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Effect of fiber on physiochemical properties of digesta and fecal microbial composition in pigs

Amirsam Banino

Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **395**

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Handledare:
Supervisor: Emma Ivarsson
Bitr. handledare:
Assistant supervisor:
Examinator:
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Abstract

This study was conducted to investigate the effects of different dietary fiber sources on physicochemical properties of digesta and fecal microbial composition in growing pigs. Seven castrated Yorkshire pigs with an initial weight of 24.8 kg (SD 3.0) fitted with a post valve t-caecum (PVTC) cannula were used in a change-over experiment with four periods and four diets. The diets consisted of a basal part and a fibre part, including either a soluble ((Chicory (CH) and Sugar beet pulp (SBP)) or an insoluble ((Wheat bran (WB) and Grass meal (GM)) fibre source. Each experimental period was two weeks and consisted of one week for adaptation to each diet followed by one collection week. Fecal sampling occurred the four first days of each collection period and the ileal digesta samples were collected during fifth and seventh day of the second week. Overall, the effect of various diets on ileal and fecal pH were significant ($P< 0.05$). Pigs fed with GM diets had a higher ileal pH compared to pigs that were fed SBP and WB diets. Pigs fed with CH diets showed higher fecal pH compared to pigs fed with WB and GM diets. Different dietary fiber diets had no significant effect on ileal digesta viscosity ($P> 0.05$).

The effect of different type of dietary fiber on fecal and ileal dry matter (DM) was significant ($P< 0.05$). The pigs fed with SBP diet had highest fecal DM followed by pigs fed with WB diet. The pigs fed with GM and CH diets showed similar fecal DM which was lower than SBP and WB diets. The ileal DM was higher in the pigs fed with WB diet than the pigs fed with the other diets.

The effect of different type of dietary fiber diet on fecal microbial composition was analyzed by Terminal Restriction Fraction Length Polymorphyism (T-RFLP) and showed significant differences among the diets, however the total diversity did not differ due to diet ($P> 0.05$).

This study showed that pigs fed by CH, have more unique fecal microbial composition compared to the pigs fed with the other diets. TRF 160 and TRF 412 identified as *Prevotella* had higher relative abundance in pigs fed with the CH diet.TRF 275 identified as *Megasphaera elsdenii* had the highest relative abundance in pigs fed with WB diets. Generally, the effects of the studied fiber sources on physicochemical properties and gut microbiota seems to be ingredient specific. All animals stayed healthy on all diets which mean that all of our experimental dietary fiber sources can be used in pig nutrition at the inclusion level tested in our experiment.

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List of abbreviations

ADF	Acid-detergent fiber
ADL	Acid-detergent lignin
CH	Chicory
DF	Dietary fiber
DM	Dry matter
DNA	Deoxyribonucleic acid
E.Coli	Escherichia coli
GALT	Gut associated lymphoid tissue
GI	Gastrointestinal
GM	Grass meal
NDF	Neutral-detergent fiber
NDO_S	Non digestible oligosaccharides
NSP	Non starch polysaccharides
PCR	Polymerase chain reaction
PVTC	Post valve t-caecum
r RNA	Ribosomal Ribonucleic acid
SBP	Sugar beet pulp
SCFA	Short chain fatty acid
SD	Standard deviation
SEM	Standard error of the mean
TiO_2	Titanium dioxide
T-RFLP	Terminal restriction fraction length polymorphism
TRF	Terminal restriction fragment
VFA	Volatile fatty acids
WB	Wheat bran

1. Introduction

During the past years, there has been a great interest in alternative feedstuffs in pig diets with higher dietary fiber content compared to traditional crops. This shift toward the usage of alternative feedstuffs is due to their availability as a cheap byproduct and energy source, as well as their possibility to stimulate gut health and improve pig well being (De Leeuw *et al*., 2008).

Dietary fiber is generally from physiological aspects defined as the dietary components resistant to degradation by mammalian enzymes (Bach Knudsen, 2001), while they chemically are defined as the sum of non-starch polysaccharides (NSP) and lignin (Theander *et al*., 1994). NSP can further be divided into soluble and insoluble NSP, based on its solubility in water. The fiber part of each plant compromises of both soluble and insoluble NSP, the ratio of these two is an effective way to differentiate between characteristics of plants. Pigs do not have any enzymes to hydrolyze the NSP part of carbohydrates, and thus bacterial fermentation play the main role for digestion of this part of dietary carbohydrates (Choct *et al*., 2010). Inclusion of NSP in a diet stimulates bacterial fermentation that could be either beneficial or harmful to the gut environment. An anti-nutritive effect of NSP by decreasing nutrient digestion and absorption in pigs should also be considered when the dietary fiber inclusion level in a diet for beneficial effects is discussed.

Generally, for making decision about the use of new alternative dietary fiber feedstuffs to growing pigs, more knowledge and studies is needed to evaluate the effects of different dietary fiber sources on the physical and chemical properties of ileal digesta and feces as well as fecal microbial composition.

2. Literature review

2.1 Dietary fiber- definition

Despite extensive research during the last century, the definition of dietary fiber is continuously debated (De Vries *et al*., 1999; Cummings *et al*., 1997). A general agreement of the definition was stated by CODEX (2009).
CODEX defines dietary fiber as carbohydrate polymers with ten or more monomeric units, which are resistant to hydrolize by the endogenous enzymes of humans small intestine and belong to the one of following categories: Edible natural carbohydrate polymers, synthetic carbohydrate polymers or carbohydrate polymers which have been derived from food raw material by physical, enzymatic or chemical tools (De Vries, 2011).

Dietary fiber is generally from a physiological aspect defined as the dietary components resistant to degradation by mammalian enzymes (Bach Knudsen, 2001), while the chemical definition is the sum of NSP and lignin (Theander *et al*., 1994) which are the main compounds of plant cell walls (Bach Knudsen, 2001). The principle constituents of NSP are cellulose, hemicelluloses, pectins and fructans (De Leeuw *et al*., 2008). According to De Leeuw *et al*. (2008), dietary fiber includes resistant starch, non digestible oligosaccharides (NDO_S), NSP and lignin. This definition is more complete and contains the constitutes

(resistant starch and non digestible oligosaccharides (NDO_S)) that are not part of the cell wall structure but have similar physiological effects as NSP and lignin (Table 1).

