Fermentation in the gut to prolong satiety

Exploring mechanisms by which dietary fibres affect satiety in pigs

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Thesis committee

Promotor
Prof. dr. ir. B. Kemp
Professor of Adaptation Physiology
Wageningen University

Co-promotors
Dr. ir. J.E. Bolhuis
Researcher, Adaptation Physiology Group
Wageningen University

Dr. ir. W.J.J. Gerrits
Associate professor, Animal Nutrition Group
Wageningen University

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Prof. dr. K.E. Bach Knudsen, Aarhus University, Denmark
Dr. M.C. Meunier-Salaün, Institut National de la Recherche Agronomique (INRA), Saint-Gilles, France

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“Start by doing what is necessary; then do what is possible; and suddenly you are doing the impossible”

St. Francis of Assisi

To my beloved parents
Dalci and Zermira
and partner Dennis,
for always being there for me

Abstract

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Obesity has become a major health problem in humans and companion animals. Although obesity is not common in farm animals, food restriction is often used to maintain low feeding costs and performance of, for instance, pregnant sows and fattening pigs. Food restriction may result in hunger and increased feeding motivation, which are associated with behavioural problems. Knowledge on the regulation of satiety is thus crucial to aid in the control of food intake in humans, and to improve welfare in food-restricted farm animals. Dietary fibres are believed to enhance satiety, but the effectiveness varies with the physicochemical properties of the fibre sources concerned. Therefore, the objective of this thesis was to identify whether and how dietary fibres with different physicochemical properties, such as bulkiness, viscosity, gelling and fermentability, affect satiety in the domestic pig, which was used both as a model for humans and as a target animal. In a study focusing on behavioural measures of satiety, pectin (viscous fibre) was the least satiating, whereas lignocellulose (bulking fibre) and resistant starch (fermentable fibre) were the most satiating fibres tested. In a subsequent study, increasing levels of guar gum, inulin, and resistant starch (all fermentable fibres), when replacing digestible starch, enhanced satiety throughout the day. Resistant starch was the most satiating fibre among all fibres tested, and used, in a subsequent study, to assess possible physiological and molecular mechanisms by which fermentation may affect satiety. Also in this study, resistant starch appeared to enhance satiety based on behavioural observations, i.e. reduced feeder-directed and drinking behaviours during 24 h. As expected, the satiating effects of resistant starch coincided with increased 24 h plasma short-chain fatty acids (SCFA) levels and decreased postprandial glucose and insulin plasma levels. Glucagon-like peptide-1 (GLP-1) plasma levels were lower in pigs fed resistant starch, whereas peptide tyrosine tyrosine (PYY) plasma levels were not affected by resistant starch, suggesting that these hormones do not play a role in the increased satiety induced by fermentation. Resistant starch consumption led to downregulation of genes involved in immune responses, and upregulation of genes involved in metabolic processes such as fatty acid and energy metabolism in the proximal colon. Moreover, correlation analysis inversely linked potential pathogenic microbial groups with plasma SCFA concentrations and with genes involved in fatty acid metabolism. These findings suggest that besides satiating effects, resistant starch has a beneficial effect on colonic health. In the last study, the long-term effects of a

gelling fibre promoting satiation (alginate) and a fermentable fibre promoting satiety (resistant starch) on feeding patterns and growth performance were assessed. In the long-term, growing-finishing pigs compensated for a reduced dietary energy content by increasing voluntary food intake (alginate), or they became more efficient in the use of digestible energy (resistant starch). Moreover, dietary fibres increased the relative weight of the gastrointestinal tract and led to changes in body composition (less fat more muscle), which may be relevant for the maintenance of lean weight in humans. In conclusion, fermentable fibres are more satiating than viscous and bulking fibres. The satiating effects of fermentable fibres are likely mediated by an increased SCFA production, and a reduced and attenuated glucose supply. Under unrestricted feeding conditions, dietary fibres promoting satiation (alginate) and satiety (resistant starch) did not reduce long-term food intake and total body weight gain, yet, colon empty weight was increased and carcass growth was reduced. This implies that changes in body composition and intestinal weight or content, rather than body weight and body mass index (BMI) alone may be relevant to fully acknowledge the effects of fibres to aid in maintaining or promoting healthy body weight in humans.

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Chapter 1

General introduction

According to the World Health Organization (WHO, 2013), obesity worldwide has almost doubled over the last three decades. In 2008, more than 1.4 billion adults (age ≥ 20 years) were overweight, of which at least 500 million were obese (Finucane et al., 2011). Obesity has become a major public health problem in humans due to the increased risk of cardiovascular and metabolic diseases, and associated mortality (O'Rahilly and Farooqi, 2008). Also, in companion animals, the incidence of overweight and obesity has increased resulting in similar health problems as in humans (German, 2006). Although obesity is not a common problem in farm animals, food restriction is used, for instance in pregnant sows and broiler breeders (the parent stock of meat pigs and chickens, respectively) to maintain low feeding costs, and an optimal body condition and reproductive performance. As in humans, food restriction may prevent obesity and associated health problems in farm animals, but it may impair the welfare of these animals due to hunger and frustration of feeding motivation (Meunier-Salaun et al., 2001). The unfulfilled feeding motivation of these animals, even just after the meal, leads to increased levels of activity and the development of abnormal, redirected oral behaviours that may evolve into stereotypies (D'Eath et al., 2009). These are an indicator of poor welfare (Broom, 1983). Dietary fibres are believed to enhance satiety (Burton-Freeman, 2000; Howarth et al., 2001; Slavin and Green, 2007; De Leeuw et al., 2008; Bolhuis et al., 2010), and could potentially be used to control energy intake and body weight gain in humans (Wanders et al., 2011), and to reduce hunger and improve welfare of food restricted animals (D'Eath et al., 2009). Many studies have, therefore, been conducted to examine the influence of dietary fibres on satiety, but the results are inconsistent (reviewed by Wanders et al., 2011). To improve understanding of the role of dietary fibre in satiety, it is necessary to identify whether and how dietary fibres with different physicochemical properties, such as bulkiness, viscosity, gelling, and fermentability, affect satiety. This chapter starts with the description of satiety and dietary fibre. This is followed by an overview of the putative mechanisms by which dietary fibre may affect satiety, and methods for studying satiety. Finally, the aim and the outline of the thesis are presented.

Satiety

The word ‘satiety’ originates from the Latin ‘satis’ meaning ‘enough’, and refers to the process that leads to inhibition of further eating, decline in hunger, and increase in fullness after a meal (Blundell et al., 2010). In human nutrition, Blundell and co-workers (1987) developed the concept of the satiety cascade, to describe a series of events that occur preceding or following a meal and that inhibit further eating until the return of hunger signals. The satiety cascade is characterised

by three main phases: sensory and cognitive, post-ingestive and post-absorptive phases (Fig. 1). The first phase is related to the taste, texture and smell of the food during consumption (sensory), and the knowledge of when a last meal was eaten, how much food was consumed, and early experiences associated with that particular food (cognitive). The second phase starts when the food reaches the stomach and involves post-ingestive signals originating from the gastrointestinal tract (neural or hormonal), which inform the brain that food is being processed. In the third phase, metabolites (e.g. fatty acids, glucose, amino acids) become available in peripheral blood and oxidation of nutrients (partly reflected in thermogenesis) occurs in the liver and other metabolically active tissues, so that satiety is maintained until the energy brought by the previous meal has been used and/or stored. At the end of this phase, hunger signals return to inform the brain that more nutrient sources should be ingested. This is the end of the satiety cascade and the beginning of a new ingestive event (Bellisle, 2008; Van Kleef et al., 2011).

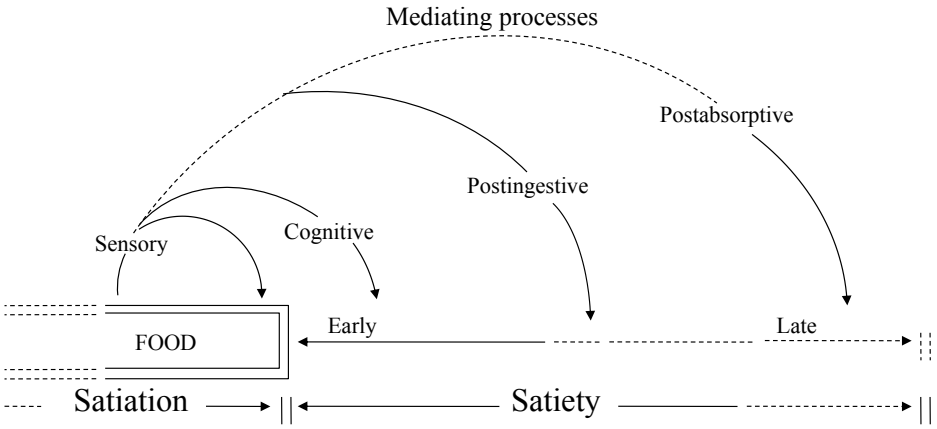

Fig. 1. Satiety cascade (Blundell et al., 1987).

Satiety develops within and between meals and contributes to the regulation of adequate food intake needed to maintain energy balance and body weight (Bellisle, 2008). The satiety that develops within a meal corresponds to the first phase of the satiety cascade (short-term satiety), which leads to satiation or meal termination, whereas the satiety that develops between meals corresponds to the second (mid-term satiety) and third (long-term satiety) phases of the satiety cascade, which lead to changes in inter-meal intervals and subsequent meal initiation (Woods et al., 1998; Van Kleef et al., 2011). The distinction between short-, mid- and long-term satiety is, however, not very sharp. Therefore, preferred terms are satiation and satiety, which are often used to distinguish between short- and long-term regulation

of food intake within a day, which will ultimately affect energy balance and body weight over longer periods of time.

Over the course of the day, animals with free access to food will have a number of meals. Satiation is important in controlling the amount of food consumed at each of these meals, while satiety affects the period of time between meals and potentially the amount consumed at the next meal. Overall, total daily energy intake is a function of both the number of meals per day, and their size and energy content. As control of energy intake is one of the key factors for body weight management, insight in the regulation of both satiation and satiety processes is important (Benelam, 2009).

Satiation or meal termination, and meal initiation related to satiety contribute to energy intake in various ways. Feeling satiated results from a diverse array of neural, hormonal and metabolic signals generated by the gastrointestinal tract in interaction with the brain (Woods, 2004). The integration of these signals in the brain to affect satiation and satiety occurs within the hypothalamus (Benelam, 2009). The hypothalamus contains multiple neural systems important in the regulation of energy balance and body weight. For example, neuropeptides expressed in the arcuate nucleus (ARC) of the hypothalamus are involved in appetite regulation. In this area of the brain, neuropeptide Y/agouti-related peptide (NPY/AgRP) and pro-opiomelanocortin/cocaine-amphetamine-regulated transcript (POMC/CART) neurons respond to peripheral signals, such as hormones and nutrients, and regulate energy balance (Schwartz et al., 2003). Generally, activation of NPY/AgRP neurons stimulates food intake and promotes weight gain (anabolic pathways), and activation of POMC/CART neurons reduces food intake and promotes weight loss (catabolic pathways) (Woods et al., 1998). In the short-term, energy balance is controlled by meal-generated signals, such as satiety hormones and other neural signals via the vagal nerve (e.g. stomach distension). These signals are integrated in the brain, and will thereby affect satiation and satiety and adjust food intake per meal to maintain sufficient energy intake. Short-term signals have limited influence on fat storage and body weight maintenance. The hormones leptin and insulin are secreted proportionally to the amount of fat stored in the body over long periods of time, which ultimately interact with meal-generated signals to alter body weight (Woods et al., 1998; Schwartz et al., 2003).

Satiety signals start to occur already when food is smelled, seen or taken into the mouth, and continue as it reaches the stomach and undergoes further digestion and absorption in the intestine (Benelam, 2009). According to De Graaf and co-workers (2004), meal termination depends on short-term, meal-generated signals such as pleasantness of food, sensory-specific satiety, stomach distension, and on blood concentrations of gastrointestinal hormones such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1).

The characteristics of food, such as palatability (tastiness of food) and texture, determine the sensory pleasantness of food in the mouth, and have been associated with satiation. Most studies that assessed effects of palatability on food intake during a meal indicate that more palatable food result in higher food intake (Sørensen et al., 2003). Palatability is an important factor at the beginning of the meal, but its importance tends to decrease during eating (Bellisle et al., 2000), likely due to the increased secretion of saliva and gastric juices that may contribute to stomach distension (Van Kleef et al., 2011). A coarse texture of food may, however, reduce food intake by intensifying chewing, increasing eating time and stimulating mechanoreceptors in the gastrointestinal tract. These could enhance satiation and promote meal termination via sensory-specific satiety (Rolls and Rolls, 1997). Sensory-specific satiety is a phenomenon that refers to the declining pleasantness of taste generated by the consumption of a certain type of food as compared to a new type of food. This decline in pleasantness of taste is greatest within the first 20 min after food consumption, suggesting that this effect is primarily based on sensory cues, and less on post-ingestive or post-absorptive cues (Smeets and Westerterp-Plantenga, 2006). In recent studies, longer sensory exposure time in the mouth has been also indicated to enhance satiation (Zijlstra et al., 2009; Bolhuis et al., 2011).

In the stomach, increased distension may induce satiation by activation of stretch receptors in the smooth muscles (Hoad et al., 2004) and afferent vagal signals of fullness (Howarth et al., 2001). There is evidence for an inverse relationship between gastric distension and appetite, with CCK and GLP-1 being key-hormones regulating the influx of nutrients from the stomach into the small intestine. CCK and GLP-1 act via effects on pyloric pressure and stomach motility, causing a delay in gastric emptying that reduces appetite and enhances satiation (De Graaf et al., 2004). In summary, satiation depends on short-term signals such as stomach distension and on hormones such as CCK and GLP-1. The sensitivity to these short-term signals is affected by signals that work in the long-term, such as leptin, insulin, and ghrelin (De Graaf et al., 2004). This is because short-term signals serve to adjust food intake per meal, but they have limited influence on long-term fat storage and body weight maintenance, as previously described.

According to Forbes (1988), meal initiation (related to satiety) depends mainly on post-absorptive metabolic changes that follow a meal. In rats, it was shown that there is a correlation between the amount of food eaten in a meal and the interval to the next meal (Forbes, 1988). In this context, glucose was mentioned to play a major role in the regulation of food intake (Mayer, 1952), as a temporary decline (6 to 8%) in blood glucose levels has been shown to precede voluntary meals in rats

(Louis-Sylvestre and Le Magnen, 1980). Therefore, one currently available biomarker for satiety in the short-term is the decrease (both transient and dynamic[1]) in blood glucose within a short time frame (<5 min), which has been shown to be involved in meal initiation. Other biomarkers may include leptin changes during long periods of negative energy balance (>2–4 days), and ghrelin concentrations, which have been implicated in both short-term and long-term energy balance (De Graaf et al., 2004).

In the long-term, satiety is regulated by various gastrointestinal hormones that are secreted into the stomach (e.g. ghrelin, also called the "hunger hormone"), the small intestine and colon (e.g. GLP-1 and peptide tyrosine tyrosine – PYY), and the pancreas (e.g. insulin). They are secreted into the blood where they have a variety of targets, such as, the liver and the brain (Van Kleef et al., 2011). The endocrine mechanisms by which these hormones regulate satiety have been extensively reviewed in recent years (Woods, 2004; Cummings and Overduin, 2007; Benelam, 2009) and are, therefore, only partially discussed in this chapter. Briefly, the vast majority of the gastrointestinal hormones induce satiety, and therefore inhibit food intake following a previous meal and/or postpone initiation of a next meal. Ghrelin is the only hormone currently known to increase food intake. Ghrelin is produced by the stomach, and its rise leads to meal initiation, whereas its suppression after a meal is proportional to the energy intake during that meal, therefore, it is also important for the onset of satiety (De Graaf et al., 2004). Ghrelin may also play a role in long-term regulation of energy balance, as in humans ghrelin levels are inversely correlated with levels of body fat (Benelam, 2009). GLP-1 is a proglucagon-derived hormone produced in intestinal enteroendocrine L-cells and in the brain (Benelam, 2009). In addition to its effects on gastric emptying and satiation, GLP-1 affects also satiety, likely via its effects on the ileal brake (see page 11 for explanation) and glucose homeostasis. In general, enhancement of insulin secretion, suppression of glucagon secretion, and inhibition of gastric emptying are the main determinants controlling glucose homeostasis by GLP-1 (Schirra and Göke, 2005). PYY is also produced by L-cells in the ileum, colon and rectum; it inhibits gastric emptying, increases transit time through the intestine and reduces gastric secretions, indicating that it may play a role in the ileal brake mechanism, and affect satiety in a similar way as GLP-1 (Benelam, 2009). Leptin is mainly produced by adipose tissues; it provides information on the availability of body fat storage to the brain. When subjects are

[1]The endogenous transient decline in blood glucose is defined as a deviation of >5% from a stable baseline blood glucose concentration that lasts ≥5 min. A dynamic decline is a rapid drop in blood glucose after a peak induced by macronutrient ingestion (Melanson et al., 1999).

in negative energy balance, reduced levels of leptin are strongly correlated with increased appetite and food intake, whereas during positive energy balance the relationship between leptin and appetite seems to be less strong (Benelam, 2009). At least in humans, this has been confirmed by a stronger negative correlation between leptin and appetite ratings after 2 to 4 days of 66% energy restriction than before the energy restriction protocol (Mars et al., 2002). Therefore, leptin seems to play a role in the regulation of food intake and satiety, but only when subjects are in negative energy balance (De Graaf et al., 2004). Insulin is a metabolic hormone produced by the pancreas. Unlike leptin, which does not rise immediately in response to food intake, insulin secretion increases rapidly after a meal (Benelam, 2009), as it acts to stabilize blood glucose levels by stimulating the uptake of glucose by peripheral tissues and by suppressing hepatic glucose production (De Graaf et al., 2004). Although blood insulin levels are mostly dependent on blood glucose levels (Woods et al., 2006), insulin responses do not always parallel glucose absorption. An earlier peak of insulin secretion has been shown, for instance, in animals consuming rapidly digestible starch, which was attributed to a conditioned insulin secretion (likely in anticipation to the meal), or stimulation of chemosensory proteins in the tongue, stomach, and proximal small intestine in the presence of free glucose (Regmi et al., 2011). The relationship between blood insulin levels and appetite is usually confounded or moderated by many metabolic processes, therefore, insulin is not recognized as a biomarker for satiety (De Graaf et al., 2004). Altogether, insulin seems to be mostly recognized by its central role in long-term energy balance, as levels of plasma insulin are directly related to changes in body fat stores, and have been found to be increased with obesity (Benelam, 2009).

Dietary fibre

The definition of dietary fibre as non-digestible constituents that make up the plant cell wall was first introduced by Hipsley (1953). In the past decades, the definition of dietary fibre has been extensively discussed, and there are several nationally recognized definitions from experts and organizations being widely used. Recently, a final definition of dietary fibre has been adopted in the 2009 meeting of the Codex Alimentarius Commission (Phillips and Cui, 2011). This definition describes dietary fibre as *carbohydrate polymers with ten or more monomeric units which are not hydrolysed by the endogenous enzymes in the small intestine of humans*. According to this definition, resistance to digestion and absorption in the human small intestine is the key characteristic of dietary fibre. In this thesis, fermentation in the colon will be considered as an important part of dietary fibre metabolism.

Composition and physicochemical properties of dietary fibre

The different components of dietary fibre, modified after the Van Soest analysis (Van Soest et al., 1991), are shown in Fig. 2. According to this analysis, the fraction of dietary fibre that is insoluble in neutral detergents (NDF: neutral detergent fibre) and in acid detergents (ADF: acid detergent fibre) is measured. NDF consists of cellulose, hemicellulose and lignin while ADF consists of cellulose and lignin. However, NDF and ADF analysis are primarily used for the analysis of roughages in ruminant nutrition, and they do not necessarily quantify all components of dietary fibre in monogastric diets. As based on more recent analytical methods that properly quantify other possible fibre components (Champ et al., 2003), dietary fibre corresponds to non-digestible carbohydrates, including non-starch polysaccharides (NSP) (such as cellulose, hemicellulose, fructans and pectins), non-digestible oligosaccharides, and resistant starch (RS). Dietary fibres are resistant to enzymatic degradation by endogenous enzymes and can potentially be broken down by microbial enzymes. Lignin is not considered as part of the dietary fibre fraction, as it is entirely non-digestible, and as such is unchanged during transit through the gastrointestinal tract (Graham et al., 1986). Nevertheless, lignin may be embedded in some dietary fibre where it forms an integral part of secondary plant cell walls (e.g. lignocellulose). The degree of lignification of a dietary fibre source is negatively related to its fermentability (Bach Knudsen, 2001).

Dietary fibres vary in the polysaccharides that make up the plant cell wall and their intermolecular association. Due to this variation, dietary fibres may be classified in accordance to different physicochemical properties, such as solubility in water, viscosity, gel formation, water-binding capacity, and fermentability (Blackwood et al., 2000). Commonly, fibres have been classified as either soluble or insoluble, based on whether they form a dispersion when mixed with water (soluble fibre), or not (insoluble fibre) (Jiménez-Escrig and Sánchez-Muniz, 2000). The molecular structure and especially the type of linkage between polysaccharides determine whether a polysaccharide will dissolve or not. Thus, linear polysaccharides such as cellulose are insoluble whereas polysaccharides with some irregularities in their molecular structure, in the backbone or as side chains tend to be soluble (e.g. pectins, fructans, guar gum, marine polysaccharides) (Guillon and Champ, 2000). A characteristic of insoluble fibres is their faecal-bulking capacity, whereas soluble fibres can be distinguished by their ability to increase the viscosity of digesta and, sometimes, to form viscous gels in the stomach (Jiménez-Escrig and Sánchez-Muniz, 2000; Bach Knudsen, 2001). Soluble and insoluble fibres share many physical properties, such as the ability to bind water or bile-acids, and may be used by colonic microbiota as fermentable substrate (Jiménez-Escrig and

Sánchez-Muniz, 2000). Fibres can be bulking as a result of their coarse structure (volume) or a high water-binding capacity (=water and volume) (De Leeuw et al., 2008). Generally, all fibres have bulking properties, because inclusion of dietary fibre in isocaloric diets reduces dietary energy density (Wanders et al., 2012).

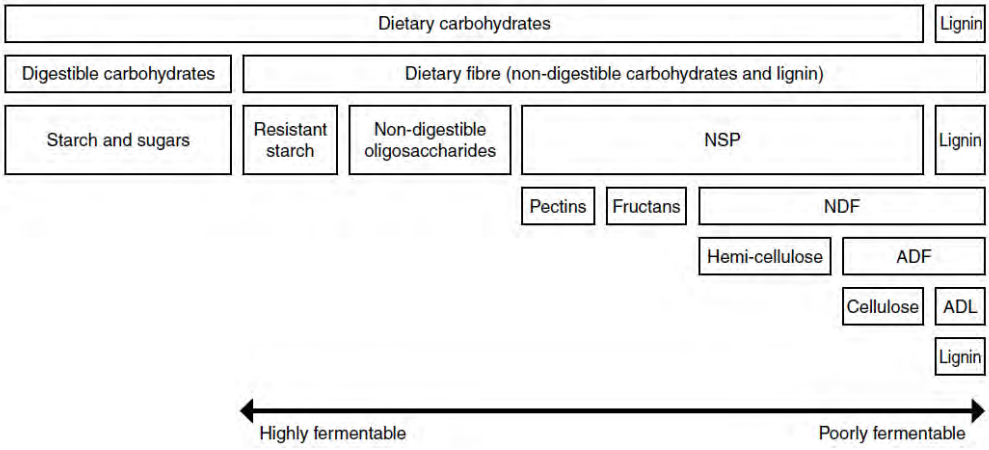

Fig. 2. Schematic representation of dietary fibre (modified after the Van Soest analysis, 1991). NSP, non-starch polysaccharides; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin (De Leeuw et al., 2008).

Dietary fibre and satiety

Dietary fibres may enhance satiety in humans (Slavin and Green, 2007) and animals, including pigs (De Leeuw et al., 2008). Many studies have been conducted to examine the influence of dietary fibres on satiety, but results are variable and sometimes contradictory (Wanders et al., 2011). For example, in pigs fed restrictedly, fermentable fibres clearly reduce physical activity and appetitive behaviours for several hours after the meal, indicating prolonged satiety (De Leeuw et al., 2008). Nevertheless, some fibre sources, e.g. sugar beet pulp (Schrama et al., 1996), and resistant starch (Schrama and Bakker, 1999) seemed more effective to enhance satiety than other sources such as soybean hulls, and solvent-extracted coconut meal (Rijnen et al., 2003). The variable efficacy by which dietary fibres enhance satiety could be related to the physicochemical properties of dietary fibre (Wanders et al., 2011), leading to different effects in the gastrointestinal tract (Benelam, 2009). In addition, satiety enhancing effects of fibres may also be strongly dependent on the level of fibre used. The major physicochemical properties that have been associated with satiety, particularly in pigs, are bulkiness (related to volume or water-binding capacity), viscosity, gelling, and fermentability

(De Leeuw et al., 2008). Based on these properties, putative effects of dietary fibre on satiety throughout the gastrointestinal tract are described below.

Mechanisms by which dietary fibre may affect satiety

In the mouth, sensory information is used as signal initiating the development of satiety within a meal, i.e. satiation (Mattes, 2006). Bulking and viscous fibres may increase chewing effort and time, and saliva production (Howarth et al., 2001), which may lead to early satiation and reduced energy intake (Burton-Freeman, 2000; Zijlstra et al., 2008). This is likely due to the reduced pleasantness of food in the mouth (De Graaf et al., 2004), and aspects of sensory-specific satiety (Rolls and Rolls, 1997) previously described in this chapter. Further down in the gastrointestinal tract, the sensory information obtained in the mouth is thought to enhance the satiating effects of food in the stomach and intestine (Mattes, 2006; Smeets and Westerterp-Plantenga, 2006). This is likely a result of anticipatory responses (e.g. digestive secretions) that prepare animals to digest, absorb and metabolize nutrients (Power and Schulkin, 2008).

In the stomach, distension is used as a signal of satiety within a meal. There is evidence that with increasing gastric distension, appetite decreases (De Graaf et al., 2004; Benelam, 2009). Bulking and viscous fibres could promote gastric distension due to the extra production of saliva and secretion of gastric juices (Howarth et al., 2001). Bulking fibres with a high water-binding capacity may further increase gastric distension, by expanding their volume from, for instance, 3-fold (e.g. alginate, see Wanders et al., 2012) up to 8-fold (e.g. lignocellulose, see Büttner, 2006) in the stomach. This will increase satiety feelings (De Graaf et al., 2004; Benelam, 2009), likely via afferent vagal signals of fullness (Howarth et al., 2001). Apart from distension, gastric emptying also plays a role in satiety signalling, both within and between meals (Delzenne et al., 2010). A delay in gastric emptying accompanied by an increase in gastric distension is often associated with enhanced satiety (Bergmann et al., 1992; De Graaf et al., 2004). It has been hypothesized that viscous fibres could delay gastric emptying via a direct effect of the food nutrients trapped within a viscous or gelled matrix that will more slowly leave the stomach (Howarth et al., 2001), or via an indirect effect of hormones released from the gastrointestinal tract when food passes from the stomach into the small intestine (Schneeman, 1998). As previously described, the hormones CCK and GLP-1 regulate the influx of digesta from the stomach into the small intestine (De Graaf et al., 2004). These hormones may contribute to the ability that viscous fibres have to influence satiety both within and between meals.

From the stomach, food reaches the small intestine where information about digestion and absorption of nutrients serves to signal satiety already within a meal,

but mostly between meals (Benelam, 2009; Delzenne et al., 2010). In the small intestine, the presence of unabsorbed macronutrients (fat, carbohydrates and proteins) and viscous fibres is associated with delayed gastric emptying and slower ileal transit time, known as ileal brake (Howarth et al., 2001). The ileal brake is a mechanism by which motility and secretion become reduced in the distal portion of the small intestine, so that digestion is optimized along the intestine (Maljaars et al., 2008). Next to GLP-1, PYY and oxyntomodulin (OXM) are hormones that may mediate the ileal brake (Maljaars et al., 2008). Apart from macronutrients, fibres could also influence the activation of the ileal brake (Maljaars et al., 2008). Especially viscous or gel-forming fibres are associated with slower transit time of digesta, and a reduced rate of nutrient absorption (Brownlee, 2011), which could contribute to enhanced feelings of satiety, because they will increase the time that digesta stay in contact with intestinal absorptive surfaces (Howarth et al., 2001). This process is likely resulting in increased secretion of satiety-related hormones, such as the ones involved in the ileal brake (Brownlee, 2011).

Also in the colon, a braking mechanism referred to as the colonic brake, can be influenced by fibres which are fermented by microbiota in the colon (Maljaars et al., 2008). The colonic brake seems to be caused by a local peak of GLP-1 secretion, triggered by end-products of fermentation such as short-chain fatty acids (SCFA). In face of that, GLP-1 exhibits a biphasic response after food ingestion, with the first peak occurring before nutrients reach the distal portion of the small intestine (about 15 to 30 min after food ingestion, likely via neural regulation), followed by the second peak occurring in the colon several hours after food ingestion (Delzenne et al., 2010), likely coinciding with a significant rise in fermentation and SCFA production. It is still unclear if signals arising from the colon add to the ileal brake effect (Maljaars et al., 2008). SCFA (mainly acetate, propionate and butyrate) may contribute to enhanced feelings of satiety between meals because of two main aspects: 1) SCFA serve as an additional energy source, especially at moments when glucose absorption is decreasing (or completed) in the small intestine (De Leeuw et al., 2008); and 2) SCFA may increase production and secretion of satiety-related hormones GLP-1 and PYY (Delzenne and Cani, 2005), possibly via G-protein coupled receptors expressed by L-cells (Darzi et al., 2011). Moreover, the exchange of starch for fibre results in lower postprandial glucose and insulin responses due to a decreased influx of glucose via enzymatic digestion of starch (Higgins, 2004).Throughout the day, a combination of a reduced glucose supply and an increased SCFA production may enhance satiety, likely because SCFA prolong the energy supply to the body, which may bridge the energy gap during the interprandial period (Rérat, 1996; Darzi et al., 2011). It is likely that dietary fibres with different physicochemical properties operate via various

mechanisms to induce feelings of satiation and satiety, and many of these are interconnected.

Methods for studying satiating properties of dietary fibres: using the pig as a model animal

In humans, satiating effects of dietary fibre are usually investigated through short-term studies where humans are given diets supplemented with dietary fibre. Measurements include changes in hunger or fullness ratings, voluntary food intake and body weight gain (Wanders et al., 2011). In addition, associations between different dietary fibre types and long-term changes in energy intake and body weight gain may also originate from epidemiological studies (e.g. Rienks et al., 2013). Although these studies indicate whether or not different types of fibre affect satiety or body weight gain, the mechanisms by which dietary fibres may affect satiety remain to be identified. Animal studies could help identify the mechanisms by which fibres influence satiety (Van Kleef et al., 2011). Factors involved in food intake and satiety regulation, such as social and physical environment, physical activity and environmental cues, can be better standardized in animals than in humans. Moreover, invasive studies in animals, as a model for humans, are considered to be ethically acceptable, which allows collection of body tissues, for instance for measurement of gene expression in specific organs that may be involved in fibre-induced satiety. Rodents, mainly mice and rats, have been widely used as models for humans due to their low-cost maintenance and sequenced genome, which made genetic modification easier (Spurlock and Gabler, 2008). In spite of that, physiological and metabolic differences with humans have complicated the translation of research findings in mice and rats into the human situation (Spurlock and Gabler, 2008). For example, rats and pigs are omnivorous like humans, but mice are granivorous (feeding on seeds and grain). The stomach of mice and rats is divided into a glandular and a non-glandular portion, whereas in humans and pigs the stomach is only of glandular origin, and is lined with cardiac, gastric, and pyloric mucosa (Kararli, 1995). Mice and rats practice coprophagy, whereas humans and pigs do not (Baker, 2008). Humans and pigs obtain usable energy from fermentable fibre predominantly via colonic fermentation, whereas mice and rats are caecal fermenters, and their colon, neither sacculated nor long, differs substantially from that of humans and pigs (Kararli, 1995). Furthermore, numerous contrasting results between rodent models and humans, for example in adipsin, leptin, resistin, tumor necrosis factor-α, and other adipokines illustrating important differences related to obesity, insulin resistance, and inflammation, have hindered the translation of rodent data for humans (Spurlock and Gabler, 2008).

Alternatively, the domestic pig has been increasingly advocated as a more suitable animal model, because of similarities in genome (3x closer to the human than the mouse, see Wernersson et al., 2005), anatomy, digestive physiology, body size and diet (Graham and Aman, 1987; Guilloteau et al., 2010; Clouard et al., 2012). Moreover, obese pigs are also more similar to obese humans than obese mice and rats, as the major contribution to body fat mass is, in both humans and pigs, the subcutaneous adipose tissue (Spurlock and Gabler, 2008). Pigs are omnivorous colonic fermenters, with the distal small intestine also serving as a site of fermentation, like in humans (Topping and Clifton, 2001), and are therefore increasingly used as models to study human digestive function (Guilloteau et al., 2010). Their larger body size and blood volume allows the use of more frequent blood sampling for measurement of postprandial plasma responses of satiety-related hormones and metabolites than is possible in rodents. Moreover, intestinal cannulation is a standard technique for studying nutrient and energy digestibility in pigs, which may be used for multiple live tissue and digesta samplings. In this thesis, the domestic pig is used both as a model for humans, and as a target animal, as more knowledge on satiating properties of fibres and mechanisms involved in fibre-induced satiety can also be applied in pig nutrition to reduce hunger, and thereby improve welfare of restrictedly-fed pigs.

To study satiety in humans, often a fixed amount of a defined food is consumed and after a time interval, the effect of the food on subsequent energy intake is measured. Visual analogue scales (VAS) are used to assess ratings of fullness (Rolls, 2009; Blundell et al., 2010), usually at one or more time points after consumption of a test meal. VAS have been shown to correlate with energy intake, but do not reliably predict energy intake to the extent that they could be used as a proxy of energy intake (Stubbs et al., 2000). Therefore, these measurements, VAS and energy intake, are used in conjunction. Contrary to humans, animals cannot be directly asked a number of questions relating to their motivation to eat and satiety feeling. Alternatively, to study satiety in animals, feeding motivation tests can be used to assess the strength of the motivation to obtain food (i.e. reversal satiety) (Dawkins, 1990). Generally, feeding motivation (as an indicator of satiety) is expected to decrease when the animal's feeling of satiety, reflecting metabolic processes, is increased (Dawkins, 1990; D'Eath et al., 2009). Feeding motivation tests are also used in humans, for instance to assess factors (other than metabolic processes) that influence the reinforcing value of food and the motivation to eat (Raynor and Epstein, 2003; Epstein et al., 2007) during disorders of ingestive behaviour, such as obesity and anorexia nervosa. In this thesis, feeding motivation is used as proxy measure of satiety in animals.

Feeding motivation tests can be applied at various time points after consumption of a test meal (i.e. dietary treatment of interest). These repeated

measurements may give a better indication of physiological mechanisms by which dietary fibres affect satiety over time than a single measurement, because signals may originate from various sites along the gastrointestinal tract, and the time taken for food to transit through the gastrointestinal tract takes hours within the stomach and small intestine up to days within the colon (Brownlee, 2011). In addition, observations of individual pigs, particularly of behaviours related to food search and food consumption (De Leeuw et al., 2008; D'Eath et al., 2009), may be used as an extra indicator of feeding motivation over the day. For example, a shorter meal or a shorter duration of the first bout of food consumption may indicate earlier satiation, whereas explorative and foraging behaviours may indicate reduced satiety, but also stereotypic behaviours, like sham chewing, nosing, rooting or chewing the trough, floor, chains and other fixtures in the pen, may indicate that animals are not fully satiated (D'Eath et al., 2009). General physical activity could also be a measurement of reduced satiety, because in animals that are not fully satiated there is often an increase in activity associated to the increase in food search (Beattie and O'Connellt, 2002). Thus, feeding motivation tests combined with behavioural observations are considered valuable tools in the assessment of hunger and satiety in animals (Day et al., 1997). In the first two studies presented in this thesis, feeding motivation tests were applied at different time points after the meal to investigate whether dietary fibres with different physicochemical properties would affect satiety in pigs, used as model for humans and as a target animal. Furthermore, to be able to identify how some dietary fibres would regulate satiety, the approach used was integrative, combining measurements at various levels: intestine, microbiota, blood, liver and the whole organism (e.g. feeding patterns, behaviour and growth).

Aim and outline of the thesis

The research described in this thesis is part of the IP/OP strategic research program 'Satiety & Satisfaction' of Wageningen UR. The primary goal of this program is to combat obesity by changing the satiating properties of food products. The subprojects included in this program deal with a variety of aspects such as plant derived foods, food patterns of consumers, health effects of making fibre-rich foods more satiating, and satiety regulation. As part of satiety regulation, the project entitled 'Fermentation in the gut to prolong satiety' aimed to identify whether and how dietary fibres with different physicochemical properties, such as bulkiness, viscosity, gelling, and fermentability, affect satiety in the pig, which is used as a model for humans and as a target animal.

Fig. 3 gives a schematic presentation of the outline of this thesis. The first study (**Chapter 2**) was aimed at studying the effectiveness of three fibres with different

physicochemical properties (viscous vs. bulking vs. fermentable) to affect long-term satiety in adult pigs. Several behavioural tests, including an operant test and a runway test, were developed and subsequently used to assess feeding motivation at different times of the day (1 h, 3 h, and 7 h after the meal). Among the fibres tested in Chapter 2, the fermentable fibre reduced feeding motivation and enhanced satiety for up to 7 hours after a meal. Therefore, a second study (**Chapter 3**) was conducted to assess effects of three other fermentable fibres with different fermentation characteristics (i.e. fermentation kinetics and SCFA-profile) on satiety in the same adult pigs used in Chapter 2. To facilitate interpretation of the results with regard to putative satiety mechanisms related to fibre properties, purified dietary fibre components were used in the studies described in Chapter 2 and 3. All fibres studied in Chapters 2 and 3 were tested at two inclusion levels to study dosage effects. Individual differences in response to experimental conditions are inevitable in behavioural research. Thus, in the studies described in Chapters 2 and 3, adult sows were assigned to all dietary treatments in a Latin square design. In this way, the animals served as their own controls, which increased power by better control of variation in individual response. Among the fermentable fibres tested in Chapter 3, resistant starch gave most pronounced effects on feeding motivation and satiety. Therefore, resistant starch was selected to identify physiological and molecular mechanisms by which fermentation may regulate satiety at the gut and systemic level (**Chapters 4 and 5**, carried out together with the Nutrition, Metabolism & Genomics Group at the Division of Human Nutrition from Wageningen University). A final study was conducted to match human studies on satiation (project entitled 'Food, fibre and health – an integrated approach', Wanders, 2013) with pig studies on satiety. The fibres studied in the first 5 chapters were provided to food restricted animals. Ultimately, any fibre source contributing to satiation and satiety in function of body weight management should affect voluntary food intake, and also energy intake and body weight gain. To this end, a study was designed (**Chapter 6**) to study the long-term effects of a gelling fibre (alginate) demonstrated to increase satiation in humans (Wanders et al., 2012) and pigs (see Chapter 7, general discussion) in the presence or absence of a fibre promoting satiety in pigs (resistant starch, findings in Chapters 2 to 5). Effects of these fibres, fed ad libitum during 12 weeks, on satiation, satiety (via feeding patterns), energy intake, and body weight development of growing pigs were assessed. In **Chapter 7**, the general discussion, the methods for studying satiety and the major findings of studies are discussed. Moreover, an overview of the main conclusions with their implications is given.

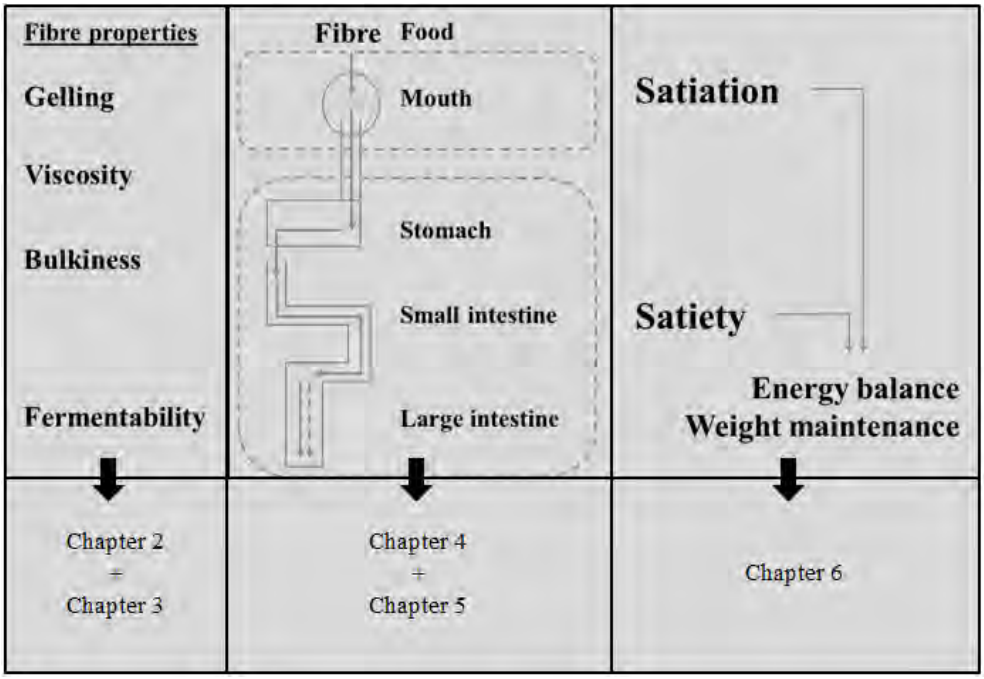

Fig. 3. Schematic presentation of the outline of this thesis.

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Chapter 2

Effects of dietary fibres with different physicochemical properties on feeding motivation in adult female pigs

Satiating properties of bulking, viscous and fermentable fibres

Carol Souza da Silva[1,2], Joost J.G.C. van den Borne[2], Walter J.J. Gerrits[2], Bas Kemp[1], J. Elizabeth Bolhuis[1]

[1] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

[2] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

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Abstract

The satiating effects of dietary fibre may depend more on physicochemical properties of the fibre than on total fibre intake. These properties are expected to affect satiety feelings and feeding motivation due to different effects in the gastrointestinal tract. The aim of the current study was to assess the effects of fibres with varying physicochemical properties (bulkiness, viscosity and fermentability) on feeding motivation in adult female pigs. Sixteen pair-housed pigs received four diets: lignocellulose (LC), pectin (PEC), resistant starch (RS), and control (C) without fibre, in four periods in a Latin square design. Each fibre was fed at a low (L) followed by a high (H) inclusion level (7 days each). At 1 h, 3 h, and 7 h after the morning meal, feeding motivation was assessed in an operant test, where turning a wheel yielded multiple food rewards, and in a runway test, where walking a fixed U-shaped track yielded one food reward. Pigs were observed in their home pen for 6 h, using 90-s instantaneous scan sampling. In the operant test, throughout the day feeding motivation was higher for pigs on PEC compared with pigs on LC. In the runway, feeding motivation increased particularly at 1 h after the meal for pigs on PEC compared with pigs on RS. Also at 7 h, feeding motivation tended to decrease for pigs on RS compared with pigs fed other diets. In their home pen, pigs on PEC showed more feeder-directed behaviour compared with pigs on RS. In conclusion, PEC was the least satiating fibre. LC and RS, despite a lower supply of available energy, were the most satiating fibres, possibly due to their bulky and fermentation properties, respectively.

Keywords: dietary fibre, satiety, feeding motivation, pigs, operant test, runway

Introduction

Dietary fibres are believed to enhance satiety in humans (Delzenne and Cani, 2005; Slavin and Green, 2007) and animals, including pigs (Bolhuis et al., 2010; De Leeuw et al., 2008), cats (Bissot et al., 2010; Servet et al., 2008), dogs (Bosch et al., 2009a and 2009b), and rats (Cani et al., 2005; Zhou et al., 2008). This could be relevant not only for reducing energy intake and ultimately body weight in humans, where obesity is an increasing problem (O'Rahilly and Farooqi, 2008), but also for improving welfare in restrictedly fed animals, such as adult pigs, which may suffer from hunger and related welfare problems (D'Eath et al., 2009; De Leeuw et al., 2008).

The efficacy by which different types of dietary fibre induce satiety is variable (Slavin, 2010; Willis et al., 2009). This could be due to the physicochemical properties of dietary fibres, including bulkiness (or water-binding capacity), viscosity and fermentability, which are expected to influence digestive physiology in different ways throughout the gastrointestinal tract (Benelam, 2009).

In the mouth, bulky and viscous fibres increase chewing activity and saliva production (Howarth et al., 2001), which can result in early satiety and reduced energy intake (Burton-Freeman, 2000; Zijlstra et al., 2008). In the stomach, fibres may cause gastric distension due to the extra production of saliva and secretion of gastric juices (Howarth et al., 2001). Bulky fibres with a high water-binding capacity may further increase gastric distension, by expanding their volume up to 8-fold in the stomach (Büttner, 2006), which will increase satiety feelings (Benelam, 2009; De Graaf et al., 2004), probably via afferent vagal signals of fullness (Howarth et al., 2001). Moreover, a delay in gastric emptying accompanied by an increase in gastric distension is often associated with enhanced satiety between meals (Bergmann et al., 1992; De Graaf et al., 2004). It has been hypothesized that viscous fibres may delay gastric emptying via a direct effect of the nutrients trapped within a viscous matrix that will more slowly exit the stomach (Howarth et al., 2001), or via an indirect effect of hormones released from gastrointestinal tissues when food passes from the stomach into the small intestine (Schneeman, 1998). Glucagon-like peptide (GLP-1) and cholecystokinin (CCK) are key hormones responsible for maintaining a constant flux of nutrients leaving the stomach (De Graaf et al., 2004). In the small intestine, the presence of unabsorbed macronutrients and viscous fibres delays gastric emptying and prolongs transit time (Howarth et al., 2001). This mechanism, which is mediated by GLP-1 and peptide tyrosine tyrosine (PYY), is also known as ‘ileal brake’ and ultimately enhances digestion and absorption of food (Maljaars et al., 2008). The ileal brake could contribute to enhanced feelings of satiety, because it will increase the time that digesta stays in contact with intestinal absorptive surfaces (Howarth et

al., 2001), which is likely to result in increased secretion of satiety-related hormones (Brownlee, 2011). Finally, dietary fibres can be fermented in the distal small intestine but predominantly in the colon, thereby increasing the production of short-chain fatty acids (SCFA), which may also enhance satiety (Sleeth et al., 2010). SCFA serve as an additional energy source, especially at moments when glucose absorption is decreasing (or completed) in the small intestine (De Leeuw et al., 2005), and they stabilize levels of glucose and insulin in blood (Higgins, 2004). In addition, SCFA stimulate the production and secretion of satiety-related hormones, such as GLP-1 and PYY (Delzenne and Cani, 2005), possibly via G-protein coupled receptors (Darzi et al., 2011). Therefore, it seems likely that dietary fibres with different physicochemical properties operate via various mechanisms to induce feelings of satiety.

Studies in humans have revealed variable and sometimes contradictory effects of fibre intake on appetite and body weight development (e.g. Wanders et al., 2011), which may partly originate from methodological issues. Most studies have not included multiple levels of fibre when comparing fibres with different properties, whereas effects of fibres on satiety are often dose-dependent (Delargy et al., 1997; Serena et al., 2008). Optimal levels may, therefore, differ between types of dietary fibre.

Moreover, the time at which feelings of satiety are assessed may be crucial, because satiety signals may originate from various sites along the gastrointestinal tract, and the time taken for food to transit through the gastrointestinal tract takes hours within the stomach and small intestine up to days within the colon (Brownlee, 2011). This means that the satiating effects of fibre will vary according to the time relative to eating, and ideally, they should be assessed at different time points after the meal, instead of only at one particular time point. Similarly, interpretation of single test meal studies is often complicated by the lack of long-term (e.g. second meal) effects on feeding motivation and subjective appetite. Such adaptation may especially be required for fermentable fibres to allow measurements at steady state. According to Zhou et al. (2008), the gut microbiota needs time to adapt to a new fibre source before the full effects of fermentation can occur. This may explain contradicting results found with fermentable fibres in some human studies (Raben et al., 1994; De Roos et al., 1995).

Finally, external factors (availability and palatability of food, environment) vs. internal factors (metabolic state and feelings of satiety) (Barbano and Cador, 2005), and large variation between individual subjects complicate standardization of human studies. Alternatively, the domestic pig, which is increasingly advocated as a suitable model for digestive function in humans because of similarities in genome, gastrointestinal function, body size and (omnivorous) diet (Clouard et al., 2012; Guilloteau et al., 2010; Moughan et al., 1994), can be used. A major

advantage of using the adult pig as a model for humans is the better standardization of experimental factors involved in satiety regulation, such as age, body weight, gender, and food intake. Moreover, knowledge of satiety-enhancing fibres can also be applied in pig nutrition to reduce hunger and thereby improve welfare of restrictedly fed sows (De Leeuw et al., 2008).

The aim of our study was to assess the effects of three types of fibre with different physicochemical properties (bulking vs. viscous vs. fermentable), each at two levels (low vs. high), on feeding motivation throughout the day in adult female pigs in a Latin square design. The fibres were exchanged for pregelatinized starch from a control diet based on equal gross energy (GE) content. This means that the satiating effects of the fibres had to overcome effects of a reduced available energy supply resulting from the removal of starch from the control diet. Satiety and hunger are subjective states that cannot be directly measured (see D'Eath et al., 2009), but they are reflected by the individual's feeding motivation, i.e. the strength of the motivation to obtain food (Dawkins, 1990). Feeding motivation tests are therefore considered valuable tools in the assessment of hunger and satiety (Day et al., 1997), and were applied at different time points after the meal in the present study. In addition, behavioural observations of individual pigs, particularly of behaviours related to food search and food consumption (D'Eath et al., 2009; De Leeuw et al., 2008), were used as an indicator of feeding motivation over the day.

Materials and methods

The effects of fibre type (3 types and a control) and fibre level (2 levels) on the feeding motivation of 16 adult female pigs were assessed. Each pig received four dietary treatments (i.e. one of three fibre types or control, each at two inclusion levels) in four periods according to a Latin Square design. Each period lasted 14 days. During the first 7 days of each period, pigs were fed a low fibre diet (L), and during the second 7 days they were fed a high fibre diet (H) (for details see *Diets and feeding*). The protocol for this experiment was approved by the Animal Care and Use Committee of Wageningen University.

Animals and housing

One-year-old female pigs (gilts) with an initial body weight of 199±2.3 kg (n=16; PIC Benelux B.V.) were used in this experiment. They were housed in pairs in 11 m^2 partially slatted pens in two climate controlled rooms at the experimental facilities of Wageningen University, The Netherlands. The two pigs within a pen received the same diet within each period. They were individually fed using two separate feeding places with closing gates. Each feeding place was equipped with a

feeder and a water barrel connected to a nipple drinker next to the feeder. Room temperature was maintained at 20±1 °C and pens were cleaned daily. Artificial lights were on from 0630 until 2200 h and dimmed during the dark period. Pigs were provided with a variety of toys, which were changed daily.

Diets and feeding

Pigs were assigned to four dietary treatments differing in fibre type: lignocellulose (LC), resistant starch from native potato starch (RS), highly methylated citrus pectin (PEC), and a high-starch control (C). Fibres were selected based on different physicochemical properties that were expected to have various effects in the gastrointestinal tract (which can be subsequently related to satiety): LC is a bulky fibre, RS is a fermentable fibre and PEC is a viscous fibre. The different fibres were exchanged for pregelatinized starch (i.e. readily digestible starch) from the C diet based on GE content (Table 1), implying that the satiating effects of the fibres were measured by their ability to overcome the effects of a reduced available energy supply from the fibre diets compared to the C diet. For studying dose-response relationships, each fibre was fed at two inclusion levels, i.e. a low inclusion level (L) during the first 7 days and a high inclusion level (H) during the last 7 days of each period. Animals received the two inclusion levels always in this order (L–H) to avoid upsetting their digestive system by abrupt introduction of high levels of dietary fibres. The maximum level of fibre that could be provided to the pigs without causing digestive problems or feed refusals was determined, based on previous studies (Backers, 2005; Bolhuis et al., 2008; Drochner et al., 2004), and used for formulating the high fibre diets. For the low fibre diets, 50% of the high level was used. Effective dosages for inducing satiety were expected to differ between fibre sources, due to different working mechanisms (bulking, viscous, fermentable). Therefore, levels were specific for each fibre type: 50 g/kg and 100 g/kg for LC (LC-L and LC-H, respectively), 197 g/kg and 394 g/kg for RS (RS-L and RS-H, respectively), and 75 g/kg and 149 g/kg for PEC (PEC-L and PEC-H, respectively). Because all fibre sources were exchanged for pregelatinized starch, diets were isoenergetic on GE basis, but not on metabolizable energy (ME) basis. To assess the effect of ME intake loss on feeding motivation, two C diets (C-L and C-H) were formulated with different inclusion levels of pregelatinized starch, which means that total ME intake differed between the two C diets. In the C-L diet, a lower inclusion level of pregelatinized starch (−79 g/kg of the C-H diet) was used to reach a similar level of ME as in the diet with the highest level of fibre, and hence the lowest in ME, i.e. the RS-H diet, as estimated from indirect calorimetry data (Bolhuis et al., 2008). Diets were manufactured, as mash, at a research feed mill (Research Diet Services B.V., Wijk

bij Duurstede, The Netherlands), and were given a cherry honey flavour (Luctarom Advance Cherry Honey, Lucta S.A., Barcelona, Spain) to mask differences in palatability as much as possible.

Table 1. Dietary treatments.

Ingredient, g/kg[1]	Diet							
	Control		Lignocellulose		Resistant starch		Pectin	
	Low	High	Low	High	Low	High	Low	High
Basal diet[2]	650	650	650	650	650	650	650	650
Pregelatinized potato starch[3]	271	350	300	250	175	–	275	200
Native potato starch[4]	–	–	–	–	197	394	–	–
Pectin[5]	–	–	–	–	–	–	75	149
Ligno-cellulose[6]	–	–	50	100	–	–	–	–
Total[7]	921	1000	1000	1000	1022	1044	1000	1000

[1] Ingredients are expressed in g/kg of the Control-High diet and sum up to 1000 for all diets in which the gross energy (GE) content of the fibre sources is identical to that of the pregelatinized potato starch. Deviations from 1000 in other treatment groups, therefore, reflect the relative difference in feed allowance of these groups (see footnote 7).

[2] Composition basal diet (per kg diet): potato protein, 50 g; maize gluten meal, 100 g; wheat, 249 g; soy oil, 15 g; animal fat, 15 g; barley, 150 g; vitamin and mineral premix, 10 g; calcium carbonate, 17 g; monocalcium phosphate, 11 g; potassium chloride, 10 g; sodium bicarbonate, 15 g; salt, 5 g; L-lysine HCl, 1.5 g; flavour Luctarom Advance Cherry Honey (LUCTA S.A., Barcelona, Spain), 1.5 g. The vitamin and mineral premix supplied (per kg diet): retinol, 10000 IU; cholecalciferol, 2000 IU; DL-α-tocopherol, 25 mg; menadione, 1 mg; thiamin, 0.75 mg; riboflavin, 4 mg; D-pantothenic acid, 13 mg; niacin, 20 mg; cyanocobalamin, 15 µg; folic acid, 2.5 mg; biotin, 0.1 mg; pyridoxine chloride, 1 mg; choline chloride, 300 mg; Fe as $Fe2SO_4 \cdot H_2O$, 80 mg; Cu as $CuSO_4 \cdot 5H_2O$, 10 mg; Zn as $ZnO_4 \cdot H_2O$, 60 mg; Mn as MnO, 30 mg; Co as $CoCO_4 \cdot 7H_2O$, 0.2 mg; I as KI, 0.75 mg; Se as Na_2SeO_3, 0.2 mg.

[3] Paselli™ WA 4 (Avebe Food, The Netherlands).

[4] Native potato starch (Avebe Food, The Netherlands).

[5] Unipectine™ RS 150 Citrus (Cargill, Belgium).

[6] Arbocel® RC Fine (J. Rettenmaier & Söhne, Germany).

[7] Pigs were fed equal amounts of basal diet. As the GE content of resistant starch was lower than that of pregelatinized potato starch, this was compensated by increasing the feed allowance for the resistant starch treatments (i.e. 102.2 and 104.4% of that of the Control-High diet). The difference between the Control-High and Control-Low treatments was designed to provide a reduced metabolizable energy (ME) intake, identical to the estimated drop in ME intake in the treatment with the lowest estimated ME intake (i.e. the Resistant starch-High diet), see text for details. Therefore, the feed allowance of the Control-Low treatment was 92.1% of that of the Control-High treatment.

Pigs were fed 1150 g per meal twice daily, i.e. at 0700 h and 1700 h. All pigs received a similar amount of food at approximately 1.5 times the energy requirements for maintenance (net energy=293 kJ/$kg^{0.75}$/day), based on the average metabolic body weight ($kg^{0.75}$) of the pigs at the start of the experiment. At a maximum time of 1 h after food was provided, gates of the individual feeding places were reopened and food refusals (if present) were collected. Water was continuously available throughout the study, via a nipple drinker located next to the feeder.

Prior to the start of the experiment, pigs were gradually adapted, in 6 days, to each of the high fibre diets to prevent food neophobia during the experimental phase. Pigs received a mixture of C-H and high fibre (LC-H, RS-H or PEC-H) diets in different proportions: 75% C-H and 25% H-fibre diet on day 1; 50% C-H and 50% H-fibre diet on day 2; 25% C-H and 75% H-fibre diet on day 3; and 100% H-fibre diet on days 4 to 6. The order of diets during this habituation was randomized over the pens. Then, all pigs were adapted to the experimental feeding level by receiving the C-H diet for 18 days before the start of the dietary treatments.

Measurements

In each period (day 1 to day 14, Fig. 1), effects of the different diets on feeding motivation were assessed via behavioural observations of individual pigs in their home pen, and via two feeding motivation tests: an operant test, and a runway test performed at different time points after ingesting the diets.

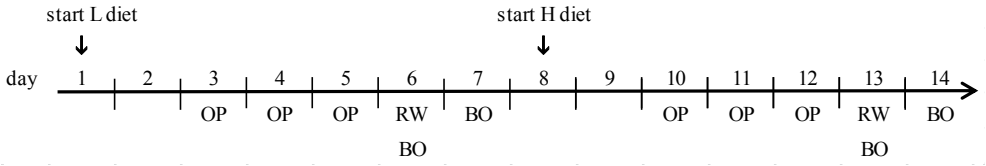

Fig. 1. Schedule of measurements of feeding motivation applied to adult female pigs fed four dietary treatments (i.e. one of three fibre types or control), each at a low (L) followed by a high (H) inclusion level for a period of 14 days. OP=operant test, RW=runway test, BO=behavioural observations.

Before the start of the experiment, pigs were habituated to the experimenter, to the test room, and to the feeding motivation tests. Pigs were trained for the operant test daily. They were first habituated to the operant apparatus and test room in groups of three (five days), followed by habituation in pairs (five days) and finally trained individually until the experiment started (36 days). During the individual training, one pig per pen was trained to turn a wheel (pushing it with the snout) to receive multiple food rewards. During a training session, the number of wheel turns per reward was adjusted according to a progressive ratio (PR) schedule (see *Operant test*). Each training session ended when the pig had obtained 10 rewards (equal to 55 wheel turns), or at a maximum time of 5 min. For the runway test, all pigs were individually trained to walk a fixed route (see *Runway test*) to receive one food reward (25 days). Each training session ended when the pig had reached the food reward, or at a maximum time of 5 min. In both tests, animals were tested individually, and the floor of the test room was cleaned whenever a pig defecated and/or urinated. Five days prior to the start of the dietary treatments, pigs were

exposed to the complete schedule of testing and measurements were taken according to the same schedule as used in the experiment (Fig. 1).

Operant test

On days 3, 4, 5 (all at low inclusion level), 10, 11 and 12 (all at high inclusion level) in each period, one pig per pen (n=8) was subjected to an operant test (Fig. 2A).

The test took place in a separate, climate-controlled test room (13 m^2, temperature 20±1 °C) containing a tailor-made feeder (Fig. 3) operated by a computer (Verbakel B.V., Sint Oedenrode, The Netherlands). This feeder contained a wheel and turning the wheel 360° in forward direction resulted in delivery of a food reward from a dispenser. The higher the number of rewards obtained, the higher the feeding motivation of the pigs. The force needed to turn the wheel was kept constant (0.02±0.006 N m).

Each reward was 10 g (0.43% w/w of daily food allowance) of a commercial pelleted feed (per kg: crude protein, 135 g; crude fat, 49 g; crude fibre, 79 g). The delivery of food was automatically controlled via a tailor-made software package designed for operating the feeder. Via a sensor positioned next to the wheel, the number of wheel turns performed was detected, and the food reward was delivered after performance of the required number of wheel turns in a PR schedule. In this schedule the response contingency was increased in a prearranged way for successive rewards. In the present study, the pigs were required to turn the wheel once for the first reward, then twice for the second reward, then three times for the third reward, and so on. A PR schedule usually leads to large variation in operant response between individuals (Lawrence and Illius, 1989). In spite of this variation, previous studies in growing (Lawrence and Illius, 1989) and adult (Souza da Silva et al., 2010) pigs, in which a contrast in feeding level was created, have demonstrated that a PR schedule is more sensitive to measure changes in feeding motivation than a fixed ratio (FR) schedule.

The computer recorded the cumulative number of rewards from which the cumulative number of turns was calculated. The number of turns was used as main indicator for feeding motivation in this test. In addition, the latency time to turn the wheel for the first time after entering the test room, the total time spent in the test room (duration), and the time spent at the feeder were recorded.

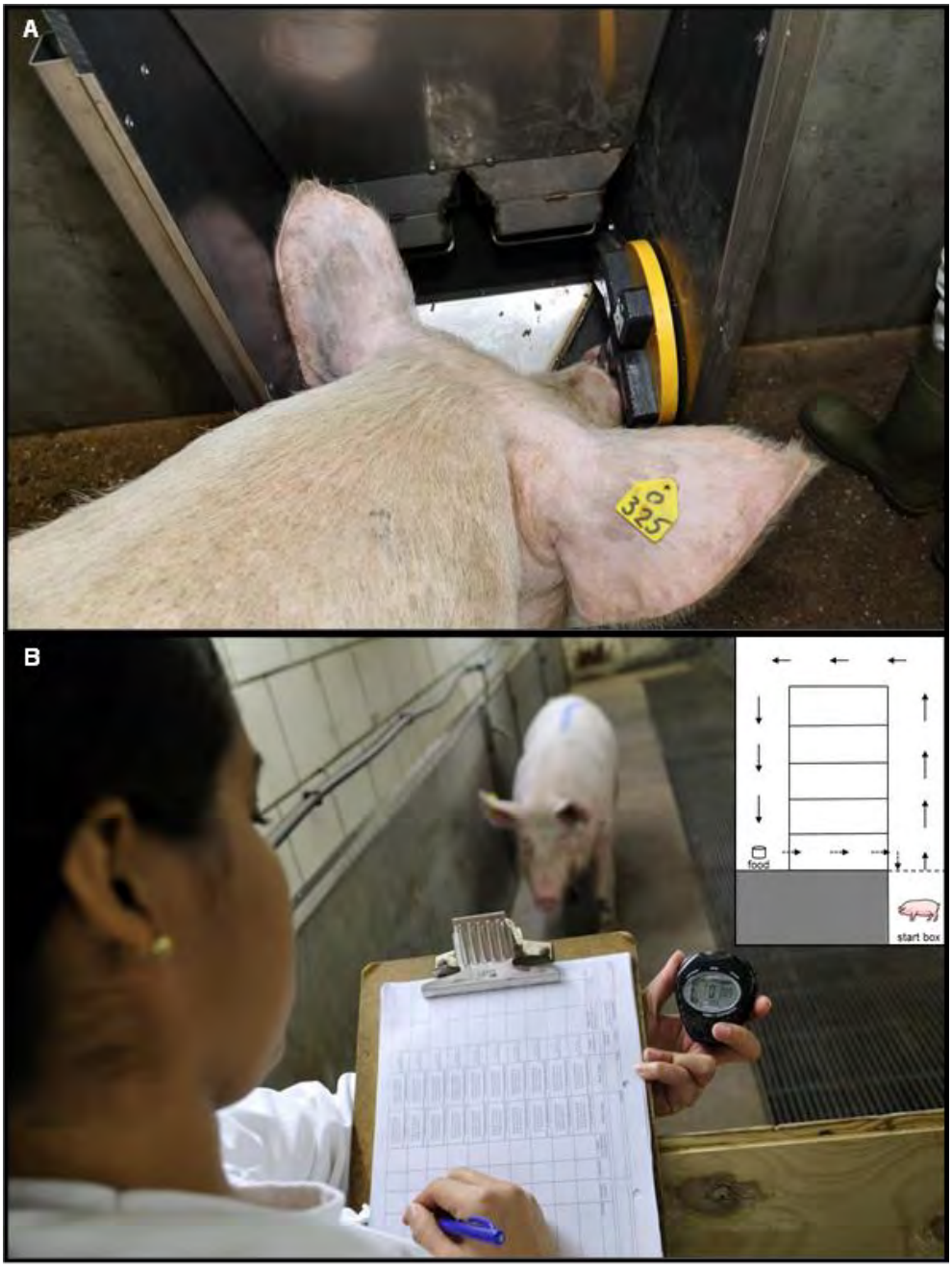

Fig. 2. Photographs of the two feeding motivation tests. Panel A shows the operant test, with a pig turning the wheel by pushing it with the snout in order to obtain a food reward. Panel B gives an impression of the runway test, with a pig almost reaching the end of the track (where it would find a food reward). The drawing given in panel B shows the setup for the runway test, solid arrows indicate the route that pigs had to walk to reach the food, and dotted arrows indicate the route through which pigs left the test room. Photographs taken by Bart Nijs fotografie.

To assess feeding motivation after ingestion of the different fibre diets, operant responding was measured once at ~1 h (0800 h), 3 h (1000 h) and 7 h (1400 h) after the morning meal on three separate days, so that each pig was tested at a different time on each day. The order of testing was balanced for diets. The test session ended when the pig was not turning the wheel for ≥2 min, or when the maximum time of 20 min was reached, after which an audio cue (horn sound) was introduced, and the pig was gently guided back to her home pen.

