al.*, 2002). Moreover, the SCFA which is the product in the fermentation process of AX in the large intestine also decrease the level of cholesterol-transporting low-density lipoprotein (LDL) produced in the liver (Lopez *et al.*, 1999).

### $\beta$ -glucan

$\beta$ -glucan, a linear polysaccharide of glucose monomer with  $\beta(1\rightarrow4)$  and  $\beta(1\rightarrow3)$  linkages is abundant in the endosperm of cereal grains.  $\beta$ -glucan also has a property like AX, it can be fermented in both small and large intestine by the dwelling bacteria that produce SCFA (Bartłomiel *et al.*, 2012). Earlier studies revealed that  $\beta$ -glucan reduced the occurrence of coronary heart disease due to the decline of lipids and level of LDL in blood serum, as well as reducing the risk of type 2 diabetes mellitus by improving the insulin sensitivity, hence they contribute to control blood glucose levels (Klopfenstein, 1988; Anderson *et al.*, 1990; Liu, 2002; McKeown *et al.*, 2002).

### Alkylresorcinol

Alkylresorcinol (AR) is one of the phytochemical components in both rye and wheat. This phenolic lipid is present in the outer parts of rye and wheat kernels. In rye grains, it can be found (dry basis) at 360-3200  $\mu\text{g/g}$  and in wheat grains at 317-1430  $\mu\text{g/g}$  db (Kulawinek and Kozubek, 2007; Ross *et al.*, 2003c). Consumption of AR is beneficial for their ability to reduce the cholesterol absorption and regulate triglycerol metabolism (Kozubek, 2009). Animal fed rye had a poor feed intake and growth performance compared to animal fed by wheat or maize (Wieringa, 1967). However, the anti-nutritional effect of AR and how AR relates to food intake suppression still remain unclear.

### Fructan

Fructan is  $\beta$ -D-linked polymers of fructose (Karppinen *et al.*, 2003). In cereals, the fructan content is related to the composition of water soluble pentosan. Rye appears to be the cereal with highest fructan content which is 3.6-6.6% of dry weight in wholegrain rye flour (Karppinen *et al.*, 2003; Haskå *et al.*, 2008), compared with 2.2% in triticale (Nardi *et al.*, 2003), and 1.6-2.1% in barley flour (Vieter *et al.*, 1991); Huynh *et al.*, 2008). For wheat, with a genotypic variation of the content of fructan, it has been reported to the range between 0.7 to 2.9% by Huynh *et al.* (2008) and between 0.8 to 1.9% by Andersson *et al.* (2009). Fructan plays a role as dietary fiber and has prebiotic effect by increasing bifidobacteria in the gastrointestinal tract (Meyer and Stasse-Wolthuis, 2009), as well as increasing mineral absorption such as calcium and magnesium (Ritsemá and Smeekens, 2003; Scholz-Ahrens *et al.* 2007) and reduce appetite (Cani *et al.*, 2009).

## Gut hormones and their mechanisms

As food is consumed and moved down to the gastrointestinal tract, satiation is initiated as a result of the gastric distention which communicates with the brain through the vagus nerve. Satiety is induced by hormones, released in response to food components in the stomach and small intestine along with nutrient absorption into blood circulation. As satiation arises within a meal this leads to termination of intake (Isaksson *et al.*, 2008) and both mechanisms are essential in limiting total energy intake (Benelam, 2009).

Cholecystokinin (CCK) is a gut hormone secreted from L cells in the duodenum and jejunum and function on areas of the brain that are involved in appetite regulation (Wren and Bloom, 2007).

Leptin is secreted by white adipose tissue and the concentration of leptin that circulates in plasma is proportional to the fat mass (Murphy and Bloom, 2004). After the administration of leptin to rodents via the peripheral part of the CNS the food intake and body weight is decreased and energy expenditure is increased. Leptin also plays a crucial role during starvation. It has been shown that the absence of leptin has effects in preventing of excess of body and the regulation of appetite (Friedman and Halaas, 1998).

Insulin is secreted from pancreas and will affect the regulation of satiety and satiation in the brain (Wynne *et al.*, 2005). This hormone has a similar role as leptin as an adiposity signal. Insulin is released cephalically at the smell, sight and taste of the food via the vagus nerve (Wood and Bernstein, 1980). Several studies have demonstrated that a slightly increasing insulin level decrease food intake and body weight gain and an impaired insulin release is related to a satiety deficiency (Van derWeele, 1988; 1990; 1994).

Ghrelin is a peptide hormone which is composed of 28 amino acids attached by an acyl side-chain at the serine residue at position 3 (Kojima *et al.*, 1999). This hormone is mainly secreted from the stomach, and function to stimulate appetite and increase food intake (Tschöp *et al.*, 2000). The concentration of ghrelin in blood circulation increases during fasting and rapidly decreases after a meal (Cummings *et al.*, 2001). However, the mechanism behind the release of ghrelin is still unknown, although the caloric intake appears to be the primary factor that which regulate the plasma ghrelin level (Tschöp *et al.*, 2000). It has been suggested that the release of ghrelin might be suppressed by glucose and/or insulin (Yoshihara *et al.*, 2002).

Glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM) are released into blood circulation in response to nutrients in the GI tract (Le Quiellec *et al.*, 1992; Herrman *et al.*, 1995). These hormones are produced by pre-proglucagon gene which can be found in the brain, pancreas and intestine. Glucagon is produced by the pre-proglucagon gene product in the pancreas, whereas, GLP-1 and OXM are produced in intestine and brain respectively (Murphy and Bloom, 2004). GLP-1 receptors are also found in the brain area which is involved in appetite control, and it is assumed that GLP-1 also has an effect on satiety (Yamamoto *et al.*, 2003). Moreover, GLP-1 also slows down the rate of gastric emptying and controls the secretion of gastric acid, resulting in the “ileal break” mechanism of the upper GI tract, these combined effects modulate the food transit from the stomach into intestine (Näslund *et al.*, 1999). With an iso-caloric diet, the level of

satiety and satiation rely on various factors of food composition. Several studies have found that among the dietary components, protein, carbohydrate and fat, protein is the macronutrient which generates the most satiation per calorie (Eisenstein *et al.*, 2002). The capacity of satiation from carbohydrate-rich food depends largely upon energy density and dietary fiber content where both soluble and insoluble fiber increases satiety (Isaksson *et al.*, 2008).

### H-NMR technique

Nuclear Magnetic Resonance (NMR) spectroscopy has been used since 1950s. The H-NMR technique has been used for several years to study the metabolite profiling of nutrients and biochemical effect of food. Proton (H)-NMR spectroscopy provides the detection of all hydrogen-containing molecules in a sample. The NMR technique is widely accepted as an excellent tool for studies on the metabolome in an organism (Lindon *et al.*, 2006).

Metabolomics facilitate make it possible to study metabolic change in humans and animals related to changes in nutrition, genetics, gut microbiota and environment. The H-NMR technique has become increasingly important since it profoundly provide information that may help to understanding various biological processes. The information obtained from NMR can provide a quantitative analysis and metabolic profiles simultaneously. Besides getting a general understanding of the metabolic effect to the diet administration (Bertram *et al.*, 2006), nutritional metabolomics can also illustrate the metabolic response of the host to the nutrients in term of health status, physiology and molecular pathways (Orešič, 2009).

## Material and Method

### Diet, feeding and experimental design

#### Breads

In total three breads were used in the study. Two breads were made of whole grain cereals, namely whole grain rye bread (WGRB), and whole grain wheat bread (WGWB). The third bread were made of white wheat flour (WB). All breads were baked at 225 °C for 35 minutes in a local bakery. The ingredient of breads were flour from respective cereal type, cellulose, rape seed oil, sugar, salt, yeast, casein and vitamin-mineral mixture. Titanium dioxide (TiO<sub>2</sub>) was added as an inert marker for the calculation of digestibility. Dietary ME content was optimized to 12 MJ/kg dry matter.

The pigs were fed 3% of body weight (dry matter basis) per day and the daily portion was divided into two equal portions fed at 09.00 and at 15.00. If any feed residues were left, they were collected and weighted daily at 12.00

#### Animal and housing

The pigs used in this study were from the swine herd of the Swedish Livestock Research Center, SLU. Five 7-weeks-old female growing pigs (Swedish Landrace × Yorkshire) were kept individually in pens (1.5 × 1.8 m), the pigs originated from the same litter. The experiment was



performed as a change-over experiment with five pigs, three diets and three periods. Each experimental period lasted for 7 days. Three of the pigs were surgically fitted with catheters and randomly allocated to the three diets in a 3×3 Latin square design and two pigs were considered as a replicates following the same feeding order as two of the pigs in the Latin square (Table 5). The two non-catheterized pigs were only used for fecal and urinary sample purpose.

Table 3. Ingredients (kg/100 kg) of the experimental diets

Ingredients	Bread		
	WGWB	WGRB	WB
Whole wheat	79.75	0	0
Rye	0	80.25	0
White wheat flour	0	0	70.00
Casein	3.00	6.00	5.00
Vegetable fat	4.00	4.00	4.00
Cellulose	3.50	00	11.25
Sugar	1.50	1.50	1.50
TiO <sub>2</sub>	0.25	0.25	0.25
Yeast	5.00	5.00	5.00
Premix	3.00	3.00	3.00

Table 4. Analyzed chemical composition (g/kg DM) of the breads used as the experimental diets

Nutrient	Bread			Unit
	WB	WGWB	WGRB	
Ash	39	46	49	g/kg DM
CP	92.91	92.96	93.06	g/kg DM
Fat	50	67	63	g/kg DM
DM	65.00	66.69	65.80	g/kg DM
NSP	16.38	12.76	13.49	g/kg DM
<b>Arabinose</b>				
Total	0.62	1.70	2.66	g/kg DM
Insoluble	0.38	1.36	1.70	g/kg DM
<b>Xylose</b>				
Total	2.70	3.32	4.46	g/kg DM
Insoluble	2.35	2.80	2.91	g/kg DM
<b>Uronic acid</b>				
Total	0.28	0.41	0.35	g/kg DM
Insoluble	0.22	0.35	0.27	
<b>Glucose</b>				
Total	11.86	6.38	4.97	g/kg DM
Insoluble	11.74	6.13	4.02	
Klason lignin	0.74	1.31	1.49	g/kg DM
Dietary fiber with fructan	17.25	14.28	16.56	g/kg DM

<b>NDF</b>	10.7	9.3	9.2	g/kg DM
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Table 5. Feeding order of the pigs in different experimental periods.