In summary, the definition for dietary fiber should include and show, the constituents that give dietary fiber the unique chemical structure that characterize the physiological effect, (Kritchesvsky, 1998), like resistance to enzymatic digestion and absorption in small intestine of humans and instead be the main substrate for bacterial fermentation in the large intestine (Lunn and Buttriss, 2007; Montagne *et al*., 2003).

Table1. Schematic classification of dietary carbohydrate and lignin according to Van Soest *et al.* (1991).

<table>
<tr><td colspan="6">Dietary carbohydrates</td><td>Lignin</td></tr>
<tr><td>Digestible carbohydrates</td><td colspan="6">Dietary fibre (non-digestible carbohydrate and lignin)</td></tr>
<tr><td>Starch and sugars</td><td>Resistant starch</td><td>NDO_S</td><td colspan="3">NSP (FIBER)</td><td>Lignin</td></tr>
<tr><td colspan="3"></td><td>pectins</td><td>Fructans βglucans</td><td colspan="2">NDF</td></tr>
<tr><td colspan="4"></td><td>Hemi-cellulose</td><td>ADF</td></tr>
<tr><td colspan="5"></td><td>cellulose</td><td>ADL</td></tr>
<tr><td colspan="6"></td><td>lignin</td></tr>
</table>

2.2 Dietary fiber in plant

The functional properties of dietary fiber are usually shaped by the monomeric composition of NSP. Dietary fibers are basically classified as two physiochemical groups according to their solubility in water: the insoluble and soluble fiber (Bach Knudsen, 2001).

Plants generally contain a mixture of both soluble and insoluble fiber in a ratio that varies between species of plant and also depends on the stage of maturity of each plant (Montagne *et al*., 2003). Within the same plant the variety of cellular tissue is also important to characterize the amount and type of NSP, for example there is more insoluble fiber in husk and pericarp/testa than endosperm (Bach Knudsen, 2001).

Identical monomeric composition of a certain NSP does not mean that the NSP have the same solubility. For example, cellulose and mixed linked β-glucan are both polymers of glucose, cellulose is insoluble in water because of the presence of only one β-D -1,4 linkage while β-glucan is far more soluble in water because of the presence of interrupting β-D -1,3 linkage (Bach Knudsen, 2001). The physical and chemical locations of polymers (polysaccharides)

within the plant cell wall affect the solubility of the NSP and that affect their action in the gastrointestinal (GI)-tract (McDougall *et al*., 1996). Arabinoxylans are part of NSP that are mostly insoluble with a small portion of soluble NSP. Arabinoxylan is a hemicellulose that is composed mainly by xylose in their backbone and arabinose in their side chains (Zhong *et al*., 2000).

Pectins are the main NSP portion of cell wall in the dicotyledonous plants like chicory and sugar beet pulp. Pectins are water soluble and their main role is to functioning like cementing material in the cell walls of all plant tissues. Pectins are polymers of α-galacturonic acid with a variable number of methyl ester groups (Bemiller, 1986).

The physicochemical properties of dietary fibre that are important during the passage of the digestive tract are hydration, viscosity, water holding capacity, cation exchange capacity and absorption of organic material. These properties are linked to the type of polymers that builds up the cell wall and their intermolecular association (McDougall *et al*., 1996).

2.3 Anatomy and physiology of gastrointestinal (GI) tract in pigs

The pig's digestive tract starts with the mouth that plays an important role in mechanical digestion by reducing the feed particle size by chewing and start the digestion by secretion of saliva. The chewing mix the saliva with feed that cause salivary enzymes like amylase to start the digestion of starch to a limited extent and simplify the movement of feed through esophagus to the stomach of the pig that is a place for both digestion and storage of feed. The stomach epithelium is divided into four distinct areas with different mucosal structure and different capability to participate in the feed digestion process. The four areas are the esophagus, the cardiac, the fundic and the pyloric region. The esophagus region does not secrete digestive enzymes and is just an extension of esophagus to stomach. The cardiac gland region is the next, this is responsible for alkaline and mucus secretion as well as the mixing of digestive food and protection of the epithelial cell in an acidic environment. The fundic region is the third, and is the first part where the digestive process is started and the pepsinogen is secreted. This region is the place of hydrolic acid secretion that reduces pH to 1.5 – 2.5; this kills the bacteria that entered with the feed. The last part of stomach is pyloric region that is responsible to increase the low pH of digesta before it passes into the small intestine and some pepsinogens are also secreted from the pyloric region too (Argenizo, 1993; Högberg, 2003). The pyloric sphincter at the end of stomach regulates the amount of digesta (chyme) that passes into the small intestine and is undoubtedly an important function for proper digestion and absorption.

The small intestine can be divided into duodenum, jejunum and ileum and is the major site of nutrient absorption. The duodenum is the part of small intestine where the secretion ducts from pancreas and liver enters and their enzymes are mixed with the chyme. The cells of duodenum have exocrine ability and secreting digestive enzymes and sodium bicarbonate that are vital to breakdown of hydrolyze fats, proteins and carbohydrates in the chyme. By production of the sodium bicarbonate an alkaline environment is created that prevent damage to epithelial cell that would be caused by low pH. The jejunum is the part where break down of nutrients continues and absorption of nutrient starts. The absorption of nutrients occurs

with villi that are finger-like projection in jejunum and ileum that increase the absorption capacity. The ileo-caecal junction in the end of small intestine also decreases the passage rate that is beneficial for bile salt recirculation by active transport (Argenizo, 1993).

The watery chyme passes to large intestine that consists of the caecum and colon. Pigs have a short caecum and long colon compared to the other monogastric omnivores. The large intestine main functions are the absorption of nutrients, water and electrolytes from chyme. The large intestine is not the place of enzymatic digestion but limited microbial enzymatic activity occurred and produces volatile fatty acids (VFA) that are easily absorbed in the large intestine and contribute as an energy supplement for the pig. Bacterial action in the large intestine consists of complex populations of aerobic and obligate anaerobic bacteria (Conway, 1994) and affects the synthesis of B-vitamins, which may be absorbed and utilized by the host. The stool or waste material excreted from the large intestine via the anus includes the water, undigested food residues, digestive secretion, and separated epithelial cells from digestive tract, inorganic salts, bacteria and products of microbial decomposition (McDonald *et al*., 1995).