Runway test

On days 6 (at low inclusion level) and 13 (at high inclusion level) in each period, pigs were subjected to a runway test (Fig. 2B). In this test, all pigs (n=16) had to individually walk a fixed U-shaped track (22.5 m) for obtaining 20 g (0.86% w/w of daily food allowance) of a commercial pelleted feed (see *Operant test* for details) as reward at the end of the runway. Before the start of the test session, the box at the start of the runway was closed, and the feeder at the end of the runway was baited with food. In a pilot study on adult pigs subjected to different feeding levels, the speed of walking or running toward the food was positively correlated to feeding motivation (Souza da Silva et al., 2010).

The runway test was carried out in a separate, climate-controlled test room (temperature 20±1 °C). After opening the start box, each pig was given a maximum time of 3 min to reach the feeder. When the pig reached the feeder, it was allowed to eat the food reward without time limits, after which an audio cue (horn sound) was introduced and the pig was gently guided back to her home pen. During each session, the time elapsed from opening the start box to first contact with the feeder was recorded and the average speed was calculated (length of track divided by time) and expressed in km/h. The calculated speed was used as main indicator for feeding motivation in this test. In addition, latency time to leave the start box after opening and time spent at the feeder were recorded.

To assess feeding motivation after ingestion of the different fibre diets, pigs were tested at the same time points as in the operant test, i.e. at ~1 h, 3 h, and 7 h after the morning meal, all on one day.

Fig. 3. Tailor-made feeder developed by Verbakel B.V. (Sint Oedenrode, The Netherlands). The feeder was fixed to the wall of the test room, and consisted of a food dispenser, a wheel and a food trough beneath the wheel. By turning the wheel multiple times, the pig would obtain a food reward, which was automatically delivered in the food trough after the required number of wheel turns (detected via the sensor) was performed. Photograph taken by Bart Nijs fotografie.

Behavioural observations

Pigs were observed in their home pen on days 6, 7 (all at low inclusion level), 13 and 14 (all at high inclusion level) in each period, during 6 intervals of 1 h using 90 s-instantaneous scan sampling. Live observations started at 0800 h, 1000 h, 1200 h, 1400 h, 1600 h and 1800 h. The posture of each pig and the activity it performed were recorded for each scan, using the following ethogram–Postures: lying; standing and walking; sitting and kneeling; Behavioural activities: explorative behaviour (sniffing or rooting floor, pen fixtures or toys); chewing (chewing air or pen fixtures); stereotypic chewing (chewing air or pen fixtures in a stereotypic manner, i.e. repetitively and without an obvious goal or function); feeder-directed behaviour (climbing, nosing or lifting the feeder, and eating); manipulative behaviour directed at pen mates (nibbling, sucking or chewing any part of the body of a pen mate); drinking; other (all other behavioural activities). Explorative behaviour, (stereotypic) chewing, feeder-directed behaviour, manipulative behaviour and drinking were also summed into a score of 'total oral behaviour'.

Behaviours that are part of foraging strategies are often referred to as indicators of feeding motivation and level of satiety in pigs, because they reflect the animal's repertoire of food search and food consumption (D'Eath et al., 2009; De Leeuw et al., 2008). Behaviours related to food search include general activity (lying, standing, walking), foraging, redirected foraging (manipulation of non-food components of the pen), whereas behaviours related to food consumption include eating and drinking, together with stereotypies (e.g. chewing air). Stereotypies, in particular, are thought to reflect short-term satiety because they are usually initiated shortly after the meal; whereas general activity and other foraging related behaviours are more related to long-term satiety because they are performed for many hours after the meal, or in anticipation to the next meal (De Leeuw et al., 2008).

For behavioural recordings the Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on a Psion Workabout MX was used. Behaviours were averaged per pen per observation hour and expressed as percentages of observation time.

Statistical analysis

All data were averaged per pen before data analysis, so that the experimental unit was pen. In the operant test only one pig per pen was tested, so in this test the experimental unit pen corresponded to pig. The data of one pig in the second week of period 3 (PEC-H diet) were excluded from analyses because the animal showed acute vomiting after the morning meal. The speed in the runway test, and the

proportions of time spent on the various behaviours were log-, and arcsine square root-transformed, respectively, if the residuals were not normally distributed.

For each variable, fixed effects of fibre type (C, LC, RS, PEC), fibre level (L, H), time point (1 h, 3 h, 7 h), and their interactions were analysed using a split–split–plot mixed model in SAS (version 9.1; SAS Institute) with values in time and treatment of individual pens taken as repeated measurements. This mixed model included the fixed effect of period and the random effects of pen, pen nested within fibre type, and pen nested within fibre type and fibre level. Thus, main effects of fibre type were tested against the effect of pen nested within fibre type, and effects of fibre level and its interaction with fibre type were tested against the effects of pen nested within fibre type and fibre level. In addition, effects of fibre type, fibre level, and their interaction were also analysed per time point in a model that contained the fixed effect of period and the random effects of pen, and pen nested within fibre type.

The different fibre types, LC, RS and PEC, were exchanged for pregelatinized starch from the C diet at various inclusion levels, to achieve similar GE intake. This implies that treatments were not equal in their ME intake. To facilitate interpretation of the effects of inclusion level per fibre type on feeding motivation of the pigs, an additional analysis was conducted. In this analysis, effects of fibre inclusion level (high, low or zero, i.e. the C-H diet) on behavioural responses of the pigs were assessed per fibre type using a mixed model that included the fixed effects of inclusion level, time point and their interaction, and random effects of pen and pen within inclusion level.

For analysing the relationship between variables recorded in the operant and runway tests, residuals of the transformed means were calculated using a GLM model with period, fibre type, fibre level and time point. Correlation coefficients were generated using Pearson's correlation procedure for both raw and residual correlations, with the latter being reported.

In case of significant fibre type or fibre type×fibre level effects, least square means adjusted with the Tukey–Kramer correction were used to estimate pairwise differences. Data are presented as (untransformed) least square means±SEM on pen averages.

Results

Correlations within and between tests

There were large inter-individual differences in both operant and runway tests, irrespective of fibre type and fibre level, with significant effects of pig and pair of

pigs for the number of wheel turns in the operant test and for speed in the runway test, respectively (both $P < 0.05$).

Within the operant test, the number of rewards obtained per test ranged from 0 to 49 (mean: 24±13), whereas the number of wheel turns obtained per test session ranged from 0 to 1225 (mean: 398±334), and was strongly correlated with the time spent in the test room (duration) (r=0.68, $P < 0.001$), with the time spent at the feeder (r=0.75, $P < 0.001$), and with the latency time to turn the wheel (r=−0.42, $P < 0.001$).

Within the runway test, the speed per test session ranged from 0.45 to 10.1 km/h (2.6±0.1 km/h). In 3 out of the 384 sessions, pigs did not walk toward the food and were given the maximum time score of 3 min (resulting in a speed of 0.45 km/h). Speed was negatively correlated with the latency time to leave the start box (r=−0.32, $P < 0.001$), and positively correlated with the time spent at the feeder (r=0.48, $P < 0.001$).

Between tests, the speed in the runway test was positively correlated with the number of wheel turns and with the number of rewards (both r=0.47, $P < 0.001$) in the operant test.

Operant test

Table 2 shows the number of wheel turns for each fibre type and fibre level combination at the three time points and averaged over the day. The number of wheel turns performed in the operant test was highly affected by time point ($F(2,110)=8.1$, $P < 0.001$), with higher levels at 3 h (402) and 7 h (425) than at 1 h (360, SEM=110) after the morning meal. Fibre type and its interaction with fibre level tended to influence the number of wheel turns over the day ($F(3,18)=3.1$ and $F(3,27)=2.6$, both: $P < 0.10$). Over the three time points, the number of wheel turns tended to be higher for pigs on the PEC diet (470) than for pigs on the LC diet (326), with levels of C (397) and RS (389, SEM=113) in between ($P < 0.05$ Tukey–Kramer). For the PEC and LC diets, the number of wheel turns tended to increase with fibre level, but this was not the case for the C and RS diets (fibre type×fibre level interaction, $F(3,27)=2.6$, $P < 0.10$, Table 2).

Analysis per time point revealed that at 1 h after the meal the number of wheel turns was influenced by both fibre type and fibre level ($F(3,18)=3.4$ and $F(1,27)=5.8$, both: $P < 0.05$). At this time point, the number of wheel turns was higher ($P < 0.05$ Tukey–Kramer) for pigs on the PEC diet (452) than for pigs on the LC diet (284), with levels of C (352) and RS (356, SEM=112) in between, and with higher levels ($P < 0.05$ Tukey–Kramer) for pigs on high fibre diets (393) than for pigs on low fibre diets (329, SEM=109). At 3 h after the meal, the number of wheel turns was higher for pigs on the PEC-H diet than for pigs on the LC-L diet

(fibre type×fibre level interaction, $F(3,27)=4.2$, $P < 0.05$). At 7 h after the meal, fibre type ($F(3,18)=2.5$, $P < 0.10$) tended to influence the number of wheel turns, with higher levels ($P < 0.10$ Tukey–Kramer) for pigs on the PEC diet (494) than for pigs on the LC diet (355), and levels of C (413) and RS (433, SEM=119) in between (Table 2).

Effects of fibre inclusion level

Within fibre type, fibre inclusion level (high, low or zero, i.e. the C-H diet) influenced the number of wheel turns of pigs fed the PEC ($F(2,11)=4.2$, $P < 0.05$) and LC ($F(2,11)=5.5$, $P < 0.05$) diets. For the PEC diet, the number of wheel turns increased with inclusion level: pigs turned the wheel more often at the high than at the zero inclusion level, with the low inclusion level being intermediate (Fig. 4B). For the LC diet, the number of wheel turns was higher for pigs at the zero inclusion level than for pigs at the low inclusion level, with the high inclusion level being intermediate (Fig. 4A). Time point ($F(2,42)=3.7$, $P < 0.05$) also affected the number of wheel turns within the LC diet, with higher levels at 3 h (364) and 7 h (374) than at 1 h (316, SEM=112) after the morning meal. Inclusion of RS in the diet did not affect the number of wheel turns in the operant test ($F(2,11)=0.6$, $P=0.55$, Fig. 4C).

Runway test

Table 2 shows the speed to reach the food reward in the runway for each fibre type and fibre level combination at the three time points and averaged over the day. The speed to reach the food reward in the runway test was affected by time point ($F(2,112)=23.5$, $P < 0.001$), with higher levels ($P < 0.01$ Tukey–Kramer) at 1 h (3.2 km/h) and at 7 h (2.7 km/h) than at 3 h (1.9 km/h, SEM=0.3) after the morning meal (Table 2). Fibre type influenced the speed in this test ($F(3,18)=3.5$, $P < 0.05$). Over the three time points, the speed was higher ($P < 0.01$ Tukey–Kramer) for pigs on the PEC diet (3.1 km/h) than for pigs on the RS diet (2.2 km/h), with levels of C (2.5 km/h) and LC (2.6 km/h, SEM=0.3) in between. Analysis per time point revealed that pigs were particularly faster ($P < 0.05$ Tukey–Kramer) to reach the food on the PEC diet as compared to the RS diet at 1 h after the morning meal ($F(3,18)=3.5$, $P < 0.05$; PEC: 3.8 km/h; RS: 2.6 km/h, SEM=0.5). Also at 7 h after the morning meal, PEC-fed pigs tended to run faster ($P < 0.10$ Tukey–Kramer) to reach the food compared to RS-fed pigs ($F(3,18)=2.7$, $P < 0.10$; PEC: 3.0 km/h; RS: 2.3 km/h, SEM=0.4).

Fibre level influenced the speed in the runway ($F(1,28)=5.1$, $P < 0.05$). Over the three time points, pigs on low fibre diets (2.8 km/h) had a higher speed ($P < 0.05$ Tukey–Kramer) than pigs on high fibre diets (2.4 km/h, SEM=0.3) (Table 2).

Analysis per time point revealed that the speed was particularly higher at 7 h after the meal (P=0.05 Tukey–Kramer) for pigs on low fibre diets than on high fibre diets (F(1,28)=4.1, P=0.05; low: 3.0 km/h; high 2.5 km/h, SEM=0.4). Also at 3 h after the morning meal, pigs fed low fibre diets tended to run faster (P=0.10 Tukey–Kramer) to reach the food than pigs fed high fibre diets (F(1,28)=2.8, P=0.10; low: 2.1 km/h; high 1.7 km/h, SEM=0.2).

Effects of fibre inclusion level

Within fibre type, fibre inclusion level (high, low or zero, i.e. the C-H diet) influenced the speed of pigs fed PEC (F(2,12)=4.8, $P < 0.05$) and tended to influence the speed of pigs fed LC (F(2,53)=2.8, $P < 0.10$). For the PEC diet, the speed was higher for pigs at both inclusion levels of PEC than for pigs on the diet without fibre (Fig. 5B). The speed within the PEC diet was affected by time point (F(2,42)=8.0, $P < 0.01$), with higher levels at 1 h (3.4 km/h) and 7 h (2.9 km/h) than at 3 h (2.1 km/h, SEM=0.3) after the morning meal. For the LC diet, the speed tended to be higher for pigs at the low inclusion level than for pigs on the diet without fibre, with pigs at the high inclusion level being intermediate (Fig. 5A). The speed within the LC diet was also affected by time point (F(2,53)=13.6, $P <$ 0.01), with higher levels at 1 h (3.1 km/h) and 7 h (2.8 km/h) than at 3 h (1.7 km/h, SEM=0.3) after the morning meal. For the RS diet, inclusion level did not affect the speed in the runway test (F(2,11)=0.5, P=0.64, Fig. 5C), but time point (F(2,42)=3.9, $P < 0.05$) did. Pigs on the RS diet were faster to reach the food at 1 h (2.6 km/h) and 7 h (2.5 km/h) than at 3 h (1.7 km/h, SEM=0.3) after the morning meal.

Behavioural observations

Table 3 shows the proportions of time spent on the various behaviours for each fibre type and fibre level combination averaged over the day. In Fig. 6, patterns of behaviours over the day are shown.

Proportions of time spent on lying and on oral behaviours were strongly affected by time of the day (F(5,326)=38.4 and F(5,333)=30.3, both $P < 0.001$) (Table 3). In the middle of the day, i.e. from 1200 h to 1300 h, pigs spent 95% of the time lying and only 12% on oral behaviours, whereas between 1600 h and 1700 h, i.e. 1 h before the afternoon meal, pigs were lying only 74% of the time, and spent 44% of the time on oral behaviours (Fig. 6) with values of other observation times in between. Other behaviours were generally affected in a similar way by time of the day, i.e. pigs were active around feeding, and low activity levels were seen in the middle of the day. Therefore, time effects are not described for each individual behaviour separately.

Pigs on the PEC diet tended to spend more time on stereotypic chewing (14%) than pigs on the C diet (9%), with levels of LC and RS (both 11%, SEM=2.0) in between ($F(3,326)=2.2$, $P < 0.10$) (Table 3). Feeder-directed behaviour, which peaked before the second meal (Fig. 6), was affected by fibre type ($F(3,326)=4.8$, $P < 0.01$). PEC-fed pigs spent more time on feeder-directed behaviours (3.2%) than pigs on the RS diet (1.6%), with levels of C and LC (both 2.3%, SEM=0.4) in between. Pigs fed the high level of PEC spent more time manipulating their pen mates (0.31%) than pigs on most of other diets, including the PEC-L diet (0.05%, SEM=0.1) (fibre type×level effect, ($F(3,326)=3.4$, $P < 0.05$). It should be noted, though, that levels of oral manipulative behaviours directed at pen mates were generally very low (< 0.4%). No other effects of fibre type, fibre level and their interaction on behaviours were found.

Table 2. Number of wheel turns in the operant test and speed in the runway test performed by adult female pigs at 1 h, 3 h and 7 h after the morning meal. Pigs were fed four experimental diets (Control, Lignocellulose, Resistant starch and Pectin) at two levels (Low vs. High). Results are expressed as least square means±SEM, n=8 for operant test and n=16 for runway test.

	Diet									Effects[1]			
	Control		Lignocellulose		Resistant starch		Pectin		Pooled				
	Low	High	Low	High	Low	High	Low	High	SEM	T	F	L	F×L
Average over the three time points													
Turns, no.	391	403	291	360	411	366	436	503	114	***	#	ns	#
Speed, km/h	2.7	2.3	2.9	2.3	2.4	2.1	3.1	3.0	0.4	***	*	*	ns
At 1h after the meal													
Turns, no.	324	381	239	329	362	349	392	512	115	–	*	*	ns
Speed, km/h	3.0	2.5	3.5	3.3	2.5	2.7	3.8	3.9	0.6	–	*	ns	ns
At 3h after the meal													
Turns, no.	436	416	282	396	449	305	437	499	116	–	ns	ns	*
Speed, km/h	2.0	1.6	2.1	1.4	2.0	1.5	2.4	2.3	0.3	–	ns	#	ns
At 7h after the meal													
Turns, no.	414	413	354	355	422	444	480	507	120	–	#	ns	ns
Speed, km/h	3.1	2.8	3.2	2.3	2.5	2.1	3.2	2.8	0.5	–	#	*	ns

[1] Significance of effects of time (T), fibre type (F), fibre level (L), and interaction fibre type and level (F × L) are indicated. Interactions between fibre type, fibre level and time point did not influence the number of wheel turns in the operant test and the speed in the runway test. # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

Table 3. Behaviour of adult female pigs fed four experimental diets (Control, Lignocellulose, Resistant starch and Pectin) at two levels (Low vs. High), based on live observations using 90 s-instantaneous scan sampling (six intervals of 1-h during daytime). Results are expressed as least square means±SEM, n=16.

	Diet									Effects[1]				
Behavior	Control		Lignocellulose		Resistant starch		Pectin		Pooled					
(% of observation time)	Low	High	Low	High	Low	High	Low	High	SEM	T	F	L	F×L	L×T
Posture														
Lying	86.4	86.6	85.5	87.1	87.2	86.3	85.7	84.9	1.9	***	ns	ns	ns	ns
Standing and walking	9.2	9.6	10.6	9.9	9.4	10.6	10.9	10.6	1.5	***	ns	ns	ns	ns
Sitting and kneeling	4.1	3.6	3.6	2.9	3.1	2.9	3.2	4.3	0.7	***	ns	ns	ns	ns
Behavioural activity														
Total oral behaviour	27.1	26.5	29.1	27.3	29.6	27.4	30.7	31.1	2.7	***	ns	ns	ns	ns
Explorative behaviour	7.0	8.7	8.5	7.6	7.6	8.2	9.0	8.3	1.0	***	ns	ns	ns	ns
Chewing	16.1	14.0	16.3	15.6	18.3	15.7	16.8	17.5	2.4	***	ns	ns	ns	ns
Stereotypic chewing	10.3	8.6	10.8	11.0	12.7	9.9	13.1	14.0	2.3	***	#	ns	ns	ns
Feeder-directed behaviour	2.1	2.4	2.2	2.4	1.6	1.6	3.2	3.2	0.5	***	**	ns	ns	ns

[1] Significance of effects time (T), fibre type (F), fibre level (L), interaction fibre type and level (F×L), and interaction fibre level and time (L×T) is indicated. Interactions between fibre type and time, and between fibre type, fibre level and time did not influence the various behaviours observed. # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

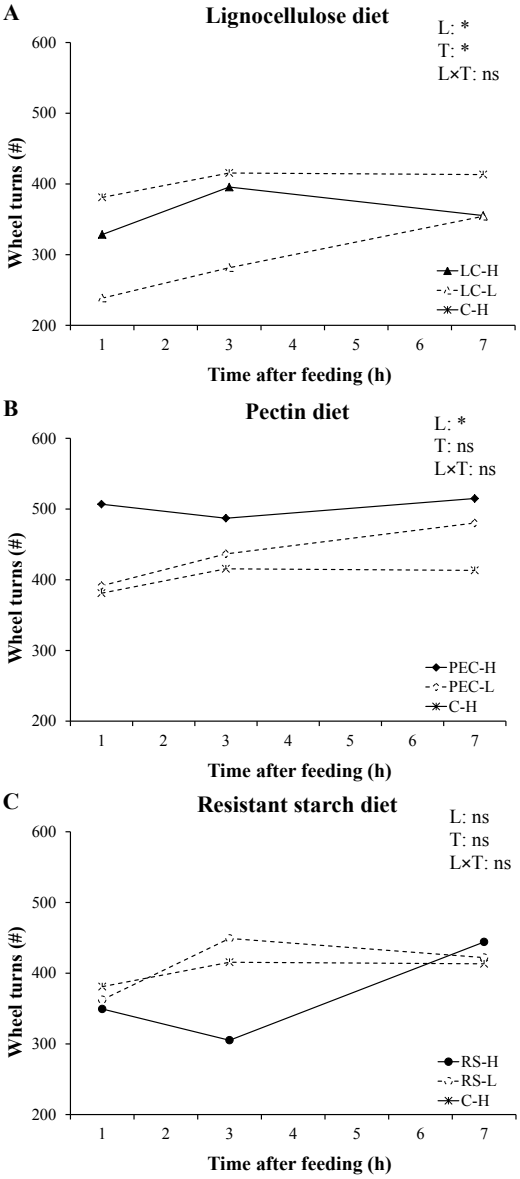

Fig. 4. Number of wheel turns during operant test at 1 h, 3 h and 7 h after the morning meal performed by adult female pigs (n=8) fed three fibre types: lignocellulose (panel A, triangles), pectin (panel B, diamonds), and resistant starch (panel C, circles). Each fibre type, at two inclusion levels: low (dotted line) and high (solid line), is compared with the isoenergetic control high diet (solid line, asterisk). Results are expressed as least square means. Pooled SEMs were 113 for lignocellulose, 122 for pectin and 115 for resistant starch. Statistical significance of effects of inclusion level (L), time point (T) and their interaction (L×T) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

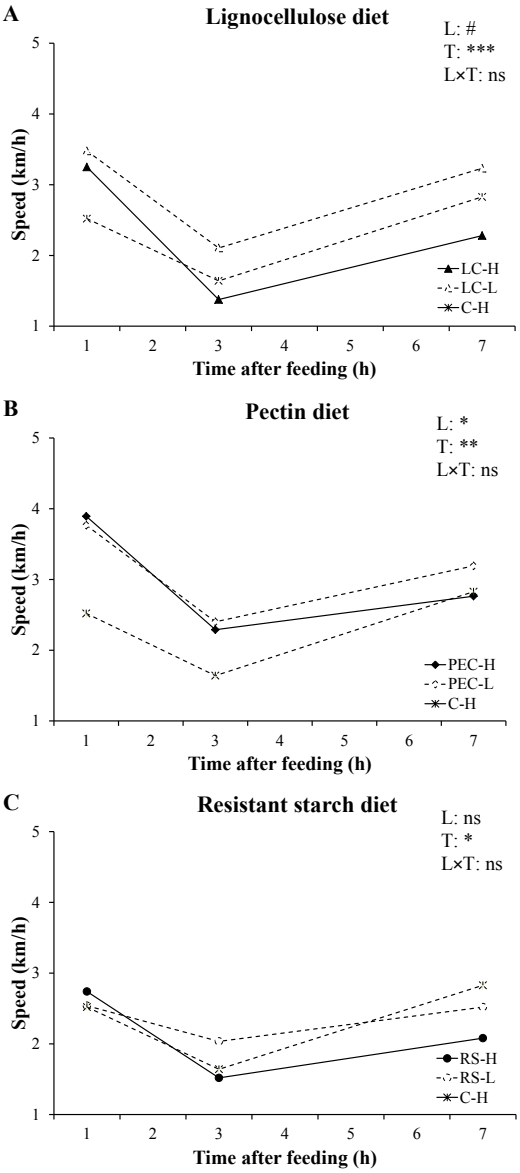

Fig. 5. Speed (km/h) to reach food reward during runway test at 1 h, 3 h and 7 h after the morning meal performed by adult female pigs (n=16) fed three fibre types: lignocellulose (panel A, triangles), pectin (panel B, diamonds), and resistant starch (panel C, circles). Each fibre type, at two inclusion levels: low (dotted line) and high (solid line), is compared with the isoenergetic control high diet (solid line, asterisk). Results are expressed as least square means. Pooled SEM were equal to 0.33 for lignocellulose, 0.34 for pectin and 0.31 for resistant starch. Statistical significance of effects of inclusion level (L), time point (T) and their interaction (L×T) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

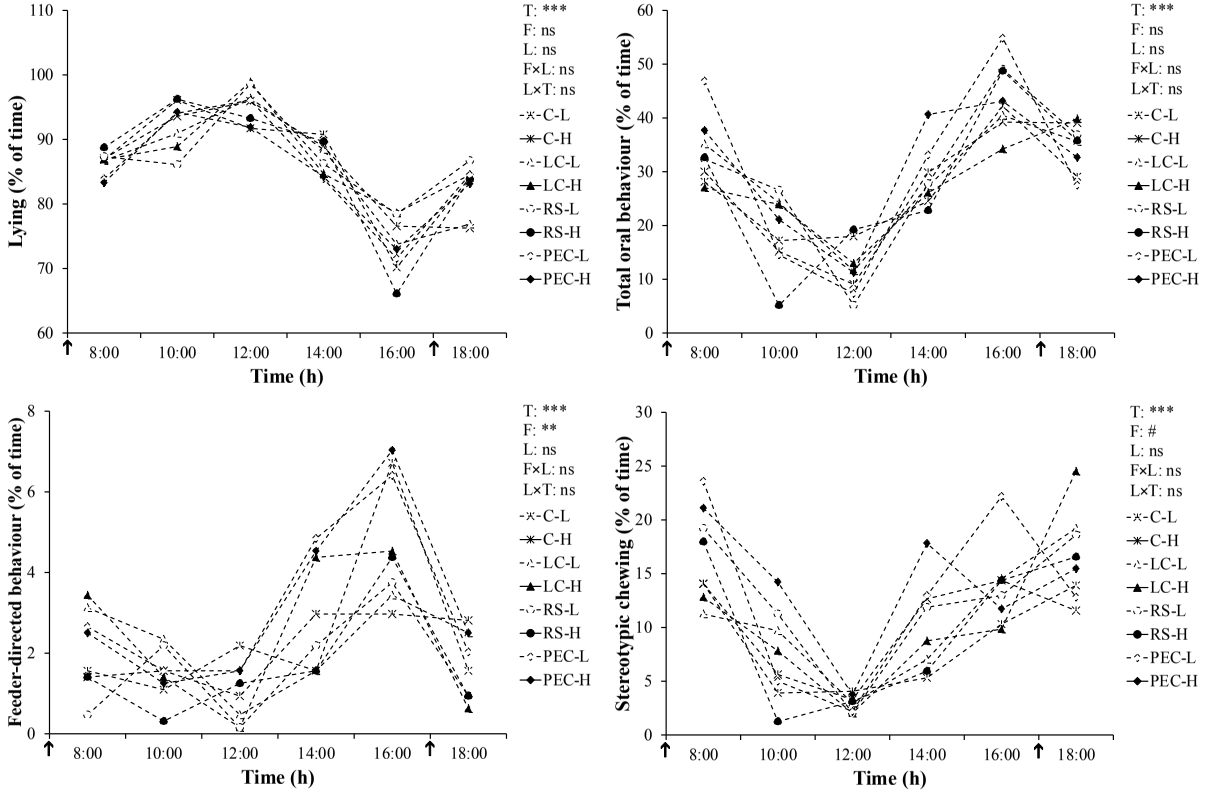

Fig. 6. Percentages of time spent on lying, total oral behaviour, feeder-directed behaviour and stereotypic chewing during behavioural observations started at 0800 h, 1000 h, 1200 h, 1400 h, 1600 h and 1800 h performed by adult female pigs (n=16) fed four experimental diets: control (asterisks), lignocellulose (triangles), resistant starch (circles) and pectin (diamonds), each at two levels: low (dotted line) and high (solid line). Arrows indicate feeding times. Results are expressed as least square means. Pooled SEMs were 4.0 for lying, 6.6 for total oral behaviour, 1.2 for feeder-directed behaviour and 4.3 for stereotypic chewing. Statistical significance of effects of time point (T), fibre type (F), inclusion level (L), interaction fibre type and level (F×L), and interaction level and time (L×T) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

Discussion

In this study, feeding motivation and behaviour of adult female pigs were assessed at different times of the day to evaluate the satiating properties of dietary fibres with distinctive physicochemical properties that could affect satiety by different physiological mechanisms.

Measurements of feeding motivation and satiety

Our expectation, at least for the C diet, was that animals would show the lowest feeding motivation at 1 h after the meal, and the highest at 7 h after the meal, shortly before their afternoon meal. In the runway test, however, pigs ran faster for food during the first run of the day than during the second run (3 h after the meal). Furthermore, in the operant test, at 1 h after the meal, pigs fed the C diet did unexpectedly not reduce the number of turns with increasing feeding level.

Pigs tend to synchronize their activity with feeding, and often show a diurnal activity pattern characterized by peaks of activity in the morning and in the afternoon, interspersed by resting periods (Jensen, 2002; Schouten, 1985). This means that the tests applied 1 h after the meal may have coincided with the morning peak of activity. Responses of animals in feeding motivation tests can, apart from being affected by metabolic state and level of satiety, also be influenced by factors like the appreciation of the food (i.e. its incentive properties) provided during the tests (Barbano and Cador, 2005; Day et al., 1996; Saper et al., 2002). In this study, we chose to use a commercial pelleted feed as a reward in the operant and runway tests, because using the experimental, mash, diets would have led to technical (e.g. electronic operant feeder malfunction) and methodological (e.g. difficulty to compare treatments, see D'Eath et al., 2009) problems. The commercial pelleted feed used, however, might have been preferred by the pigs over their experimental diets, and a contrast in perceived food quality may have led to high levels of responding irrespective of metabolic state (Ramonet et al., 2000; Saper et al., 2002). The potential contrast between the experimental and reward diets may have been most easily noticed just after the meal, because, in humans, palatability has been demonstrated to particularly affect satiation, i.e. short term food intake, but not long term satiety (De Graaf et al., 1999).

It should be noticed, however, that also the behavioural observations in the home pen point to a relatively high feeding motivation shortly after the meal, as activity levels of the pigs and the time spent on oral activities, including stereotypic behaviour, were high during the first observation hour. Also others have reported postprandial peaks in activity and oral behaviours in pigs (Bolhuis et al., 2010; De Leeuw et al., 2008).This high postprandial feeding motivation might indicate that

the present feeding level, though sufficient for maintenance and some growth, was insufficient to make pigs fully satiated, as other authors have found higher daily voluntary food intakes in sows fed ad libitum (van der Peet-Schwering et al., 2004). Another explanation could relate to the natural foraging strategy of pigs, which forage for patchily distributed food, and alternate walking plus rooting in search for food with food consumption. Therefore, appetitive behaviour is probably stimulated by the ingestion of food (Wiepkema, 1971), which could probably explain high postprandial activity and stereotypy levels, until satiety signals provide a negative feedback (Hughes and Duncan, 1988). In addition, it has been suggested that the execution of appetitive foraging behaviours is not only driven by metabolic state, but is also rewarding in itself (Inglis et al., 1997), i.e. pigs have an inherent motivation to forage (Scott et al., 2007). In support of this, it has been demonstrated that some pigs continue to put effort in foraging activities despite having food freely available, i.e. contrafreeloading (de Jonge et al., 2008); but see (Young and Lawrence, 2003). Also in motivation tests in rats, an increased speed during the first test of the day, regardless of previous treatment, has been reported, which was suggested to result from the animal's innate explorative behaviour, overruling the effect of a drug or a food reinforcement (Wakonigg et al., 2003).

The runway and operant tests consistently revealed PEC to be the least satiating fibre. The apparent satiating effects of LC and RS diets over different times of the day were, however, not completely the same for the runway and operant tests. In both tests pigs had to pay a price for obtaining food. It is generally stated that for feeding motivation tests, the imposed cost should be in line with the natural foraging behaviour of the animal under study (Verbeek et al., 2011), which is true for both the snout movements to turn the wheel and for the walking requested in the operant and runway tests, respectively. The imposed costs may both have been appetitive behaviours, but the impact they had on the willingness to work for food might have been different in the tests used. Moreover, during the operant test, animals could obtain multiple rewards, i.e. the benefits were different from the runway, and the cost-to-benefit ratio increased during the course of the test due to the progressive ratio schedule chosen. So, the trade-off between the benefits of food consumption and the costs of obtaining food was different for the operant test as compared with the runway test. In addition, the runway is a short test in which only the appetitive response of pigs, as reflected in their speed, was measured, whereas in the operant test food incentive properties might have played a bigger role as the appetitive responses of pigs were alternated with consummatory phases (*sensu* Barbano and Cador, 2005). Each operant session ended when pigs had paid their maximum price, and this break-point varied largely and consistently between individuals, partly irrespective of their diet and irrespective of the fact that they were all of the same breed, origin, and weight at the start of the study. This large

inter-individual variation suggests that a within-subject design would be valuable in future studies. Despite the large individual variation in operant responding, and despite the differences in both tests used, responses in the operant and runway tests correlated well, suggesting that they, at least partly, reflect the same, i.e. feeding motivation.

In addition to feeding motivation tests, behavioural observations were used for indicating feeding motivation and satiety of pigs. In general, all behaviours were strongly affected by time of the day, whereas dietary treatments affected only feeder-directed behaviour, stereotypic chewing and manipulative behaviour. These behaviours showed increased occurrence in the PEC fed pigs compared with the RS fed pigs, and reinforced the findings of the feeding motivation tests regarding the PEC treatment in particular. Although there was no effect of dietary treatment on behaviours reflecting physical activity, which may also reflect feeding motivation (De Leeuw et al., 2008), the effects found on oral behaviours are in accordance with previous studies (D'Eath et al., 2009; De Leeuw et al., 2008; Verbeek et al., 2011). Physical activity, appetitive behaviours and stereotypies may indicate the existence of a motivation to search for food, but can also be masked by motivation for other resources or may be indicators of other types of frustration (Verbeek et al., 2011). For that reason, we believe that behavioural observations, when used to measure feeding motivation in animals, should be preferably combined with feeding motivation tests.

Fibre effects

Diets were formulated to be isoenergetic in their GE content, with fibres being exchanged for pregelatinized starch from the C diet. It was not possible to design diets with equal metabolizable or net energy contents (D'Eath et al., 2009), because calorimetric data are not available for all fibre sources. This implies that the satiating effects of the fibres had to overcome the effects of a reduced available energy supply compared to the C diet. As this reduction in available energy could not be precisely quantified, the C diet was fed at two levels of intake in the present study. The formulation of the C-L diet, which was similar in ME content to the RS-H diet, was based on calorimetric data for RS utilization in pigs (Bolhuis et al., 2008).

The alternative approach, often used in human studies, of adding the fibre on top of a diet was not used in the present study, because variable levels of the three fibres used would result in variable GE intake and meal size. This would complicate interpretation of treatment effects, because the effects of fibres would be confounded with the effects of GE intake and meal size (Wanders et al., 2011).

Compared with the C diet, the reduction in ME content of our high fibre diets was estimated from previous studies to be approximately 8–10% (Bolhuis et al., 2008; Gerrits et al., 2012; Hansen et al., 2006; Jonathan et al., 2012; Lange et al., 2006; Noblet and Le Goff, 2001; Schrama and Bakker, 1999; Serena et al., 2008). Total energy available is, however, not the only factor affecting satiety, as in the present study C fed pigs were not the most satiated. A reduction in ME content is a consequence of replacing pregelatinized starch with fibre, and consists of a reduction in digestible carbohydrate intake (direct effect), and a decrease in fat and protein digestibility (indirect effect) by interacting with dietary fibre (Fernandez and Jorgensen, 1986; Hooda et al., 2011; Le Gall et al., 2009). Because available or net energy intake directly affects feeding motivation (D'Eath et al., 2009), and such effects are fibre dependent, the details of ME reduction will be discussed for each fibre separately.

Pectin

Viscous fibres, such as PEC, are generally thought to induce satiety by delaying gastric emptying and total intestinal transit (also measured as passage rate), which prolongs the time for enzymatic digestion, thereby improving digestibility (Brownlee, 2011; Dikeman and Fahey, 2006; Eastwood and Kay, 1979; Hooda et al., 2011), and these actions may affect the release of intestinal satiety-related hormones such as CCK, PYY and GLP-1 (Wanders et al., 2011). Therefore, PEC was expected to reduce feeding motivation from 3 h up to 7 h after the meal. This was, however, not the case. Over the entire day, PEC fed pigs showed an increased feeding motivation in the runway and operant tests compared with pigs fed the other diets (including the C diet). In addition, the PEC diet increased time spent on feeder-directed behaviour, stereotypic chewing and manipulation of pen mates. Moreover, feeding motivation increased with inclusion level of PEC. This suggests a low satiating capacity of PEC in adult pigs, which does not correspond with studies in humans demonstrating enhanced satiety feelings after consumption of viscous fibres (Burton-Freeman, 2000; Wanders et al., 2011; Zijlstra et al., 2008). These human studies were, however, short-term feeding studies where usually a single level of fibre was fed as a supplement, thus, not exchanged with a nutrient (e.g. starch) on an equal energy basis, implying that consumption of the treatment will result in extra energy intake and change in meal volume (Wanders et al., 2011).

In our study, pigs were fed diets with equal GE content. The exchange of starch for pectin likely reduced the available energy supply in the PEC diets. Calculated from the measured decrease in ME intake of growing pigs (11%, Hansen et al., 2006) or sows (8%, Serena et al., 2008) fed pectin rich diets, and adjusted for the

size of the experimental contrast, it appears that the pigs in our study consumed approximately 8% less ME compared with the C-H diet. Possibly, the lower intake of available energy with the PEC diet than with the C diet may have increased feeding motivation in the present study, because when ME intake was maintained at a constant level (33–35 MJ/day), pectin (Jensen et al., 2011) or sugar beet pulp (a pectin-rich by-product) (Ramonet et al., 2000) did not alter feeding motivation in adult pigs compared to control diets.

In addition, the physical form of the diet (i.e. solid feed) may have prevented the satiating effects of PEC, as in human studies, viscous fibres appeared more satiating when provided as liquids compared to solids (Wanders et al., 2011). This variation may be explained by the increase in viscosity related to the rate of hydration of the fibre, which means that the increase in viscosity is usually higher in liquid diets than in solid diets, probably due to a complete hydration of the fibre prior to consumption of the liquid (Kristensen and Jensen, 2011). In addition, a viscous liquid gives a different feeling in the mouth compared with a non-viscous liquid, and may induce satiation, i.e. short-term satiety as suggested by Lyly et al. (2009).

Lignocellulose

Lignocellulose has a high water-binding capacity (eight times its original weight, Büttner, 2006) and is therefore considered a bulky fibre (De Leeuw et al., 2008). Bulky fibres may increase stomach distension (Howarth et al., 2001), and could thereby enhance satiation (Geliebter et al., 1992), which may result in meal termination. Therefore, the bulking properties of LC were expected to promote early satiety feelings specifically from 1 h up to 3 h after the meal. An interaction between time and inclusion level of LC on feeding motivation was, however, not found. In the operant test, not only at 1 h and 3 h after the meal, but also at 7 h, pigs fed LC (at both inclusion levels) generally showed a reduced feeding motivation compared with pigs fed the other diets, including the isoenergetic C diet. Surprisingly, LC, particularly at low inclusion level, affected long term (i.e. more than 3 h after the meal) satiety in the operant test more than we expected. At 3 h after feeding LC-L pigs showed a lower feeding motivation than LC-H pigs, possibly because the strong reduction in ME counteracted the effects of bulkiness for the LC-H diet. Nevertheless, results from the runway test were contradictory, because LC fed pigs (especially at the low inclusion level) showed a higher feeding motivation than pigs fed the C diet. Moreover, LC did not affect time spent on behaviours related to feeding motivation at any time relative to feeding.

It is not very likely that the long term satiating effects of LC, as found in the operant test, were due to fermentation, as data from an in vitro fermentation study

showed that lignocellulose is not fermented by inoculum from adult pigs (Jonathan et al., 2012), and human studies indicate that only minor amounts (0–5%) of LC are fermented (Cook and Sellin, 1998). However, long term effects may be explained by increased swelling and water-binding capacity in the stomach, which in turn could have delayed the gastric emptying rate of the liquid phase (referred to as more important for satiety than the solid phase) of gastric contents (Jørgensen et al., 2010), and delayed the transit of digesta in the small intestine (van Leeuwen and Jansman, 2007). Fibres that have a high water-binding capacity and that are not well fermented by colonic bacteria are also thought to be best for increasing luminal bulk in the colon (Brownlee, 2011), which tends to increase passage rate due to increased intestinal filling (van Leeuwen and Jansman, 2007). Filling or bulk, besides affecting gastric emptying and intestinal transit time, affects distension in the stomach (De Graaf et al., 2004), and perhaps also in the intestine, which may have contributed to the long term satiating effect of LC found in the operant test.

In the runway test, LC-L fed pigs showed a higher apparent feeding motivation than pigs fed the C diet, with levels of LC-H pigs in between. This suggests that a high inclusion level of LC could reduce feeding motivation, despite a lower energy supply compared to the C diet. The contribution of lignocellulose to ME intake is unknown, but that of other highly lignified fibre sources like straw seems to be negligible (Lange et al., 2006; Schrama and Bakker, 1999), even though total tract digestibility coefficients of non-starch polysaccharides from wheat straw of 16% were reported (review Noblet and Le Goff, 2001). It is therefore likely that 10% inclusion of lignocellulose at the expense of starch reduced ME intake by nearly 10%.

Resistant starch

Resistant starch is a highly fermentable fibre source, and its ingestion results in production of SCFA (Sleeth et al., 2010), with relatively high levels of butyrate (Regmi et al., 2011), in the distal part of the gastrointestinal tract. As a consequence, longer term satiety (i.e. more than 3 h after the meal) can develop as indicated by a reduction in spontaneous physical activity in growing pigs (Bolhuis et al., 2010; Schrama and Bakker, 1999). This may be related to a more gradual supply of energy during the day (De Leeuw et al., 2005; De Leeuw et al., 2004; Regmi et al., 2011), or to SCFA-induced specific stimulation of GLP-1 and PYY, which are gastrointestinal peptides enhancing satiety (Delzenne and Cani, 2005). In the present study, the fermentation properties of RS were expected to promote prolonged satiety feelings from 3 h up to 7 h after the meal. This hypothesis was partly accepted, as suggested by the tendency for a lower speed in the runway test

(particularly at 7 h) and by the lower proportion of time spent on feeder-directed behaviour over the day. Nevertheless, RS-fed pigs showed a reduced feeding motivation compared only with pigs fed the PEC diet, thus, not compared with pigs fed the C diet. In addition, in the operant test, RS did not reduce the number of wheel turns as we expected. This could, however, be related to a different cost-to-benefit ratio in the operant test than in the runway test as previously discussed. Moreover, at the time of the runway test and home pen behavioural observations, pigs were longer on the diets, thereby allowing better adaptation of the microbiota and intestinal tissues to RS intake, which may explain the effect of RS on feeding motivation only in the runway test and behavioural measurements.

In the analysis by fibre type, inclusion level of RS did not significantly affect responses in the feeding motivation tests. Nonetheless, the level of responding by RS-fed pigs was numerically very close to the level shown by pigs fed the C-H diet in both runway and operant tests. Calculated from measured ME/GE ratios in comparable studies using either 35% native potato starch (Bolhuis et al., 2008) or 45% retrograded maize starch (Gerrits et al., 2012), both of which were almost completely fermented, we estimated that pigs on the RS-H treatment consumed 8% less ME compared with pigs fed the C-H diet. This suggests that, the RS diet, despite a low ME supply when exchanged for digestible starch, seemed to be more (or equally) satiating than the C diet. We expected mainly long-term effects of RS on feeding motivation, but its satiating capacity was most pronounced at 1 h after the meal. This could reflect an effect of the previous meal, as it has been shown in humans that fermentation of resistant starch may be slow in the small intestine and can continue in the colon for at least 10–13 h after the meal (Achour et al., 1997).

With regard to the utilization of absorbed energy obtained from SCFA, it is expected that SCFA-derived energy will be less efficiently used for body fat deposition than energy obtained from glucose (Gerrits et al., 2012; Jorgensen et al., 1996; Noblet and van Milgen, 2004). Hence, consumption of RS may not only reduce feeding motivation, possibly resulting in reduced long-term energy intake (Wanders et al., 2011), but could also prevent weight gain by modifications in post-absorptive energy utilization.

Conclusions

In conclusion, despite a lower supply of available energy, LC and RS did not result in a greater feeding motivation compared to the C diet (higher in ME), whereas PEC increased feeding motivation in the operant and runway tests, and increased time spent on feeder-directed behaviour, stereotypic chewing and manipulative behaviour during behavioural observations. This means that PEC was the least satiating fibre, whereas both LC and RS were successful in overcoming the effects of a reduced available energy supply compared to the C diet, possibly due to their bulky and fermentation properties, respectively. The physiological mechanisms by which bulkiness and fermentation reduce feeding motivation and promote satiety, and the long term effects of fibre intake on voluntary energy intake and body weight merit further research.

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Chapter 3

Effects of dietary fibres with different fermentation characteristics on feeding motivation in adult female pigs

Satiating properties of fermentable fibres

Carol Souza da Silva[1,2], J. Elizabeth Bolhuis[1], Walter J.J. Gerrits[2], Bas Kemp[1], Joost J.G.C. van den Borne[2]

[1] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[2] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

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Abstract

Dietary fibres can be fermented in the colon, resulting in production of short-chain fatty acids (SCFA) and secretion of satiety-related peptides. Fermentation characteristics (fermentation kinetics and SCFA-profile) differ between fibres and could impact their satiating potential. We investigated the effects of fibres with varying fermentation characteristics on feeding motivation in adult female pigs. Sixteen pair-housed pigs received four diets in four periods in a Latin square design. Starch from a control (C) diet was exchanged, based on gross energy, for inulin (INU), guar gum (GG), or retrograded tapioca starch (RS), each at a low (L) and a high (H) inclusion level. This resulted in a decreased metabolizable energy intake when feeding fibre diets as compared with the C diet. According to in vitro fermentation measurements, INU is rapidly fermentable and yields relatively high amounts of propionate, GG is moderately rapidly fermentable and yields relatively high amounts of acetate, and RS is slowly fermentable and yields relatively high amounts of butyrate. Feeding motivation was assessed using behavioural tests at 1 h, 3 h and 7 h after the morning meal, and home pen behavioural observations throughout the day. The number of wheel turns paid for a food reward in an operant test was unaffected by diet. Pigs on H-diets ran 25% slower for a food reward in a runway test than pigs on L-diets, and showed less spontaneous physical activity and less stereotypic behaviour in the hours before the afternoon meal, reflecting increased interprandial satiety. Reduced feeding motivation with increasing inclusion level was most pronounced for RS, as pigs decreased speed in the runway test and tended to have a lower voluntary food intake in an ad libitum food intake test when fed RS-H. In conclusion, increasing levels of fermentable fibres in the diet seemed to enhance satiety in adult pigs, despite a reduction in metabolizable energy supply. RS was the most satiating fibre, possibly due to its slow rate of fermentation and high production of butyrate.

Keywords: dietary fibre, fermentability, satiety, feeding motivation, pigs, operant test

Introduction

Diets high in dietary fibre have been suggested to increase satiety (Burton-Freeman, 2000; Howarth et al., 2001; Slavin and Green, 2007) and to reduce energy intake (Pereira and Ludwig, 2001; Wanders et al., 2011). Nevertheless, studies on the satiating properties of dietary fibre have yielded variable and sometimes contradictory results (reviewed by Wanders et al., 2011). Dietary fibre includes a variety of non-starch polysaccharides (Topping and Clifton, 2001) that may affect satiety in humans (Delzenne and Cani, 2005; Slavin and Green, 2007) and monogastric animals, including pigs (Bolhuis et al., 2010; De Leeuw et al., 2008), but not all fibre types influence satiety to the same extent (Slavin, 2010).

The physicochemical properties of dietary fibre such as bulkiness, viscosity and fermentability may contribute to the satiating potential of high-fibre diets in humans and pigs (Wanders et al., 2011; Souza da Silva et al., 2012). Putative underlying mechanisms for the satiating effect of dietary fibre include dilution of the energy content of food items (Burton-Freeman, 2000), increased chewing activity, saliva production and gastric juice production (Benelam, 2009), delayed gastric emptying, and reduced intestinal transit (Brownlee, 2011). In addition, some types of dietary fibre are fermented by intestinal microbiota, yielding short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate, that can impact feelings of satiety (Darzi et al., 2011). SCFA can stimulate the release of satiety-related peptides, such as peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) (Delzenne and Cani, 2005) from entero-endocrine cells. Both peptides are likely to affect satiety via a direct effect in the central nervous system, and via their effects on gut motility (De Silva and Bloom, 2012), delaying gastric emptying and slowing transit time ('ileal brake') to enhance digestion and nutrient absorption while reducing appetite (Sleeth et al., 2010). GLP-1 is also an incretin hormone, enhancing glucose dependent insulin release after a meal (De Silva and Bloom, 2012). In humans and rats, free fatty acid (FFA) receptors (co-localized with PYY secreting cells) are activated by SCFA, and may thereby cause hormonal secretion to induce satiety (Darzi et al., 2011). Moreover, despite a lower energetic utilization of SCFA as compared with that of glucose (Gerrits et al., 2012), SCFA can contribute significantly to the energy supply of pigs (Jørgensen et al., 1997). In humans, SCFA have been estimated to contribute approximately 10% of daily energy requirements (Conterno et al., 2011). Due to the timing of fermentation, which can continue for more than 10 h after fibre ingestion (Achour et al., 1997), SCFA are useful for covering energy requirements, especially when postprandial glucose absorption from the small intestine is decreased or completed (De Leeuw et al., 2005). Furthermore, fermentable fibres, such as resistant starch, when replacing the available carbohydrate fraction of a meal, clearly reduce postprandial

glycemic and insulinemic responses (Higgins, 2004; Robertson, 2012). In this way, SCFA seem to stabilize plasma glucose and insulin concentrations, which may potentially enhance satiety up to several hours after a meal rich in fermentable fibre (De Leeuw et al., 2005; Higgins, 2004).

In humans, only a few studies have evaluated the effects of fermentable fibre on satiety, and their results were contradictory (Higgins, 2004; Howarth et al., 2001; Papathanasopoulos and Camilleri, 2010). Such studies are, however, often complicated by the time required for microbial adaptation to fermentable fibre, and the difficulty standardizing external factors affecting satiety regulation (e.g. age, body weight, gender, food intake). Therefore, rodents are frequently used as models, but they are coprophagic and cecal fermenters, and their colon, neither sacculated nor long (Kararli, 1995), differs substantially from that of humans. Pigs, which are omnivorous colonic fermenters, like humans, are therefore increasingly advocated as models to study digestive function (Guilloteau et al., 2010; Topping and Clifton, 2001). Using adult pigs, we have recently shown that a fermentable fibre was more satiating than viscous and bulky fibres (Souza da Silva et al., 2012). The fermentation characteristics (fermentation kinetics and SCFA-profile) differ substantially between fibre types. For instance, resistant starch yields relatively high amounts of butyrate as compared with other fibres (Champ et al., 2003). Relatively high amounts of butyrate could differentially affect satiety through increased secretion of satiety-related peptides (Zhou et al., 2006). Apart from differing in fermentation end-products, fibres also differ in fermentation kinetics. For instance, fructans are rapidly fermented, whereas resistant starches are more slowly fermented (Williams et al., 2005). These differential characteristics could impact the satiating potential of fermentable fibres, but it is unknown how the fermentation kinetics and end-products of fermentation affect satiety.

The aim of our study was to assess the effects of three highly fermentable fibres, each included at two levels and varying in fermentation characteristics, on feeding motivation in adult female pigs throughout the day. Fibres were selected based on different fermentation characteristics: inulin is rapidly fermentable and yields relatively high amounts of propionate, guar gum is moderately rapidly fermentable and yields relatively high amounts of acetate, and retrograded tapioca starch is slowly fermentable and yields relatively high amounts of butyrate. Observations of diurnal behavioural patterns and feeding motivation tests, which are considered valuable tools in the assessment of hunger and satiety (Day et al., 1997), were conducted at different time intervals after the meal. Foraging-related behaviours may be indicators of feeding motivation in pigs (D'Eath et al., 2009; De Leeuw et al., 2008), but may be occasionally masked by motivation for other resources or may be indicators of other types of frustration (Verbeek et al., 2011). Therefore, in our previous (Souza da Silva et al., 2012) and present studies

behavioural observations were combined with feeding motivation tests to assess satiety. The fibres were exchanged for pregelatinized starch from a control diet based on gross energy (GE) content. This implies that satiating effects of the fibres had to overcome the effects of a reduced metabolizable energy (ME) supply resulting from the removal of starch from the control diet.

Materials and methods

Feeding motivation was assessed in 16 adult female pigs fed three different fibre types at two inclusion levels. Each pig received four dietary treatments (i.e. one of three fibre diets and a control diet, each at two levels) in four 14-day periods in a Latin Square design. During the first 7 days of each period, pigs were fed a low fibre diet (L), and during the second 7 days they were fed a high fibre diet (H) (for details see *Diets and feeding*). The protocol for this experiment was approved by the Animal Care and Use Committee of Wageningen University.

Animals and housing

Adult female pigs (18 months of age) with an initial body weight of 250±3.1 kg (n=16; PIC Benelux B.V.) were used in this experiment. They were housed in pairs in 11 m^2 partially slatted pens in one of two air conditioned rooms at the experimental facilities of Wageningen University, The Netherlands. Pairs received the same diet within each period. Pigs were individually fed using separate feeding places with closing gates. Each feeding place was equipped with a feeder and a water barrel connected to a nipple drinker next to the feeder. Pigs had free access to the feeding places to access water at all times. Room temperature was maintained at 20±1 °C and pens were cleaned daily. Lights were on from 0630 until 2200 h and dimmed during the dark period. Pigs were provided with a variety of toys, which were changed daily.

Diets and feeding

Pigs received four dietary treatments differing in fibre type: inulin (INU), guar gum (GG), resistant starch from retrograded tapioca starch (RS), and a low-fibre control (C). Fibres were selected based on different fermentation characteristics, i.e. fermentation kinetics and SCFA-profile, which were expected to differently affect satiety. Based on literature (Champ et al., 2003; Regmi et al., 2011; Williams et al., 2005), and confirmed by fermentation in vitro (see below), INU is rapidly fermentable and yields relatively high amounts of propionate, GG is moderately rapidly fermentable and yields relatively high amounts of acetate, and RS is slowly

fermentable and yields relatively high amounts of butyrate. The different fibres were exchanged for pregelatinized potato starch (i.e. readily digestible starch) from the C diet based on GE content (Table 1). For studying dose–response relationships, each fibre was fed at two inclusion levels, i.e. a low fibre level (L) during the first 7 days and a high fibre level (H) during the last 7 days of each period. Inclusion levels were provided in the order L-H to avoid upsetting the digestive system of the pigs by sudden introduction of high levels of fibre, and because adaptation of the intestinal microbiota seems to occur more rapidly when animals are changed from a low to a high fibre diet (Sappok, 2012). The maximum level of fibre provided to the pigs without causing digestive problems or feed refusals was determined based on previous studies (Bolhuis et al., 2008; Petkevicius et al., 2007; Pluske et al., 1998), and used for formulating the H-diets. For the L-diets, 50% of this maximum level was used. Effective dosages for inducing satiety were expected to differ between fibre sources, due to different physicochemical properties and fermentation characteristics. Therefore, dietary levels differed between fibre types: 70 g/kg and 140 g/kg for INU (INU-L and INU-H, respectively), 50 g/kg and 100 g/kg for GG (GG-L and GG-H, respectively), and 170 g/kg and 340 g/kg for RS (RS-L and RS-H, respectively). Because all fibres were exchanged for pregelatinized starch, diets were isoenergetic on GE but not on ME basis. Therefore, the satiating effects of the fibres had to overcome the effects of a reduced ME supply from the fibre diets as compared with the C diet. To separate effects of ME intake from fibre-specific effects on feeding motivation, two C diets (C-L and C-H) were formulated with different inclusion levels of pregelatinized starch. In the C-L diet, a lower inclusion level of pregelatinized starch (−79 g/kg of the C-H diet) was used to reach a similar level of ME as in the diet with the lowest ME, i.e. the RS-H diet, as estimated from ME/GE ratios measured in a previous study (Bolhuis et al., 2008).

Diets were manufactured, as pellets, at a research feed mill (Research Diet Services B.V., Wijk bij Duurstede, The Netherlands), and were given a flavour (Luctarom Advance Cherry Honey, Lucta S.A., Barcelona, Spain) to mask differences in palatability as much as possible. Pigs were fed 1050 g per meal at 0700 h and 1700 h, which corresponded with approximately 1.2 times the energy requirements for maintenance (net energy=293 kJ/$kg^{0.75}$/day), based on the average metabolic body weight ($kg^{0.75}$) of the pigs at the start of the experiment. At a maximum time of 1 h after food was manually provided, gates of the individual feeding places were reopened and food refusals (if present) were collected. Water was continuously available throughout the study, via a nipple drinker located next to the feeder. Before the start of the experiment, pigs were habituated to each of the H-diets during 3 days to avoid neophobic responses during the experiment.

Table 1. Ingredient composition and analysed nutrient content of the dietary treatments.

	Diet							
	Control		Inulin		Guar gum		Resistant starch	
	Low	High	Low	High	Low	High	Low	High
Ingredient, g/kg[1]								
Basal diet[2]	650	650	650	650	650	650	650	650
Pregelatinized potato starch[3]	271	350	275	200	300	250	175	–
Retrograded tapioca starch[4]	–	–	–	–	–	–	170	340
Inulin[5]	–	–	70	140	–	–	–	–
Guar gum[6]	–	–	–	–	50	100	–	–
Total[7]	921	1000	995	990	1000	1000	995	990
Analyzed composition, g/kg								
Dry matter	881	883	–	887	–	882	–	906
Crude ash	62	58	–	57	–	58	–	57
Crude protein	153	146	–	139	–	145	–	141
Crude fat	27	22	–	23	–	24	–	28
Starch	453	505	–	340	–	408	–	513
Sugars	12	11	–	145	–	14	–	31
Total GE, MJ/kg	16.3	16.3	–	16.2	–	16.2	–	16.6

[1] Ingredients are expressed in g per kg of the Control-High (C-H) diet and sum up to 1000 for all diets in which the gross energy (GE) content of the fibre sources is identical to that of the pregelatinized potato starch. Deviations from 1000 in other treatment groups, therefore, reflect the relative difference in feed allowance of these groups (see footnote 7).

[2] Composition basal diet (per kg): potato protein, 50 g; maize gluten meal, 100 g; wheat, 249 g; soy oil, 15 g; animal fat, 15 g; barley, 150 g; vitamin and mineral premix, 10 g; calcium carbonate, 17 g; monocalcium phosphate, 11 g; potassium chloride, 10 g; sodium bicarbonate, 15 g; salt, 5 g; L-lysine HCl, 1.5 g; flavour Luctarom Advance Cherry Honey (Lucta S.A., Barcelona, Spain), 1.5 g. The vitamin and mineral premix supplied (per kg diet): retinol, 3 mg; cholecalciferol, 50 µg; DL-α-tocopherol, 25 mg; menadione, 1 mg; thiamin, 0.75 mg; riboflavin, 4 mg; D-pantothenic acid, 13 mg; niacin, 20 mg; cyanocobalamin, 15 µg; folic acid, 2.5 mg; biotin, 0.1 mg; pyridoxine chloride, 1 mg; choline chloride, 300 mg; Fe, 80 mg; Cu, 10 mg; Zn, 60 mg; Mn, 30 mg; Co, 0.2 mg; I, 0.75 mg; Se, 0.2 mg.

[3] Paselli™ WA 4 (Avebe Food, The Netherlands).

[4] ActiStar™ (Cargill, Belgium).

[5] Orafti IPS™ (Beneo-Orafti, Belgium).

[6] Viscogum MP 41210™ (Cargill, Belgium).

[7] To reach a similar level of metabolizable energy (ME) in the Control-Low (C-L) diet as in the diet with the lowest ME, i.e. the Resistant starch-High diet, 27.1% potato starch was included in the C-L diet. Consequently, the feeding level for the C-L diet was 7.9% lower than that for the C-H diet. The feeding level for inulin (Inulin-Low: −0.5%; Inulin-High: −1.0%) and resistant starch (Resistant starch-Low: −0.5%; Resistant starch-High: −1.0%) was slightly lower than that for the C-H diet, because the DM content of these fibre types was higher than that of pregelatinized potato starch (exchange was based on DM or GE).

Measurements

Fermentation characteristics of the three fibre types were assessed in vitro. Procedures as previously described (Jonathan et al., 2012) were followed, but each fibre type was fermented in triplicate, fermentation was continued for 72 h, and intermediate samples were not taken.

For each experimental period (day 1 to 14, Fig. 1), treatment effects on feeding motivation were assessed via live behavioural observations of pigs in their pen, and via feeding motivation tests, including an operant test, a runway test, and an ad libitum food intake test. The operant and runway tests were applied at different time intervals relative to feeding. Before the start of the experiment, pigs were habituated to the experimenter, the test room, and the feeding motivation tests (see Souza da Silva et al., 2012). Five days before the start of the experiment, pigs were subjected to the complete schedule of testing and observations that was also used during each experimental period.

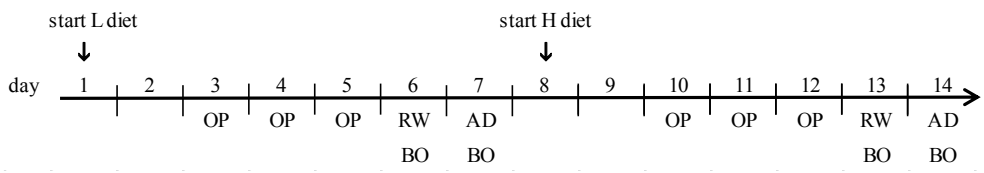

Fig. 1. Schedule of measurements on feeding motivation applied to adult female pigs fed four experimental diets (one of three fibre types or control), each at a low (L) followed by a high (H) inclusion level for a period of 14 days. OP=operant test, RW=runway test, BO=behavioural observations, AD=ad libitum food intake test.

Operant test

On days 3, 4, 5 (all at low fibre level), 10, 11 and 12 (all at high fibre level) in each period, one pig per pen (n=8) was subjected to an operant test. Operant responding was measured at 1 h, 3 h and 7 h after the morning meal on three successive days, so that each pig was tested at a different time on each day. The order of testing was balanced for diets. The operant test was performed in an air-conditioned test room (13 m^2, temperature 20±1 °C) containing a tailor-made feeder operated by a computer (Verbakel B.V., Sint Oedenrode, The Netherlands). This feeder contained a wheel and turning the wheel 360° in the forward direction a required number of times resulted in delivery of a 10-g food reward (see Souza da Silva et al., 2012 for details), which was a mixture of the three L-diets and the C-L diet. The reward schedule used during tests was a progressive ratio schedule (Lawrence and Illius, 1989; Souza da Silva et al., 2010), in which the requirements were systematically increased with one extra turn for successive rewards, i.e. pigs

were required to turn the wheel one time for the first reward, then two times for the second reward, then three times for the third reward, etc. A test session ended when the pig was not turning the wheel for ≥2 min, or when the maximum time of 20 min was reached, after which an audio cue (horn sound) was introduced, and the pig was gently guided back to her home pen. The cumulative number of turns was used as indicator of feeding motivation.

Runway test

In the runway test, conducted on days 6 (at low fibre level) and 13 (at high fibre level) in each period, all pigs (n=16) had to individually walk a fixed U-shaped track (22.5 m) for obtaining a 20-g food reward (a mixture of the three L-diets and the C-L diet) at the end of the runway. Pigs were tested at 1 h, 3 h and 7 h after the morning meal. The runway test was performed in an air-conditioned test room (temperature 20±1 °C). Before the start of each test session, the start box of the runway was closed, and the feeder at the end of the runway was baited with food. After opening the start box, each pig was given a maximum time of 3 min to reach the feeder. When the pig reached the feeder, it was allowed to eat the food reward without time limits, after which an audio cue (horn sound) was introduced and the pig was gently guided back to her home pen. During each session, the time elapsed from opening the start box to first contact with the feeder was recorded and the average speed (km/h) was calculated and used as main indicator of feeding motivation (Souza da Silva et al., 2010).

Ad libitum food intake test

In the ad libitum food intake test, conducted on days 7 (at low fibre level) and 14 (at high fibre level) in each period, all pigs (n=16) were individually offered an unlimited amount of food, which was a mixture of the three L-diets and the C-L diet, during 60 min. Pigs were tested at 3 h after the afternoon meal. The total voluntary food intake was used as indicator of feeding motivation. In addition, eating behaviour was scored during the test using continuous sampling, and used to calculate the duration of the first bout of food consumption, which was considered the moment at which a pig stopped eating for the first time (i.e. the latency to change eating behaviour to any other behaviour). The Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on a Psion Workabout MX was used for recording eating behaviour.

Behavioural observations

Pigs were observed in their home pen on days 6, 7 (all at low fibre level), 13 and 14 (all at high fibre level) in each period during six intervals of 1 h using 90 s-

instantaneous scan sampling. Live observations started at 1800 h (days 6 and 13), 0800 h, 1000 h, 1200 h, 1400 h and 1600 h (days 7 and 14). Every 90 s, the posture of each pig and its behaviour were scored. Postures were standing and walking, sitting and kneeling, and total lying, which was differentiated into lateral lying (lying on the side with all legs stretched horizontally), and ventral lying (all other lying) (Zonderland et al., 2004). Lateral lying is a more inactive form of lying than ventral lying due to the total absence of activity and reduced alertness, and therefore better represents reduced physical activity, which reflects enhanced satiety (D'Eath et al., 2009; De Leeuw et al., 2008). Behavioural activities scored were explorative behaviour (rooting or sniffing floor or pen fixtures), chewing, stereotypic chewing (repetitively chewing of pen fixtures or sham chewing) (De Leeuw et al., 2008), feeder-directed behaviour (climbing, nosing or lifting the feeder, and eating), manipulative behaviour (nibbling, suckling or chewing any part of the body of a pen mate), drinking, and other (all other behavioural activities). Explorative behaviour, (stereotypic) chewing, feeder-directed behaviour, manipulative behaviour and drinking were also summed as 'total oral behaviour'. Oral behaviours, particularly stereotypic chewing, are indicative of reduced satiety because they reflect redirected foraging behaviour, often displayed very close to feeding (D'Eath et al., 2009; De Leeuw et al., 2008). For these and all other behavioural observations, observers were blind to the treatment that the pigs had. The Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on a Psion Workabout MX was used for behavioural recordings. Behaviours were averaged per pen per observation h and expressed as percentages of observation time.