	<b>Pig number</b>				
	849	850	851	852	856
<b>Period 1</b>	WGRB	WB	WGWB	WB	WGWB
<b>Period 2</b>	WB	WGWB	WGRB	WGWB	WB
<b>Period 3</b>	WGWB	WGRB	WB	WGRB	WGWB

The pigs were adapted to the pen for two weeks and to avoid stressing the pigs, they were adapted to close contact to humans before the surgery. Then the pigs were surgically fitted with the aforementioned chronic indwelling catheter in portal vein (PV), ileal vein (IV) and carotid artery (CA). Pigs were fasted for 16 hours before the surgery and had free access to water. General anesthesia was induced by using an intramuscular (i.m.) combination of Domitor<sup>®</sup> (medetomidine HCL 1 mg/ml; Orion Pharma, Espoo, Finland) and Zoletil<sup>®</sup> (tiletamine hydrochloride 25 mg/ml and zolazepam hydrochloride 25mg/ml, Virbac S.A., Carros, France) at a dose of 0.05 ml/kg. Thereafter, the animals were intubated and kept anaesthetized through inhalation of isofluran plus oxygen before surgery. Analgesia was induced before surgery through epidural injection with buprenorfin (Temgesic<sup>®</sup> 0.3 mg/ml). Aseptic techniques and sterile conditions were applied throughout the whole surgical procedure. The surgical procedure has been described by Rodríguez-López *et al.* (2012).

Pre- and post-operative care included antibiotic treatment (procain penicillin, Penovet<sup>®</sup>) injected once a day starting on the surgical day and then for 5 consecutive days post-surgery. Pain relief was provided by buprenorfin injection for 3 days post-surgery. After the surgery, the pigs were allowed 10 days for recovery before starting the 21 days of experiment (three consecutive experimental weeks). Three days before starting the experiment the straw bedding was removed and replaced with rubber mats. The pigs were weighted every week to calculate the allowance bread in each experimental period.

The entire experiment was conducted at the Center of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU) and was approved by the ethical committee of the Uppsala region.

At the end of the experiment, all pigs were euthanized and necropsy was performed to evaluate the effect on the tissues surrounding the catheters.

### Sample collection

*Blood:* Blood samples were collected from the CA and PV at the same time starting just before feeding the pigs (0). Then the samples were collected at 15, 45, 60, 90, 120, 180 and 240 min

post start of feeding. A total of 4 ml blood was collected at each collection time from the CA and PV catheter, respectively. Two ml of the blood sample were collected in EDTA vacutainers and 2 ml in heparinized vacutainers. Serum insulin was analyzed by radioimmunoassay (Phadaseph Insulin RIA 100: Phamacia Diagnostica, Uppsala, Sweden). Plasma was separated from whole blood sample by centrifugation at 1000×g and kept in 1 ml aliquots at -80°C for NMR analysis and further biochemical analysis.

*Feces*: Feces samples were collected immediately after defecation on day 4-7 of the experimental period and kept in sterile plastic bags and stored in -20 °C. The samples were pooled for each pig and period.

*Urine*: Spot urine samples for metabolomics analysis was collected twice a week on day 4 and day 6 in each experimental period.

### Sample Analyses

Feed and fecal samples were thawed at room temperature, homogenized and freeze-dried and milled through a 1-mm sieve before analysis. Samples were analyzed for dry matter (DM) by drying at 103°C for 16 h and for ash after ignition at 600°C for 3 h. Crude protein (CP; N × 6.25) was determined by the Kjeldahl method according to the Nordic Committee on Feed Analysis (2003) using a Kjeltec Auto 1030 analyzer (Tecator, Höganäs, Sweden). Crude fat was determined according to European Communities (1984) using a hydrolyzing unit (Soxtec System 1047 Hydrolysing Unit, Tecator AB) and an extraction unit (Soxtec System HT6, Tecator AB). Total and insoluble NSPs and their constituent sugars, Klason lignin and total dietary fiber were determined using a modified Uppsala method (Bach Knudsen, 1997). TiO<sub>2</sub> concentration was analyzed according to Short *et al.* (1996). Fructan content was determined according to Association of Official Analytical Chemists with a Fructan Assay Kit (Megazyme Cat. no. K-FRUC, Bray County, Ireland).

*pH measurement*: Five grams of pooled fecal sample were homogenized in 10 ml of deionized water and measured and pH with a Metrohm 654 pH meter.

### NMR measurements

Fecal and urinary samples were analyzed with H-NMR to measure the impact of the diet on metabolic profiles of the three diets. Prior to measurements, frozen urine samples from each pig were thawed at room temperature and 500 µl urine was mixed with 500 µl phosphate buffer including the reference substance TSP (Trimethylsilyl propanoic acid) . TSP functions as an internal standard and reference for the chemical shift but can also be used for quantitative analyses. For analysis of fecal metabolic profiles 0.1g of stool was mixed 0.9 ml phosphate buffer and the mixture was thoroughly homogenized. After vortexing for 2 minutes, samples were centrifuged at 17800g for 10 minutes and the supernatant was subsequently collected and filtrated through steroid filter (Filtropur<sup>®</sup>, 2 µm pore size) to remove free particles from the samples.

The H-NMR measurement was performed on Bruker spectrometer operated at 600 MHz (Karlsruhe, Germany). The NMR spectral data were processed through Bruker Topspin 1.3

software. Baseline and spectral phase were corrected manually corrected and residue water was removed. Then, each spectral region was normalized to the intensity of internal standard (TSP).

## Calculation and Statistical Analyses

The fecal digestibility was calculated by using the indicator (TiO<sub>2</sub>) technique according to the following equation (Wilfart *et al.*, 2007):

$$\text{Apparent digestibility} = 1 - \left\{ \frac{(\text{TiD} \times \text{DCF})}{(\text{DCD} \times \text{TiF})} \right\}$$

Where DCD and DCF are energy concentration in the diet and the feces, respectively; and TiD and TiF represent the titanium dioxide concentration of the diet and the feces, respectively.

Statistical analysis was performed by the Statistical Analysis System package version 9.3 (SAS Institute, Cary, NC, USA) by using the MIXED procedure. A model including the fixed effect of the diet (WB v. WGWB v. WGRB), the effect of period (period I v. period II v. period III) and the random effect of pig was applied. The results are showed as least square means  $\pm$  SEM. Significant differences are considered at  $p < 0.05$ .

Principal component analysis (PCA) was applied to elucidate the effects of three diets on metabolic profiles. PCA was analyzed using the SIMCA-P+ 12.01 software (UMERTRICS, Umeå, Sweden) on log-transformed and centered data.

## Results

All 5 pigs completed the experiment according to the protocol. The pathological evaluation showed traces of inflammation around the portal vein and some nodules on the liver surface.

### *Digestibility*

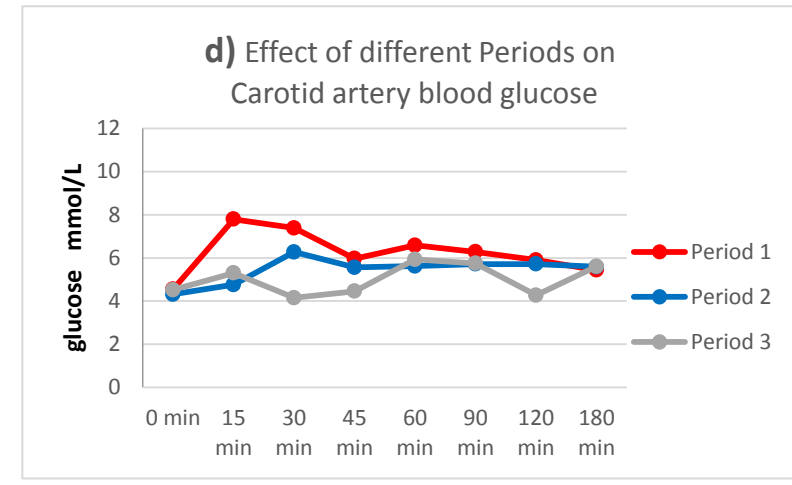
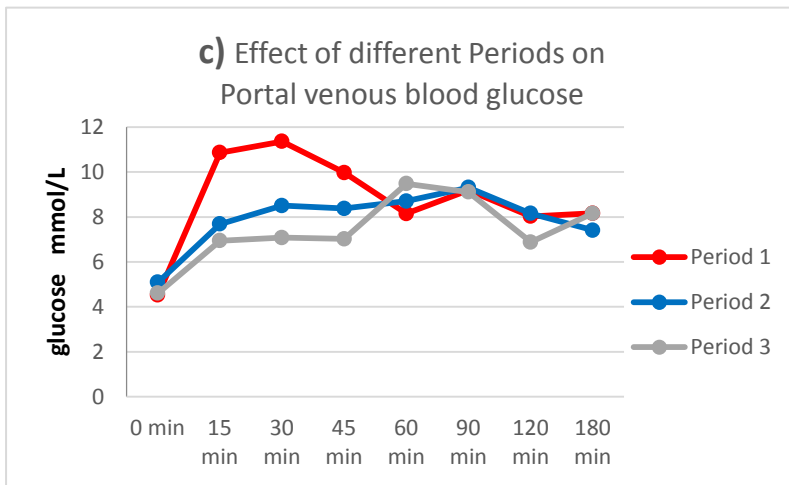
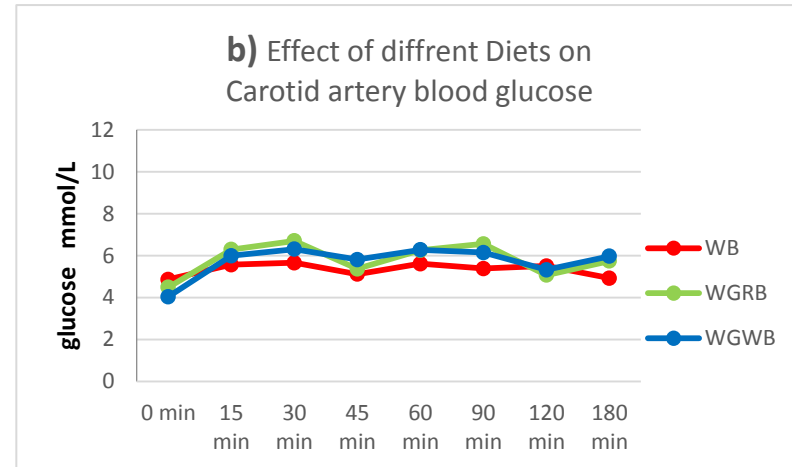
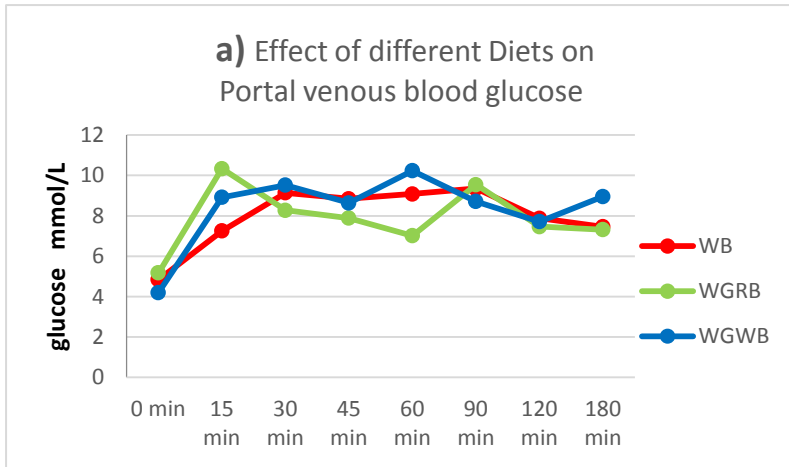
The effects of different diets on the total tract apparent digestibility are shown in Table 6.