2.4 Dietary fiber in pig diets

The majority of feed ingredients used in pig diets have botanical origin. Thus, carbohydrates constitute quantitatively as the most important energy source for pigs (Church and Pond, 1982), and comprise approximately 60-70% of the diet, of which 14-22% is dietary fiber (Canibe and Knudsen, 2001). The impact of dietary fiber level in the diet on gut environment and digestibility may differ with fiber properties (soluble vs. insoluble) and with age (Högberg *et al*., 2006).

In the past, dietary fiber has generally been illustrated just as an anti-nutritive substance for non-ruminant animals like pigs. This theory based on their action as a effective factor on decreasing the ileal and fecal digestibility of energy and nutrients including starch, proteins and lipids that leads to increased dry matter flow and endogenous losses from both endogenous and exogenous sources (Eggum, 1995; Souffrant, 2001). However, positive effects of increasing the dietary fiber content in a diet are argued nowadays and a minimum level of fiber in a pig diet is necessary to maintain and support normal physiological GI function and gut health (Wenk, 2001). The increasing interest to use dietary fiber in pig diets is due to an economical point and animal welfare prospective. Feedstuffs with a high content of dietary fiber could be supplied as a cheap by-product from food production (De Leeuw *et al*, 2008) or as roughage. Increased dietary fiber content in a diet has the potential to enhance gut health and feeding roughage increase the natural feeding behavior of pig like rooting and chewing (Meunier *et al*., 2001), which can reduce the incidence of stereotypic behavior as well as increase reproductive performance (Meunier *et al*., 2001).

However, a too high level of dietary fiber in the diet could be harmful and cause an unbalanced GI function as well as decreased digestibility and energy value of the diet (Le Goff *et al*., 2002). The negative effect of dietary fiber as anti-nutritive portion of feed is more pronounced in chicken than in piglets and growing pigs respectively, and consequently growing pigs can tolerate a higher inclusion level (Blaak and Saris, 1995; Potty, 1996).

Soluble NSP can be degraded to a higher extent than insoluble NSP in the small intestine (Bach Knudsen, 2001) and consequently plays a more important role in the regulation of digestion and absorption in the small intestine. Soluble NSP affect the physical and chemical properties in the digestive tract by increasing the viscosity of the digesta, increasing intestinal transit time, delaying gastric emptying, delaying glucose absorption, increasing pancreatic secretion and lowering the absorption rate (Stephan and Cumming, 1980). Those changes could impair the digestibility and the nutritive content of the diet. In contrast, the insoluble fiber mainly act in the large intestine due to its physical effects with decreasing transit time and enhancing water holding capacity, increasing fecal bulk and dilution of colonic content (Stephan and Cumming, 1980). Soluble NSP mainly includes pectin and hemi-cellulose that could be digested by fermentation easier and more complete than insoluble NSP that contains mainly cellulose, lignin and hemicelluloses (Lunn and Buttriss, 2007).

2.5 Utilization of dietary fiber

The small and large intestines of pigs, are both carbohydrate digestion sites and the chemical composition of carbohydrates determines if they are degraded by enzymes or microbes (Bach Knudsen and Jorgensen, 2001). The cereal starch digestibility ranges from 84 to 100% in the end of small intestine (Bach Knudsen and Jorgensen, 2001). Although there are no enzyme secreted from stomach and upper intestinal tract of pigs to hydrolyze the glycosidic linkages in NSP, small amount of NSP is digested by fermentation of the microflora that colonizing this upper intestinal tract (Bach Knudsen and Jorgensen, 2001). Microbial fermentation produce lactic acid, short chain fatty acids (SCFA), several gases (hydrogen, carbon dioxide, methane) and heat (Bach Knudsen *et al*., 1991). The SCFA are absorbed rapidly from large intestine and contribute up to 24% of the maintenance energy supply in growing pigs (Yen *et al*., 1991).

The amount of NSP digested in the small intestine varies from -10 to 62%, and differ between types of NSP like β-glucans, arabinoxylans and cellulose (Bach Knudsen and Jorgensen, 2001).The caecum and proximal colon are the major sites of NSP degradation (Gdala *et al*., 1997). NSP degradation depends on the botanical origin of the fiber (Graham et al., 1986). For instance hemicelloluse and pectic substance are generally more completely digested than cellulose and lignified material (Bach Knudsen & Jorgensen, 2001). Drochner (1993) showed that the digestibility of isolated pectins in the large intestine of pigs are around 80 to 90%, while β-glucan actually is totally digested in the total tract (Bach Knudsen *et al*., 2001).

There are several factors known to influence the digestibility of fibers in pigs like restricted or *ad libitum* feeding, adaption, age and live weight of the pig (Cunningham *et al*., 1962 ; Henry and Etienne, 1969; Gargallo and Zimmerman, 1981), amount and type of fiber in diet (Farrell and Johnson, 1972; Gargallo and Zimmerman, 1981) and existence and level of the other ingredients like fats, sugars and antibiotics in diet (Skipitaris *et al*., 1957; Kennelly and Aherne, 1980; Gargallo and Zimmerman, 1981). Fiber from different source influences the digestibility with their variation in solubility and degree of lignifications. The dietary fiber digestibility increases with the body weight of the pig, for example adult sows have higher digestibility values than growing pigs (Noblet and Shi., 1993; 1994). These changes are due to several factors like increased ability of the bacterial flora to digest fiber because increased

amount and diversity of the bacteria, increasing transit time and generally a reduction of relative feeding level with exception for lactating sows (Dierick *et al*., 1989; Noblet and Shi 1993; 1994; Varel and Yen, 1997).

Consequently, soluble dietary fibers are generally more easily, rapidly and completely fermented when enter the large intestine compared with insoluble dietary fiber (Nyman *et al*., 1986). The degradation and fermentation of insoluble dietary fiber in the gut takes longer time and as a result the fermentation occurs in the full length of large intestine (Fernandez *et al*., 1986; Noblet and Shi, 1993).

2.6 Different sources of carbohydrates

Cereal grains like corn, wheat, oat, barely and sorghum are the main feed ingredients of pig diets in intensive energy demanding systems because of their energy and nutrient content that supply most of the pigs' requirements. Different cereals have different carbohydrate composition, therefore their influence on the digestive tract function varies. Although cereals are the main portion of the diet, they cannot satisfy all animal demands because of the lack of some protein and essential amino acids that are necessary for health and growth of pigs (NRC, 1998).