Statistical analysis

All data were averaged per pen before data analysis, so that the experimental unit was pen. The data of four pigs, equally spread over treatments across the experiment, were excluded from analysis of the runway and ad libitum food intake tests, because the pigs showed signs of lameness and were treated with painkillers on test days. Data of two other pigs (one in the runway and one in the operant test) at a specific day of testing were excluded from analysis because the pigs behaved peculiarly. Including these data, however, did not change the treatment effects found. The speed in the runway test, and the proportions of time spent on the various behaviours were log-, and arcsine square root-transformed, respectively, if the residuals of the mixed model (see below) were not normally distributed.

For the behaviours and variables in the operant and runway tests, the fixed effects of fibre type (C, INU, GG, RS), fibre level (L, H), time point (1 h, 3 h, 7 h), and their interactions were analysed using a split–split–plot mixed model in SAS

(version 9.1; SAS Institute, Cary, NC) with values in time and treatment of individual pens taken as repeated measurements. This mixed model included the fixed effect of period and the random effects of pen, pen nested within fibre type, and pen nested within fibre type and fibre level. In addition, the effects of fibre type, fibre level, and their interaction were analysed per time point in a model that contained the fixed effect of period and the random effects of pen, and pen nested within fibre type. For the variables in the ad libitum food intake test, the fixed effects of fibre type, fibre level, and their interaction were analysed using the same model.

To facilitate the interpretation of the effects of each fibre type at different levels, an additional analysis was conducted per fibre type. In this analysis, the effects of fibre inclusion level (high, low or zero, i.e. the C-H diet) on behavioural responses of the pigs were assessed per fibre type using a mixed model that included the fixed effects of inclusion level, time point and their interaction, and random effects of pen and pen within inclusion level.

In case of significant fibre type or fibre type×level effects, least square means adjusted with the Tukey–Kramer correction were used to estimate pairwise differences. Data are presented as (untransformed) least-square means±SEM on pen averages.

Results

In vitro fermentation

INU was rapidly fermentable (t_{max}=8.4 h) and yielded relatively high amounts of propionate (53% acetate, 34% propionate, 13% butyrate), GG was moderately rapidly fermentable (t_{max}=15.2 h) and yielded relatively high amounts of acetate (64% acetate, 27% propionate, 9% butyrate), and RS was slowly fermentable (t_{max}=35.3 h), following a biphasic pattern, and yielded relatively high amounts of butyrate (56% acetate, 22% propionate, 22% butyrate).

Operant test

Over the three time points, the number of wheel turns was unaffected by fibre type or fibre level (Fig. 2A), but it highly differed between time points ($F(2,108)=8.8$, $P < 0.001$), with higher levels at 7 h (556) than at 3 h (495) and 1 h (467, SEM=115) after the morning meal (see Table 2). Analysis per time point revealed that the number of wheel turns tended to be higher for H-pigs (576) than for L-pigs (536, SEM=120, $F(1,28)=3.2$, $P < 0.10$; Table 2) at 7 h after the meal.

Fibre inclusion level (high, low or zero, i.e. C-H diet) within fibre type did not influence the number of wheel turns of pigs fed GG, INU and RS.

Runway test

The speed to reach the food reward in the runway was affected by fibre level ($F(1,46)=21.4$, $P < 0.001$; Fig. 2B), time point ($F(2,112)=3.8$, $P < 0.05$) and their interaction ($F(2,112)=4.0$, $P < 0.05$) (Table 2). The speed was higher when pigs were fed L-diets, particularly at 1 h (H=2.0 km/h, L=2.7 km/h) and at 3 h (H=1.5 km/h, L=2.5 km/h, SEM=0.2) after the morning meal. Fibre inclusion level within fibre type influenced the speed of pigs when fed RS ($F(2,11)=7.4$, $P < 0.01$): the speed was higher for pigs at the low inclusion level than for pigs at the high inclusion level, with the C-H diet in between. For GG and INU, inclusion level did not affect speed.

Ad libitum food intake test

Pigs tended to have a lower voluntary food intake (fibre type×level, $F(3,28)=2.6$, $P < 0.10$; Fig. 2C) when fed RS-H (2.4 kg) than for most of the other diets, including RS-L (3.1 kg, SEM=0.3). The duration of the first bout of food consumption in this test was shorter ($F(3,46)=2.9$, $P < 0.05$) for pigs on the RS diet (29 min) than for pigs on the GG diet (35 min), with levels of C and INU in between (both 33 min, SEM=2.4, see Table 2). Fibre inclusion level within fibre type did not influence the duration of the first bout and the voluntary food intake of pigs fed GG, INU and RS.

Behavioural observations

Behaviours (except manipulative behaviour) were strongly affected by time of the day (all $P < 0.001$; Table 3). Generally, pigs were less active at the middle of the day (e.g. between 1200 h and 1300 h, 96% lying and 8% oral behaviours), but increased activity before the afternoon meal (e.g. between 1600 h and 1700 h, 79% lying and 27% oral behaviours).

Over the day, GG-fed pigs spent more time lying (90%) than C-fed pigs (85%), with levels of INU and RS (both 88%, SEM=2.0) in between ($F(3,18)=3.2$, $P < 0.05$, Fig. 2D). Sitting and kneeling occurred more in C-fed pigs (4.3%) than in RS-fed pigs (1.9%), with levels of INU (2.7%) and GG (2.4%, SEM=0.7) in between ($F(3,46)=4.4$, $P < 0.01$, Table 3). Time spent standing and walking was not affected by fibre type or level. Explorative behaviour tended to occur more in C-fed pigs (6.3%) than in GG-fed pigs (3.5%), with levels of INU and RS (both 4.0%, SEM=1.0) in between ($F(3,18)=2.7$, $P < 0.10$, Table 3). Levels of feeder-directed behaviour and manipulative behaviour were generally low. GG-fed pigs

tended to spend more time on feeder-directed behaviours (0.9%) than INU-fed pigs (0.4%), with levels of C (0.6%) and RS (0.7%, SEM=0.1) in between ($F(3,46)=2.4$, $P < 0.10$, Table 3). C-fed pigs tended to spend more time manipulating their pen mates (0.3%) than pigs on the other diets (all 0.1%, SEM=0.07), particularly between 1400 h and 1500 h (0.9%), just before the afternoon meal (interaction fibre type×time, $F(15,333)=1.5$, $P=0.10$; Table 3).

Lateral lying and stereotypic chewing, which are indicators of enhanced and reduced satiety, respectively (D'Eath et al., 2009; De Leeuw et al., 2008; Zonderland et al., 2004), were strongly affected by the interaction between fibre level and time in the present study. In general, fibre level effects on lateral lying and stereotypic chewing were most pronounced before the afternoon meal (interaction fibre level×time, $F(5,326)=4.1$ and $F(5,326)=4.3$, both $P < 0.01$; Table 3). Lateral lying occurred more in H-pigs at 1600 h, and H-pigs spent less time on stereotypic chewing than L-pigs at 1400 h and 1600 h. At 1200 h, however, lateral lying occurred more in L-pigs.

Within fibre type, lateral lying increased (interaction inclusion level×time, $F(10,117)=2.3$, $P < 0.05$) with inclusion level of RS (Fig. 3C), particularly at 1200 h, 1400 h and 1600 h, whereas stereotypic chewing decreased (interaction inclusion level×time, $F(10,117)=2.0$, $P < 0.05$) with inclusion level (Fig. 4C), particularly at 1400 h. For GG, inclusion level did not influence lateral lying (Fig. 3A) and stereotypic chewing (Fig. 4A). For INU, pigs at the low inclusion level tended to spend more time lying laterally ($F(2,117)=2.5$, $P < 0.10$) than pigs at the high inclusion level (Fig. 3B). Stereotypic chewing, however, was not affected by inclusion level of INU (Fig. 4B).

Table 2. Number of wheel turns in the operant test and speed in the runway test at 1 h, 3 h and 7 h after the morning meal, and voluntary food intake and duration of first bout of food consumption in the ad libitum food intake test at 3 h after the afternoon meal, performed by adult female pigs fed four experimental diets (Control, Guar gum, Inulin and Resistant starch) at two levels (Low vs. High). Results are expressed as least square means±SEM, n=8 for operant test and n=16 for runway and ad libitum food intake tests.

	Diet									Effects[1]				
	Control		Guar gum		Inulin		Resistant starch		Pooled					
	Low	High	Low	High	Low	High	Low	High	SEM	T	F	L	F×L	L×T
Average over the day														
Turns, no.	514	470	517	553	505	569	423	498	121	***	ns	ns	ns	ns
Speed, km/h	2.4	2.1	2.4	1.9	2.5	1.9	2.6	1.5	0.2	*	ns	***	ns	*
At 1 h after the meal														
Turns, no.	460	519	472	514	429	519	399	424	129	–	ns	ns	ns	–
Speed, km/h	2.7	2.3	2.7	1.7	2.8	2.1	2.7	1.7	0.4	–	ns	**	ns	–
At 3 h after the meal														
Turns, no.	516	325	516	557	534	545	404	524	125	–	ns	ns	ns	–
Speed, km/h	2.5	1.9	2.5	1.8	2.2	1.3	2.7	1.1	0.4	–	ns	***	ns	–
Voluntary food intake, kg	2.7	2.9	2.8	2.8	2.8	2.8	3.1	2.4	0.3	–	ns	ns	#	–
First bout duration, min	34	31	37	33	33	33	29	28	3	–	*	ns	ns	–
At 7 h after the meal														
Turns, no.	567	538	562	594	550	634	466	538	124	–	ns	#	ns	–
Speed, km/h	2.0	2.2	2.1	2.1	2.6	2.2	2.4	1.4	0.3	–	ns	ns	ns	–

[1] Significance of effects time (T), fibre type (F), fibre level (L), interaction fibre type and level (F×L), and interaction fibre level and time (L×T) is indicated. Interactions between fibre type and time, and between fibre type, fibre level and time did not influence the variables measured in the tests. # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

Table 3. Behaviour of adult female pigs fed four experimental diets (Control, Guar gum, Inulin and Resistant starch) at two levels (Low vs. High), based on live observations using 90 s-instantaneous scan sampling (six intervals of 1-h during daytime). Results are expressed as least square means±SEM, n=16.

Behavior	Diet									Effects[1]				
	Control		Guar gum		Inulin		Resistant starch							
(% of observation time)	Low	High	Low	High	Low	High	Low	High	SEM	T	F	L	F×T	L×T
Posture														
Total lying	85.0	84.1	91.0	89.0	88.5	88.0	87.6	88.4	2.4	***	*	ns	ns	ns
Ventral lying	43.5	48.1	40.7	46.6	42.8	54.9	46.5	41.7	3.9	***	ns	#	ns	**
Lateral lying	41.5	36.1	50.3	42.4	45.7	33.0	41.1	46.6	4.5	***	ns	ns	ns	**
Standing and walking	9.7	12.1	6.8	7.6	8.8	8.7	10.2	9.4	2.1	***	ns	ns	ns	ns
Sitting and kneeling	5.1	3.5	1.8	3.0	2.6	2.9	1.8	1.9	0.9	***	**	ns	ns	ns
Behavioural activity														
Total oral behaviour	23.4	23.7	20.5	19.0	23.4	23.2	24.7	20.9	3.1	***	ns	ns	ns	ns
Explorative behaviour	6.0	6.6	3.7	3.4	4.2	3.9	4.1	3.9	1.1	***	#	ns	ns	ns
Chewing	14.0	13.9	14.4	12.9	16.7	16.8	18.0	14.2	2.5	***	ns	ns	ns	ns
Stereotypic chewing	11.0	11.0	12.1	9.7	9.9	13.2	14.5	10.8	2.0	***	ns	ns	ns	***
Feeder-directed behaviour	0.6	0.6	0.8	1.0	0.5	0.3	0.6	0.7	0.2	***	#	ns	*	ns
Manipulative behaviour	0.3	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	ns	ns	ns	#	ns
Drinking	2.5	2.4	1.4	1.7	1.8	2.1	1.9	2.0	0.5	***	ns	ns	ns	ns

[1] Significance of effects time (T), fibre type (F), fibre level (L), interaction fibre type and time (F×T), and interaction fibre level and time (L×T) is indicated. Interactions between fibre type and fibre level, and between fibre type, fibre level and time did not influence the various behaviours observed. # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

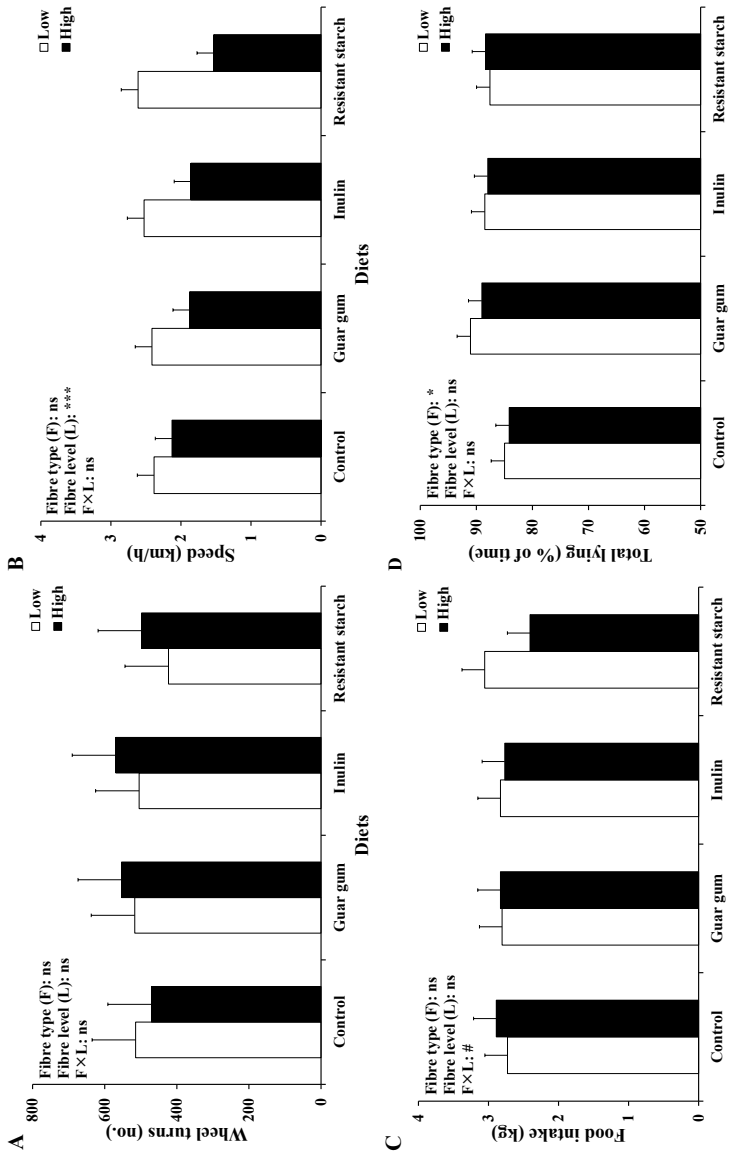

Fig. 2. Number of wheel turns in the operant test (A), speed in the runway test (B), voluntary food intake in the ad libitum food intake test (C), and percentage of time spent lying (D) performed by adult female pigs fed four experimental diets: control, guar gum, inulin and resistant starch, each at two levels: low and high. Results were averaged over the day and are expressed as least square means±SEM, n=8 for operant test and n=16 for runway test, ad libitum food intake test and behavioural observations. Pooled SEMs were 121 for wheel turns, 0.2 for speed, 0.3 for food intake and 2.4 for total lying. Statistical significance of effects of fibre type (F), fibre level (L), and interaction fibre type×level (F×L) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

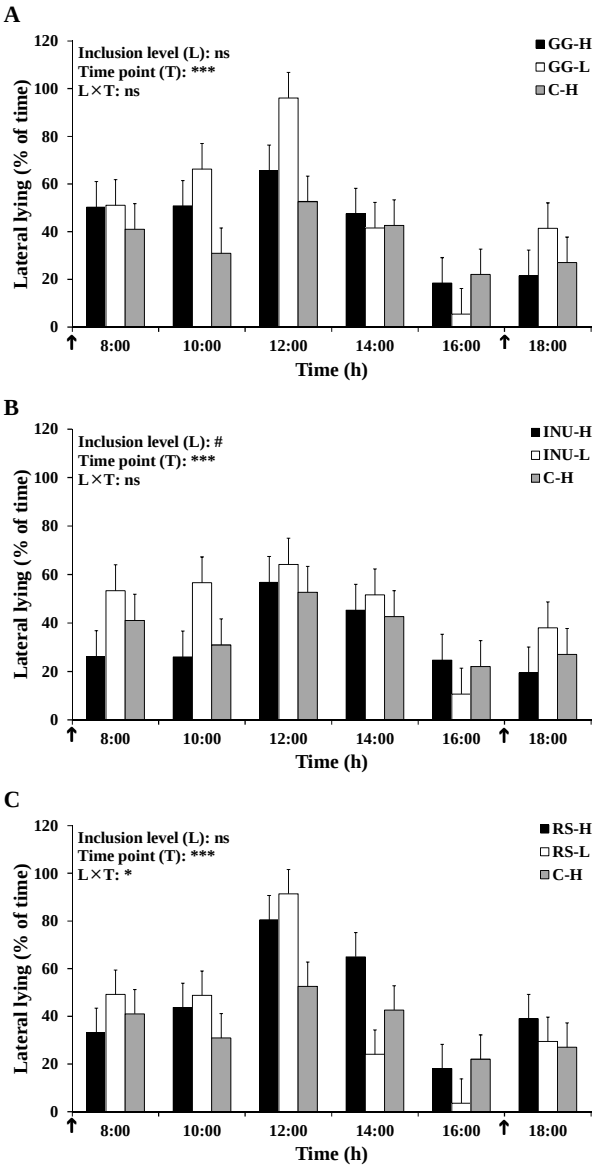

Fig. 3. Percentage of time that adult female pigs (n=16) spent on lateral lying during 1-h behavioural observations that started at 0800 h, 1000 h, 1200 h, 1400 h, 1600 h and 1800 h. Pigs were fed three fibre types: guar gum (A), inulin (B) and resistant starch (C). Each fibre type, at two inclusion levels: low (L) and high (H), is compared with the isoenergetic control high diet (C-H). Arrows indicate feeding times. Results are expressed as least square means. Pooled SEMs were 10.7 for guar gum, 10.7 for inulin, and 10.2 for resistant starch. Statistical significance of effects of inclusion level (L), time point (T) and their interaction (L×T) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

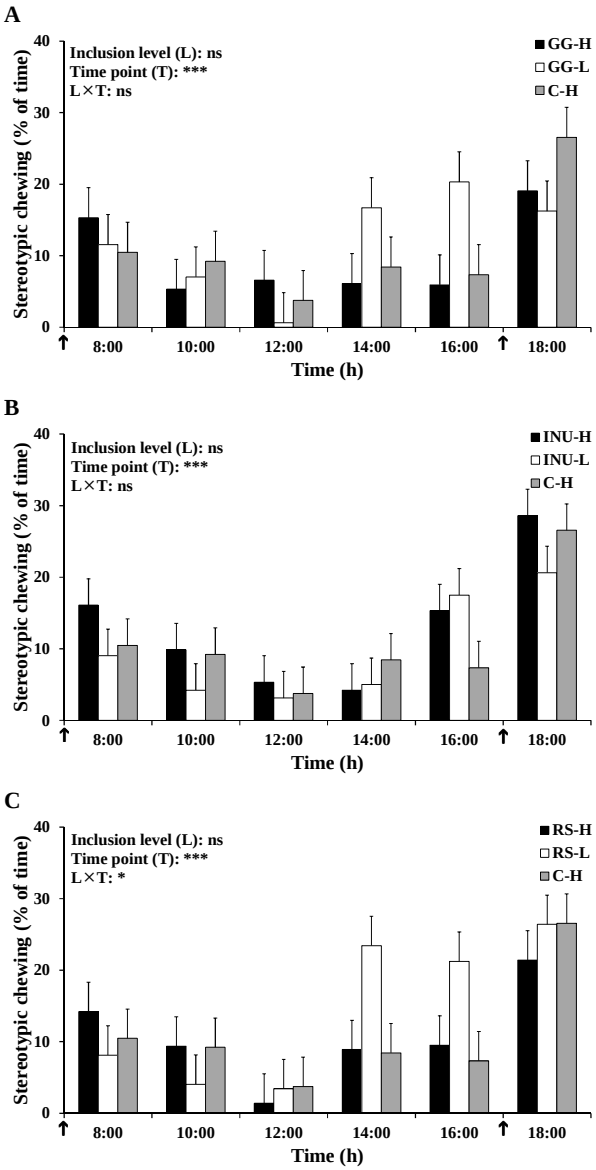

Fig. 4. Percentage of time that adult female pigs (n=16) spent on stereotypic chewing during 1-h behavioural observations that started at 0800 h, 1000 h, 1200 h, 1400 h, 1600 h and 1800 h. Pigs were fed three fibre types: guar gum(A), inulin (B) and resistant starch (C). Each fibre type, at two inclusion levels: low (L) and high (H), is compared with the isoenergetic control high diet (C-H). Arrows indicate feeding times. Results are expressed as least square means. Pooled SEMs were 4.2 for guar gum, 3.7 for inulin, and 4.1 for resistant starch. Statistical significance of effects of inclusion level (L), time point (T) and their interaction (L×T) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

Discussion

In this study, feeding motivation and behaviour of adult female pigs were assessed at different times of the day to evaluate the satiating properties of dietary fibres with distinctive fermentation characteristics (fermentation kinetics and SCFA-profile). Diets were formulated with equal GE content, implying that the satiating effects of the fibres had to overcome the effects of a reduced ME supply compared to the C diet. The drop in ME content was expected to be proportional to the fibre inclusion level in the diet. Calculated from measured ME/GE ratios in a study using 45% retrograded maize starch (Gerrits et al., 2012), which was almost completely fermented, we estimated that pigs fed the RS-H diet consumed 8% less ME than pigs fed the C-H diet. The contribution of GG and INU to ME intake is unknown, but considering that they are almost completely fermented, we assumed that the contribution of fermentable fibre to ME is constant and independent of fibre source; therefore the impact of the exchange with starch is proportional to the fibre inclusion level in the diet, i.e. 4% for GG-H and 5% for INU-H. Compared with glucose, SCFA are less efficiently converted into body fat, and yield approximately 70% of the net energy value of enzymatically digestible starch in pigs (Gerrits et al., 2012), which help to explain the drop in ME found with our diets.

Effect of fibre level

Based on behavioural observations and on speed in the runway test, pigs showed a lower feeding motivation throughout the day when fed the H-diets. This corresponds with other studies in pigs (De Leeuw et al., 2008) and humans (Slavin and Green, 2007), suggesting enhanced satiety feelings with increasing levels of fermentable fibre. H-pigs showed more lateral lying than L-pigs and displayed less stereotypic chewing, particularly just before the afternoon meal. As stereotypic chewing and increased physical activity are strong indicators of reduced satiety in pigs (D'Eath et al., 2009; De Leeuw et al., 2008), this indicates that fermentable fibres may exert a long-term satiating effect up to at least 9 h after the meal. In the runway test, H-fed pigs ran slower for food at 1 h and 3 h after the meal, which could be due to a satiating effect of the previous meal. Fermentation may indeed continue for more than 10 h after ingestion of a RS-diet in humans (Achour et al., 1997) and pigs (Gerrits et al., 2012). Second meal effects of RS were not considered in human studies (Achour et al., 1997; Raben et al., 1994), because only a single meal (approximately 50 g RS added to a meal) was provided. This resulted in reduced satiety feelings at 1 h after the meal, which was likely due to the reduced ME supply from the fermentable fibre as fermentation does not yet occur

immediately after the meal (Raben et al., 1994). It therefore seems important to have longer experimental periods, to fully evaluate the satiating potential of fermentable fibres.

It should be noted, though, that there were no fibre level effects found in the operant test. The operant procedure reflects feeding motivation in pigs (Jensen et al., 2012; Lawrence and Illius, 1989; Souza da Silva et al., 2010; Souza da Silva et al., 2012), as indicated by the strong effect of time after feeding on the number of turns (higher levels of responding with increasing time), but sensitivity of the test may be reduced when feeding levels are substantially lower than the voluntary food intake (Lawrence and Illius, 1989). In the present study, the average number of wheel turns per test session was profoundly higher (502 vs. 398) than that in our previous study with a similar design (Souza da Silva et al., 2012). The speed in the runway and the number of wheel turns in the operant test did not correlate ($r=-0.05$, $P=0.54$) in the current study, whereas they were highly correlated in our previous study where pigs were fed at 1.5 times the energy requirements for maintenance. This might indicate that the present feeding level (1.2 times maintenance), although sufficient for maintenance and some growth, resulted in relatively high levels of feeding motivation, as indicated from high levels of stereotypic chewing and a high voluntary food intake during the ad libitum food intake test (133% of daily food allowance). This increased feeding motivation may have overruled the fibre level effects in the operant test (Lawrence and Illius, 1989). Also, the reward (a mixture of L-diets) in the operant test may have been preferred (due to palatability or energy content) by the pigs over their single fibre diets, thereby increasing levels of responding (D'Eath et al., 2009). The relative contrast in ME content between the H-diets and the food reward might have influenced responses of pigs more in the operant test than in the runway test, because the trade-off between the benefits of food consumption (multiple vs. single reward) and the costs of obtaining food was greater for the operant test than for the runway test (see Souza da Silva et al., 2012). Therefore, the runway test, and to some extent also the ad libitum food intake test and behavioural observations, may have been more effective than the operant test to measure the effects of fibre level on feeding motivation in the present study. Increasing levels of fermentable fibre may thus result in diets that are at least as satiating as control diets, in spite of the reduction in ME supply. Generally, for the runway test and behavioural observations, an increase in fermentable dietary fibre content apparently successfully compensated for a reduced ME supply as compared with the C diet. Another explanation for this finding could be that pigs were longer on diets at the time of these measurements, which probably allowed better adaptation of the intestinal microbiota and tissues to fibre intake. The time required for the intestinal microbiota to adapt to a new diet in terms of fermentation capacity is not well

defined, but for various studies with pigs, a dietary adaptation time of 7 to 14 days has been often used (Anguita et al., 2006; Bauer et al., 2004; Bindelle et al., 2009), and is comparable to the experimental periods used in the current study.

Effect of fibre type

The fibres tested in the present study were characterized by different in vitro fermentation kinetics and SCFA-profile. The fermentation kinetics may determine the possible site in the gastrointestinal tract where the fibre will be fermented, while the production of specific end-products, such as SCFA, may provide insight into its potential to affect physiological mechanisms (Williams et al., 2005). The diurnal pattern of energy supply from the gastrointestinal tract may be less pulsatile and more prolonged when fermentable fibres are included in the diet. For example, a reduced passage rate through the gastrointestinal tract (applies for GG only) (Papathanasopoulos and Camilleri, 2010), or a reduced availability of glucose (from digestible starch), but increased and more uniform uptake of SCFA from the intestine (Serena et al., 2009), could maintain a continuous energy supply to the body (De Leeuw et al., 2005). SCFA indeed are associated often with a decreased variation in interprandial blood glucose and insulin concentrations (De Leeuw et al., 2005; Serena et al., 2009), but they also stimulate secretion of satiety related peptides such as PYY and GLP-1 (Delzenne and Cani, 2005), through activation of FFA receptors expressed in L-cells (Darzi et al., 2011). Furthermore, diets containing fermentable fibres such as resistant starch are effective models for studying the exchange of glucose by SCFA (Achour et al., 1997), which also leads to increased insulin sensitivity (Maki et al., 2012). Because putative mechanisms may differ per fibre type, they are discussed for each fibre separately.

Inulin

Due to its rapid rate of fermentation (t_{max}=8.4 h), INU was expected to be fermented in the small intestine and proximal colon (Williams et al., 2005), and to induce satiety more quickly after the meal than the other two fibres. However, INU did not affect feeding motivation much compared with the C diet, and at the highest inclusion level even tended to decrease lateral lying, which may indicate reduced satiety. Our results are not in accordance with studies in rats (Cani et al., 2004; Delzenne et al., 2005) and humans (Cani et al., 2009; Verhoef et al., 2011) that reported a satiating effect of inulin-type fructans. An explanation for this discrepancy could be the type of fructans used, because the chain length (degree of polymerization, DP) of fructans may affect the site of fermentation. In humans, short-chain fructans were suggested to induce satiety more than long-chain fructans, as oligofructose increased fermentation by 3-fold and enhanced satiety

feelings by increasing plasma GLP-1 and PYY (Cani et al., 2009). In that study, however, oligofructose was fed as supplement, which results in extra energy intake and meal volume (Wanders et al., 2011). In rats, inulin (DP=25) is mainly fermented in the distal colon, whereas oligofructose (DP=4.5) is predominantly fermented in the proximal colon (Cani et al., 2004). In growing pigs, a different type of inulin (DP=12), comparable to the INU in the current study, was to a large extent (20–50%) already fermented in the small intestine, with less than 0.02% being recovered in the colon (Loh et al., 2006). In contrast with humans, where >80% of inulin seems to be fermented in the colon (Ellegård et al., 1997; Knudsen and Hessov, 1995; Molis et al., 1996), in pigs >90% of inulin seems to be fermented before the colon (Branner et al., 2004; Houdijk et al., 2000; Yasuda et al., 2007). This precolonic fermentation may not sufficiently stimulate GLP-1 secretion, as GLP-1 secretion occurs mainly from L-cells in the proximal colon, at least in rats (Cani et al., 2004).

Guar gum

GG has a moderate rate of fermentation (t_{max}=15.2 h) compared to INU and RS, and was, therefore, expected to prolong satiety throughout the day. GG-fed pigs spent more time lying and less time performing explorative behaviours than C pigs, which may reflect a satiating effect of GG (De Leeuw et al., 2008). These results are in accordance with human studies, where GG consumption enhanced satiety feelings (Kovacs et al., 2001; Kovacs et al., 2002). The viscous properties of GG may reduce gastric emptying and intestinal transit time, thereby delaying glucose absorption and prolonging feelings of satiety (Kovacs et al., 2002). In growing pigs, GG also increases the viscosity of ileal digesta and prolongs the intestinal transit time (Owusu-Asiedu et al., 2006). In addition, fermentation of GG yields relatively high amounts of acetate (64%), which may enhance satiety, as in humans increased blood acetate levels were associated with enhanced satiety feelings at 4 h after ingestion of partially indigestible corn starch (Achour et al., 1997). Acetate does not directly influence glucose and insulin metabolism (Achour et al., 1997; Akanji and Hockaday, 1990; Freeland and Wolever, 2010), but may increase the secretion of satiety related peptides because intravenous and rectal acetate administration increased plasma PYY and GLP-1 levels in humans (Freeland and Wolever, 2010). This effect is thought to be mediated via a calcitonin gene related peptide (CGRP), as in rats acetate increased colonic CGRP secretion (Grider and Piland, 2007) and ileal secretion of PYY and GLP-1 (Dumoulin et al., 1995).

Resistant starch

RS was expected to yield the most pronounced effects on long-term satiety due to its slow and prolonged rate of fermentation (t_{max}=35.3 h), which may reach up to the more distal colon, as indicated by previous studies in humans (Achour et al., 1997; Molis et al., 1992) and pigs (Regmi et al., 2011; Williams et al., 2005). In addition, ingestion of RS results in relatively high levels of butyrate (Regmi et al., 2011), which may induce satiety via activation of FFA receptors (Darzi et al., 2011). In the present study, pigs decreased speed in the runway test and tended to have a lower voluntary food intake in the ad libitum food intake test with increasing level of RS. Particularly before the afternoon meal, pigs were also lying more laterally at 1200 h, 1400 h and 1600 h, and performed less stereotypic chewing at 1400 h with increasing level of RS. The combination of these findings suggests that RS was the most satiating fibre in our study, which corresponds with previous findings that a diet containing 40% native potato starch reduced feeding motivation in adult pigs up to 7 h after the meal (Souza da Silva et al., 2012). It should be noted, though, that RS did not significantly reduce responding in the operant test, albeit RS-fed pigs showed less wheel turns than pigs fed other diets, particularly at 7 h after the meal (RS: 502 vs. C: 553, GG: 578 and INU: 592). This is in line with Jensen et al. (2012) who reported a (non-significant) reduction in operant responding in pigs fed potato pulp, which even contains more fermentable material than RS alone (Mayer and Hillebrandt, 1997). In future studies using operant tests, it would be valuable to apply higher feeding levels (closer to ad libitum) or higher levels of fibre.

A putative mechanism for the satiating effect of RS is that the slow rate of fermentation promotes a more gradual and prolonged energy supply with stable blood glucose and insulin levels (De Leeuw et al., 2005; Regmi et al., 2011; Serena et al., 2009). In growing pigs, starch with a high amylose content and low in vitro digestibility delayed glucose absorption and decreased plasma insulin concentrations, and increased GLP-1 secretion from 4 h to 10 h after the meal (Regmi et al., 2011). In that study, glucose and SCFA acted as potent stimulators of GLP-1 production: during the early postprandial period GLP-1 production was predominantly sustained via glucose, but after 4 h SCFA were mainly responsible for an increased GLP-1 secretion (Regmi et al., 2011). GLP-1 secretion is stimulated by nutrient availability in the intestine (Zhou et al., 2008), and a more gradual release of glucose and SCFA from RS probably prolongs the production and secretion of GLP-1, which could result in sustained satiety feelings during the day.

In addition, fermentation of RS yields relatively high amounts of butyrate (22%), which could have influenced satiety in the adult pigs. Butyrate is an

important energy source for intestinal epithelial cells and plays a role in the maintenance of colonic homeostasis (Hamer et al., 2008), but may also promote the release of satiety-related peptides. For example, in rat epithelial cells tested in vitro, butyrate increased the expression of PYY and proglucagon mRNA (Zhou et al., 2006) and in the isolated colon of rats, it increased PYY secretion (Plaisancie et al., 1996). These effects are probably mediated via the FFA receptor 3 expressed by L-cells and activated by SCFA in the order propionate≥butyrate>acetate for PYY secretion (Darzi et al., 2011).

Conclusions

Generally, higher levels of fermentable fibre reduced feeding motivation, which means that fermentable fibres successfully compensated for the lower ME supply compared with the C diet in the current study. This leads to the conclusion that fermentable fibres, particularly RS, enhance satiety in adult pigs, which may affect long-term energy intake and body weight development. The physiological and molecular mechanisms by which RS promotes satiety merit further research, and may thereby help to reveal the effects of long-term intake of RS on body weight management and voluntary food intake.

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Chapter 4

Effects of resistant starch on behaviour, satiety-related hormones and metabolites in growing pigs

Effects of resistant starch on satiety in pigs

Carol Souza da Silva[1,2], Daniëlle Haenen[3], Sietse Jan Koopmans[1], Guido J.E.J. Hooiveld[3], Guido Bosch[2], J. Elizabeth Bolhuis[1], Bas Kemp[1], Michael Müller[3], Walter J.J. Gerrits[2]

[1] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[2] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[3] Nutrition, Metabolism and Genomics Group, Division of Human Nutrition, Wageningen University, Bomenweg 2, 6703 HD, Wageningen, The Netherlands

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Abstract

Resistant starch (RS) has been suggested to prolong satiety in adult pigs. The present study investigated the RS-induced changes in behaviour, satiety-related hormones and metabolites in catheterised growing pigs to explore the possible underlying mechanisms for RS-induced satiety. In a cross-over design with two 14-day periods, 10 pigs (approximately 58 kg) were assigned to two dietary treatments comprising diets containing either 35% pregelatinized potato starch (PS) or 34% retrograded tapioca starch (RS). Diets were isoenergetic on gross energy basis. Pigs were fed at 2.8 × maintenance. The postprandial plasma response of satiety-related hormones and metabolites was measured at the end of each period using frequent blood sampling. Faecal and urinary energy losses were measured. Behaviour was scored from video recordings using instantaneous scan sampling for 24 h. Energy digestibility and metabolisability were ~6% lower in the RS compared with the PS diet ($P < 0.001$), and metabolizable energy (ME) intake was ~3% lower in RS-fed than in PS-fed pigs ($P < 0.001$). RS-fed pigs showed less feeder-directed ($P=0.001$) and drinking ($P=0.10$) behaviours than PS-fed pigs, and higher peripheral short-chain fatty acid (SCFA) levels ($P < 0.001$) throughout the day. Postprandial glucose and insulin responses were lower in RS-fed than in PS-fed pigs ($P < 0.001$). Triglyceride levels were higher in RS-fed than in PS-fed pigs ($P < 0.01$), and nonesterified fatty acid levels did not differ between diets ($P=0.90$). Glucagon-like peptide-1 (GLP-1) levels were lower in RS-fed than in PS-fed pigs ($P < 0.001$), and peptide tyrosine tyrosine (PYY) levels did not differ between diets ($P=0.90$). Blood serotonin levels were lower ($P < 0.001$), whereas monoamine oxidase activity ($P < 0.05$) and tryptophan ($P < 0.01$) levels were higher in RS-fed than in PS-fed pigs. Despite a lower ME intake, RS seemed to enhance satiety based on behavioural observations. Possible underlying mechanisms for RS-induced satiety include increased 24 h plasma SCFA levels, and decreased postprandial glucose and insulin responses. GLP-1 and PYY seemed not to play a role in RS-induced satiety. Low blood serotonin levels suggested a difference in intestinal serotonin release between treatments. The role of intestinal serotonin on motility and transit, and immunity in relation to RS requires additional research. It is unknown if higher plasma tryptophan levels led to higher brain serotonin levels in RS-fed pigs, which could support the reduced feeder-directed behaviours after the RS diet. Increased postprandial plasma triglyceride levels corresponded with increased SCFA levels, but it is unclear if triglycerides may have signalled satiety in RS-fed pigs.

Keywords: resistant starch, pigs, hunger, physical activity, satiety-related hormones

Introduction

Resistant starch (RS) escapes enzymatic digestion in the small intestine and is largely fermented in the caecum and colon into short-chain fatty acids (SCFA) (Darzi et al., 2011). Behavioural studies in pigs suggest that RS prolongs the duration of satiety (Bolhuis et al., 2010, Souza da Silva et al., 2013, 2012). For adult pigs, RS appeared to be more satiating than other types of fermentable fibre, likely due to its slow rate of fermentation, which may impact feelings of satiety (Souza da Silva et al., 2013).

In humans, however, studies on the satiating properties of RS have yielded inconsistent results (Higgins, 2004, Wanders et al., 2011), which could be related to the difficulty to standardize external factors affecting satiety regulation (age, BW, gender, food intake) and the time required for microbial adaptation to fermentable fibre. Therefore, pigs, which are also omnivorous colonic fermenters (Topping and Clifton, 2001) are increasingly used as models for human digestive function. Moreover, the fat content of a meal influences satiety regulation, as consumption of high fat diets reduces plasma glucose levels (Higgins, 2004). Most RS diets used in human studies had a low fat content (0-5%) or were not matched for fat content, which may influence satiating effects of RS (Higgins, 2004).

Putative mechanisms for the satiating effects of RS are related to the increased microbial production of SCFA. First, increased absorption of SCFA may prolong postprandial energy supply to the body (De Leeuw et al., 2005, Darzi et al., 2011), which may cover energy requirements particularly after intestinal absorption of glucose is completed, and thereby, prolong satiety (De Leeuw et al., 2005). It has been shown that exchange of enzymatically digestible starch by RS in the diet, indeed, resulted in stabilized postprandial levels of glucose and insulin, which prolongs satiety likely by preventing drops in glucose levels below basal levels (De Leeuw et al., 2005, Serena et al., 2009). Second, SCFA may stimulate the release of the satiety-related hormones peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) from entero-endocrine cells (Keenan et al., 2012). Both hormones affect satiety via an effect in the brain (either through the circulation or through vagal afferent signals, or both) and via the 'ileal brake' (Sleeth et al., 2010). Finally, SCFA may stimulate the release of serotonin (5-hydroxytryptamine, 5-HT) in the colon through activation of free fatty acid 2 receptors expressed in 5-HT-containing cells, which affects colonic motility and overall transit time of digesta, thus contributing to satiety regulation, independently of PYY and GLP-1 (Sleeth et al., 2010).

Postprandial changes in metabolic and hormonal profiles and physical activity induced by RS are not well characterized over time, and are important to study the mechanisms by which RS promotes long-term satiety. Therefore, the present study

aimed to assess the time-course of RS-induced changes in general physical activity and behaviour (as an indicator of hunger/satiety), satiety-related hormones and metabolites in growing pigs, and relate those to feeding behaviour and meal size during an ad libitum meal. In addition, satiating effects of RS were evaluated in the presence of extra dietary fat.

Materials and methods

Animals and housing

Ten Landrace barrows (initial BW: 58±1.6 kg; age: 4 months) from eight litters (1-2 pigs per litter) were assigned to two dietary treatments in a 2×2 cross-over design with two identical 14-day experimental periods. Treatments differed in the type of starch in the diet: pregelatinized potato starch (PS) or retrograded tapioca starch (RS). Pigs were individually housed in metabolism pens (2×1 m) within a temperature controlled room (20±2 °C). Lights were on from 0500 h until 1900 h and dimmed during the night. Pens were equipped with a feeder and cleaned daily. A metal tray and funnel system beneath a rubberized metal-grid floor (0.5 cm oval holes) allowed urine collection with minimal faecal contamination. The Animal Care and Use Committee of Wageningen University and Research Centre (Lelystad, The Netherlands) approved the experiment.

Diets, feeding and surgery

The two experimental diets contained either 35% PS (Paselli^{TM} WA4, Avebe Food) or 34% RS (C*Actistar 11700, Cargill), and were designed to meet nutrient requirements according to the Dutch feed evaluation system for pigs (Centraal Veevoeder Bureau, 2007). Diets were formulated to provide equal amounts of gross energy (GE) (~17 MJ GE/kg diet). Table 1 shows the ingredient and analysed chemical composition of the diets. Diets were produced as a single batch of a basal diet to which starch sources were added. Diets were flavoured to mask differences in palatability, and TiO_2 was added as an indigestible marker. Two isocaloric high-fat (HF) diets were formulated with wheat and barley starch being exchanged for 20% soy oil resulting in a HF-PS and a HF-RS diet (Table 1). Diets were fed as mash, and mixed with water (water:feed=2.5:1) in the feeders just before feeding. Pigs were fed at 0700 h and 1600 h at 2.8× the energy requirements for maintenance (MEm=450 kJ/kg$^{0.75}$ per day). The amount of feed was adjusted daily according to the metabolic BW (kg$^{0.75}$) of the pigs and an anticipated daily gain of about 500 g. Water was continuously available.

Table 1. Ingredient and analysed chemical composition of experimental diets.

	Diet			
	PS	RS	HF-PS	HF-RS
Ingredient, g/kg				
Pregelatinized purified potato starch[1]	350.0	0.0	452.0	0.0
Retrograded tapioca starch[2]	0.0	342.6	0.0	444.0
Soy oil	29.2	29.5	198.2	201.2
Wheat	200.0	202.3	0.0	0.0
Beet pulp (sugar<100 g/kg)	50.0	50.6	64.6	65.5
Barley	150.0	151.7	0.0	0.0
Wheat gluten meal	60.0	60.7	77.5	78.6
Potato protein[3]	100.0	101.1	129.1	131.0
Premix[4]	10.0	10.1	12.9	13.1
$CaCO_3$	13.5	13.7	17.4	17.7
$Ca(H_2PO_4)_2$	11.0	11.1	14.2	14.4
NaCl	3.0	3.0	3.9	3.9
L-lysine HCL	2.2	2.2	2.8	2.9
L-tryptophan	0.2	0.2	0.3	0.3
MgO (80%)	0.4	0.4	0.5	0.5
$NaHCO_3$	14.0	14.2	18.1	18.3
KCl	3.0	3.0	3.9	3.9
TiO_2	2.0	2.0	2.6	2.6
Flavour[5]	1.5	1.5	1.9	2.0
Chemical composition, g/kg dry matter				
Dry matter (g/kg as is)	894.5	910.0	924.3	952.1
Organic matter	941.4	941.9	937.4	936.9
Crude protein (N × 6.25)	190.9	194.3	186.2	188.7
Crude fat	16.1	28.6	197.0	201.0
Starch	524.7	477.1	411.1	337.5
Sugar	13.1	69.4	6.0	70.7
Ti	1.6	1.6	–	–
Energy content, MJ/kg				
GE	16.5	16.8	–	–

PS=pregelatinized potato starch diet; RS=retrograded tapioca starch diet; HF-PS=high-fat pregelatinized potato starch diet; HF-RS=high-fat retrograded tapioca starch diet.

[1] Paselli™ WA4, Avebe Food, Veendam, The Netherlands.

[2] C*Actistar 11700, Cargill, Amsterdam, The Netherlands.

[3] Protostar, Avebe Food, Veendam, The Netherlands.

[4] Provided the following per kg of feed: vitamin A: 7500 IU; vitamin D_3: 1500 IU; vitamin E: 60 mg; vitamin K_3: 1.0 mg; vitamin B_1: 1.0 mg; vitamin B_2: 4.0 mg; vitamin B_6: 1.0 mg; vitamin B_{12}: 20 µg; niacin: 20 mg; calcium-D pantothenate: 10.5 mg; choline chloride: 100 mg; folic acid: 0.4 mg; Fe: 120 mg ($FeSO_4 \cdot H_2O$); Cu: 15 mg ($CuSO_4 \cdot 5H_2O$); Mn: 60 mg (MnO); Zn: 75 mg ($ZnSO_4 \cdot H_2O$); I: 4.0 mg (KI); Se: 0.30 mg (Na_2SeO_3); anti-oxidant: 75 mg.

[5] Luctarom Advance Cherry Honey, Lucta S.A., Barcelona, Spain.

During 1-week habituation, pigs were fed a 50:50 mix of the PS and RS diets, and adapted to individual housing and feeding regime. Furthermore, each of the two HF diets was fed twice to prevent feed neophobia during the experiment. After the habituation period, pigs were provided with two permanent blood vessel catheters in the carotid artery for blood sampling and in the jugular vein for back-up in case of a malfunctioning arterial catheter (see Koopmans et al., 2006 for details). In the week after surgery, pigs were habituated to blood sampling. After 4 to 6 days of postsurgical recovery, pigs were gradually switched to the experimental diets. In each experimental period (days 1 to 14), pigs were daily fed a restricted meal of the PS and RS diets, with the exception of the morning meal on day 8, when a restricted meal of the HF-PS and HF-RS diets was provided, and the morning meal on day 10, when an ad libitum meal of the PS and RS diets was provided.

Measurements and sample collection

BW was measured weekly. Faeces (for GE and Ti analyses) and urine (for GE analysis) were collected quantitatively per pig during the last 2 and 3 days, respectively, of each experimental period. Faecal samples were collected directly from the pen floor twice daily and urine samples were collected daily from the total urine output of each pig, and stored at -20 °C until analysis. In each period, satiating effects of the diets were assessed when pigs were fed a restricted morning meal of the HF-PS and HF-RS diets (day 8), an ad libitum morning meal (day 10), and a restricted morning meal (day 14), both of the PS and RS diets. An additional ad libitum meal of a 50:50 mix of the PS and RS diets was provided 7 h after the HF morning meals. During the ad libitum meals (days 8 and 10), feeding behaviour and voluntary feed intake were measured.

Blood samples (6 mL) were collected in EDTA tubes with protease and dipeptidyl peptidase-IV inhibitors before (at -30 and 0 min) and after (at 20, 40, 60, 90, 120, 180, 240 and 300 min) the restricted meal of the PS and RS diets on day 14, placed in ice water and centrifuged at 1300× g for 10 min at 4 °C within 20 min after collection. Plasma was stored at -80 °C until analysis. Extra blood samples (9 mL) were collected in EDTA tubes before (at -30 min) and after (at 20 and 300 min) the restricted meal on day 14. For measuring 5-HT levels, tubes with blood were placed in ice water and centrifuged at 160× g for 10 min at room temperature to obtain platelet-rich plasma (PRP). The extracted PRP (1 mL) was centrifuged at 16100× g for 15 min at room temperature to obtain platelet pellets. The washed (with 1 mL of 0.9% NaCl solution, followed by centrifugation at 13000× g for 5 min) pellets were stored at -80 °C until analysis. For measuring monoamine

oxidase (MAO) activity, blood samples (1.2 mL) were stored at -80 °C until analysis.

Chemical analyses and calculations

Diets were analysed for dry matter (ISO 6469/NEN 3332), ash (ISO 5984/NEN 3329), Kjeldahl nitrogen (ISO 5983/NEN 3145), crude fat (ISO-DIS 6492), starch and sugars (NIKO-MEMO 93–302) as previously described (Goelema et al., 1998), and for gross energy (GE) using an adiabatic bomb calorimetry (IKA-C700, Janke & Kunkel, Heitersheim, Germany). Ti was analysed using a method based on Short et al. (1996) and Myers et al. (2004). Faeces were dried at 70 °C and ground in a centrifugal mill to pass a 1.0-mm mesh screen (ZM100, Retsch B.V., Ochten, The Netherlands) prior to analyses. Faeces and urine were analysed for GE and faeces for Ti as described above. All analyses were carried out in duplicate.

Apparent faecal energy digestibility coefficient was calculated as previously described (Bosch et al., 2009) and the digestible energy (DE) content of the diet as its GE content multiplied by this coefficient. The metabolizable energy (ME) content was calculated as GE intake minus energy lost in faeces and urine.

Blood plasma was analysed for glucose (Glucose PAP SL; ELITech Group, Sees, France), insulin (Porcine/Canine Insulin EIA kit; ALPCO Diagnostics, New Hampshire, USA), triglycerides (Triglycerides liquicolor kit; Instruchemie, Delfzijl, The Netherlands), nonesterified fatty acids (NEFA) (NEFAc-kit, Wako; Instruchemie, Delfzijl, The Netherlands), active GLP-1 (GLP-1 ELISA kit; Millipore, Linco Research, Missouri, USA), active PYY (PYY EIA kit; Phoenix Pharmaceuticals, California, USA), tryptophan (Trp) (^{1}H-NMR spectroscopy), large neutral amino acids (LNAA; sum of isoleucine, leucine, valine, phenylalanine, and tyrosine) (^{1}H-NMR spectroscopy) and SCFA (^{1}H-NMR spectroscopy).

For NMR measurements, plasma samples were filtered using Nanosep® Centrifugal Devices with Omega™ Membrane (Pall Corporation) with a 10K molecular weight cut-off. To remove trace amounts of glycerine and sodium azide, all filters were washed six times (centrifuged at 14000× g for 5 min) with MQ water (500 µL), and centrifuged for 10 min after the 6th wash to make sure the filters were water free. Plasma samples were diluted 1:1 in a 75 mM phosphate buffer (pH 7.4). The diluted plasma (300 µL) was transferred to the filter and centrifuged at 14000× g for 60 min at 4 °C. The extracted solution (200 µL) was transferred to a 3 mm NMR tube (Bruker match system), and samples were stored at -20 °C until analysis. For ^{1}H-NMR spectroscopy, samples were slowly warmed up to room temperature and measured at 310K (calibrated temperature) in an Avance III NMR spectrometer operated at 600.13 MHz. Each sample was

transferred into the magnet, and equilibrated at 310K for 5 min. Subsequently, automated locking, shimming and 90° pulse angle determination was performed. For each sample ^{1}H NMR NOESY datasets were acquired, and processed and aligned using the alanine signal (upfield resonance of the alanine doublet signal) at 1.49 ppm. From the aligned spectra, integrals for resonances of the metabolites of interest were selected and quantified. Concentrations of metabolites were calculated based on the number of hydrogen atoms for each metabolite selected.

The 5-HT concentration in platelet pellets was determined using a protocol adapted from Kluge et al. (1999). Results were expressed in μmol/10^9 platelets (i.e. platelet 5-HT) and subsequently over total blood platelets in whole blood in μmol/L (i.e. blood 5-HT) by multiplying platelet 5-HT by the number of platelets counted in whole blood using a Sysmex (10^9 platelets/L).

MAO activity in whole blood was determined using a protocol adapted from Van Kempen et al. (1985), which represents MAO activity that is almost completely (>95%) found on blood platelets. Results were expressed as total amount of formed 4-hydroxyquinoline in whole blood in μmol/L/h.

Behavioural observations

On day 12 of each experimental period, the pigs' postures and behaviours were scored from video recordings using 10 min-instantaneous scan sampling for 24 h and expressed as percentages of observation time. Postures were standing and walking, kneeling and sitting, lateral lying, and ventral lying (Souza da Silva et al., 2013). Behavioural oral activities were explorative behaviour (rooting or nosing floor or pen fixtures), chewing (repetitively chewing of pen fixtures or sham chewing) (Bolhuis et al., 2010), feeder-directed behaviour (eating, and sniffing, licking or touching the feeder with the snout), drinking, and other (all other behavioural activities).

Furthermore, pigs were observed continuously for 1 h during the ad libitum meal of a 50:50 mix of the PS and RS diets 7 h after the HF-PS and HF-RS diets (day 8) and during the ad libitum meal of the PS and RS diets (day 10). Behaviours and postures scored were the same as described above, except that eating was scored separately, and used to determine the duration of the first bout of eating. The Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands) was used for all behavioural recordings.

Statistical analyses

Data were analysed using a mixed model in SAS (version 9.1; SAS Institute) with values in time and treatment of individual pigs taken as repeated measurements. For feed intake and BW data, the model included period and diet as

fixed effects and pig as random effect. For the behaviours (days 8, 10 and 12), plasma hormones and metabolites (day 14) the model included period, diet, (sampling or observation) time, and interaction of diet and time as fixed effects, and pig and pig (diet) as random effects. Sequence of treatments effect was removed from the final model if not significant ($P > 0.10$). Data are presented as least-square means±SEM.

Results

Catheters functioned well during the experiment and were accurately placed as confirmed after section. None of the diets offered was refused during the experiment. All pigs remained healthy, and had a normal growth throughout the experiment. Pigs' BW at the start (57.9±1.6 kg) and end (79.7±2.0 kg) of the experiment did not differ between diets.

Diets and feed intake

Energy digestibility (DE:GE) and metabolisability (ME:GE) were approximately 6% lower ($P < 0.001$, Table 2), and ME intake was approximately 27 kJ/kg$^{0.75}$ per day (~3%) lower in the RS compared with the PS diet ($P < 0.001$, Table 2). When a mixture of the PS and RS diets was provided ad libitum 7 h after providing a restricted meal of either HF-PS or HF-RS diets in the morning of day 8, pigs consumed 70.3 and 58.9 g/kg$^{0.75}$ (SEM=5.1), respectively, corresponding to a difference in ME intake of 165 kJ/kg$^{0.75}$ (P=0.12, Table 2). When the PS and RS diets were fed ad libitum in the morning of day 10, feed intake was about twice the normal meal size: 70.3 and 68.8 g/kg$^{0.75}$ (SEM=5.0) in PS-fed and RS-fed pigs, respectively, with no difference between diets (P=0.83). ME intake of the ad libitum meal was 69 kJ/kg$^{0.75}$ lower in RS-fed than in PS-fed pigs (P=0.52, Table 2). There was an effect of the sequence of treatments on the size of the ad libitum meal consumed on days 8 and 10. Pigs receiving the PS-RS sequence ate 23 and 15 g/kg$^{0.75}$ less compared with pigs receiving the RS-PS sequence ($P < 0.05$ and P=0.07 on days 8 and 10, respectively). This corresponded to a difference in ME intake of 332 kJ/kg$^{0.75}$ and 214 kJ/kg$^{0.75}$ ($P < 0.05$ and P=0.08 on days 8 and 10, respectively).

Table 2. Digestible and metabolizable energy intake (in kJ/kg$^{0.75}$ per day, unless indicated otherwise) in growing pigs fed an ad libitum meal of a 50:50 mix of the pregelatinized potato starch (PS) and retrograded tapioca starch (RS) diets at 7 h after a restricted high fat (HF) meal of either PS or RS (day 8), an ad libitum morning meal (day 10), and a restricted morning meal (day 14), both of the PS and RS diets[1].

	Diet			Effects[2]	
	PS	RS	s.e.[3]	D	S
Day 8, kJ/kg$^{0.75}$ per meal[4]					
ME intake	1015.3	850.2	73.8	ns	*
Day 10, kJ/kg$^{0.75}$ per meal					
ME intake	1039.0	970.1	72.9	ns	#
Day 14					
GE intake	1095.3	1137.3	4.0	***	ns
DE intake	988.9	963.1	3.5	***	ns
ME intake	982.3	955.6	3.5	***	ns
DE:GE, %	90.1	84.5	0.3	***	ns
ME:GE, %	89.6	84.0	0.3	***	ns

DE=digestible energy; GE=gross energy; ME=metabolizable energy.

[1] Throughout the experiment, pigs were daily fed a restricted meal of the PS and RS diets, with the exception of the morning meals on days 8 and 10.

[2] Statistical significance of effects of diet (D), and sequence of treatment (S) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant. Period (P) did not influence energy intake.

[3] Pooled standard error of the least-square means.

[4] Pigs were fed a HF meal containing PS or RS in the morning of day 8, and ME intake corresponds to the consumption of a 50:50 mix of the PS and RS diets 7 h after each HF meal.

Physical activity (24 h)

Behaviours on day 12 were affected by time of the day (all $P < 0.001$; Table 3), except for drinking behaviour. Generally, pigs showed a daily activity pattern characterized by peaks of activity around feeding in the morning (between 0700 h and 0900 h) and in the afternoon (between 1600 h and 1800 h), interspersed by resting periods. Lying did not differ between diets (P=0.30). RS-fed pigs spent more time lying ventrally (45% vs. 37%) and less time lying laterally (44% vs. 51%, SEM=2) than PS-fed pigs (both $P < 0.05$, Table 3). PS-fed pigs performed more feeder-directed behaviours (5% vs. 3%, SEM=1, P=0.001), and tended to spend more time drinking (1.3% vs. 0.6%, SEM=0.3, P=0.10) than RS-fed pigs (Table 3).

Table 3. Daily physical activity (in % of observation time) of growing pigs fed a restricted meal of a diet containing either pregelatinized potato starch (PS) or retrograded tapioca starch (RS), based on video observations using 10 min-instantaneous scan sampling.

	Diet			Effects[1]	
Behaviour	PS	RS	s.e.[2]	D	T
Posture					
Lying	88.0	90.0	1.0	ns	***
Ventrally	37.0	45.0	2.0	*	***
Laterally	51.0	44.0	2.0	*	***
Standing and walking	9.0	8.0	1.0	ns	***
Kneeling and sitting	3.0	3.0	1.0	ns	***
Behavioural oral activities	23.0	19.0	2.0	ns	***
Explorative behaviour	10.0	10.0	2.0	ns	***
Chewing	7.0	6.0	2.0	ns	***
Feeder-directed behaviour	5.0	3.0	1.0	**	***
Drinking	1.3	0.6	0.3	#	ns

[1] Statistical significance of effects of diet (D), and observation time (T) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant. Interaction between diet and time (D×T), and period (P) did not influence daily physical activity.

[2] Pooled standard error of the least-square means.

Behaviour during ad libitum meals

During the ad libitum meal of the PS and RS diets (day 10), all behaviours were affected by time (all $P < 0.001$). Generally, activity of the pigs decreased after the first 15 min of the meal. The duration of the first bout of eating was shorter ($P < 0.01$) in RS-fed (452 s) than in PS-fed pigs (957 s, SEM=109). RS-fed pigs spent more time on non-feeder directed explorative behaviour (77 s vs. 42 s, SEM=13, $P < 0.05$), and less time on feeder-directed behaviour, including eating, than PS-fed pigs (74 s vs. 130 s, SEM=11, $P < 0.01$). RS-fed pigs (63 s) spent less time eating than PS-fed pigs (116 s, SEM=9, $P < 0.001$), and this effect tended to be most pronounced during the first 30 min of the meal (diet×time interaction, P=0.06). During the ad libitum meal of a 50:50 mix of the PS and RS diets provided 7 h after the HF-PS and HF-RS diets (day 8), all behaviours were affected by time (all $P < 0.001$), but were unaffected by diet (data not shown).

Blood parameters

Plasma SCFA, glucose, insulin, triglycerides, NEFA, GLP-1, and PYY levels around ingestion of the restricted PS and RS meals on day 14 are presented in Fig. 1-4. 5-HT, MAO, Trp, LNAA, and Trp:LNAA ratio are given in Table 4. Plasma

SCFA levels were higher in RS-fed than in PS-fed pigs (all $P < 0.001$). Basal glucose and insulin levels did not differ between diets. When pigs were fed the restricted meal, plasma glucose levels were affected by diet ($P < 0.001$), sampling time ($P < 0.001$) and their interaction ($P < 0.01$), with lower levels in RS-fed than in PS-fed pigs, particularly at 20, 40, 60, 120 and 240 min postprandial. The peak in glucose levels at approximately 20 min postprandial was lower in RS-fed than in PS-fed pigs ($P < 0.01$). Plasma insulin levels were affected by diet ($P < 0.001$), sampling time ($P < 0.001$) and their interaction ($P < 0.001$), with lower levels in RS-fed than in PS-fed pigs, particularly at 20, 40 and 60 min postprandial. The peak in insulin at approximately 40 min postprandial was lower in RS-fed than in PS-fed pigs ($P < 0.01$). Plasma triglyceride levels were higher in RS-fed pigs than in PS-fed pigs ($P < 0.01$). Triglyceride levels differed between sampling times ($P < 0.001$), with higher levels at 90 min postprandial than at 0 min. Plasma NEFA levels were unaffected by diet ($P=0.90$), but were affected by sampling time ($P < 0.05$), with higher levels at 90, 120, 180 and 240 min than at 40 min postprandial. Plasma GLP-1 levels were lower in RS-fed than in PS-fed pigs ($P < 0.001$). GLP-1 levels differed between sampling times ($P < 0.001$), with higher levels at 60 min than at 90, 120 and 180 min postprandial. Plasma PYY levels were unaffected by diet ($P=0.90$), but tended to be affected by sampling time ($P=0.06$), with higher levels at -30 min than at 120 min postprandial.

Platelet 5-HT was affected by sampling time ($P < 0.05$) with higher levels at -30 min than at 20 min postprandial, and tended to be lower in RS-fed pigs ($P=0.08$). Blood 5-HT levels were lower ($P < 0.001$) and MAO activity was higher in RS-fed than in PS-fed pigs ($P < 0.05$). Plasma Trp levels were affected by diet ($P < 0.01$), sampling time ($P < 0.001$) and there was a trend for the effect of their interaction ($P=0.07$). Trp levels were higher in RS-fed than in PS-fed pigs, particularly at 300 min postprandial. Plasma LNAA levels and Trp:LNAA ratio tended to be higher in RS-fed than in PS-fed pigs ($P=0.08$ and $P=0.07$, respectively). LNAA levels were affected by sampling time ($P < 0.001$), with higher levels at 300 min postprandial than at -30 min. The Trp:LNAA ratio was affected by sampling time ($P=0.001$), with higher levels at -30 min than at 20 and 300 min postprandial.