**Table 6. Effect of the diet on the CTTAD<sup>1</sup> of dietary components. Least square means  $\pm$  standard error (SEM)**

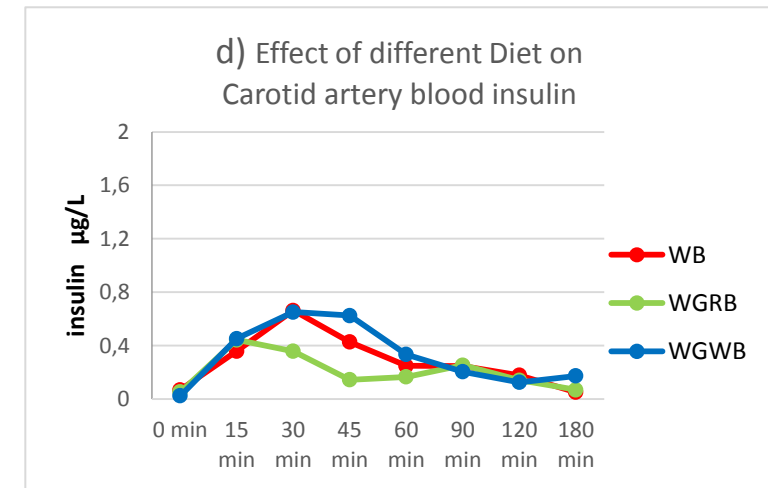
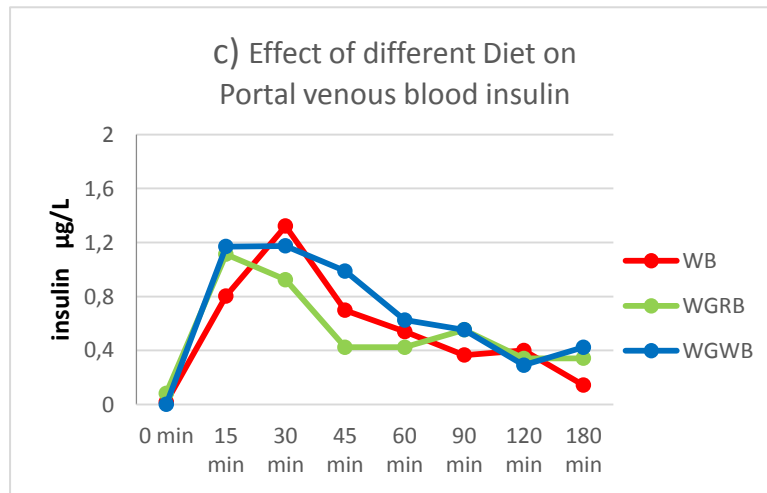
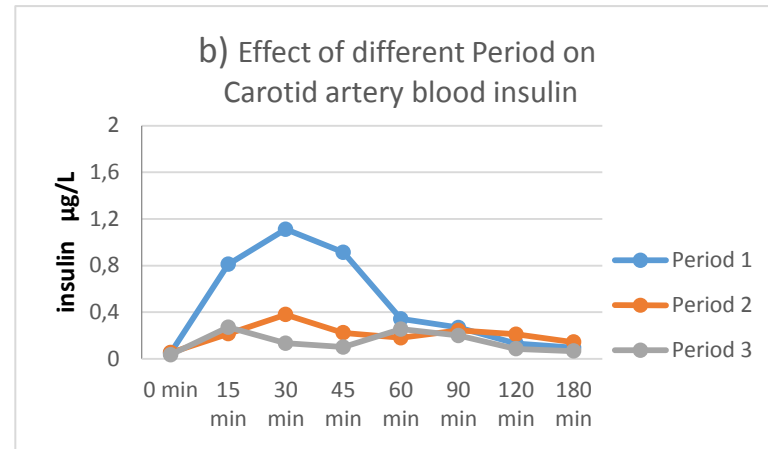
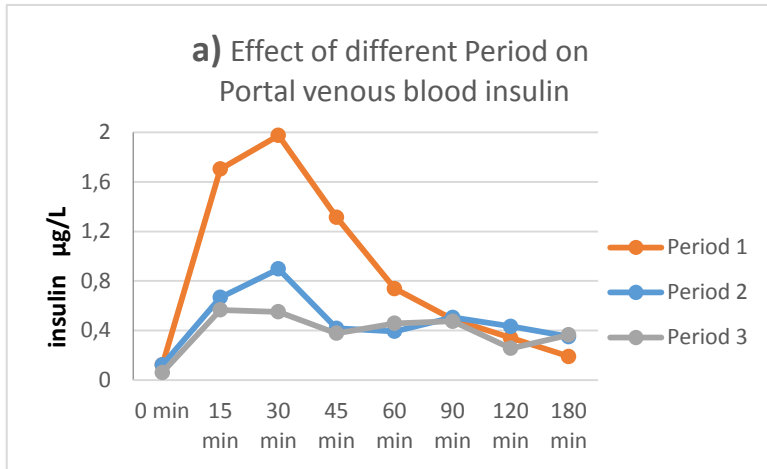
	Diets			SEM	P-value
	WB	WGRB	WGWB		
OM <sup>2</sup>	0.87	0.89	0.88	0.006	0.4314
Total NSP <sup>3</sup>	0.57	0.67	0.51	0.063	0.2956
Total arabinose	0.72	0.60	0.53	0.087	0.3615
Total xylose	0.53	0.67	0.63	0.052	0.2466
Total uronic acid	0.32	0.37	0.46	0.879	0.5357
Total dietary fiber	0.55	0.63	0.50	0.049	0.2720
Glucose	0.62	0.68	0.59	0.098	0.8231

<sup>1</sup>CTTAD = coefficient of the total tract apparent digestibility; <sup>2</sup>OM = organic matter; <sup>3</sup>NSP = non-starch polysaccharide

There was no significant difference between the diets ( $p>0.05$ ). However, WGBR had a numerically higher CTTAD of NSP, xylose, dietary fiber and glucose than WB and WGWB. The CCTAD of arabinose was numerically higher in pigs fed WB than pigs fed WGRB and WGWB. Pigs fed WGWB had a numerically higher CTTAD of uronic acid than pigs fed WB and WGRB.



**Figure 3. Postprandial plasma glucose responses; to (a) portal venous blood glucose-diet effect, (b) carotid artery blood glucose- diet effect, (c) portal venous blood glucose-period effect, and (d) carotid artery blood glucose- period effect ( -WB : refined wheat bread; -WGRB: whole grain rye bread; - WGWB; whole grain wheat bread). All data were present as means  $\pm$  SEM., n=3. There was no significant time  $\times$  treatment (diet) for the plasma glucose response in the portal vein or in the carotid artery.**



**Figure 4. Postprandial plasma insulin responses; to (a) portal venous blood insulin-diet effect, (b) carotid artery blood insulin-diet effect, (c) portal venous blood insulin-period effect, and (d) carotid artery blood insulin- period effect ( - WB : refined wheat bread; - WGRB: whole grain rye bread; - WGWB; whole grain wheat bread). All data were present as means  $\pm$  SEM, n=3. There was no significant time  $\times$  treatment (diet) for the plasma insulin response in the portal vein or in the carotid artery.**

## Postprandial glucose and insulin response

The effect of diet on plasma glucose and insulin concentration in the carotid artery and portal vein is shown in Figure 3 and 4. Neither the glucose nor the insulin concentration level in the portal vein or carotid artery differed between pigs fed different diets ( $P > 0.005$ ) at any time. However, for both glucose and insulin a period effect was observed both in the portal vein and carotid artery with higher values at period I compared to period II and III.

At 30 min, pigs fed WB had numerically higher insulin concentration level than pigs fed WGWB and WGRB. At 30, 45 and 60 min pigs fed WGRB had a numerically lower insulin concentration compared to WB and WGWB. At 45 min, WGWB had a numerically lower level of insulin and kept this constant until at 60 min where after then it slightly increased at 90 min.

**Table 7. NMR data for SCFA, monosaccharides, disaccharides and ethanol in fecal sample. Least square means  $\pm$  standard error (SEM)**

Metabolites	Diet			SEM	P-Value
	WB	WGWB	WGRB		
<i>SCFA (mmol/L)</i>					
Acetate	58.02	68.37	57.24	7.840	0.4553
Propionate	25.22	30.39	29.29	4.809	0.7344
Butyrate	14.85	16.87	15.26	2.571	0.8380
Lactate	5.39 <sup>b</sup>	5.86 <sup>ab</sup>	7.75 <sup>a</sup>	0.585	0.0428
<i>SCFA mol (%)</i>					
Acetate	56.46 <sup>b</sup>	56.10 <sup>b</sup>	50.96 <sup>a</sup>	1.318	0.0315
Propionate	23.70	25.06	27.02	1.598	0.3809
Butyrate	14.54	13.35	14.11	1.026	0.5759
Lactate	5.30	5.49	7.90	1.142	0.2575
<i>Monosaccharides (mmol/L)</i>					
Fucose	1.1643	0.9317	0.4509	0.2079	0.0647
Galactose	0.7839	0.6691	0.7881	0.1501	0.8207
Glucose	4.4658 <sup>a</sup>	2.7569 <sup>b</sup>	2.6373 <sup>b</sup>	0.5629	0.0361
Xylose	4.0135 <sup>a</sup>	2.1664 <sup>b</sup>	1.4577 <sup>b</sup>	0.4869	0.0106
<i>Disaccharides (mmol/L)</i>					
Maltose	0.2141	0.5906	0.1525	0.2768	0.5097
Sucrose	0.2247	0.2765	0.1348	0.0714	0.4074
Ethanol	0.7218	0.4981	0.7719	0.1182	0.2086
pH	6.28	6.63	6.51	0.1452	0.3638



## Effect on metabolites in fecal sample

In fecal samples, 31 metabolites were identified in the NMR analysis. Their content was quantified using TSP as the internal standard and it was statistically evaluated if the metabolites were influenced by the diet type. Acetate was the most abundant metabolite in all samples. The dietary effect on SCFA, mono- and disaccharides are shown in Table 7. Lactate had the highest concentration ( $p < 0.05$ ) in pigs fed WGRB compared with pigs fed WB and WGWB (7.75, 5.86 and 5.39 mmol/L respectively). However, comparison of the molar proportion of four SCFA (acetate, propionate, butyrate and lactate) showed that acetate was lower for WGRB than for WB and WGWB. Glucose and xylose were higher for WB than for WGRB and WGWB ( $p < 0.05$ ). However, no significant differences in fucose, galactose, maltose, sucrose, ethanol and pH were observed between three different diets.

