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$_2$ 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 lifestyle diseases such as type 2 diabetes mellitus (T2D) and cardiovascular diseases (CVD). Recent studies conclude that body weight is inversely 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, β-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 β-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).

| Macronutrient compositions of rye and wheat grains (% of dry matter)*# |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | Whole grain rye flour (100) | Whole grain wheat flour (100) | White wheat flour (66) |
| Fat                             | 8-13             | 12-14           | 13              |
| Protein                         | 2-3              | 3               | 1               |
| Starch                          | 56-70            | 67-70           | 84              |
| Ash                             | 2                | 20.5            |                 |
| Total fiber                     | 15-17            | 10-13           | 3               |
| Of which soluble fiber          | 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 β-glucan are present in both soluble and insoluble form. Several studies have shown beneficial effect of arabinoxylan and β-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)

<table>
<thead>
<tr>
<th>Classification</th>
<th>GI range</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low GI</td>
<td>&lt; 55</td>
<td>Whole grains, pasta, lentils, beans, most fruit and vegetables</td>
</tr>
<tr>
<td>Medium GI</td>
<td>56-69</td>
<td>Sucrose, brown rice, basmati rice</td>
</tr>
<tr>
<td>High GI</td>
<td>&gt; 70</td>
<td>Baked potato, white bread, white rice (e.g. jasmine), corn</td>
</tr>
</tbody>
</table>
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 % β-glucan, 1.8 % cellulose and 1.0 % fructan (Karppinen et al., 2003; Vitaglioneet al., 2008; Stone and Morell, 2009).

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 β (1-3;1-4)-D-glucans (β-glucans) (Knudsen et al., 2005).The fiber composition in rye grains are approximately 8-10 % arabinoxylan, 1.7-2 % β-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).

β-glucan
β-glucan, a linear polysaccharide of glucose monomer with β(1→4) and β(1→3) linkages is abundant in the endosperm of cereal grains. β-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 β-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 µg/g and in wheat grains at 317-1430 µg/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 (Kozubel, 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 β-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 (Vietor et al., 1991; Huynh et al., 2008). For wheat, with a genotypic variation of the content of fructan, it has was 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 (Ritsema and Smeeke, 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 as 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 der Weele, 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 Quellec 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 (WGW). 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 (TiO₂) 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 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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>WGWB</th>
<th>WGRB</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole wheat</td>
<td>79.75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rye</td>
<td>0</td>
<td>80.25</td>
<td>0</td>
</tr>
<tr>
<td>White wheat flour</td>
<td>0</td>
<td>0</td>
<td>70.00</td>
</tr>
<tr>
<td>Casein</td>
<td>3.00</td>
<td>6.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Vegetable fat</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3.50</td>
<td>0.0</td>
<td>11.25</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Yeast</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Premix</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>WB</th>
<th>WGWB</th>
<th>WGRB</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>39</td>
<td>46</td>
<td>49</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>CP</td>
<td>92.91</td>
<td>92.96</td>
<td>93.06</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Fat</td>
<td>50</td>
<td>67</td>
<td>63</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>DM</td>
<td>65.00</td>
<td>66.69</td>
<td>65.80</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>NSP</td>
<td>16.38</td>
<td>12.76</td>
<td>13.49</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Arabinose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.62</td>
<td>1.70</td>
<td>2.66</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Insoluble</td>
<td>0.38</td>
<td>1.36</td>
<td>1.70</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Xylose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.70</td>
<td>3.32</td>
<td>4.46</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Insoluble</td>
<td>2.35</td>
<td>2.80</td>
<td>2.91</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Uronic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.28</td>
<td>0.41</td>
<td>0.35</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Insoluble</td>
<td>0.22</td>
<td>0.35</td>
<td>0.27</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11.86</td>
<td>6.38</td>
<td>4.97</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Insoluble</td>
<td>11.74</td>
<td>6.13</td>
<td>4.02</td>
<td></td>
</tr>
<tr>
<td>Klason lignin</td>
<td>0.74</td>
<td>1.31</td>
<td>1.49</td>
<td>g/kg DM</td>
</tr>
<tr>
<td>Dietary fiber with fructan</td>
<td>17.25</td>
<td>14.28</td>
<td>16.56</td>
<td>g/kg DM</td>
</tr>
</tbody>
</table>
Table 5. Feeding order of the pigs in different experimental periods.