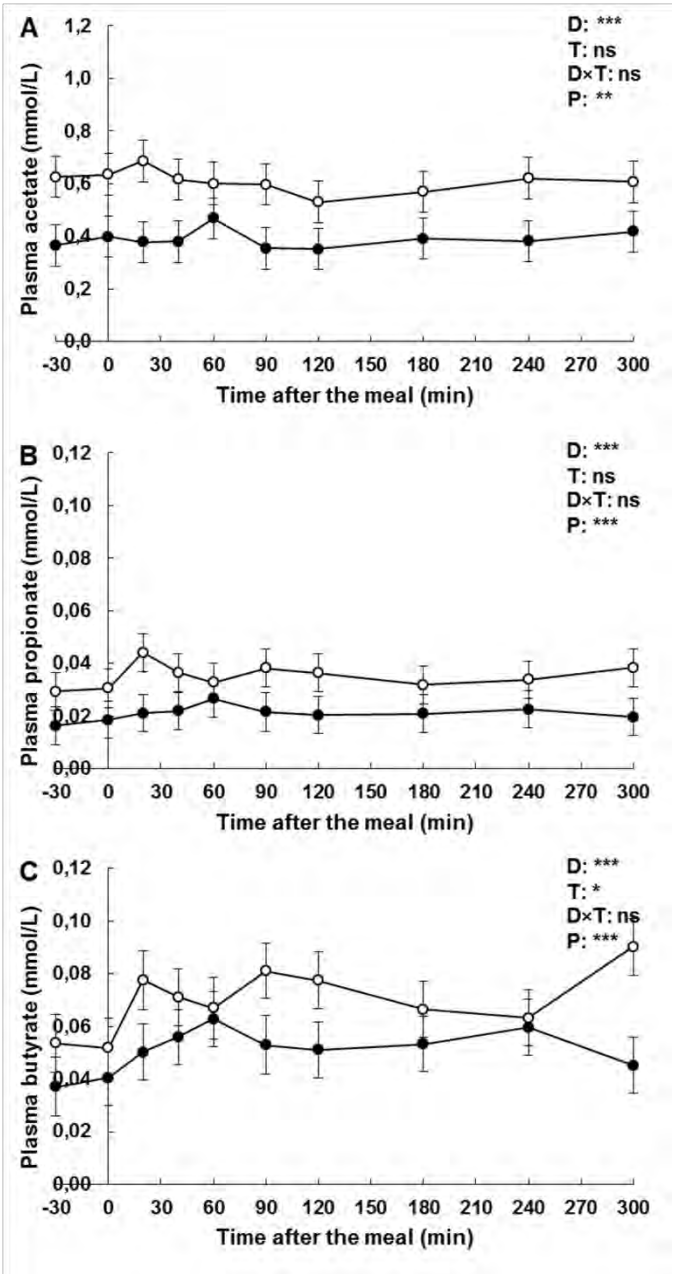

Fig. 1. Plasma concentrations of short-chain fatty acids: acetate (panel A), propionate (panel B) and butyrate (panel C), in peripheral blood collected before (-30 min), during (0 min) and after (20 to 300 min) feeding growing pigs a restricted meal of the pregelatinized potato starch (PS) diet (●) and retrograded tapioca starch (RS) diet (○) in the morning of day 14. Statistical significance of effects of diet (D), sampling time (T), their interaction(D×T), and period (P) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

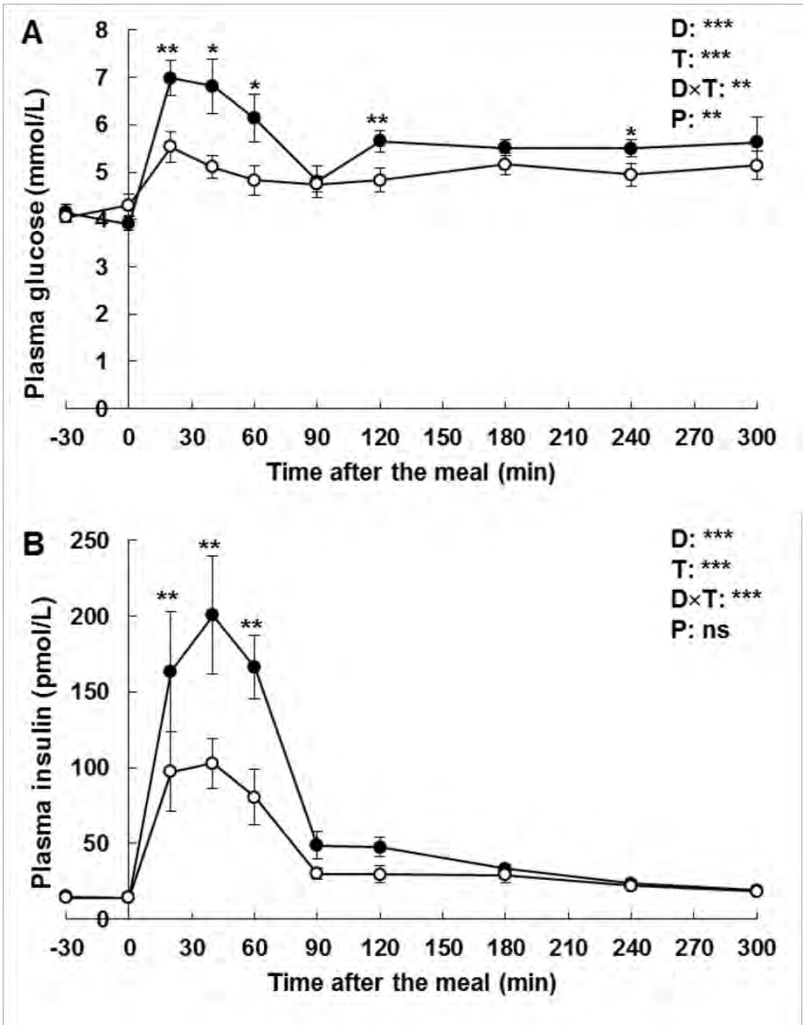

Fig. 2. Plasma concentrations of glucose (panel A) and insulin (panel B) in peripheral blood collected before (-30 min), during (0 min) and after (20 to 300 min) feeding growing pigs a restricted meal of the pregelatinized potato starch (PS) diet (●) and retrograded tapioca starch (RS) diet (○) in the morning of day 14. Statistical significance of effects of diet (D), sampling time (T), their interaction (D×T), and period (P) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

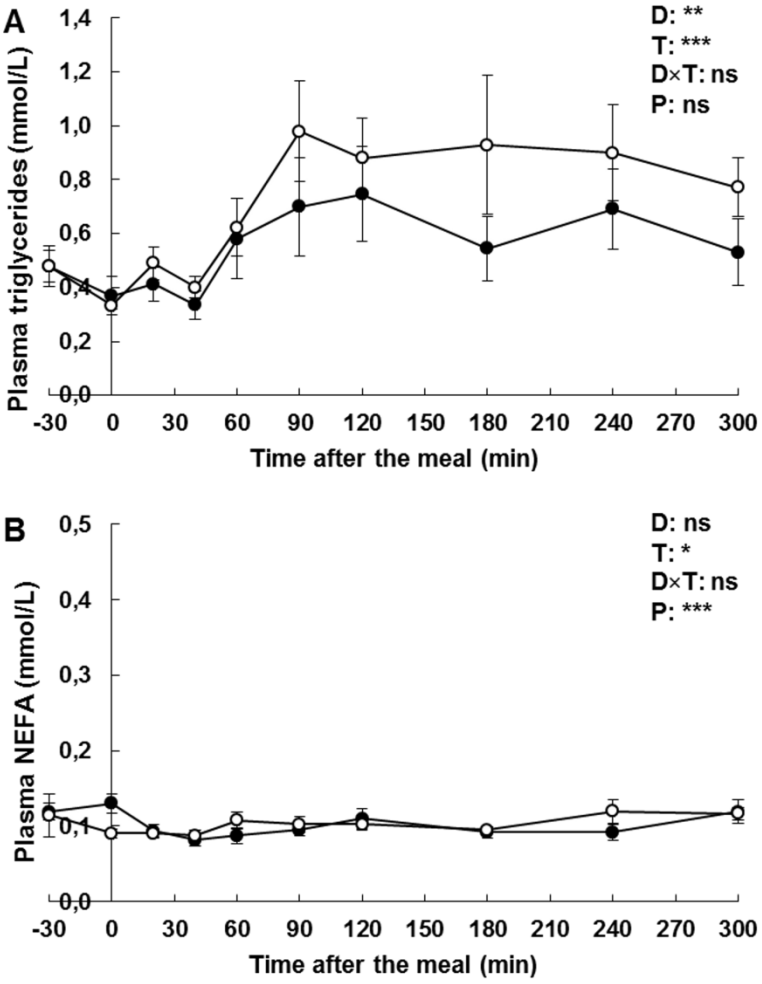

Fig. 3. Plasma concentrations of triglycerides (panel A) and nonesterified fatty acids (NEFA, panel B) in peripheral blood collected before (-30 min), during (0 min) and after (20 to 300 min) feeding growing pigs a restricted meal of the pregelatinized potato starch (PS) diet (●) and retrograded tapioca starch (RS) diet (○) in the morning of day 14. Statistical significance of effects of diet (D), sampling time (T), their interaction (D×T), and period (P) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

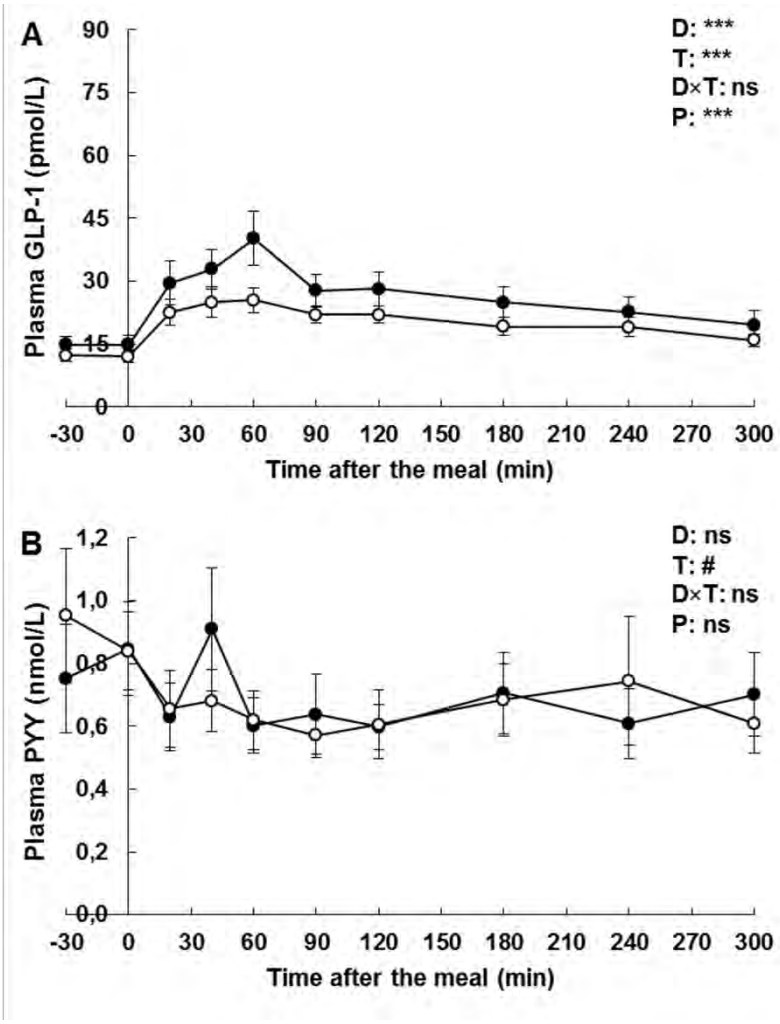

Fig. 4. Plasma concentrations of glucagon-like peptide 1 (GLP-1, panel A) and peptide tyrosine tyrosine (PYY, panel B) in peripheral blood collected before (-30 min), during (0 min) and after (20 to 300 min) feeding growing pigs a restricted meal of the pregelatinized potato starch (PS) diet (●) and retrograded tapioca starch (RS) diet (○) in the morning of day 14. Statistical significance of effects of diet (D), sampling time (T), their interaction (D×T), and period (P) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

Table 4. Platelet serotonin (platelet 5-HT, in μmol/10^9 platelets), whole blood serotonin (blood 5-HT, in μmol/L), monoamine oxidase activity (MAO, in μmol/L/h), tryptophan (Trp, in mmol/L), large neutral amino acids (LNAA, in mmol/L), and Trp:LNAA ratio in peripheral blood of growing pigs fed a restricted meal of a diet containing either pregelatinized potato starch (PS) or retrograded tapioca starch (RS).

	Diet			Effects[1]			
	PS	RS	s.e.[2]	D	T	D×T	P
Average over sampling times							
Platelet 5-HT	0.024	0.021	0.002	#	*	ns	***
Blood 5-HT	6.46	5.03	0.46	***	ns	ns	ns
MAO activity	82.94	89.34	7.70	*	ns	ns	ns
Trp	0.060	0.068	0.002	**	***	#	ns
LNAA	1.22	1.32	0.05	#	***	ns	ns
Trp:LNAA	0.050	0.053	0.002	#	**	ns	ns
30 min before the meal							
Platelet 5-HT	0.026	0.023	0.003	ns	–	–	**
Blood 5-HT	5.95	4.91	0.58	#	–	–	#
MAO activity	84.78	85.93	8.29	ns	–	–	ns
Trp	0.043	0.046	0.003	#	–	–	ns
LNAA	0.80	0.79	0.08	ns	–	–	ns
Trp:LNAA	0.054	0.059	0.002	ns	–	–	ns
20 min after the meal							
Platelet 5-HT	0.021	0.018	0.003	ns	–	–	**
Blood 5-HT	6.86	5.18	0.58	*	–	–	ns
MAO activity	79.81	91.78	8.29	*	–	–	ns
Trp	0.070	0.074	0.003	ns	–	–	ns
LNAA	1.46	1.50	0.08	ns	–	–	ns
Trp:LNAA	0.048	0.050	0.002	ns	–	–	ns
300 min after the meal							
Platelet 5-HT	0.023	0.020	0.003	ns	–	–	*
Blood 5-HT	6.56	5.01	0.58	**	–	–	ns
MAO activity	84.23	90.31	8.29	ns	–	–	ns
Trp	0.067	0.084	0.003	*	–	–	ns
LNAA	1.39	1.66	0.08	#	–	–	ns
Trp:LNAA	0.049	0.051	0.002	ns	–	–	ns

[1] Significance of effects of diet (D), sampling time (T), their interaction (D T), and period (P) is indicated: # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns=non-significant.

[2] Pooled standard error of the least-square means.

Discussion

To assess satiating effects of RS, we studied general physical activity during 24 h, and feeding behaviour and meal size during an ad libitum meal in growing pigs. In addition, we studied the effect of feeding HF diets containing RS or PS on feeding behaviour and meal size 7 h postprandial, to test whether satiating effects of RS are dependent on fat intake. RS-fed pigs showed less feeder-directed and drinking behaviours than PS-fed pigs over 24 h, indicating a long-term satiating effect of RS, which corresponds with studies reporting reduced signs of hunger such as decreased feeding motivation (Souza da Silva et al., 2013, 2012), reduced physical activity and preprandial restlessness, and enhanced satiety with dietary RS in restrictedly-fed pigs (Bolhuis et al., 2010).

When the PS and RS diets were fed ad libitum, RS-fed pigs decreased duration of the first bout of eating and reduced feeder-directed behaviour (including eating), suggesting that RS enhanced also short-term satiety in pigs, likely reflecting an effect of the previous RS meal (Souza da Silva et al., 2013). These effects of RS were found with RS-fed pigs having a 3% lower ME intake than PS-fed pigs. During an ad libitum meal, however, feed intake was similar for RS-fed and PS-fed pigs. Voluntary feed intake of a mixed diet (PS:RS=50:50) 7 h after ingestion of a HF diet was numerically reduced in the HF-RS-fed compared with the HF-PS-fed pigs, although the ME supply from the HF-RS diet was expected to be lower than that of the HF-PS diet. Thus, satiating effects of RS diets successfully compensated for a reduced ME content of the diet, but were apparently not able to further reduce voluntary feed intake during an ad libitum meal. Possibly, the drive for lean growth in the growing pigs used in the present study partly overruled the satiety-enhancing effect of RS previously observed in adult pigs (Souza da Silva et al., 2013). Alternatively, the ad libitum meal test may be less sensitive after a prolonged period of restricted feeding (~9 h inter-meal interval), resulting in temporary over-feeding and large variation in voluntary feed intake. Remarkably, feed intake during ad libitum meals on days 8 and 10 was lower for pigs receiving PS in period 1 followed by RS in period 2, than for pigs receiving RS-PS (sequence effect), which could be due to a decrease in the time needed for adaptation to the RS after feeding PS. Adaptation of the intestinal microbiota composition may occur more rapidly when pigs are changed from a low to a high fibre diet, which is accompanied with increased fermentation activity (Sappok, 2012). This increased fermentation likely contributed to the decreased voluntary feed intake in pigs receiving RS as last treatment.

Mechanisms for satiating effects of RS

To explore mechanisms by which RS may promote long-term satiety, we evaluated the effect of dietary RS on postprandial satiety-related hormones and metabolites. Mechanisms for the satiating effects of RS may be related to increased production of SCFA (De Leeuw et al., 2005, Darzi et al., 2011), which can prolong energy supply to the body (De Leeuw et al., 2005). In the present study, peripheral SCFA levels were elevated throughout the day (both pre- and postprandial) in RS-fed pigs compared to PS-fed pigs, in accordance with previous studies (Topping and Clifton, 2001, Regmi et al., 2011). Moreover, postprandial glucose and insulin responses were lower in RS-fed pigs than in PS-fed pigs, reflecting a decreased influx of glucose by enzymatic digestion of RS as compared with PS, in line with other studies (Regmi et al., 2011, Giuberti et al., 2012).

In rats, SCFA from RS stimulate the release of satiety-related hormones, such as GLP-1 and PYY from entero-endocrine cells (Keenan et al., 2012), which could be a biological mechanism by which RS promotes satiety. In the present pig study, however, postprandial GLP-1 levels were lower for RS-fed than for PS-fed pigs, and PYY levels were similar for both diets throughout the day. The effect found on GLP-1 could be attributed to the lower amount of glucose released from the RS diet as compared with the PS diet, as it has been shown in pigs that GLP-1 secretion is maintained by glucose until 4 h postprandial, and thereafter by SCFA until up to 10 h postprandial (Regmi et al., 2011). In our pigs it was only possible to relate GLP-1 secretion to the differences in glucose response, and not to the differences in SCFA levels. SCFA and glucose can stimulate PYY secretion in rat intestine, but both to a lesser extent as fatty acids, which are considered the most potent stimulators of PYY secretion (Onaga et al., 2002). In the present study, the intake of long-chain fatty acids was identical between diets, thus the similar postprandial PYY levels likely resulted from reduced glucose and increased SCFA concentrations in RS-fed pigs. It is unknown whether the PYY stimulating potential of SCFA equals that of glucose in pigs, but studies in rats suggest that the PYY response may be less strong with normal luminal physiological concentrations of SCFA than with supra-physiological intestinal infusions (Onaga et al., 2002). The absence of a preprandial treatment difference in plasma PYY implies that the influence of SCFA on plasma PYY is limited, as preprandial SCFA uptake likely exceeds that of glucose in RS-fed pigs (Gerrits et al., 2012). Alternatively, a negative feedback of GLP-1 on PYY secretion may exist, as also demonstrated in humans (Näslund et al., 1999), and may have contributed to the PYY responses observed in our study.

Over the three sampling times, blood 5-HT levels were lower, whereas MAO activity and Trp levels were higher in RS-fed than in PS-fed pigs, indicating dietary

effects on 5-HT metabolism. In the present study 5-HT was quantified in blood platelets, which possess a high-affinity uptake system and accumulate high concentrations of excess 5-HT produced by the intestine (Keszthelyi et al., 2009). Dietary manipulation can influence intestinal 5-HT release (e.g. Bertrand et al., 2011), which in turn changes intestinal motility and transit (Kemperman et al., 2007), and thereby potentially affects satiety (Sleeth et al., 2010). Intestinal motility could be reduced in RS-fed pigs, possibly leading to a reduced passage rate of digesta in the colon, in line with a previous study demonstrating increased full weights of the caecum and colon in pigs fed native potato starch (Bolhuis et al., 2007). It should be noted though, that the relationship between intestinal 5-HT release and motility is not always in the same direction (see Bertrand et al., 2011). The role of locally produced 5-HT on colonic motility, transit time and satiety in relation to dietary RS remains to be elucidated.

Intestinal 5-HT release may also be related to the activity of the gastrointestinal immune system. Some of the 5-HT secreting cells (e.g. enterochromaffin and mucosal mast cells) and 5-HT itself are involved in pro-inflammatory immune responses in the gastrointestinal tract (Manocha and Khan, 2012). The low blood 5-HT levels found in the RS-fed pigs could be associated with a downregulation of genes involved in both innate and adaptive immune responses found in the colon of RS-fed pigs in this study (Haenen et al., 2013a). Changes in microbiota composition likely also play a role in the downregulated immune response observed in RS-fed pigs (Haenen et al., 2013a).

The increased plasma Trp levels (5-HT precursor) and Trp:LNAA ratios in RS-fed pigs, combined with the reduced blood 5-HT levels found in RS-fed pigs may reflect a decreased uptake of Trp into peripheral tissues. Plasma Trp:LNAA ratio has been shown to be a major determinant of brain 5-HT concentration (Fernstrom and Wurtman, 1972), which has been reported to be involved in the regulation of feeding behaviour (Magalhaes et al., 2010). For instance, it has been demonstrated that food-seeking and food-taking behaviours are usually reduced in non-human primates with increased brain 5-HT turnover (Foltin, 2001). Although it is unknown whether the higher Trp levels and Trp:LNAA ratios in the RS-fed pigs resulted in higher brain 5-HT levels, this would be consistent with the lower level of feeder-directed behaviours in these pigs.

NEFA levels measured in blood reflect mobilized fatty acids from adipose tissues, and were expected to be increased just before the morning meal, particularly in PS-fed pigs, likely coinciding with a drop in respiration quotient (RQ) (Bolhuis et al., 2008) due to increased fatty acid oxidation. There were no differences, though, in NEFA levels between treatments, likely because in growing animals the majority of nutrients is used for muscle and adipose tissues growth, with minimal rates of lipolysis. Particularly in pigs, most body lipids originate from

de novo fatty acid synthesis, and mainly glucose is used as substrate by adipose tissue (major site of fatty acid synthesis) for de novo lipogenesis (O'Hea and Leveille, 1969).

Plasma triglyceride levels were increased after feeding RS-diets, particularly between 90 and 300 min postprandial, corresponding with our previous observations in pigs fed similar diets (Haenen et al., 2013b). An explanation is that RS may have increased plasma levels of angiopoietin-related protein 4 (Angptl4), which is related to an upregulated expression of Angptl4 in the distal small intestine of RS-fed pigs (Haenen et al., 2013b). Angptl4 inhibits lipoprotein lipase (LPL), which is responsible for the hydrolysis of triglycerides, thereby increasing plasma triglyceride levels (Delzenne et al., 2011). The large difference in postprandial glucose and insulin responses between treatments occurred predominantly before 90 min postprandial. It is generally assumed that insulin stimulates triglyceride uptake in peripheral tissues and inhibits triglyceride production by the liver (Morand et al., 1992). Consequently this would have led to reduced plasma triglyceride responses in PS fed pigs, particularly in the first 90 min postprandial. Such a response was not observed. Moreover, no inverse relationship between the area under the curve (AUC) was observed between plasma triglycerides and insulin until 300 min postprandial ($r=0.04$, $P=0.88$). In contrast, AUC for triglycerides and SCFA (particularly acetate) were highly correlated ($r=0.62$, $P < 0.01$), which suggests that increased plasma triglyceride levels are mainly driven by increased SCFA levels. The expression of two genes responsible for fatty acid transport (fatty acid-binding protein 1, FABP1) and fatty acid synthesis (Acetyl-CoA carboxylase alpha, ACACA) were indeed found to be upregulated in the liver of RS-fed pigs (both $P < 0.05$, 2.58 and 1.33 fold increase, respectively) (Haenen, Souza da Silva et al., unpublished results). Inhibition of hepatic fatty acid oxidation seems to increase food intake in rats, mice and humans, although a suppressive effect of an enhanced hepatic fatty acid oxidation on feeding has not been demonstrated (Leonhardt and Langhans, 2004). Further studies are required to investigate whether triglyceride levels signal satiety.

Conclusions

In conclusion, although RS-fed pigs showed behavioural signs of increased satiety and reduced postprandial glucose and insulin responses as compared with PS-fed pigs, we did not find an increase in GLP-1 and PYY plasma levels. Dietary RS did, however, affect SCFA and triglyceride plasma levels throughout the day, and 5-HT metabolism. The involvement of these in the satiating effects of RS merits further research.

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Chapter 5

Dietary resistant starch improves mucosal gene expression profile and luminal microbiota composition in porcine colon

Effects of resistant starch on pig intestine

Daniëlle Haenen[1], Carol Souza da Silva[2,3], Jing Zhang[4], Sietse Jan Koopmans[2], Guido Bosch[3], Jacques Vervoort[5], Walter J.J. Gerrits[3], Bas Kemp[2], Hauke Smidt[4], Michael Müller[1], Guido J.E.J. Hooiveld[1]

[1] Nutrition, Metabolism and Genomics Group, Division of Human Nutrition, Wageningen University, Bomenweg 2, 6703 HD, Wageningen, The Netherlands
[2] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[3] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[4] Laboratory of Microbiology, Wageningen UR, Dreijenplein 10, 6703 HB Wageningen, The Netherlands
[5] Laboratory of Biochemistry, Wageningen UR, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

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Abstract

Dietary resistant starch (RS) favours both colonic and whole-body metabolism and health, mainly via its fermentation by microbiota in the colon that results in the production of short-chain fatty acids (SCFA). However, knowledge on the affected processes and underlying mechanisms responsible for the beneficial effects of RS is limited. The aim of the current study was to identify genes regulated by RS in the proximal colon, to elucidate which metabolic pathways were affected, and to link gene expression changes to alterations in microbiota composition and SCFA concentrations. Ten pigs, fitted with a cannula in the proximal colon for repeated collection of tissue biopsies and luminal content, were fed a digestible starch (DS) or a RS diet for two consecutive periods of 14 days in a crossover design. Colonic gene expression profiling revealed a shift upon RS consumption from the regulation of immune response towards lipid and energy metabolism. The nuclear receptor PPARG was identified as a potential key upstream regulator. RS increased the abundance of healthy gut-associated bacteria, and decreased potentially pathogenic microbial groups. Correlation analysis inversely linked potential pathogens with SCFA concentrations and genes involved in lipid metabolism. Overall, this study provides novel molecular insights on the beneficial effects of RS.

Keywords: resistant starch, whole-genome expression profiling, microbiome, short-chain fatty acids, pigs

Introduction

Dietary resistant starch (RS) is a naturally occurring starch that resists digestion in the small intestine (SI). The effects and potential health benefits of RS have been extensively studied (Topping and Clifton, 2001; Higgins, 2004; Nugent, 2005). In the first place, effects are found in the large intestine, i.e. caecum (CE) and colon (C), where RS is highly fermentable by microbiota, resulting in the production of short-chain fatty acids (SCFAs). Several animal studies have indeed shown that RS increases the caecal and faecal production of total SCFAs and the main individual SCFAs acetate, propionate and butyrate (Topping and Clifton, 2001; Bird et al., 2007; Haenen et al., 2013). Furthermore, changes in caecal and faecal microbiota composition have been demonstrated with RS (Kleessen et al., 1997; Martinez et al., 2010; Haenen et al., 2013), and it has been reported that RS plays a role in the prevention of colorectal cancer (Conlon et al., 2012) and inflammatory bowel disease (Jacobasch et al., 1999).

Besides its local effects on intestinal function, RS may affect whole-body metabolism and health. An increasing amount of literature provides evidence that RS improves insulin sensitivity (Robertson et al., 2005; Johnston et al., 2010) and blood lipid profile (Lopez et al., 2001; Park et al., 2004), reduces body fat (Keenan et al., 2006; So et al., 2007) and food intake (Bodinham et al., 2010) and enhances satiety (Raben et al., 1994; Souza da Silva et al., 2013a).

A large proportion of these beneficial effects is thought to be mediated through the actions of SCFAs (Nugent, 2005). In addition to serve as metabolic substrate, both locally in the colon and in other tissues (Bergman, 1990; Bloemen et al., 2009), it is known that SCFAs can influence whole-body metabolism by acting as signalling molecules, e.g. via G protein-coupled receptors FFAR2 and FFAR3 that are expressed broadly throughout the body (Ichimura et al., 2009; Layden et al., 2013).

Genome-wide transcriptional profiling, or transcriptomics, is extensively used to study how cells respond to certain stimuli or to diagnose and predict clinical outcomes (Quackenbush, 2006; Schadt, 2009). Similarly, there is a major interest in characterizing the genes and networks that are regulated by food components, since this contributes to our understanding of a healthy diet (Muller and Kersten, 2003; Afman and Muller, 2006). Remarkably, data on the genome-wide effects of RS in the intestinal tract is scarce. It has only been reported that differential gene expression due to consumption of type 2 RS for 4 weeks suggested improvement of structure and function of the GI tract in rats compared to a corn starch diet with the same energy density (Keenan et al., 2012). In addition, the effect of colonic butyrate administration on gene expression profile in distal colonic mucosa has been investigated, showing that butyrate regulates fatty acid metabolism, electron

transport and oxidative stress pathways in healthy humans (Vanhoutvin et al., 2009).

In this study we set out to identify genes regulated by RS in the proximal colon (pCO), to elucidate which metabolic pathways were affected, and to link gene expression changes to alterations in microbiota composition and SCFA concentrations. To this end, a cross-over study was performed in pigs that were fitted with a permanent cannula in the pCO for repeated collection of luminal content and tissue biopsies. The full colonic gene expression profile and luminal microbiota composition were obtained by microarray techniques. Pigs were used as a model for humans, because the anatomy and physiology of the gastrointestinal tract of pigs and the pig genome bear a lot of similarities with those in humans (Miller and Ullrey, 1987; Guilloteau et al., 2010).

Materials and methods

Experimental design, pigs and housing

Ten Landrace barrows (17 wk of age; initial body weight of 57.9 ±1.61 kg) from eight litters were fitted with cannulas and catheters and assigned to two dietary treatments in a 2 x 2 cross-over design. Each treatment lasted for 14 days, and differed with regard to the type of starch in the diet: pregelatinized digestible starch (DS) or retrograded resistant starch (RS). Pigs were individually housed in metabolism pens of 2 m^2, equipped with a feeder. Artificial lights were on from 0500 h until 1900 h and dimmed during the dark period. All experimental protocols describing the management, surgical procedures, and animal care were reviewed and approved by the Animal Care and Use Committee of Wageningen University and Research Centre (Lelystad, The Netherlands).

Diets and feeding

The 2 experimental diets used were identical except for the type of starch. The main source of starch in the DS diet was pregelatinized potato starch (Paselli WA4, AVEBE), which was replaced on dry matter basis in the RS diet by retrograded tapioca starch (Actistar, Cargill). According to the supplier, this starch was at least 50% resistant to digestion in the SI. Based on physical and chemical characteristics the RS used in this study can be classified as RS type 3 (Nugent, 2005). Composition of the experimental diets is presented in Supplemental Table 1. Diets were fed twice a day at 0700 h and 1600 h as a mash, and mixed with water (ratio water:feed=2.5:1) in the feeders immediately before feeding. The diets were isoenergetic on gross energy (GE) basis. The daily feed allowance was adjusted to

2.8 times the energy required for maintenance (MEm=450 kJ/$kg^{0.75}$ per day) and was based on metabolic body weight ($kg^{0.75}$). Pigs were weighed every week to allow the adjustment of their feeding level in accordance to metabolic body weight. All pigs had free access to water throughout the study. During the 1-week adaptation period, before surgery, pigs were fed a 50:50 mix of the DS and RS diet, and adapted to the feeding pattern and individual housing.

Surgery

In the second week after arrival, pigs underwent surgery for the placement of a cannula in the pCO and a catheter in the carotid artery. After an overnight fast, all pigs were sedated with intramuscular Ketamine 10 mg/kg (Ketamine; Alfasan) and Midazolam 0.75 mg/kg (Dormicum; Roche) and anesthesia was intravenously induced with the anodyne Sufentanil 1 µg/kg (Sufenta; Janssen-Cilag). Pigs were intubated and anaesthesia was maintained by inhalation of 2% Sevoflurane (Abbott) combined with 40% oxygen and nitrous oxide. A Sufentanil infusion was maintained at 1 µg/kg.hour.

Pigs were surgically fitted with a simple T-cannula in the pCO 1.45 ±0.16 m distal from the ileocecal sphincter, as was confirmed at section. The cannula was inserted in the intestinal lumen, exteriorized through a hole, fastened to the exterior part and closed with a stopper (Mroz et al., 1996). In addition, pigs were fitted with a permanent blood vessel catheter (Tygon, Norton) as described previously (Koopmans et al., 2006). The catheter for blood sampling was placed in the carotid artery, fixed firmly at the site of insertion, tunnelled subcutaneously to the back of the pig and exteriorized between the shoulder blades. The catheters were filled and sealed off with saline containing heparin and penicillin (Procpen) and kept in a backpack which was glued to the skin.

The first 3 days after surgery, pigs were fed a restricted amount of the 50:50 mix of the DS and RS diet, i.e. 25%, 50% and 75% of their daily feed allowance on days 1, 2 and 3 respectively, to allow a gradual recovery and to avoid problems with the gut cannula. Pigs were habituated to digesta collection from the cannula and blood sampling in the first week after surgery. After 4 to 6 days of postsurgical recovery, pigs were gradually switched to one of two dietary treatments (DS and RS).

Colon biopsies

Biopsies from the intestinal wall of the pCO were collected 300 min after the morning meal on day 14 of each dietary treatment. Pigs were acutely sedated by intravenous injection with propofol (Alfasan). The stopper was unscrewed from the cannula and digesta was removed to expose the mucosal wall of the pCO. An

endoscope (OES Colonofiberscope, Olympus CF type ITIOL/1, Olympus Optical Co; LTD) was inserted via the permanent cannula into the lumen of the pCO, the intestinal wall was illuminated (OES Halogen light source with flash, Model CLE-F10, Olympus Optical Co; LTD) and visualized on a monitor (Endovision 538; Karl Storz). Biopsies were taken by an endoscopic biopsy forceps (Olympus FB-28U-1, 2 mm, 225 cm, Olympus Optical Co; LTD) and collected in screw cap tubes, after which they were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

Digesta collection

Digesta were collected via the gut cannula on day 14 of each dietary treatment, both 30 min before and 300 min after feeding.

After sampling of digesta and biopsies on day 14 of the last treatment period, pigs were anesthetized and exsanguinated, after which the abdominal cavity was opened. The gastrointestinal tract from stomach to anus was removed from the cavity and the length of the SI, CE and CO was determined. The SI was divided into 10 parts of equal length and the CO into 3 parts. Digesta were collected from 5 areas of the gastrointestinal tract: ileum (IL; SI part 7), CE, pCO, middle colon (mCO) and distal colon (dCO).

Collection and storage of the digesta samples, either taken from the cannula or at section, for determination of microbiota composition, SCFA concentration, and dry matter content was performed as follows. To determine microbiota composition, digesta were collected in 1.5 mL Eppendorf tubes, after which the tubes were immediately frozen in liquid nitrogen and stored at -80°C until further analyses. In addition, digesta was collected in pre-weighed 2 mL Eppendorf tubes with 0.75 mL H_3PO_4 for determining SCFA concentrations. These tubes were weighed again, thoroughly mixed on a vortex and stored at -20°C until further analysis. For measuring dry matter content, digesta were collected in empty pre-weighed Eppendorf tubes and stored at -20°C until further analysis. SCFA concentrations and dry matter content were determined in the luminal content as described before (Haenen et al., 2013).

Blood collection

Blood was drawn from the carotid artery on day 14 of each dietary treatment, 300 min after feeding. Blood was collected in 6 mL Vacutainer EDTA tubes (Becton Dickinson) supplemented with protease (Complete, EDTA-free; Roche) and dipeptidyl peptidase-4 (Millipore) inhibitors and then placed in ice water. Tubes were centrifuged for 10 min at 1300 g at 4°C within 20 min after blood collection. Plasma was aliquoted and stored at -80°C.

RNA isolation and quality control

Total RNA was isolated from CO biopsies using TRIzol reagent (Life Technologies) according to the manufacturer's instructions, followed by RNA Cleanup using the RNeasy Micro kit (Qiagen). Concentrations and purity of RNA samples were determined on a NanoDrop ND-1000 spectrophotometer (Isogen Life Science). RNA quality was verified on an Agilent 2100 Bioanalyzer (Agilent Technologies) by using 6000 Nano Chips (Agilent Technologies) according to the manufacturer's instructions. RNA was judged as suitable for array hybridization only if samples exhibited intact bands corresponding to the 18S and 28S ribosomal RNA subunits, and displayed no chromosomal peaks or RNA degradation products (RNA Integrity Number > 8.0).

Microarray hybridization and analysis

The pCO biopsies of all 10 pigs, collected on day 14 of both dietary treatments, were subjected to genome-wide expression profiling. To this end, total RNA (100 ng) was used for whole transcript cDNA synthesis using the Ambion WT expression kit (Life Technologies) and subsequently labelled using the Affymetrix GeneChip WT Terminal Labelling Kit (Affymetrix). Samples were hybridized on Porcine Gene 1.0 ST arrays (Affymetrix), washed, stained, and scanned on an Affymetrix GeneChip 3000 7G scanner. Detailed protocols for array handling can be found in the GeneChip WT Terminal Labelling and Hybridization User Manual (Affymetrix; P/N 702808, Rev. 4) and are also available on request. Packages from the Bioconductor project (Gentleman et al., 2004), integrated in an online pipeline (Lin et al., 2011), were used to analyse the array data. Various advanced quality metrics, diagnostic plots, pseudoimages, and classification methods were used to determine the quality of the arrays prior statistical analysis (Heber and Sick, 2006). Array data have been submitted to the Gene Expression Omnibus under accession number GSE45554.

The approximately 600,000 probes on the Porcine Gene 1.0 ST array were redefined utilizing current genome information (Dai et al., 2005). In this study probes were reorganized based on the gene definitions as available in the NCBI Sus scrofa Entrez Gene database, build 4.1 (Sscrofa10.2 genome assembly)[1] as well as the gene predictions made by the AUGUSTUST software[2]. Since the annotation of the pig genome is still poor, the functional annotation was improved by mapping the AUGUSTUST gene predictions to the human RefSeq database[3].

[1] ftp://ftp.ncbi.nih.gov/genomes/MapView/Sus_scrofa/sequence/BUILD.4.1/
[2] http://gbi.agrsci.dk/pig/sscrofa10_2_annotation/
[3] ssc10.2.RNA.hints.augustus.gff.gffreads.fna.vs.hsa_refseqHsa_2011_10_03.txt.gz

Out of 17,118 pig gene predictions, 14,505 were found to have a human orthologous gene. Unless otherwise stated, the functional interpretation of the transcriptome data was performed using the human orthologs.

Normalized gene expression estimates were obtained from the raw intensity values using the robust multiarray analysis (RMA) preprocessing algorithm available in the library 'AffyPLM' using default settings (Irizarry et al., 2003). Differentially expressed probe sets (genes) were identified using linear models, applying moderated t-statistics that implemented empirical Bayes regularization of standard errors (library 'limma'). To adjust for both the degree of independence of variances relative to the degree of identity and the relationship between variance and signal intensity, the moderated t-statistic was extended by a Bayesian hierarchical model to define a intensity-based moderated T-statistic (IBMT) (Sartor et al., 2006). Probe sets that satisfied the criterion of $P < 0.01$ were considered to be significantly regulated.

Changes in gene expression were related to functional changes using gene set enrichment analysis (GSEA) (Subramanian et al., 2005). GSEA has the advantage that it is unbiased, because no gene selection step is used, and a score is computed based on all genes in a gene set. Briefly, genes were ranked based on the paired IBMT-statistic and subsequently analysed for over- or underrepresentation in predefined gene sets derived from Gene Ontology, KEGG, National Cancer Institute, PFAM, Biocarta, Reactome and WikiPathways pathway databases. Only gene sets consisting of more than 15 and fewer than 500 genes were taken into account. Statistical significance of GSEA results was determined using 1000 permutations. The Enrichment Map plugin for Cytoscape was used for visualization and interpretation of the GSEA results (Merico et al., 2010; Smoot et al., 2011).

Upstream Regulator Analysis in IPA (Ingenuity Systems) was used to identify the cascade of upstream transcriptional regulators that may explain the observed gene expression changes in the dataset, and whether they are likely activated or inhibited.

To identify potentially secreted proteins, all significantly regulated genes were mapped to determine the corresponding protein IDs and peptide sequences via the UniProt database[4]. The obtained sequences were used as input for ngLOC[5] to predict the subcellular localization of these proteins.

[4] http://www.uniprot.org/
[5] http://ngloc.unmc.edu/

Microbiota analysis

Microbiota composition was determined in the luminal content collected from the pCO via the cannula 300 min after feeding on day 14 of each dietary treatment, essentially as described before (Haenen et al., 2013). Due to technical issues, samples from only 9 of the 10 pigs could be analysed. Samples were analysed on the second generation Pig Intestinal Tract Chip (PITChip), an improved version of the original phylogenetic microarray (Pérez Gutiérrez et al., 2010) which is comprised of more than 3,200 tiled oligonucleotides targeting the 16S rRNA gene sequences of 781 porcine intestinal microbial phylotypes. PITChip images were processed using Agilent's Feature Extraction Software version 9.5 and further processed in R using the library 'microbiome'[6].

SCFA determination by NMR spectroscopy

SCFA concentrations were determined in plasma samples obtained from the carotid artery 300 min after feeding on day 14 of each dietary treatment. Plasma samples were diluted 1:1 in a 75 mM phosphate buffer (pH 7.4) and filtered using Nanosep centrifugal devices with Omega Membrane (Pall Corporation). The molecular weight cut-off of the filter was 10K. Subsequently, 200 µL of the eluate was transferred to a 3 mm NMR tube (Bruker match system). Samples were stored at -20 °C until analysis using NMR spectroscopy. Before NMR measurements, samples were slowly warmed up to room temperature and measured at 310 K (calibrated temperature) in an Avance III NMR spectrometer operated at 600.13 MHz. After transfer of each sample into the magnet, the sample was equilibrated at 310 K for 5 min. Subsequently automated locking, automated shimming and automated 90 degree pulse angle determination was performed. ^{1}H NMR NOESY datasets were acquired for each sample. In addition, each dataset was automatically processed and aligned using the alanine signal (upfield resonance of the alanine doublet signal) at 1.49 ppm. From the aligned spectra, integrals for resonances of the metabolites of interest were selected and quantified. Concentrations of metabolites were calculated based on the number of hydrogens for each metabolite selected.

Multivariate correlation analysis

To get insight into the mutual interactions between the gene expression, microbiota composition and plasma SCFA data, these datasets were pair-wise combined using the linear multivariate method partial least squares (PLS), taking

[6] http://microbiome.github.com/

into account the repeated measures (multilevel) structure of the data (Liquet et al., 2012). We used the canonical correlation framework of multilevel-PLS since we deliberately did not want to make any prior assumption on the relationship between the two sets of variables that were analysed (Le Cao et al., 2009b). Analyses were performed in R using the library mixOmics (Le Cao et al., 2009a).

Statistical methods

Univariate testing of differences for individual microbial groups was performed using the Mann–Whitney U signed rank test. P values were corrected for multiple testing using the Benjamini–Hochberg's approach (Benjamini and Hochberg, 1995). Multivariate analysis was applied for PITChip data interpretation as follows. To relate changes in total bacterial community composition to diet (DS or RS), period, and the interaction of period and diet, redundancy analysis (RDA) and Principal response curves (PRC) were used as implemented in the CANOCO 5 software package (Biometris). RDA is the canonical form of principle component analysis and is a multivariate linear regression method where several response parameters are related to the same set of environmental variables. The signal intensities for 151 genus-level phylogenetic groups of PITChip were used as responsive variables. Partial RDA was employed to analyse the effect of diet on microbiota. RDA was performed by centering the species and samples, using freely exchangeable whole-plot Permutations.

SCFA concentrations measured in digesta were analysed using a mixed model in SAS (version 9.1; SAS Institute). For samples derived from the pCO cannula, time and individual pigs were included as repeated measurements. The model included period, diet, time, and interaction of diet and time as fixed effects, and pig as random effect. For samples taken at section, segment and individual pigs were included as repeated measurements. The model included diet, segment, and interaction of diet and segment as fixed effects, and pig as random effect. SCFA concentrations measured in plasma were analysed using a paired samples t-test in IBM SPSS Statistics 19. Differences were considered significant if $P < 0.05$. Results were expressed as means ± SEM.

Results

Anthropometric variables

All pigs remained healthy during the experiment and showed normal growth and appetite. Average body weight at the start of the experiment was 57.9 ± 1.6 kg and increased with 21.8 ± 1.1 kg during the study period. No significant effect of

treatment order was found with respect to body weight development (data not shown). The average lengths of the SI and CO, determined at section, were similar for both treatment groups (16.1 ± 0.62 m and 3.96 ± 0.18 m respectively).

Differentially expressed genes in colon

Microarray hybridization was performed to identify genes that were differentially expressed in pCO by RS compared to DS. The expression of 748 genes was significantly changed by RS ($P < 0.01$). Of these genes, 459 were significantly induced, whereas 289 genes were significantly suppressed by RS. The top induced gene was Intestinal-type alkaline phosphatase-like (*LOC100521756*), showing a 2.9 fold increase on RS (Fig. 1), whereas the most suppressed gene was Chitinase 3-like 1 (*CHI3L1*), with a 3.7 fold decrease on RS (Fig. 1).

Functional implications of differential gene expression

To gain insight into the underlying biological phenomena affected by RS, gene set enrichment analysis (GSEA) was performed. Results of GSEA were summarized in enrichment maps to enable the functional interpretation of enriched gene sets upon RS consumption. Using conservative significance thresholds, an enrichment map was generated that consisted of 331 nodes (gene sets), of which 57 were positively and 274 were negatively enriched (Supplemental Fig. 1). We observed that gene sets describing processes related to *lipid metabolism*, *tricarboxylic acid (TCA) cycle*, *compound sensing*, *barrier function*, and *metabolism of xenobiotics* were significantly enriched in the RS group, while gene sets involved in *immune response*, *transcription and translation*, *post-translational modification* and *intracellular processing* were enriched in the DS group (Supplemental Fig. 1). Next, expression changes of genes that contributed to the differential regulation of the 3 largest clusters of processes, i.e. *lipid metabolism*, *TCA cycle* and *immune response* were visualized (Fig. 2). These results showed a modest yet consistent regulation of genes involved in the before-mentioned processes, supporting the robustness of the analysis.

Top induced genes				Individual log2 FC									
Gene Description	**Gene symbol**	**Entrez ID**	**Mean log2 FC**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Intestinal-type alkaline phosphatase-like	LOC100521756	100521756	1.55										
Carbonic anhydrase II	CA2	100154873	1.35										
Uncharacterized LOC100621113	LOC100621113	100621113	1.01										
Hydroxysteroid (17-beta) dehydrogenase 2	HSD17B2	100312973	1.00										
Transmembrane 4 L6 family member 20-like	LOC100513630	100513630	0.96										
Uncharacterized protein C5orf4 homolog	LOC100525263	100525263	0.95										
Solute carrier family 30, member 10	SLC30A10	100623097	0.92										
Angiotensinogen-like	LOC100157073	100157073	0.86										
Carbonic anhydrase 12-like	LOC100152749	100152749	0.83										
Carcinoembryonic antigen-related cell adhesion molecule 7-like	LOC100524810	100524810	0.81										
ATP-binding cassette, sub-family A (ABC1), member 6	ABCA6	100520861	0.79										
Agmatinase, mitochondrial-like	LOC100519548	100519548	0.74										
ADAM metallopeptidase domain 23	ADAM23	100518044	0.74										
Claudin 10	CLDN10	100153752	0.73										
Thiopurine S-methyltransferase	TPMT	100157630	0.72										
Ubiquitin carboxyl-terminal hydrolase 2-like	LOC100520041	100520041	0.72										
Transient receptor potential cation channel subfamily M member 6-like	LOC100157775	100157775	0.71										
Transmembrane protein 117	TMEM117	100524623	0.71										
Poly (ADP-ribose) polymerase family, member 15	PARP15	100520273	0.70										
Serine palmitoyltransferase, long chain base subunit 3	SPTLC3	100519280	0.68										
Collagen alpha-6(VI) chain-like	LOC100516642	100516642	0.67										
Guanylate cyclase activator 2A (guanylin)	GUCA2A	100301560	0.66										
EGF containing fibulin-like extracellular matrix protein 1	EFEMP1	100512046	0.66										
Pancreatic lipase-related protein 2	PNLIPRP2	100462755	0.66										
Olfactory receptor 9K2-like	LOC100737562	100737562	0.66										
Top suppressed genes				**Individual log2 FC**									
Gene Description	**Gene Symbol**	**Entrez ID**	**Mean log2 FC**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Chitinase 3-like 1 (cartilage glycoprotein-39)	CHI3L1	396865	-1.88										
C4b-binding protein alpha chain-like	LOC100520761	100520761	-1.42										
Secretory leukocyte peptidase inhibitor	SLPI	396886	-1.13										
C-type lectin domain family 7, member A	CLEC7A	100038025	-0.94										
Immunoresponsive 1 homolog (mouse)	IRG1	100524951	-0.93										
Chemokine (C-X-C motif) receptor 4	CXCR4	396659	-0.89										
P2Y purinoceptor 13-like	LOC100524766	100524766	-0.86										
BPI fold containing family B, member 2	BPIFB2	100113424	-0.84										
Tryptophan hydroxylase 1	TPH1	100511002	-0.80										
Chromosome 1 open reading frame 162 ortholog	C4H1orf162	100627962	-0.78										
SLAM family member 7-like	LOC100154053	100154053	-0.78										
Placenta-specific gene 8 protein-like	LOC100525175	100525175	-0.78										
Antileukoproteinase-like	LOC100512873	100512873	-0.74										
Rho GTPase activating protein 15	ARHGAP15	100520808	-0.74										
Monocarboxylate transporter 7-like	LOC100739042	100739042	-0.71										
Lymphoid enhancer-binding factor 1	LEF1	100170126	-0.70										
Transmembrane protein 156-like	LOC100525349	100525349	-0.69										
Membrane-spanning 4-domains, subfamily A, member 1	MS4A1	100627952	-0.68										
SLAM family member 6	SLAMF6	100156912	-0.67										
Sorting nexin-10-like	LOC100520876	100520876	-0.66										
Acyloxyacyl hydrolase (neutrophil)	AOAH	100522290	-0.64										
Clusterin	CLU	397025	-0.63										
CD1B antigen	PCD1B	100038007	-0.62										
Interleukin 2 receptor, gamma	IL2RG	397156	-0.60										
A-kinase anchor protein 5-like	LOC100153460	100153460	-0.60										

Fig. 1. Top 25 induced genes and bottom 25 suppressed genes on the RS diet, based on the gene definitions of the NCBI Sus scrofa Entrez Gene database, build 4.1. Average log2 fold changes of the signal intensity of RS compared with DS were determined from the individual response of the 10 pigs, which are expressed as a heatmap. The intensity of the red and green color indicates the degree of induction or suppression per pig, respectively.

Microbiota analysis

Consumption of RS did not significantly change the microbial diversity as indicated by Shannon's index for diversity (data not shown). PRC analysis showed that diet (DS or RS) explained 15.7% of the total variation in microbiota, while period and the interaction of period and diet only explained 4.8% and 6.8% respectively (data not shown). This indicated that diet was the main factor driving the microbial variation. Partial RDA of the PITChip data confirmed that diet had a significant effect on microbial variation ($P < 0.05$) (Fig. 3).

At the phylum level, we observed a significant increase in the relative abundance of Bacteroidetes (P=0.036) (Supplemental Fig. 2A). As a result, we found a significantly lower Firmicutes/Bacteroidetes ratio (P=0.017) in RS-fed pigs (10.3 ± 2.0) compared to DS-fed pigs (20.1 ± 3.4) (Supplemental Fig. 2B).

At the approximate genus level (90% 16S ribosomal RNA similarity threshold), the relative abundance of *Prevotella melaninogenica*-like, *uncultured Prevotella, Enterococcus*-like, *Faecalibacterium prausnitzii*-like, *Dialister*-like, *Megasphaera elsdenii*-like, *Mitsuokella multacida*-like and *Clostridium ramosum*-like bacteria strongly increased upon RS feeding compared to DS feeding ($P < 0.05$) (Table 1). On the other hand, *Eggerthella*-like, *uncultured Bacteroidetes*, *Turicibacter*-like, *Clostridium perfringens*-like, *Anaerovorax*-like, *Peptoniphilus*-like, *uncultured Clostridia XIVa, uncultured Planctomycetacia, Sphingomonas*-like, *uncultured Betaproteobacteria, Actinobacillus indolicus*-like, *Aeromonas, Alishewanel*-like, *Halomonas*-like, *Pasteurella*-like, *Pseudomonas*-like, *Psychrobacter*-like, *Ruminobacter amylophilus*-like, *Vibrio*-like, *Xanthomonas*-like and *Leptospira* bacteria strongly decreased on the RS diet ($P < 0.05$) (Table 2).

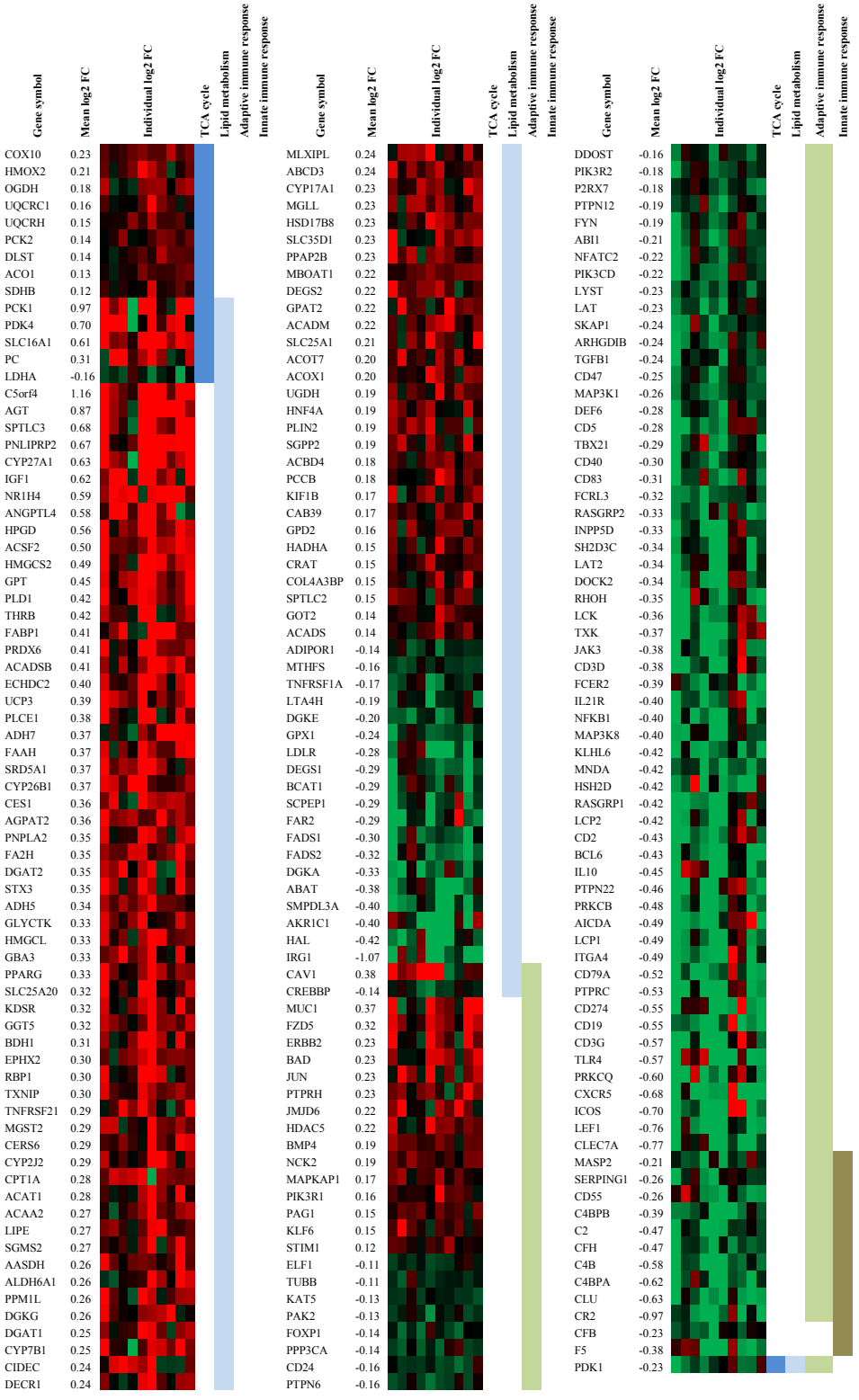

Gene symbol
Mean log2 FC
Individual log2 FC
TCA cycle
Lipid metabolism
Adaptive immune response
Innate immune response
COX10 0.23
HMOX2 0.21
OGDH 0.18
UQCRC1 0.16
UQCRH 0.15
PCK2 0.14
DLST 0.14
ACO1 0.13
SDHB 0.12
PCK1 0.97
PDK4 0.70
SLC16A1 0.61
PC 0.31
LDHA -0.16
C5orf4 1.16
AGT 0.87
SPTLC3 0.68
PNLIPRP2 0.67
CYP27A1 0.63
IGF1 0.62
NR1H4 0.59
ANGPTL4 0.58
HPGD 0.56
ACSF2 0.50
HMGCS2 0.49
GPT 0.45
PLD1 0.42
THRB 0.42
FABP1 0.41
PRDX6 0.41
ACADSB 0.41
ECHDC2 0.40
UCP3 0.39
PLCE1 0.38
ADH7 0.37
FAAH 0.37
SRD5A1 0.37
CYP26B1 0.37
CES1 0.36
AGPAT2 0.36
PNPLA2 0.35
FA2H 0.35
DGAT2 0.35
STX3 0.35
ADH5 0.34
GLYCTK 0.33
HMGCL 0.33
GBA3 0.33
PPARG 0.33
SLC25A20 0.32
KDSR 0.32
GGT5 0.32
BDH1 0.31
EPHX2 0.30
RBP1 0.30
TXNIP 0.30
TNFRSF21 0.29
MGST2 0.29
CERS6 0.29
CYP2J2 0.29
CPT1A 0.28
ACAT1 0.28
ACAA2 0.27
LIPE 0.27
SGMS2 0.27
AASDH 0.26
ALDH6A1 0.26
PPM1L 0.26
DGKG 0.26
DGAT1 0.25
CYP7B1 0.25
CIDEC 0.24
DECR1 0.24
MLXIPL 0.24
ABCD3 0.24
CYP17A1 0.23
MGLL 0.23
HSD17B8 0.23
SLC35D1 0.23
PPAP2B 0.23
MBOAT1 0.22
DEGS2 0.22
GPAT2 0.22
ACADM 0.22
SLC25A1 0.21
ACOT7 0.20
ACOX1 0.20
UGDH 0.19
HNF4A 0.19
PLIN2 0.19
SGPP2 0.19
ACBD4 0.18
PCCB 0.18
KIF1B 0.17
CAB39 0.17
GPD2 0.16
HADHA 0.15
CRAT 0.15
COL4A3BP 0.15
SPTLC2 0.15
GOT2 0.14
ACADS 0.14
ADIPOR1 -0.14
MTHFS -0.16
TNFRSF1A -0.17
LTA4H -0.19
DGKE -0.20
GPX1 -0.24
LDLR -0.28
DEGS1 -0.29
BCAT1 -0.29
SCPEP1 -0.29
FAR2 -0.29
FADS1 -0.30
FADS2 -0.32
DGKA -0.33
ABAT -0.38
SMPDL3A -0.40
AKR1C1 -0.40
HAL -0.42
IRG1 -1.07
CAV1 0.38
CREBBP -0.14
MUC1 0.37
FZD5 0.32
ERBB2 0.23
BAD 0.23
JUN 0.23
PTPRH 0.23
JMJD6 0.22
HDAC5 0.22
BMP4 0.19
NCK2 0.19
MAPKAP1 0.17
PIK3R1 0.16
PAG1 0.15
KLF6 0.15
STIM1 0.12
ELF1 -0.11
TUBB -0.11
KAT5 -0.13
PAK2 -0.13
FOXP1 -0.14
PPP3CA -0.14
CD24 -0.16
PTPN6 -0.16
DDOST -0.16
PIK3R2 -0.18
P2RX7 -0.18
PTPN12 -0.19
FYN -0.19
ABI1 -0.21
NFATC2 -0.22
PIK3CD -0.22
LYST -0.23
LAT -0.23
SKAP1 -0.24
ARHGDIB -0.24
TGFB1 -0.24
CD47 -0.25
MAP3K1 -0.26
DEF6 -0.28
CD5 -0.28
TBX21 -0.29
CD40 -0.30
CD83 -0.31
FCRL3 -0.32
RASGRP2 -0.33
INPP5D -0.33
SH2D3C -0.34
LAT2 -0.34
DOCK2 -0.34
RHOH -0.35
LCK -0.36
TXK -0.37
JAK3 -0.38
CD3D -0.38
FCER2 -0.39
IL21R -0.40
NFKB1 -0.40
MAP3K8 -0.40
KLHL6 -0.42
MNDA -0.42
HSH2D -0.42
RASGRP1 -0.42
LCP2 -0.42
CD2 -0.43
BCL6 -0.43
IL10 -0.45
PTPN22 -0.46
PRKCB -0.48
AICDA -0.49
LCP1 -0.49
ITGA4 -0.49
CD79A -0.52
PTPRC -0.53
CD274 -0.55
CD19 -0.55
CD3G -0.57
TLR4 -0.57
PRKCQ -0.60
CXCR5 -0.68
ICOS -0.70
LEF1 -0.76
CLEC7A -0.77
MASP2 -0.21
SERPING1 -0.26
CD55 -0.26
C4BPB -0.39
C2 -0.47
CFH -0.47
C4B -0.58
C4BPA -0.62
CLU -0.63
CR2 -0.97
CFB -0.23
F5 -0.38
PDK1 -0.23

Fig. 2. Heatmap of significantly regulated genes (P <0.05) present in the positively enriched gene sets describing TCA cycle or lipid metabolism, or present in the negatively enriched gene sets describing processes involved in adaptive or innate immune response. Average and individual log2 fold changes are presented. The intensity of the red and green color indicates the degree of induction or suppression per pig, respectively, and ranged from -0.65 (green) to 0.65 (red). Pigs are ranked based on their identifier. The vertical color bars indicate gene set membership; dark blue: TCA cycle, light blue: lipid metabolism, olive green: adaptive immune response, tan: innate immune response.

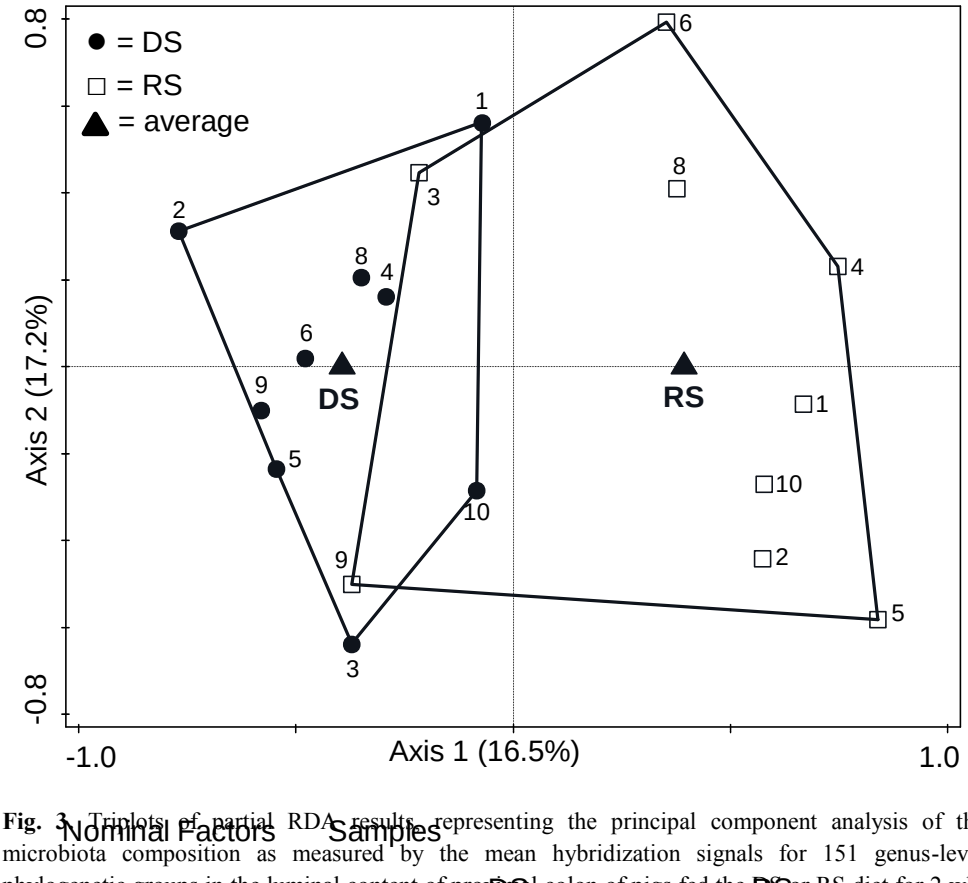


Fig. 3. Triplots of partial RDA results, representing the principal component analysis of the microbiota composition as measured by the mean hybridization signals for 151 genus-level phylogenetic groups in the luminal content of proximal colon of pigs fed the DS or RS diet for 2 wk. Samples are grouped by diet. Each symbol represents 1 pig, with numbers indicating pig identifiers. The average of the nominal environmental variables DS and RS are represented by triangles. DS, digestible starch; RS, resistant starch.

SCFA concentrations

Total SCFA concentration in the luminal content of pCO, collected 30 min before and 300 min after feeding, was not affected by dietary treatment (Supplemental Fig. 3A). However, the percentage of branched-chain fatty acids

(BCFA), i.e. isobutyrate and isovalerate, of total SCFA was significantly lower in pigs fed RS (Supplemental Fig. 3B). Total SCFA concentration in the luminal content of CE and dCO obtained at sacrifice was significantly higher with RS consumption (Supplemental Fig. 4A). In pCO, mCO and dCO, significantly lower percentages of BCFAs were observed with RS consumption (Supplemental Fig. 4B).

Acetate, propionate, and butyrate concentrations determined in peripheral plasma collected 300 min after feeding were significantly higher with RS consumption compared to DS consumption (Fig. 4).

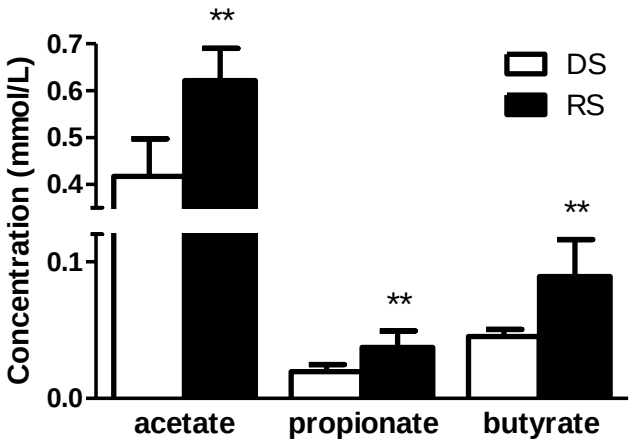


Fig. 4. Acetate, propionate, and butyrate concentrations in peripheral plasma of pigs fed the DS or the RS diet for 2 wk. Samples were collected 300 min after feeding. Values are means ± SEM, n=10 for DS-fed pigs and n=9 for RS-fed pigs. ** indicates P <0.01. DS, digestible starch; RS, resistant starch.

Correlation analysis

The expression of the 618 significantly regulated genes (AUGUSTUST annotation) was integrated with the relative abundance of the 37 significantly changed microbial groups using multivariate correlation analysis. A network was generated that revealed gene-microbiota combinations that were strongly correlated (correlation coefficient cutoff > 0.9). This network contained 123 genes and 17 microbial groups, with each gene correlating with 1 up to 14 microbial groups (Fig. 5A). Most genes (i.e. 96) were significantly correlated with *Vibrio*-like bacteria, of which 75 genes were negatively correlated including the SCFA transporter *SLC16A1*, *PPARG* and the *PPARG* target gene *GPT*.

In addition, we determined which of the 618 genes correlated with concentrations of acetate, propionate and butyrate measured in peripheral plasma 300 mins after feeding. The resulting network showed that 26 genes were

correlated (correlation coefficient > 0.9) with acetate and 48 genes were correlated with propionate and/or butyrate (Fig. 5B).

Correlation analysis of the 37 microbial groups with plasma acetate, propionate and butyrate (correlation coefficient > 0.8) revealed 20 highly correlating microbial groups (Fig. 5C). Whereas the majority of correlations included negative correlations of microbial groups reduced in the presence of RS, we also observed positive correlations with known acid producing bacteria, including *Megasphaera elsdenii*, a known producer of acetate, propionate and butyrate, as well as *Mitsuokella multacida*, which has been shown to predominantly produce lactate, succinate and smaller amounts of acetate from carbohydrate fermentation. The latter compounds are in turn used as substrates by producers of propionate and butyrate (Flint et al., 2012).

Upstream regulators

A set of upstream transcriptional regulators that could explain the observed shift in gene expression profile was identified. The transcription factors *PPARG*, *ERG* and *HTT* were predicted to be significantly activated on RS, while *XBP1* was predicted to be inhibited (Table 2).