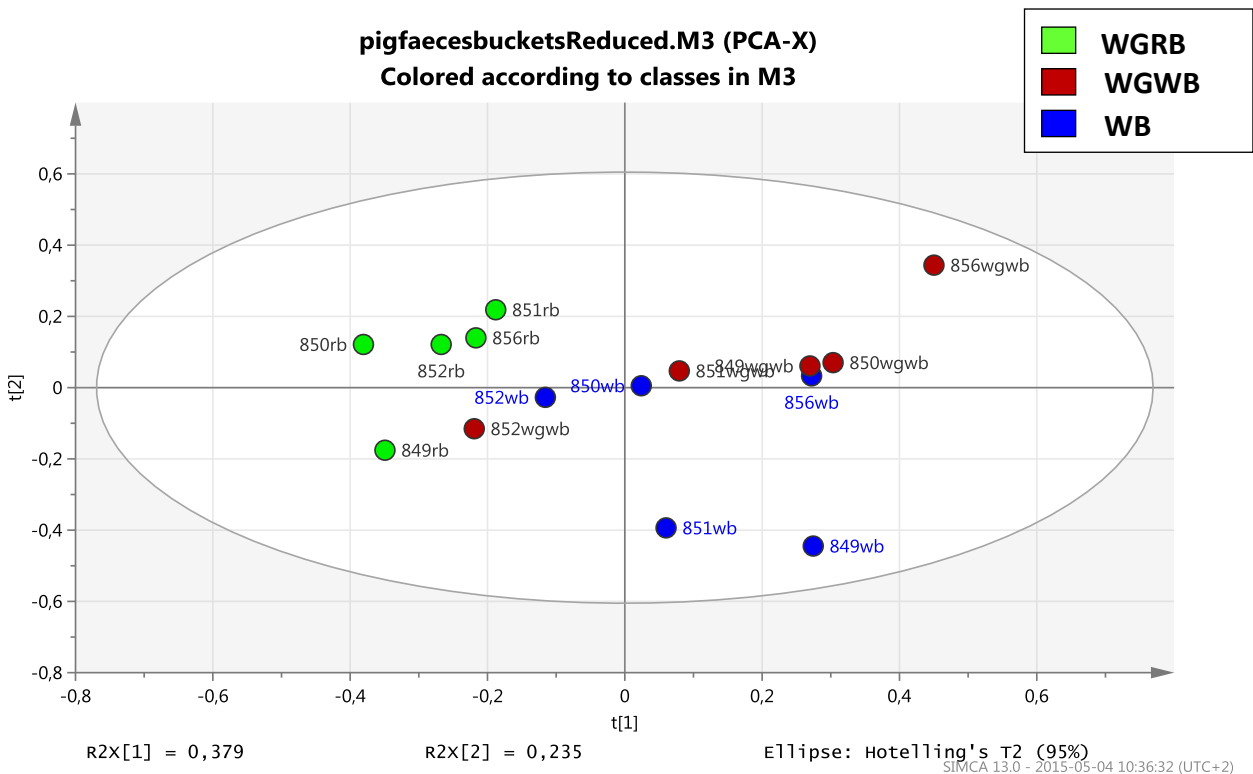


Figure 5. The results from faeces metabolic profile. WB: refined wheat bread; WGWB: whole grain wheat bread; WGRB: whole grain rye bread.

*Fecal samples:* PCA was performed on the pre-processed H-NMR spectra. The result plot for centred- data is shown in Figure 5 where the metabolic profiles were represented by a single data point. WGRB showed a clear tendency to clustering differently than WGWB and WB.

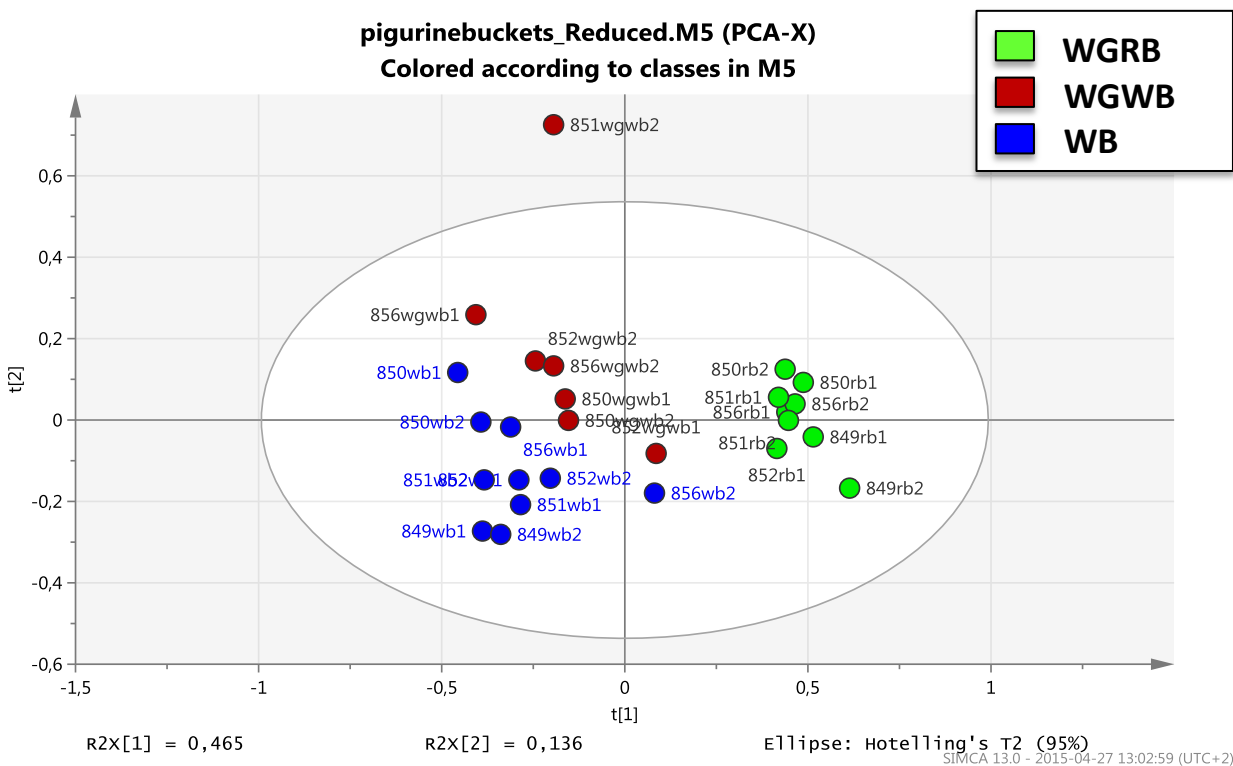


Figure 6. The results from urine metabolic profile WB: refined wheat bread; WGWB: whole grain wheat bread; WGRB: whole grain rye bread.

*Urinary samples:* PCA was performed on the pre-processed H-NMR spectra. The result plot for centred- data is shown in Figure 6 and illustrates that diet had a clear effect on the urine metabolic profiles and in particular for the WGRB diet.

## Discussion

The present study was conducted to investigate the effect of whole grain wheat and rye- bread compared to a refined wheat bread on glucose and insulin response in catheterized pigs and to elucidate the metabolic changes from a diet rich in whole grain by using  $^1\text{H}$  NMR on urine and fecal samples. The present study focused on a specific multi-catheters pig model and it was the first time that this model was used in Sweden. The choice of, the study design result in limitations in the number of pigs that could be used due to ethical consideration.

Insulin is secreted from the pancreas and absorbed into the portal vein. Insulin and glucose are partially metabolized in the liver before reaching to the carotid artery. Their concentrations are therefore different in the carotid artery than in the portal vein. In addition, glucose is absorbed in the GI tract. (Farmer *et al.*, 1998). Fasting blood glucose level did not significantly differ between the diets. The portal glucose concentrations immediately rose after feeding. Previous studies have shown that the time of glucose peak is 46-60 minutes (Freckman *et al.*, 2007; Theil *et al.*, 2011) from the start of feeding session. The rapid increase of portal blood glucose in present study after fasting for 12 hr indicates rapid starch hydrolysis of the diet and the response of insulin is due to the metabolism change from catabolism to anabolism stage (Anderson, 1974).

The results on blood glucose level did not show a clear effect of WB, WGRB and WGWB which is in disagreement with other studies (Hartvigsen *et al.*, 2014; Forsberg *et al.*, 1985) in which the level of glucose response from WGRB were significantly different from WB and WGWB. The unexpected effect of period has of course contributed to the lack of diet effect in the present study. We speculate that the lower response of blood glucose and insulin level from period II and period III can be due to the effect of heparin as the anticoagulant. Heparin was used to flush the catheters during the blood collection procedure. The effect of heparin can be explained by the polyanionic nature of heparin and might interfere with the interaction of antigen-antibody in the analysis (Iglesias *et al.*, 1985). A lower insulin level when heparin was used has previously been reported in a human experiment (Soeldner and Slone, 1965).

Numerically, WB and WGWB had lower blood glucose concentration than WGRB, which could indicate an effect of modified cellulose. To balance the level of dietary fiber of the experimental diet, cellulose was added to the WGWB (35g/kg and WB (112.5g/kg). Studies in pigs (Low *et al.*, 1985) and rats (Takahashi *et al.*, 2005) have shown the ability of modified cellulose to lower the blood glucose level.