<table>
<thead>
<tr>
<th>Pig number</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>849</td>
<td>WGRB</td>
<td>WB</td>
<td>WGWB</td>
</tr>
<tr>
<td>850</td>
<td>WB</td>
<td>WGWB</td>
<td>WGRB</td>
</tr>
<tr>
<td>851</td>
<td>WGWB</td>
<td>WGRB</td>
<td>WB</td>
</tr>
<tr>
<td>852</td>
<td>WB</td>
<td>WGWB</td>
<td>WB</td>
</tr>
<tr>
<td>856</td>
<td>WGRB</td>
<td>WB</td>
<td>WGWB</td>
</tr>
</tbody>
</table>

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® (medetomidine HCL 1 mg/ml; Orion Pharma, Espoo, Finland) and Zoletil® (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® 0.3 mg/ml). Aseptic techniques and sterile conditions were applied throughout the whole surgical procedure. The surgical procedure has been described by Rodrígues-López et al. (2012).

Pre- and post-operative care included antibiotic treatment (procain penicillin, Penovet®) 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, Höganas, 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). TiO₂ 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 mixed0.9 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®, 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$_2$) technique according to the following equation (Wilfart *et al.*, 2007):

\[
\text{Apparent digestibility} = 1 - \frac{(TiD \times DCF)}{(DCD \times TiF)}
\]

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 ± 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$^1$ of dietary components. Least square means ± standard error (SEM)**

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

$^1$CTTAD = coefficient of the total tract apparent digestibility; $^2$OM = organic matter; $^3$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 ± SEM., n=3. There was no significant time × 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 ± SEM, n=3. There was no significant time × 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 Figure3 and4. 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 ± standard error (SEM)

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

In fecal samples, 31 metabolites from 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 $^1$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 shown 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 studied are performed. This study shows that wheat and rye generates different metabolic profiles. However, to get a deeper understanding of possible mechanism behind the different profiles and how this can be linked to health issue will require further studies with more pigs and longer experimental periods.
Acknowledgements

I would like to express my deep gratitude to my master thesis advisor, Dr. Emma Ivarsson for spending very much time instructing me patiently how to write my thesis. She takes efforts to correct my endless grammatical mistakes with a consistent notation in my writings and for carefully reading and commenting on countless revisions of this thesis. I am so fortunate to have her as a supervisor. Without her kindness and patient instruction, it is impossible for me to finish this thesis.

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To all my Thai friends, thank you for your help and encouragement during I studied in Sweden. Your friendship makes my life a wonderful experience. I can’t list all the names here, but you are always on my mind.

My gratitude also goes to SLU for giving me a scholarship during my two years at SLU.

Finally, my deepest gratitude goes to my parents for their love and support throughout my life. Thank you both for giving me a big support to chase my dreams. My little sister for taking care of our parents while I was miles away from home.
References


Appendix
Blood glucose level

Table 1. Effect of the different diets on blood glucose level (µg/l) from portal vein. Least square means ± standard error

<table>
<thead>
<tr>
<th>Diet</th>
<th>WB</th>
<th>WGRB</th>
<th>WGWB</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
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<td>4.20</td>
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Table 2. Effect of the different diets on blood glucose level (µg/l) from carotid artery. Least square means ± standard error

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<th>WGWB</th>
<th>SEM</th>
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Table 3. Effect from different period on blood glucose level (µg/l) from carotid artery. Least square means ± standard error.

<table>
<thead>
<tr>
<th>Time</th>
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Table 4. Effect from different period on blood glucose level (µg/l) from portal vein. Least square means ± standard error

<table>
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<th>Period 3</th>
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</table>

Blood insulin level

Table 5. Effect different period on blood insulin level (µg/l) from portal vein. Least square means ± standard error.

<table>
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<th>Period 3</th>
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<th>P-value</th>
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</tr>
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<tr>
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</tr>
<tr>
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<td>0.37</td>
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</table>

Table 6. Effect of the different diets on blood insulin level (µg/l) from portal vein. Least square means ± standard error

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<th>Time</th>
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<th>WGWB</th>
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Table 7. Effect from different diets on blood insulin level (µg/l) from carotid artery. Least square means ± standard error

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Table 8. Effect from different period on blood insulin level (µg/l) from carotid artery. Least square means ± standard error

<table>
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<th>Period 3</th>
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<th>P-value</th>
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