Because the highest z-score (3.069) was found for PPARG, we had a closer look at the downstream *PPARG* target genes. Out of 21 PPARG-target genes present in our dataset, 15 genes had an expression change consistent with activation of PPARG (Table 3). Since *TGFBR1* and *CXCL14* are known to be down-regulated by PPARG and we indeed observed reduced expression of these target genes, our data suggested that *PPARG* was activated by RS. The other 13 target genes were induced in our dataset, which corresponds with observations from literature showing that these genes are up-regulated by *PPARG*.

Potentially secreted proteins

The transcriptome data were mined to identify potentially secreted proteins that may mediate effects of RS in liver or other tissues and organs. From the 655 significantly regulated genes in our dataset, we identified 849 unique encoding proteins for which the predicted locations were determined. The number of proteins is larger than the number of genes due to alternatively splicing of the mRNA molecules of the regulated gens. Fifty-three proteins were predicted to be located extracellular, and therefore secreted, with a probability above 0.20 (Supplemental Table 2). Of these proteins, 31 were encoded by genes induced on RS and 22 proteins were encoded by suppressed genes. Among the potentially secreted proteins that, based on the transcriptome data, are expected to increase on RS were IGF1, AGT, VEGFA and ANGPTL4. These proteins are all derived from PPARG

target genes. The proteins that are expected to go down on RS include 2 more proteins derived from PPARG target genes, namely SERPINA1 and CXCL14.

Table 1. Phylogenetic groups in the luminal content of proximal colon of pigs fed DS or RS diet for 2 wk, which were significantly affected by diet according to univariate analysis of PITChip data.

Phylogenetic group	*P* value	FDR[1]	ARC[2]		Effect[3]
			DS	RS	(RS-DS)
Actinobacteria					
Actinobacteria					
Eggerthella et rel.	0.008	0.08	0.109 ± 0.050	0.033 ± 0.043	-
Microbacterium et rel.	0.055	0.22	0.586 ± 0.100	0.709 ± 0.193	+
Bacteroidetes					
Bacteroidetes					
Parabacteroides distasonis et rel.	0.055	0.22	0.411 ± 0.278	0.622 ± 0.278	+
Prevotella melaninogenica et rel.	0.012	0.09	0.056 ± 0.055	3.079 ± 3.194	+
Uncultured *Bacteroidetes*	0.039	0.20	0.605 ± 0.934	0.080 ± 0.241	-
Uncultured *Prevotella*	0.008	0.08	0.836 ± 0.531	2.501 ± 1.679	+
Firmicutes					
Bacilli					
Enterococcus et rel.	0.039	0.20	2.480 ± 0.576	3.025 ± 0.657	+
Lactobacillus amylovorus et rel.	0.055	0.22	1.094 ± 0.963	0.400 ± 0.458	-
Lactobacillus salivarius et rel.	0.055	0.22	0.926 ± 0.356	1.267 ± 0.426	+
Turicibacter et rel.	0.004	0.08	0.197 ± 0.069	0.062 ± 0.036	-
Clostridium cluster I					
Clostridium perfringens et rel.	0.008	0.08	2.398 ± 1.763	0.403 ± 0.810	-
Clostridium cluster IV					
Faecalibacterium prausnitzii et rel.	0.020	0.12	0.969 ± 0.398	1.925 ± 1.142	+
Uncultured *Clostridia* IV	0.055	0.22	2.404 ± 0.863	1.697 ± 1.005	-
Clostridium cluster IX					
Dialister et rel.	0.004	0.08	0.453 ± 0.248	0.852 ± 0.328	+
Megasphaera elsdenii et rel.	0.008	0.08	0.850 ± 0.210	1.386 ± 0.600	+
Mitsuokella multacida et rel.	0.008	0.08	1.084 ± 0.301	1.776 ± 0.743	+
Clostridium cluster XI					
Anaerovorax et rel.	0.004	0.08	1.811 ± 0.681	0.495 ± 0.973	-
Clostridium cluster XIII					
Peptoniphilus et rel.	0.008	0.08	0.601 ± 0.350	0.176 ± 0.364	-
Clostridium cluster XIVa					
Uncultured *Clostridia* XIVa	0.039	0.20	2.412 ± 0.828	1.657 ± 0.928	-
Clostridium cluster XVIII					
Clostridium ramosum et rel.	0.008	0.08	0.213 ± 0.057	0.296 ± 0.101	+
Planctomycetes					
Planctomycetacia					
Uncultured *Planctomycetacia*	0.012	0.09	0.159 ± 0.114	0.037 ± 0.048	-
Proteobacteria					
Alphaproteobacteria					
Rhizobium et rel.	0.055	0.22	0.552 ± 0.085	0.382 ± 0.153	-
Sphingomonas et rel.	0.027	0.17	0.214 ± 0.203	0.059 ± 0.154	-
Betaproteobacteria					
Uncultured *Betaproteobacteria*	0.012	0.09	0.329 ± 0.046	0.246 ± 0.066	-
Gammaproteobacteria					
Actinobacillus indolicus et rel.	0.008	0.08	0.332 ± 0.058	0.227 ± 0.079	-
Aeromonas	0.020	0.12	0.671 ± 0.147	0.389 ± 0.268	-
Alishewanel et rel.	0.020	0.12	0.472 ± 0.093	0.255 ± 0.176	-
Escherichia coli et rel.	0.055	0.22	0.397 ± 0.190	0.166 ± 0.196	-
Halomonas et rel.	0.004	0.08	0.747 ± 0.204	0.448 ± 0.225	-
Pasteurella et rel.	0.012	0.09	0.145 ± 0.041	0.093 ± 0.037	-
Pseudomonas et rel.	0.008	0.08	0.697 ± 0.253	0.279 ± 0.340	-
Psychrobacter et rel.	0.008	0.08	0.629 ± 0.126	0.408 ± 0.173	-

Ruminobacter amylophilus et rel.	0.039	0.20	0.211 ± 0.022	0.153 ± 0.057	-
Thiocapsa et rel.	0.055	0.22	0.675 ± 0.163	0.450 ± 0.202	-
Vibrio et rel.	0.008	0.08	0.690 ± 0.149	0.438 ± 0.207	-
Xanthomonas et rel.	0.020	0.12	0.852 ± 0.063	0.682 ± 0.140	-
Spirochaetes					
Spirochaetes					
Leptospira	0.012	0.09	0.717 ± 0.172	0.402 ± 0.208	-

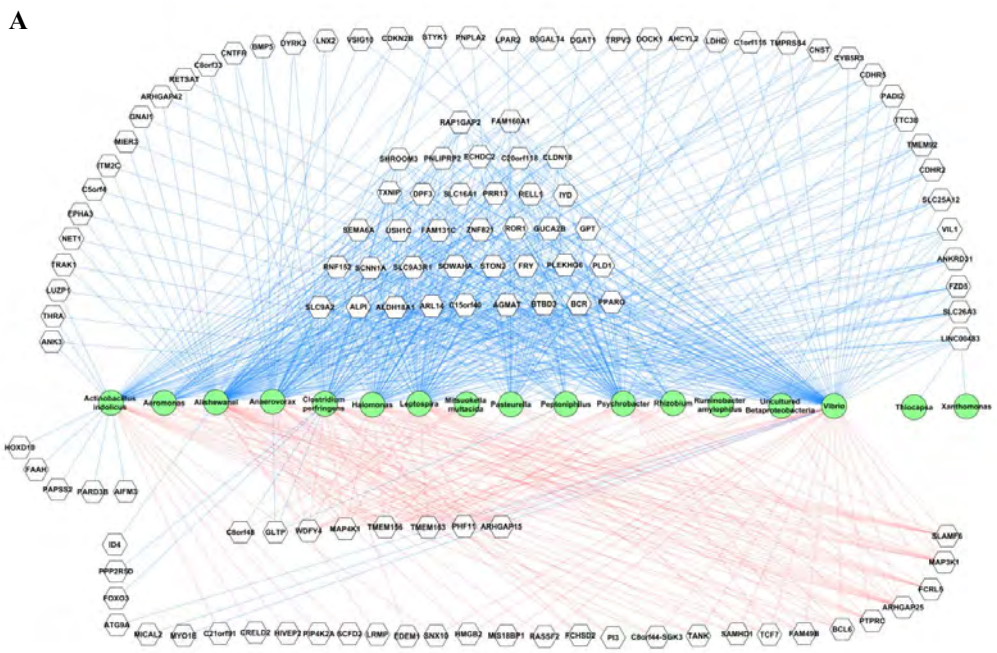
[1] FDR: false discovery rate.
[2] ARC: average relative contribution [%] of a microbial group. Values represented means ± SEM, n=9 per treatment.
[3] "+" or "-" indicates whether the average relative contribution of the microbial group was increased or decreased by the RS treatment.

Table 2. Upstream regulators, determined by Ingenuity Systems Pathway Analysis Software.

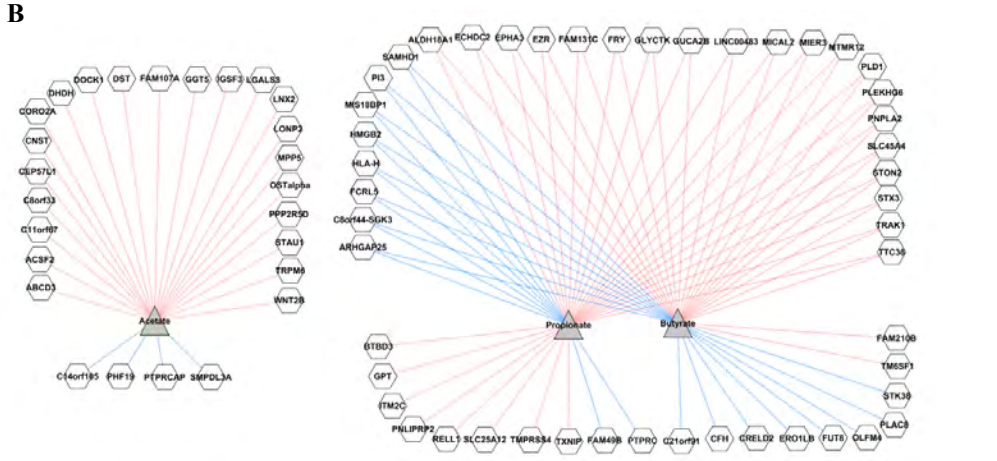
Upstream Regulator	Average FC[1]	Molecular Type	Predicted Activation State	Activation z-score[2]
XBP1		Transcription regulator	Inhibited	-2.041
HTT	-1.05	Transcription regulator	Activated	2.186
ERG	1.01	Transcription regulator	Activated	2.828
PPARG	1.26	Ligand-dependent nuclear receptor	Activated	3.069

XBP1, X-box binding protein 1; *HTT*, Huntingtin; *ERG*, V-ets erythroblastosis virus E26 oncogene homolog (avian); *PPARG*, Peroxisome proliferator-activated receptor gamma.
[1] Average fold changes of the signal intensity of RS compared with DS. No fold change is given for *XBP1* because this gene is not present in the dataset.
[2] Activation z-score predicts the activation state of the upstream regulator, using the gene expression pattern of its downstream genes. Upstream regulators with z-score ≥ 2 are considered to be significantly activated, if z-score ≤ -2 the upstream regulator is significantly inhibited.

A


Actinobacillus indolicus
Aeromonas
Alishewanel
Anaerovorax
Clostridium perfringens
Halomonas
Leptospira
Mitsuokella multacida
Pasteurella
Peptoniphilus
Psychrobacter
Rhizobium
Ruminobacter amylophilus
Uncultured Betaproteobacteria
Vibrio
Thiocapsa
Xanthomonas

B


Acetate
Propionate
Butyrate

C

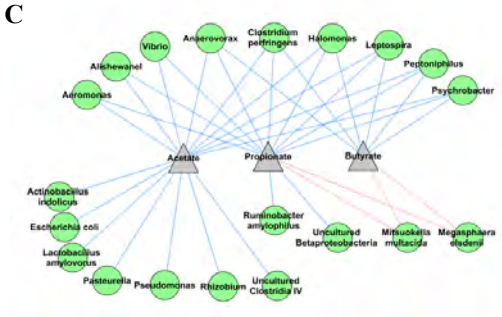

Vibrio
Anaerovorax
Clostridium perfringens
Halomonas
Leptospira
Alishewanel
Peptoniphilus
Aeromonas
Psychrobacter
Acetate
Propionate
Butyrate
Actinobacillus indolicus
Escherichia coli
Ruminobacter amylophilus
Uncultured Betaproteobacteria
Mitsuokella multacida
Megasphaera elsdenii
Lactobacillus amylovorus
Pasteurella
Pseudomonas
Rhizobium
Uncultured Clostridia IV

Fig. 5. Correlation networks of genes correlating with microbial groups with correlation coefficient > 0.9 (A), genes correlating with plasma acetate, propionate and butyrate concentrations with correlation coefficient > 0.9 (B), and microbial groups correlating with plasma acetate, propionate and butyrate concentrations with correlation coefficient > 0.8 (C). Genes are indicated as white hexagons, microbial groups as green circles, and SCFAs as grey triangles. Blue and red lines respectively represent negative and positive correlations.

Table 3. *PPARG* target genes, determined by Ingenuity Systems Pathway Analysis Software.

Name	Gene Symbol	Entrez ID	Prediction[1]	Average FC[2]	Findings[3]
Transforming growth factor, beta receptor 1	*TGFBR1*	7046	Activated	-1.19	Down
Chemokine (C-X-C motif) ligand 14	*CXCL14*	9547	Activated	-1.20	Down
Carbonic anhydrase II	*CA2*	760	Activated	2.56	Up
Angiopoietin-like 4	*ANGPTL4*	51129	Activated	1.49	Up
3-Hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)	*HMGCS2*	3158	Activated	1.41	Up
Uncoupling protein 3 (mitochondrial, proton carrier)	*UCP3*	7352	Activated	1.31	Up
Caveolin 1, caveolae protein, 22kDa	*CAV1*	857	Activated	1.30	Up
Vascular endothelial growth factor A	*VEGFA*	7422	Activated	1.30	Up
Peroxisome proliferator-activated receptor gamma	*PPARG*	5468	Activated	1.26	Up
Solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	*SLC25A20*	788	Activated	1.25	Up
3-Hydroxybutyrate dehydrogenase, type 1	*BDH1*	622	Activated	1.24	Up
Lipase, hormone-sensitive	*LIPE*	3991	Activated	1.21	Up
Diacylglycerol O-acyltransferase 1	*DGAT1*	8694	Activated	1.19	Up
Monoglyceride lipase	*MGLL*	11343	Activated	1.17	Up
Acyl-CoA dehydrogenase, C-4 to C-12 straight chain	*ACADM*	34	Activated	1.16	Up
Phosphodiesterase 3B, cGMP-inhibited	*PDE3B*	5140	Inhibited	-1.36	Up
Serpin peptidase inhibitor, clade A, member 1	*SERPINA1*	5265	Inhibited	-1.52	Up
Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	*AGT*	183	-	1.82	Regulates
Insulin-like growth factor 1 (somatomedin C)	*IGF1*	3479	-	1.53	Regulates
Glutamic-pyruvate transaminase (alanine aminotransferase)	*GPT*	2875	-	1.37	Regulates
Protein tyrosine phosphatase, receptor type, F	*PTPRF*	5792	-	1.21	Regulates

[1] Column indicates the predicted activation state of *PPARG* (either Activated or Inhibited), based on the direction of the gene expression change in the uploaded dataset.
[2] Average fold change of the signal intensity of RS compared to DS.
[3] Column indicates whether literature findings support the prediction. 'Down' and 'up' indicate whether literature supports a down- or an up-regulation of the target gene by *PPARG*, respectively. 'Regulates' indicates that there is insufficient support from literature that the target gene is either up- or down-regulated by *PPARG*.

Discussion

In the present study, we examined the effects of 2-wk-consumption of a diet high in RS on the mucosal transcriptome and luminal microbiota composition and SCFA concentrations in proximal colon of pigs. Our results showed that compared to a DS diet, the RS diet shifted colonic gene expression profile of the host from immune regulation towards metabolic regulation and reduced the abundance of several potentially pathogenic bacteria in the colonic lumen. In addition, plasma SCFA concentrations increased on the RS diet.

Microbiota composition

Evidence suggesting that gut microbiota are highly important in the regulation of energy homeostasis and fat storage is accumulating (Delzenne et al., 2011; Grootaert et al., 2011). Supporting this view, the Firmicutes/Bacteroidetes ratio has been linked to adiposity in humans, since obese people were found to have fewer Bacteroidetes compared to lean controls. In addition, the relative abundance of Bacteroidetes increased while the abundance of Firmicutes decreased with weight loss in obese subjects (Ley et al., 2006). Because we found a significantly lower Firmicutes/Bacteroidetes ratio in RS-fed pigs compared to DS-fed pigs, the microbial profile might have shifted towards a healthier phenotype in RS-fed pigs. Although the relative abundance of Firmicutes slightly, albeit not significantly, decreased (Supplementary Fig. 2), we observed significant increase in the relative abundance of several microbial groups previously shown to produce butyrate, including *Faecalibacterium prausnitzii* and *Megasphaera elsdenii* (Tsukahara et al., 2002; Louis and Flint, 2009; Flint et al., 2012). Other microbial populations stimulated by RS included fermenting microorganisms such as members of the *Parabacteroides*, *Prevotella*, *Mitsuokella multacida*, and lactic acid bacteria, that produce organic acids such as acetate, lactate and succinate that are in turn used as main substrates for the production of propionate and butyrate(Flint et al., 2012).

Fermentation products

In contrast with our previous study in pigs consuming the same RS diet (Haenen et al., 2013), in the current study no significant difference in SCFA concentration was observed in the pCO digesta 300 min after feeding. However, the changed percentage of BCFAs in colonic digesta confirms the applied dietary contrast. These branched-chain products are formed by amino acid fermenting microbial species that metabolize undigested and endogenous proteins, peptides, and amino acids (Hendriks et al., 2012), particularly when carbohydrates as preferential energy source are absent. The lower percentage of BCFAs reflects the use of RS as

energy source by microbiota whereas in the DS diet the microbiota used proteinaceous energy sources due to the digestion and fermentation of starch in the upper gastrointestinal tract and the caecum. Moreover, plasma SCFA concentrations are known to be a more reliable measure of colonic SCFA production (Cummings et al., 1987). As expected, we observed increased plasma SCFA concentrations upon RS feeding.

Functional implications colon

Genome-wide expression profiling is an unbiased approach for identifying genes regulated by RS. Our microarray analysis resulted in a dataset with the expression levels of 17,118 unique genes measured in 20 colonic samples from 10 pigs on 2 dietary conditions (DS and RS). From these data we extracted the significantly regulated genes and the main processes that are expected to change based on the gene expression profile.

In RS-fed pigs, we observed an increased expression of genes involved in the TCA cycle, the pathway responsible for generating energy through the oxidization of acetyl-CoA. We previously showed that RS increases SCFA concentrations in the colonic lumen (Haenen et al., 2013). These SCFAs are subsequently taken up by the colonocytes, where mainly butyrate serves as an energy source for these cells as a precursor to the TCA cycle (Donohoe et al., 2011). Therefore, the increased energy generation in colonocytes upon RS feeding was in line with our expectations.

In addition, genes involved in lipid metabolism were found to be induced by RS. More specifically, GSEA revealed increased uptake and release of fatty acids, fatty acid beta-oxidation and triglyceride (TG) synthesis. We hypothesize that these processes are a consequence of increased SCFA production, having a direct link with energy metabolism resulting in increased energy harvest from the dietary.

Gene expression profiling also showed that RS suppressed genes involved in both the innate and the adaptive immune response, which indicates that the CO of RS-fed pigs is less exposed to potential pathogens as was confirmed by microbiota analysis of luminal content, where especially members of the Proteobacteria were found reduced in relative abundance in the RS group. This observation, combined with a less pro-inflammatory state, might thus indicate a healthier condition of RS-fed pigs as compared to DS. This observation is in line with previous studies on RS in relation to immune regulation, showing amelioration of inflammatory bowel disease upon RS feeding (Bassaganya-Riera et al., 2011).

Microbiota are known to play a crucial role in the immune system. Therefore, it is likely that the changed microbiota composition we observed on RS directly reflects changes in expression level of genes involved in immunity. Furthermore,

immune signalling can affect microbiota composition, as shown by altered gut microbiota in mice lacking toll-like receptor 5, an essential protein for pathogen recognition and activation of innate immunity (Vijay-Kumar et al., 2010).

In addition to the microbiota, we hypothesize that PPARG, a ligand-activated transcription factor found to be significantly activated by RS, plays a role in the suppressed immune response. This is in line with a recent report that showed that SCFA, and especially butyrate, are agonists for PPARG (Alex et al., 2013). PPARG is also known to be involved in the prevention of inflammatory bowel disease in mice (Hontecillas and Bassaganya-Riera, 2007), pigs (Bassaganya-Riera and Hontecillas, 2006), and humans (Lewis et al., 2008). Moreover, PPARG activation suppresses the activity of NF-κB, thereby blocking pro-inflammatory gene transcription (Bassaganya-Riera et al., 2004).

Functional implications whole-body metabolism

To determine how colonic gene expression changes affect inter-organ crosstalk, we identified potentially secreted proteins. Among these proteins were angiopoietin-like 4 (ANGPTL4) and apolipoprotein D (APOD), which both play an important role in lipid metabolism. ANGPTL4 provides a link between microbiota and SCFAs in CO on one hand and adiposity on the other hand. Studies have suggested that there is a relationship between microbiota composition and ANGPTL4, as observed by strong suppression of *ANGPTL4* after conventionalization of germ free mice (Backhed et al., 2004; Backhed et al., 2007). Furthermore, microbiota are able to induce ANGPTL4 production in colonocytes via production of SCFAs and subsequent activation of PPARG (Alex et al., 2013). Because we found ANGPTL4 to be a potentially secreted protein, we hypothesize that ANGPTL4 is secreted from the colonocytes into the circulation, where it inhibits lipoprotein lipase (LPL), an enzyme that hydrolyses TGs in lipoproteins into free fatty acids for uptake by tissues (Yoshida et al., 2002). Therefore, SCFAs may inhibit fat storage by stimulating ANGPTL4 release.

Based on the direction of the expression of its encoding gene, we expect APOD to be decreased with RS consumption. Elevated APOD production was found to significantly reduce plasma TG levels in mice, due to enhanced LPL activity and improved catabolism of TG-rich particles (Perdomo et al., 2010). Plasma TG levels measured in the pigs from our experiment were significantly higher after RS consumption (Souza da Silva et al., 2013b). Assuming that APOD decreases and ANGPTL4 increases on RS as suggested by the transcriptome data, we expect to find a decrease in LPL activity, resulting in increased plasma TG levels.

Based on our data, TG uptake by adipose tissue is expected to be inhibited by RS, indicating that long-term RS consumption might decrease adiposity. However, the final destination of the circulating TGs is yet unknown.

Conclusions

This is the first study to show that major changes in colonic gene expression occur upon 2-wk-consumption of a diet high in RS. Compared to the isocaloric DS diet, the RS diet shifted the transcriptional profile from the regulation of immune response towards metabolic processes such as lipid and energy metabolism, emphasizing the high metabolic resilience of the colon. The direction of gene expression changes combined with the decreased abundance of several pathogenic bacteria, and stimulation of SCFA-producing populations in the colonic lumen supports the belief that RS has a beneficial impact on colonic health. Because pigs are known to be a good model for humans, our study outcomes are highly relevant to human health.

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Supplemental materials

Supplemental Table 1. Ingredient and analysed chemical composition of experimental diets.

	Diet	
	DS	RS
Ingredient composition, g/kg		
Pregelatinized purified potato starch[1]	350.0	0.0
Retrograded tapioca starch[2]	0.0	342.6
Soy oil	29.2	29.5
Wheat	200.0	202.3
Beet pulp (sugar<100 g/kg)	50.0	50.6
Barley	150.0	151.7
Wheat gluten meal	60.0	60.7
Potato protein[3]	100.0	101.1
Premix[4]	10.0	10.1
$CaCO_3$	13.5	13.7
$Ca(H_2PO_4)_2$	11.0	11.1
NaCl	3.0	3.0
L-lysine HCl	2.2	2.2
L-tryptophan	0.2	0.2
MgO (80%)	0.4	0.4
$NaHCO_3$	14.0	14.2
KCl	3.0	3.0
TiO_2	2.0	2.0
Flavour[5]	1.5	1.5
Chemical composition, g/kg dry matter		
Dry matter (g/kg)	894.5	910.0
Organic matter	941.4	941.9
Crude protein (N x 6.25)	190.9	194.3
Crude fat	16.1	28.6
Starch	524.7	477.1
Sugar	13.1	69.4
TiO_2	1.6	1.6
Energy content, MJ/kg		
GE	16.48	16.78

DS=digestible starch diet; RS=resistant starch diet.

[1] Paselli™ WA4, Avebe Food, Veendam, The Netherlands.

[2] C*Actistar 11700, Cargill, Amsterdam, The Netherlands.

[3] Protostar, Avebe Food, Veendam, The Netherlands.

[4] Provided the following per kg of feed: vitamin A: 7500 IU; vitamin D_3: 1500 IU; vitamin E: 60 mg; vitamin K_3: 1.0 mg; vitamin B_1: 1.0 mg; vitamin B_2: 4.0 mg; vitamin B_6: 1.0 mg; vitamin B_{12}: 20 µg; niacin: 20 mg; calcium-D pantothenate: 10.5 mg; choline chloride: 100 mg; folic acid: 0.4 mg; Fe: 120 mg ($FeSO_4.H_2O$); Cu: 15 mg ($CuSO_4.5H_2O$); Mn: 60 mg (MnO); Zn: 75 mg ($ZnSO_4.H_2O$); I: 4.0 mg (KI); Se: 0.30 mg (Na_2SeO_3); anti-oxidant: 75 mg.

[5] Luctarom Advance Cherry Honey, Lucta S.A., Barcelona, Spain.

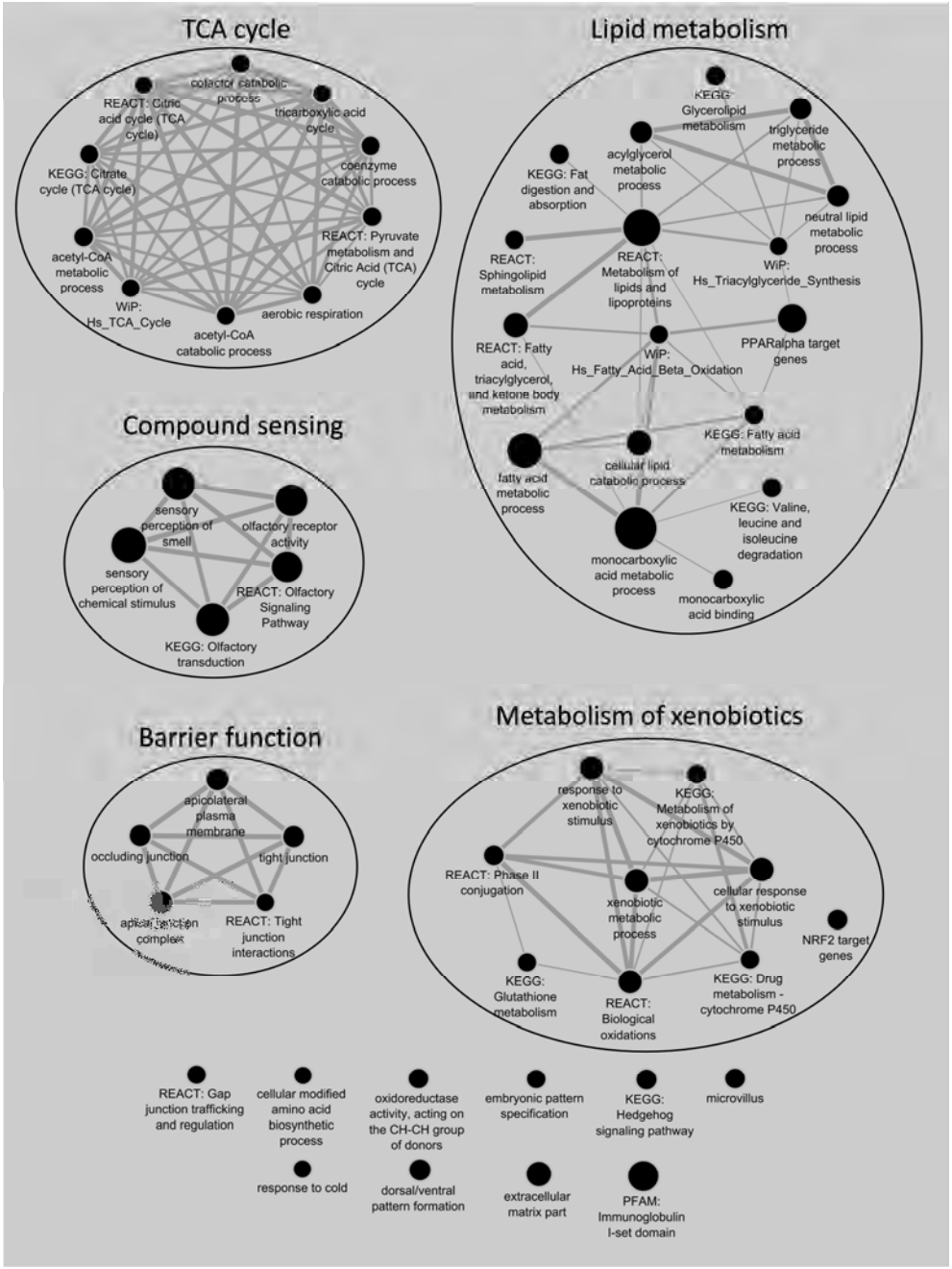
Supplemental Table 2. Potentially secreted proteins.

Name	Gene Symbol	Entrez ID	Average FC[1]	Protein ID	Prob[2]
Insulin-like growth factor 1 (somatomedin C)	*IGF1*	3479	1.53	Q5U743	0.78
Bone morphogenetic protein 4	*BMP4*	652	1.14	P12644	0.63
Pancreatic lipase-related protein 2	*PNLIPRP2*	5408	1.59	P54317	0.62
Wingless-type MMTV integration site family, member 2B	*WNT2B*	7482	1.36	Q93097	0.61
Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, 3C	*SEMA3C*	10512	1.28	Q99985	0.60
Dermatopontin	*DPT*	1805	1.50	Q07507	0.60
Bone morphogenetic protein 2	*BMP2*	650	1.26	P12643	0.57
Resistin like beta	*RETNLB*	84666	1.56	Q9BQ08	0.57
Proline/arginine-rich end leucine-rich repeat protein	*PRELP*	5549	1.50	P51888	0.57
Bone morphogenetic protein 5	*BMP5*	653	1.33	P22003	0.57
Tubulointerstitial nephritis antigen	*TINAG*	27283	1.43	Q9UJW2	0.54
Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	*AGT*	183	1.82	B2R5S1	0.54
Gastric intrinsic factor (vitamin B synthesis)	*GIF*	2694	1.41	P27352	0.54
ADAM metallopeptidase domain 23	*ADAM23*	8745	1.69	O75077	0.53
Vascular endothelial growth factor A	*VEGFA*	7422	1.30	P15692	0.52
Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	*CILP*	8483	1.48	O75339	0.51
EGF containing fibulin-like extracellular matrix protein 1	*EFEMP1*	2202	1.59	Q12805	0.47
Guanylate cyclase activator 2A (guanylin)	*GUCA2A*	2980	1.58	Q02747	0.44
Signal peptide, CUB domain, EGF-like 1	*SCUBE1*	80274	1.28	A0JP65	0.43
Peptidase domain containing associated with muscle regeneration 1	*PAMR1*	25891	1.26	Q6UXH9	0.42
Guanylate cyclase activator 2B (uroguanylin)	*GUCA2B*	2981	2.03	Q16661	0.42
Protocadherin-related 15	*PCDH15*	65217	1.32	A2A3E3	0.42
Transmembrane protease, serine 2	*TMPRSS2*	7113	1.12	O15393	0.37
Collagen, type VI, alpha 6	*COL6A6*	131873	1.80	A6NMZ7	0.33
Lysophosphatidic acid receptor 2	*LPAR2*	9170	1.24	Q9HBW0	0.31
Collagen, type XIV, alpha 1	*COL14A1*	7373	1.37	Q05707	0.31
Transmembrane protease, serine 4	*TMPRSS4*	56649	1.39	B7Z8C5	0.30
EPH receptor A3	*EPHA3*	2042	1.44	P29320	0.29
Angiopoietin-like 4	*ANGPTL4*	51129	1.49	Q9BY76	0.27
Thrombospondin 3	*THBS3*	7059	1.39	B4DQ20	0.22
Slit homolog 3 (Drosophila)	*SLIT3*	6586	1.33	O75094	0.20
Complement component 2	*C2*	717	-1.38	E9PFN7	0.66
Granulin	*GRN*	2896	-1.14	P28799	0.61
Complement component 4B (Chido blood group)	*C4B*	721	-1.50	P0C0L5	0.60
Complement factor H	*CFH*	3075	-1.39	F8WDX4	0.59
Serpin peptidase inhibitor, clade A, member 1	*SERPINA1*	5265	-1.52	P01009	0.58
Peptidase inhibitor 3, skin-derived	*PI3*	5266	-1.41	P19957	0.57
Lymphocyte antigen 96	*LY96*	23643	-1.50	Q9Y6Y9	0.57

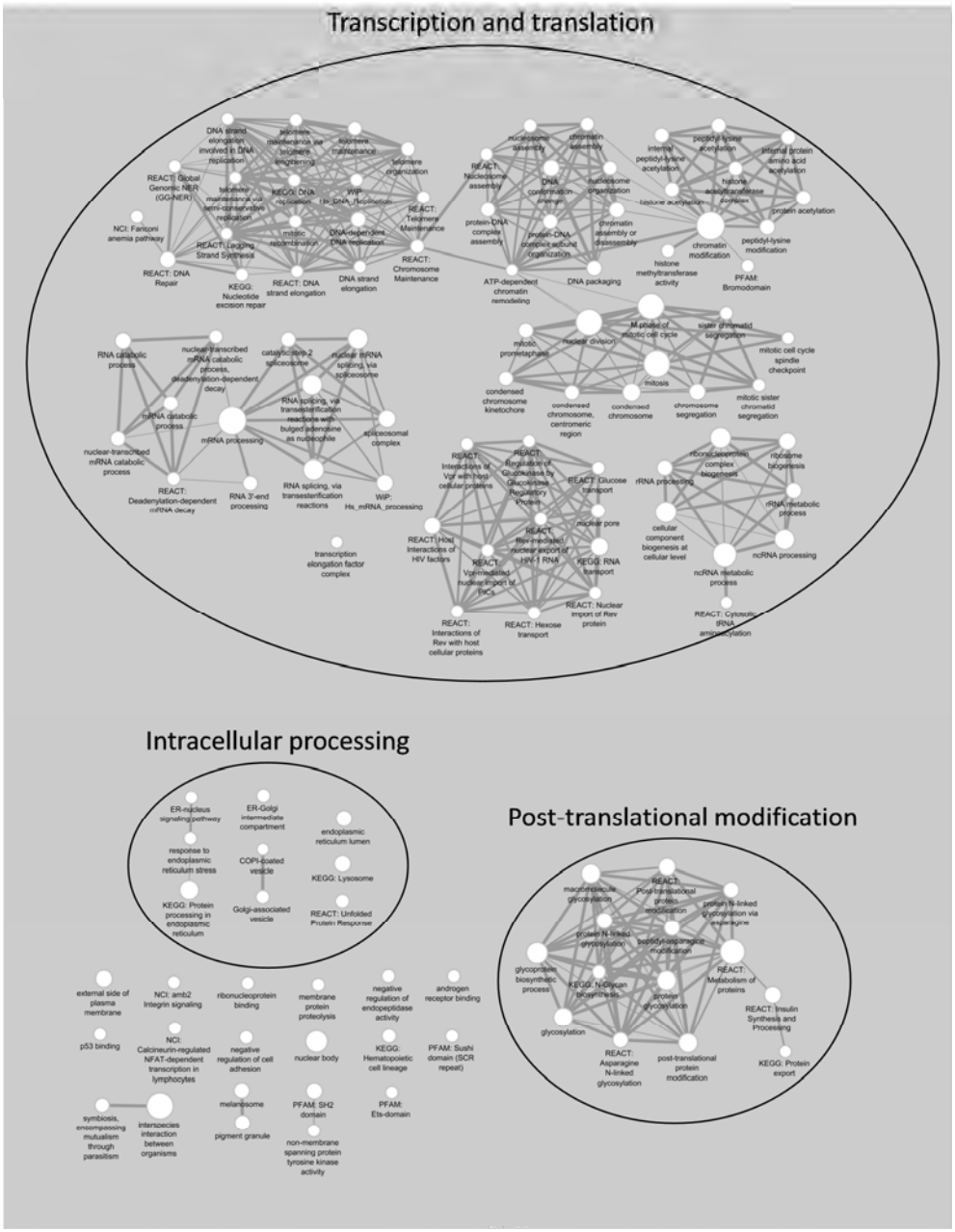
Apolipoprotein D	*APOD*	347	-1.35	P05090	0.56
Complement component 4 binding protein, alpha	*C4BPA*	722	-1.53	P04003	0.53
Complement component 4 binding protein, beta	*C4BPB*	725	-1.31	P20851	0.53
Acyloxyacyl hydrolase (neutrophil)	*AOAH*	313	-1.54	P28039	0.50
Clusterin	*CLU*	1191	-1.55	P10909	0.49
Chromogranin B (secretogranin 1)	*CHGB*	1114	-1.46	P05060	0.46
EMI domain containing 1	*EMID1*	129080	-1.32	Q96A84	0.43
Olfactomedin 4	*OLFM4*	10562	-1.16	Q6UX06	0.42
Poliovirus receptor-related 4	*PVRL4*	81607	-1.68	Q96NY8	0.41
Chemokine (C-X-C motif) ligand 14	*CXCL14*	9547	-1.20	O95715	0.36
Polymeric immunoglobulin receptor	*PIGR*	5284	-1.24	P01833	0.35
Bactericidal/permeability-increasing protein	*BPI*	671	-1.31	P17213	0.34
Serine peptidase inhibitor, Kazal type 4	*SPINK4*	27290	-1.27	O60575	0.33
Cathepsin K	*CTSK*	1513	-1.19	P43235	0.25
ADAM metallopeptidase domain 17	*ADAM17*	6868	-1.16	P78536	0.23

[1] Average fold changes of the signal intensity of RS compared with DS.
[2] Probability that the listed protein is present extracellularly (secreted), as determined by ngLOC. Only proteins with a probability above 0.20 are listed.

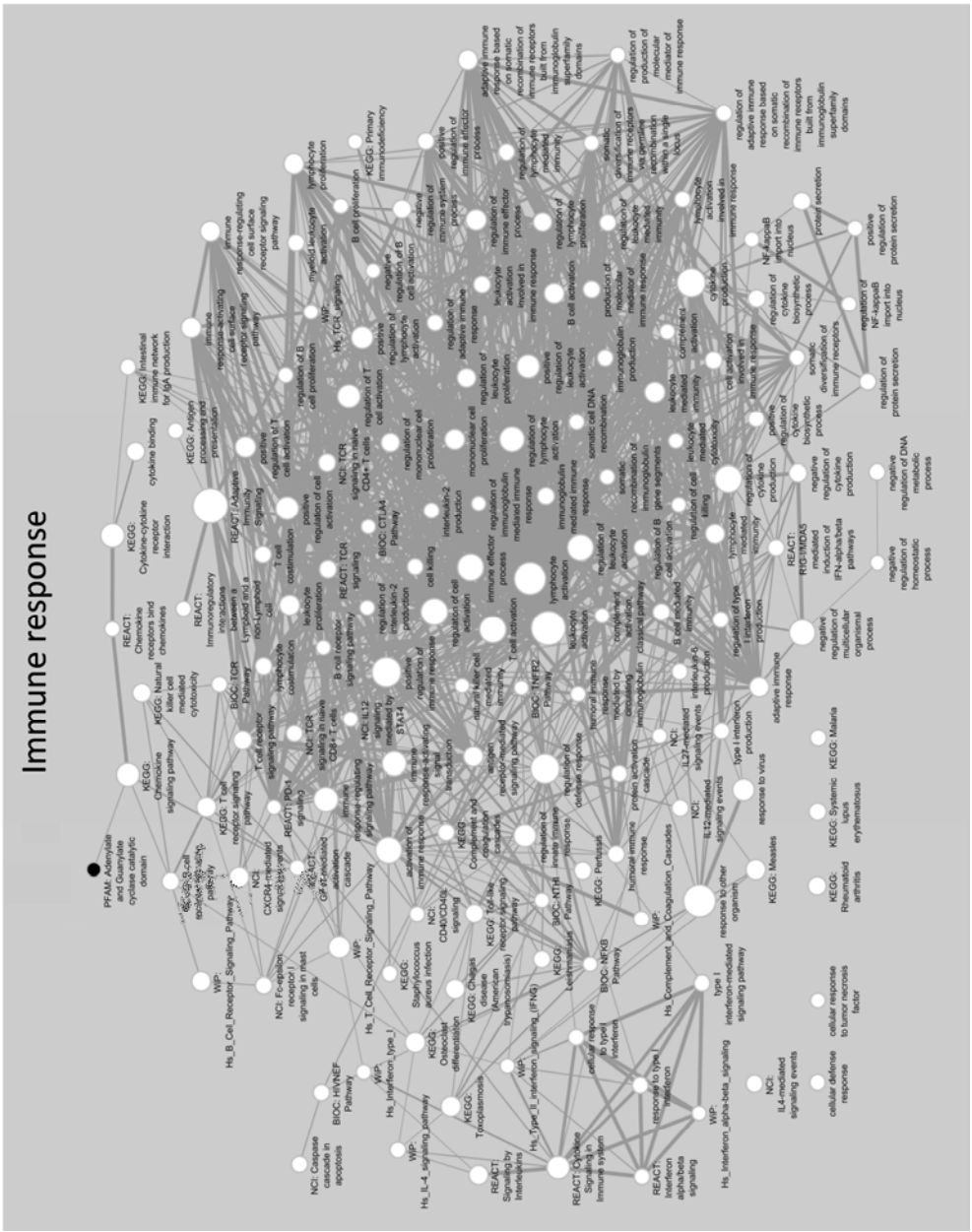
Supplemental Fig. 1.



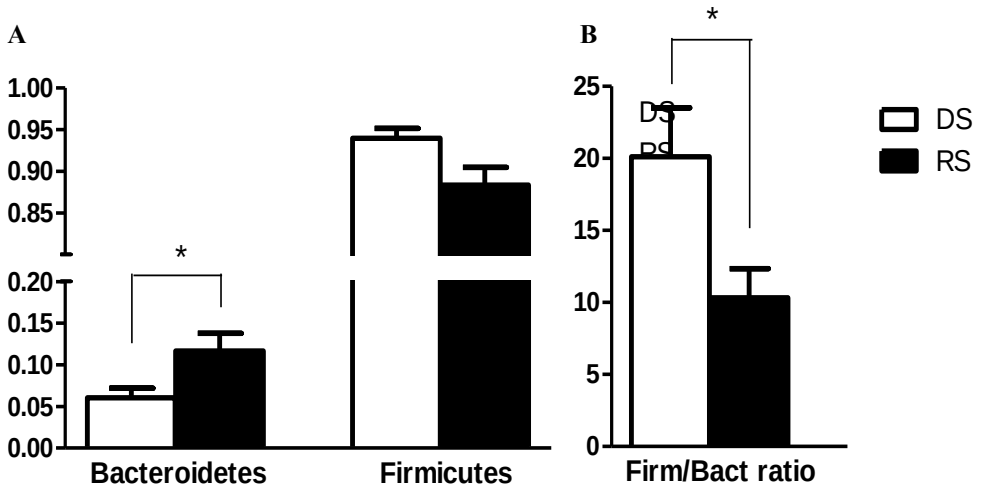
Supplemental Fig. 1. (continued)



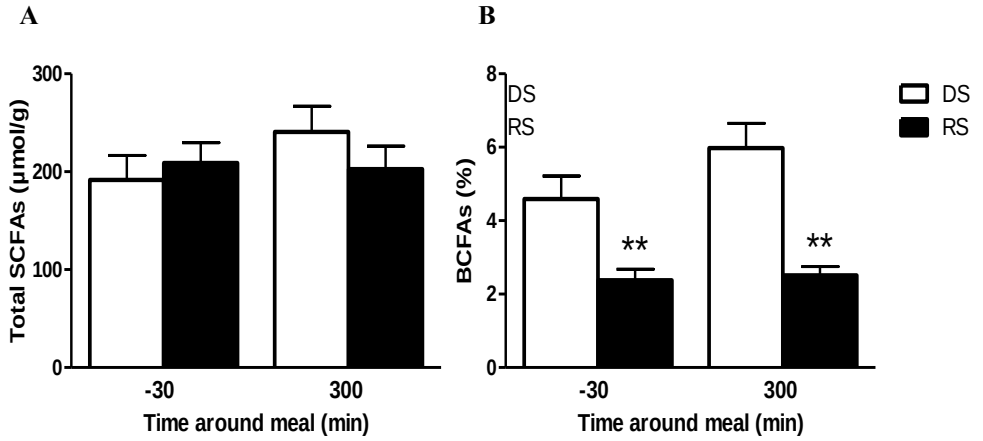
Supplemental Fig. 1. (continued)



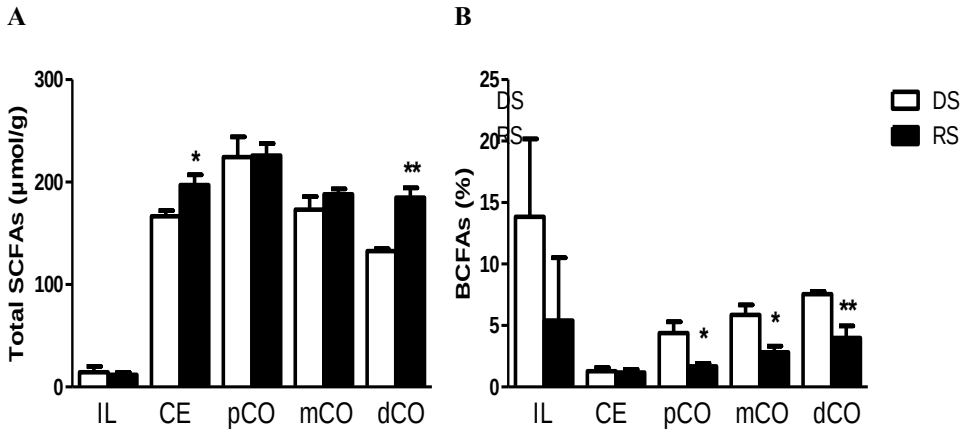
Enrichment map indicating positively and negatively enriched gene sets. Nodes represent functional gene sets, and edges between nodes their similarity. Black indicates increased and white indicates suppressed gene sets in RS group compared with DS group. Node size represents the gene set size, and edge thickness represents degree of overlap between two connected gene sets. Clusters were manually circled and labeled to highlight the prevalent biological functions among related gene sets.



Supplemental Fig. 2. The abundance of the phyla Bacteroidetes and Firmicutes (A) and the ratio Firmicutes/Bacteroidetes (B) in pigs fed DS or RS for 2 wk, as determined by PITChip. Data are presented as means ± SEM, n=9 per treatment. * indicates $P < 0.05$. DS, digestible starch; RS, resistant starch.



Supplemental Fig. 3. Total SCFAs (A) and percentage of BCFAs from total SCFAs (B) measured in luminal content from proximal colon, collected 30 min before feeding (-30) and 300 min after feeding in pigs fed the DS and the RS diet. Data are presented as means ± SEM, n=10 per treatment. ** indicates $P < 0.01$. BCFAs, branched-chain fatty acids; DS, digestible starch; RS, resistant starch.



Supplemental Fig. 4. Total SCFAs (A) and percentage of BCFAs from total SCFAs (B) measured in luminal content from different segments of the gastrointestinal tract in pigs fed the DS or the RS diet. Data are presented as means ± SEM, n=5 per treatment. * indicates $P < 0.05$, ** indicates $P < 0.01$. BCFAs, branched-chain fatty acids; DS, digestible starch; RS, resistant starch; ileum, IL; caecum, CE; proximal colon, pCO; middle colon, mCO; distal colon, dCO.

Chapter 6

Potential of alginate and resistant starch to modify feeding patterns and performance in ad libitum-fed growing pigs

Alginate and resistant starch in growing pigs

Carol Souza da Silva[1,2], Guido Bosch[2], J. Elizabeth Bolhuis[1], Lian J.N. Stappers[1], Hubèrt M.J. van Hees[3], Walter J.J. Gerrits[2], Bas Kemp[1]

[1] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[2] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[3] Nutreco Research and Development, 5830 AE Boxmeer, The Netherlands

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Abstract

This study assessed the long-term effects of diets containing either a gelling fibre (alginate, ALG), or a fermentable fibre (resistant starch, RS), or both (RSALG), on feeding patterns and performance of growing pigs fed ad libitum for 12 weeks. The experiment was set up as a 2 × 2 factorial arrangement: presence (5% of DM) or absence of ALG, and presence (34% of DM) or absence of RS in the diet, resulting in 4 dietary treatments, i.e. control (CON), ALG, RS, and RSALG. Both RS and ALG were exchanged for pregelatinized potato starch. A total of 240 pigs in 40 pens were used. From all visits to an electronic feeding station, feed intake and detailed feeding patterns were calculated. Apparent total tract digestibility of energy, DM, and CP was determined in week 6. Pigs' postures and behaviours were scored from live observations in weeks 7 and 12. Dietary treatments did not affect final BW and ADG. Energy and DM digestibility were reduced by ALG ($P < 0.01$). Average daily DM intake was increased, and backfat thickness and carcass gain:DE intake were reduced by ALG ($P < 0.05$). Resistant starch increased feed intake per meal, meal duration ($P < 0.05$) and inter-meal intervals ($P=0.05$), and reduced the number of meals per day ($P < 0.01$), but did not affect daily DM intake. Energy, DM, and CP digestibility were reduced by RS ($P < 0.001$). Average daily DE intake was reduced ($P < 0.05$), and gain:DE intake tended to be increased ($P=0.08$), whereas carcass gain:DE intake was not affected by RS. In week 12, RSALG decreased standing and walking, aggressive, feeder-directed, and drinking behaviours (RS × ALG interaction, $P < 0.05$) as compared with ALG, with CON and RS in between. No other RS × ALG interactions were found. In conclusion, pigs fed ALG compensated for the reduced dietary DE content by increasing their feed intake, achieving similar DE intake and ADG as CON pigs. Backfat thickness and carcass efficiency were yet reduced in pigs fed ALG, which also showed increased activity. Pigs fed RS changed feeding patterns, but did not increase feed intake. Despite a lower DE intake, pigs fed RS achieved similar ADG as CON pigs by increasing efficiency in DE use. This indicates that the energetic utilization of RS in pigs with ad libitum access to feed is close to that of enzymatically digestible starch. The increased efficiency may relate to a more efficient utilization of VFA, and reduced energy expenditure on immune responses.

Keywords: dietary fibre, digestible energy, feeding behaviour, growth, pigs, satiety

Introduction

Dietary fibre is a generic term for a range of non-starch polysaccharides (NSP) (Topping and Clifton, 2001) that vary considerably in their physicochemical properties and can potentially affect energy intake by different working mechanisms (Wanders et al., 2011). Viscous and gelling fibres have been associated with enhanced satiation, i.e. meal termination (Georg Jensen et al., 2013), which is related to reduced gastric emptying rate (Hoebler et al., 2000). Fibres that are fermented in the caecum and colon have been associated with prolonged satiety, i.e. postprandial inhibition of feeding motivation (Souza da Silva et al., 2012, 2013a), which is often attributed to a more gradual energy supply during the day (Regmi et al., 2011). Satiating properties of fibres may potentially be used to limit energy intake in breeding sows or fattening pigs without the need for quantitative feed restriction, while improving welfare by reducing hunger. However, given that increasing dietary fibre level generally reduces dietary DE content, and decreases nutrient and energy digestibility (Noblet and Le Goff, 2001), pigs may increase their voluntary feed intake to try to meet their energy requirements (Cole et al., 1968). Anticipated satiating properties of viscous and fermentable fibres originate mainly from short-term studies in restrictedly-fed pigs (Souza da Silva et al., 2012, 2013a). It is yet unclear if satiating properties of these fibres lead to a reduced energy intake in pigs fed ad libitum during a long period of time. Therefore, the objective of the present study was to assess the potential of a gelling fibre (alginate, ALG), a fermentable fibre (resistant starch, RS), and the combination of both, to modify feeding patterns (via satiation and satiety), and thereby long-term feed intake and growth performance of growing pigs fed ad libitum during 12 weeks. In addition, the extent to which pigs compensated for the reduced DE content of the fibre diets was evaluated.

Materials and methods

Experimental design

The protocol for this experiment was approved by the Animal Care and Use Committee of Wageningen University. The experiment was set up as a 2 × 2 factorial arrangement: presence (5% of DM, exchanged for digestible starch) or absence of ALG, and presence (34% of DM, exchanged for digestible starch) or absence of RS in the diet, resulting in 4 dietary treatments, i.e. control (CON), ALG, RS, and RSALG. The experiment was carried out in 2 successive batches. Forty pens in total were allocated to treatments (n=10 pens per treatment) with an

equal number of barrows and gilts in each pen (n=6 pigs per pen). Pigs had ad libitum access to their dietary treatments for 12 weeks.

Animals, housing, and diets

Per batch, 120 growing Shade Oak Duroc × Hypor gilts and barrows with an initial BW of approximately 37.8 ± 0.3 kg (age: 3 months) were used. Pigs were selected 4 weeks before the start of the experiment and were allocated to groups of 8 (4 barrows and 4 gilts) balanced for BW at birth, BW at weaning (age: 3 weeks), gender, and litter. After 3 weeks of habituation, 2 pigs were removed from each pen, and pigs were housed in treatment groups of 6 (3 barrows and 3 gilts) 1 week before the start of the experiment on the basis of similar average pen BW across treatments. Pens (12 m^2) had partly solid and partly slatted floors, and were evenly distributed over 3 climate-controlled rooms at the experimental facilities of the Nutreco Swine Research Centre, Sint Anthonis, The Netherlands. The room temperature was maintained at 20 ± 2 ºC, and artificial lights were on from 0600 h until 2200 h and dimmed during the night. Pens were equipped with an electronic feeding station (Compident MLP, model 1, Schauer Agrotronic GmbH, Prambachkirchen, Austria) to monitor individual feed intake. Water was continuously available throughout the experiment, via 2 nipple drinkers located opposite to the feeding station. Pigs were provided with a variety of toys (3 toys per pen), which remained in the pen throughout the experiment.

The composition of the diets is presented in Table 1. A wheat- and soybean meal-based CON diet containing 40% pregelatinized potato starch (Avebe Food, Veendam, The Netherlands) was formulated. In the ALG, RS, and RSALG diets, pregelatinized potato starch from the CON diet was replaced on a DM basis by either 5% sodium alginate (Acatris, Bunschoten, The Netherlands), or 34% retrograded tapioca starch (Cargill, Amsterdam, The Netherlands), or both, respectively. Diets were designed to meet nutrient requirements according to the Centraal Veevoeder Bureau (2007), and were isoenergetic in their GE content. Diets were flavoured to mask possible differences in palatability, and titanium dioxide (2.5 g/kg) was added as an indigestible marker. Diets were pelleted to guarantee homogeneous distribution of ingredients and marker, and to avoid malfunctioning of the feeding stations. Pigs had ad libitum access to feed throughout the experiment.

Table 1. Composition of the experimental diets[1], g/kg DM.

Item	Diet			
	CON	ALG	RS	RSALG
Ingredient composition				
Pregelatinized potato starch[2]	400.0	349.5	51.0	–
Retrograded tapioca starch[3]	–	–	336.5	336.0
Sodium alginate[4]	–	51.4	–	52.4
Soybean meal	350.0	349.5	357.3	356.7
Wheat	79.5	79.4	81.1	81.0
Wheat middlings	53.0	52.9	54.1	54.0
Animal fat	70.0	69.9	71.5	71.3
Vitamin-mineral premix[5]	10.0	10.0	10.2	10.2
Calcium carbonate	12.5	12.5	12.8	12.7
Monocalcium phosphate	13.0	13.0	13.3	13.2
Salt	3.5	3.5	3.6	3.6
L-Lysine·HCl	1.5	1.5	1.5	1.5
DL-Methionine	2.0	2.0	2.0	2.0
L-Threonine	1.0	1.0	1.0	1.0
Titanium dioxide	2.5	2.5	2.6	2.5
Flavor[6]	1.5	1.5	1.5	1.5
Chemical composition				
DM, g/kg as is	900.4	896.0	908.4	902.8
Starch	406.3	362.9	373.9	329.9
Sugar	42.9	41.7	55.3	55.6
Starch and sugar	449.2	404.5	429.2	385.5
CP (N × 6.25)	187.5	184.8	191.4	193.1
Crude fat	80.1	80.5	77.4	79.5
Ash	52.5	60.5	54.2	62.0
ADF	31.3	53.4	31.1	48.0
ADL	4.9	5.5	4.9	5.2
NSP[7]	131.2	165.6	156.2	182.7
Titanium	2.0	1.9	2.0	1.9
Energy, MJ GE/kg	17.5	17.1	17.6	17.2

[1] CON, control diet; ALG, alginate diet; RS, retrograded tapioca starch diet; RSALG, retrograded tapioca starch plus alginate diet.
[2] Avebe Food, Veendam, The Netherlands.
[3] Cargill, Amsterdam, The Netherlands.
[4] Acatris, Bunschoten, The Netherlands.
[5] Provided per kilogram of diet: retinol, 6,000 IU; cholecalciferol, 1200 IU; DL-α-tocopherol, 40 mg; menadione, 1.5 mg; thiamine, 1 mg; riboflavin, 3 mg; D-pantothenic acid, 10 mg; niacin, 20 mg; cyanocobalamin, 15 µg; folic acid, 0.2 mg; pyridoxine hydrochloride, 1 mg; choline chloride, 150 mg; Fe as ferrous sulphate, 80 mg; Cu as copper sulphate, 15 mg; Zn as zinc sulphate, 50 mg; Mn as manganese oxide, 30 mg; Co as cobalt sulphate, 0.2 mg; I as potassium iodide, 0.7 mg; Se as sodium selenite, 0.2 mg.
[6] Luctarom Advance Cherry Honey, Lucta S.A., Barcelona, Spain.
[7] NSP=non-starch polysaccharides, calculated as DM − ash − CP − crude fat − starch − sugar content.

Before the start of the experiment, all pigs were fed a commercial pelleted feed, and were allowed a 3-week habituation to their pen, group, and electronic feeding station, followed by a period of gradual adaptation to the experimental diets to prevent feed neophobia during the experiment. The change to the experimental diets took place during 5 days in which the commercial feed was step-wise exchanged for one of the experimental diets. The experiment was carried out over a 12-week period.

Chemical analyses

A diet sample was taken at the start of every batch for chemical analyses. The diets were analysed for DM, ash, starch, sugar, CP, crude fat, ADF, ADL, GE, and titanium. The content of NSP was calculated by subtracting the ash, starch, sugar, CP, and crude fat content from the DM content. Diets were ground to pass a 1 mm sieve. Dry matter and ash contents were determined by drying to a constant weight at 103 °C (ISO, 1999b) and combustion at 550 °C (ISO, 2002), respectively. Crude protein (N × 6.25) was determined by the Kjeldahl-method (ISO, 2005) and crude fat by petroleum-ether extraction after acid hydrolysis (ISO, 1999a). Starch content was analysed enzymatically and additionally extracted with 40% ethanol to determine reducing sugars (Brunt, 1993). Acid detergent fibre and ADL were analysed according to the Van Soest-method (Van Soest, 1973). Gross energy was determined using a bomb-calorimeter (model IKA calorimeter C7000, IKA Werke GmbH, Staufen, Germany) and titanium according to a modified standard method (Short et al., 1996; Myers et al., 2004). All analyses were carried out in duplicate.

Feeding patterns

Feed intake was recorded via the electronic feeding station, consisting of a feed dispenser, a feed trough connected to a load cell to measure the weight of the feed consumed, and a receiving equipment to identify radio signals from a transponder carried by the pigs. Each pig was fitted with an ear tag transponder with an unique electronic identification number, by which each visit to the feeding station, its duration, and the feed consumption per visit were recorded in the receiving equipment (Hoy et al., 2012). Access to the feeding station was restricted to 1 pig at a time, and a maximum of 1800 g of feed was available for consumption per visit. After each feeding, the feeding station was closed and the feed trough was lifted to enable automatic weighing (with ± 3 g accuracy) of the amount of feed consumed. When less than 1000 g of feed was left in the feed trough, it was automatically refilled to 1800 g. After 30 s, which included the weighing and refilling, the next pig could access the feeding station. Pigs had 24-h access to the feeding station. All data was continuously stored in a data file with the pen number, the pig's identification number, the date, the time of entry and exit per visit, and amount of feed consumed per visit. Data on feed intake characteristics for individual pigs were accumulated over the 12-week period of each batch, and were used to estimate mean values for feeding pattern variables per pig per day. Intervals between successive visits of the same pig shorter than 5 min (which was used as meal criterion) were grouped into the same meal (De Haer and Merks, 1992), and then used to estimate mean values for cumulative feed intake (kg) during the whole experiment, daily feed intake (kg), feed intake per meal (g), meal duration (min;

time from start first visit to end last visit belonging to a meal), inter-meal interval (min; time between the end of one meal and the initiation of next), daily time feeding (min; sum of duration of all meals on a day), and number of meals per day. All scales of the electronic feeding stations were calibrated at the start of each batch and thereafter only in case of malfunction, using a 2-kg test weight.

Apparent total tract digestibility

Faeces were collected from 3 randomly selected pigs in each pen during 3 days in week 6 of each batch for the determination of apparent total tract digestibility of energy, DM, and CP. Faecal samples were collected directly from the rectum of each pig twice a day and weighed. All collected faeces were stored at −20 °C until further analyses. The total faeces of each pen within each batch (n=10 pens per treatment) was dried (70 °C), pooled, mixed, sampled, and analysed for DM, CP, GE and titanium as described before. Apparent total tract digestibility for energy and CP was calculated as previously described (Bosch et al., 2009). The DE content of the diet was calculated as its GE content multiplied by the digestibility coefficient for the energy.

Growth performance

Pigs were weighed throughout the experiment in weeks 1, 2, 4, 6, 8, 10, 11 and 12, and had backfat and muscle thickness estimated in week 12. Backfat and muscle estimates were recorded approximately 6.5 cm off the dorsal midline near the last rib (P2 location) using a portable real-time ultrasound scanner (Vetko Plus, Noveko, Quebec, Canada). The growth rate data combined with information about cumulative feed intake were used for calculations of feed efficiency, which was expressed in two ways: gain to feed ratio (gain:DM intake), based on DM intake, and gain to energy ratio (gain:DE intake), based on DE intake calculated in GJ.

All pigs (age: 6 months) were slaughtered at a commercial abattoir at the end of the 12-week period of each batch. Pigs were electrically stunned, exsanguinated, scalded, dehaired, and eviscerated according to standard procedures. After evisceration, carcasses were weighed. The carcass growth was defined as the final carcass weight measured for a pig at slaughter minus the estimated initial carcass weight (week 1) of that pig, assuming the carcass:BW ratio at the start of the experiment to be similar to that of the CON-fed pigs at slaughter. These data combined with information about cumulative feed intake were used for calculations of carcass efficiency, which was expressed as carcass gain to feed ratio (carcass gain:DM intake), based on DM intake, and carcass gain to energy ratio (carcass gain:DE intake), based on DE intake calculated in GJ.

Behavioural observations

Pigs were observed in their home pen during 1 day at the start of weeks 7 and 12 in each batch during 6 intervals of 1 h using 4 min-instantaneous scan sampling. Live observations started at 0800 h, 0915 h, 1030 h, 1400 h, 1515 h, and 1630 h. Every 4 min, the posture of each pig and its behaviour were scored. Postures were lying, standing and walking, and sitting and kneeling. Behavioural activities scored were social behaviour (touching or sniffing any part of the body of a pen mate), play behaviour (pivoting, rolling, sliding, play with toy, gambolling), aggressive behaviour (ramming or pushing a pen mate, with or without biting), fighting at feeder (push, head knock or bite given at a pen mate near the feeding station), manipulative behaviour (belly nosing or nibbling, sucking or chewing any other part of the body of a pen mate), explorative behaviour (rooting or sniffing floor, toys or pen fixtures), chewing (repetitively chewing of toys or pen fixtures, or sham chewing) (De Leeuw et al., 2008), feeder-directed behaviour (sniffing or touching the feeding station, with or without eating), drinking, and other (all other behavioural activities). Fighting at feeder was considered as part of 'aggressive behaviour'. Explorative behaviour, chewing, feeder-directed behaviour, and drinking were also summed as 'total oral behaviour'. For all behavioural observations, observers were blind to the dietary treatment that the pigs had. The Observer software package (Noldus Information Technology B.V., Wageningen, The Netherlands) installed on a handheld computer (Psion Workabout MX, Psion PLC, London, UK) was used for behavioural recordings. Behaviours were averaged per pen per observation h and expressed as percentages of observation time.

Blood sampling and plasma analyses

Three days after the behavioural observations in week 7 and 12 of each batch, a blood sample (9 mL) was collected by jugular venipuncture from 2 pigs (1 barrow and 1 gilt) per pen using disposable vacuum containers containing liquid K3EDTA (Becton Dickinson, Franklin Lakes, NJ) and protease inhibitor cocktail (Complete, Roche Diagnostics GmbH, Mannheim, Germany). The 2 pigs from each pen were selected with a BW close to the average of the pen at the start of the experiment (10 pigs per dietary treatment per batch). Blood samples were taken at 0830 h after an overnight fast. After centrifugation (1300 × g for 10 min at 4 °C), plasma samples were stored at −80 °C and later analysed for concentrations of glucose, insulin, and leptin. Plasma glucose concentrations were determined using an enzymatic method (Gluco-quant Glucose/HK, Roche/Hitachi Modular P800 Autoanalyser, Roche Diagnostics GmbH, Mannheim, Germany) based on hexokinase activity (Peterson and Young, 1968). For the glucose assay, the

sensitivity was 0.11 mmol/L, and the intra- and interassay CV were 1.0% (n=63) and 1.7% (n=63), respectively. Plasma insulin concentrations were determined using a Porcine Insulin RIA kit (PI-12K, Millipore, St. Charles, MO). For the insulin assay, the sensitivity was 1.61 μU/mL, and intra- and interassay CV were 4.4% (n=199) and 13.1% (n=51), respectively. Plasma leptin concentrations were determined using a Multispecies Leptin RIA kit (KL-85K, Millipore, St. Charles, MO). For the leptin assay, the sensitivity was 0.80 ng/mL, and intra- and interassay CV were 3.2% (n=16) and 7.8% (n=4), respectively.