The insulinaemic response from our study showed no significant differences between the diets. However, pigs fed by WGRB had numerically a lower insulin concentration compared to WGWB and WB. Again, the unexpected period effect explains the lack of diet effect and previous studies have shown that rye has the ability to lower glucose (Hartvigsen *et al.*, 2014) and insulinaemic response (Juntanen *et al.*, 2003; Rosén *et al.*, 2009). The observed effects of rye may be the result from the fiber property in whole grain, the outer part of kernel protects the starch in the endosperm from hydrolytic enzymes (Juntanen *et al.*, 2002). Moreover, arabinoxylan and in particular the soluble part in rye hinder amylolysis Lu *et al.* (2000), due to increased digesta viscosity (Lattimer and Haub, 2010), leading to a less glucose being absorbed into the blood circulation (Rosén *et al.*, 2009). Furthermore, the production of SCFA such as

propionate and butyrate and other bioactive compound can influence the insulinaemic response (Brockman, 1982; Haslam, 1989; Faulds and Williamson, 1999). Therefore, the former structure and the bioactive compounds of rye have a more important role in attenuating insulinaemic response, whereas the amount of dietary fiber is less important.

Non-digested dietary fiber in the colon provides substrate for the fermentation process of micro-organism that resides in the colon (Hudson and Marsh, 1995). About 60% of the energy produced from fermentation of fiber is constrained in SCFAs which are absorbed then taken up as energy in the colon (Isaksson *et al.*, 2013). The primary SCFAs are acetate, propionate, butyrate and lactate. Among the three diets in the present study, the production of acetate was significantly higher in the wheat-based diets ( $p < 0.05$ ). The difference of the fermentation pattern may be explained by the type of carbohydrates present in the different cereals which affect the metabolism of intestinal microflora contributing to SCFA production (Bach Knudsen *et al.*, 2005). In wheat, the major carbohydrate that enters the large intestine is cellulose, which stimulates cellulolytic bacteria and the acetate production (Giusi-Perier *et al.*, 1989) whereas in rye the major carbohydrate is arabinoxylan which previous studies have shown to stimulate the production of butyrate (Bach Knudsen *et al.*, 1993). However, no effects on butyrate were observed in the present study.

Numerically, the total fecal SCFA concentration was lowest in the WB diet. This may be a result of modified cellulose which was highest in the WB diet. The WB diet also had the numerically lowest dietary fiber digestibility, indicating a lower fermentability. The results are in agreement with Topping *et al.* (1988) who studied the effect of level of dietary fiber by increasing the level of cellulose from 50 to 100 g/kg feed, and found a lowered SCFA production with increasing cellulose level.

The data of fecal NMR quantification revealed that the molar ratio of xylose and glucose from WB was significantly higher than WGWB and WGRB. This may be explained by higher digestibility of xylose and glucose of the whole grain than the modified cellulose added in the WB. The digestibility was not significantly different between diets, due to high variation, but numerically, diet WB had about ten percent digestibility coefficient of xylose and glucose lower than WGRB and WGWB.

The principal component analysis results from urine and fecal metabolic profiles showed a clear clustering according to the diets in the urine samples where the rye bread was separated from the wheat based diets. A similar but not as clear tendency was observed on fecal samples. This indicates that there is a significant effect of rye on the metabolic profile. Similar observations have been reported by Bertram *et al.* (2006) who studied the biochemical effect of whole grain rye and non-whole grain wheat bread.

In conclusion, the present study revealed that by using H-NMR analysis distinct clustering pattern among different diets could be observed. The effects was clearer on the urine compared to fecal samples. The urine metabolic profile from rye bread had distinct clustering pattern from wheat-based bread. Quantification of fecal metabolites showed that pigs fed on wheat-based diets had a significantly higher acetate concentration than pigs fed on the rye diet. The analysis

of plasma insulin and plasma glucose did not show any clear effect related to diet but an unexpected period effect was observed, which can explain the lack of diet effect. The period effect needs to be sorted out before further studies are performed. This study shows that wheat and rye generate different metabolic profiles. However, to get a deeper understanding of possible mechanisms behind the different profiles and how this can be linked to health issues will require further studies with more pigs and longer experimental periods.

## Acknowledgements

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## References

- Anderson, D.M. (1974). The effect of feeding on the concentration of glucose and insulin in the portal and insulin in the portal and arterial plasma in pigs. *Journal of Agricultural Science (Camb)* 82, 29-36.
- Anderson, J., Deakins, D.A., Floore, T.L., Smith, B.M. & Whitis, S.E. (1990). Whole grains and coronary heart disease. *Critical Reviews in Food Science and Nutrition* 29(2), 95-147.
- Andersson, U., Rosén, L., Tech, M., Östman, E., Ström, K., Wierup, N., Björck, I., & Holm, C. (2010). Metabolic effects of whole grain wheat and whole grain rye in the C57BL/6J mouse. *Nutrition* 26(2), 230-239.
- Andersson, A.A.M., Andersson, R., Piironen, V., Lampi, A.M., Nyström, L., Boros, D., Fras, A., Gebruers, K., Courtin, C.M., Delcour, J.A., Rakszegi, M., Bedő, Z., Ward, J.J., Shewry, P.R., & Åman, P. (2013). Content of dietary fiber components and their relation to associated bioactive components in whole grain wheat samples from the HEALTHGARIN diversity screen. *Food Chemistry* 136, 1243-1248.
- Åman, P., Nilsson, M. & Andersson, R. (1997). Positive health effect of rye. *Cereal Foods World* 42, 684-688.
- Bach Knudsen, K.E., Jensen, B.B. & Hansen, I. (1993). Oat bran but not oat gum enhance butyrate production in the large intestine of pigs. *Journal of Nutrition* 123, 1235-1247.
- Bach Knudsen, K.E., Johansen, H.N., & Glitsø, V. (1997). Rye dietary fiber and fermentation in colon. *Cereal Food World* 42, 690-694.
- Bach Knudsen, K.E. (2001). The nutritional significance of “dietary fiber” analysis. *Animal Feed and Science and Technology* 90, 3-20.
- Bach Knudsen, K.E.B., Serena, A., Kjær, A.K.B., Jørgensen, H., & Engberg, R. (2005). Rye bread enhance the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. *Journal of Nutrition* 135(7), 1696-17-4.
- Bake info (2014) <http://www.bakeinfo.co.nz/Facts/Wheat-Milling/Wheat/Wheat-grain>
- Ball, S.D., Keller, K.R., Moyer-Mileur, L.J., Ding, Y.W., Donaldson, D. & Jackson, W.D. (2003). Prolongation of satiety after low versus moderately high glycemic index meals in obese adolescents. *Pediatrics* 111, 488-494.
- Bartłomiej, S., Justyna, R-K., & Ewa, N. (2012). Bioactive compound in cereal grains-occurrence. Structure technological significance and nutritional benefits—a review. *Food Science and Technology International* 18(6), 559-568.
- Bertram, H.C., Bach Knudsen, K.E., Serena, A., Malmendal, A. Nielsen, N.C., Fretté, X.C. & Andersen, H.J. (2006). NMR-based metabolomics studies reveal changes in the

- biochemical profile of plasma and urine pigs fed high-fiber rye bread. *British Journal of Nutrition* 95, 955-962.
- Benelam, B. (2009). Satiation, satiety and their effects on eating behavior. *Nutrition Bulletin* 34, 126-173.
- Bjorck, I., Ostman, I. & Nilsson, A. (2007). Modulating glycemic with cereal products. In: Whole grains & health. pp. 177-184. Chichester. United Kingdom: Blackwell Publisher.
- Blundell, J.E. & Burley, V.J. (1987). Satiation, satiety and the action of fiber on food intake. *International Journal of Obesity* 11, 9-25.
- Blundell, J.E., Lawton, C.L., Cotton, J.R., & Macdiarmid, J.I. (1996). Control of human appetite: Implications for the intake of Dietary fat. *Annual Review of Nutrition* 16, 285-319.
- Brand-Miller, J., Wolever, T.M., Foster-Powell, K. & Colagiuri, S. (2003a). The new glucose revolution. Marlowe & Company: New York.
- Brownlee, I.A. (2011). The physiological roles of dietary fiber. *Food Hydrocolloids* 25, 238-250.
- Brockman, R.P.(1982). Insulin and glucose responses in plasma to intraportal infusions of propionate and butyrate in sheep (*Ovis aries*). *Comparative Biochemistry and Physiology* 73, 237-238.
- Burton-Freeman, B. (2000). Dietary fiber and energy regulation. *Journal of Nutrition* 130, 272s-5s.
- Bolhuis, J.E., van den Brand, H., Bartels, A.C., Oostindjer, M., van den Borne, J.J.G.C., & Kemp, B. (2010). Effect of fermentable starch on behavioral of growing pigs in barren or enriched housing. *Applied Animal Behavior Science* 123, 77-86.
- Cani, P.D., Lecourt, E., Dewulf, E.M., Sohet, F.M., Pachikian, B.D., Naslain, D. De Backer, F., Neyrinck, A.M., Delzenne, N.M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *American Journal of Clinical Nutrition* 90, 1236-1243.
- Cees de Graaf, Blom, W.A.M., Smeets, P.A.M., Stafleu, A. & Hendriks, H.F.J. (2004). Biomarkers of satiety and satiety. *American Journal of Clinical Nutrition* 79(6), 949-961.
- Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M., Fagerstorm, R., & Matto, J. (2002). In vitro fermentation of cereal dietary fiber carbohydrates by probiotic and intestinal bacteria. *Journal of the Science of Food and Agriculture* 82(8), 781-789.
- Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E. & Weigle, D.S. (2001). A pre-prandial rise in plasma ghrelin levels suggests a role in meal initiation in human. *Diabetes* 50, 1714-1719.