Chicory (*Cicorium intybus L.)* is a perennial herb with a high content of uronic acid that is the building block in pectin (Voragen *et al*., 2001). The usage of chicory as a palatable forage crop in sheep, deer, and cattle diets is common (Li and Kemp, 2005). Favorable traits like high mineral content and drought resistance in chicory has also been reported (Foster, 1988). Positive effect of inclusion of chicory in cereal-based diet to weaned pigs with increasing feed intake and growth performance is reported together with very small negative effect on nutrient and energy digestibility (Ivarsson *et al*., 2011). Thus, the forage of chicory is of interest as an alternative fiber sources to regular forage in pig nutrition.

Sugar beet pulp (SBP) (*Beta vulgaris)* is a by-product that has a high soluble dietary fiber level with a high portion of soluble pectin polysaccharides. SBP cell walls contain arabinogalactans and cellulose that are embedded in a pectic matrix (Bertin *et al*., 1988). This SBP homogeneous network structure and composition present high water retention capacity and hence high fermentability by colonic bacteria (Graham *et al*., 1986; Stevens and Selvendran, 1988).

Grass meal can be made from different grass plants like timothy and meadow fescue. Grass meal includes high portion of insoluble arabinoxylans and insoluble cellulose (Hayes, 2011).

Wheat bran is the rough outer layer of the wheat kernel that has a high portion of NSP as arabinoxylans and insoluble cellulose (Bach Knudsen, 1997). Wheat bran is less digestible compared to soluble dietary fibers because the existence of more structural polysaccharides (Graham *et al*., 1986; Chabeauti *et al*., 1991).

2.7 Microbes in GI tract of adult pig

The bacterial composition of the GI tract is species-specific (Moore *et al.*, 1987). The bacterial population and species composition depend on age and physiological stage of each animal and differs between gut sites with a general increase in population and composition from upper to lower of GI tract (Richard *et al.*, 2005). Different nutritional and environmental factors also affect the number and composition of bacteria (Richard *et al*., 2005).

The GI tract of pigs contains a huge and diverse microbial population that is mostly colonized by a diverse population of aerobic and facultative anaerobic (including *Escherichia coli* (*E.coli*), *lactobacilli and streptococci*) and strictly anaerobic species (Conway, 1994). The numbers of bacterial species in the different GI sites depends on the different conditions of the GI tract.

The proximal region of the GI tract in pigs harbours a microbiota that mainly consists of lactobacilli and streptococci. Different strains of streptococci are commonly found in the mouth that originates from oral cavity or feed bacterial content (Gibbson and van Houte, 1971). The stomach and proximal small intestine provide a harsh condition (acid pH and rapid transport of feed content) for bacterial growth. Thus, the acid tolerant bacterial species like lactobacilli and streptococci with relatively low numbers are dominating the stomach and proximal small intestine (Jensen, 2001) while other bacterial species like *E. coli, Clostridia, Eubacterium, Bifidobacterium, Staphylococcus, Actinomyces* and *Klebsiella* also are observed but with lower abundance (Conway, 1994; Melin, 2001). The ileum with neutral pH environment and slower feed passage rate host greater number and variety of species of bacteria (Zoric *et al*., 2002). *Lactobacilli, Streptococci, Clostridia* and *Eubacterium* are the most common species in ileum but the presence of *E.coli* and *Bacteroides* has also been observed (Conway, 1994; Jensen, 2001). The ceacum and colon host both higher number of bacteria, higher species diversity and strict anaerobic bacteria (Moore *et al*., 1987; Gaskins, 2001). This is because of slower feed passage rate and the anti-peristaltic movements in the large intestine that make a favorable environment for bacterial growth (Fonty *et al*., 1989). *Bacteroides, Prevotella, Colostridia, Lactobacilli, Streptococci, Megasphera, Sellenomona, Mitsoukella, Fusobacteria* and *Eubacteria* have been reported as dominant microbial groups of this site (Conway, 1994; Jensen, 2001).

One of the many functions of the microbiota is to cause competitive exclusions with pathogens bacteria and prevent the colonization of them in GI tract (Asplund *et al*., 1996) and another is contributing with energy supply by fermentation of ingested nutrient and production of VFA (Kass *et al*., 1980).

2.8 Methods to study the microbial composition

There are many different methods to study the existence, the amount and identity of the microbiota. These methods are usually divided into traditional methods like culturing and modern methods like molecular ecology techniques. The 16S rRNA gene sequencing, 16S rRNA hybridization and Terminal Restriction Fraction Length Polymorphyism (T-RFLP) methods are example of molecular ecology techniques.

Culturing methods are more dependent to phenotypic characterization of bacteria and could be used for both aerobic and anaerobic population of bacteria. Culturing methods need previous knowledge of nutritional and growth requirements of bacterial species and is a labor intensive method (Zoetendal, *et al*., 2004; Amann *et al*., 1995).

The new molecular techniques are alternatives to the traditional methods to classify, quantify and determine the bacterial species according to their evolutionary phylogenetic relationships. Different molecular-based techniques are choosen based on the goal of the study according to pros and cons of each method. For instance the 16S rRNA hybridization method is more suitable to identify and quantify bacterial species while T-RFLP is more suitable for comparing the bacterial community composition (Richards et al., 2005). The T-RFLP method is a marriage of three technologies including comparative genomics/RFLP, PCR, and nucleic acid electrophoresis. T-RFLP is a suitable tool to analysis whole microbial ecosystems (March, 1999). Advantages with the method is that T-RFLP is a sensitive method, the sample requirement is small, and the T-RFLP analysis is rapid and the output is digital (March *et al*., 2000). This method also have some disadvantages like the lost of information about bacterial species with less abundance than 0.05% of the total community and difficulties of identification of bacterial group that are not yet established in open databases (Zoetendal *et al*., 2004; Spiegelman *et al*., 2005).

2.9 Dietary fiber and gut health

Gut health is often referred to the balance and interaction between the diet, the commensal bacterial flora and the gut mucosa in the digestive epithelium and overlying mucus layer (Conway, 1994). A schematic picture of the gut health ecosystem (modified from Conway, 1994) is show in Figure 1.