Extra blood samples (9 mL) were collected using disposable vacuum containers containing liquid K3EDTA in week 12 to measure levels of serotonin (5-hydroxytryptamine, 5-HT) in blood platelets, and monoamine oxidase (MAO) activity in whole blood as previously described (Souza da Silva et al., 2013b).

Empty weights of the gastrointestinal tract

From 48 pigs (12 pigs per dietary treatment in a 1:1 gender ratio) in batch 2, the gastrointestinal tract (GIT) was separated into stomach, small intestine, caecum, and colon after slaughter. These parts were cut open and emptied by gently squeezing digesta out. The colon was also washed with water to remove remaining digesta. The empty weights of the different parts were recorded and expressed in g/kg BW. The weight of mesenteric tissue was also recorded as a separate part.

Statistical analyses

Three pigs died during the experiment due to respiratory problems. Data were analysed as a 2 × 2 factorial arrangement using PROC MIXED in SAS 9.2 (SAS Institute, Cary, NC). For the feeding patterns and growth performance data measured throughout the experiment, the model included ALG (yes/no), RS (yes/no), their interaction, gender and batch as fixed effects, and pen within ALG, RS and batch, and pig within pen, ALG, RS, gender and batch as random effects. Thus, effects of dietary treatments, batch and their interaction were tested against the random effect of pen, and effects of gender and its interaction with dietary treatments were tested against the random effect of pig. The daily patterns of feeding were estimated for each pig by calculating the means for the number of meals and DM intake for each h of every 24-h period for the 12-week period of each batch. For the daily patterns data, the model was extended with h of day as fixed effect, and pen within ALG, RS, batch and h as random effect. For the behaviours and plasma metabolites data measured in weeks 7 and 12 of the experiment, the model was extended with week of sampling as fixed effect, and pen within ALG, RS, batch and week as random effect. For the energy and CP digestibility data measured once in week 6 of the experiment, the model included

ALG (yes/no), RS (yes/no), their interaction and batch as fixed effects, and pen within ALG, RS and batch as random effect. For the GIT weights data measured only in batch 2, the model included ALG (yes/no), RS (yes/no), their interaction and gender as fixed effects, and pen within ALG and RS as random effect. Interactions between dietary treatments and gender or batch were removed from the final model if not significant ($P > 0.10$). Data are presented as means ± SEM. Differences were considered significant if $P \leq 0.05$, whereas $P \leq 0.10$ was considered a trend.

Results

Feeding patterns

The feeding pattern variables for each dietary treatment are presented in Table 2. Adding ALG to the diets increased daily and cumulative feed intake (both $P < 0.05$), and tended to increase daily time spent feeding ($P=0.08$). Adding RS to the diets increased feed intake per meal and meal duration (both $P < 0.05$), and tended to increase inter-meal intervals ($P=0.05$). This resulted in a decreased number of meals per day for the RS diets as compared with ALG and CON diets ($P < 0.001$). RS and ALG tended to interact to affect the meal duration ($P=0.06$), which was greatest in the combination treatment as compared with other treatments. Most feeding pattern variables were affected by gender (all $P < 0.01$), except for inter-meal interval and number of meals per day. Average daily feed intake (2.1 ± 0.02 kg vs. 2.3 ± 0.03 kg, $P < 0.001$), cumulative feed intake (173.1 ± 1.83 kg vs. 190.1 ± 2.28 kg, $P < 0.001$), feed intake per meal (132.2 ± 4.01 g vs. 147.2 ± 5.07 g, $P < 0.01$), meal duration (6.1 ± 0.14 min vs. 6.7 ± 0.14 min, $P < 0.01$), and daily time spent feeding (78.2 ± 1.14 min vs. 86.2 ± 1.64 min, $P < 0.001$) were lower in gilts than in barrows. Dietary treatments and gender did not interact to affect feeding pattern outcomes.

Table 2. Feeding patterns of growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks.

	Diet				*P*-value		
Item	CON	ALG	RS	RSALG	ALG	RS	RS × ALG
Cumulative feed intake, kg	179.3 ± 3.21	186.9 ± 3.85	176.6 ± 3.35	183.7 ± 3.24	**0.037**	0.329	0.988
Average daily feed intake, kg	2.19 ± 0.04	2.29 ± 0.04	2.15 ± 0.04	2.24 ± 0.04	**0.023**	0.173	0.930
Feed intake per meal, g	134.0 ± 7.03	128.0 ± 6.14	146.8 ± 5.87	150.0 ± 6.67	0.854	**0.015**	0.490
Meal duration, min	6.28 ± 0.14	5.98 ± 0.18	6.39 ± 0.24	6.94 ± 0.23	0.578	**0.015**	0.056
Inter-meal interval, min	83.3 ± 3.46	78.6 ± 3.55	92.2 ± 2.65	85.9 ± 3.64	0.190	**0.051**	0.846
Daily time feeding, min	81.4 ± 2.41	82.8 ± 2.05	79.3 ± 2.04	84.4 ± 2.15	0.077	0.650	0.511
Number of meals per day	17.5 ± 0.90	19.0 ± 0.68	15.3 ± 0.47	16.0 ± 0.61	0.138	**< 0.001**	0.655

Daily patterns

The daily patterns of feeding for each dietary treatment are presented in the number of meals per h (Fig. 1a) and DM intake per h (Fig. 1b) averaged over 12 weeks. During daytime (0600 h until 2200 h, equal to light schedule) 68.3% of the meals were consumed, with peaks between approximately 0600 h and 1000 h, and between 1500 h and 1900 h. The peaks in DM intake were similar to the peaks in the number of meals, i.e. between 0600 h and 1000 h, and between 1600 h and 2000 h. During daytime 69.3% of the DM intake occurred. Although the effect of ALG on the average number of meals per day was not significant (P=0.14, Table 2), there was an effect of ALG and RS on the number of meals per h over 24 h when means for each h of every 24-h period were taken as repeated measurements. Adding ALG to the diets increased the number of meals per h, whereas adding RS to the diets decreased the number of meals per h ($P < 0.05$ and $P < 0.01$, respectively, Fig. 1a). Although the effect of RS on the average daily feed intake was not significant (P=0.17, Table 2), adding RS to the diets tended to increase the DM intake per h over 24 h when analyzed in a repeated model (P=0.06, Fig. 1b). Alginate and h interacted to affect DM intake ($P < 0.001$, Fig. 1b). Analysis per h revealed that adding ALG to the diets decreased DM intake per h, particularly at 0200 h ($P < 0.05$), 0400 h ($P < 0.01$), 0500 h, and 0900 h (both $P < 0.05$), although average daily feed intake was increased by adding ALG to the diets ($P < 0.05$, Table 2). The DM intake per h was lower in gilts than in barrows (165.5 ± 2.88 g vs. 185.9 ± 3.97 g, $P < 0.001$). The number of meals per h was unaffected by

gender. Dietary treatments and gender did not interact to affect daily pattern outcomes.

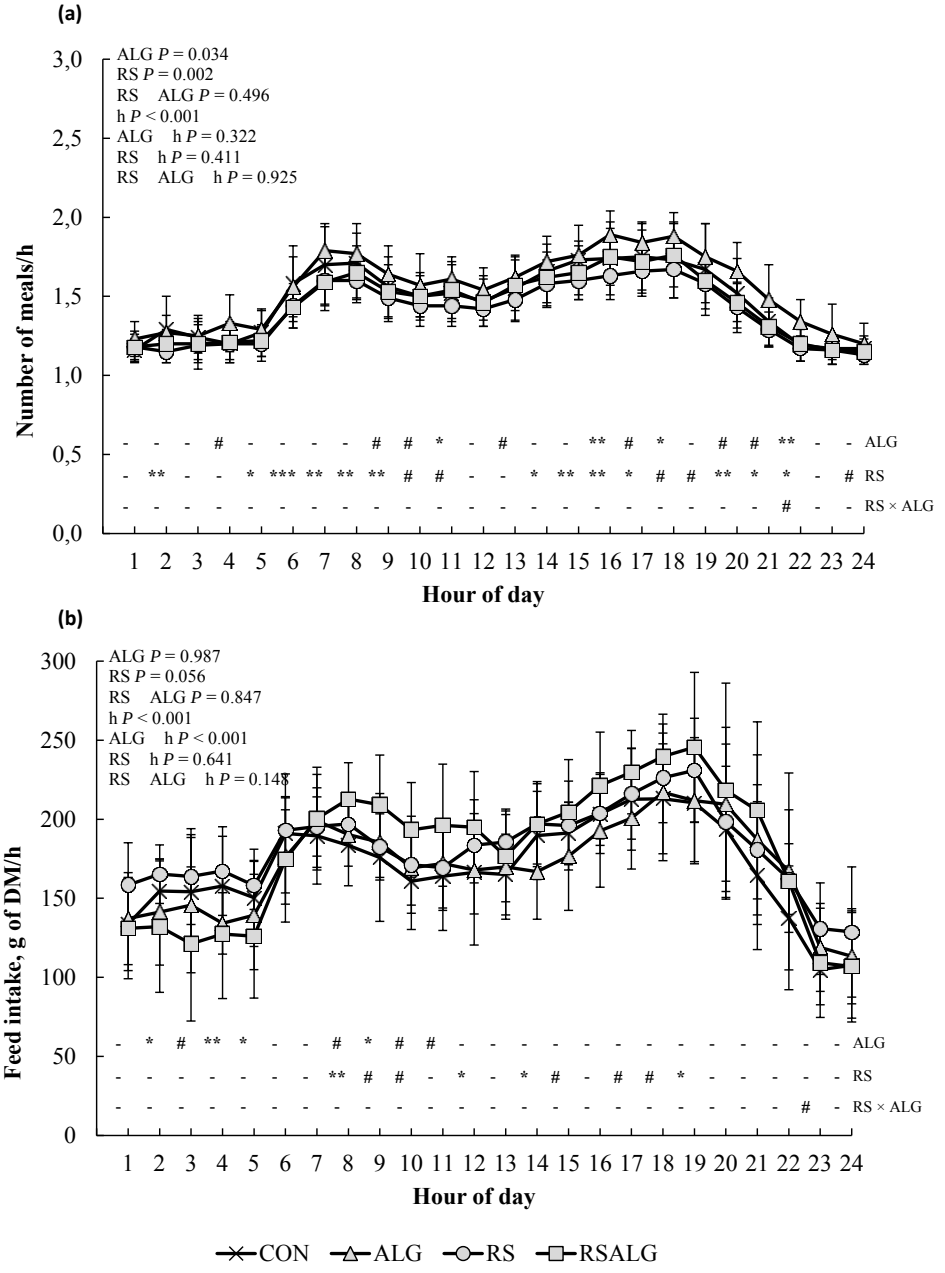

Fig. 1. Distribution of feeding patterns by hour of day for growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks: (a) number of meals per h, and (b) DM intake per h averaged over the 12-week period of each batch.

Lights were on from 0600 h until 2200 h and dimmed during the night. Statistical significance of effects of RS (yes/no), ALG (yes/no), hour of day (h), and their interaction is indicated in the upper left corner of each figure. In addition, statistical significance of effects of RS, ALG, and their interaction is indicated per h: #$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Apparent total tract digestibility

The digestibility coefficients for each dietary treatment are presented in Table 3. Diets with ALG had a lower digestibility of DM and GE ($P < 0.001$ and $P < 0.01$, respectively) than diets without ALG. Diets with RS had a lower digestibility of DM, CP, and GE than diets without RS (all $P < 0.001$). Alginate and RS did not interact to affect digestibility of DM, CP or GE.

Table 3. Apparent total tract digestibility measured in week 6 in growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks.

	Diet				*P*-value		
Item	CON	ALG	RS	RSALG	ALG	RS	RS × ALG
DM	0.85 ± 0.003	0.84 ± 0.003	0.83 ± 0.007	0.81 ± 0.003	**< 0.001**	**< 0.001**	0.819
CP	0.82 ± 0.006	0.80 ± 0.010	0.77 ± 0.014	0.77 ± 0.006	0.481	**< 0.001**	0.134
GE	0.86 ± 0.003	0.85 ± 0.003	0.84 ± 0.007	0.83 ± 0.004	**0.007**	**< 0.001**	0.526

Growth performance

The growth performance parameters for each dietary treatment are presented in Table 4. Initial BW, final BW, and ADG did not differ between treatments. There was a trend (P=0.06) for pigs fed RS-containing diets to be heavier than those fed ALG and CON diets at the start of the experiment (d 0), which was not sustained and final BW was similar for all treatments. Average daily DM intake was greater and gain:DM intake ratio was lower for pigs fed ALG-containing diets than for pigs fed diets without ALG (both $P < 0.05$). The DE intake or gain:DE intake ratio did not differ between pigs fed diets with or without ALG. Resistant starch did not affect DM intake or gain:DM intake ratio. Average daily DE intake was lower ($P < 0.05$) and gain:DE intake ratio tended to be greater (P=0.08) for pigs fed RS-containing diets than for pigs fed diets without RS. Muscle thickness did not differ between treatments. At the end of the experiment, pigs fed ALG-containing diets had thinner backfat than those fed diets without ALG ($P < 0.05$). Pigs fed ALG-containing diets had a lower carcass growth ($P < 0.05$) and a lower carcass gain:DM intake ratio ($P < 0.001$) than pigs fed diets without ALG. Pigs fed RS-containing diets had a lower carcass growth ($P < 0.05$) and tended to have a lower carcass gain:DM intake ratio (P=0.09) than pigs fed diets without RS. The carcass

gain:DE intake ratio was lower for pigs fed ALG-containing diets than for pigs fed diets without ALG ($P < 0.05$), and tended to be lower for pigs fed RSALG diets than for pigs fed other diets (RS × ALG interaction, P=0.09).

Table 4. Growth performance of growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks.

	Diet				*P*-value		
Item	CON	ALG	RS	RSALG	ALG	RS	RS × ALG
Initial BW, kg	37.4 ± 0.32	37.1 ± 0.38	37.7 ± 0.46	38.8 ± 0.52	0.459	0.060	0.213
Final BW, kg	110.9 ± 1.16	110.1 ± 1.77	111.6 ± 1.45	111.7 ± 1.59	0.792	0.403	0.753
ADG, kg	0.92 ± 0.012	0.91 ± 0.024	0.92 ± 0.017	0.91 ± 0.020	0.678	0.900	0.926
Average daily DM intake, kg	1.98 ± 0.031	2.05 ± 0.037	1.95 ± 0.029	2.02 ± 0.036	**0.041**	0.372	0.912
Average daily DE intake, MJ	29.7 ± 0.46	29.8 ± 0.57	28.7 ± 0.28	28.9 ± 0.47	0.811	**0.041**	0.945
Gain:DM intake, kg/kg	0.45 ± 0.006	0.44 ± 0.011	0.46 ± 0.009	0.44 ± 0.009	**0.031**	0.493	0.918
Gain:DE intake, kg/GJ	27.2 ± 0.36	26.9 ± 0.69	28.3 ± 0.52	27.8 ± 0.60	0.462	0.082	0.778
Backfat thickness, mm	17.4 ± 0.35	16.3 ± 0.47	17.1 ± 0.49	16.1 ± 0.33	**0.023**	0.638	0.769
Muscle thickness, mm	57.2 ± 0.63	57.1 ± 0.87	57.0 ± 0.73	56.8 ± 0.74	0.768	0.639	0.936
Carcass growth, kg	57.8 ± 0.84	57.2 ± 0.86	56.8 ± 1.11	53.7 ± 0.73	**0.046**	**0.019**	0.156
Carcass gain:DM intake, kg/kg	0.36 ± 0.005	0.34 ± 0.005	0.35 ± 0.007	0.32 ± 0.004	**< 0.001**	0.086	0.165
Carcass gain:DE intake, kg/GJ	21.4 ± 0.31	21.1 ± 0.32	21.9 ± 0.43	20.4 ± 0.28	**0.015**	0.744	0.094

Final BW tended to be affected by gender (P=0.06), with barrows (112.21 ± 0.89 kg) being heavier than gilts (109.97 ± 0.90 kg). Average daily DM intake (1.9 ± 0.02 kg vs. 2.1 ± 0.02 kg) and DE intake (27.9 ± 0.27 MJ vs. 30.6 ± 0.33 MJ) were lower in gilts than in barrows (both $P < 0.001$). Backfat thickness was affected by gender ($P < 0.001$), with barrows (17.95 ± 0.32 mm) having a thicker backfat than gilts (15.54 ± 0.27 mm), this effect was stronger in RS-fed barrows (RS × gender interaction, $P < 0.05$, data not shown). Resistant starch and batch interacted to affect muscle thickness ($P < 0.01$). Muscle thickness was lower for pigs fed RS-containing diets (56.26 ± 0.81 mm) than for pigs fed diets without RS in batch 2 (58.88 ± 0.64 mm), whereas in batch 1 muscle thickness was not affected by RS (57.52 ± 0.60 mm for diets with RS vs. 55.50 ± 0.55 mm for diets without RS). Carcass growth tended to be affected by gender ($P < 0.10$), with

barrows (57.19 ± 0.67 kg) growing more than gilts (55.63 ± 0.61 kg). The carcass gain:DM intake ratio and carcass gain:DE intake ratio also tended to be affected by gender (both $P < 0.10$), with greater ratios in barrows (0.35 ± 0.004 and 21.49 ± 0.25) than in gilts (0.34 ± 0.004 and 20.92 ± 0.23).

Behavioural observations

Over time, sitting and kneeling (2.4 ± 0.1 % vs. 1.9 ± 0.1 %, $P < 0.05$), exploration (10.0 ± 0.3 % vs. 8.8 ± 0.3 %, $P < 0.05$), and chewing (5.5 ± 0.2 % vs. 3.9 ± 0.2 %, $P < 0.001$) were increased in week 12 as compared with week 7. Resistant starch and ALG interacted to affect chewing ($P < 0.05$): RSALG-fed (4.9 ± 0.3 %) pigs spent more time chewing than pigs fed ALG (4.0 ± 0.3 %), but less than CON-fed pigs (5.4 ± 0.3 %). Moreover, RSALG-fed pigs (0.48 ± 0.1 %) tended to spend the least time on aggressive behaviours as compared with pigs fed other diets (CON: 0.64 ± 0.2 %, ALG: 0.94 ± 0.2 %, RS: 0.55 ± 0.1 %, P=0.10).

Dietary treatments and week interactions were found for most behavioural traits. Therefore the behavioural traits for each dietary treatment are presented per week in Table 5. Two-way interaction between RS and week was found for play behaviour ($P < 0.05$). Analysis per week revealed that pigs fed diets with RS (0.23 ± 0.05 %) tended to spend less time playing than pigs fed diets without RS (0.56 ± 0.09 %) in week 7 (P=0.06), whereas in week 12 play behaviour was not affected by RS (0.29 ± 0.06 % for diets with RS vs. 0.16 ± 0.04 % for diets without RS). Three-way interactions between RS, ALG and week were found for lying ($P < 0.01$), standing and walking ($P < 0.05$), total oral behaviours ($P < 0.05$), feeder-directed behaviours ($P < 0.01$), and drinking (P=0.08). Generally, differences between RSALG and ALG treatments were more pronounced in week 12 as compared with week 7. Analysis per week revealed that in week 12, RSALG-fed pigs spend more time lying (74.5 ± 1.6 % vs. 69.6 ± 1.4 %, P=0.08), and less time standing and walking (21.7 ± 1.0 % vs. 27.8 ± 1.4 %, $P < 0.05$) as compared with ALG-fed pigs. In addition, RSALG-fed pigs showed less aggressive behaviours (0.35 ± 0.08 % vs. 1.09 ± 0.15 %, $P < 0.05$), and less oral behaviours (24.8 ± 1.0 % vs. 30.0 ± 1.3 %, P=0.08), particularly less feeder-directed oral behaviours (7.4 ± 0.5 % vs. 11.1 ± 0.8 %, $P < 0.05$) and less drinking (2.5 ± 0.2 % vs. 3.3 ± 0.2 %, $P < 0.05$) than ALG-fed pigs, with the CON- and RS-fed pigs in between. Social and manipulative behaviours did not differ between treatments. Generally, gilts spent more time in standing and walking, social, playful, aggressive, manipulative, and explorative behaviours than barrows (all $P < 0.05$, data not shown).

Table 5. Daily physical activity measured in weeks 7 and 12 in growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks.

Behaviour[1], % of observation time	Diet				*P*-value		
	CON	ALG	RS	RSALG	ALG	RS	RS × ALG
Week 7							
Lying	73.4 ± 1.62	75.3 ± 2.21	74.9 ± 1.05	71.5 ± 1.89	0.667	0.515	0.136
Standing and walking	22.6 ± 1.55	23.0 ± 2.07	22.9 ± 1.06	25.0 ± 1.77	0.478	0.513	0.618
Sitting and kneeling	2.2 ± 0.46	1.7 ± 0.22	2.1 ± 0.17	1.8 ± 0.25	0.254	0.672	0.846
Social	0.9 ± 0.16	0.7 ± 0.14	0.9 ± 0.13	0.9 ± 0.18	0.580	0.508	0.661
Play	0.4 ± 0.17	0.7 ± 0.26	0.3 ± 0.12	0.2 ± 0.07	0.653	0.057	0.292
Aggression	0.9 ± 0.31	0.8 ± 0.20	0.7 ± 0.13	0.6 ± 0.14	0.847	0.671	0.724
Manipulation	1.3 ± 0.22	1.5 ± 0.39	2.0 ± 0.34	1.6 ± 0.50	0.465	0.570	0.487
Total oral behaviour	23.8 ± 1.18	23.1 ± 2.09	24.2 ± 1.20	25.5 ± 1.71	0.873	0.383	0.540
Exploration	8.7 ± 0.71	9.0 ± 1.07	9.0 ± 0.82	8.6 ± 0.77	0.971	0.919	0.658
Chewing	4.4 ± 0.36	3.2 ± 0.49	3.7 ± 0.48	4.4 ± 0.59	0.644	0.583	0.051
Feeder-directed	8.8 ± 0.68	8.6 ± 0.82	9.3 ± 0.52	10.2 ± 0.72	0.585	0.148	0.467
Drinking	2.0 ± 0.12	2.2 ± 0.14	2.3 ± 0.27	2.3 ± 0.33	0.617	0.331	0.824
Week 12							
Lying	73.8 ± 1.59	69.6 ± 2.67	72.1 ± 1.45	74.5 ± 1.67	0.657	0.392	0.080
Standing and walking	22.0 ± 1.22	27.8 ± 2.71	23.2 ± 0.84	21.7 ± 1.38	0.211	0.141	**0.034**
Sitting and kneeling	2.3 ± 0.27	2.3 ± 0.21	2.9 ± 0.29	2.0 ± 0.38	0.171	0.587	0.263
Social	0.8 ± 0.23	1.0 ± 0.20	1.3 ± 0.15	0.9 ± 0.17	0.723	0.302	0.212
Play	0.1 ± 0.06	0.2 ± 0.10	0.3 ± 0.14	0.3 ± 0.12	0.897	0.320	0.962
Aggression	0.4 ± 0.13	1.1 ± 0.26	0.4 ± 0.09	0.4 ± 0.06	0.067	**0.030**	**0.018**
Manipulation	1.0 ± 0.23	1.4 ± 0.19	1.2 ± 0.27	1.4 ± 0.42	0.161	0.924	0.489
Total oral behaviour	26.8 ± 1.54	30.0 ± 2.48	28.0 ± 1.06	24.8 ± 1.69	0.998	0.269	0.080
Exploration	9.1 ± 1.02	10.9 ± 1.01	10.4 ± 0.57	9.5 ± 1.05	0.641	0.921	0.130
Chewing	6.5 ± 0.58	4.7 ± 0.47	5.6 ± 0.58	5.4 ± 0.36	0.059	0.869	0.122

Feeder-directed	8.8 ± 0.63	11.1 ± 1.13	9.0 ± 0.65	7.4 ± 0.85	0.700	**0.050**	**0.030**
Drinking	2.4 ± 0.27	3.3 ± 0.22	2.9 ± 0.34	2.5 ± 0.25	0.398	0.737	**0.022**

[1] Aggression=sum of aggressive behaviour and fighting at feeder; Manipulation=sum of belly nosing and nibbling, sucking or chewing any other part of the body of a pen mate; Exploration=sum of rooting and sniffing floor, toys or pen fixtures; Chewing=sum of chewing toys or pen fixtures, and sham chewing; Feeder-directed=sum of sniffing and touching the feeding station, and eating; Total oral=sum of exploration, chewing, feeder-directed and drinking.

Plasma metabolites

Treatment effects were consistent for both weeks, and only few interactions were found between treatments and week due to small variations in the size of treatment effects. Therefore, the plasma metabolites fasting concentrations for each dietary treatment were averaged over weeks in Table 6. The ALG-containing diets tended to decrease insulin levels (P=0.07) and increase MAO levels as compared with diets without ALG (P=0.07). The RS-containing diets increased plasma glucose and insulin levels (both $P < 0.001$) as compared with diets without RS. Moreover, RS-containing diets tended to lower 5-HT levels as compared with diets without RS (P=0.09). In batch 1 only, MAO levels were lower in RS-fed pigs than in pigs fed diets without RS (74.56 ± 4.25 $\mu mol \cdot L^{-1} \cdot h^{-1}$ vs. 89.24 ± 4.72 $\mu mol \cdot L^{-1} \cdot h^{-1}$, RS × batch interaction, $P < 0.05$). Plasma leptin levels did not differ between treatments, but increased in week 12 as compared with week 7 (2.09 ± 0.12 mmol/L vs. 1.71 ± 0.11, mmol/L, $P < 0.001$). Plasma glucose and insulin levels were greater, whereas MAO levels were lower in gilts than in barrows (all $P < 0.05$, except for insulin P=0.06, data not shown).

Empty weights of the gastrointestinal tract

The empty weights of the GIT parts for each dietary treatment are presented in Table 7. The ALG-containing diets increased the empty weight of the colon as compared with diets without ALG ($P < 0.01$). The RS-containing diets increased the empty weight of the colon and total GIT as compared with diets without RS ($P < 0.001$ and $P < 0.05$, respectively). There was also a trend for increased empty weights of the stomach and caecum with RS-containing diets (P=0.10 and P=0.07, respectively). The weight of the mesenteric tissue was affected by gender, with barrows having heavier mesenteric tissue than gilts ($P < 0.01$, data not shown).

Table 6. Concentrations of glucose, insulin, leptin, serotonin (5-hydroxytryptamine, 5-HT), and monoamine oxidase (MAO) in peripheral blood collected after an overnight fast in growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks.

	Diet				*P*-value		
Item	CON	ALG	RS	RSALG	ALG	RS	RS × ALG
Glucose, mmol/L	3.9 ± 0.09	3.9 ± 0.17	4.5 ± 0.04	4.4 ± 0.07	0.552	**< 0.001**	0.420
Insulin, µU/mL	3.8 ± 0.46	3.7 ± 0.56	6.7 ± 0.57	4.8 ± 0.49	0.066	**< 0.001**	0.128
Leptin, ng/mL HE	1.9 ± 0.13	2.3 ± 0.41	1.7 ± 0.06	1.7 ± 0.10	0.427	0.123	0.408
5-HT, µmol/L	3.8 ± 0.87	5.6 ± 0.94	3.0 ± 0.33	3.7 ± 0.76	0.133	0.088	0.483
MAO, µmol·L^{-1}·h^{-1}	84.6 ± 4.88	87.2 ± 5.27	74.0 ± 5.30	89.8 ± 4.97	0.068	0.414	0.187

Table 7. Empty weights of the gastrointestinal tract (GIT) parts (in g/kg BW) of growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), and a diet containing both RS and ALG (RSALG) ad libitum during 12 weeks.

	Diet				*P*-value		
Item	CON	ALG	RS	RSALG	ALG	RS	RS × ALG
Mesentery	8.1 ± 0.25	7.9 ± 0.13	7.7 ± 0.21	7.2 ± 0.19	0.422	0.262	0.723
Stomach	4.9 ± 0.28	4.7 ± 0.27	4.9 ± 0.42	5.9 ± 0.06	0.217	0.103	0.113
Small intestine	17.1 ± 0.54	16.1 ± 0.48	16.4 ± 0.10	15.3 ± 0.86	0.290	0.424	0.996
Caecum	1.7 ± 0.08	1.5 ± 0.16	2.0 ± 0.23	1.9 ± 0.03	0.346	0.066	0.892
Colon	11.9 ± 0.28	12.9 ± 0.21	14.7 ± 0.20	17.4 ± 0.53	**0.010**	**< 0.001**	0.096
GIT	35.6 ± 0.08	35.2 ± 1.12	38.0 ± 0.56	40.5 ± 1.38	0.365	**0.022**	0.243

Discussion

Anticipated satiating properties of the fibres used in the present study originated from previous studies conducted in restrictedly-fed adult pigs fed 2 daily meals during 8 weeks (Souza da Silva et al., 2012, 2013a). It was yet unclear if dietary fibres would lead to a reduced energy intake in ad libitum-fed pigs, allowed to feed to satiety, during a long period of time. We addressed this by feeding diets containing either a gelling fibre (ALG), or a fermentable fibre (RS), or both (RSALG), ad libitum to growing pigs during 12 weeks, and studied the long-term effects of these diets on feeding patterns, feed intake and growth rates. Alginate and RS were used as fibres with satiation and satiety enhancing properties, respectively. In adult restrictedly-fed pigs, RS was reported to be more satiating than other types of fermentable fibres with different fermentation or physicochemical properties up to 7 h after the meal (Souza da Silva et al., 2013a). In the present study, the fermentation properties of RS were expected to increase the inter-meal interval, and thereby decrease the number of meals per day as compared with diets without RS. Indeed, adding RS to the diet resulted in a lower number of meals per day and tended to increase inter-meal intervals, which could be related to prolonged satiety after a RS-meal. Nevertheless, pigs fed RS-containing diets also increased feed intake per meal and meal duration, which also contributed to greater inter-meal intervals and suggests reduced satiation. This confirms observations in meal-fed humans who reported reduced satiety up to 5 h after a first meal of resistant corn starch, and then enhanced satiety 10-13 h after a second meal of resistant corn starch as compared with digestible corn starch (Achour et al., 1997). Resistant starch is less bulking than digestible starch (Nugent, 2005) and other types of fibre, such as viscous and gelling fibres, that have been reported to enhance satiation (Georg Jensen et al., 2013). Short-term feed intake, which reflects satiation, may be closely related to postprandial blood glucose levels (Higgins, 2004). In humans, it has been demonstrated that blood glucose levels are inversely correlated with appetite and food intake up to 60 min after different carbohydrate meals (Anderson et al., 2002). Thus, reduced blood glucose levels after a high-RS meal (Souza da Silva et al., 2013b), as has been reported for humans (Raben et al., 1994; Achour et al., 1997), may have delayed the onset of satiation, thereby leading to larger meals in pigs fed RS-containing diets in the present study. Throughout the day, a combination of a reduced glucose supply and an increased VFA production (Regmi et al., 2011) supports the less pronounced satiation, but enhanced satiety after the RS-containing diets. Production of VFA in the colon prolongs the energy supply to the body, which may bridge the energy gap during the interprandial period (Rérat, 1996; Darzi et al., 2011). In the present study, microbial production of VFA was not quantified, but

an increase in fermentation activity, and thereby an increase in VFA production (Haenen et al., 2013; Souza da Silva et al., 2013b) could be confirmed by adaptations found in the GIT of pigs fed diets with RS. The empty weight of the colon and total GIT were greater in pigs fed diets with RS than in pigs fed diets without RS, in line with previous studies (Martinez-Puig et al., 2003; Bolhuis et al., 2007).

Alginate was expected to yield most pronounced effects on satiation rather than on satiety, due to its physicochemical properties, such as viscosity and gelling in the stomach, leading to increased gastric distension, reduced gastric emptying rate, slowed passage rate, and slowed overall nutrient absorption in the small intestine (see Georg Jensen et al., 2013 for review). This is in line with the observed numerical decrease in feed intake per meal and shorter meal duration. However, pigs fed ALG-containing diets increased their daily and cumulative feed intake, which indicates a compensatory mechanism to overcome the reduction in dietary DE content associated with the exchange of starch for ALG. This corresponds with the theory of Cole et al. (1968), which asserts that when reducing dietary energy content, pigs may increase their voluntary feed intake to meet their energy requirements.

Growth performance

From previous studies in restrictedly-fed pigs, growth performance is expected to be reduced in pigs fed fibre diets due to the reduced digestibility and metabolisability of these diets (see Bindelle et al., 2008 for review). Indeed, in the present study, RS-diets reduced digestibility of DM, GE, and CP (likely as a consequence of microbial growth, see Shriver et al., 2003) as compared with ALG and CON diets, whereas ALG-diets reduced digestibility of DM and GE, but not of CP. Despite lower digestibility coefficients, final BW and ADG were not affected by diet. Moreover, DE intake was similar, but the carcass gain:DE intake ratio was lower for pigs fed ALG-containing diets than for pigs fed diets without ALG, whereas DE intake was lower, but the carcass gain:DE intake ratio was similar for pigs fed RS-containing diets than for pigs fed diets without RS. This suggests a more efficient use of DE in RS-fed pigs as compared with ALG-pigs and CON-pigs.

Alginate

In accordance with Cole et al. (1968), pigs fed ALG-containing diets compensated for the dietary DE dilution by increasing their DM intake, achieving a similar daily DE intake as the CON diet. Consequently, ADG and final BW were also not affected by ALG. Nevertheless, backfat thickness and carcass efficiency

were reduced in pigs fed ALG-containing diets as compared with pigs fed diets without ALG. Generally, fibre diets may reduce growth performance, not only due to reduced digestibility, but also due to constraints on food processing by the GIT, for example because of the greater volume or slower digestion of such diets (Henry, 1985). Furthermore, besides reducing ileal fat absorption (Aller et al., 2004), highly viscous ALG could reduce or delay intestinal absorption of glucose, as previously shown in pigs fed seaweed fibres of different viscosities (Vaugelade et al., 2000). Over an 8 h period, glucose absorption expressed as a percentage of ingested starch was reduced by half after highly viscous ALG supplementation. An overall reduction in nutrient absorption in the small intestine of pigs fed ALG-containing diets would result in lowered energy available for fat deposition in these animals, in line with the reduced backfat thickness and carcass gain. Fermentation did not seem to contribute substantially to nutrient supply in pigs fed ALG-containing diets. Although ALG has been reported to lead to a significant increase in fermentation activity and VFA production in pigs (Hoebler et al., 2000), according to in vitro fermentation measurements, ALG is slowly fermented by faecal microbiota from pigs (Jonathan et al., 2012). Moreover, a low inclusion level of ALG in the diets may have further contributed to its lower VFA supply. In the present study, the empty weight of the colon was increased in pigs fed the ALG diet, which is probably an adaptation of the GIT to fermentation, but not as much as found in pigs fed RS diets. More energy needed for GIT development may have further contributed to a reduced backfat thickness and carcass gain in ALG-pigs. Alternatively, a lower efficiency could be related to an increase in physical activity found in pigs fed ALG diets, which showed the least lying, and the most standing and walking, aggressive, feeder-directed and drinking behaviours as compared with pigs fed RSALG diets. These changes could be related to the numerically increased number of meals per day or to the increased daily feed intake that might have predisposed ALG-pigs to social constraints at the feeding station, and thereby increasing energy expenditure on physical activity.

Resistant starch

Although adding RS to the diet resulted in larger meals and longer inter-meal intervals, pigs fed diets with RS did not increase their DM intake to compensate for the dietary DE dilution associated with the exchange of starch for RS. Surprisingly, despite the lower DE intake, ADG and final BW were not reduced in pigs fed RS-containing diets. Contrary to pigs fed ALG-containing diets, pigs fed RS-containing diets enhanced efficiency in the use of DE. Although carcass growth was reduced, colon and total GIT empty weight were heavier in pigs fed diets with RS, reflecting increased stimulation of fermentation in the GIT (Bolhuis et al.,

2007). Moreover, it is also remarkable that the carcass gain:DE intake ratio was not affected by RS. The latter would imply a similar efficiency of utilization of fermentable starch for energy retention compared with that of enzymatically digestible starch, which is considerably greater than the 70% assumed for fermentable fibres in some NE systems (Jørgensen et al., 1996; Noblet and van Milgen, 2004), and the 83% recently reported by Gerrits et al. (2012) for fermentable corn starch in restrictedly-fed growing pigs.

The efficiency of utilization of fermentable fibres for energy retention in restrictedly-fed growing pigs has been reported to be close to that of enzymatically digestible starch in previous studies with sugar beet pulp (Schrama et al., 1996, 1998) and fermentable starch (Gerrits et al., 2012), but these values were to a large extent related to a reduction in physical activity caused by these fibres, in line with previous studies (Schrama and Bakker, 1999; Bolhuis et al., 2008). This reduction in physical activity in restrictedly-fed pigs, which likely reflects increased satiety (De Leeuw et al., 2008) was, however, not observed in the pigs fed the RS-containing diets ad libitum in the present study. The behavioural patterns of pigs fed ALG and RSALG diets differed the most, whereas the behaviours of pigs fed RS and CON diets were very similar. Thus, the reduction in physical activity caused by RS found in restrictedly-fed pigs is apparently not so strong in ad libitum-fed pigs (RS vs. CON), likely because pigs can consume feed when their feeding motivation increases, instead of becoming more active performing foraging behaviours.

The efficiency of utilization of RS for energy retention was not measured in the present study. It can, therefore, not be excluded that efficiency was indeed lower in pigs fed RS-diets. Nevertheless, the observed similar ADG, muscle thickness, and carcass gain:DE intake ratio suggest this was not the case. Furthermore, a difference in energy retention, as affected by a reduction in activity-related heat production in relation to RS, was expected to be reflected in fat retention (Gerrits et al., 2012). This was, however, not observed in pigs fed RS-containing diets, as fat deposition, measured by backfat thickness and weight of mesenteric tissue at slaughter, was not affected by RS. Also leptin levels, secreted proportionally to adiposity over long periods of time (see Woods et al., 1998 for review), were not significantly different between diets. An alternative explanation for the similar efficiency of utilization of RS vs. enzymatically digestible starch would be an increased availability of VFA, which prolongs energy supply to the body throughout the day. Previous findings in pigs restrictedly fed native potato starch, compared with digestible starch, revealed relatively small fluctuations in heat production and RQ within the 24 h cycle, and an increase in RQ particularly during the nightly hours before the morning meal, which likely relates to a general increase in the oxidation of absorbed VFA (Bolhuis et al., 2008; Gerrits et al.,

2012). This may have increased the *de novo* gluconeogenesis in pigs fed diets with RS, in line with increased fasting levels of glucose and insulin found in these animals. Thus, in spite of the reduced glucose supply from enzymatic digestion, RS provides a large amount of VFA to be oxidized in the postabsorptive phase (Haenen et al., 2013; Souza da Silva et al., 2013b). According to Zijlstra et al. (2012), absorbed VFA are metabolized in various cell types, including colonocytes, hepatocytes, and skeletal and cardiac muscle cells. A prolonged energy supply from VFA throughout the day may have reduced the mobilization of glucose from cell storages and likely lead to a more efficient mechanism for maintenance of blood glucose between meals in pigs fed RS-containing diets. This would be consistent with the enhanced efficiency in these pigs, although it is yet unclear whether and to what extent VFA may affect rates of glucose mobilization from cell storages in pigs.

Moreover, a reduced energy expenditure on immune responses may have partly contributed to the similar efficiency of RS-fed pigs when compared with the CON pigs. In the present study, white blood cell count tended to be lower in pigs fed diets with RS ($16.1 \pm 1.05\ 10^9$ cells/L) as compared with ALG ($18.2 \pm 0.78\ 10^9$ cells/L) and CON ($18.9 \pm 0.83\ 10^9$ cells/L) diets (P=0.09), with the RSALG diet in between ($17.8 \pm 1.14\ 10^9$ cells/L). This is in line with Haenen et al. (2013), who reported lowered immune-related gene expression in the colon of 58 kg growing pigs fed the same source and quantity of RS as in the present study, and Nofrarías et al. (2007), who found lower proliferation and production of anti-inflammatory cytokines in RS-fed pigs. Further, RS-containing diets tended to lower blood 5-HT levels as compared with diets without RS, which may be related to a reduced activity of the gastrointestinal immune system, as previously suggested (see Manocha and Khan, 2012 for review). Although it remains to be described, changes in microbiota composition observed in RS-fed pigs by Haenen et al. (2013) may indirectly play a role in immune regulation in the intestine (Wikoff et al., 2009; Collins et al., 2012).

Combination of alginate and resistant starch

In the present study, the combination treatment was hypothesized to have greatest satiety enhancing effects as compared to the other treatments. This appeared, however, not to be the case, as RS and ALG rarely interacted to affect the feeding pattern variables. In spite of that, some behaviours related to reduced satiety (see De Leeuw et al., 2008), including active behaviours such as standing and walking, aggressive behaviours, and oral behaviours, particularly feeder-directed and drinking, were generally reduced in RSALG-fed as compared with ALG-fed pigs. Particularly in week 12, there were indications that RS counteracted

the increased activity found in ALG-fed pigs. A similar change in behavioural patterns was observed in restrictedly-fed growing pigs when RS was added to their diet (Schrama and Bakker, 1999; Bolhuis et al., 2010), but in the present study RS was not able to change behavioural traits much compared to CON. Notably, in the present study, the effect of RS on behavioural patterns depended strongly on ALG. Thus, a combination of the viscous and gelling properties of ALG in the stomach, and the more gradual supply of energy from fermentation processes, as previously discussed for RS, seemed to better compensate for the increased activity in pigs fed ALG diets. Alternatively, a substantial reduction in physical activity may be observed only in response to high inclusion level of fibres in the diet.

Conclusions

In conclusion, dietary fibres did not reduce voluntary feed intake in ad libitum-fed growing pigs. Pigs fed ALG, a gelling fibre, compensated for the reduced DE content of their diet by increasing feed intake and, thereby, achieved similar DE intake and ADG as pigs fed CON. Backfat thickness and carcass efficiency were yet reduced by ALG. A combination of RS and ALG counteracted the increased activity caused by ALG. Pigs fed RS changed feeding patterns, but did not increase feed intake. Despite a reduction in DE intake, pigs fed RS achieved similar ADG as pigs fed CON suggesting a more efficient use of DE. Although carcass growth was reduced, colon and total GIT empty weight were increased in pigs fed RS, reflecting increased fermentation. A more gradual energy supply via the increased availability of VFA and reduced activation of the immune system may underlie the increased efficiency found in pigs fed RS, but require further study.

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Chapter 7

General discussion

The objective of this thesis was to identify to what extent and how dietary fibres with different physicochemical properties, such as bulkiness, viscosity, gelling, and fermentability, affect satiety in the domestic pig, used as a model for humans and as a target animal. The effects of different fibres on satiety and the potential underlying mechanisms were investigated in four studies. First, satiating properties of fibres with variable physicochemical properties were assessed in two behavioural studies. From these studies, resistant starch was selected based on its satiating properties, and used to assess the possible physiological and molecular mechanisms by which fermentation may affect satiety in a subsequent invasive study. In the last study, the long-term effects of two fibres, a gelling fibre potentially promoting satiation and a fermentable fibre promoting satiety, on feeding patterns and growth performance were assessed. In the present chapter, first the methodology that was used to assess satiety and satiation will be discussed. Secondly, an overview of the short- and long-term effects of dietary fibres on satiety and satiation will be given. This is followed by interpretation and discussion of the potential underlying mechanisms by which dietary fibres affect satiety and satiation. Thirdly, long-term effects of dietary fibres on energy intake and body weight development are discussed in the perspective of their application in animal and human nutrition. This chapter will end with an overview of the main conclusions.

Methodological considerations

Before discussing the main findings, it is important to reflect on the methodology that was used to measure satiety and satiation in this thesis. In this section, first an overview of the different measurements of satiety and satiation will be given. This is followed by a discussion of possible factors that may interfere with the potential of different measurements to assess satiety and satiation in animals.

Measurements of satiety and satiation

Although the research described in this thesis focused mainly on satiety, in Chapter 6 both satiety and satiation were studied. From a human nutrition perspective, satiation concerns the satisfaction of appetite that develops during the course of eating and leads to meal termination. Satiety is the feeling of fullness after a meal, which decreases in time and ultimately leads to initiation of a new meal (De Graaf et al., 2004). Satiety and satiation are subjective states that cannot be directly measured in animals (see D'Eath et al., 2009), but they are reflected by the individual's feeding motivation, i.e. the strength of the motivation to obtain food (Dawkins, 1990). Generally, feeding motivation is expected to decrease when

the feeling of satiety is increased (Dawkins, 1990; D'Eath et al., 2009). Feeding motivation tests combined with behavioural observations are therefore considered valuable tools in the assessment of satiety and satiation in animals (Day et al., 1997). In the studies presented in this thesis, these measurements were done at different time points after or around the meal to assess satiating properties of dietary fibres with different physicochemical properties. Table 1 gives an overview of the different measurements of satiety and satiation used.

Table 1. Different measurements of satiety and satiation.

Measurement	Variable measured	Interpretation of an increase in the variable measured	Time	Meaning[1]	Chapter
Feeding motivation tests					
Operant test	Number of turns	↑ feeding motivation	After test meal	Satiety	2, 3
Runway test	Speed (km/h)	↑ feeding motivation	After test meal	Satiety	2, 3
Ad libitum food intake test	Voluntary food intake (kg)	↑ feeding motivation	After test meal	Satiety	3, 4
			During test meal	Satiation	7
	Feeding rate (g/min)	↑ feeding motivation	During test meal	Satiation	7
	Duration of first bout (min)	↑ feeding motivation	After test meal	Satiety	3, 4
Behavioural observations					
	Physical activity (% of observation time)	↑ feeding motivation	After test meal	Satiety	2, 3, 4, 6, 7
	Aggression (% of observation time)	↑ feeding motivation	After test meal	Satiety	6
	Stereotypic chewing (% of observation time)	↑ feeding motivation	After test meal	Satiety	2, 3, 4, 6, 7
	Feeder-directed behaviour (% of observation time)	↑ feeding motivation	After test meal	Satiety	2, 3, 4, 6, 7
	Drinking (% of observation time)	↑ feeding motivation	After test meal	Satiety	4, 6
Feeding patterns					
	Food intake per meal (g)	↑ feeding motivation	During test meal	Satiation	6
	Meal duration (min)	↑ feeding motivation	During test meal	Satiation	6
	Inter-meal interval (min)	↓ feeding motivation	After test meal	Satiety	6
	Daily time feeding (min)	↑ feeding motivation	Over test meals	Satiety and Satiation	6
	Number of meals per day	↑ feeding motivation	Over test meals	Satiety and Satiation	6
	Cumulative food intake (kg)	↑ feeding motivation	Over test meals	Satiety and Satiation	6
	Daily food intake (kg)	↑ feeding motivation	Over test meals	Satiety and Satiation	6

[1] For the measurement of satiation it is necessary to provide animals with the test food of interest (i.e. dietary treatment), whereas this is not necessary (or preferred) for the estimation of satiety.

1

Feeding motivation was measured using (1) feeding motivation tests, including an operant test and a runway test, where food reward(s) could be obtained by turning a wheel and running a track, respectively, and an ad libitum food intake test, where an unlimited amount of food reward (i.e. commercial diet or mix of experimental diets) or test food (i.e. dietary treatment) was offered for a limited amount of time (60 min). In these tests the cumulative number of turns (operant test), the average speed in km/h (runway test), the total voluntary food intake, the feeding rate, and the duration of the first bout of food consumption (ad libitum food intake test) were used as indicators of feeding motivation. All of these

variables are expected to increase as feeding motivation increases. Increased feeding motivation measured after a test meal is expected to reflect reduced satiety, whereas increased feeding motivation measured during a test meal in which the dietary treatment is offered is expected to reflect delayed satiation. Thus, for most studies in this thesis, the operant test (Chapters 2 and 3), the runway test (Chapters 2 and 3), and the ad libitum food intake test (Chapters 3 and 4) were used for the measurement of satiety, whereas in a short-term pilot study (see next section), the ad libitum food intake test was also used for the measurement of satiation, for which animals needed to be fed their actual dietary treatments. In addition, (2) behavioural observations of individual pigs, particularly of behaviours related to foraging and feeding, as for instance postures related to general physical activity and feeder-directed behaviours (De Leeuw et al., 2008; D'Eath et al., 2009), were used as an extra indicator of feeding motivation. Also stereotypic behaviours, such as chewing air or biting pen fixtures (bar biting), were studied. These stereotypies, shown repetitively, invariably, and without an obvious goal or function (Lawrence and Terlouw, 1993) are thought to be redirected foraging behaviours that reflect an unsatisfied feeding motivation (D'Eath et al., 2009). Over the day, mostly after test meals, pigs' postures and behaviours were scored from live observations or video recordings. In general, the occurrence of foraging behaviours, like nosing (sniffing or touching), rooting (forceful snout movements) and scraping the ground or an object, stereotypic behaviours, and physical activity is expected to increase as feeding motivation increases (De Leeuw et al., 2008). Physical activity (which is part of appetitive foraging) and foraging behaviours may be shown just after a meal until several hours later in anticipation to the next meal, whereas stereotypies appear to be mainly elicited by a meal (Terlouw et al., 1993). This could mean that stereotypies may also reflect delayed satiation. In spite of that, the stereotypies described in this thesis are related to satiety, as changes in the occurrence of stereotypies were mostly detected throughout the day (Chapter 2) or in anticipation to a next meal (Chapter 3). Drinking behaviour could also represent redirected foraging behaviour, as both excessive drinking and water spillage have been reported in restrictedly-fed sows (D'Eath et al., 2009), and therefore may also reflect increased feeding motivation. Moreover, individual animals with an unsatisfied feeding motivation may act out on pen mates, for instance via aggressive and manipulative oral behaviours directed towards pen mates, either because of increased activity levels or due to frustration (Bolhuis et al., 2010). Thus, the occurrence of aggression and manipulation of pen mates is also expected to increase as feeding motivation increases, which may reflect reduced satiety. Finally, (3) feeding patterns were used as measures of satiety and satiation in pigs with free access to food over a long period of time, to assess whether satiating properties of the fibres originated from studies described in Chapters 2 to 4

conducted in restrictedly-fed pigs would lead to a reduced energy intake and body weight gain in ad libitum-fed pigs in Chapter 6. Briefly, from all visits to an electronic feeding station, the food intake per meal, meal duration, inter-meal interval, daily time feeding, number of meals per day, and daily and cumulative food intake were calculated. From these variables, satiation is mainly reflected by the food intake per meal and meal duration, as meal termination normally depends on short-term, meal-generated signals (see Chapter 1). Satiety is mainly reflected by the duration of the intervals between meals (Blundell et al., 1996), as the longer the intervals, the longer the animal did not experience hunger. Longer intervals could also lead to fewer meals per day, which may reflect enhanced satiety. Overall, total food intake, time spent feeding or number of meals per day is expected to be a result of both satiety and satiation.

Factors affecting sensitivity of the measurements

Although there might be a clear picture regarding the satiating properties of fibres given all measurements together (see Chapters 2 and 3), there are also some discrepancies in the results of different measurements of satiety suggesting that the discriminating power of the measurements may be sometimes reduced due to for instance experimental conditions and animal inherent characteristics.

According to previous studies on the effect of food restriction on hunger and feeding motivation in adult (Lawrence et al., 1988) and growing male pigs (Lawrence and Illius, 1989), restricting pigs to low feeding levels results in quite high levels of feeding motivation (hunger) throughout the day, so that a maximum and relatively constant level of responding is found in the feeding motivation tests. In these studies, responses in an operant test were found to increase with food restriction, but only to a point, as further restriction below 60% of ad libitum intake produced no further increase in responding (Lawrence et al., 1988). This means that minor differences between dietary treatments may become difficult to detect when pigs have a maximum level of feeding motivation. For example, in Chapter 2, where pigs were fed at 1.5 times the energy requirements for maintenance, the results from the runway and operant tests were highly correlated ($r=0.47$, $P < 0.001$), whereas in Chapter 3, where pigs were fed at 1.2 times maintenance, results did not correlate ($r=-0.05$, $P=0.54$). Moreover, levels of stereotypic chewing were higher in Chapter 3 than in Chapter 2, and the voluntary food intake during the ad libitum food intake test (not conducted in Chapter 2) corresponded to 133% of the daily food allowance of pigs in Chapter 3. This might indicate that the lower feeding level used in Chapter 3, although sufficient for maintenance and some growth, resulted in relatively high levels of feeding motivation, which may have overruled some of the treatment effects in the feeding motivation tests in Chapter 3.

Basically, the feeding levels applied in Chapters 2 and 3 were selected to ensure that pigs would consume all the food provided per meal, so that the interpretation of the results would not be complicated by the occurrence of food refusals, which can occur when feeding animals with such high levels of fibres in the diet. In addition, a similar feeding level was used for all pigs to avoid huge inter-individual variation in body weight gain during the studies, which would also complicate the interpretation of the results. In future studies using feeding motivation tests as measurements of satiety, it would be valuable to apply higher feeding levels (closer to ad libitum), as in Chapters 2 and 3 pigs were fed 2.3 kg (initial body weight: 185 – 216 kg) and 2.1 kg (initial body weight: 226 – 272 kg) of food per day (as-fed basis) throughout the studies, respectively, whereas other authors have found average voluntary food intakes of 4.2 kg of food per day (as-fed basis) in sows fed ad libitum over 3 reproduction cycles (initial body weight: 178 – 232 kg), which remained stable in subsequent parities (van der Peet-Schwering et al., 2004).

Also, the way that pigs respond to feeding motivation tests may not be only related to short-term effects of the previous meal, but it may reflect long-term catching-up effects (Morgan et al., 1995), as voluntary food intake has been shown to be even higher than 4.2 kg of food per day in multiparous sows fed ad libitum after long periods of food restriction. In a short-term pilot study (Souza da Silva et al., 2010, unpublished results) old sows (average age: 3.5 years) that were previously food restricted for at least 4 reproduction cycles, as is common practice in pig husbandry, were given free access to food in ad libitum food intake tests conducted to measure their voluntary food intake. In this study 10 sows (parity 4 – 7) were fed a standard commercial diet (per kg: crude protein, 135 g; crude fat, 49 g; crude fibre, 79 g) ad libitum for 5 consecutive days for 60 min twice per day. Initially, the sows received a large amount of food (approximately 6 kg). When almost all food (circa 75%) was consumed some extra food was provided, so that the feeder would never be empty. After 60 min the food left in the feeder was removed and weighed. The average voluntary food intake ranged from 1.7 to 10.5 kg (mean: 6.3 ± 0.2 kg) of food per day (Table 2). This indicates that pigs submitted to food restriction for long periods of time consume excessive amounts of food during ad libitum food intake tests, even beyond (see range of voluntary food intakes in Table 2) a normal voluntary food intake of around 7.2 kg of food per day (> 3 times maintenance) when fed conventional diets (see Bergeron et al., 2000). This likely reflects an attempt to compensate for a previously constrained food intake, and consequently, to meet specific nutrient requirements needed for achieving their high genetic potential for lean growth. This is in line with changes in body weights found from the start (244 ± 17 kg) to the end (312 ± 30 kg) of the present pilot study (duration: 10 weeks), with some animals (n=8) achieving > 400 kg body weight in a subsequent pilot study. Thus, food intake of pigs in ad libitum

food intake tests may, apart from short-term satiety, also reflect the long-term feeding regime and the metabolic state of the animal resulting from this regime.

Table 2. Voluntary food intake of sows (initial body weight: 244 ± 17 kg, age: 3.5 years) fed an ad libitum meal of a standard commercial diet for 5 consecutive days during 60 min in the morning (7:30 h – 8:30 h) and 60 min in the afternoon (16:00 h – 17:00 h). Results are expressed as means ± SEM (range), n=10. Adapted from Souza da Silva and co-workers (2010), unpublished results.

Sow	Initial BW[1] (kg)	Voluntary food intake (kg/day)	Voluntary food intake (g/kg BW)
388	243.1	7.25 ± 0.46 (6.11 – 8.76)	29.8
355	216.3	5.78 ± 0.12 (5.39 – 6.11)	26.7
181	233.5	5.79 ± 0.56 (4.30 – 7.30)	24.8
325	242.9	6.11 ± 0.44 (4.67 – 6.92)	25.2
120	270.3	6.30 ± 0.37 (5.30 – 7.14)	23.3
150	271.0	7.06 ± 0.88 (5.73 – 10.50)	26.1
332	239.9	6.41 ± 1.29 (1.72 – 9.51)	26.7
327	232.3	6.26 ± 1.00 (3.83 – 9.03)	26.9
259	237.7	6.60 ± 0.51 (4.61 – 7.42)	27.8
380	251.5	5.67 ± 0.77 (4.00 – 8.11)	22.6
average	243.9	6.32 ± 0.17	26.0

[1] BW=body weight

The feeding levels used in Chapters 2 and 3 might not have been very close to the voluntary food intake level commonly reported for sows (Brouns et al., 1995; van der Peet-Schwering et al., 2004). Observations during the ad libitum food intake test in Chapter 3 suggest that amounts of food consumed were still slightly higher (2.8 kg of food per meal) than the expected 4.2 kg per day voluntary food intake reported by for instance van der Peet-Schwering and co-workers (2004). However, in contrast with the pilot study presented in Table 2, where sows that were food restricted for at least 4 reproduction cycles would consume up to 10 kg of food per day, and show acute vomiting between meals, an abnormal drive to consume more food or overconsumption was not observed in the younger gilts that were food restricted over a 8-week period in Chapter 3. This could mean that in spite of the food restriction, pigs' responses to feeding motivation tests in Chapters 2 and particularly in Chapter 3 were likely more related to short-term effects of the previous meal than to long-term catching-up effects, but the latter should be always considered for the interpretation of feeding motivation tests in future studies.

Another aspect that should be considered while interpreting feeding motivation tests concerns the differences between the tests used. As explained in Chapter 2, in both the operant and runway tests pigs had to pay a price for obtaining food. It is generally stated that the imposed cost should be in line with the natural foraging behaviour of the animal under study (Verbeek et al., 2011), which is true for both the snout movements to turn the wheel and for the walking requested in the operant and runway tests, respectively. Thus, the imposed costs were both related to

appetitive behaviours, but the impact they had on the willingness to work for food might have been different in the tests used.

In the wild, pigs can spend up to 70% of their daily activity budget foraging (Young and Lawrence, 2003), during which they continuously have to make decisions on where and how to forage (Verbeek et al., 2011). According to the optimal foraging theory, animals are expected to maximize the ratio of benefit to cost (Inglis et al., 1997). Particularly in the operant test, animals could attempt to maximize this ratio during the course of the test. However, by using a progressive ratio (PR) schedule only highly motivated animals would maintain food consumption with an increasing cost of wheel turning, but eventually had to stop when the maximum price paid or break-point was reached (Verbeek et al., 2011). Thus, during the operant test, animals could obtain multiple rewards, i.e. the benefits were different from the runway, but the costs increased during the course of the test due to the PR schedule chosen. This means the trade-off between the benefits of food consumption and the costs of obtaining food was different for the operant test as compared with the runway test. In addition, the runway is a short test in which only the appetitive response of pigs, as reflected in their speed, was measured, whereas in the operant test food incentive properties might have played a bigger role as the appetitive responses of pigs were alternated with consummatory phases (Barbano and Cador, 2005). Each operant session ended when pigs had paid their maximum price, and this break-point varied largely and consistently between individuals, partly irrespective of their diet and irrespective of the fact that they were all of the same breed, sex, origin (farm), age, and weight at the start of the study. Despite the large individual variation in operant responding, and despite the differences in both tests used, responses in the operant and runway tests correlated well (at least in Chapter 2), suggesting that they, at least partly, reflect the same, i.e. feeding motivation.

Responses of animals in feeding motivation tests can, apart from being affected by metabolic processes and level of satiety, also be influenced by factors like the appreciation of the food provided during the tests (Day et al., 1996; Saper et al., 2002; Barbano and Cador, 2005). In Chapter 2, a commercial pelleted food was used as a reward in the operant and runway tests, because using the experimental, mash (i.e. finely ground solid food), diets would have led to technical (e.g. electronic operant feeder malfunction) and methodological (e.g. difficulty to compare treatments, see D'Eath et al., 2009) problems. The commercial pelleted food used, however, might have been preferred by the pigs over their experimental diets, and a contrast in perceived food palatability or quality may have led to high levels of responding irrespective of metabolic processes and level of satiety (Ramonet et al., 2000; Saper et al., 2002). Therefore, in Chapter 3 a different food reward (i.e. a mixture of low fibre diets) was used to minimize the contrast in

perceived palatability between the experimental diets and food reward, and thereby to avoid as much as possible that using a different food reward from the experimental diet would lead to increased levels of responding, particularly in the operant test. In spite of that, the relative contrast in energy content between the high fibre diets and the food reward might have influenced responses of pigs in the operant test, as suggested by the results given in Chapter 3. Thus, it seems that differences in responses due to differences in perceived food palatability or quality is something inevitable in feeding motivation tests where different food than the experimental diet is used as reward. Alternatively, the pigs' usual experimental diets could be used in future studies, yet it has been suggested that this should be avoided, because when the animal receives its experimental diet, it becomes then difficult to define what is 'equivalent responding' when comparing treatments (D'Eath et al., 2009).

Regarding animal inherent characteristics, pigs are patch feeders, alternating appetitive and consummatory bouts of foraging and feeding. There are indications that pigs are particularly thwarted in the expression of appetitive behaviours (food searching) under standard housing conditions, and some pigs seem to be willing to work for food even when free food is available, i.e. contrafreeloading (de Jonge et al., 2008; but see Young and Lawrence, 2003). This may also have affected responses of pigs in the operant test, as it has been suggested that the execution of appetitive foraging behaviours is not only driven by metabolic processes, but is also rewarding in itself (Inglis et al., 1997), i.e. pigs have an inherent motivation to forage (Scott et al., 2007). It is difficult to indicate to what extent feeding motivation tests reveal pigs' motivation for food consumption rather than for showing appetitive (food searching) behaviours, and there are also individual differences in this, such as individual coping or personality characteristics of pigs (Bolhuis, 2004). Individual differences (e.g. regarding personality) in response to experimental conditions are actually inevitable in behavioural research. Therefore, in Chapters 2 and 3 pigs were assigned to all dietary treatments in a Latin square design. In this way, the animals served as their own controls, which increased power by better control of variation in individual response. Also, behaviours may reflect motivation for other resources or may indicate frustrations, other than motivation for food consumption. For example, the occurrence of stereotypies (e.g. oral abnormal behaviours) may reflect hunger and frustration of feeding motivation (Meunier-Salaun et al., 2001) due to food restriction in pregnant sows, but may also indicate frustration in the expression of natural foraging behaviours, as in intensive husbandry pigs are mostly kept in barren environments (De Leeuw et al., 2008). The occurrence of stereotypies is also generally higher in sows kept under individual housing than under group housing conditions (Arellano et al., 1992). The time spent on stereotypic behaviour may be also highly variable between

individuals and dependent on their personality (Geverink et al., 2002, 2003; Ijichi et al., 2013). Moreover, when sows are fed for a long time restrictedly it seems that time spent on stereotypies increases and they become even more invariable and difficult to reduce (Rushen, 1984; Terlouw and Lawrence, 1993). Therefore, feeding motivation tests were combined with behavioural observations in Chapters 2 and 3, and these reliably reflected, for the greatest part, the satiating properties of the different fibres described.

Table 3. Voluntary food intake, feeding rate, chewing rate, and meal duration of sows (initial body weight: 312 ± 30 kg, age: 3.7 years) fed an ad libitum meal of diets differing in dietary fibre type: cellulose[1] (bulking), guar gum[2] (viscous), alginate[3] (gelling), each at different inclusion levels, and a high fat-low fibre control, at 1 h after a pre-load of control diet (50% of voluntary food intake measured at start of the experiment). Results are expressed as means ± SEM, n=8, except for diets 1 and 3, where n=7. Adapted from van Merwijk, Souza da Silva, and van den Borne (2009), unpublished results.

Diet	Fibre	Inclusion level (%)	Voluntary food intake (g DM)	Feeding rate (g DM/min)	Chewing rate (#/30 s)	Meal duration (min)
1	Cellulose	10.0	1621 ± 247 ^{de}	128 ± 16 ^{abc}	57.8 ± 2.0 ^{bcd}	15.7 ± 2.4
2	Cellulose	5.0	1751 ± 306 ^{def}	153 ± 34 ^{bc}	59.3 ± 2.1 ^{cd}	16.3 ± 3.9
3	Cellulose	2.5	2162 ± 292 ^{def}	179 ± 33 ^{cd}	60.7 ± 1.8 ^{d}	16.6 ± 4.0
4	Guar gum	5.0	791 ± 304 ^{a}	91 ± 26 ^{a}	52.4 ± 2.2 ^{ab}	10.6 ± 2.0
5	Guar gum	2.5	1653 ± 328 ^{bd}	125 ± 20 ^{ab}	54.6 ± 1.9 ^{abc}	15.6 ± 3.4
6	Alginate	5.0	1207 ± 282 ^{abc}	112 ± 29 ^{ab}	51.8 ± 1.8 ^{a}	14.5 ± 2.4
7	Alginate	2.5	1821 ± 341 ^{cdef}	148 ± 18 ^{ab}	54.6 ± 1.8 ^{abc}	14.5 ± 2.1
8	Control	-	2747 ± 362 ^{f}	240 ± 21 ^{d}	60.2 ± 1.9 ^{d}	13.5 ± 2.6
			P-value	*P*-value	*P*-value	*P*-value
	Fibre (F)		0.001	0.001	0.001	0.575
	Inclusion (I)		0.537	0.019	0.531	0.554
	F × I		0.754	0.990	0.491	0.471

[1] Vitacel L 00 (J. Rettenmaier & Söhne, Germany).
[2] Viscogum MP 41230™ (Cargill, Belgium).
[3] Protanal LF 5/60 (FMC BioPolymer, Norway).
^{abcdef} Means lacking a common superscript letter within columns indicate a significant ($P < 0.05$) difference.