- Cummings, J.H. & Stephen, A.M. (2007). Carbohydrate terminology and classification. *European Journal of Clinical Nutrition* 61, 5-18.
- Dannis, A.V. (1994). Insulin is a prandial satiety hormone. *Physiology and Behavior* 56(3), 619-622.
- Darzi, J., Frost, G.S. & Robertson, M.D. (2011). Postgraduate Symposium. Do SCFA have a role in appetite regulation? *Proceedings of the Nutrition Society* 70, 119-128.
- Delzenne, N.M. & Cani, P.D. (2005). A place for dietary fiber in management of the metabolic syndrome. *Current Opinion in Clinical Nutrition and Metabolic Care* 8, 636-640.
- Dunn, C.J. & Faulds, D. (2000). Nateglinide. *Drugs* 60, 607-617.
- Eisenstein, J., Roberts, S.B., Dallas, G., & Saltzman, E. (2002). High-protein weight-loss diets: are they safe and do they work? A review of the experiment and epidemiologic data. *Nutrition Review* 60, 189-200.
- Ellis, P.R., Roberts, F.G., Low, A.G., & Morgan, L.M. (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. *British Journal of Nutrition* 74, 539-556.
- Farmer, C., Roy, N., Rushen, J. & Robert, S. (1998). Feeding motivation in swine: Relation with insulin, glucose and free fatty acids in portal and jugular blood, and involvement of cholecystokinin. *Canadian Journal of Animal Science* 98, 730
- Faulds, C.B. & Williamsom, G. (1999). The role of hydroxycinnamates in the plant cell wall; *Review. Journal of the Science of Food and Agriculture* 79, 393-395.
- Fraser, K.A., Raizada, E. & Davidson, J.S. (1995). Oral-pharyngeal-esophageal and gastric cues contribute to meal-induced c-fos expression. *American Journal of Physiology* 268, R223-R230.
- Freckmann, G., Hagenlocher, S., Baumstark, A., Jandrike, N., Gillen, R.C., Rössner, K. & Haug, C. (2007). Continuous glucose profiles in human healthy subjects under everyday life condition and after different meal. *Journal of Diabetes Sciences and Technology* 1(5), 695-703.
- Friedman, J.M. & Halaas, J.L. (1998). Leptin and the regulation of body weight in mammals. *Nature* 395, 763-770.
- Forsberg, N.E., Baldwin, R.L. & Smoth, N.E. (1985a). Roles of glucose and its interactions with acetate in maintenance and biosynthesis in bovine mammary tissue. *Journal of Dairy Science* 68, 2544-2549.
- Jenkins, D.J., Wolever, T.M., Taylor, R.H., Barker, H., Fielden, H., Baldwin, J.M., Bowling, A.C., Newman, H.C. & Jenkins, A.L. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal Clinical of Nutrition* 34, 362-366.

- Jenkins. D.J., Kendall. C.W., Axelxen. M., Augustin. L.S. & Vuksan. V. (2000). Viscous and non-viscous fibers, non-absorbable and low glycemic index carbohydrate, blood lipids and coronary heart disease. *Current Opinion in Lipidology* 11, 49-56.
- Jennische, P & Larsson, K. (1990). *Traditionella svenska analysmetoder för foder och växtmaterial*. Uppsala. Sweden: Staten Lantbrukskemiska.
- Jørgensen, H., Larsen, T., Zhao, X.Q. & Eggum. B.O. (1997). The energy value of short-chain fatty acids infused into caecum of pigs. *British Journal of Nutrition* 77, 745-756.
- Juntunen. K.S., Niskanen. L.K., Liukkonen. K.H., Poutanen, K.S., Holst. J.J. & Mykkänen. M. (2002). Postprandial glucose, insulin, and incretin responses to grain product in healthy subjects. *American Journal of Clinical Nutrition* 75, 254-262.
- Juntanen. K.S., Laaksonen. D.E. & Autio. K. (2003). Structural differences between rye and wheat breads but not total total fiber content may explain the lower postprandial insulin responses to rye bread. *American Journal of Clinical Nutrition* 91, 90-97.
- Juntanen. K.S., Laaksonen. D.E., Poutanen. K.S., Niskanen. L.K. & Mykkänen. H.M. (2003). High-fiber rye bread and insulin secretion and sensitivity in healthy postmenopausal women. *American Journal of Clinical Nutrition* 77, 385-391.
- Garcia, A.L., Otto, B., Reich, S.C., Weickert, M, O., Steiniger, J., & Machowetz, A. (2007). Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance. *European Journal of Clinical Nutrition* 61, 334-341.
- Gaesser, G.A. (2007). Carbohydrate quantity and quality in relation to body mass index. *Journal of the American Dietetic Association* 107, 1768-1780.
- Gerrits, W.J.J., Bosch, M.W. & van den Borne, J.J.G.C. (2012). Quantifying resistant starch using novelin vivo methodology and the energetic utilization of fermented starch in pigs. *Journal of Nutrition* 142, 238-244.
- Giusi-Perier, A., Fiszlelewicz, M. & Rérat, A. (1989). Influence of diet composition on intestinal volatile fatty acids and nutrient absorption in unanesthezied pigs. *Journal of Animal Science* 67, 386-402.
- Gråsten, S., Juntunen, K.S., Poutanen, K.S., Gylling, H.K., Miettinen, T.A. & Mykkänen, H.M. (2000). Rye bread improves bowel function and decreases the concentrations of some compounds that are putative colon cancer markers in middle-aged women and men. *Journal of Nutrition* 130, 2215-2221.
- Hartvigsen, M.L., Gregersen, S., Lærke, H.N., Holst, J.J., Bach Knudsen, K.E. & Hermansen, K. (2014). Effect of concentrated arabinoxylan and  $\beta$ -glucan compared with refined wheat and whole grain rye on glucose and appetite in subject with metabolic syndrome: a randomized study. *European Journal of Clinical Nutrition* 68, 84-90.

- Haskå, L., Nyman, M. & Andersson, R. (2008). Distribution and characterization of Fructan in wheat milling fraction. *Journal of Cereal Science* 48, 768-774.
- Haslam, E. (1989). Plant polyphenols. Vegetable tannins. Cambridge, United Kingdom: Cambridge University Press.
- Havel, P.L. (2001). Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. *Experimental Biology and Medicine* 226, 963-977.
- Herrmann, C., Goke, R., Richter, G. (1995). Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 56, 117-126.
- Howarth, N.C., Saltzman, E. & Robert, S.B. (2001). Dietary fiber and weight regulation. *Nutrition Reviews* 59, 129-139.
- Hudson, M.J. & Marsh, P.D. (1995). Carbohydrate metabolism in the colon. In: Gibson, G.R. & Macfarlane, G.T. (eds). *Human Colonic Bacteria. Role in Nutrition, Physiology, and Pathology*. Boca Raton: CRC Press. Pp.61-74
- Huynh, B.L., Plamer, L., Mather, D.E., Wallwork, H., Graham, R.D., Welch, R.M. & Stangoulis, J.C.R. (2008). Genotypic variation in wheat grain fructan content revealed by simplified HPLC method. *Journal of Cereal Science* 48, 369-378.
- Iglesias, R., Villarroya, F. & Alemany, M. (1985). Comparison of the effects of different anticoagulants and sample handling procedures on rat insulin radioimmunoassay. *Comparative Biochemistry and Physiology* 82:4, 863-866.
- Isaksson, H., Sundberg, B., Åman, P., Fredriksson, H., & Olsson, J., (2008). Whole grain rye porridge breakfast improves satiety compared to refined wheat bread breakfast. *Food & Nutrition Research* 52: 10.3402/fnr.v52i0.1809
- Isaksson, H., Lanberg, R., Sundberg, B., Lundin, E., Hallmans, G., Zhang, J.X., Tidehag, P., Bach Knudsen, K.E., Moazzami, A.A. & Åman, P. (2013). High-fiber rye diet increase ileal excretion of energy and macronutrients compared with low-fiber wheat diet independent of meal frequency in ileostomy subjects. *Food and Nutrition Research* 57.
- Kay, R.M. (1982). Dietary fiber. *Journal of Lipid Research* 23,221-242.
- Karppinen, S., Liukkonen, K., Aura, A.N., Forssej, Pirkko. & Poutanen, K. (2000). Wheat and oat bran and inulin by human fecal bacteria. *Journal of the Science of Food and Agriculture* 80, 1469-1476.
- Karppinen, S., Myllymäki, O., Forsell, P. & Poutanen, K. (2003). Fructan content of rye and rye products. *Cereal Chemistry* 80(2), 168-171.

- Khoury, D.E., Cuda, C., Luhovyy, B.L., Anderson, G.H. (2012). Beta Glucan: Health benefits in obesity and metabolic syndrome. *Journal of Nutrition and Metabolism*. Article ID 851362, 28 pages. Doi: 10.1155/2012/851362
- Klopfenstein, C.F. (1998). The role of cereal beta-glucan in nutrition and health. *Cereal Foods World* 33(10), 865-869.
- Koh-Banerjee, P. & Rimm, E.R. (2003). Whole grain consumption and weight gain: a review of the epidemiological evidence potential mechanisms and opportunities for future research. *Proceedings of Nutrition Society* 62, 25-9.
- Kojima, M., Pi-Sunyer, F.X., Date, Y., Nakazato, M., Matsuo, O. & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656-660.
- Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal Medicine* 113 (Suppl 9B). 71S-88S.
- Kulawinek, M., Jaromin, A. & Kozubek, A. (2007). Mechanizmy dzialania skladnikow pelnych ziaren zboz *Lek w Polsce* 17. 85-96
- Lattimer, J.M. & Haub, M.D. (2010). Effect of dietary fiber and its Components on Metabolic health. *Nutrients* 2, 1266-1289.
- de Leeuw, J.A., Bolhuis, J.E., Bosch, G. & Gerrits, W.J.J. (2008). Effect of dietary fiber on behavior and satiety in pigs. *Proceedings of Nutrition Society* 67, 334-342.
- Leinonen, K., Liukkonen, K., Poutanen, K., Uusiyupa, M. & Mykkanen, H. (1999). Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. *European Journal of Clinical Nutrition* 53, 262-267.
- Le Queelec, A., Karvran, A., Blache, P. (1992). Oxyntomodulin-like immune-reactivity: diurnal profile of a new potential enterogastrone. *The Journal of Clinical Endocrinology and Metabolism* 74, 1405-1409.
- Lindon, J., Holmes, E. & Nicholson, J.K. (2006). Metabolomics techniques and application to pharmaceutical research& development. *Pharmaceutical Research* 23, 1075-1088.
- Liu, S. (2002). Intake of refined carbohydrates and whole grain foods in relation to risk of type 2 diabetes mellitus and coronary heart disease. *Journal of American Collage Nutrition* 21(4), 298-306.
- Lopez, H.W., Levrat, M.A., Guy, C., Messenger, A., Deminge, C. & Remesy, C. (1999). Effect of soluble corn bran arabinoxylans on cereal digestion lipid metabolism and mineral balance (Ca, Mg) in rats. *Journal of Nutritional Biochemistry* 10(9), 500-509.