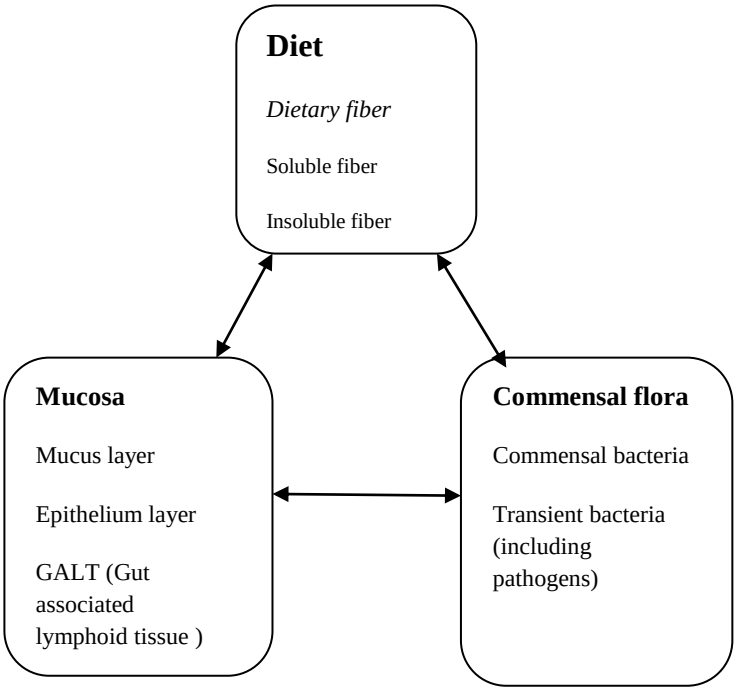

Figure 1. The interrelationship between the effective factors in gut health (modified from Conway, 1994).

The diet has a significant role in the maintenance of gut health and could have both beneficial and harmful effects by providing substrate that either prevent or increase the proliferation of pathogenic bacteria. Dietary fiber has a major influence as part of diet in this regard (Montagne *et al*., 2003) because dietary fiber is the main substrate for bacterial fermentation especially in the large intestine of pigs and interact with both the gut mucosa and the gut microbiota (Montagne *et al*., 2003).

2.10 Dietary fiber and microbial changes

External and internal environment of pigs affect the structure and function of the microbiota. The microbiota can remain in the GI tract by attachment to epithelial cells or by growing at a faster rate than the perialistic movement that washing them. Soluble dietary fiber increase the microbial population and activity in the ileum and large intestine of pigs more than insoluble DF (Wenk, 2001) because of their easier and faster fermentation in the GI tract (Nyman *et al*., 1986; Bach Knudsen *et al*., 1993).

Digesta samples from stomach of the pigs fed a diet with a high content of soluble dietary fiber showed higher diversity of cultured bacteria compared with a group fed with low dietary fiber diet content (Jensen and Jorgensen, 1994). Roca-Canudas *et al*. (2007) showed that growing pigs fed a diet including SBP as a soluble fiber source had a more stable colonial microbial diversity throughout the experimental period than pigs fed a diet including WB as an insoluble fiber source that caused a lower bacterial diversity.

Wang *et al*. (2004) showed the counts of bacteria (*coliform, yeast, lactobacilli, lactic acid bacteria and total anaerobes*) excreted in feces was higher in pigs fed WB as an insoluble source of dietary fiber, than pigs fed by SBP as a soluble dietary fiber source, whereas both SBP and WB diets had higher counts than pigs fed a standard feed.

2.11 Dietary fiber and gut mucosa

Gut mucosa include the digestive epithelium and mucosa overlying the epithelium and gut-associated lymphoid tissue (GALT). Dietary fiber interacts with gut mucosa by stimulating the gut size and physiological function, regulation of enzymatic activity and mucin secretion of GI tract.

Dietary fiber ingestion usually causes increasing size and length of small intestine, caecum and colon of pigs (Jin *et al*., 1994; McDonald *et al*., 2001; Jorgensen *et al*., 1996). Moreover, dietary fiber affects the gut epithelium morphology by changing the hydrolytic and absorptive ability of the epithelium (Montagne *et al*., 2003). Dietary fiber also provides an important energy source for epithelial cells due to a higher bacterial fermentation and hence increased production of SCFA and specifically butyrate (Pryde *et al*., 2002; Barbara *et al*., 2010).

The ability of dietary fiber to affect the gut epithelial anatomy and function seems to depend on the digesta viscosity. Increased digesta viscosity has a negative impact on the gut epithelium with increased villus cell losses that leads to villus atrophy and generally

increasing crypt depth (Montagne *et al*., 2003). Increased viscosity is more associated with a diet including more soluble dietary fiber (Montagne *et al*., 2003). However, there are research that shows completely different result and indicate that feeding high or low levels of fermentable dietary fiber have no or very small effects on the morphology of large and small intestine of pigs (Vahouny *et al*., 1986; Anugwa *et al*., 1989; McCracken *et al*., 1995; Glitso *et al*., 1998) .

Dietary fiber also interact with gut mucosa by fysical abrasion that influence on the production and regulation of mucin that is the main glycoprotein of the mucus layer that cover and protect the gut from physical, chemical and enzymatic injuries and bacterial infections (Montagne *et al*., 2003).The insoluble dietary fiber have more scratch action during their passage in digestive tract that cause an increasing mucin production (Montagne *et al*., 2003).

Dietary fiber has the ability to modulate the balance between secretion, synthesis of mucin and its composition. In the chemical structure of mucin there is a carbohydrate chain that plays an important role as a particular receptor for attachment of adhesions of pathogenic and commensal bacteria. The modification of the composition of carbohydrate chain in mucin leads to changes in its ability to attach with different commensal bacteria, this might also destroy the balance and interaction between commensal and pathogenic bacteria (Montagne *et al*., 2003). Dietary fiber increases the production of acidic mucin that is more resistant to pathogenic bacteria enzymatic attack and easing the elimination of pathogenic bacteria (Rhodes, 1989).

3. Aim of the study and hypothesis

The aim of our study was to investigate how fiber of different types; soluble (Chicory and Sugar beet pulp) and insoluble (Grass meal and Wheat bran) affect the physicochemical properties of digesta and fecal microbial composition.

Our hypotheses were:

Feeding diets with a high content of soluble fiber will in comparison to pigs fed diets with insoluble fiber result in higher ileal viscosity and water binding capacity in the intestine, a higher bacterial activity and fermentation which will result in a higher microbial diversity, and a higher production of short chain fatty acids (SCFA) which will result in a lower pH in feces and ileal digesta.