Short- and long-term effects of dietary fibres on satiety and satiation

In this thesis, feeding motivation and behaviour of restrictedly-fed pigs were assessed at different times of the day (short-term), and feeding patterns of ad libitum-fed pigs were assessed over 12 weeks (long-term), to evaluate the satiating properties of dietary fibres with variable physicochemical properties, which were expected to affect satiety and satiation in different ways, because satiety signals may originate from various sites along the gastrointestinal tract (GIT). The effects of dietary fibres on satiety and satiation found in adult and growing pigs in this thesis are summarized in Tables 6a and 6b. In addition, the effects of similar dietary fibres as the ones used in this thesis on satiety and satiation were investigated in adult humans, these are summarized in Table 7 (Wanders, 2013).

Alginate (gelling)

Alginate is a viscous fibre that can form gels when in contact with calcium and under acidic conditions (pH < 4) in the stomach (Georg Jensen et al., 2013). Viscous fibres without gelling effect remain one solution in the stomach; whereas viscous fibres with gelling effect form lumps (Fig. 1) in the stomach (Hoad et al., 2004). In a short-term pilot study (van Merwijk, Souza da Silva, and van den Borne, 2009, unpublished results), where sows that were previously food restricted for at least 4 reproduction cycles were given free access to food in ad libitum food intake tests, a gelling fibre seemed to impact satiation more than bulking and viscous fibres, as suggested by measures of voluntary food intake, feeding rate, and chewing rate (Table 6a). Compared with a high fat-low fibre control diet, diets containing 5% alginate (gelling fibre) reduced voluntary food intake by 56% (Table 3). In addition, diets containing 2.5% and 5% alginate reduced the feeding rate (g of DM/min) and the chewing rate (number of chews/30 seconds), while the meal duration in the ad libitum food intake test was not affected (Table 3). From these results, it was hypothesized that gelling fibres result in earlier satiation. This effect might be related to an increased oral exposure time, as shown in humans (Wanders et al., 2012, Table 7), in combination with a reduced rate of gastric emptying, as indicated in restrictedly-fed sows by a lower ^{13}C enrichment in expired CO_2 (originated from ^{13}C-octanoate in the food) up to 3 h after consumption of a 5% alginate diet compared with a high fat-low fibre control diet (Fig. 2). Based on the combination of these results, in Chapter 6 the gelling properties of alginate were expected to yield most pronounced effects on satiation. In the long-term, diets containing 5% alginate led to a numerical decrease in food intake per meal, meal duration and inter-meal intervals, which likely led to a significant increase in the number of meals per hour throughout the day in a repeated model. This is in line with the expected effects of alginate on satiation, likely due to satiation signals originating mostly from the mouth and the stomach. Nevertheless, pigs fed alginate diets increased their daily and cumulative food intake (Table 6a), which indicates a compensatory mechanism (Cole et al., 1968) to overcome the reduction in dietary energy content associated with the exchange of starch for alginate. Moreover, under free-feeding conditions satiety and satiation may interact to affect total energy intake over the day (Mattes, 2007) (see *Long-term effects of dietary fibres on energy intake and body weight development* for further details). This means that earlier satiation observed in pigs fed alginate diets may have coincided with reduced duration of satiety, as in Chapter 6 alginate also decreased inter-meal intervals and increased the number of meals per day.

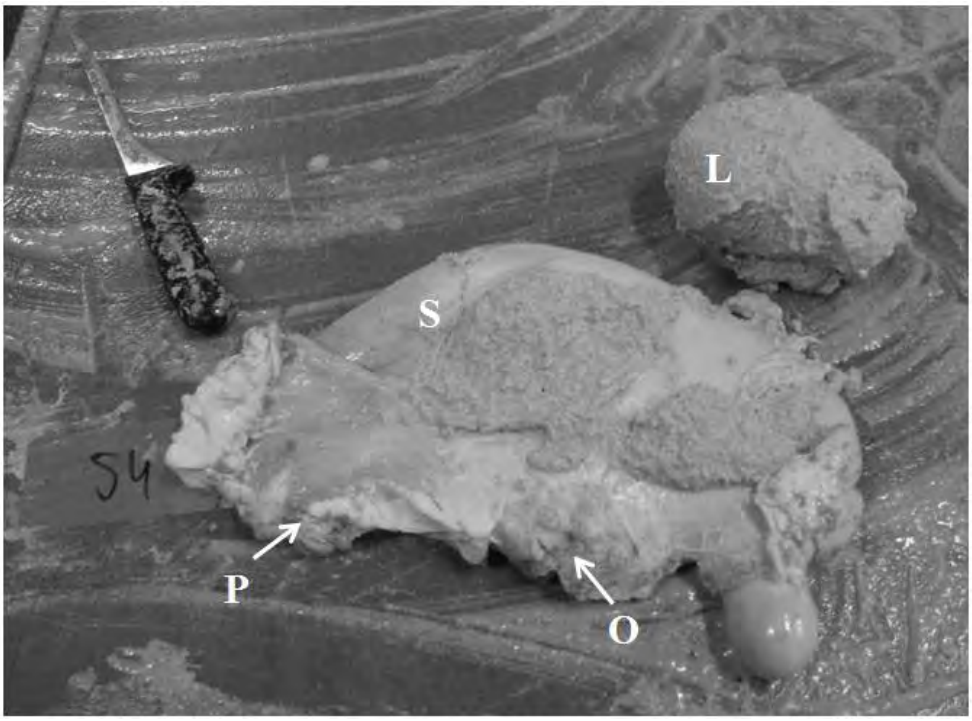

Fig. 1. Photograph of a lump removed from the stomach of a pig fed a diet containing both a gelling fibre (5% alginate) and a fermentable fibre (34% resistant starch) ad libitum during 12 weeks (Chapter 6). (S) Stomach cut open along its body, (O) Oesophageal region, (P) Pyloric region, (L) large lump of gastric content. Photograph taken by Anne Burgers.

Pectin (viscous)

Viscous fibres, such as pectin, were expected to affect short- to mid-term satiety by reducing gastric emptying, increasing gastric distension and thereby reducing intestinal passage rate, which is thought to prolong the time for enzymatic digestion and to improve digestibility (Hooda et al., 2011), affecting the release of intestinal satiety-related hormones such as cholecystokinin (CCK), peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1). Therefore, in Chapter 2 the viscous properties of pectin (Unipectine™ RS 150 Citrus, high methoxyl (HM) pectin from citrus origin, degree of esterification (DE)=70–74%, manufactured by Cargill, Belgium) were expected to promote satiety feelings from 3 h up to 7 h after the meal, but this was not observed. In Chapter 2, pectin appeared to be the least satiating fibre over the entire day, as suggested by the higher number of wheel turns in the operant test, the increased speed to reach a food reward in the runway test, and the higher proportion of time spent on feeder-directed behaviour, stereotypic behaviour and manipulation of pen mates (Table 6a).

These results obtained with pectin may be surprising, because previous studies with pectin-rich by-products, such as sugar beet pulp, have indicated satiety enhancing effects of pectin in pigs (see De Leeuw et al., 2008 for review). In Chapter 2, it was concluded that the satiating capacity of pectin was not sufficient to compensate for an estimated 8-11% reduction in metabolizable energy (ME) intake. Moreover, a low satiating capacity of pectin could be attributed to the physical form of the diet (i.e. mash), as liquids with added viscous fibre could be more satiating than solid ones, at least in humans (Lyly et al., 2009). Furthermore, despite the fact that human microbiota could degrade pectin more than pig microbiota, fermentability of citrus pectin is generally considered to be high (Jonathan et al., 2012). Nevertheless, the level of colonic fermentation of pectin was apparently not sufficient to compensate for the lower ME intake compared with the control diet in Chapter 2. This was further confirmed by unaffected pre- and post-prandial concentrations of short-chain fatty acids (SCFA) in portal blood of minipigs fed the same source and quantity (149 g/kg) of pectin as in the study presented in Chapter 2 (Haenen et al., 2013a, unpublished results, Table 4). Also in humans, Wanders and co-workers (2013b) showed that an increase in breath hydrogen excretion (suggesting an increase in colonic fermentation, see Topping and Clifton, 2001) did not persist over time after sustained consumption (16 days) of a gelling pectin (Classic CU 901, low methoxyl (LM) pectin from citrus origin, DE=10%, manufactured by Herbstreith & Fox, Germany), expected to be quickly fermented by human microbiota (Gulfi et al., 2006). This is in line with unaffected fasting concentrations of SCFA found in peripheral blood (Wanders et al., 2013b). In humans it was suggested that adaptation of microbial metabolism towards efficient fermentation pathways not producing hydrogen or SCFA may have occurred (Wanders et al., 2013b). In pigs it could be that a low rate of fermentation is a result of a small pectin dosage (75 – 149 g/kg) used in Chapter 2, whereas in the case of the minipigs, it could be an indirect result of a reduced voluntary food intake of the pectin diet (Table 4). The lower voluntary food intake of the pectin diet as compared with other fibre diets suggests that viscous pectin may have led to earlier satiation, rather than prolonged satiety. This aspect will be further discussed in the section on potential underlying mechanisms.

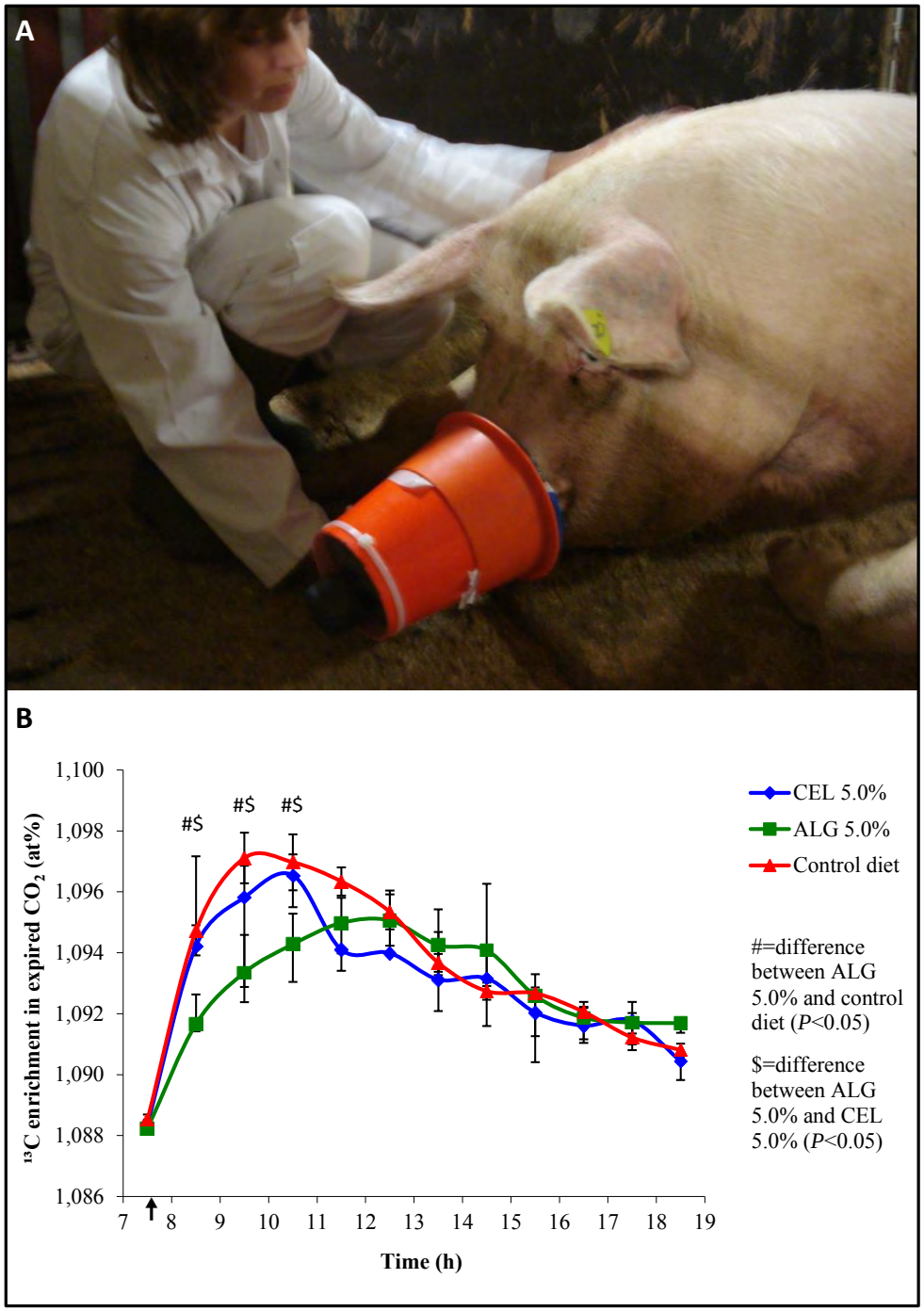

Fig. 2. Panel A gives an impression of the collection of breath samples into a plastic bag (11 x 31 cm, not shown) via a tailor-made cone-shaped mask (20 cm diameter), which was snugly fitted around the sow's snout, and allowed her to breath normally during 1-2 minutes until ~75% of the bag was filled with exhaled air. From the bag, breath samples were transferred into a 10 mL Exetainer tube (Labco

Ltd, United Kingdom) via a drinking straw, and then the tube was closed with a cap. Samples were stored at room temperature and were analysed for ^{13}C enrichment in expired CO_2 on a Finnigan Delta C continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Germany). Data obtained from the mass spectrometer were expressed as atom% $^{13}CO_2$. Photograph taken by Carol Souza da Silva. Panel B gives the gastric emptying, measured in ^{13}C enrichment in expired CO_2 (at%, originated from ^{13}C-octanoate in the diet) of sows (initial body weight: 312 ± 30 kg, age: 3.7 years) fed a restricted morning meal (indicated by arrow) of diets differing in dietary fibre type: cellulose[1] (CEL, bulking), alginate[2] (ALG, gelling), each at 5% inclusion level, and a high fat-low fibre control. Arrow indicates feeding time at 7:45 h. Results are expressed as means ± SEM, n=7. Adapted from van Merwijk, Souza da Silva, and van den Borne (2009), unpublished results. [1]Vitacel L 00 (J. Rettenmaier & Söhne, Germany), [2]Protanal LF 5/60 (FMC BioPolymer, Norway).

It should be noted that pectin is a good example of a fibre with varying physicochemical properties, such as viscosity, gelling and fermentability, depending on the type of pectin. This may also underlie the discrepancy in the results obtained in this thesis and in the literature. Recently the effects of different physicochemical properties of pectin on satiety (and possible underlying mechanisms) were investigated in humans (Wanders et al., 2013a, Table 7). The different types of pectin were: non-viscous/non-gelling pectin (called bulking) (Herbapekt SF 50-A-LV, HM pectin from apple origin, DE=62%); viscous pectin (Classic AU 201 USP, HM pectin from apple origin, DE=72%); gelling pectin (Classic CU 901, LM pectin from citrus origin, DE=10%, all manufactured by Herbstreith & Fox, Germany); and no pectin (control), which were all provided added to a liquid dairy-based product. Satiety was enhanced after gelling pectin compared to bulking, viscous, and no pectin (Table 7). Moreover, gastric emptying rate was delayed after gelling pectin compared to no pectin, and associations with metabolic responses (glucose and insulin) were inconsistent (Table 7). According to the authors of this study, by selecting only pectins, a reasonable comparison between different physicochemical properties could be made. Overall, results suggest that gelling fibres are more satiating than viscous and bulking fibres, likely due to the reduced rate of gastric emptying, in line with previous findings in sows by van Merwijk, Souza da Silva, and van den Borne (2009, unpublished results).

Lignocellulose (bulking)

Bulking fibres with a high water-binding capacity, such as lignocellulose that can expand its original weight up to 8-fold in the stomach (Büttner, 2006), were expected to yield the most pronounced effects on short-term satiety due to a reduced rate of gastric emptying and a concurrently increase in gastric distension, which will increase satiety feelings (De Graaf et al., 2004; Benelam, 2009) via afferent vagal signals of fullness (Howarth et al., 2001). Therefore, in Chapter 2 the bulking properties of lignocellulose were expected to promote early satiety feelings

from 1 h up to 3 h after the meal. This was, however, not the case. In Chapter 2, lignocellulose seemed to enhance satiety for more than 3 h after the meal, as suggested by the lower number of wheel turns in the operant test for pigs fed the diet with a low level of lignocellulose (Table 6a). Nevertheless, results from the runway test were contradictory. A long-term satiating effect of lignocellulose within the day, if present, could not be attributed to fermentation, as data from an in vitro fermentation study showed that lignocellulose is not fermented by inoculum from adult pigs (Jonathan et al., 2012). This is in line with lowered plasma SCFA levels in portal blood of minipigs fed the same source (Arbocel® RC Fine, manufactured by Rettenmaier & Söhne, Germany) and quantity (100 g/kg) of lignocellulose as in the study presented in Chapter 2 (Haenen et al., 2013a, unpublished results, Table 4). Although it remains to be elucidated, the satiating effects of lignocellulose could be attributed to increased luminal bulk in the small intestine and colon. This aspect will be briefly discussed in the section on potential underlying mechanisms.

Table 4. Mean glucose and SCFA concentrations in portal blood collected before (-30 and 0 min) and after (30 to 480 min) feeding adult female Göttingen minipigs (initial body weight: 44 ± 1.5 kg, age: 2 years) an ad libitum morning meal of four experimental diets (control, lignocellulose, resistant starch and pectin) in a Latin square design. Results are expressed as means ± SEM, n=5. Adapted from Haenen and co-workers (2013a), unpublished results.

	Diet				*P*-value
Parameter	Control no fibre	Lignocellulose[4] 100 g/kg	Resistant starch[5] 394 g/kg	Pectin[6] 149 g/kg	Diet
Pre-prandial[1] glucose (mmol/L)	4.65 ± 0.56	5.19 ± 0.58	4.49 ± 0.41	4.14 ± 0.27	0.353
Post-prandial[2] glucose (mmol/L)	7.63 ± 0.64	7.34 ± 0.39	5.40 ± 0.32	5.37 ± 0.23	0.062
Pre-prandial SCFA (mmol/L)	0.82 ± 0.10	0.79 ± 0.12	1.57 ± 0.17	1.42 ± 0.33	0.128
Post-prandial SCFA (mmol/L)	0.70± 0.06	0.69 ± 0.08	1.98 ± 0.23	1.06± 0.11	0.004
Voluntary food intake[3] (g/meal)	732 ± 180	995 ± 210	1394 ± 262	503 ± 106	0.033

[1] Pre-prandial concentrations correspond to the average of the samples collected before the meal (-30 and 0 min).
[2] Post-prandial concentrations correspond to the average of the samples collected after the meal (30 to 480 min).
[3] Pigs were fed an ad libitum meal at 7:00 h and 15:00 h; the voluntary food intake given per meal corresponds to the morning meal.
[4] Arbocel® RC Fine (J. Rettenmaier & Söhne, Germany).
[5] Native potato starch (Avebe Food, The Netherlands).
[6] Unipectine™ RS 150 Citrus (Cargill, Belgium).

Resistant starch (fermentable)

Dietary fibres that are fermented in the distal intestine, resulting in increased production of SCFA, were expected to yield the most pronounced effects on satiety due to a more gradual and prolonged energy supply to the body during the day (Regmi et al., 2011), or to SCFA-induced specific stimulation of GLP-1 and PYY secretion (Zhou et al., 2008; Keenan et al., 2012). Therefore, in Chapters 2 and 3

the fermentation properties of resistant starch were expected to promote prolonged satiety feelings for more than 3 h after the meal. In the short-term (Table 6a), resistant starch was, indeed, more satiating than other types of dietary fibres with different physicochemical or fermentation properties up to 7 h after the meal, as indicated by the lower speed to reach a food reward in the runway test (Chapters 2 and 3), the lower voluntary food intake (Chapter 3) and shorter duration of the first bout of food consumption in the ad libitum food intake test (Chapter 3 and 4), the lower proportion of time spent on spontaneous physical activity and stereotypic behaviour in the hours before the afternoon meal (Chapter 3), and the lower proportion of time spent on feeder-directed behaviour (Chapters 2 and 4) and drinking behaviour (which may reflect redirected foraging behaviour, see D'Eath et al., 2009) over the entire day (Chapter 4). Moreover, in the long-term study (Table 6a), resistant starch increased inter-meal intervals, and thereby reduced the number of meals per day in ad libitum-fed pigs (Chapter 6). These findings further confirm the potential role of resistant starch to prolong satiety, despite the fact that under free-feeding conditions satiety and satiation may interact to affect total energy intake over the day (Mattes, 2007) (see *Long-term effects of dietary fibres on energy intake and body weight development* for further details). This means that prolonged duration of satiety observed in pigs fed resistant starch diets may have coincided with delayed satiation, as in Chapter 6 resistant starch also increased food intake per meal and meal duration.

Results from the study in Chapter 2 showed that not all fibres influence satiety equally, particularly resistant starch seemed to affect satiety more than lignocellulose (bulking) and pectin (viscous) (see Table 6a). Moreover, in Chapter 3 there were indications that satiety-enhancing effects of fermentable fibres were more dependent on fibre level than on fibre type, as increasing levels of fermentable fibres in the diet enhanced satiety in restrictedly-fed pigs, despite a reduction in calculated ME supply compared with the control diet. This could mean that fermentable fibres (resistant starch, inulin and guar gum) may have affected satiety in a more similar way than other types of fibres (with physicochemical properties different from fermentability) do. The contribution of fermentable fibres to ME intake was not measured in Chapter 3, but considering that they are almost completely fermented, the contribution of fermentable fibre to ME intake, and likely to satiety, would be constant and independent of fibre type; therefore the impact of the exchange with starch may have been proportional to the fibre inclusion level in the diet. Moreover, resistant starch was consistently most satiating in Chapters 2 and 3, likely because among all fibres tested, it was the only fibre that was only fermentable, i.e. it was not bulking (lignocellulose, cellulose) nor viscous (pectin, guar gum) or gelling (alginate, pectin). Thus, with resistant starch the fermentability property could be well differentiated from other

properties, as for most other fibres tested there were always overlapping properties (see Table 5). Finally, in Chapter 3 it was hypothesized that the fermentation characteristics (fermentation kinetics and SCFA-profile) that differ substantially between dietary fibres could play a role in satiety. From the results, it was concluded that the slow rate of fermentation of resistant starch may have played a role in its satiety enhancing effects.

Besides satiating effects, resistant starch may affect colonic health as suggested by the findings in Chapter 5. Briefly, consumption of resistant starch was associated with various changes in metabolism. For example, a downregulation of genes involved in both innate and adaptive immune responses, and an upregulation of genes involved in metabolic processes such as fatty acid and energy metabolism were found in the proximal colon of pigs fed the same source and quantity of resistant starch as in the studies described in Chapters 3, 4 and 6. Moreover, resistant starch decreased the abundance of potentially pathogenic bacteria such as *Actinobacillus indolicus* and *Pseudomonas*, and increased the abundance of healthy gut-associated bacteria such as *Faecalibacterium prausnitzii* and *Megasphaera elsdenii* that have been previously shown to produce butyrate in the colon. With regard to butyrate, it is considered an important energy source for intestinal epithelial cells, and has been also implicated to help maintain colonic health (Hamer et al., 2008). No difference in SCFA concentration was observed in digesta collected from the proximal colon of pigs fed resistant starch as compared to pigs fed pregelatinized starch in Chapter 5. Nevertheless, the percentage of branched-chain fatty acids (BCFA), i.e. iso-butyrate and iso-valerate, was lower in these animals, which implies the predominant use of resistant starch as energy source by microbiota. As expected, SCFA concentrations determined in peripheral blood collected 300 min after feeding were significantly higher with resistant starch consumption compared to pregelatinized starch consumption. These were inversely correlated with potential pathogenic microbial groups, which further supports the belief that resistant starch has a beneficial impact on colonic health. This may provide some benefits to humans, as well as to animals.

Table 5. Comparison of dietary fibres used in this thesis, based on physicochemical properties*.

Fibre source	Bulkiness**	Viscosity	Gelling	Fermentability	References
Alginate[1]	+	+	+++	–	Hoad et al., 2004 Wanders et al., 2012
Guar gum[2]	+	+	–	+++	Bosch et al., 2008 Jonathan et al., 2012
Inulin[3]	–	–/+	–	+++	Alexiou and Franck, 2008 Bosch et al., 2008 Jonathan et al., 2012
Lignocellulose[4]	+++	–	–	–	Büttner, 2006 Jonathan et al., 2012
Pectin[5]	+	+++	–	++	Slavin and Green, 2007 Jonathan et al., 2012
Resistant starch[6]	–	–	–	+++	Fuentes-Zaragoza et al., 2010 Jonathan et al., 2012 Nugent, 2005

– = low, +, ++, +++ =increasingly higher
* Estimation based on comparison of literature sources and product specifications obtained from the supplier.
** Based on water-binding capacity.
[1] Pectacon M-5761 (Acatris, The Netherlands).
[2] Viscogum MP 41210™ (Cargill, Belgium).
[3] Orafti IPS™ (BENEO-Orafti, Belgium).
[4] Arbocel® RC Fine (J. Rettenmaier & Söhne, Germany).
[5] Unipectine™ RS 150 Citrus (Cargill, Belgium).
[6] Native potato starch (Avebe Food, The Netherlands) in Chapter 2; Retrograded tapioca starch, ActiStar™ (Cargill, Belgium) in Chapters 3 to 6.

Potential underlying mechanisms

1. Slow eating and a reduced rate of gastric emptying as mechanisms to reach earlier satiation

The research described in this thesis showed that fibres reducing feeding rates (see Table 3) were associated with earlier satiation (van Merwijk, Souza da Silva, and van den Borne, 2009, unpublished results). A reduced chewing rate concurrently with a reduced feeding rate were likely induced by the thickening of gelling or viscous fibres during oral food processing, resulting in reduced voluntary food intake and earlier satiation in sows in a pilot study (Table 6a). This is in line with earlier animal studies, such as the one of Brouns and co-workers (1997) where sows receiving a diet with a high inclusion (500 g/kg) of sugar beet pulp (rich in pectins) consumed their daily food allowance more slowly than sows receiving a conventional cereal-based diet (control). Also in pigs fed viscous fibre in the study shown in Chapter 2, observations around feeding suggested that pectin diets became very viscous and sticky in the mouth, leading to a substantial increase in eating time, likely related to the increased chewing effort required for pectin diets than for other diets. This finding is in line with the earlier satiation found in sows fed a viscous guar gum diet in the pilot study (van Merwijk, Souza da Silva, and van den Borne, 2009, unpublished results) previously described in this chapter (Table 6a). Moreover, enhanced satiation feelings were found in humans after

consumption of a test product added a similar viscous guar gum fibre (Wanders et al., 2012, Table 7). In humans, a reduced feeding rate is often associated with an increased oral exposure to food (Viskaal-van Dongen et al., 2011). Oral exposure time was not measured in the studies described in this thesis, but in the study of Wanders and co-workers (2012) in humans, oral exposure time was indeed longer and voluntary food intake was lower for a solid test product (chocolate cookie) with 5 g/100 g gelling fibre as compared with a cookie with no added fibre (Wanders et al., 2012, Table 7). This is in line with other studies in humans demonstrating reduced voluntary food intake and earlier satiation as a result of increased oral exposure to food (Zijlstra et al., 2009; Bolhuis et al., 2011). Andrade and co-workers (2008) listed several explanations for the relationship between slow eating (reduced feeding rates in pigs or increased oral exposure time in humans) and reduced voluntary food intake (earlier satiation). First, it has been stated that the physiological feedback from ingested food (primarily based on sensory cues, see Smeets and Westerterp-Plantenga, 2006) takes at least 20 minutes to develop in humans, and this delay is independent of the amount of food consumed. This would imply that when feeding rate is low or oral exposure time is long after consumption of gelling or viscous fibres, more sensory signals from the oral cavity reach the brain, leaving sufficient time for satiation signals to induce meal termination (Rolls, 2007). Secondly, the process of chewing per se may also stimulate satiation signals, as in rats it has been shown that chewing induces activation of histamine neurons in the hypothalamus that may suppress food intake by affecting both eating volume and eating speed (Sakata et al., 2003). Finally, characteristics of food such as palatability, which determine the sensory pleasantness of food in the mouth, have been associated with satiation, as for instance more palatable food often results in higher food intake (Sørensen et al., 2003). Humans consuming cookies with added fibres in the study of Wanders and co-workers (2012) have rated the palatability of cookies with added gelling and viscous fibres as being lower than that of cookies with no added fibre. Although in all studies presented in this thesis, diets were given a cherry-honey flavour to mask differences in palatability as much as possible, the possibility that gelling and/or viscous fibres may have reduced palatability in the fibre diets cannot be discarded. A reduced palatability could have further contributed to a reduced feeding rate or increased oral exposure time associated with earlier satiation in pig and human studies.

Furthermore, a reduced rate of gastric emptying may be one of the key mechanisms for induced earlier satiation. In a pilot study with sows, we observed a reduced gastric emptying rate induced particularly by gelling in the stomach (Tables 6b), resulting in reduced voluntary food intake and earlier satiation. This is in line with the hypothesis that gelling fibres increase viscosity and water-binding

capacity of digesta, and more importantly lead to formation of gel particles in the stomach (Hoad et al., 2004). These, in turn, could promote earlier satiation by reducing gastric emptying rate and increasing gastric distension, as there is evidence that gastric distension is one of the causal factors leading to satiation (De Graaf et al., 2004; Benelam, 2009). To date, only few studies have investigated effects of dietary fibres on gastric emptying rates in pigs under normal meal conditions and results were sometimes contradicting (Guerin et al., 2001; Miquel et al., 2001). The pilot study presented in this chapter is the first to report an effect of a gelling fibre in reducing gastric emptying rate in sows (Table 6b), as measured by the validated non-invasive ^{13}C breath test (Jørgensen et al., 2010a). This is in line with a previous study (Jørgensen et al., 2010b) showing a general reduced gastric emptying rate of pigs fed diets containing fibres with variable physicochemical properties (swelling and water-binding capacity, but not gelling). Also in humans, in another study of Wanders and co-workers (2013a), gastric emptying rate was indeed reduced by a gelling fibre added to a liquid test product (Table 7). In addition, the potential of gelling fibres to reduce gastric emptying rate in humans may be dependent on the rate of hydration in the mouth related to the physical form of the diet. This is because in one earlier study of Wanders and co-workers (2012) an increased gastric emptying rate was found in humans fed a solid test product added with the same source (Protanal LF 5/60, FMC BioPolymer, Norway) and quantity (5%) of gelling fibre as in the pilot study presented in this chapter (see Table 7). This was explained by the fact that the gelling fibre added to a solid food might not have been fully hydrated in the mouth, and this might have reduced its gelling properties in the stomach of humans (Wanders et al., 2012). In sows, the physical form of the diet (i.e. solid food) seemed not to affect the physicochemical properties of the gelling fibre in the stomach, as gastric emptying rate was still found to be reduced. Despite the conflicting effects of gelling fibres on gastric emptying rate in pigs and humans due to the physical form in which the fibre is supplied, satiation was consistently enhanced by gelling fibres in both pig and human studies. Particularly in pigs, gelling fibres affected satiation via a reduced feeding rate and via a reduced gastric emptying rate, which seemed to be independent of the physical form of the diet, whereas in humans, gelling fibres affected satiation via an increased oral exposure to food and via a reduced gastric emptying rate, the latter being most pronounced after adding gelling fibres to a liquid product. The processes by which gelling fibres lead to a reduced gastric emptying rate are not yet known, but it may be related to the more difficult movement of digesta within the stomach due to gel particles or to the ileal brake, a mechanism which reduces gastric emptying rate and reduces intestinal passage rate when undigested nutrients and viscous fibres reach the distal portion of the small intestine (Maljaars et al., 2008). Moreover, the ileal brake could contribute to

enhanced feelings of satiety, because it will increase the time that digesta stay in contact with intestinal absorptive surfaces (Howarth et al., 2001), which likely results in increased secretion of satiety-related hormones (Brownlee, 2011). The role of viscous fibres in reducing gastric emptying rates was less clear than that of gelling fibres, because in the pilot study presented in this chapter viscous fibres were not tested, and in the human studies of Wanders and co-workers (Table 7), viscous fibres (guar gum, pectin) did not affect gastric emptying rates (Wanders et al., 2012; Wanders et al., 2013a).

2. Increased luminal bulk and reduced intestinal passage rate to enhance satiety

The research described in this thesis showed that fibres affecting swelling and particularly water-binding capacity (bulking) of digesta in the stomach did not induce earlier satiation, nor enhanced short-term satiety. Instead, in Chapter 2 a bulking fibre prolonged the duration of satiety beyond 3 h after the meal (Table 6a). Data from literature about passage rate of digesta (expressed as transit time and retention time) in different segments of the GIT in pigs fed fibre diets vary strongly among studies. For example, Wilfart and co-workers (2007) found mean retention times of 1 h from the mouth to the proximal duodenum (stomach), 4 h from the proximal duodenum to the distal ileum (small intestine), and 35 h from the distal ileum to the faecal excretion in growing pigs fed variable fibre diets. In another study, Van Leeuwen and Jansman (2007) found mean transit times of 3-6 h in the stomach, 5-20 h in the small intestine, and 60-82 h in the colon. Based on this variation, it is hypothesized that bulking fibres may have reduced intestinal passage rate more than gastric emptying rate in Chapter 2. The role of fibre-rich diets on gastric emptying and intestinal passage rate is yet unclear and controversial, as it has been reported that fibres in general can reduce (Miquel et al., 2001), not affect (Potkins et al., 1991) or even increase (Guerin et al., 2001) gastric emptying or intestinal passage rates. It is generally accepted that large intestinal passage rate is often accelerated by fibres, because fibres exert a direct physical action in the pigs' intestine, which stimulates propulsive colonic motility due to a greater bulk of digesta (Wilfart et al., 2007). Studies reporting effects of bulking fibres, such as lignocellulose that possess a very high water-binding capacity, on passage rates or mean retention times along the GIT of pigs or humans are scarce. Nevertheless, as based on the study of Miquel and co-workers (2001), fibres that have a high water-binding capacity such as wheat bran, will indeed reduce the gastric emptying rate (from 2 h after a meal onwards) more than fibres that have a high viscosity such as sugar-beet pulp, but likely to a lesser extent as gelling fibres do (see previous section). This means that bulking fibres in Chapter 2 may have reduced gastric emptying rate, but not sufficiently for promoting earlier

satiation. Although it is unknown whether a reduced gastric emptying rate after lignocellulose ingestion resulted in reduced intestinal passage rate, this would be in line with the enhanced satiety found in these pigs. Moreover, passage rate might have been reduced particularly in the small intestine, in line with the ileal brake mechanism (Maljaars et al., 2008), to allow for proper digestion and nutrient absorption, coinciding with an enhanced satiety feeling for more than 3 h after the meal in pigs in Chapter 2. Although it remains to be elucidated, the satiating effects of lignocellulose could thus be attributed to increased luminal bulk and reduced passage rate in the intestine. Alternatively, increased luminal bulk may have increased distension in the small intestine, and thereby have led to activation of stretch receptors in the smooth muscles in the intestinal wall signalling satiety to the brain via vagal afferents, in line with previous studies in rats (Davis and Collins, 1978; Fox et al., 2001). Moreover, in sham-fed Rhesus monkeys this mechanism has been shown to occur independently of gastric emptying rate or gastric distension (Gibbs et al., 1981).

3. Fermentation in the gut as a mechanism to enhance satiety

Pigs fed resistant starch showed elevated peripheral SCFA levels throughout the day (both pre- and post-prandial) compared to pigs fed pregelatinized starch (Chapter 4) (Table 6b). This confirms the observation that resistant starch escapes enzymatic digestion in the small intestine, and is largely fermented in the distal small intestine, caecum and colon into SCFA (Topping and Clifton, 2001). Moreover, because fermentation of resistant starch is slow in the small intestine, and continues in the caecum and colon for several hours after a meal (Achour et al., 1997), SCFA have been hypothesized to be involved in the long-term satiating effect of resistant starch shown in pigs in this thesis. Various studies have shown indeed that the prolonged production and absorption of SCFA prolongs the energy supply to the body (Rérat, 1996; Serena et al., 2009; Regmi et al., 2011), which bridges the energy gap during the inter-prandial period, where declines in blood glucose levels below basal levels have been consistently shown to precede meal initiation in animals and humans (for review, see Campfield and Smith, 2003). This corresponds to observations in humans by Speechly and co-workers, where the spreading of an isocaloric amount of food over five hourly portions reduced the amount consumed at a subsequent test meal in comparison to a single portion (Speechly and Buffenstein, 1999; Speechly et al., 1999). The greater satiety when increasing meal frequency in humans has been attributed to attenuation of the insulin response, where plasma insulin levels were maintained slightly above basal levels. Previous studies in pigs have failed to show a relationship between increasing meal frequency and satiety, likely because the number of meals provided varied only between one or two daily meals (see for example Farmer et

al., 2002; Robert et al., 2002). It has, however, been shown that fibre ingredients, as for instance purified starches differing in the rate of digestion, can affect the metabolite and hormonal meal responses in pigs (Regmi et al., 2011). In humans, increasing meal frequency maintained plasma glucose levels at basal (Speechly and Buffenstein, 1999), or slightly above basal levels (Speechly et al., 1999) throughout the treatment, thereby also contributing to an attenuated glucose response (Campfield and Smith, 2003; Chapelot et al., 2006). In the research described in this thesis (Chapter 4), satiating effects of resistant starch may also have been associated with decreased (more stable) post-prandial glucose and insulin responses as compared with responses induced by pregelatinized starch (Table 6b), in line with previous studies (De Leeuw et al., 2004; De Leeuw et al., 2005; Regmi et al., 2011). It can be concluded that in addition to the SCFA supply obtained with resistant starch, the kinetics of the glucose supply contributes to the properties of resistant starch as a more satiating fibre than other (non-fermentable) fibres tested in this thesis. In allusion to the theory of "nibblers" and "gorgers" in human nutrition, a gorging pattern of energy intake (i.e. low frequency pattern) results in carbohydrate oxidation being significantly high between meals, while in the fasting period reduced carbohydrate oxidation is compensated for by increased fat oxidation to cover energy needs (Westerterp-Plantenga et al., 1994). In a nibbling pattern of energy intake (i.e. high frequency pattern) carbohydrate and fat oxidation remain relatively constant throughout the day. This implies that nibblers are continuously using the nutrients they have ingested, whereas gorgers are more often using body reserves. In the case of resistant starch, an increased SCFA production and a more stable postprandial response of glucose and insulin could be compared to the nibbler situation, where nutrients obtained from food are continuously made available and used by the body (Jenkins et al., 1989; Jenkins et al., 1992), thereby sustaining energy balance and satiety (Smeets and Westerterp-Plantenga, 2008). Under free-feeding conditions (Chapter 6), despite the fact that pigs consuming resistant starch diets had larger meals than pigs consuming alginate or control diets, over treatments all pigs may be still considered as nibblers. On the basis of a meal criterion of 5 min (i.e. intervals between visits to the feeder < 5 min were considered as within-meal intervals and these visits were grouped into meals), De Haer and Merks (1992) found an average of 9.2 ± 2.4 meals per day in group-housed pigs (n=8) fed a commercial diet for growing pigs ad libitum, whereas in Chapter 6 over treatments the average number of meals per day corresponded to 17.3 ± 0.4. This is higher than the average found by De Haer and Merks, and also much higher than the average number of 2 meals imposed to restrictedly-fed pigs in daily practice.

Surprisingly, the putative hormonal regulators of satiety GLP-1 and PYY were not increased in response to resistant starch (Table 6b). This would imply that

hormonal secretion is contributing to a lesser extent to the net satiating effects of exchanging digestible for resistant starch in pigs, as compared to the kinetics of nutrient absorption. This could be explained in different ways. First, apart from SCFA, glucose is a potent stimulator of GLP-1 secretion (Regmi et al., 2011). In the study in Chapter 4 differences found in GLP-1 secretion were attributed to differences in intestinal glucose levels, but not to differences in SCFA levels. This indicates that a SCFA-induced GLP-1 production was not present, or it was insufficient to balance the glucose-induced GLP-1 production. In the study of Regmi and co-workers (2011), where both glucose and SCFA were found to stimulate GLP-1 secretion, the net portal appearance (NPA) of starch-derived nutrients and hormones was used instead of their peripheral concentrations as a measure of starch effects in pigs. In this way the net absorption of nutrients and secretion of hormones was measured before their hepatic metabolism. In the study in Chapter 4, meal responses of nutrients and hormones were conducted in peripheral circulation, instead of in the portal circulation. This may help explain the absence of a GLP-1 response to feeding in pigs fed resistant starch, as affected by SCFA production. In Chapter 4, measurement of biologically active GLP-1 in peripheral blood was likely useful for estimating at least part of GLP-1 derived from the intestine, but not all because a significant amount of newly secreted GLP-1 is degraded before it is actually released from the intestine (Hansen et al., 1999). For that reason the NPA seems a better measurement than peripheral concentrations. Secondly, SCFA, but also glucose can stimulate PYY secretion in rat intestine. Similar PYY levels were probably a result of simultaneously reduced glucose and increased SCFA concentrations in pigs fed resistant starch in Chapter 4 (Table 6b). The responsiveness of PYY secreting cells to luminal supply of glucose and SCFA has not been studied in pigs, but studies in rats and humans suggest variable responsiveness. For example, the sensitivity of PYY secreting cells to glucose seems to be less strong in humans than in rats after supra-physiological glucose intestinal infusions (Onaga et al., 2002). Moreover, studies in rats suggest that the PYY response may be less strong with normal luminal physiological concentrations of SCFA than after supra-physiological intestinal infusions (Onaga et al., 2002). The combination of these results would imply that in pigs PYY secreting cells are also not very sensitive to intestinal glucose and SCFA, as PYY levels (unchanged) found in pigs fed resistant or pregelatinized starch seemed not to be related to glucose levels or to SCFA levels. Furthermore, in humans there is evidence for a negative feedback of GLP-1 on PYY secretion (Näslund et al., 1999), which may have contributed to the PYY responses observed in the study in Chapter 4. Also preprandial GLP-1 and PYY levels (~15 h after previous meal) were similar for both resistant and pregelatinized starch diets, implying that secretion of both GLP-1 and PYY was likely not directly modulated by SCFA.

This is in line with Voortman and co-workers (2012), who also did not find effects of SCFA on GLP-1 and PYY release in a pig perfused intestine model. The authors suggested that effects of SCFA were probably more pronounced on protein expression level than on hormone release, as SCFA have been shown to increase proglucagon mRNA levels in the intestine of rats (Tappenden et al., 1998) and pigs (Haenen et al., 2013b). More studies are needed to test this hypothesis.

According to Mars and co-workers (2012), GLP-1 and PYY show effects on satiety mostly in exogenous infusion studies using supra-physiological dosages, whereas relatively small effects were found in response to food. This is in line with unchanged levels of GLP-1 and PYY found in pigs fed resistant starch in this thesis. As indicated by Mars and co-workers (2012), GLP-1 and PYY responses should be considered in conjunction with other biomarkers in studies on satiety. Various hormones have been shown to be involved in satiety regulation (see Chapter 1), whereas in this thesis only two of these were measured, i.e. GLP-1 and PYY in Chapter 4. It could be that other hormones might have contributed to a satiating effect of resistant starch, but these were not measured. For example, other biomarkers of satiety that have been mentioned in humans include glucose-dependent insulinotropic polypeptide (GIP), which shares insulinotropic effects with GLP-1 (De Graaf et al., 2004). Oxyntomodulin (OXM), which is co-secreted with PYY and GLP-1 by L-cells, has been shown to reduce gastric acid secretion, reduce gastric emptying rate and reduce intestinal motility, contributing to the ileal brake in humans (Maljaars et al., 2008). Moreover, OXM has been associated with enhanced satiety and reduced food intake in healthy humans (Cohen et al., 2003). Pancreatic polypeptide (PP), which is mainly produced by the pancreas but also in small amounts by intestinal tissue, has been shown to be released into the circulation after eating in proportion to the amount of energy consumed (Benelam, 2009). Studies have also shown that PP administration reduces food intake in humans (Batterham et al., 2003). Specifically in pigs, ghrelin, orexin, neuropeptide Y (NPY) and agouti-related protein (AgRP) have been suggested to stimulate appetite, thereby associated with reduced satiety, whereas leptin and urocortin have been suggested to suppress appetite, thereby associated with enhanced satiety (Carroll and Allee, 2009). Ghrelin is produced by the stomach and leads to meal initiation, whereas its suppression after a meal may be also important for the onset of satiety (De Graaf et al., 2004). Orexin is a hypothalamic peptide that has been used as means to enhance food intake in weaned pigs. Despite the results reported by Dyer and co-workers (1999), no further published studies have evaluated effects of orexin on satiety regulation in pigs. Also little is known about the appetite suppressive effects of urocortin in pigs, whereas leptin is often referred as an appetite suppressor (Carroll and Allee, 2009). In addition, neuropeptides expressed in the arcuate nucleus (ARC) of the hypothalamus are involved in appetite

regulation. In this area of the brain NPY/AgRP and pro-opiomelanocortin (POMC) neurons respond to peripheral signals, such as hormones and nutrients, and regulate energy balance. Activation of NPY/AgRP neurons and activation of POMC neurons will increase and decrease food intake, respectively (Schwartz et al., 2003). It has been shown that PYY and GLP-1 affect the activities of these two sets of neurons. Because resistant starch was thought to influence satiety via an increase in the production and secretion of PYY and GLP-1, it could be hypothesized that the expression of NPY, AgRP or POMC in the hypothalamus of animals fed resistant starch would also be altered. This has been shown in rats by Shen and co-workers (2009), who found that resistant starch elevated plasma PYY and GLP-1, and increased hypothalamic POMC expression in rats. Besides investigating changes in plasma concentrations of specific hormones after a meal, it would be valuable to try to combine hormonal measurements with other measurements, as for instance, measurements of gene expression in the brain, or even measurements of associated actions of specific hormones (e.g. gastric emptying, passage rate, etc.) in future studies on satiety, as means to obtain more elaborate measures of mechanisms involved in the satiating effects of resistant starch. Moreover, resistant starch reduced blood serotonin levels, increased plasma tryptophan levels, and increased plasma triglyceride levels in Chapter 4 (Table 6b). The involvement of these in the satiating effects of resistant starch still needs further testing.

Table 6a. Overview of effects of dietary fibres on satiety and satiation in adult and growing pigs in this thesis.

Fibre source	Inclusion level	Feeding regime	Subjects (BW, age)	Operant responding	Speed	Voluntary FI	Feeding rate	First bout duration	Physical activity	Aggression	Stereotypic chewing	FD behaviour	Drinking	Feeding patterns	Reference
Alginate (gelling)	2.5 and 5%	Ad libitum (1 h)	Sows, (312 ± 30 kg, 3.7 y)	n/a	↔	↓	↓	n/a	↔	n/a	↔	↔	n/a	n/a	Unpublished data
Alginate (gelling)	5%	Ad libitum (12 weeks)	Growing male and female pigs (37.8 ± 0.3 kg, 3 m)	n/a	n/a	↑	n/a	n/a	↑	↑	↔	↑	↑	↑ ADFI ↑ cumulative FI ↑ daily time feeding	Chapter 6
Guar gum (viscous)	2.5 and 5%	Ad libitum (1 h)	Sows, (312 ± 30 kg, 3.7 y)	n/a	↔	↓	↓	n/a	↔	n/a	↔	↔	n/a	n/a	Unpublished data
Guar gum (fermentable)	5 and 10%	Restricted (twice daily for 14 days)	Gilts (250 ± 3.1 kg, 1.5 y)	↔	↔	↔	n/a	↑	↓	n/a	↔	↑	n/a	n/a	Chapter 3
Inulin (fermentable)	7 and 14%	Restricted (twice daily for 14 days)	Gilts (250 ± 3.1 kg, 1.5 y)	↔	↔	↔	n/a	↔	**↓ with inulin 7%**	n/a	↔	↔	n/a	n/a	Chapter 3
Lignocellulose (bulking)	5 and 10%	Restricted (twice daily for 14 days)	Gilts (199 ± 2.3 kg, 1 y)	**↓ with lignocellulose 5%**	**↑ with lignocellulose 5%**	n/a	n/a	n/a	↔	n/a	↔	↔	n/a	n/a	Chapter 2
Pectin (viscous)	7.5 and 15%	Restricted (twice daily for 14 days)	Gilts (199 ± 2.3 kg, 1 y)	**↑ with pectin 15%**	↑	n/a	n/a	n/a	↔	n/a	↑	↑	n/a	n/a	Chapter 2
Resistant starch (fermentable)	20 and 40%	Restricted (twice daily for 14 days)	Gilts (199 ± 2.3 kg, 1 y)	↔	↓	n/a	n/a	n/a	↔	n/a	↔	↓	n/a	n/a	Chapter 2
Resistant starch (fermentable)	17 and 34%	Restricted (twice daily for 14 days)	Gilts (250 ± 3.1 kg, 1.5 y)	↔	**↓ with resistant starch 34%**	**↓ with resistant starch 34%**	n/a	↓	**↓ with resistant starch 34%**	n/a	**↓ with resistant starch 34%**	↔	n/a	n/a	Chapter 3
Resistant starch (fermentable)	34%	Restricted (twice daily for 14 days)	Growing male pigs (58 ± 1.6 kg, 4 m)	n/a	n/a	↔	n/a	↓	↔	n/a	↔	↓	↓	n/a	Chapter 4
Resistant starch (fermentable)	34%	Ad libitum (12 weeks)	Growing male and female pigs (37.8 ± 0.3 kg, 3 m)	n/a	n/a	↔	n/a	n/a	↔	↔	↔	↔	↔	↑ FI per meal ↑ meal duration ↑ inter-meal intervals ↓ number of meals per day	Chapter 6

ADFI, average daily food intake; BW, body weight; FD, feeder-directed; FI, food intake; n/a, not applicable.

Table 6b. Overview of effects of dietary fibres on physiological processes in adult and growing pigs in this thesis.

Fibre source	Inclusion level	Feeding regime	Subjects (BW, age)	Gastric emptying rate	DE/GE	ME/GE	SCFA levels	Glucose levels	Insulin levels	Leptin levels	GLP-1 levels	PYY levels	TG levels	5-HT levels	Trp levels	MAO levels	Body weight	Reference
Alginate (gelling)	2.5 and 5%	Ad libitum (1 h)	Sows, (312 ± 30 kg, 3.7 y)	↓ with alginate 5%	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Unpublished data
Alginate (gelling)	5%	Ad libitum (12 weeks)	Growing male and female pigs (37.8 ± 0.3 kg, 3 m)	n/a	↓	n/a	n/a	⇔	↓	⇔	n/a	n/a	n/a	⇔	n/a	↑	⇔ final BW ⇔ ADG ⇔ gain:DE intake ↓ carcass gain:DE intake ↓ backfat thickness ⇔ muscle thickness ↑ colon empty weight	Chapter 6
Resistant starch (fermentable)	34%	Restricted (twice daily for 14 days)	Growing male pigs (58 ± 1.6 kg, 4 m)	n/a	↓	↓	↑	↓	↓	n/a	↓	⇔	↑	↓	↑	↑	n/a	Chapter 4
Resistant starch (fermentable)	34%	Ad libitum (12 weeks)	Growing male and female pigs (37.8 ± 0.3 kg, 3 m)	n/a	↓	n/a	n/a	↑	↑	⇔	⇔	⇔	⇔	↓	n/a	↓ (batch 1 only)	⇔ final BW ⇔ ADG ↑ gain:DE intake ⇔ carcass gain:DE intake ⇔ backfat thickness ⇔ muscle thickness ↑ colon empty weight	Chapter 6

ADG, average daily gain; BW, body weight; DE, digestible energy; GE, gross energy; GLP-1, glucagon-like peptide-1; MAO, monoamine oxidase; ME, metabolizable energy; n/a, not applicable; PYY, peptide tyrosine tyrosine; SCFA, short-chain fatty acids; TG, triglycerides; Trp, tryptophan; 5-HT, 5-hydroxytryptamine (serotonin).

Table 7. Overview of effects of dietary fibres on satiety and satiation and accompanying physiological processes in adult humans fed similar dietary fibres as the ones used in this thesis (Wanders, 2013).

Fibre source	Inclusion level	Feeding regime	Subjects (BMI, age)	Appetite sensations	Voluntary food intake	Oral exposure time	Gastric emptying rate	Glucose levels	Insulin levels	Body weight	Reference
Alginate (gelling)	2.5 and 5%	Ad libitum (1.5 h)	Healthy men and women, non-restrained eaters (18.5–25 kg/m^2, 18–50 y)	↓ hunger ↑ fullness	↓ by 17% with alginate 5%	↑ by 48% with alginate 5%	↑ with alginate 5%	n/a	n/a	n/a	Wanders et al., 2012
Guar gum (viscous)	1.25 and 2.5%	Ad libitum (1.5 h)	Healthy men and women, non-restrained eaters (18.5–25 kg/m^2, 18–50 y)	↓ hunger ↑ fullness	⇔	⇔	⇔	n/a	n/a	n/a	Wanders et al., 2012
Pectin (bulking)	10 g (dairy based liquid test product)	Restricted (once daily)	Healthy men and women, non-restrained eaters (18.5–25 kg/m^2, 18–50 y)	⇔	⇔	n/a	⇔	⇔	⇔	n/a	Wanders et al., 2013a
Pectin (viscous)	10 g (dairy based liquid test product)	Restricted (once daily)	Healthy men and women, non-restrained eaters (18.5–25 kg/m^2, 18–50 y)	↑ fullness (↑ thirst)	⇔	n/a	⇔	↓	↓	n/a	Wanders et al., 2013a
Pectin (gelling)	10 g (dairy based liquid test product)	Restricted (once daily)	Healthy men and women, non-restrained eaters (18.5–25 kg/m^2, 18–50 y)	↓ hunger ↑ fullness	⇔	n/a	↓	↓	↓	n/a	Wanders et al., 2013a
Pectin (gelling)	10 g	Restricted (once daily for 16 days)	Healthy men, non-restrained eaters (18.5–25 kg/m^2, 18–30 y)	↓ hunger ↑ fullness	↓ by 6% after single exposure ⇔ after repeated exposure	n/a	n/a	↑ (fasting levels)	⇔	⇔	Wanders et al., 2013b

BMI, body-mass index; n/a, not applicable.

Long-term effects of dietary fibres on energy intake and body weight development

In Chapter 6 of this thesis, the potential of a gelling fibre (alginate), a fermentable fibre (resistant starch), and the combination of both, to modify feeding patterns (via satiation and satiety), and thereby long-term food intake and growth performance of ad libitum-fed growing pigs was assessed over 12 weeks. As previously indicated, resistant starch increased inter-meal intervals and reduced the number of meals per day, which was related to prolonged satiety. Alginate numerically reduced food intake per meal, meal duration and inter-meal intervals, which significantly increased the number of meals per hour throughout the day, and was related to earlier satiation. Pigs fed alginate diets increased, however, their dry matter (DM) intake, and achieved a similar digestible energy (DE) intake as in pigs fed diets with no added fibre (control). This is in line with the theory of Cole and co-workers (1968), stating that when dietary energy content is reduced pigs will increase their voluntary food intake to meet their energy requirements. Alginate-fed pigs achieved similar final body weights and average daily gains as pigs fed the control diet. Backfat thickness and the carcass gain:DE intake ratio were, however, reduced by alginate. It should be noted that although daily gains were similar to those of control-fed pigs, alginate-fed pigs had a lower carcass weight gain, but the empty weight of their colon was increased, which is probably an adaptation of the GIT to fermentation, but not as much as found in pigs fed resistant starch diets (Table 6b).

Table 8. Feeding patterns of growing pigs fed a control diet (CON) or a diet containing a novel corn fibre (12.5%, NCF) ad libitum during 12 weeks.

	Diet		*P*-value
Item	CON	NCF	CON vs. NCF
Cumulative feed intake, kg	179.3 ± 3.21	195.6 ± 4.32	0.005
Average feed intake, kg/d	2.19 ± 0.04	2.38 ± 0.05	0.004
Meal size, g/meal	134.0 ± 7.03	130.4 ± 4.06	0.715
Meal duration, min	6.28 ± 0.14	5.71 ± 0.16	0.065
Inter-meal interval, min	83.3 ± 3.46	75.7 ± 2.76	0.195
Time feeding, min/d	81.4 ± 2.41	81.4 ± 2.35	0.961
Number of meals/d	17.5 ± 0.90	19.2 ± 0.59	0.108

In addition to alginate and resistant starch, a novel corn fibre (NCF) manufactured by Cargill (Belgium) was included in the study described in Chapter 6, but results were not presented in this chapter. The extent to which pigs compensated for the reduced dietary energy content of this fibre diet was also

evaluated. Typically, the fibre from corn seeds is characterized by a slow rate of fermentation in vitro (t_{max} 28.3h) and a low total tract digestibility in vivo (37%), as found in a study investigating effects of fermentable feed ingredients (potato fibre, chicory pulp, pea hulls, corn fibre, and palm kernel) on the energetic utilization of fermentable fibre in pigs (Rijnen et al. 2004, unpublished results). Similarly to alginate, pigs fed the NCF diet increased their voluntary food intake, but without an effect on the other feeding pattern variables (Table 8). Average daily food intake and cumulative food intake were higher in pigs fed NCF than in pigs fed the control diet. The NCF diet decreased DM and gross energy (GE) digestibility, and tended to reduce crude protein (CP) digestibility. Moreover, pigs fed the NCF diet had higher DM intake, thinner backfat, and lower gain:DM intake and carcass gain:DM intake than pigs fed the control diet (Table 9). A combination of an increased DM intake and a similar DE intake found in pigs fed NCF as compared with control pigs, which was also found for the alginate diet, confirms the presence of a compensatory mechanism. Dietary effects seemed stronger with the NCF diet than with the alginate diet, likely due to the differences in inclusion levels (5% for alginate vs. 12.5% for NCF), but possibly also due to chemical characteristics that make NCF very difficult to ferment. Generally, corn fibres such as other lignocellulosic materials are mostly insoluble in water. This insoluble fraction consists mostly of cellulose (15–20%) and hemicellulose (30–50%) (Gáspár et al., 2007). Hemicellulose is a highly-branched complex polymer having five and six carbon atoms in its sugar structure (Gáspár et al., 2005). Due to this complexity, many different enzymes are required to degrade the corn fibre by converting the carbohydrate polymers into fermentable sugars (Gáspár et al., 2007). The corn fibre tested in Chapter 6 contained 12% of protein, 14% of starch and 58% of non-starch polysaccharides (NSP). These NSP were not characterized in terms of oligosaccharides composition. According to literature, corn fibre NSP likely consisted mainly of complex and highly substituted glucuronoarabinoxylans (Appeldoorn et al., 2010). Only certain species of bacteria from the human intestine produce the enzymes needed for the degradation of arabinoxylans. In general, soluble arabinoxylans are readily fermented in the colon, whereas arabinoxylans characterized by a low degree of substitution (i.e. the extent of substitution and distribution of substituents along the xylan backbone) are slowly degraded at more distal locations, and remaining arabinoxylans characterized by a high degree of substitution, are not degraded at all (Knudsen and Lærke, 2010). The empty weights of the GIT parts, particularly caecum and colon, for pigs fed NCF did not differ from control. This would imply that NCF was indeed not well fermented by bacteria from pig intestine. Thus, the compensatory mechanism of increasing voluntary food intake to meet energy requirements found in NCF pigs

was similar to the one found in alginate pigs, however, these were elicited by different fibre characteristics. NCF could be considered a true bulking fibre.

Table 9. Performance of growing pigs fed a control diet (CON) or a diet containing a novel corn fibre (12.5%, NCF) ad libitum during 12 weeks.

	Diet		*P*-value
Item	CON	NCF	CON vs. NCF
Initial BW, kg	37.4 ± 0.32	37.5 ± 0.30	0.924
Final BW, kg	110.9 ± 1.16	111.0 ± 0.55	0.967
Average gain, kg/d	0.92 ± 0.012	0.92 ± 0.006	0.963
Average DM intake, kg/d	1.98 ± 0.035	2.14 ± 0.049	0.006
Average DE intake, MJ/d	29.7 ± 0.52	29.7 ± 0.59	0.942
Gain:DM intake, kg/kg	0.45 ± 0.006	0.42 ± 0.003	0.005
Gain:DE intake, kg/GJ	27.2 ± 0.36	27.0 ± 0.17	0.819
Backfat thickness, mm	17.4 ± 0.35	15.6 ± 0.45	0.008
Muscle thickness, mm	57.2 ± 0.63	58.0 ± 0.59	0.505
Carcass growth, kg	57.8 ± 0.84	56.8 ± 0.59	0.443
Carcass gain:DM intake, kg/kg	0.36 ± 0.005	0.32 ± 0.004	< 0.001
Carcass gain:DE intake, kg/GJ	21.4 ± 0.31	20.9 ± 0.23	0.286

It should be noted, though, that this compensatory mechanism was not observed with resistant starch. Pigs fed resistant starch changed feeding patterns, but did not increase voluntary food intake, resulting in a lower DE intake. Despite their lower DE intake, pigs fed resistant starch achieved similar final body weights and average daily gains as pigs fed the control diet by increasing efficiency in DE use. In Chapter 6, the increased efficiency in energetic utilization found in pigs fed resistant starch was attributed to a more gradual energy supply via the increased availability of SCFA and, possibly, partly to a reduced activation of the immune system (see Chapter 5). These aspects have been discussed in Chapter 6 and are, therefore, not discussed in this chapter.

Despite the fact that dietary fibres did not reduce voluntary food intake or average daily gain in ad libitum-fed growing pigs, they led to changes in tissue distribution (carcass vs. intestine) and body composition (fat vs. muscle), which may be relevant for the maintenance of lean weight in humans. Pigs fed resistant starch diets had a lower carcass gain, but more intestinal gain, as suggested by an increased gain of non-carcass and higher colon and total GIT empty weight in comparison with pigs fed diets without resistant starch (Table 10). Also pigs fed alginate diets had a lower carcass gain, with no effects on non-carcass gain but a slightly higher empty weight of the colon at slaughter. Moreover, pigs fed alginate

had a lower backfat:muscle ratio as compared with pigs fed diets without alginate. The combination of these findings would imply that pigs fed alginate and resistant starch diets in the study described in Chapter 6 deposited less carcass mass than pigs fed control diets. Moreover, fat mass deposition was reduced particularly in pigs fed alginate (and corn fibre) diets (Table 10). Thus, even in the absence of a dietary effect on average daily gain and ultimate body weight, changes in body composition following prolonged fibre intake were found. This suggests that investigation of changes in tissue distribution or body composition, rather than changes in body-mass index (BMI), body weight, or waist circumference alone, may be also useful in future studies investigating the role of dietary fibres to maintain or promote lean weight in humans. For example, it can be speculated that in the study of Rienks and co-workers (2013) in a cohort of elderly Dutch men and women, the lack of effect of the intake of total dietary fibre, dietary fibre from variable food sources, and dietary fibre types on BMI, body weight, or waist circumference may be caused at least partly by an increase in intestinal weight at the expense of body fat. Thus, considering the particular role of fermentable fibres to reduce carcass gain while increasing intestinal weight gain in pigs, and, in the case of alginate, to change backfat:muscle ratio (Chapter 6), it seems highly relevant to investigate fibre-induced changes in tissue distribution that may potentially impact human health, even in the absence of a net effect on body weight gain. There are currently no studies available in humans reporting changes in intestinal weight or adaptations in length or size of (part of) the GIT to fibrous diets, not even in vegetarians, who generally consume diets based on plant-derived foods such as grains, beans, fruits and vegetables, and may be thus extreme in their fibre intake (see Berkow and Barnard, 2006 for review). Humans may show some degree of plasticity in intestinal size, as it has been reported that there are both intra- and inter-population differences in characteristics of the human GIT such as the length of the colon and small intestine, and the size of the caecum (Milton, 2003). For instance, colon length may range from 109 to 181 cm in adult humans. This variation has been in part explained by differences in body weight (Hounnou et al., 2002), however, a thorough analysis of the influence of diet composition has not been performed to date.

Table 10. Average daily gain, carcass gain, non-carcass gain, and backfat to muscle thickness ratio (B:M) in growing pigs fed a control diet (CON), a diet containing a gelling fibre (5% alginate, ALG), a diet containing a fermentable fibre (34% resistant starch, RS), a diet containing both RS (34%) and ALG (5%) (RSALG), or a diet containing a novel corn fibre (12.5%, NCF) ad libitum during 12 weeks.

	Diet					*P*-value[1]			
Item	CON	ALG	RS	RSALG	NCF	ALG	RS	RS × ALG	CON vs. NCF
Average daily gain, kg/d	0.92 ± 0.012	0.91 ± 0.024	0.92 ± 0.017	0.91 ± 0.020	0.92 ± 0.006	0.678	0.900	0.926	0.963
Average daily carcass gain, kg/d	0.72 ± 0.010	0.72 ± 0.011	0.71 ± 0.014	0.67 ± 0.009	0.71 ± 0.008	0.042	0.017	0.149	0.449
Average non-carcass gain, kg/d	0.20 ± 0.007	0.20 ± 0.016	0.21 ± 0.010	0.24 ± 0.013	0.21 ± 0.006	0.192	0.016	0.225	0.394
B:M, mm/mm	0.31 ± 0.006	0.29 ± 0.005	0.31 ± 0.015	0.29 ± 0.006	0.27 ± 0.007	0.041	0.900	0.983	0.014

[1] As described in Chapter 6, data were analysed as a 2 × 2 factorial arrangement in a model including ALG (yes/no), RS (yes/no), their interaction, gender and batch as fixed effects, and pen (ALG, RS and batch) and pig (pen, ALG, RS, gender and batch) as random effects. Differences between CON diet and NCF diet were tested by including a contrast statement in the statistical procedures.