- Low, A.G., Pittman, R.J. & Elliott, R.J. (1985). Gastric-emptying of barley soybean diets in the pig-effects of feeding level, supplementary maize oil, sucrose or cellulose, and water-intake. *British Journal of Nutrition* 54, 437-447.
- Lu, Z.X, Walker, K.Z., Muir, J.G., Mascara, T., O'Dea, K. (2000). Arabinoxylan fiber, a by-product of wheat flour processing, reduces the pro-prandial glucose response in normoglycemic subjects. *American Journal of Clinical Nutrition* 71, 1123-1128.
- Ludwig, D.S., Majzoub, J.A., Al-Zahrani, A., Dallal, G.E., Blanco, I. & Robert, S.B. (1999). High glycemic index foods overeating and obesity. *Pediatrics* 103. E26.
- Maires, D.J. & Stone, B.A. (1973). Studies on wheat endosperm. I. Chemical composition and ultrastructure of cell wall. *Australian Journal of Biology Sciences* 26, 793-812.
- Marlett, J.A. & Cheung, T. (1997). Database and quick methods of assessing typical dietary fiber intakes using data for 228 commonly consumed foods. *Journal of American Dietary Association* 97, 1139-1148.
- Mathis, C., Moran, T.H. & Schwartz, G.J. (1998). Load-sensitive rat gastric vagal afferents encode volume but not gastric nutrients. *American Journal of Physiology* 274, R280-R286.
- McKee, L.H. & Latner, T.A. (2000). Underutilized sources of dietary fiber: a review. *Plants Foods of Human Nutrition* 55, 285-304.
- McKeown, N.M., Meigs, J.B., Liu, S., Wilson, P.W.F. & Jacques, P.F. (2002). Whole-grain intake is favorably associated with metabolic risk factor for type 2 diabetes and coronary heart disease in the Framingham offspring study. *American Journal of Clinical Nutrition* 76(2), 390-398.
- McMinn, J.E., Sindelar, D.K., Havel, P.J. & Schwartz, M.W. (2000). Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology* 141, 4442-4448.
- Meunier-Salaün, M.C., Edwards, S.A. & Robert, S. (2001). Effect of dietary fiber on the behavior and health of the restricted fed sow. *Animal Feed Science and Technology* 90, 53-69.
- de Munter, J.S., Hu, F.B., Spiegelman, D., Franz, M. & van Dam, R.M. (2007). Whole grain, bran and germ intake and risk of type 2 diabetes: a prospective cohort study and systemic review. *PloS Medicine* 4:e261.
- Murphy, K.G. & Bloom, S.R. (2004). Gut hormones in the control of appetite. *Experimental Physiology* 89, 507-516.
- Meyer, D., Stasse-Wolthuis, M. (2009). The bifido genetic effect of inulin and oligo fructose and its consequences for gut health. *European Journal of Clinical Nutrition* 63, 1277-1289.

- Nardi, S., Calcagno, C., Zunin, P., D'Egidio, M.G., Cecchini, C., Boggia, R., & Evangelisti, F. (2003). Nutritional benefits of developing cereals for functional foods. *Cereal Research Communications* 31, 445-452.
- Näslund, E., Bogefors, J., Skogar, S. (1999). Glucagon-like peptide slow solid gastric emptying with inhibition of insulin. C-peptide and peptide YY release in humans. *The American Journal of Physiology* 277, R910-916.
- Nattrass, M. & Lauritzen, T. (2000). Review of postprandial regulation with repaglinide: a solution to the problem of hypoglycemia in the treatment of Type 2 diabetes. *International Journal of Obesity and Related Metabolic Disorder* 24(suppl), S21-31.
- Nilsson, M., Åman, P., Härkönen, H., Hallmans, G., Bach Knudsen, K.E., Mazur, W. & Adlercreutz, H. (1997). Content of nutrients and lignans in roller-milled fraction of rye. *Journal of the Science of Food and Agriculture* 73, 143-148.
- Orešič, M. (2009). Metabolomics, a novel tool for studies of nutrition, metabolism and lipid dysfunction. *Nutrition, Metabolism and Cardiovascular Diseases* 19, 816-824.
- Peters, H.P.F. & Mela, D.J. (2008). The role of the gastrointestinal tract in satiation, satiety and food intake: evidence from research in humans. In: Harris RBS, Mattes, R.D. eds. *Appetite and Food intake: behavioral and physiological considerations*. Boca Raton, F.L.: Taylor & Francis; 2008, Pp187-211.
- Prentice, N., Babler, S. & Faber, S. (1980). Enzymatic analysis of beta-D- glucans in cereals. *Cereal Chemistry* 57(3), 198-202.
- Priebe, M.G., van Binsbergen, J.J., de Vos, R. & Vonk, R.J. (2008). Whole grain food for the prevention of type 2 diabetes mellitus. *Cochrane Database of Systemic Review* 23: CD006061.
- Rakha, A., Åman, P. & Andersson R. (2009). Characterization of dietary fiber component in rye product. *Food Chemistry* 119(3), 859-867.
- Ritsema, T., Smeeckens, S. (2003). Fructans: Beneficial for plants and humans. *Current Opinion in Plant Biology* 6, 223-230.
- Rodríguez-López, J.M., Lachica, M., González-Valero, L. & Ferná-Fígares, I. (2013). Approaches for quantifying gastrointestinal nutrient absorption and metabolism in a native and a modern pig breed. *The Journal of Agricultural Science* 151, 434-443.
- Rosén, L.A.H., Silva, L. O. B., Andersson, U.K., Holm, C., Östman, E. M. & Björck, I. M.E. (2009). Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile. *Nutrition Journal* 8:42
- Ross, A.B., Shepard, M.J., Schupphaus, M., Sinclair, V., Alfaro, B. & Kamal-Eldin, A. (2003c). Alkylresorcinols in cereal and cereal products. *Journal of Agricultural and Food Chemistry* 51(14), 4111-4118.

- Scholz-Ahrens, A.E., Ade, P., Marten, B., Weber, P., Timm, W., Açil, Y., Glüer, C.C., Schrezenmeir, J. (2007). Prebiotics, probiotics and synbiotics affect mineral absorption, bone mineral content and bone structure. *Journal of Nutrition* 137(Suppl 2), 838S-846S.
- Schulze, M.B., Schulz, M., Heidemann, C., Schienkiewitz, A., Hoffman, K. & Boeing, H. (2007). Fiber and magnesium intake and incidence of type 2 diabetes: A prospective study and meta-analysis. *Archives of Internal Medicine* 167, 956-965.
- Schroeder, N., Gallaher, D.D., Arndt, E.A. & Marquart, L. (2009). Influence of whole grain barley, whole grain wheat and refined rice based foods on short-term satiety and energy intake. *Appetite* 53(3), 363-369.
- Sedlet, K., Mathias, M. & Lorenz, K. (1984). Growth-depressing effects of 5-n-pentadecylresorcinol: a model for cereal alkylresorcinols. *Cereal Chemistry* 61, 239-241.
- Slavin, J.L. (2003). Why whole grains are protective: biological mechanism. *Proceeding of the Nutritional Society* 62, 129-134.
- Slavin, J.L. & Green, H. (2007). Dietary fiber and satiety. *Nutrition Bulletin* 32(Suppl 1), 32-42.
- Slavin, J.L. (2008). Position of the American Dietetic Association: health implication of dietary fiber. *Journal of American Dietetic Association* 108, 1716-1731.
- Slavin, J.L. (2010). Dietary fiber and satiety-not all fiber is alike. In: Williams P.A., Phillips G.O., editors. Gums and stabilizers for the food industry 15; Proceedings of the 15<sup>th</sup> Gums and stabilizers for the food industry conference; 2009 Jun 22-26; Wareham. United Kingdom. Cambridge: Royal Society of Chemistry, P.333.
- Slavin, J.L. & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition* 3, 506-516.
- Sleeth, M.L., Thompson, E.L., Ford, H.E., Zac-Varghese, S.E.K. & Frost, G. (2010). Free fatty acid receptor 2 and nutrient sensing: a proposed role for fiber fermentable carbohydrates and short-chain fatty acids in appetite regulation. *Nutrition Research Reviews* 23, 135-145.
- Soeldner, J.S. & Slone, D. (1965). Critical variables in the radioimmunoassay of serum insulin using the double antibody technique. *Diabetes* 14, 771-779.
- Southgate, D. & Durnin, J. (1970). Calorie conversion factors – an experimental reassessment of factors used in calculation of energy value of human diets. *British Journal of Nutrition* 24, 517-535.
- Souza da Silva, C., van den Borne, J.J.G.C., Gerrits, W.J.J., Kemp, B. & Bolhuis, J.E. (2012). Effect of dietary fibers with different physiochemical properties on feeding motivation on adult female pigs. *Physiological Behavior* 107, 218-230.
- Spurlock, M.E. & Gabler, N.K. (2008). The development of porcine model of obesity and the metabolic syndrome. *The Journal of Nutrition* 138, 397-402.

- Stone, B. & Morell, M.K. (2009). Carbohydrates. Khan, K. & Shewry, P.R. (EDs). Wheat: Chemistry and Technology (fourth ed.). American Association of Cereal Chemist. St Paul. MN. Pp. 299-362.
- Takahashi, T., Karita, S., Ogawa, N. & Goto, M. (2005). Crystalline cellulose reduces plasma glucose concentrations and stimulates absorption by increasing the digesta viscosity in the rats. *Journal of Nutrition* 135, 2405-2410.
- Theil, P.K., Jørgensen, H., Serena, A., Hendrickson, J & Bach Knudsen, K.E. (2011). Products deriving from microbial fermentation are linked to insulinaemic response in pigs fed breads prepared from whole-wheat grain and wheat and rye ingredients. *British journal of Nutrition* 105, 373-383.
- Topping, D.I., Mock, S., Trimble, R.P. & Illman, R.J. (1988). Effect of varying the content and proportions of gum Arabic and cellulose on ceecal volatile fatty acids in the rat. *Nutrition Research* 8, 1013-1020.
- Topping, D.L. (1991). Soluble fiber polysaccharides: effects on plasma cholesterol and colonic fermentation. *Nutrition Reviews* 49(7), 195-203.
- Topping, D.L. & Clifton, P.M. (2001). Short-chain fatty acids and human colonic function: roles of resistance starch and non-starch polysaccharides. *Physiological Review* 81, 1031-1064.
- Trowell, H. (1972). Dietary fiber and coronary heart disease. *European Journal of Clinical Biology Research* 17, 345-349.
- Tschöp, M., Smiley, D.L. & Heiman, M.L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407, 908-913.
- Tunland, B.C. & Meyer, D. (2002). Non-digestible oligo and polysaccharides (dietary fiber): their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety* 1, 73-92.
- Van Soest, P.J., Roberftson, J.D. & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. *Journal of Dietary Science* 74, 3583-3597.
- Vietor, R.J., Voragen, A.G.J., Angelino, S. & Pilnik, W. (1991). Non-starch polysaccharides in barley and malt – A mass balance of flour fractionation. *Journal of Cereal Science* 14, 73-83.
- Vitaglione, P., Napolitano, A. & Fogliano, V. (2008). Cereal dietary fiber as natural functional ingredient to deliver phenolic compounds into gut. *Trends Food Science and Technology* 19 (9), 451-463.
- Wanders, A.J., van den Borne, J.J.G.C., de Graaf, C., Hulshof, T., Jonathan, M.C. & Kristensen, M. (2011). Effect of dietary fiber on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. *Obesity Reviews* 12, 724-739.