4. Material and method

4.1 Experimental design

The experiment was carried out at the Clinical center, SLU, Uppsala, Sweden from 1st of March to 24th of May 2010. The experiment was performed as a change-over experiment with seven cannulated growing pigs, four diets and four periods. Four of the pigs were randomly allocated to the four diets, and three pigs were considered as replicates. Before the change-

over experiment started, a pre-period was performed, during this time all pigs were fed the same diet.

4.2 Animals and housing

Seven castrated male Yorkshire pigs with an initial weight of 24.8 kg (SD 3.0) and a final weight of 79.4 kg (SD 8.6) were used in the experiment. Pigs were transported from Lövsta research Station, 10 km east of Uppsala to the Clinical center one week before the surgery. The pigs were fitted with a post valve t-caecum (PVTC) cannula as described by van Leeuwen et al. (1991) at an average weight of 22.8 kg (SD 0.79). The pigs were housed individually in pens (147 x 189 cm) with solid wooden walls between each pen and metal bars in front of the pens to allow eye contact between pigs. The pigs did not have access to bedding, but the pens were equipped with a rubber mat. Each pen had one water nipple and one water cup and the water was available *ad libitum* throughout the experiment. Some plastic toys were distributed in each pen as environmental compliments to avoid stereotypic behavior. The temperature was maintained at 19.2 ^{0}C (min. at 16.4 and max at 22.4), an extra heat lamp was provided in each pen until the start of period II. Artificial light was provided for 8 h/day.

4.3 Experimental diets and feeding

Four experimental diets with four different fiber sources differing in physicochemical properties and botanical origin were formulated. The four fiber sources were chicory forage (CH), grass meal (GM), sugar beet pulp (SBP) and wheat bran (WB), giving two diets with a high content of pectins (CH and SBP) and two diets with a high content of arabinoxylans (GM and WB). The chemical composition (g/kg DM) of the fiber sources used in experiment is shown in Table 2. The experimental diets were balanced to have a similar NSP content and comprised of a basal diet, and one of the dietary fiber sources, the proportion of fiber sources and basal diet are shown in Table 3. The basal diet included maize starch, casein, vegetable fat, cellulose, premix and sugar and was formulated to fulfill the minimum nutritional requirements of pigs (Evans, 1985). Titanium dioxide (TiO_2) was included in the basal portion of experimental diets as an inert marker for digestibility calculation. The basal diet was fed to all pig during the pre-period.

Table 2.Chemical composition (g/kg DM) of the fiber sources used in the experimental diets

	Wheat bran	Grass meal	Sugar beet pulp	Chicory
Dietary fiber	449	595	814	513
NSP-Total	374	426	779	349
NSP-Insoluble	286	215	216	236
Arabinose-Total	90	25	189	19
Arabinose-insoluble	83	22	90	8
Xylose-Total	148	114	14	37
Xylose-Insoluble	138	114	13	36
Uronic Acid-Total	15	35	304	171
Uronic Acid- Insoluble	13	23	39	24

Table 3. Ingredients, kg/100 kg of the basal and experimental diets

	Basal diet	CH	SBP	WB	GM
Maize starch	66.75	56.44	62.11	57.15	58.3
Casein	17.5	14.78	16.29	14.97	15.28
Vegetable fat	3	2.53	2.79	2.56	2.61
Cellulose	5	4.22	4.65	4.27	4.36
Sugar	5	4.22	4.65	4.27	4.36
Ti02	0.25	0.25	0.25	0.25	0.25
Premix	2.5	2.5	2.5	2.5	2.5
Chicory	0	15			
Sugar Beet pulp	0		6.7		
Wheat bran	0			14	
Grass meal	0				12.3

The experimental diets were fed to the pigs as a meal mixed with water (1:2) in a feed trough twice a day. Pigs were fed equal portions at 8:00 and 16:00 h daily. The feed allowance was 4% of the body weight per day until the pigs reached 60 kg then the feeding level was kept at 2.4 kg feed per day. The feed rations were adjusted weekly because the pigs were weighed once a week. The seven pigs were allocated to the different experimental diets in the order

shown in Table 4. Pig 1, 7, 3 and 4 were randomly distributed to the diets whereas pig 8, 9 and 2 were considered as replicates, pig 7 did not have a replicate.

Table 4. Feeding order of the pigs in different experimental periods

	Pig number						
	1	7	3	4	8	9	2
Pre-period	Basal	Basal	Basal	Basal	Basal	Basal	Basal
Period I	CH	SBP	WB	GM	CH	GM	WB
Period II	SBP	WB	GM	CH	SBP	CH	GM
Period III	GM	CH	SBP	WB	GM	WB	SBP
Period IV	WB	GM	CH	SBP	WB	SBP	CH

4.4 Sample collection and analytical procedure

The first week of each period was used to adapt the pigs to the new diets, and the second week was the collection period. Fecal sampling occurred the four first days (Friday-Monday) of each collection period. The samples were collected in plastic bags and immediately frozen (-20^{0}C), samples were pooled for each pig and collection period. Every Monday and Friday freshly made fecal samples, from each pig, were collected. A part of the sample was collected in eppendorf tubes and stored (-80^{0}C) until molecular analysis of the microbiota. The rest of the sample was collected in falcon tubes, placed on ice and brought to the lab. About 2 grams of each sample was mixed with 20 ml distilled water and vortex before pH was measured (PHM210 Radiometer).

Ileal digesta samples were collected during two days (Tuesday and Thursday) with one day rest between the collections. During the collection days digesta was collected at 8.00-9.00 h, 10.00-11.00 h, 12.00-13.00 h and 14-15.00 h (Thursdays) and 9.00-10.00 h, 11.00-12.00 h, 13.00-14.00 h and 15.00- 16.00 h (Tuesdays). Digesta was collected in polyethylene bags (8x30 cm) while the pigs were in the pens, no restriction of the pigs occurred during the sampling.

4.5 Viscosity and pH measurement

The first digesta samples of each collecting day, approximately 5 ml, was transferred to a falcon tube, immediately put on ice and brought to the lab for measuring of pH (PHM210 Radiometer) and viscosity. These measurements were performed within two hours from sampling, The rest of digesta samples in the collection bags was emptied in a plastic bucket and immediately frozen (-20 ^{0}C). The viscosity measurement was done by Brookfield Programmable DV-II+ Viscometer (Brookfield Engineering Laboratories Inc., Middleboro, USA) at 38^{0}C with spindle CPE-40 over the shear rate 1/s. Before viscosity measurement started, a calibration of the viscometer with standard oil (9.3 and 48.4 cP) was done at 25 ^{0}C.