By considering changes in carcass weight and tissue distribution instead of changes in whole body weight alone, the hypothesis that fibrous diets are able to maintain or promote lean weight in humans might still be valid. This may be achieved via effects on satiety (Burton-Freeman, 2000; Howarth et al., 2001). The satiety-promoting effects of fibres, in this thesis mainly the fermentable fibres, may be most effective to maintain lean weight in a food restriction scheme (Chapters 2 and 3), while, on the long term in ad libitum conditions, compensatory food intake may occur (Chapter 6). In addition, consumption of high fibre diets is associated with reduced postprandial glucose and insulin responses (Chapter 4), which will lead to improved whole body insulin sensitivity (Maki et al., 2012), and may aid to control body weight in the long-term. Moreover, fibrous diets are generally thought to reduce digestibility of fat and other energy-providing nutrients, also in humans (Sanders and Manning, 1992), which would be in line with the reduced fat deposition found in pigs fed alginate and corn fibre, and may also further aid in the maintenance of lean weight. Lastly, it can be speculated that changes in GIT mass following long-term fibre intake could affect metabolic rate. For instance, vegetarians have been shown to have a higher resting metabolic rate as compared to omnivorous humans (Toth and Poehlman, 1994; Sabaté and Wien, 2010), although it is not clear to what extent this can be attributed to the intake of fibre. In the pigs in Chapter 6, due to the unrestrained feeding regime, satiating properties of fibres were likely not contributing much to changes in body weight, because pigs could simply compensate for the reduced energy density of the fibre diets by increasing their voluntary food intake. An increased energy expenditure on the

maintenance of GIT tissues (due to fermentation) would be in line with a reduced carcass gain. There is evidence that the contribution of visceral tissues maintenance energy exceeds that of muscle, despite the fact that the muscle mass exceeds to a large extent the visceral mass (see for example Noblet et al., 1999). With regard to the energetic utilization of absorbed SCFA, it is expected that SCFA-derived energy will be less efficiently used for body fat deposition than energy obtained from glucose. For example 70% efficiency has been assumed for fermentable fibres in some net energy (NE) systems (Noblet and van Milgen, 2004), and recently 83% efficiency has been reported by Gerrits and co-workers (2012) for fermentable corn starch in restrictedly-fed growing pigs. Hence, consumption of fermentable fibres may still prevent weight gain by modifications in post-absorptive energy utilization when animals are fed restricted.

In studies using restricted feeding (e.g. Gerrits et al., 2012) a considerable part of the reduction in ME intake on resistant starch diets was compensated for by a reduction in energy expenditure on physical activity, saving energy to be retained. It should be noted, though, that when feeding unrestricted (Chapter 6), differences in energy expenditure on physical activity are unlikely to occur. Under these conditions, however, we found the efficiency of DE utilization for body weight gain to be remarkably similar for digestible starch and resistant starch. The reasons for this are not clear, but likely include an increase in energetic utilization when energy absorbed is better spread throughout the day (see Aggett et al., 2003 for human comparison), and a potential reduction in energy expenditure on immune responses (Chapter 5, see Benzie and Wachtel-Galor, 2009 for human comparison). Due to this energetic compensation, fibrous diets may become less effective to control body weight gain in the long term in humans, but if fibres are still able to enhance satiety feelings they may help humans to feel less hungry while on an energy-restricted diet. For the formulation and manufacturing of pig diets, an increased efficiency of DE utilization could mean a reduced need for starch gelatinization, whereas in NE systems used for the evaluation of feed ingredients, the incremental efficiency of conversion of fermentable starch into retained energy could be reconsidered.

From studies in restrictedly-fed pigs, growth performance is expected to be reduced in pigs fed high-fibre diets, which is generally attributed to the reduced digestibility and metabolisability of these diets. In the research described in Chapter 6 of this thesis, the digestibility of diets containing gelling, bulking and fermentable fibres was reduced, but growth performance was overall not reduced, and efficiency of using DE for growth was even enhanced in pigs fed the fermentable fibre resistant starch. There are a number of high fibre ingredients available to pig producers. These ingredients are often available at relatively low costs, and are therefore attractive in feed formulation. If efficiency in energy

utilization can be maintained or enhanced on some high-fibre ingredients, e.g. resistant starch, high-fibre ingredients can be included in diets for growing-finishing pigs without negatively affecting growth. Carcass gain may be still reduced due to increased fermentation of such high-fibre ingredients. This would imply lower gains to the pig producer, but these may be compensated for by reductions in feed costs, and/or, depending on fibre type, reductions in fat deposition (i.e. more favourable meat to fat ratio).

Main conclusions

From the behavioural studies, it is concluded that fermentable fibres were more satiating than bulking and viscous fibres in food restricted adult pigs. Moreover, diets with higher amounts of fermentable fibres were more satiating throughout the day, with resistant starch being the most satiating fibre among all fibres tested. Possible underlying mechanisms for satiating effects of resistant starch included decreased and attenuated glucose and insulin responses after the meal, in conjunction with an increased availability of SCFA, spread throughout the day. Hormonal regulators of satiety GLP-1 and PYY appeared not to contribute to the satiating effects of exchanging digestible for resistant starch in pigs. Apart from satiating effects, we found indications that resistant starch also changed the gene expression profile in proximal colonic epithelial cells. A downregulated expression of genes involved in immune responses together with increases in healthy gut-associated bacteria and decreases in potentially pathogenic bacteria abundance in the colon of pigs fed resistant starch suggests beneficial effects of resistant starch on colonic health. In the long-term, growing pigs fed alginate, a gelling fibre, exchanged for digestible starch compensated for a reduced dietary energy content by increasing their voluntary food intake, thereby achieving similar digestible energy intake and growth rates. Backfat thickness and carcass gain were, however, reduced in these animals. Pigs fed resistant starch in exchange for digestible starch took less and larger meals, but did not increase voluntary food intake, effectively reducing their digestible energy intake. These pigs maintained similar growth rates, suggesting that they became more efficient in the use of digestible energy. Finally, pigs fed resistant starch had a lower carcass gain, but more intestinal gain, reflecting increased fermentation. This implies that changes in body composition and intestinal weight (tissue or content), rather than body weight and BMI alone are relevant to fully acknowledge the potential of fibres to aid in maintaining or promoting healthy body weight in humans.

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Summary

Obesity has become a major public health problem in humans due to the increased risk for cardiovascular and metabolic diseases. Also in companion animals, the incidence of obesity has increased resulting in similar health problems as in humans. Although obesity is not a common problem in farm animals, food restriction is used to maintain low feeding costs and reproductive performance of, for instance, pregnant sows, or to ensure a desirable fat:lean ratio in fattening pigs. Food restriction may result in hunger and increased feeding motivation, which are associated with behavioural problems. Knowledge on the regulation of satiety (i.e. postprandial inhibition of feeding motivation) is crucial to aid in the control of food intake in humans, and to improve welfare in food-restricted farm animals. Dietary fibres are believed to enhance satiety, but there are inconsistencies in the results. The variable efficacy by which dietary fibres enhance satiety could be related to the physicochemical properties of dietary fibre, leading to different effects in the gastrointestinal tract. Therefore, the objective of this thesis was to identify whether and how dietary fibres with different physicochemical properties, such as bulkiness, viscosity, gelling and fermentability, affect satiety in the domestic pig, which was used both as a model for humans and as a target animal.

The effects of different fibres on satiety and possible underlying mechanisms were investigated in four studies. First, satiating properties of fibres with variable physicochemical properties were assessed in two studies focusing on behavioural measures of satiety (Chapters 2 and 3). Based on the results of these studies, one fermentable fibre (resistant starch) was selected based on its satiating potential, and used to assess possible physiological and molecular mechanisms by which fermentation may affect satiety in a subsequent study (Chapters 4 and 5). In the last study, the long-term effects of a gelling fibre promoting satiation (alginate) and a fermentable fibre promoting satiety (resistant starch) on feeding patterns and growth performance were assessed (Chapter 6).

In Chapters 2 and 3, the feeding motivation of adult sows fed different dietary fibres at two levels was assessed in a Latin square arrangement, at different times of the day using behavioural observations and tests. In both studies, animals were restrictedly fed diets in which purified fibre sources were included in the diet at the expense of readily digestible starch, thus reducing metabolizable energy intake. In Chapter 2, diets containing a viscous fibre (pectin) increased feeding motivation during the entire day, whereas diets containing a bulking fibre (lignocellulose) reduced feeding motivation for more than 3 h after the meal, and diets containing a fermentable fibre (resistant starch) reduced feeding motivation up to 7 h after the meal. Both lignocellulose and resistant starch were successful in overcoming the effects of the reduced dietary energy supply compared to the control diet. The combination of these findings suggests that pectin was the least satiating fibre,

whereas lignocellulose and resistant starch were the most satiating fibres, used in this study.

In Chapter 3, diets containing a high level of fermentable fibre (guar gum, inulin, and resistant starch) reduced feeding motivation during the entire day, which means that all fermentable fibres successfully compensated for the effects of a reduced dietary energy supply compared to the control diet. Reduced feeding motivation with an increasing fibre inclusion level was most pronounced for resistant starch, as pigs decreased speed in the runway test and tended to have a lower voluntary food intake in an ad libitum food intake test when fed diets containing a high level of resistant starch. The combination of these findings suggests that increasing levels of fermentable fibres in the diet enhanced satiety throughout the day, and resistant starch was the most satiating fibre among all fibres tested.

The increased satiety found with lignocellulose in Chapter 2 was likely mediated by reduced intestinal passage rates, resulting in an increased luminal bulk in the small intestine and colon. Nevertheless, more studies will be needed to challenge this hypothesis. The increased satiety found with resistant starch in Chapter 2, and particularly in Chapter 3, was partly attributed to a slow rate of fermentation and a high production of short-chain fatty acids (SCFA) for resistant starch as compared to other fermentable fibres. Based on these findings and on findings from previous studies, in Chapter 4 it was hypothesized that resistant starch-induced satiety could be attributed to (1) an increased SCFA production and a reduced glucose supply, (2) SCFA-stimulated release of satiety-related hormones glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) from entero-endocrine cells, or (3) perhaps via effects on serotonin metabolism.

In Chapter 4, the physiological and molecular mechanisms by which fermentation may affect satiety were investigated in an invasive study. Growing pigs were fed a control diet with pregelatinized starch or a diet high in resistant starch in a crossover arrangement. Energy digestibility and metabolizability were 6% lower in the resistant starch compared with the pregelatinized starch diet, and metabolizable energy (ME) intake was 3% lower in pigs fed resistant starch than in pigs fed the pregelatinized starch diet. Despite a lower ME intake, resistant starch appeared to enhance satiety based on behavioural observations, i.e. reduced feeder-directed and drinking behaviours during 24 h. As expected, the satiating effects of resistant starch coincided with increased 24 h plasma SCFA levels and decreased postprandial glucose and insulin plasma levels. GLP-1 plasma levels were lower in pigs fed resistant starch, whereas PYY plasma levels were not affected by resistant starch. Thus, meal responses of GLP-1 and PYY were likely not related to the increased satiety found with resistant starch. Low blood serotonin levels after resistant starch consumption suggested a difference in intestinal serotonin release

between diets. Moreover, pigs fed resistant starch had higher plasma tryptophan levels potentially leading to higher brain serotonin levels, which could support the reduced feeder-directed behaviours in these animals. Interestingly, high plasma triglyceride levels corresponded with increased SCFA levels in pigs fed resistant starch. The involvement of serotonin, tryptophan and triglycerides in the satiating effects of resistant starch are yet unclear and need further testing.

At a molecular level (described in Chapter 5), the gene expression profile of epithelial cells of the proximal colon revealed a downregulation of genes involved in immune responses, and an upregulation of genes involved in metabolic processes such as fatty acid and energy metabolism upon resistant starch consumption. With regard to microbial variation, resistant starch increased the abundance of healthy gut-associated bacteria (e.g. *Faecalibacterium prausnitzii* and *Megasphaera elsdenii* previously shown to produce butyrate), and decreased potentially pathogenic bacteria (e.g. *Actinobacillus indolicus* and *Pseudomonas*) in the proximal colon. No difference in SCFA concentration was observed in digesta from the proximal colon of pigs fed resistant starch as compared to pigs fed pregelatinized starch. However, the percentage of branched-chain fatty acids (BCFA), i.e. iso-butyrate and iso-valerate, was lower in pigs fed resistant starch, which implies the predominant use of resistant starch as energy source by microbiota, whereas in the pregelatinized starch diet the microbiota used more proteinaceous energy sources. As previously mentioned, increased plasma SCFA concentrations were observed following resistant starch consumption. Moreover, correlation analysis inversely linked potential pathogenic microbial groups with plasma SCFA concentrations and genes involved in fatty acid metabolism. The combination of these findings suggests that besides satiating effects, resistant starch has a beneficial effect on colonic health. Overall, the study described in Chapter 5 provided novel insights in the molecular mechanisms of the potential beneficial effects of resistant starch.

In Chapter 6, the potential of a gelling fibre (alginate), a fermentable fibre (resistant starch), and the combination of both, to modify feeding patterns (via satiation and satiety), and thereby long-term food intake and growth performance of ad libitum-fed growing pigs were assessed over 12 weeks. Pigs in all treatments achieved similar rates of weight gain. Pigs fed alginate compensated for the reduced dietary digestible energy (DE) supply by increasing their voluntary food intake, achieving similar DE intake as control pigs. Backfat thickness and efficiency of carcass growth were yet reduced in these animals, likely partly related to their increase in physical activity. Pigs fed resistant starch changed their feeding patterns, and took less, but larger meals than control pigs. Voluntary food intake was unaffected, but DE intake was reduced in pigs fed resistant starch. As rates of body weight gain were similar, this indicates that the energetic utilization of

resistant starch in pigs with ad libitum access to feed is closer to that of enzymatically digestible starch than usually assumed. Moreover, while carcass weight gain was reduced, colon and total gastro-intestinal tract (GIT) empty weight were increased in pigs fed resistant starch, reflecting increased fermentation. A more gradual energy supply via the increased availability of SCFA (as shown in Chapter 4) and potentially reduced activation of the immune system (as shown in Chapter 5) may underlie the increased efficiency found in pigs fed resistant starch. Effects of the interaction between resistant starch and alginate were rare. In spite of that, active behaviours such as standing and walking, aggressive behaviours, and oral behaviours, particularly feeder-directed and drinking, were generally reduced in pigs fed the combination treatment as compared with pigs fed alginate alone. Overall, in the long-term, pigs may compensate for a reduced dietary energy intake by increasing voluntary food intake (alginate), or they may become more efficient in the use of DE from resistant starch. Moreover, dietary fibres increase the contribution of the weight of the gastrointestinal tract to body weight and lead to changes in body composition (less fat more muscle), which may be relevant for the maintenance of lean weight in humans.

In conclusion, fermentable fibres are more satiating than viscous and bulking fibres. The effects are likely mediated by an increased SCFA production, and a reduced and attenuated glucose supply. The satiety-related hormones GLP-1 and PYY did not seem to play a role in the increased satiety induced by fermentation. Under unrestricted feeding conditions, dietary fibres promoting satiation (alginate) and satiety (resistant starch) did not reduce long-term food intake and total body weight gain, yet, colon empty weight was increased and carcass growth was reduced. This implies that changes in body composition and intestinal weight or content, rather than body weight and body mass index (BMI) alone may be relevant to fully acknowledge the effects of fibres to aid in maintaining or promoting healthy body weight in humans.

Samenvatting

Obesitas is een groot probleem voor de volksgezondheid. Het verhoogt het risico op cardiovasculaire en metabole ziekten. Ook bij gezelschapsdieren komt obesitas steeds meer voor, wat resulteert in soortgelijke gezondheidsproblemen als bij de mens. Hoewel obesitas niet een veel voorkomend probleem is bij landbouwhuisdieren, wordt voerbeperking gebruikt om, bijvoorbeeld bij dragende zeugen, de voerkosten laag te houden en reproductieve prestaties te bevorderen of, om bij bijvoorbeeld vleesvarkens, vervetting aan het einde van het mestperiode tegen te gaan. Voerrestrictie bij landbouwhuisdieren kan leiden tot honger en een verhoogde motivatie voor voeropname, wat kan resulteren in gedragsproblemen. Kennis over de regulering van lange-termijn verzadiging (bijvoorbeeld post-prandiale remming van de motivatie om te eten) is cruciaal om te helpen bij de beheersing van voedselinname bij de mens, en om het welzijn van beperkt gevoerde landbouwhuisdieren te verbeteren. Van voedingsvezels wordt vaak aangenomen dat ze de lange-termijn verzadiging stimuleren, maar resultaten van onderzoek naar de verzadigende effecten van voedingsvezels zijn inconsistent. De doeltreffendheid waarmee voedingsvezels lange-termijn verzadiging verbeteren varieert tussen studies. Dit kan mogelijk worden gerelateerd aan de variatie in fysisch-chemische eigenschappen van deze vezels, die ervoor zorgen dat de darminhoud zich anders gedraagt in het maagdarmkanaal of andere fysiologische effecten bewerkstelligen. Daarom was het doel van dit onderzoek om te bepalen in welke mate en hoe voedingsvezels met verschillende fysisch-chemische eigenschappen, zoals het vergroten van volume, viscositeit, gel vorming en fermenteerbaarheid, de lange-termijn verzadiging beïnvloeden. Hierbij werd het varken gebruikt als doeldier en als model voor de mens.

De invloed van verschillende typen vezels op lange-termijn verzadiging en mogelijke onderliggende mechanismen voor deze effecten werden onderzocht in vier studies. Als eerste werden verzadigende eigenschappen van vezels met verschillende fysisch-chemische eigenschappen onderzocht in twee gedragsstudies (hoofdstukken 2 en 3), bij beperkte voedering. Op basis van deze studies werd een fermenteerbare vezel (resistent zetmeel) geselecteerd voor een vervolgstudie naar de fysiologische en moleculaire mechanismen waarmee fermentatie de lange termijn-verzadiging zou kunnen beïnvloeden (hoofdstukken 4 en 5). In de laatste studie werden de lange-termijn effecten van een gelvormende vezel (alginaat) en een fermenteerbare vezel (resistent zetmeel), die naar verwachting, respectievelijk, verzadiging op korte en lange termijn, zouden bevorderen, op voeropnamepatronen en groei bestudeerd (hoofdstuk 6).

In hoofdstukken 2 en 3 werden volwassen zeugen gevoerd met verschillende typen voedingsvezels, in verschillende doseringen. In beide studies werden de dieren beperkt gevoerd. De voedingsvezels werden uitgewisseld tegen verteerbaar zetmeel. Hierdoor nam metaboliseerbare energieopname af met een toenemende

opname van de voedingsvezels. De proefopzet was een Latijns vierkant, dus dieren waren hun eigen controle. De motivatie om te eten werd op verschillende tijdstippen van de dag bepaald door middel van gedragsobservaties en gedragstesten. In hoofdstuk 2 werd gevonden dat voer met een visceuze vezel (pectine) de motivatie om te eten gedurende de hele dag verhoogde, terwijl een voer met een volume-vergrotende vezel (lignocellulose) de motivatie om te eten verminderde tot meer dan 3 uur na de maaltijd, en een voer met een fermenteerbare vezel (resistent zetmeel) de motivatie om te eten verminderde tot 7 uur na de maaltijd. Zowel lignocellulose als resistent zetmeel waren in staat de effecten van de verminderde energieopname op verzadiging te compenseren. Uit hoofdstuk 2 blijkt dat pectine het minst lang een verzadigend effect had, terwijl lignocellulose en resistent zetmeel de meest verzadigende vezels waren.

In de studie beschreven in hoofdstuk 3 verminderden voeders met een hoog gehalte aan fermenteerbare vezels (guargom, inuline, en resistent zetmeel) de motivatie van de zeugen om te eten gedurende de hele dag. Dit betekent dat al deze fermenteerbare vezels het effect van een verminderde metaboliseerbare energie in vergelijking met het controle voer compenseerden. Resistent zetmeel had het sterkste remmende effect op de motivatie om te eten. Bij een hoog gehalte aan resistent zetmeel in het voer liepen varkens minder snel naar een voerbeloning in een zogenaamde 'runway test' en bovendien aten ze minder in de onbeperkte voerinname test. De combinatie van deze resultaten suggereert dat fermenteerbare vezels in het voer de lange-termijn verzadiging gedurende de gehele dag verhoogden en dat resistent zetmeel de meest lange-termijn verzadigende vezel was van alle geteste vezels.

De verhoogde lange-termijn verzadiging van lignocellulose (hoofdstuk 2) werd waarschijnlijk veroorzaakt door een vertraagde darmpassagesnelheid, resulterend in een toename in vulling van de dunne en dikke darm. Meer onderzoek is nodig om deze hypothese te testen. De verlenging van de verzadiging gevonden met resistent zetmeel in hoofdstuk 2 en 3, kan gedeeltelijk worden toegeschreven aan een langzame fermentatie en een hoge productie van vluchtige vetzuren uit resistent zetmeel in vergelijking met andere fermenteerbare vezels. Op basis van deze resultaten en die van eerdere studies, was de hypothese in hoofdstuk 4 dat de door resistent zetmeel-geïnduceerde lange-termijn verzadiging kan worden toegeschreven aan een (1) combinatie van een verhoogde productie van vluchtige vetzuren en een verminderd glucose-aanbod, (2) door vluchtige vetzuren gestimuleerde afgifte van hormonen zoals glucagon-like peptide-1 (GLP-1) en peptide tyrosine tyrosine (PYY) door entero-endocriene cellen die lange-termijn verzadiging bevorderen, of (3) effect op serotonine metabolisme.

In hoofdstuk 4 werden de fysiologische en moleculaire mechanismen die ten grondslag liggen aan de relatie tussen fermentatie en de lange-termijn verzadiging

onderzocht in een cross-over studie. Vleesvarkens kregen een controle voer met ontsloten (gemakkelijk verteerbaar) zetmeel of een voer met een hoog gehalte aan resistent zetmeel. De energie verteerbaarheid en metaboliseerbaarheid waren 6% lager bij varkens die resistent zetmeel kregen dan van varkens die het ontsloten zetmeel voer kregen, en hun metaboliseerbare energie (ME) opname was 3% lager. Ondanks de lagere ME opname, bleek op basis van gedragsobservaties dat resistent zetmeel de lange-termijn verzadiging verbeterde: we vonden bijvoorbeeld een afname in voer-gericht gedrag en drinkgedrag. Zoals verwacht ging het lange-termijn verzadigende effect van resistent zetmeel gepaard met verhoogde vluchtige vetzuren concentraties in het plasma over de hele dag en verminderde postprandiale plasma glucose en insuline concentraties. GLP-1 plasmaspiegels waren lager bij varkens gevoerd met resistent zetmeel, terwijl PYY plasmaspiegels niet werden beïnvloed. GLP-1 en PYY spiegels na de maaltijd lijken dus niet gerelateerd aan de verhoogde lange-termijn verzadiging van resistent zetmeel. Lage bloedserotonine spiegels na inname van voer met resistent zetmeel suggereerden een verschil tussen verschillende voertypen in afgifte van serotonine door de darmwand. Bovendien hadden varkens gevoerd met resistent zetmeel hogere plasma tryptofaan spiegels. Deze zouden mogelijk kunnen leiden tot hogere serotonine niveaus in het brein, wat correspondeert met het verminderde voer-gericht gedrag van deze dieren. Interessant is dat hoge plasma triglyceride spiegels gerelateerd zijn aan verhoogde vluchtige vetzuur-spiegels bij varkens gevoerd met resistent zetmeel. De rol van serotonine, tryptofaan en triglyceriden in het lange-termijn verzadigende effect van resistent zetmeel zijn nog onduidelijk en moeten verder onderzocht worden.

Darmepitheelcellen in het begin van de dikke darm van varkens die resistent zetmeel kregen hadden een verlaagde expressie van genen betrokken bij immuunreacties en een verhoogde expressie van genen betrokken bij metabole processen zoals vetzuur- en energiemetabolisme (hoofdstuk 5). Resistent zetmeel verhoogde de aanwezigheid van een aantal soorten darm-geassocieerde bacteriën waarvan wordt aangenomen dat ze bijdragen aan darmgezondheid, bijvoorbeeld omdat ze boterzuur produceren (bijv. *Faecalibacterium prausnitzii* en *Megasphaera elsdenii*). Het voeren van resistent zetmeel verminderde het aantal soorten potentieel pathogene bacteriën (bijv. *Actinobacillus indolicus* en *Pseudomonas*) aan het begin van de dikke darm. We vonden geen verschil in de concentratie vluchtige vetzuren in de inhoud van het begin van de dikke darm. Echter, het aandeel vluchtige vetzuren met vertakte-ketens, bv. iso-butyraat en iso-valeraat, was lager in varkens gevoerd met resistent zetmeel, wat impliceert dat deze microbiota voornamelijk resistent zetmeel als energiebron gebruiken, terwijl microbiota van varkens die ontsloten zetmeel aten meer eiwit als energiebron gebruiken. Zoals eerder vermeld werden verhoogde vluchtige vetzuur-concentraties

in het plasma waargenomen na consumptie van resistent zetmeel. Bovendien bleken potentieel pathogene microbiële groepen negatief gecorreleerd met plasma concentraties van vluchtige vetzuren en met genen betrokken bij vetzuurmetabolisme. Deze resultaten geven aan dat resistent zetmeel, naast het lange-termijn verzadigend effect, mogelijk een gunstige invloed heeft op de gezondheid van de dikke darm. De in hoofdstuk 5 beschreven studie geeft nieuwe inzichten in de moleculaire mechanismen achter de gunstige effecten van resistent zetmeel.

In hoofdstuk 6 werden de effecten van een gelvormende vezel (alginaat), een fermenteerbare vezel (resistent zetmeel), en de combinatie van beide op voeropnamepatronen, voeropname en groei van onbeperkt gevoerde vleesvarkens bestudeerd over een periode van 12 weken. Varkens in alle behandelingsgroepen groeiden even snel. Varkens gevoerd met alginaat compenseerden de verminderde verteerbare energie uit voer door het verhogen van hun voeropname, waardoor ze een vergelijkbare verteerbare energieopname hadden als de controle varkens. Spekdikte en de efficiëntie van karkasgroei waren verlaagd bij de alginaat-gevoerde dieren, wat waarschijnlijk deels kwam doordat ze actiever waren. Varkens gevoerd met resistent zetmeel veranderden hun voeropnamepatroon; ze aten minder frequent, maar wel grotere maaltijden dan de controle varkens. De voeropname werd niet beïnvloed, maar hun verteerbare energie inname was daardoor wel lager. De groeisnelheid was echter vergelijkbaar met die van de controle varkens. Dit betekent dat de energetische benutting van resistent zetmeel bij onbeperkt gevoerde varkens dichter bij die van enzymatisch verteerbaar zetmeel ligt dan gewoonlijk aangenomen wordt. De gewichtstoename van het karkas verminderde, terwijl het leeggewicht van dikke darm en totale maagdarmkanaal hoger was bij varkens gevoerd met resistent zetmeel. Een beter gespreide energievoorziening binnen de dag en de verminderde activatie van het immuunsysteem (zie hoofdstuk 5) vormen mogelijke verklaringen voor de observatie dat resistent zetmeel efficiënter gebruikt wordt voor groei dan tot nu toe werd aangenomen. Er waren weinig interacties tussen resistent zetmeel en alginaat. Echter, varkens gevoerd met de combinatie behandeling vertoonden minder actief gedrag, zoals staan en lopen, minder agressief gedrag en minder oraal gedrag, in het bijzonder voer-gericht gedrag en drinken, in vergelijking met varkens gevoerd met alleen alginaat. Kortom, op de lange termijn compenseren varkens voor een verminderd energiegehalte van het voer door hun voeropname te verhogen (alginaat), of ze kunnen efficiënter gebruik maken van verteerbare energie uit resistent zetmeel. Bovendien verhogen voedingsvezels het aandeel van het maagdarmgewicht in het lichaamsgewicht en veranderen ze de lichaamssamenstelling (minder vet t.o.v. spier), wat voor gewichtsbeheersing bij mensen relevant kan zijn.

Concluderend zijn fermenteerbare vezels, als ze verteerbaar zetmeel vervangen, meer verzadigend op lange-termijn dan visceuze en volume-vergrotende vezels. De effecten worden waarschijnlijk gemedieerd door een verhoogde productie van vluchtige vetzuren en terwijl de glucose-absorptie afneemt. De energievoorziening wordt dan ook meer gespreid over de dag. De hormonen GLP-1 en PYY, gerelateerd aan lange-termijn verzadiging, lijken geen rol te spelen in dit proces. Bij onbeperkte voerverstrekking leveren voedingsvezels die zorgen voor korte-termijn verzadiging (alginaat) en lange-termijn verzadiging (resistent zetmeel) geen verlaging op van de voeropname en groeisnelheid. Desondanks werd het leeggewicht van de dikke darm verhoogd en karkasgroei verlaagd. Dit impliceert dat gegevens over darmgewicht en lichaamssamenstelling een belangrijke aanvulling zijn op lichaamsgewicht en body mass index (BMI) als je de mogelijkheden wilt bestuderen om met vezels de handhaving van een gezond lichaamsgewicht te bevorderen.

Sumário

A obesidade se tornou um importante problema de saúde pública em humanos, devido ao risco elevado para desenvolvimento de doenças cardiovasculares e metabólicas. A ocorrência de obesidade em animais de companhia também aumentou, resultando em problemas de saúde similares aos que ocorrem em seres humanos. Embora a obesidade não seja um problema comum em animais de produção, a restrição alimentar é utilizada para reduzir o custo de alimentação e manter o desempenho reprodutivo de, por exemplo, porcas gestantes, ou para assegurar deposição desejável de gordura e de carne magra na carcaça de suínos de engorda. A restrição alimentar pode resultar em fome e aumento da motivação alimentar, que estão associados à problemas comportamentais. O entendimento da regulação da saciedade (ou seja, da inibição da motivação alimentar pós-prandial) é fundamental para auxiliar no controle da ingestão alimentar em humanos, e melhorar o bem-estar de animais de produção submetidos a restrição alimentar. Vários estudos têm relacionado o consumo de fibras alimentares ao aumento da saciedade, mas existem algumas inconsistências nos resultados. A variável eficácia das fibras alimentares no aumento da sensação de saciedade pode estar relacionada às propriedades físico-químicas da fibra alimentar, que levam a diferentes efeitos no trato gastrointestinal. Portanto, o objetivo deste trabalho foi identificar se e como fibras alimentares com diferentes propriedades físico-químicas, tais como capacidade de reter água (CRA), viscosidade, gelificação e fermentação, afetam a saciedade no porco doméstico, que foi utilizado como um modelo para o ser humano e como um animal alvo.

Os efeitos de diferentes fibras alimentares sobre a saciedade e possíveis mecanismos subjacentes a estas ações foram investigados em quatro estudos. Em primeiro lugar, a capacidade de prolongar a sensação de saciedade pós-prandial de fibras com propriedades físico-químicas variáveis foi avaliada em dois estudos abordando medidas comportamentais da saciedade (Capítulos 2 e 3). Com base nos resultados destes estudos, uma fibra fermentável (amido resistente) foi selecionada com base no seu potencial de prolongar a saciedade, e utilizada num estudo subsequente para avaliar possíveis mecanismos fisiológicos e moleculares pelos quais a fermentação colônica pode afetar a saciedade (Capítulos 4 e 5). No último estudo, os efeitos a longo prazo de uma fibra gelificante (alginato) capaz de promover saciedade de curto prazo ou saciação, i.e. inibição do consumo alimentar durante a refeição, e uma fibra fermentável (amido resistente) capaz de promover saciedade de longo prazo, i.e. após a refeição, sobre os padrões de alimentação e desempenho de suínos na fase de crescimento foram avaliados (Capítulo 6).

Nos Capítulos 2 e 3, a motivação alimentar de porcas adultas alimentadas com diferentes fibras alimentares testadas em dois conteúdos foi avaliada em um delineamento Quadrado Latino, por meio de observações e testes comportamentais aplicados em diferentes momentos do dia. Em ambos os estudos, os animais foram

submetidos a restrição alimentar qualitativa, sendo alimentados com dietas em que fontes de fibras purificadas foram trocadas por amido pré-gelatinizado com base no teor médio de matéria seca, reduzindo assim o consumo de energia metabolizável. No Capítulo 2, as dietas de fibra viscosa (pectina) aumentaram a motivação alimentar ao longo do dia, enquanto que as dietas de fibra de 'enchimento' ou alta CRA (lignocelulose) reduziram a motivação alimentar por mais de 3 horas após a alimentação, e dietas de fibra fermentável (amido resistente) reduziram a motivação alimentar por até 7 horas após a alimentação. Em relação à dieta controle, ambos lignocelulose e amido resistente foram capazes de superar os efeitos da redução da oferta de energia dietética. A combinação destes resultados sugere que a pectina foi a fibra menos eficaz, ao passo que a lignocelulose e o amido resistente foram as fibras mais eficazes no aumento da saciedade.

No Capítulo 3, as dietas de alto conteúdo de fibras fermentáveis (goma guar, inulina, e amido resistente) reduziram a motivação alimentar ao longo do dia, o que significa que todas as fibras fermentáveis contrabalancearam com sucesso os efeitos da redução da oferta de energia dietética em comparação com a dieta controle. A redução da motivação alimentar com um aumento do conteúdo de fibra na dieta foi mais pronunciada com o amido resistente, pois porcas caminharam com menor velocidade até uma pequena recompensa alimentar no teste de corrida ('runway test') e mostraram tendência de redução do consumo alimentar no teste de consumo alimentar voluntário quando alimentadas com dietas de alto conteúdo de amido resistente. A combinação destes resultados sugere que um conteúdo elevado de fibras fermentáveis na dieta resulta em maior saciedade ao longo do dia, sendo o amido resistente a fibra mais eficaz no aumento da saciedade entre todas as fibras testadas.

O aumento da saciedade encontrado com lignocelulose no Capítulo 2 foi provavelmente mediado pela diminuição da velocidade de passagem intestinal, resultando num aumento de volume no lúmen dos intestinos delgado e grosso. Não obstante, mais estudos serão necessários para testar esta hipótese. O aumento da saciedade encontrado com o amido resistente no Capítulo 2, e em particular no Capítulo 3, foi atribuído em parte a uma taxa de fermentação lenta e a uma elevada produção de ácidos graxos de cadeia curta (AGCC) observadas com o amido resistente em comparação com outras fibras fermentáveis. Com base nestes resultados e nos resultados de estudos anteriores, no Capítulo 4, foi levantada a hipótese de que a saciedade induzida pelo amido resistente poderia ser atribuída a (1) aumento da produção de AGCC combinado a diminuição das concentrações de glicose plasmática, (2) estimulação por via de AGCC da secreção dos hormônios anorexígenos GLP-1 (peptídeo 1 semelhante ao glucagon) e PYY (peptídeo tirosina-tirosina) a partir de células enteroendócrinas, ou (3) efeitos sobre o metabolismo da serotonina.

No Capítulo 4, os mecanismos fisiológicos e moleculares pelos quais a fermentação pode afetar a saciedade foram investigados em um estudo invasivo. Suínos em crescimento foram alimentados com uma dieta controle rica em amido pré-gelatinizado ou uma dieta rica em amido resistente em um delineamento cruzado ('crossover'). Digestibilidade e metabolizabilidade de energia foram 6% inferiores na dieta de amido resistente em comparação com a dieta controle, e o consumo de energia metabolizável foi 3% menor em suínos alimentados com a dieta de amido resistente do que em suínos alimentados com a dieta controle. Apesar de um baixo consumo de energia metabolizável, o amido resistente parece ter aumentado a saciedade com base em observações de comportamento, ou seja, houve redução dos comportamentos direcionados ao comedouro e do comportamento de bebida durante 24 horas. Como esperado, os efeitos do amido resistente sobre a saciedade coincidiram com o aumento das concentrações plasmáticas de AGCC durante 24 horas e com a redução das concentrações plasmáticas de glicose e insulina pós-prandiais. As concentrações plasmáticas de GLP-1 foram menores nos suínos alimentados com amido resistente, ao passo que as concentrações plasmáticas de PYY não foram afetadas pelo amido resistente. Portanto, as respostas pós-prandiais de GLP-1 e PYY provavelmente não foram causativas do aumento da saciedade encontrado com o amido resistente. Baixas concentrações sanguíneas de serotonina após o consumo de amido resistente sugeriram uma diferença na secreção intestinal de serotonina entre as dietas. Além disso, os suínos alimentados com amido resistente tiveram concentrações plasmáticas de triptofano elevadas, potencialmente levando a maiores concentrações de serotonina no cérebro, o que poderia reforçar a redução do comportamento alimentar encontrada nesses animais. Curiosamente, elevadas concentrações plasmáticas de triglicerídeos foram relacionadas ao aumento das concentrações plasmáticas de AGCC em suínos alimentados com amido resistente. O envolvimento da serotonina, do triptofano e de triglicerídeos nos efeitos do amido resistente sobre a saciedade ainda é pouco entendido e necessita de mais estudo.

No nível molecular (descrito no Capítulo 5), análises de expressão gênica em células epiteliais do início do intestino grosso revelaram uma expressão reprimida dos genes envolvidos em respostas imunes, e uma expressão estimulada dos genes envolvidos em processos metabólicos, tais como o metabolismo energético e o de ácidos graxos, após o consumo de amido resistente. No que diz respeito à variação microbiana, o amido resistente aumentou a abundância de bactérias associadas a saúde do intestino (por exemplo, *Faecalibacterium prausnitzii* e *Megasphaera elsdenii*, reconhecidas anteriormente como produtoras de butirato), e diminuiu a abundância de bactérias potencialmente patogênicas (por exemplo, *Actinobacillus indolicus* e *Pseudomonas*) no início do intestino grosso. Não houve diferença nas

concentrações de AGCC encontradas nas digestas de intestino grosso de suínos alimentados com amido resistente em comparação com os suínos alimentados com amido pré-gelatinizado. No entanto, a percentagem de ácidos graxos de cadeia ramificada (ou seja, iso-butirato e iso-valerato) foi inferior nas digestas de suínos alimentados com amido resistente, o que implica a utilização predominante de amido resistente como fonte de energia por bactérias, enquanto que na dieta de amido pré-gelatinizado a fonte de energia utilizada por bactérias foi predominantemente protéica. Como mencionado anteriormente, foi observado um aumento das concentrações plasmáticas de AGCC após o consumo de amido resistente. Além disso, foi observada correlação inversa e significativa entre grupos de bactérias potencialmente patogênicas e concentrações plasmáticas de AGCC, e entre grupos de bactérias potencialmente patogênicas e genes envolvidos no metabolismo de ácidos graxos. A combinação destes resultados sugere que, além dos efeitos sobre a saciedade, o amido resistente tem um efeito benéfico sobre a saúde do intestino. Em geral, o estudo descrito no Capítulo 5 forneceu novas percepções dos mecanismos moleculares envolvidos nos potenciais efeitos benéficos do amido resistente à saúde.

No capítulo 6, o potencial de uma fibra gelificante (alginato), uma fibra fermentável (amido resistente), e a combinação de ambas, para modificar os padrões de alimentação (via saciação e saciedade), e assim também o consumo alimentar de longo prazo e o desempenho produtivo na fase de crescimento de suínos com alimentação à vontade foi avaliado ao longo de 12 semanas. Em todas as dietas os animais alcançaram taxas similares de ganho de peso. Suínos alimentados com alginato compensaram a oferta dietética reduzida de energia digestível (ED) aumentando o consumo alimentar voluntário, atingindo semelhante consumo de ED como suínos alimentados com dieta controle (sem fibra). Espessura de toucinho e eficiência de ganho de carcaça foram, contudo, reduzidas nestes animais, provavelmente, relacionado em parte ao aumento na atividade física destes animais. Suínos alimentados com amido resistente mudaram seus padrões de alimentação, e consumiram ao longo do dia menos, mas maiores (maior quantidade) porções de alimento do que os suínos alimentados com dieta controle. O consumo alimentar voluntário não foi afetado, mas o consumo de ED foi reduzido nos suínos alimentados com amido resistente. Como as taxas de ganho de peso foram semelhantes, isso indica que a utilização energética do amido resistente em suínos com alimentação à vontade pode estar mais perto daquela do amido digerível através da hidrólise enzimática. Além disso, enquanto o ganho de peso de carcaça foi reduzido, o peso total do intestino grosso e do trato gastrointestinal (TGI) vazios foram aumentados em suínos alimentados com amido resistente, o que reflete o aumento de fermentação colônica. Uma oferta de energia mais gradual através do aumento da disponibilidade de AGCC (como mostrado no

Capítulo 4) e, potencialmente, uma reduzida ativação do sistema imunitário (como mostrado no Capítulo 5) pode constituir a base do aumento da eficiência energética encontrada em suínos alimentados com amido resistente. Efeitos da interação entre amido resistente e alginato foram raras. Apesar disso, os comportamentos ativos, tais como das atividades de pé e de caminhar, comportamentos agressivos, comportamentos direcionados ao comedouro e comportamento de bebida, foram geralmente reduzidos em suínos alimentados com a combinação de amido resistente e alginato, mas apenas quando comparado com suínos alimentados com alginato sozinho. Em geral, a longo prazo, os animais podem compensar uma diminuição da oferta de energia dietética por via do aumento da ingestão voluntária de alimento (dieta de alginato), ou podem se tornar mais eficientes na utilização de ED obtida a partir de amido resistente. Além disso, as fibras dietéticas podem aumentar a contribuição do peso do TGI para o peso corporal total, e levar a alterações na composição corporal (menos gordura mais músculo), que podem ser importantes para a manutenção de peso saudável e a prevenção da obesidade em seres humanos.

Em conclusão, as fibras fermentáveis resultaram em maior saciedade em comparação a fibras viscosas e de alta CRA. Os efeitos são provavelmente mediados por um aumento da produção de AGCC e uma diminuição da oferta de glicose. Os hormônios anorexígenos GLP-1 e PYY aparentemente não estão relacionados ao aumento da saciedade induzida pela fermentação colônica. Sob condições de alimentação irrestritas, fibras alimentares que promovem a saciação (alginato) e a saciedade (amido resistente) não reduziram o consumo alimentar de longo prazo e nem o ganho de peso total, no entanto, o peso do intestino grosso vazio foi aumentado e o ganho de carcaça foi reduzido. Isto implica que as mudanças na composição corporal e no peso ou conteúdo intestinal, ao invés de peso corporal e índice de massa corporal (IMC) sozinhos podem ser relevantes para o reconhecimento pleno dos efeitos das fibras para ajudar na manutenção ou promoção de peso corporal saudável em humanos.

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[1]I assumed that my academic educational background would not adequately match the background needed to put such a multidisciplinary PhD project into practice, combining knowledge from animal nutrition, physiology and behaviour. Later I realized that I would not need to do a PhD if I already knew everything about it in advance.

extreme patience. Bas, your more distant supervision with well-timed discussions to assess the progress of my research gave me a feeling of independence which made my life as a PhD student very pleasant. With your outstanding ability to have a positive view at disappointing results, and to manage the difficulties encountered during the IP/OP project, you kept me motivated to finish the task. I am very grateful for that, and I am proud having you both as a supervisor and as a promotor. Thank you also for your great pragmatism, enthusiasm, trust and commitment at all times. Finally, thanks for always having a 'minute' for me!

Special thanks go to all other research groups involved in the "Satiety & Satisfaction" programme, including the colleagues of the Human Nutrition & Epidemiology Group plus Eating Behaviour Group at the Division of Human Nutrition: Anne Wanders, Monica Mars, Edith Feskens and Kees de Graaf; the colleagues of the Laboratory of Food Chemistry: Melliana Jonathan and Henk Schols; the colleagues of the Laboratory of Microbiology: Odette Pérez Gutiérrez, Jing Zhang and Hauke Smidt; the colleagues of Plant Research International: Ingrid van der Meer and Jeroen van Arkel; and particularly the colleagues of the Nutrition, Metabolism & Genomics Group at the Division of Human Nutrition: Daniëlle Haenen, Guido Hooiveld and Michael Müller. Thanks to your expertise and collaboration we could apply an integral approach from molecule to organism to try to increase understanding of mechanisms in which dietary fibres would regulate satiety in pigs. Daniëlle, thanks for all the help during the behavioural studies in Wageningen and during the invasive physiological study in Lelystad. The assistance from Katja Lange (PhD student) of the Nutrition, Metabolism & Genomics Group at the end of the invasive study in Lelystad is also greatly appreciated. Daniëlle and Guido, the invasive study in Lelystad was really unforgettable! Thanks for all the support and guidance during the process of planning, performing and finalizing this study. My special thanks are extended to the colleagues of the Animal Science Group in Lelystad. Sietse Jan Koopmans, Jan van der Meulen, Dirk Anjema, John Jansen, Gerrit Jan Deetman, Gerald Lok, Ruud Dekker, and all other personnel of experimental facilities in Lelystad, thank you so much for all the help carrying out this invasive study. Your help and expertise were indispensable! Sietse Jan, thanks for the critical comments and valuable suggestions during the various meetings and discussions about the results obtained from the invasive study. You were also my supervisor for some time, which I really appreciated. I have learned a lot from you, particularly about Animal Physiology and Biochemistry. Without your knowledge and help I would never have got explanations for the intriguing increased plasma triglyceride levels found in pigs fed resistant starch. You also have a great sense of humour, thanks for that.

Not only in Lelystad, but also in Wageningen many people contributed to the preparations and performance of behavioural tests during the first two experiments

in the old experimental farm ‘de Haar’. I am particularly grateful for the assistance given by Elske de Haas, Tamara Bergstra, Monique Ooms and Fleur Bartels. Elske and Tamara, thanks for accepting the task of student assistant, and for all the help with data collection at ‘de Haar’. Monique and Fleur, how can a student of Liesbeth get started with a behavioural study without you? You are always actively supporting students in experiments and even more important, in video-recording and observing animal behaviour. Thanks to your help we could make the most of our behavioural studies. Thanks for your enthusiasm and flexibility. I also want to thank all other colleagues and students, in particular Inonge Reimert, Sven Alferink, Tamme Zandstra, Carolina Kyriacou, Chantal van Merwijk, Patricia van Wiechen and Suzan Vleeming for their help during the behavioural tests. My special thanks are extended to Ben van den Top, Ries Verkerk and all other animal caretakers of ‘de Haar’ for taking care of the pigs and assisting in the stables.

In addition to the practical work in the stables, I have received help from many people, directly or indirectly, with laboratory work. I would like to offer my special thanks to the colleagues of the Division of Human Nutrition: Shohreh Keshtkar and Jenny Jansen for their assistance with microarray and laboratory analyses; to the colleagues of the Adaptation Physiology Group: Rudie Koopmanschap, Ger Reilingh, Merel Verhoeven, Iris van der Heden, Monique Ooms and Marije Oostindjer for their assistance with serotonin and MAO measurements, plus insulin and leptin measurements (for the latter two particularly Rudie); to the colleagues of the Animal Nutrition Group: Saskia van Laar-van Schuppen, Jane Martine Muijlaert, Michel Breuer, Tamme Zandstra, Niels Wever and Erika Beukers-van Laar for their assistance with samples preparation and laboratory analyses for feed, digesta, faeces and urine samples; to the colleague Lisette Ruuls van Stalle (Pathobiology Group, Department of Infection Biology, Central Veterinary Institute, Wageningen UR) for assistance with platelets counting in whole blood and plasma; and to the colleague Jacques Vervoort (Biochemistry Group) for assistance with analysing plasma samples using NMR spectroscopy.

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personnel of the Nutreco Swine Research Centre in Sint Anthonis for their assistance during data collection in this study. The assistance from other colleagues and students, in particular Sven Alferink, Tamme Zandstra, Mascha Sappok, Anne Burgers, Devi Hermsen and Lian Stappers is also greatly appreciated.

Throughout the studies described in this thesis, after collecting and editing all data, the next step was its statistical analysis. Thanks also to all people who helped me in this step, among which Gerrit Gort and Evert Jan Bakker (Mathematical and Statistical Methods Group, Biometris). My special thanks are extended to Lian Stappers, who did an outstanding job at her MSc thesis analysing the feeding patterns of growing pigs in the last experiment at Nutreco, which contributed significantly to this thesis.

In addition to the practical work in the stables, in the laboratory and behind the computer on my own, I have spent many hours with colleagues at Zodiac, especially with the colleagues of the Adaptation Physiology Group (ADP). All serious and less serious conversations made the PhD-research work more pleasant! Thanks for the 'gezelligheid' during coffee, lunch, drinks, and staff outings. I want to mention a number of colleagues in particular. First, my roommates and fellow PhD students at the old Zodiac building (no. 531): Annette Boerlage, Els Broens, Lia Hoving, and Anne Wientjes. Lia, you presented me to all other ADP fellows on my very first day of work, thanks for that! Anne, as PhD student, and now also as Doctor, you were always reachable at all times I needed your advice and help. Thanks a lot! In addition, my new roommates and fellow PhD students at the new Zodiac building (no. 122): Marielle Bruijnis (now Post-doctoral researcher!), Juncai Chen, Elske de Haas (one of my paranymphs!), Danny de Koning (our Statistics guru!), Conny Maatjens, Novi Mayasari, Ampai Nangsuay and Sofie van Nieuwamerongen. Thank you all for the wonderful time and fun that I experienced during and outside working hours. I wish you all good luck with your future plans. At last, Nanette van Hapert and Lora Bor-van der Kleijn, thank you for your help in administrational issues. As many others have said, it is wonderful having you as secretaries at ADP. My special thanks are extended to the colleagues of the Animal Nutrition Group (ANU), including the fellow PhD students: Harma Berends, Esther Kampman-van de Hoek (since our MSc studies!), Lotte van Rooijen, Mascha Sappok (now Doctor!) and Sonja de Vries; and secretaries: Betty Looijen and Yvonne van Holland. It is great knowing you all! Furthermore, I want to acknowledge all other fellow PhD students (including the graduated) from ADP, ANU and other chair groups I did not mention here. Thanks for the highly entertaining conversations, and sorry for not thanking you by name. I mean all of you as well ☺

Outside the university setting, there was the Brazilian community in Wageningen, to which I also would like to say a very special thanks! Queridos

colegas e amigos brasileiros, muito obrigada pelos encontros no 'De Vlaamsche Reus', jantares, churrascos, festas e comemorações de aniversários maravilhosos, e principalemente pela companhia, carinho e amizade! Infelizmente não será possível nomear todos, mas agradeço a Anabele & Wiebe, Ana Carolina & William, Ana Paula & Rodrigo, Aníbal, Beatriz, Betânia, Bruno & Fernanda, Carla, Carols (Castro, Mosca e Soares), Charles, Cinara & Janio, Claire, Daniel, Deborah, Dorys & Richard, Edilaine & Lucas, Fabio, Fernanda & Mauricio, Fhernanda, Filipe, Flavia & Rijk, Flavia Talarico, Gislene, Glaciela, Gustavo & Simone, Haissa, Isabel (portuguesa), Isabela, Julio, Jullyana & Flávio, Kleibe & Lívia, Larissa, Luciana, Marcio, Marcos, Maria Fernanda, Maria Marta, Marina & Felipe, Martha (cubana), Murilo, Odair, Priscilas (Rossetto e Sabadin), Rafael, Rita & Celso, Sandra & Omar, Sandrine, Saulo & Suzanne, Sidnei, Tamara, Tatiana & Andre, Thiago, Ulisses, Vania & Manoel, Zany & Anderson, e muitos outros que conheci em Wagenigen por estarem presentes, mesmo que fosse por pouco tempo, na minha vida em Wageningen nesses último anos.

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Curriculum vitae

Carol Souza da Silva was born on August 8, 1981 in Belém, Pará, Brazil. After completing secondary school at 'Colégio Gentil Bittencourt' in Belém, she obtained her BSc degree in Veterinary Medicine at the Universidade Federal Rural da Amazônia (UFRA) in 2005. During her BSc studies, Carol was student assistant at the Laboratory of Pharmacology of the Institute of Animal Health and Production at UFRA for nearly two years. In this laboratory she worked on her BSc thesis entitled "Evaluation of the therapeutic efficacy of *Mentha crispa* L. in the treatment of giardiasis in dogs". In 2006, Carol went to The Netherlands to pursue her MSc degree in Animal Sciences at Wageningen University. Her first master thesis investigated the effects of housing conditions on the behaviour and welfare of growing pigs in The Netherlands. Her second master thesis investigated the reasons why smallholder dairy farmers do or do not organize in cooperatives in the southeast region of Brazil. After receiving her MSc degree in 2008, Carol started her PhD research at the Adaptation Physiology Group (ADP) and Animal Nutrition Group (ANU) of Wageningen University aimed at investigating whether and how dietary fibres with different physicochemical properties affect satiety in pigs, used both as a model for humans and as a target animal. Her research was part of the IP/OP strategic research programme "Satiety & Satisfaction" of Wageningen University and Research Centre. The results of her PhD research are presented in this thesis. Carol presented her work at 18 (inter)national conferences and workshops and has a broad international network. Her work is of very high quality, and she is (co)author of 5 papers in scientific journals, and has 3 papers submitted. Carol won several prizes with her PhD-thesis work such as best poster presentation, best young researcher, and best oral presentation. Besides research, Carol has been active in education (supervisor of 6 MSc and 1 BSc theses) and within WIAS, she attended the WIAS Science Day yearly (2009-2013), and was one of the organisers of the WIAS Science Day (2010-2011) and of the WIAS Seminar 'Scientific Research in Animal Welfare: Do we make a difference?' (2011). Carol is a member of the International Society for Applied Ethology (ISAE) since 2010, and this year she won a travel grant to participate in the 47th ISAE Congress in Brazil. After obtaining her PhD degree Carol is keen on pursuing a career in science, preferably with animal behaviour and welfare in relation to nutrition as main expertise. She was granted a WIAS fellowship to write a collaborative (ADP and ANU) Postdoc proposal. She will remain working at Wageningen University for at least 3 months. She can be reached by e-mail at: carol.souza@live.nl.

Publications

Refereed scientific journals

Haenen, D., C. **Souza da Silva**, J. Zhang, S. J. Koopmans, G. Bosch, J. Vervoort, W. J. J. Gerrits, B. Kemp, H. Smidt, M. Müller, and G. J. E. J. Hooiveld (2013). Dietary resistant starch improves mucosal gene expression profile and luminal microbiota composition in porcine colon. Journal of Nutrition (Submitted for publication).

Haenen, D., J. Zhang, C. **Souza da Silva**, G. Bosch, I. M. van der Meer, J. van Arkel, J. J. G. C. van den Borne, O. Pérez Gutiérrez, H. Smidt, B. Kemp, M. Müller, and G. J. E. J. Hooiveld (2013). A diet high in resistant starch modulates microbiota composition, SCFA concentrations, and gene expression in pig intestine. Journal of Nutrition 143: 274-283.

Jonathan, M. C., D. Haenen, C. **Souza da Silva**, G. Bosch, H. A. Schols, and H. Gruppen (2013). Influence of a diet rich in resistant starch on the degradation of non-starch polysaccharides in the large intestine of pigs. Carbohydrate Polymers 93: 232-239.

Souza da Silva, C., G. Bosch, J. E. Bolhuis, L. J. N. Stappers, H. M. J. van Hees, W. J. J. Gerrits, and B. Kemp (2013). Potential of alginate and resistant starch to modify feeding patterns and performance in ad libitum-fed growing pigs. Journal of Animal Science (Submitted for publication).

Souza da Silva, C., D. Haenen, S. J. Koopmans, G. J. E. J. Hooiveld, G. Bosch, J. E. Bolhuis, B. Kemp, M. Müller, and W. J. J. Gerrits (2013). Effects of resistant starch on behaviour, satiety-related hormones and metabolites in growing pigs. Animal (Submitted for publication).

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2013). Effects of dietary fibers with different fermentation characteristics on feeding motivation in adult female pigs. Physiology & Behavior 110–111: 148-157.

Jonathan, M. C., J. J. G. C. van den Borne, P. van Wiechen, C. **Souza da Silva**, H. A. Schols, and H. Gruppen (2012). In vitro fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. Food Chemistry 133: 889-897.

Souza da Silva, C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2012). Effects of dietary fibers with different physicochemical properties on feeding motivation in adult female pigs. Physiology & Behavior 107: 218-230.

Conference proceedings and abstracts

Souza da Silva, C., G. Bosch, J.E. Bolhuis, L.J.N. Stappers, H.M.J. van Hees, W.J.J. Gerrits, and B. Kemp (2013). Effects of alginate and resistant starch on feeding patterns, behavior and growth in growing pigs. In: Proceedings of the 47th Congress of the International Society for Applied Ethology (ISAE), Florianópolis, Brazil: 02-06-2013/ 06-06-2013. Wageningen Academic Publishers, p. 62.

Haenen, D., C. **Souza da Silva**, G. Bosch, S. J. Koopmans, W. J. J. Gerrits, B. Kemp, M. Müller, and G. J. E. J. Hooiveld (2012). Resistant starch modulates colonic gene expression and plasma responses in pigs. In: Proceedings of the 5th International Dietary Fibre Conference, Rome, Italy: 07-05-2012/ 09-05-2012.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2012). Effects of fibers with different fermentation characteristics on feeding motivation in adult pigs. In: Proceedings of the 12th International Symposium on Digestive Physiology in Pigs, Colorado, USA: 29-05-2012/ 01-06-2012.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2012). Effects of dietary fibre on satiety in pigs. Wageningen, The Netherlands: WIAS (Wageningen Institute of Animal Sciences) Science Day 2012, 02-02-2012.

Pérez Gutiérrez, O., G. Bosch, D. Haenen, C. **Souza da Silva**, G. J. E. J Hooiveld, and H. Smidt (2011). Influence of dietary resistant starch on the porcine microbial composition and activity in relation to satiety. In: Proceedings of the 13th Gut Day Symposium, Wageningen, The Netherlands, 20-10-2011.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2011). Eating like a pig: Effects of dietary fibre on satiety in pigs. Wageningen, The Netherlands: Seminar Learning how to eat like a pig, 22-09-2011.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2011). Satiating properties of fermentable dietary fibre sources in adult pigs. In: Proceedings of the Oskar Kellner Symposium 2011 Metabolic Flexibility in Animal and Human Nutrition, Warnemünde, Germany: 09-09-2011/ 11-09-2011.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2011). Saciedade em fêmeas suínas alimentadas com diferentes tipos de fibras dietéticas fermentáveis. Belém, Brazil: 48a Reunião Anual da Sociedade Brasileira de Zootecnia, 18-07-2011/ 21-07-2011.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2011). Dietary fibre and satiety: identifying mechanisms by which fibre regulates satiety in adult pigs. In: Proceedings of the International Symposium "Nutrition and sustainable pig reproduction", Wageningen, The Netherlands, 09-06-2011.

Souza da Silva, C., J. E. Bolhuis, W. J. J. Gerrits, B. Kemp, and J. J. G. C. van den Borne (2011). Satiating properties of fermentable dietary fibres sources in adult pigs. In: Proceedings of the II Latin American Congress of the International Society for Applied Ethology (ISAE-LA), Ilhéus, Brazil, 21-04-2011/ 23-04-2011.

Souza da Silva, C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2011). Which fibres could enhance satiety in adult pigs? Wageningen, The Netherlands: 93rd Dies Natalis of Wageningen University, 09-03-2011.

Souza da Silva[1], C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2011). Screening the satiating properties of dietary fibre sources in adult pigs. Wageningen, The Netherlands: WIAS (Wageningen Institute of Animal Sciences) Science Day 2011, 03-02-2011.

Souza da Silva, C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2010). Screening the satiating properties of dietary fibre sources in adult pigs. In: Contributed presentations of the Annual Meeting of the Netherlands Society for Behavioural Biology, Soesterberg, The Netherlands, 25-11-2010.

Souza da Silva[2], C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2010). Behavioural tests to screen satiating properties of dietary fibre sources in adult pigs. In: Book of Abstracts of the 61st Annual Meeting of the

[1]Awarded as 'Best poster presentation', WIAS (Wageningen Institute of Animal Sciences) Science Day 2011, Wageningen University, The Netherlands.

[2]Awarded as 'Best oral presentation' by the Physiology Commission (Session 45 Theatre 3). 61st Annual Meeting of the European Association for Animal Production (EAAP), Heraklion, Greece.

European Association for Animal Production (EAAP), Heraklion, Greece: 23-08-2010/ 27-08-2010. Wageningen Academic Publishers, p. 358.

Souza da Silva, C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2010). Screening the satiating properties of dietary fibre sources in adult pigs. In: Proceedings of the 44th Congress of the International Society for Applied Ethology (ISAE), Uppsala, Sweden: 04-08-2010/ 07-08-2010. Wageningen Academic Publishers, p. 94.

Souza da Silva, C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2010). Behavioural tests to screen satiating properties of dietary fibre sources in adult pigs. Lelystad, The Netherlands: Symposium "Pigs: The missing link?", 04-03-2010.

Souza da Silva[3], C., J. J. G. C. van den Borne, W. J. J. Gerrits, B. Kemp, and J. E. Bolhuis (2010). Behavioural tests to screen satiating properties of dietary fibre sources in adult pigs. Wageningen, The Netherlands: WIAS (Wageningen Institute of Animal Sciences) Science Day 2010, 28-01-2010.

Souza da Silva, C., W. J. J. Gerrits, J. J. G. C. van den Borne, B. Kemp, and J. E. Bolhuis (2009). Behavioural tests to screen the satiating properties of dietary fibre sources in adult pigs. In: Contributed presentations of the Annual Meeting of the Netherlands Society for Behavioural Biology, Dalfsen, The Netherlands, 26-11-2009.

Other Publications

Makkink, C.A.; M. Oostindjer, C. **Souza da Silva**, and J. E. Bolhuis (2011). Leren vreten als een varken. De Molenaar 15. p. 38 - 39.

Souza da Silva, C., J. J. G. C. van den Borne, J. E. Bolhuis, W. J. J. Gerrits, M. Ooms, A. C. Bartels, and B. Kemp (2009). Varkensonderzoek helpt overgewicht bij mensen voorkomen. Boerderij 94 (27). p. 41.

[3]Awarded as 'Best young researcher' on the theme human-animal-environment by NZV (Dutch Zootechnical Association), WIAS Science Day 2010, Wageningen University, The Netherlands.

Education certificate

Education certificate

Completed Training and Supervision Plan

Description	Year
The Basic Package (3.0 ECTS[1])	
WGS course Ethics and Philosophy of Animal Science	2009
WIAS Introduction course	2010
International conferences (6.9 ECTS)	
44th Congress of the International Society for Applied Ethology, Uppsala, Sweden	2010
61st Annual Meeting of the European Association for Animal Production, Heraklion, Greece	2010
II Latin American Congress of the International Society for Applied Ethology, Ilhéus, Brazil	2011
48th Annual Meeting of the Brazilian Society of Animal Science, Belém, Brazil	2011
Oskar Kellner Symposium – Metabolic Flexibility in Animal and HumanNutrition, Warnemünde, Germany	2011
12th International Symposium on Digestive Physiology in Pigs, Colorado, USA	2012
Seminars and workshops (3.9 ECTS)	
WIAS Science Day, Wageningen, The Netherlands	2009-2013
Nederlandse Vereniging voor Gedragsbiologie PhD Workshop, Dalfsen, The Netherlands	2009
Nederlandse Vereniging voor Gedragsbiologie Annual Meeting, Dalfsen/Soesterberg, The Netherlands	2009-2010
WUR Symposium "Pigs: The missing link?", Lelystad, The Netherlands	2010
35th Animal Nutrition Research Forum, Lelystad, The Netherlands	2010
WIAS Seminar "Scientific Research in Animal Welfare: Do we make a difference?", Wageningen, The Netherlands	2011
93rd Dies Natalis of Wageningen University "Food for Health", Wageningen, The Netherlands	2011
WUR Symposium "Nutrition and sustainable pig production", Wageningen, The Netherlands	2011
WUR Seminar "Learning how to eat like a pig", Wageningen, The Netherlands	2011
Presentations (16.0 ECTS)	
IPOP AIO-meeting "Satiety and Satisfaction", Wageningen, The Netherlands (oral)	2009
Nederlandse Vereniging voor Gedragsbiologie Annual Meeting, Dalfsen, The Netherlands (oral)	2009
WIAS Science Day, Wageningen, The Netherlands (poster)	2010
WUR Symposium "Pigs: The missing link?", Lelystad, The Netherlands (poster)	2010
44th Congress of the International Society for Applied Ethology, Uppsala, Sweden (oral)	2010
61st Annual Meeting of the European Association for Animal Production, Heraklion, Greece (oral)	2010
Nederlandse Vereniging voor Gedragsbiologie Annual Meeting, Soesterberg, The Netherlands (poster)	2010
WIAS Science Day, Wageningen, The Netherlands (poster)	2011

93rd Dies Natalis of Wageningen University "Food for Health", Wageningen, The Netherlands (oral)	2011
II Latin American Congress of the International Society for Applied Ethology, Ilhéus, Brazil (oral)	2011
WUR Symposium "Nutrition and sustainable pig production", Wageningen, The Netherlands (oral)	2011
48th Annual Meeting of the Brazilian Society of Animal Science, Belém, Brazil (poster)	2011
Oskar Kellner Symposium, Warnemünde, Germany (poster)	2011
WUR Seminar "Learning how to eat like a pig", Wageningen, The Netherlands (oral)	2011
WIAS Science Day, Wageningen, The Netherlands (oral)	2012
12th International Symposium on Digestive Physiology in Pigs, Colorado, USA (poster)	2012
In-Depth Studies (9.5 ECTS)	
Trends in Stress Biology: Interpretation of Animal Stress Responses, Aarhus University, Viborg, Denmark	2011
Regulation of Energy Intake: The Role of Product Properties (6th edition), VLAG, Wageningen, The Netherlands	2012
Advanced Statistics course: Design of Animal Experiments, WIAS, Wageningen, The Netherlands	2009
Statistics for Life Sciences, WIAS, Wageningen, The Netherlands	2010
PhD Animal Welfare discussion group, WIAS, Wageningen, The Netherlands	2009-2011
Analytical Work and Possibilities within Animal Nutrition Sciences, WIAS, Wageningen, The Netherlands	2009
Statutory Course (3.0 ECTS)	
Laboratory Animal Science, Utrecht University, Utrecht, The Netherlands	2009
Professional Skills Support Courses (9.9 ECTS)	
Teaching and Supervising Thesis students, ESD&PS, Wageningen, The Netherlands	2010
Techniques for Writing and Presenting a Scientific Paper, WGS, Wageningen, The Netherlands	2010
Interpersonal Communication for PhD Students, WGS, Wageningen, The Netherlands	2010
PhD Competence assessment, WGS, Wageningen, The Netherlands	2010
Project and Time Management, WGS, Wageningen, The Netherlands	2010
Workshop Scientific Publishing, WGS, Wageningen, The Netherlands	2011
Presentation Skills, Language Services, Wageningen, The Netherlands	2011
Scientific Writing, Language Services, Wageningen, The Netherlands	2011
Lecturing, ESD&PS, Wageningen, The Netherlands	2012
Career Perspectives, WGS, Wageningen, The Netherlands	2012
Didactic Skills Training (12.9 ECTS)	
Assisting practicals within course Behaviour and Welfare Assessment	2011
Supervising 1 BSc student and 6 MSc students	2009-2012
Management Skills Training (3.0 ECTS)	
Organisation WIAS Science Day	2010-2011
Organisation WIAS Seminar "Scientific Research in Animal Welfare: Do we make a difference?"	2010
Education and Training Total	**68 ECTS**

[1] one ECTS credit equals a study load of approximately 28 hours

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