- Wang,L., Barachina, M.D., Martinez, V., Wei, J.Y. & Tache, Y. (2000). Synergistic interaction between CCK and leptin to regulate food intake. *Regularly Peptide* 92, 79-85.
- Warren, J.M., Henry, C.J. & Simonite, V. (2003). Low glycemic index breakfasts and reduced food intake in preadolescent children. *Pediatrics* 112, E414
- Wenk, C. (2001). The role of dietary fiber in the digestive physiology of the pig. *Animal Feed Science and Technology* 90, 21-33.
- Wheat and Flour. (2014). [http://www.wheatandflour.com/wheat/about\\_wheat.php](http://www.wheatandflour.com/wheat/about_wheat.php)
- Weickert, M.O. & Pfeiffer, A.F.H. (2008). Metabolic effects of dietary fiber consumption and prevention of diabetes. *The Journal of Nutrition* 138, 439-442.
- Wieringa, G.W. (1967). On the occurrence of growth inhibiting substances in rye (Publication No 156). Wageningen. The Netherland: Institute of storage and processing of agricultural produce; 1967.
- Wilfart, A., Montagne, L., Simmins, P.H., van Milgen, J. & Noblet, J. (2007). Sites of nutrient digestion in growing pigs: Effect of dietary fiber. *American Society of Animal Science* 85, 976-983.
- Willing, A.E. & Berthoud, H.R. (1997). Gastric distention-induced c-fos expression in catecholaminergic neurons of rat dorsal vagal complex. *American Journal of Physiology* 272, R59-R67.
- Wirstrom, T., Hilding, A., Gu, H.F., Ostenson, C.G. & Bjorklund, A. (2013). Consumption of whole grain reduces risk of deteriorating glucose tolerance, including progression to prediabetes. *American Journal of Clinical Nutrition* 97, 179-187.
- Woods, S.C. & Bernstein, I.L. (1980). Cephalic insulin response as a test for completeness of vagotomy to the pancreas. *Physiological Behavior* 24, 485-488.
- Wood, P.J., Weisz, J. & Blackwell, B.A. (1994). Structural studies of (1→3)(1→4)-β-D-glucans by <sup>13</sup>C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharide released by lichenase. *Cereal Chemistry* 71(3), 207-225.
- Wood, P.J. (2010). REVIEW: Oat and Rye β-glucan: Properties and function. *Cereal Chemistry Journal* 87, 315-330.
- Wren, A.M. & Bloom, S.R. (2007). Gut hormone and appetite control. *Gastroenterology* 132, 2116-2130.
- Wynne, K., Stanley, S. & McGowan,B. (2005a). Appetite control. *Journal of Endocrinology* 184, 291-318.
- Yamamoto, H., Kishi, T., Lee, C.E.(2003). Glucagon-like peptide-responsive catecholamine neurons in the area postrema link peripheral glucagon peptide-1 with central autonomic sites. *Journal of Neuroscience* 23, 2939-2946.

- Ye, E.Q., Chackp, S.A., Chou, E.L., Kugizaki, M. & Liu, S. (2012). Greater whole- grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *Journal of Nutrition* 142, 1304-1313.
- Yoshihara, F., Kojima, M., Hosoda, H., Nakazato, M. & Kangawa, K. (2002). Ghrelin: a novel peptide for growth hormone release and feeding regulation. *Current opinion of Nutrition Metabolism Care* 5, 391-395.

## Appendix

### Blood glucose level

**Table 1. Effect of the different diets on blood glucose level ( $\mu\text{g/l}$ ) from portal vein. Least square means  $\pm$  standard error**

	Diet			SEM	P-value
	WB	WGRB	WGWB		
<b>0 min</b>	4.84	5.18	4.20	0.6	0.3431
<b>15 min</b>	7.25	10.34	8.92	1.5	0.4778
<b>30 min</b>	9.14	8.28	9.52	1.5	0.8497
<b>45 min</b>	8.85	7.89	8.64	1.4	0.8890
<b>60 min</b>	9.08	7.01	10.24	1.2	0.1556
<b>90 min</b>	9.35	9.55	8.71	1.0	0.8398
<b>120 min</b>	7.88	7.47	7.71	0.7	0.9088
<b>180 min</b>	7.46	7.31	8.96	1.0	0.4883

**Table2. Effect of the different diets on blood glucose level ( $\mu\text{g/l}$ ) from carotid artery. Least square means  $\pm$  standard error**

	Diet			SEM	P-value
	WB	WGRB	WGWB		
<b>0 min</b>	4.49	4.87	4.04	0.4	0.4887
<b>15 min</b>	6.29	5.57	5.99	1.0	0.8742
<b>30 min</b>	6.70	5.66	6.31	1.2	0.8427
<b>45 min</b>	5.37	5.12	5.81	0.8	0.8200
<b>60 min</b>	6.25	5.61	6.28	0.6	0.4286
<b>90 min</b>	6.55	5.38	6.15	0.8	0.5518
<b>120 min</b>	5.07	5.50	5.32	0.6	0.8759
<b>180 min</b>	5.74	4.93	5.97	0.6	0.4547

**Table3. Effect from different period on blood glucose level ( $\mu\text{g/l}$ ) from carotid artery. Least square means  $\pm$  standard error.**

Time	Diet			SEM	P-value
	Period 1	Period 2	Period 3		
<b>0 min</b>	4.55	<b>4.32</b>	<b>4.53</b>	0.5	0.9370
<b>15 min</b>	7.79 <sup>a</sup>	4.76 <sup>b</sup>	5.30 <sup>bc</sup>	0.4	0.0034
<b>30 min</b>	7.39	6.27	4.15	0.8	0.1498
<b>45 min</b>	5.97	5.56	4.46	0.7	0.4406
<b>60 min</b>	6.58	5.62	5.93	0.5	0.1681
<b>90 min</b>	6.28	5.71	5.75	0.8	0.8526
<b>120 min</b>	5.90 <sup>a</sup>	5.72 <sup>a</sup>	4.27 <sup>b</sup>	0.3	0.0410
<b>180 min</b>	5.44	5.59	5.60	0.6	0.9791

**Table 4. Effect from different period on blood glucose level ( $\mu\text{g/l}$ ) from portal vein. Least square means  $\pm$  standard error**

	Diet			SEM	P-value
	Period 1	Period 2	Period 3		
<b>0 min</b>	4.52	5.09	4.61	0.6	0.7047
<b>15 min</b>	10.86	7.69	6.95	1.2	0.1323
<b>30 min</b>	11.36	8.50	7.08	1	0.0649
<b>45 min</b>	9.97	8.38	7.02	1.2	0.3290
<b>60 min</b>	8.15	8.71	9.48	1.5	0.8070
<b>90 min</b>	9.19	9.31	9.11	1.1	0.9907
<b>120 min</b>	8.03	8.16	6.88	0.5	0.2889
<b>180 min</b>	8.16	7.40	8.16	1.1	0.8581

#### Blood insulin level

**Table 5. Effect different period on blood insulin level ( $\mu\text{g/l}$ ) from portal vein. Least square means  $\pm$  standard error.**

Time	Diet			SEM	P-value
	Period 1	Period 2	Period 3		
<b>0 min</b>	0.12	0.12	0.06	0	0.5863
<b>15 min</b>	1.70	0.67	0.57	0.2	0.0266
<b>30 min</b>	1.97	0.90	0.55	0.3	0.0356
<b>45 min</b>	1.31	0.42	0.38	0.3	0.0897
<b>60 min</b>	0.74	0.39	0.46	0.1	0.1225
<b>90 min</b>	0.49	0.51	0.48	0.3	0.9965
<b>120 min</b>	0.34	0.43	0.26	0.1	0.1442
<b>180 min</b>	0.19	0.35	0.37	0.2	0.6273

**Table 6. Effect of the different diets on blood insulin level ( $\mu\text{g/l}$ ) from portal vein. Least square means  $\pm$  standard error**

Time	Diet			SEM	P-value
	WB	WGRB	WGWB		
<b>0 min</b>	0.17	0.08	0.05	0.6	0.1410
<b>15 min</b>	0.80	1.11	1.17	1.5	0.1061
<b>30 min</b>	1.32	0.92	1.17	1.5	0.2111
<b>45 min</b>	0.70	0.42	0.99	1.4	0.2406
<b>60 min</b>	0.54	0.42	0.63	1.2	0.4434
<b>90 min</b>	0.37	0.55	0.55	1.0	0.6986
<b>120 min</b>	0.40	0.34	0.29	0.7	0.8230
<b>180 min</b>	0.14	0.34	0.42	1.0	0.3812

**Table 7. Effect from different diets on blood insulin level ( $\mu\text{g/l}$ ) from carotid artery. Least square means  $\pm$  standard error**

	Diet			SEM	P-value
	WB	WGRB	WGWB		
<b>0 min</b>	0.07	<b>0.05</b>	<b>0.02</b>	0	0.6559
<b>15 min</b>	0.36	0.44	0.45	0.2	0.8083
<b>30 min</b>	0.66	0.36	0.65	0.3	0.1567
<b>45 min</b>	0.43	0.14	0.62	0.3	0.1432
<b>60 min</b>	0.25	0.17	0.33	0.1	0.4265
<b>90 min</b>	0.25	0.25	0.20	0.1	0.9461
<b>120 min</b>	0.18	0.14	0.13	0.1	0.8746
<b>180 min</b>	0.05	0.07	0.17	0.1	0.4446

**Table 8. Effect from different period on blood insulin level ( $\mu\text{g/l}$ ) from carotid artery. Least square means  $\pm$  standard error**

	Diet			SEM	P-value
	Period 1	Period 2	Period 3		
<b>0 min</b>	0.05	<b>0.05</b>	<b>0.04</b>	0	0.9167
<b>15 min</b>	0.81	0.21	0.27	0.1	0.0064
<b>30 min</b>	1.11	0.38	0.13	0.1	0.0112
<b>45 min</b>	0.91	0.22	0.10	0.2	0.1432
<b>60 min</b>	0.34	0.18	0.25	0.1	0.5101
<b>90 min</b>	0.27	0.24	0.20	0.1	0.9218
<b>120 min</b>	0.13	0.21	0.09	0.1	0.3261
<b>180 min</b>	0.10	0.14	0.07	0.1	0.7273