The viscosity analysis started with centrifuging 2 mL of ileal digesta samples for 20 minutes at 20000 * g. In next step 0.5 mL of the supernatant from the centrifuged digesta samples were transferred to the Brookfield cup and the viscosity measurement started. The rotation speed was changed from high to low, and then back from low to high. The apparent viscosity (cP) of each digesta samples in different speed was recorded until the torque percentage was below 10% that was the lower limit for this measurement.

4.6 Dry Matter measurement

To determine the dry matter, the pooled samples of digesta and feces from each pig and collection period were mixed, weighted, freeze dried for three days and weighted again. The dry matter was calculated as the percentage of dry weight to wet weight.

4.7 T-RFLP

To monitor the influence of different dietary fiber diet on the fecal microbiota population in our study T-RFLP analysis was used.

DNA was extracted from 220 mg stool of all 70 samples (seven pigs, five period and duplicate samples) by using Qiagen mini stool DNA kit according to the manufacturer's instructions. For better result of DNA extraction, the bacteria lyses was supplemented with 3 cycles of heating of at 95 ºC for 5 min followed by quick freezing in liquid nitrogen after each heating. The extracted DNA was analysed with the T- RFLP method as previously described in detail by (Dicksved *et al*., 2008). The general steps of T-RFLP method used in the experiment are the PCR amplification of 16S r RNA genes of each DNA extract with general primers Bact-8F (5'-AGAGTTTGATCCTGGCTCAG-3') 5'end -labelled with 6-carboxyfluorescein and 926r (5'-CCGTCAATTCCTTTRAGTTT-3'), digestion of PCR products by (*Hae*III) restriction enzymes and the separation of digested fragments by capillary sequencer (ABI 3730) (Edwards *et al*., 1989; Muyzer *et al*., 1993). The next step was measurement of the size of fluorescently labeled digested fragments by comparison with the internal GS ROX-500 size standard and then the T-RFLP profiles were analyze by Peak Scanner V1.0 software. Relative peak area of each terminal restriction fragment (TRF) was found by using the formula of dividing individual peak area of each fragment on total peak area within the following size restrictions; 50 base pairs to 500 base pairs. The TRFs with a relative abundance less than 0.5% were omitted from the rest of analysis.

4.8 Statistical analysis

The statistical analysis was performed with procedure Mixed in SAS (SAS Institute, Cary, NC, USA, version 9.1). The model included diet (CH, GM, SBP, WB) and period (I, II, III, IV) as fixed factor and pig as a random factor. A carry-over effect from the previous period was tested as a fixed factor in the model, but without significance and was therefore excluded. TRFs that occurred in three or less pigs were excluded from the analysis. The effect of diet and differences between diets were tested using least square means. P-values $\leq$0.05 were considered significant.

5. Result

The pigs behaved normally in the barn during the experimental period. There was some case of lost appetite especially during the adoption period of each treatment but all animals stayed healthy on all diets.

5.1 Physicochemical properties

The effect of various type of dietary fiber diet on ileal and fecal pH of the experimental pigs are shown in Table 5.

Table 5. Effect of different type of dietary fiber on ileal and fecal ph in pig. Least square means ± standard error (s.e.)

	Diets					
	SBP	CH	WB	GM	S.E.	*P*-value
Ileal pH	6.9 ^{b}	7.1 ^{ab}	6.8 ^{b}	7.3 ^{a}	0.14	0.04
Fecal pH	6.5^{ab}	6.9^{a}	6.3 ^{b}	6.2^{b}	0.17	0.04

^{ab} Different letters in a row, indicate difference (P <0.05)

The P-value for both was P< 0.05 that means the effect of various diet on ileal and fecal pH are significant. Pigs fed with GM diets had a higher ileal pH compared to pigs fed with diets SBP and WB. Pigs fed with CH diets showed higher fecal pH compared to pigs fed with WB and GM diets.

The effect of various type of dietary fiber diet on ileal and fecal DM and ileal viscosity of the experimental pigs are shown in Table 6.

Table 6. Effect of different type of dietary fiber on ileal digesta viscosity and fecal and ileal digesta DM (%) of pigs. Least square means ± standard error (s.e.)

	Diets					
	SBP	CH	WB	GM	S.E.	*P*-value
Viscosity	1.31	1.28	1.50	1.34	0.122	0.556
Fecal DM	48^{c}	29^{a}	41^{b}	32^{a}	1.10	<0.0001
Ileal DM	8.8^{b}	8.7^{b}	15^{a}	8.7^{b}	0.60	<0.0001

^{abc} Different letters in a row, indicate difference (P <0.05).

The P-value was (P> 0.05) because of high variability of the results on ileal digesta viscosity. As a result different dietary fiber diets have no significant effect on ileal digesta viscosity.

The effect of various type of dietary fiber diet on fecal DM was significant (P< 0.05). The pigs fed with SBP diet had the highest fecal DM followed by pigs fed with WB diet. The pigs fed with GM and CH diets showed similar fecal DM which was lower than SBP and WB diets.

The effect of various type of dietary fiber diet on ileal DM was significant (P< 0.05). The ileal DM was higher in the pigs fed with WB diet than the pigs fed with other diets.

5.2 Fecal microbial composition studies

A total of 66 TRFs between 63 and 414 base pairs length were found in at least three pigs and analyzed, that 12 of these TRFs differed (P<0.05) due to diet. The TRFs that differed due to diet are shown in Table 7. The identities of TRFs are shown and investigate if their similarities to known bacteria are higher than 97%.

Table 7. Effect of diet on fecal microbial composition. Least square means ± standard error (s.e.)

			Diet					
TRF size	nearest match	Similarity%	SBP	CH	WB	GM	S.E.	*P*-value
160	***Prevotella***	**99**	**0.29 ^{b}**	**2.29 ^{a}**	**0 ^{b}**	**1.10 ^{ab}**	**0.45**	**0.017**
168	Not identified		0.38 ^{ab}	1.09 ^{a}	0 ^{b}	0.05 ^{b}	0.26	0.045
170	Not identified		0 ^{b}	2.62 ^{a}	0 ^{b}	0.06 ^{b}	0.54	0.010
199	Not identified		1.92 ^{bc}	5.50 ^{a}	1.19 ^{b}	3.82 ac	0.82	0.010
213	Not identified		0.63 a	0 a	1.97 ^{b}	0 ^{a}	0.41	0.003
261	*Prevotella*	80	3.86 ^{b}	17.89 ^{a}	1.59 ^{b}	5.51 ^{b}	0.24	0.002
275	***Megasphaera elsdenii***	**100**	**7.41 ^{ab}**	**0.37 ^{a}**	**12.93 ^{b}**	**5.92 ^{ab}**	**0.28**	**0.033**
305	*Clostridia Uncultured bacterium*	70	0.02 ^{b}	0.93 ^{a}	0 ^{b}	0.04 ^{b}	0.20	0.017
319	Not identified		1.59 ^{a}	0.12 ^{a}	3.71 ^{b}	1.46 ^{a}	0.81	0.011
408	*Porphyromonadaceae*	74	0.39 ^{b}	1.71 ^{a}	0 ^{b}	0.38 ^{b}	0.31	0.008
411	*Prevotella Uncultured bacterium*	95	0.97 ^{b}	2.57 ^{a}	0 ^{b}	0.46 ^{b}	0.52	0.021
412	***Prevotella***	**99**	**2.26 ^{b}**	**4.85 ^{a}**	**0.74 ^{b}**	**2.40 ^{b}**	**0.75**	**0.016**
Simpson's Diversity			10.06	10.23	9.99	11.54	1.44	0.851

^{abcd}Different letters in a row, indicate difference (P <0.05).

One of the TRFs that were identified is TRF 160, identified as species related to *Prevotella*, have a higher relative abundance in pigs fed diet CH than pigs fed diets WB and SBP. TRF 275, identified as *Megasphaera elsdenii*, had the highest relative abundance in pigs fed with WB diets and the lowest relative abundance in pigs fed with CH. TRF 412, identified as species related to *Prevotella*, had higher relative abundance in pigs fed CH diet than pigs fed with the other experimental diets.

6. Discussion

The effect of different types of dietary fiber on ileal viscosity was not significant. The result of our study was contrary to our hypothesis and the reports by Choct and Annison (1992) that showed increasing digesta viscosity have direct relation with soluble fraction of NSP in diets. This disagreement could be a result of other effective factors of dietary fiber that interfere with ileal viscosity like transit time, swelling, water holding capacity and fiber particle size. The other reason for no observation of viscosity change in our study could be a low inclusion of dietary fiber sources in our experimental diets. A high ileal viscosity impairs intestinal contractions and hence proper digesta and bacteria mixing (Lentle *et al*., 2008) that could be harmful for gut health (Langhout *et al*., 1999). Therefore no effect on the viscosity is a favorable result for the soluble fibre sources.

The pigs fed with the soluble dietary fiber diet CH, showed higher fecal pH compared with pigs fed with insoluble sources. That is in disagreement with our hypothesis that soluble sources cause higher fermentation activity that means higher degradation, higher production of SCFA and lower fecal pH (Bach Knudsen, 2001). Our result showed no significant difference in fecal pH between the pigs fed by SBP or WB, which is contrary to Wang *et al*. (2004) that showed a significant increase of fecal pH in pigs fed by WB compared to pigs fed SBP.

The ileal pH changes are neither following our hypothesis regarding soluble and insoluble dietary fiber. The pigs fed with WB showed lower ileal pH that is inconsistent with the fact that soluble fiber source are more fermentable and cause higher production of SCFA already in the small intestine. The disagreement of our fecal and ileal pH result with our hypothesis could have several reasons. One possible explanation can be that we did not cause big enough changes in the total microbiota. Although the diets stimulated different species of bacteria their total diversity was not changed. The activity or total bacterial numbers were not determined, but due to the fact that neither the diversity nor pH did follow any pattern related to solubility, the activity is not expected to differ between diets. Other reasons for the unexpected pH results are that some technical problems occurred with the pH electrode so this had to be changed during the measurements which also might have affected our pH results.

The fecal DM was higher in pigs fed the SBP diet that also is inconsistent with Wilfart *et al*. (2006) who showed that WB diets cause a higher fecal DM because of the faster passage rate of digesta. The disagreement between our results and the literature could be due to a low inclusion level of SBP and high digestibility of the basal diet which gives a low amount of substrate entering the hindgut.

The WB diet increased ileal DM compared to the other experimental diets, which is in agreement with our hypothesis. However, the GM diet, our other insoluble fibre source effect on ileal DM did not follow the expected pattern like WB. This might be due to separation of liquid and solid materials during the sampling that was observed only on the GM diet.

The study show we did not find any clear effect of soluble and insoluble dietary fiber diets on physicochemical properties of fecal and ileal digesta of GI tract. This could be partly due to sampling or analytical errors and high variability of results. Therefore, for reaching more accurate data higher number of samples and higher inclusion levels of the fibre sources in future studies is suggested.

The T-RFLP results that indicate the effect of different dietary fibers on fecal microbial composition showed high individual variation among the samples that is in accordance with other studies (Zoetendal *et al*., 2004; Loh *et al*., 2006). The pigs fed CH seem to have more unique fecal bacterial composition compared to pigs fed with the other diets. The abundance of bacterial species showed that each treatment stimulate the growth of different bacterial groups. The total diversity did not show any changes between different diets which is in disagreement with Roca-Canudas *et al*. (2007) who showed that wheat bran cause a lower bacterial diversity in the digestive tract than the usage of the other dietary fiber source with more soluble fractions.

TRF 160 and TRF 412 identified as *Prevotella* had higher relative abundance in pigs fed with the CH diet. *Prevotella* belong to the *Bacteroidetes* phylum that are major anaerobic bacteria species in the GI tract.TRF 275 identified as *M. elsdenii* had the highest relative abundance in pigs fed with WB diets. Overall, our result showed the effect of different diet with inclusion of different fiber source on the microbiota is not related to their fiber solubility, but was ingredient specific. The pigs fed by CH seem to have more unique fecal bacteria composition compare to the pigs fed with the other diets.

7. Conclusion

In conclusion the effects of the studied fiber sources on physicochemical properties and gut microbiota seems to be ingredient specific, where CH affected the microbiota most, with stimulation of bacteria related to *Prevotella*. Moreover, all animals stayed healthy on all diets, and the soluble fiber sources did not increase the viscosity which indicates that all the used fiber sources can be used in pig diets.

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